



## Abstracts of the 22nd Annual Meeting of the Willi Hennig Society

Dennis Wm. Stevenson

*New York Botanical Garden, Bronx, NY 10458, USA*

---

The 22nd annual meeting of the Willi Hennig Society was held at the New York Botanical Garden in the Bronx, New York from July 20–24. There were 154 participants representing 15 countries and a total of 65 talks, 16 posters, and four software presentations. There were five symposia ranging from sequence alignment to worm biology to biogeography. As with Hennig 21 at Helsinki, there was strong student participation. Student posters represented 13 of the total 16 posters and 23 of 65 oral presentations were by students. Student presentations were part of all symposia. The strength and future of the Willi Hennig Society resides in the students and recent meetings indicate a healthy society for the future.

The Student Awards Committee consisted of Jonathan A. Coddington, Arnold G. Kluge and Gunilla Ståhls. The winners were Taran Grant, the Hennig Prize (\$1000) for “Insertions and deletions in the evolution of equal-length DNA fragments”; Johannes Bergsten, the Brundin Prize (\$500) for “*Acilius* Phylogeny (Coleoptera: Dytiscidae), Problems with Long-branch attraction and Morphological Intersexual Coevolution”; and A. R. Lindgren, the Rosen Prize (\$250) for “A New Phylogeny of the Cephalopoda Using Total Evidence”.

© The Willi Hennig Society 2004.

---

### Meeting abstracts

**Comparison Between the 1928 and Current World Catalogues for False-Click Beetles (Coleoptera: Eucnemidae).** Diane Alaruikka. Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland.

A comparison between the subjectively based 1928 classification and the cladistically based current catalog, based on Muona (1993), was done for false-click beetles. Comparisons were done at the family level as well as at the subfamily level. The results of the comparison revealed that the species/genera distributions are approximately the same between the subjective 1928 world catalog and the recent classification (based on parsimony analysis and constructed using phyletic sequencing).

**Phylogenetics of Sea Spiders (Arthropoda, Pycnogonida).** Claudia Arango. Division of Invertebrate Zoology, American Museum of Natural History, New York, NY, USA.

Pycnogonids are an extraordinary and controversial group of marine arthropods of uncertain affinities: are they basal arthropods, or basal chelicerates? Their unique morphology and the meager fossil record have made difficult to resolve this issue, and the inclusion of DNA data have not yet provided robust results. The internal relationships among the lineages of Pycnogonida are also

poorly understood. The present paper shows a phylogeny for at least 20 genera of Pycnogonida based on morphological and molecular data analyzed under dynamic optimization methods. This result proposes paraphyletism in two lineages (families) and supports the hypothesis of reduction and loss of the cephalic appendages as parallel evolution events within Pycnogonida. On the other hand, although preliminary, analyses of a published arthropod data set (Giribet et al., 2001) after addition of new pycnogonid data and modification of parameters might be showing a more stable position of pycnogonids within Arthropoda.

***Acilius* Phylogeny (Coleoptera: Dytiscidae), Problems with Long-branch attraction and Morphological Intersexual Coevolution.** Johannes Bergsten\* and B. Kelly Miller. Department of Ecology and Environmental Sciences, Umeå University, Sweden; Department of Integrative Biology, Brigham Young University, Salt Lake City, UT, USA.

An ongoing taxonomic revision of the Holarctic genus *Acilius*, including the description of a new species, is accompanied with a thorough phylogenetic analysis on all presently recognized species of the genus. As all members of the subfamily Dytiscinae, males of *Acilius* possess enlarged protarsal segments equipped with large sucker-shaped adhesion discs. These are used for

gripping the female when copulating. Females of several species bear elytral and pronotal modifications of various kinds. We have argued elsewhere that these female structures are intimately connected to male tarsal characteristics and that they might be part of an evolutionary intersexual arms race. This hypothesis is here tested on the phylogeny of *Acilius*. The phylogenetic analysis is based on both adult morphological variation as well as mitochondrial (Cytochrome Oxidase I) and nuclear (Histone 3) DNA. Fresh material and thus DNA has been extracted and sequenced for all but one recognized species of the genus, thus taxon sampling is near complete even for DNA-data. The data is analyzed in two ways: (1) the DNA data is analyzed with Bayesian statistics and Markov Chain Monte Carlo methods, (2) DNA and morphological data is analyzed with parsimony, separately and ultimately in a combined analysis, under the philosophy of a total evidence combined approach. The COI-data analyzed alone gives a striking different result vs. the H3 and morphology analyses suggesting influence of the intensely debated “long-branch attraction” phenomenon. It is argued that long-branch attraction is a non-zero and very real problem in pure DNA-data parsimony analysis. The Bayesian approach, being more similar to likelihood analysis, does not suffer from the same problem but instead, just as likelihood analysis, has not yet found any justified and sound scientific foundation for its approach in the same convincing way as parsimony is firmly founded in Popperian falsificationism. Long-branch attraction artifacts can as our analysis shows be escaped from simply by adding morphological data and/or a more slowly evolving gene, to a combined analysis. Both these additional datasets reinforce the underlying phylogenetic signal in the COI dataset and together overweigh the LBA-artifact characters. The newly emerged application of Bayesian statistics to phylogenetic questions is nevertheless very interesting and has several advantages as compared to traditional likelihood analyses. Larger datasets can be analyzed in reasonable time on a personal computer and without fixed model parameter values, and clade support values are estimated simultaneously. The retrieved tree(s) was used to optimize the highly conspicuous male and female secondary sexual characters and arrive at a hypothesis of the (co)evolution of these traits across the genus. Striking male and female modifications occurred at the same nodes in the tree indicating coevolution. These characters are part of an ongoing arms race between the sexes presumably due to intersexual conflict concerning the mating rate. This is the second such example ever showing male and female morphological adaptations and counter adaptations coevolving in an antagonistic arms race as predicted by sexual conflict theory. In particular, evidence of female morphological counter adaptations is very scarce although being

critical for the theory of intersexual arms races. Diving beetles in the genus *Acilius* seem to provide one of the most elaborate and conspicuous examples of female counter adaptations.

**A New Genus and Species of Sironid from Portugal and a Morphological Phylogenetic Analysis of the Sironidae (Arachnida; Opiliones; Cyphophthalmi).** Benjamin L. de Bivort\* and Gonzalo Giribet. Department of Molecular and Cellular Biology and Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.

A new sironid genus and species from Portugal, with striking autapomorphic characters, is described based on a single male holotype. The generic classification is further justified by a phylogenetic analysis based on 46 morphological characters of 16 species of sironids, and 11 outgroup species. *Iberosiro* nov. gen. appears to be the sister taxon to the genus *Paramiopsalis* (also found in Portugal and north-western Spain). This analysis further supports the monophyly of several sironid clades, and a clade composed of *Suzukielus sauteri* plus Pettalidae. Consistent with previous studies, we find that *Metasiro americanus* (originally described as a sironid) nests outside the clade formed by Sironidae and Pettalidae. We tested the stability of these results by performing a sensitivity analysis of the clades under equal and implied character weighting. Supplementing our morphological matrix are scanning electron micrographs of the characters of all but two species used in our analysis, arranged in a fashion that allows direct comparison of characters across taxa.

**Phylogeny and Evolution of the Arhynchobdellida (Annelida: Oligochaeta: Hirudinida).** Elizabeth Borda\* and Mark E. Siddall. Department of Biology, Graduate School and University Center, City University of New York, NY; Division of Invertebrate Zoology, American Museum of Natural History, New York, NY, USA.

Unlike proboscis-bearing leeches, the Arhynchobdellida are characterized by their possession of muscular “jaws” used for feeding or cutting through skin. Arhynchobdellid leeches are among the most widely recognized and frequently encountered in historical and contemporary medical applications of bloodletting. The phylogenetic relationships, using parsimony analysis, of the Arhynchobdellida, were investigated using a total of 3644 characters, including nuclear 18S and 28S rDNA, mitochondrial 12S rDNA and cytochrome c oxidase subunit I sequence and morphological data. Thirty-nine arhynchobdellid species were chosen to represent the seven currently recognized families. Outgroup taxa included 16 species from the Glossiphoniidae and Piscicolidae. Analysis of all available data resolved one most-parsimonious tree. The cladogram yielded an hypothesis conflicting with most traditional classifications of the Arhynchobdellida. Monophyly of the

Erpobdelliformes and Hirudiniformes was supported. However, the families Haemadipsidae, Haemopidae, Hirudinidae, and the genera *Hirudo* and *Aliolimnatis* were polyphyletic. An additional 5, 112 and 108 steps, respectively, were required to make the families monophyletic. The results provide insight on the phylogenetic positions for some unusual families (Americobdellidae and Cylicobdellidae), the genera *Semiscolex*, *Patagoniobdella*, *Mesobdella*, *Xerobdella*, and genera traditionally classified under Hirudinidae. A taxonomic revision of Arhynchobdellida is proposed and evolution of life-history traits is examined.

**Systematics and Biogeography of New Zealand Harvestmen of the family Pettalidae (Arachnida, Opiliones, Cyphophthalmi).** Sarah L. Boyer\* and Gonzalo Giribet. Department of Organismic and Evolutionary Biology, and Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.

Cyphophthalmi are tiny harvestmen found in leaf litter habitats world-wide. Within this suborder, the family Pettalidae shows a typical Gondwanan distribution with a remarkable radiation in New Zealand, which is home to 60% of all described pettalid species. The poor dispersal ability of these small, slow arachnids makes them an ideal group for biogeographic studies. Preliminary results of phylogenetic analysis of Pettalidae are presented, and questions about the relationship of New Zealand species to those in Queensland, Australia are addressed. Within New Zealand, relationships of species from the North Island, South Island, and Stewart Island are explored. In addition, the status of the pettalid genera *Rakaia* and *Neopurcellia* (both of which are found both in New Zealand and in Queensland, Australia) is addressed. Sources of data include over 30 morphological characters derived from SEM and light microscopy, in addition to molecular sequences. Molecular data include sequences from 18S rRNA, 28S rRNA, 16S rRNA, cytochrome c oxidase subunit I, and histone H3. Data were analyzed via direct optimization under different analytical conditions.

**Placing a Fossil Taxon Using Phylogenetic Methods: Comparisons Across Life Stages.** Marc A. Branham. Division of Invertebrate Zoology, American Museum of Natural History, New York, NY, USA.

A phylogenetic analysis is used to place a fossil firefly larva among the present taxonomic subdivisions of the family Lampyridae. This analysis contained fossil and extant taxa as well as suites of morphological characters from different life stages (adults and larvae). As is generally the case in most groups, larvae are poorly known and well-preserved fossils of any life stage are rare. In addition, when including characters from multiple life stages in a total evidence analysis, large amounts of missing data can be introduced. Even

though a considerable amount of missing data was present in this combined analysis (~30% of the matrix), it did not drastically degrade the resolution of the consensus tree and the resulting tree contributed to placing the fossil larva.

**Identification and Characterization of Markers for Assessing the Genetic Diversity of Threatened Leech Populations.** Rebecca B. Budinoff\* and Mark E. Siddall. Cornell University, Ithaca, NY, USA; American Museum of Natural History, New York, NY, USA.

*Hirudo medicinalis*, the European medicinal leech, is threatened in light of 200 years of over-exploitation for medicinal and microsurgical uses. We are interested in assessing the genetic diversity of this species in order to better understand the effects and consequences of over-harvesting. Data from mitochondrial loci are suggestive of a lack of diversity in that genome such that microsatellite data may be better suited to our goals. We have successfully developed the first microsatellite library for an annelid, specifically for *Macrobdella decora*, the North American medicinal leech. This widespread species, which is not threatened in the wild, will serve as a test case prior to evaluation of the European counterpart. Primers generated from our microsatellite loci have found substantial variation within populations of *Macrobdella decora* from Ontario, Michigan and elsewhere. Our success in assessing genetic diversity in *Macrobdella decora*, to which we have ease of access, is promising for our goal to do the same for the real species of interest, the more elusive *Hirudo medicinalis*.

**Phylogeny of the Hirudinida (Annelida: Oligochaeta) and Evolution of Life History Strategies.** Eugene M. Bureson\* and Mark E. Siddall. Virginia Institute of Marine Science, VA, USA; American Museum of Natural History, New York, NY, USA.

Phylogenetic analysis based on 18S rDNA and mitochondrial cytochrome c oxidase support monophyly of the oligochaete order Lumbriculida with Acanthobdellida, Branchiobdellida and Hirudinea. These results suggest that leeches, branchiobdellidans and acanthobdellidans should be regarded as orders of Oligochaeta equal in rank to their closest relatives, the order Lumbriculida. Cladistic analyses of 33 leech species using molecular and morphological data supported most of the traditional higher taxa based on morphology alone. Exceptions are paraphyly of the rhynchobdellids, polyphyly of the hirudinids and problems with generic assignment in the erpobdellids. Blood-feeding behavior has been lost four times in the course of leech evolution. Evolution of the exploitation of terrestrial and aquatic habitats, vectorology, cocoon deposition and parental care will be discussed in relation to phylogeny.

**Odonate Phylogeny: A Preliminary Molecular Estimate.** Seth M. Bybee\*, T. Heath Ogden and Michael F. Whiting. Department of Integrative biology, Brigham Young University, Provo, UT 84602, USA.

Higher-level phylogenetic relationships within Odonata have been controversial for many years. Relationships among the three recognized extant suborders of Odonata are still debated, as are the phylogenetic relationships of the constituent families. Although some molecular sequence data have been generated for odonates, these data have been confined primarily to the suborder Anisoptera and consist of mitochondrial markers. DNA sequence data for six genes (12S rDNA, 16S rDNA, 18S rDNA, 28S rDNA, COII, and Histone 3) were sequenced for 90 taxa representing major odonate lineages. These data were analyzed via optimization alignment and provide new insight into the evolution and diversification of odonates.

**Diversity and Evolution of the Insect Mitochondrial Genome.** Stephen Cameron\*<sup>1</sup>, Michael Whiting<sup>1</sup> and Stephen Barker<sup>2</sup>. <sup>1</sup>Department of Integrative Biology, Brigham Young University; Department of Integrative Biology, Brigham Young University, Salt Lake City, UT, USA; <sup>2</sup>Department of Microbiology and Parasitology, University of Queensland, Queensland, Australia.

Despite being one of the better-studied groups of invertebrates for mitochondrial genome structure, huge gaps in our knowledge of genome diversity and evolution still exist for the insects. Complete genome sequences are available for only 10 of the 30 orders (Zygentoma, Orthoptera, Hemiptera, Thysanoptera, Psocoptera, Phthiraptera, Coleoptera, Hymenoptera, Lepidoptera and Diptera). Here we present the initial results of the first systematic study of mitochondrial genome diversity within the Insecta. Genomes were sequenced for 10 additional orders: Archaeognatha, Phasmatodea, Plecoptera, Grylloblattodea, Isoptera, Mantodea, Blattodea, Neuroptera, Megaloptera, and Mecoptera. The genomes of Plecoptera, Neuroptera, Megaloptera and Mecoptera match the consensus arthropod gene arrangement. Archaeognatha matches the consensus arrangement except for the deletion of two tRNA genes. Phasmatodea matches the consensus arrangement except for the deletion of one tRNA gene and its replacement by another tRNA codon type. Grylloblattodea has an enlarged genome due to the duplication of a large block of genes. Isoptera differs from consensus only by loss of two tRNA genes. Mantodea displays the first verified example of gene conversion in animal mitochondrial genomes; a single sequence encodes both a protein coding gene and a tRNA. Blattodea has two mitochondrial haplotypes that lead to ambiguities in the location of two tRNA genes and a single protein-coding gene. The evolution of genome structure will be compared against consensus

phylogenies of the insects to determine the phylogenetic utility of gene order characters.

**Molecular Phylogenetics of the Microcell Oyster Parasites: New Perspectives on a “Closely Related” Group of Species.** Ryan B. Carnegie\*<sup>1</sup>, Susan M. Bower<sup>2</sup> and Gary R. Meyer<sup>2</sup>. <sup>1</sup>Virginia Institute of Marine Science, Gloucester Point, VA, USA; <sup>2</sup>Fisheries and Oceans Canada, Science Branch, Pacific Biological Station, Nanaimo, British Columbia, Canada.

Oyster parasites of the genera *Mikrocytos* (*Mikrocytos mackini* and *M. roughleyi*) and *Bonamia* (*B. ostreae* and *B. exitiosus*) were long thought “closely related” because they look alike at the light microscope level. Primary forms are naked, uninucleate, 2–3 µm cells that have a “fried egg” appearance in histopathological sections.

*M. roughleyi* and *B. exitiosus* indeed resemble *B. ostreae* ultrastructurally and share with *B. ostreae* a specificity for oyster hemocytes. Recent molecular phylogenetic analyses confirmed the close relationship of these three species, and placed them in the Haplosporidia. *Mikrocytos mackini*, however, infects vesicular connective tissue cells and, alone among the microcells, possesses neither haplosporosomes nor mitochondria. We examined the relationship of *M. mackini* to *Bonamia* spp. and the Haplosporidia by analyzing a 1457 bp fragment of *M. mackini* SSU rDNA using maximum parsimony and evolutionary distance analyses. These analyses excluded *M. mackini* from the Haplosporidia and from every other phylum from which SSU rDNA sequences have been described, placing it alone near the base of the eukaryotic tree. *Mikrocytos mackini* may prove to be secondarily amitochondriate and attracted to the base of the tree by virtue of its divergent SSU rDNA. For now, we can only conclude that this species does not appear to be a haplosporidian. While *Bonamia* spp. and *M. roughleyi* may be described as “microcell haplosporidians”, the term “microcell” otherwise has no phylogenetic significance.

**Relationships and Biogeography of Antillean Cichlids.** Prosanta Chakrabarty. Fish Division, Museum of Zoology, University of Michigan, Ann Arbor, MI, USA.

Geological reconstruction proposed for the Greater Antilles fit into two major categories, each with different biogeographic consequences. One category of reconstructions suggests that Early Oligocene landbridge connected the Greater Antillean islands to northern South America. The other suggests a period of coalescence in the Mesozoic of some of the Antillean islands with portions of Central America. A molecular phylogenetic analysis that includes the endemic cichlid faunas of the Greater Antilles, as well as from South and

Central America, results in a phylogeny that is congruent with the later reconstruction. Also, this analysis finds that a dispersal scenario is not the simplest or most robust explanation for the presence of these cichlids on the Antilles.

**Phylogenetic and Functional Analysis of the Glutamate-Receptor-Like Gene Family in *Arabidopsis thaliana* (AtGLR).** Joanna Chiu\*, Eric Brenner and Gloria Coruzzi. New York University, New York, NY, USA.

We have identified a family of putative glutamate receptor genes in *Arabidopsis thaliana* (*AtGLR*). *AtGLRs* are similar to animal ionotropic glutamate receptors (iGluRs) in both primary and secondary structures. Their sequence similarity spans all the important functional domains. Whereas animal glutamate receptors function in synaptic transmission, preliminary data suggest that *AtGLRs* may be involved in light signal transduction and calcium homeostasis in plants. Upon the completion of the *Arabidopsis* genome-sequencing project, the *AtGLR* gene family is found to consist of 20 members. By using the amino acid sequences of all 20 *AtGLRs*, all rat iGluRs, as well as the first identified prokaryotic cyanobacteria GluR, we performed a comprehensive parsimony analysis that divides the 20 *AtGLR* genes into three clades. This parsimony analysis is used as the basis for a recently established nomenclature for the *AtGLR* gene family. Based on RT-PCR analyses as well as analyses of transgenic plants transformed with promoter-reporter gene constructs, genes from the three *AtGLR* clades have overlapping expression patterns. To examine the *in vivo* role of the different clades of *AtGLR* genes, we have generated overexpressor as well as RNAi constructs for *AtGLR* genes from each of the three clades. Whereas *GLR1.1* overexpressors do not show any phenotypic alterations when compared to wild-type plants in different growth conditions, *GLR2.2* and *GLR3.1* overexpressors appear to be hypersensitive to ionic stress. On the other hand, knocking out specific *GLR* genes by RNA interference have pleiotropic effects in growth and development.

**Phylogenetic Relationships of the Arcoidea: Initial Results Using Morphological Characters.** Louise Crowley. Graduate School and University Center, City University of New York and American Museum of Natural History, New York, NY, USA.

The Arcoidea is an extant order of bivalves with more than 300 species. These bivalves are characterized by possession of a shell microstructure suited to form a strong, interlocking hinge and a duplivinicular ligament. This is an interesting group in light of its occupying a wide range of habitats at all latitudes and depths ranging from the low water mark to the deep ocean floors. Relationships among the families of the

Arcoidea are not well understood. There have been many alternative classifications proposed for this group. While most taxonomic studies divide the order into two superfamilies: Arcoidea and Limopsoidea, others have raised the three limopsoidean families of Wilson (1998) to superfamilial status. Moreover, while recent molecular analyses of the Bivalvia support the monophyly of Arcoidea, they do not support the monophyly of the superfamily, Arcoidea, which is paraphyletic with respect to Glycymeridoidea. A preliminary cladistic analysis drawing from classic shell characters such as hinge dentition, shell sculpture and shell form and from anatomical characters such as gill and foot structure revealed interesting correspondences with classical taxonomy. Ultimately, this study will involve a comprehensive phylogenetic analysis of the Arcoidea and will explore the interrelationships of this order.

**How Many Species of Elephants in Africa? Appraisal of an Apparent Controversy Between Molecular Phylogenies.** Régis Debruyne. Muséum National d'Histoire Naturelle Département Histoire de la Terre, UMR CNRS 8569 8, Paris, France.

The systematics of the African elephant, *Loxodonta africana*, has become an issue since the publication of the first mitochondrial sequence of a forest elephant (Barriel et al., 1999). The then acknowledged subspecies, *L. africana cyclotis* (Matschie, 1900), displayed a high level of divergence the better-known savannah elephant (*L. africana africana*). Further molecular investigations have led authors to consider that forest and savannah elephants consist of two distinct species (Comstock et al., 2002; Roca et al., 2001). They rely on an extensive sampling in Africa, though poorly documented for western populations and areas where both forms of elephants co-occur. They assayed the genetic diversity through nuclear markers, sequencing of four nuclear genes and microsatellites that converge to the recognition of two highly divergent clades they assume to match the systematic division. Conversely, the analysis of 593 bp of mtDNA from a broader sample produced results at odds (Eggert et al., 2002). The reciprocal monophyly of the two lineages is not retrieved and African elephants cluster in three main groups (western, central and south-eastern), whatever their systematics. However, these results are weakly supported. Here we address the validity of these inconsistent results by reanalyzing available data and sequencing 1960 bp of mtDNA for 43 African elephants (morphologically determined as 26 savannah and 17 forest elephants). The sequenced fragment spans cytochrome b gene, Thr and Pro tRNAs and Hyper-Variable Region 1 of the control region. Fifteen sequences of Hyper-Variable Region 2, Phe tRNA and 12S rRNA were also examined. Our phylogenetic

analyses contradict both former studies. Firstly, they show that two unambiguously supported clades do exist within African elephants populations, Eggert's results (Eggert et al. 2002) being caused by an insufficient sampling of characters and a misleading attitude in the choice of the evolution model of their sequences. Secondly, this division into two main clades does not recover savannah and forest elephants as monophyletic taxa, so that they are not monophyletic species. While 21 sequences of savannah form a monophyletic group, the 5 remnants are apart within remote clades of forest elephant sequences. This conflicts with Roca et al.'s view (2001) that hybridization between the two forms can be neglected. Nevertheless, we confirm some former conclusions. Forest elephants display a stronger inner-structuration than savannah elephants. We acknowledge that some savannah elephants are mixed-up within forest elephant clades, but reciprocally, no forest elephant specimen depicted a typical savannah haplotype, so that the introgression of mitochondrial genome appears to be unidirectional. We assume that this extant phylogeographic pattern is the result of non-contemporary phenomena: (i) a prior isolation of forest elephants on the one hand and of savannah elephants on the other, leading to their molecular and morphological divergence, and (ii) secondary recurrent events of local admixture between the two forms, responsible for the geographic consistency of shared haplotypes between *L. a. cyclotis* and *L. a. africana*. Therefore we conclude that a high level of hybridization between the two forms of African elephant (with long-term effects on the genetic integrity of populations) precludes the recognition of two biological species.

**When One and One is Not Two: Parsimony Analysis of Sequence Data.** Jan De Laet. Royal Belgian Institute of Natural Sciences, Brussels, Belgium.

When analyzing DNA sequence data, it is common practice to create a multiple alignment prior to tree search, an approach that imposes unnecessary and unwarranted constraints on the analysis. Methods that do not require prior alignment have been available for over a decade. Such methods rely on a cost matrix that specifies the costs of base substitutions and on a gap function that specifies the costs of indel events. It is generally believed that these costs can only be specified or interpreted with reference to the evolutionary processes that generated the data. Observing that minimum mutation trees are not necessarily the trees that maximize similarity that can be interpreted as secondary homology, it is argued that setting substitution and gap costs such that they maximize secondary homology is the proper way to extend parsimony analysis to non-prealigned sequences instead. Some properties of this method are discussed.

**Are Hexapods Monophyletic? Or Why Would Anyone Spend So Much Money to Sequence Complete Genomes and Finally Discard 97% of It?** Cyrille A. D'Haese. FRE 2695 CNRS, Muséum National d'Histoire Naturelle, Paris, France.

Basal hexapod relationships have generated much debate. In particular, the relationships of primitively wingless hexapods in relation to winged insects (pterygotes), and the position of hexapods in arthropod remain equivocal. A recent paper based on complete mitochondrial genomes analysis went further and yielded to the rejection of hexapod's monophyly with Collembola rooted in crustaceans hence assuming several independent adaptations to life on land. That paper is discussed and new evidence is presented to address the question of hexapod phylogeny and their adaptation to terrestrial environment.

**Progress in Understanding the Phylogeny of the Juncaceae: Inferences from the Cladistic Analysis of Plastid Data Set.** Lenka Drabkova\*, Jan Kirschner and Cestmir Vlcek. Institute of Botany Academy of Sciences of the Czech Republic, Pruhonice near Prague, Czech Republic; Center for Integrated Genomics and Institute of Molecular Genetics, Academy of Sciences, Prague 6, Czech Republic.

The Juncaceae is a cosmopolitan family comprising seven genera and 442 species. Relationships within *Juncus* and *Luzula* and among the genera of Juncaceae (*Distichia*, *Marsippospermum*, *Oxychloë*, *Patosia*, and *Rostkovia*) are still unclear. RbcL sequence data solved a part of supraspecific phylogeny, but a few positions remain polytomic. For this reason, non-coding cpDNA regions, trnL intron and trnL-trnF intergenic spacer, were sequenced. In addition to creating hypotheses of relationships within Juncaceae and testing classification of the sections, a primary goal to this study was to assess relationships within *Juncus* and *Luzula*, confirmed monophyletic groups and the position of small Andean genera within the *Juncus* clade. Several separate analyses of rbcL data set and non-coding trnL intron plus trnL-trnF intergenic spacer and then combined data into one dataset consisted of 3677 characters were performed. For each individual dataset, (a) rbcL (b) trnL-F based on substitutions only (c) trnL-F based on substitutions and indels, were calculated consensus tree and basic statistic characters. Parsimony jackknife analyses were performed using PAUP 4.0 Beta 10 under the different criteria (with and without branch-swapping and multiple random addition sequences, etc.). To increase the likelihood of having explored all possible islands of shortest trees, the Ratchet procedure in WinClada 1.00.08 was applied. In summary, parsimony analyses revealed several well-supported clades. Traditionally distinguished genus *Luzula* is monophyletic and *Juncus* is non-monophyletic. *Juncus trifidus* appears in very unusual separate

position on the tree, as a sister group to *Luzula* (however, with less than 50% jackknife support, rbcL data) or as a basal taxon for whole Juncaceae according to trnL-F data, which is supported 86% and seems to be much more probable. Southern Hemisphere Clade forms not only the five Andean genera, but also it is clustered together with South African *J. lomatophylus* and *J. capensis*. Within the *Juncus* and *Luzula*, the monophyly of a number of groups was shown (e.g. sect. *Stygiopsis*, sect. *Luzula*) and the monophyly of others (e.g. sect. *Graminifolii*, subg. *Pterodes*) were questionable.

#### 18S rDNA Phylogeny of Oligochaetous Clitellata.

Christer Erséus and Mari Källersjö\*. Swedish Museum of Natural History, Stockholm, Sweden.

The phylogeny of Clitellata is analyzed, using 18S rDNA sequences of a selection of species, representing 10 oligochaetous families, Hirudinida (leeches), and the leech-like groups Acanthobdellida and Branchiobdellida. Eleven new 18S sequences of Capilloventridae (1), Haplotaxidae (1), Propappidae (1), Enchytraeidae (2), Lumbricidae (1), Almidae (1), Megascolecidae (2), Lumbriculidae (1), and Phreodrilidae (1) are reported and aligned together with corresponding sequences of 28 previously studied clitellate taxa. Twelve polychaete species are used as an outgroup. The analysis supports an earlier hypothesis based on morphological features that Capilloventridae represents a basal clade of Clitellata; in the 18S tree it shows a sistergroup relationship to all other clitellates. The remaining clitellate taxa form a basal dichotomy, one clade containing Tubificidae (including the former “Naididae”), Phreodrilidae, Haplotaxidae, and Propappidae, the other clade with two subgroups: (1) Lumbriculidae together with all leech-like taxa (Acanthobdellida, Branchiobdellida and Hirudinida), and (2) Enchytraeidae together with Crassicitellata (a taxon comprising all earthworms with a multilayered clitellum). The results support the hypothesis that the first clitellates were aquatic.

#### Sensitivity Analysis: Does it Measure Robustness?

James S. Farris. Molekylärsystematiska Laboratoriet, Natuhistoriska riksmuseet, Stockholm, Sweden.

Not in general. The approach is defensible only if combined with sincere attempts at falsification. Commendable in its brevity, no?

**So Many Worms, So Little Time...** Kristian Fauchald. Section of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

Annelida has recently undergone several major revisions at higher taxonomic levels, and have absorbed a couple of related phyla, with more to come. The evidence has been in part morphological, in part based on molecular sequences. Other kinds of evidence

such as larval/embryonic development and various ecological data have yet to be evaluated for their potential contribution to our understanding of the relationships among the taxa. A loosely organized network of interested scientists is being planned to assist in collating scattered information to guarantee that new studies are based on all available information, not only on what is most readily available. Furthermore, all results of potential importance for phylogeny should be vouchered in a permanent collection, so that the identification can be checked, and so that new information can be gathered on these specimens in the future.

**Reference.** Nico M. Franz. Cornell University, Ithaca, NY, USA.

Typically in arguments about the adequacy of alternative methods for phylogeny reconstruction, a lot of attention is directed towards the performance of each method in an increasingly large space of substitution processes. At least in simulation it is evident that inconsistencies in the abundance and directionality of evolutionary rates affect the ability of algorithms to recover previously specified phylogenies. The extent to which this is critical to practice must be determined empirically. Another set of arguments has been motivated by more epistemological considerations (e.g. “can we model evolution?”). Yet a discipline as diverse and dynamic as epistemology can hardly offer an immediate response to such questions. With this in mind, it might be informative to contrast the properties of methods with respect to a third criterion: how they align with our traditional use of language in systematics. The contention is that throughout the centuries, systematists have provided an observational terminology that continues to facilitate all kinds of inferences in biology in general. The ability of statements of (global or local) homology to refer (approximately) to characteristics of members of clades is instrumental in this context. Presumably, the precision and reliability of reference is correlated with the causally sustained properties of the corresponding evolutionary processes. By virtue of assigning probabilities to statements, contemporary implementations of likelihood inference are particularly well suited for accommodating imprecision in assessments of homology. This signifies that clades might be recovered in spite of our inability to propose and refine non-numerical terms that refer to the evolutionary properties of their members. In the case of inference under the criterion of parsimony, imprecision can be communicated through multiple equally parsimonious hypotheses of homology and phylogeny. But the contribution of reconstructions to efficient reference decreases rapidly with the number of alternatives. It will be suggested that the limitations of parsimony and those of traditional (verbal) reference are related in systematics.

**Relative Effects of Increasing Genetic Distance on Alignment and Phylogenetic Analysis.** John V. Freudenstein\* and Mark P. Simmons. Herbarium, Department of Evolution, Ecology and Organismal Biology, Ohio State University, Columbus, OH, USA; Department of Biology, Colorado State University, Fort Collins, CO, USA.

The distance between terminals for DNA sequence data can affect phylogenetic reconstruction in two ways. Problems with homology statements at the character level (errors in alignment) and problems with state-level homology due to multiple hits on particular branches. We examined these effects on the analyses of a number of nuclear ITS datasets by successive removal of sequences followed by realignment and parsimony analysis, and distinguished these effects from one another to the extent possible. Analyses that incorporated the full set of terminals were taken as our reference points. Differences in alignment seen with increasing genetic distance (progressively less dense taxon sampling) were largely due to inserting too few gaps and to their differing placement, relative to the full data set. However, problems due to multiple hits were more severe, affecting tree resolution, topology, and branch support, and suggesting that increasing distance among terminals may be more of a problem for the reconstruction of character transformations (cladistic analysis) than for alignment. Increasing taxon sampling remains the best strategy for alignment and cladistic analysis.

**Alignments and Other Comparisons of Sequence Data in Phylogenetics.** Gonzalo Giribet. Department of Organismic and Evolutionary Biology, Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.

Alignments or other types of comparisons of nucleotides across the terminals utilized in a phylogenetic analysis are viewed as an unavoidable necessity. Trivial alignments, those that are optimal under all analytical conditions, are fewer than commonly thought, especially when dealing with organisms that diverged from a common ancestor many Mya, or with fast evolving loci. Analyses of sequences with length variation are common, but obscurely presented in articles and scientific meetings. In the case of ribosomal gene alignments, “secondary structure-based” alignments are often invoked, although not explicitly explained. Concern has also been raised about coding indel events as 5th character states, and alternate codings as independent characters have been proposed instead. These alternatives are often based on criticisms to alignment methods not able to deal with secondary structure information or non-linear gap costs. Here I will show how this is not true in the case of dynamic homologies as inferred through the direct optimization (DO) method of Whee-

ler (1996; *Cladistics* 12: 1–9). Furthermore, the utilization of DO allows explicitness, repeatability, and easily incorporates data exploration via sensitivity analysis, and unlike “a posteriori” indel recoding techniques, it does not require prealigned matrices. DO and its family of related methods based on single-step phylogenetic analysis emerge as the best way to generate phylogenetic hypotheses based on unaligned DNA sequence data.

**Relationships and Phylogenesis of Host Plant Associations and Larval Behaviors within the Apameini (Lepidoptera: Noctuidae): Results from Molecular Data.** Paul Z. Goldstein. Field Museum of Natural History, Chicago, IL, USA.

With over 40 000 described species, the Noctuidae comprise the largest and one of the most systematically troublesome lepidopteran families. Having been placed in as many as five noctuid subfamilies during the last two decades, moths in the tribe Apameini represent a systematic lynchpin for understanding noctuid relationships and a touchstone for exploring the evolution of host plant associations, host specialization, and larval life histories. The Apameini comprise a diverse assemblage of subterranean cutworms and endophagous borers, many of which are associated with grass-dominated ecosystems. Expanded analyses covering a broad taxonomic range among apameine genera and putatively related groups support the monophyly of Apameini *sensu stricto* and indicate significant instances of paraphyly and polyphyly among apameine genera corresponding to observations in ongoing morphological studies. Phylogenetic analyses also suggest a single origin of endophagy followed by reversals and a primitive association with grasses and other monocots followed by subsequent radiations of exophagous and endophagous groups. Such broad-spectrum host shifts also correspond to shifts in life history strategies, most notably shifts in over-wintering diapause, a phylogenetically conservative trait in this and other insect groups.

**Cases in Which Bayesian Phylogenetic Analysis Will Be Positively Misleading.** Pablo A. Goloboff<sup>1</sup> and Diego Pol<sup>2</sup>. <sup>1</sup>Instituto Superior de Entomología Dr Abraham Willink and CONICET, SM. de Tucumán, Argentina. <sup>2</sup>American Museum of Natural History, New York, NY, USA.

Bayesian phylogenetic analysis using Monte Carlo Markov chains has become very popular in certain circles. It is based on estimating the likelihood surface of different tree topologies; the prior probabilities of different trees are generally considered as identical, with which one would expect the Bayesian analysis to produce results identical to those of standard maximum likelihood. However, there are significant differences in the results. Supposedly, Bayesian phylogenetic

analysis allows assigning confidence to hypotheses of monophyly of groups without ever finding the individual trees of highest likelihood (maximum posterior probability), thanks to the use of Monte Carlo simulations. This creates serious problems when the likelihood surface has certain characteristics. Thus, if data are generated under a given model and tree, the Bayesian analysis of the data may lead to infer that there is a high posterior (greater than 90 or 95%) of the existence of certain groups not present in the model tree. The proof of consistency of the maximum likelihood estimates does not apply in this case; those demonstrations refer to the individual trees of maximum likelihood, and Bayesian phylogenetic analysis calculates (estimates) the probability of monophyly of a given group as the sum of the likelihoods in different trees with the group. Bayesian phylogenetic analysis would be consistent if the individual tree of maximum likelihood was used, but in this case Monte Carlo Markov chains (which produce the “speed” that made the method so popular) cannot be applied and one is forced to go back to standard trial-and-error techniques to find optimal trees. In the case of likelihood, those techniques are extremely slow and inefficient.

**TNT.** Pablo A. Goloboff<sup>1</sup>, James S. Farris<sup>2</sup> and Kevin C. Nixon<sup>3</sup>. <sup>1</sup>CONICET, INSUE, Instituto Miguel Lillo, SM. de Tucumán, Argentina, <sup>2</sup>Molekylarsystematiska laboratoriet, Naturhistoriska riksmuseet, Stockholm, Sweden, <sup>3</sup>L.H. Bailey Hortorium, Cornell University, Ithaca, NY, USA.

An upgrade of the program TNT is presented. The program implements new branch swappers, which are significantly faster than the ones in use before. For example, completing a round of TBR branch swapping in Källersjö et al.’s 2500-taxon matrix, takes about 50 times shorter than in PAUP\*. Aside from the net speed of the swappers, new implementations of the ratchet seem to be more efficient than the ones in use before. In addition, TNT can now analyze characters with up to 32 states, step-matrices and character state trees, implied weighting, and different types of constraints.

**The Geometric Mean Support: A New Statistic to Describe the Distribution of Character Steps in the Tree.** P. Grandcolas\*, T. Robillard, C. D’Haese, L. Desutter-Grandcolas, E. Guilbert and J. Murienne. FRE 2695 CNRS, Département Systématique et Evolution, Muséum national d’Histoire Naturelle, 75005 Paris, France.

Most phylogenetic studies mention only three descriptive statistics: tree length, CI and RI. Trees are also published with statistics aimed at predicting robustness as bootstrap or branch support of Bremer. But the distribution of characters steps in the tree, homoplastic

or not, is generally not shown. To improve the description of the results of phylogenetic analyses, we propose a new descriptive and simple statistic, the geometric mean support (GMS), which is aimed at describing the evenness of character support in the tree.

**Insertions and deletions in the evolution of equal-length DNA fragments.** Taran Grant\* and Cyrille D’Haese. American Museum of Natural History; FR2694 CNRS, Muséum national d’Histoire Naturelle, Paris, France.

Insertion and deletion (indel) mutations are a general occurrence in the evolution of the genome that can be attributed to a variety of mechanisms. They have undoubtedly played an important role in the evolution of the tree of life, and it is important that they be studied in order to fully understand the complexities of molecular evolution. Indel formation has been identified as likely the most rapid and significant form of sequence change and a vast number of studies have found indels to provide important evidence on the phylogeny of higher groups. Although it is widely recognized that indel events must be incorporated in evolutionary studies of orthologous genome fragments composed of unequal numbers of nucleotides (unequal-length fragments), it is common practice for them to be prohibited in studies of equal-length fragments. Here we evaluate the theoretical basis for that constraint and examine the effect of its removal on several data sets composed of equal-length fragments.

**Biotic Element Analysis in Biogeography.** Bernhard Hausdorf\*<sup>1</sup> and Christian Hennig<sup>2</sup>. <sup>1</sup>Universität Hamburg, Zoologisches Museum, Hamburg, Germany. <sup>2</sup>Eidgenössische Technische Hochschule Zürich, Seminar für Statistik, Zürich, Switzerland.

Areas of endemism are not suitable as biogeographic units, because they cannot be delimited if dispersal occurred. As an alternative, we propose to use biotic elements, i.e. groups of species the ranges of which are more similar to each other than should be expected by chance, as units in biogeography. According to the vicariance model, diversification is the result of a fragmentation of an ancestral biota by emerging barriers. Such a fragmentation will result in non-random congruence of species ranges, i.e. in the formation of distinct biotic elements. Thus, we propose a statistical test for clustering of distribution areas based on a Monte Carlo simulation with a null model that generates range data sets such that their range size distribution, their species richness distribution and the spatial autocorrelation of the ranges approach the parameters in the real data set. A method for the delimitation of biotic elements which uses model based Gaussian clustering is introduced. We also tested another prediction of the vicariance model, namely that closely related species originate on different sides of an emerging

barrier and, hence, belong to different biotic elements. The ranges of north-west European land snails, Iberian land snails belonging to the Helicoidea and of species of the South African plant family Restionaceae are significantly clustered, but contrary to the prediction of the vicariance model, closely related species belong significantly more often to the same biotic element than should be expected by chance. The land snail faunas of the central Aegean Islands and of Israel and Palestine meet both predictions of the vicariance model, but only a small percentage of the species can be adequately assigned to biotic elements. The ranges of European and Mediterranean Tipulidae are not significantly clustered. These case studies indicate that speciation modes other than vicariance were frequent or that the imprint of vicariance on the ranges was more or less obscured by extensive postspeciational dispersal and/or regional extinction.

**In Support of Support.** Peter Hovenkamp. Nationaal Herbarium Nederland, Leiden Branch, Leiden, The Netherlands.

Although probabilistic branch support figures are immensely popular, their usefulness from a cladistic point of view is contentious. Arguments against such support figures stress two arguments. The first is that probabilistic statements do not fit in a falsificationist approach to science, because they do not forbid anything. The second is that they cannot apply to clades because clades are historical individuals, and that there cannot be an objective basis for assigning these probabilities. I will argue that both these arguments are misdirected. By appealing to the general requirements of objectivity and repeatability, in fact, a good case for the use of probabilistic branch supports can be made.

**No Areas.** P. Hovenkamp. Nationaal Herbarium Nederland, Leiden branch, The Netherlands.

The basic elements in a biogeographic analysis are not areas, but taxon ranges. To derive areas from taxon ranges introduces an extra step in the analysis. In this step, the study area is either exhaustively partitioned in subareas, or localities are clustered on basis of their species composition and the resulting clusters are evaluated on basis of a selection criterion. In the first case, where all subareas are uncritically accepted as basic units in the analysis, this procedure compounds the problems related to widespread and missing species. In the second case, the procedure introduces arbitrariness, error and ambiguity. For either case, I will show that the inclusion of this step may obscure signal that can easily be retrieved by comparing taxon ranges directly. This method may be a valuable alternative for methods that require the recognition of areas in advance of an analysis.

**A Phylogenetic Perspective on Larval Spine Morphology in *Leucorrhinia* (Odonata: Libellulidae).** Rasmus Hovmöller. Department of Entomology, Swedish Museum of Natural History, Stockholm, Sweden.

*Leucorrhinia* (Odonata, Anisoptera, Libellulidae) consists of 14–15 species with a holarctic distribution. We have combined the morphological characters of a previous study with sequence data from the ITS1, 5.8 S rDNA and ITS2 regions of the nuclear ribosomal repeat. Cloning was used to investigate the intraindividual variation and such variation was found in all investigated species. Parsimony jackknifing was used to identify supported groups. The effect of sequence alignment and gap coding was explored by a modified sensitivity analysis. Loss of spines in *Leucorrhinia* larvae has occurred twice: once in Europe and once in North America. The role of spines as a defense against predation is discussed in a phylogenetic context.

**Evolutionary Relationships within the Unionoid Family Margaritiferidae: A Molecular Approach.** Stephanie L. Huff\* and Gonzalo Giribet. Department of Organismic and Evolutionary Biology, and Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA.

The phylogeny of selected members of the freshwater pearl-mussel family Margaritiferidae is investigated using DNA sequence data from nuclear ribosomal (18S rRNA and partial 28S rRNA), mitochondrial ribosomal (16S rRNA), nuclear protein coding (histone H3) and mitochondrial protein coding (cytochrome c oxidase subunit I) genes. Phylogenetic analysis via direct optimization of DNA sequence data using parsimony as optimality criterion is executed for different combinations of parameter sets accounting for different indel costs and transversion/transition cost ratios in a sensitivity analysis framework. The evolutionary relationships both within Margaritiferidae and between Margaritiferidae and other unionoid families are explored. The biogeographic history of this family is also discussed.

**What is an Area?** Chris J. Humphries\* and Malte C. Ebach. Department of Botany, The Natural History Museum, London, UK.

No two areas are alike and using a statistical model to compare them (or the taxa within them) is unsuitable for determining the relationships of those taxa and areas. Endemic area analyses tend to rely on the geographical ranges of taxa as determined by specimen sampling or recording. Therefore they do not reflect a natural biotic element, but characterize the boundaries of populations or species. However treating typified areas as taxa has the advantage that the biotic elements, the organisms, act as area characters that define an endemic area. In using this typological approach along with a cladistic

method, area characters can be tested for homology and a series of areas can be tested for monophyly. The implications of this new approach are that endemic areas are treated as monophyletic (natural) biotic elements that can be classified with a consistent nomenclature. In addition, area cladistics is an innovative new method that can reconstruct the proximal positions of the biotic areas to form a biogeographical atlas that reflects the positions of the geographic areas during the time of taxic divergence. Area cladistics and the topological area concept will help create a universal system of area recognition for future biogeographical analyses.

**Evolution of SARS Associated Coronavirus.** Daniel Janies. Ohio State University, Columbus, OH, USA.

The recent outbreak of SARS and the scientific response have been dramatic. The genomes of 25 corona-virus isolates from SARS patients from Asia, Europe, and North America has been publicly released within four months of the public acknowledgment of an outbreak of atypical pneumonia by Chinese officials. The first SARS associated virus genome was released in two months. Commensurate with this burst of data were several papers declaring that the SARS associated virus is a previously uncharacterized isolate that is not closely related to any known corona-virus. There are several observational and analytical problems in these analyses. First, the SARS associated virus likely has ancestors and close relatives but they have not been observed. Second the characterization of SARS associated virus has been performed with cursory methods that consider only nucleotide substitution events in a handful of genes. I will present an analysis of all corona-virus genomes and portions of genomes available in GenBank as of June 2003. Changes that occurred in the genomes of the SARS associated corona-virus lineage since they shared a common ancestry with animal corona-viruses will be used to develop hypotheses of the novel tropism and etiology of the disease.

**The Phylogeny of Dermaptera: Molecular Evidence.** Karl J. Jarvis\* and Michael F. Whiting. Brigham Young University, Salt Lake City, UT, USA.

The Dermaptera (earwigs) is a ubiquitous order of insects, the phylogenetic relationships of which are poorly understood. A major synapomorphy of Dermaptera the presence of long, unsegmented, forceps-like cerci that are generally used for prey capture and mating. Three suborders have traditionally been recognized within the earwigs. Two of these suborders are ectoparasites; Hemimerina on murid rats and Arixeniina on molossid bats. These lineages are adapted to parasitic lifestyles, with reduced vision and cercal function. The phylogenetic relationships of these ectoparasitic lineages to members of the free-living suborder Forficulina are controversial. In this study, the

phylogeny of Dermaptera was inferred from 18S ribosomal, 28S ribosomal and Histone-3 nuclear DNA sequences. Sequence data was collected for 48 dermapteran taxa from eight families in two suborders: Hemimeridae from suborder Hemimerina: Pygidicranidae, Anisolabididae, Labiduridae, Apachyidae, Spongiphoridae, Chelisochidae and Forficulidae from suborder Forficulina. Sequence data for 17 outgroup taxa in nine orders were also included: Ephemeroptera, Odonata, Orthoptera, Phasmida, Embiidina, Mantodea, Isoptera, Blattaria and Grylloblattodea. Approximately 4600 base pairs of DNA sequence were analyzed for each of the 65 taxa included. These sequences were analyzed using Direct Optimization in POY. Results indicate a paraphyletic Forficulina, with Hemimerina as sister to Forficulina + Chelisochidae. This indicates that the ancestor to hemimerids may have had cerci that were used for prey capture and mating, but subsequent adaptations to an ectoparasitic lifestyle left the cerci in their present reduced condition. Some of the traditionally recognized families were indicated to be paraphyletic in this analysis: Spongiphoridae, Pygidicranidae, Anisolabididae, and Labiduridae.

**Origin of Polyploidy in the Frog Species *Tomopterna tandyi*.** J.P.T. Kapongo\*, A. Dawood and P. Bloomer. University of Pretoria, Department of Zoology and Entomology, Pretoria, South Africa.

*Tomopterna tandyi* is a tetraploid frog belonging to the African genus *Tomopterna* (Ranidae, Raninae). In the description of the species, it was proposed that this species originated as the result of a hybridization event between *T. cryptotis* and *T. delalandii* (Channing and Bogart 1996). Further research provided support for *T. cryptotis* as the maternal parent species of *T. tandyi* (Dawood et al., 2002) however, the identity of the putative paternal parent species remained unclear. Furthermore, the range of the species was not known. The phylogeny of *Tomopterna tandyi* therefore remained unclear in terms of its phylogenetic and geographic origins. The main aim of the present study is to identify the putative parent species of *T. tandyi* using a molecular systematic approach and to investigate its geographic origin using a phylogeographic approach. Our future research thus include the use of microsatellite markers and nuclear genes to identify both parental lineages of the tetraploid, and mitochondrial markers for the phylogeographic aspects.

**Molecular Phylogeny and Evolution of Rhabditid Nematodes.** Karin Kiontke\*, Shu-Yi Chiou, Nicholas P. Gavin, Yevgeniy Raynes and David H.A. Fitch. Department of Biology, New York University, New York, NY, USA.

To reconstruct a phylogenetic tree of “Rhabditidae” we have sequenced small subunit ribosomal RNA genes

(SSU rDNA) for more than 100 species of “Rhabditidae” and Diplogastrina representing almost every major group within these taxa. A weighted maximum parsimony analysis of our SSU rDNA sequences with 2000 jackknife replicates supports several monophyletic groups within “Rhabditidae” (e.g. *Caenorhabditis*; *Oscheius*; a clade consisting of *Pelodera*, *Mesorhabditis* and *Teratorhabditis*; Diplogastrina). Some groups previously thought to be monophyletic (“Pellioditis”, “Rhabditis” and “Rhabditoides”) are shown to be para- or polyphyletic. “Rhabditidae” itself is paraphyletic with respect to the parasitic *Strongylida* and *Heterorhabditis*, also suggesting a major taxonomic revision is required. Some relationships at the base of our phylogeny are not well resolved, as are some relationships within otherwise well resolved groups (e.g. the species most closely related to *Caenorhabditis elegans*). Therefore we are sequencing the 3' half of the largest subunit of RNA polymerase II and about 3000 bases of large subunit rDNA. Our phylogeny can be used to trace the evolution of molecular, morphological, developmental, and behavioral characters. For example, many introns in the RNA polymerase II gene were lost in the lineage leading to *Caenorhabditis*, whereas there is no unambiguous evidence for intron gain. Major evolutionary rate differences in rDNA are not matched by equivalent rate differences in RNA polymerase II, suggesting that lineage rate differences are not genome wide. The position of the vulva changed at least 4 times from median to posterior which correlates with the loss of the posterior gonad arm. Within Rhabditidae these two characters are never reversed. Hermaphroditism evolved from gonochorism at least eight times independently. In only one case, our data suggest evolution of gonochorism from hermaphroditism. Heterogony evolved twice within the group. The position of phasmids relative to genital papillae in the male tail has changed several times in a saltational manner. Phylogenetic correlation may also be made for size of the male bursa and mating type, suggesting that natural selection may have a role in diversifying male morphology.

**Event- and Pattern-based Proposals of Biogeographic Homology.** James K. Lieberr. Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY, USA.

The development of pattern-based cladistic biogeography permitted the explicit search for general biogeographic patterns founded on phylogenetic relationships. These general patterns purport to represent sequential vicariance events common to two or more taxa, thereby providing common cause for biotic diversification. The reticulate geological history of the northern hemisphere remains an impenetrable obstacle for pattern-based biogeographical analysis—i.e. assumption analysis, Brooks parsimony analysis, and reconciled tree analysis—due

to: 1, changing area relationships through geologic time; 2, extensive biotic dispersal among adjacent regions; and 3, Pleistocene-mediated extinction. An event-based biogeographical analysis (dispersal-vicariance analysis, or DIVA) undertaken for the 131 assigned species of the carabid beetle genus *Agonum* Bonelli provides hypotheses of area relationships throughout the history of *Agonum* diversification, hypothesized to have started in Paleocene based on adelphotaxon status for the African genus *Agonidium* Jeannel. Changing area relationships since Eocene are reflected in the area optimizations provided by DIVA for internal nodes of the taxon-area cladogram. The presence or absence of hypothesized amphi-Atlantic relationships for basal nodes of three subordinate clades is consistent with ecologic preferenda of the lineages. Post-Eocene hegemony of Beringia as a conduit connecting the Old and New Worlds is evident, though diversification has most commonly involved speciation events within more regional biotas. Pliocene and Pleistocene fossils mitigate extinction related biases in estimating species distributions, and permit estimates for species duration. Event-based DIVA provides primary estimates of biogeographic homology, though sequential repetition of identical area optimizations necessitates rejection of these optimizations as strictly contemporaneous. Nonetheless, by passing the roadblock caused by anastomosing area connections across the Holarctic, time-constrained event-based analysis can advance our understanding of the broader history of diversification in old Holarctic clades.

**A New Phylogeny of the Cephalopoda Using Total Evidence.** A.R. Lindgren and M.K. Nishiguchi. New Mexico State University, Las Cruces, NM, USA.

Cephalopods are a class within the Mollusca that have undergone rapid divergence and radiation, providing the basis to study evolution of familial level relationships, character development and specialization. We completed a preliminary analysis of the Cephalopoda using total evidence from four molecular loci; nuclear 18S rRNA, fragments of both 28S rRNA and Histone H3, and mitochondrial cytochrome c oxidase I (CO1) as well as 50 morphological characters to provide better resolution at the family level. Data were obtained from 40 specimens, comprising of 36 genera, representing 25 families, and analyzed using total evidence based on the parameter set that minimized overall incongruence among partitions using the direct optimization program, POY. Phylogenies generated from both total evidence and single data sets will be presented. Due to the highly divergent nature of the class, it is unlikely that only one gene or one set of characters will provide much of a solution. Therefore, by combining multiple genes and morphology, we provide a new phylogenetic framework for the evolution of the Cephalopoda.

**Web Building Behavior and the Phylogeny of Austrochilid Spiders.** Lara Lopardo\*, Martín J. Ramírez, Cristian Grismado, and Luis A. Compagnucci. Museo Argentino de Ciencias Naturales, Buenos Aires C1405DJR, Argentina. Department of Biological Sciences, The George Washington University, Washington, DC, USA.

Web architecture and the associated web building behaviors are increasingly used to reconstruct cladistic relationships among higher groups of spiders. The nature and arrangement of sticky threads is especially informative. Two basic types of sticky threads exist, each used by different groups of spiders: cribellar silk and viscid silk. The dry, cribellate threads are produced by a spinning plate, the cribellum. The araneoid viscid silk is made of an aqueous sticky glue produced by two pairs of aggregate silk glands. The cribellum is composed of hundreds of minute spigots, which produce fibrils that are combed by a comb in the fourth leg (calamistrum). It is well established that ancestral araneomorph spiders were cribellate. Two basic patterns of combing behavior are known for cribellate spiders. In type I, observed in Hypochilus (Hypochilidae, the most basal araneomorph clade) and Filistatidae (the most basal Haplogynae clade), the combing leg IV rests on the contralateral leg III, but only leg IV moves. In type II, observed in cribellate entelegynes, the combing leg IV holds on the contralateral leg IV, and both legs move together as an almost rigid unit. It has been hypothesized that type II combing behavior is a synapomorphy of Entelegynae. We present here the first observations on the combing behavior of the Austrochilinae, a subfamily that includes two genera endemic to temperate forests in Southern Chile and Argentina. The superfamily Austrochiloidea (Austrochilidae plus Gradungulidae) is the sister group of Araneoclada (which is composed of two lineages, Haplogynae + Entelegynae, that is, all araneomorph spiders except Hypochilidae). Species of these two cribellate genera construct large, two-dimensional sheet webs with a funnel retreat. When combing cribellate silk, austrochilids use both legs IV, like entelegyne spiders, and unlike Hypochilidae and Filistatidae. Furthermore, the alternation of combing legs IV is determined by the leg III involved in the attachment of a cribellate segment—the leg IV ipsilateral to the leg III that made the attachment will comb the next segment, except for the first segment. This similarity to Entelegynae in combing with both fourth legs contradicts current hypotheses of basal araneomorph relationships [(Hypochilidae (Austrochiloidea (Haplogynae Entelegynae)))]], and suggests that either type II combing behavior originated independently in Entelegynae and Austrochilinae, or type I originated independently in filistatids and Hypochilus. Furthermore, current reexamination of some internal anatomical features and current investigations on the outgroups

of the Entelegynae suggest that Austrochilidae may be its sister group, and imply different predictions for the combing behavior for cribellate gradungulids. Aerial webs are seemingly an acquisition of araneomorph spiders, but given the diversity and complexity of web building behaviors and web architecture, the details of their evolution remain so far elusive.

**Molecular Phylogenetics and Historical Biogeography of Oreobolus (Cyperaceae).** Santiago Madriñán<sup>1,2</sup>, Juliana Chacón<sup>1,2</sup>, Mark W. Chase<sup>2</sup>, <sup>1</sup>Laboratorio de Botánica y Sistemática, Universidad de los Andes, Bogotá, Colombia; <sup>2</sup>Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, UK.

Previous hypotheses on the phylogenetic relationships of the species of *Oreobolus* R. Br. (Cyperaceae) have been proposed by Mora-Osejo (1987) and Seberg (1988, 1991), the former limited to the American species, and the later embracing the whole genus. Incongruities between these phylogenies have generated different biogeographical explanations for the distribution pattern of the genus. A molecular phylogenetic study of *Oreobolus* using ITS nuclear ribosomal spacers, and the plastid trnL-F intron is presented. The results show that *Schoenoides oligocephalus*, the only species of the monotypic genus *Schoenoides* Seberg, is embedded within *Oreobolus*, thus calling for the reinstatement of *Oreobolus oligocephalus* W.M. Curtis. The designation of *O. cleefii* Mora-Osejo as a subspecies of *O. obtusangulus* by Seberg is not supported. There is a well-supported South American clade, with the Australian *Oreobolus distichus* falling well outside this clade, contrary to Seberg's hypothesis, and refuting his overly complex biogeographical story. The presence of *O. furcatus* in Hawaii allowed the calibration of a molecular clock, which indicates that *Oreobolus* began to diversify in South America approximately 3–4.5 million years ago after a long-distance dispersal event from Australia. The topology of the South American clade indicates a northward geographic progression of species along the Andes from southern Argentina to Venezuela, and finally to Central America, as proposed by Mora-Osejo in 1987.

**Taxonomy, Phylogenetics and Biogeography of Red-wing (*Scleroptila* Spp.) Francolins (Family Phasianidae, Order Galliformes).** Tshifhiwa Mandiwana. Percy FitzPatrick Institute, University of Cape Town, Rondebosch, South Africa.

The potential for using combined molecular and organismal data has opened up new avenues for taxonomy, phylogenetics and biogeography. Such a multifaceted approach is used to identify taxa, test monophyly and resolve evolutionary relationships specifically within the Orange River Francolin, *Scleroptila levaillantoides*, species complex and other mono- and polytypic species forms within the Red-winged Group of

francolins (genus = *Scleroptila sensu lato*). By this, I compare the utility of three phylogenetic methods, that is, Parsimony, Maximum Likelihood and Distance Analysis. There is a large discrepancy in the number of subspecies attributed to the polytypic species that were studied. 23 taxa of the Red-winged Group (representing all putative species and nearly all putative subspecies) from different geographic localities were studied, respectively. Mitochondrial Cytochrome b DNA sequence characters ( $\pm 250$  base pairs) and organismal characters extracted from reports in published literature were employed. These characters were analyzed separately and in combination. The trees obtained from DNA sequences, organismal and combined data sets were incongruent with respect to the position/placement of some taxa. The organismal and combined analyses probably reflects the taxon phylogeny better than the mitochondrial DNA analysis alone. Monophyly of the Red-winged Group (including the Ring-necked Francolin, *Dendroperdix streptophorus*) is well supported by all the analyses except in Distance Analysis. The bootstrap support values range from 50 to 94%. But the identity of, and interrelationships among, the subspecies and species are less well resolved. Though the Orange River Francolin was found to be non-monophyletic, distinct clades comprising the south-western and north-eastern forms of Africa were identified. The most remarkable result supported by organismal tree is that it brings a better resolution of the south-western forms of the Orange River Francolin in the Red-winged Group. It is suggested that in order to obtain a robust taxonomy and phylogeny for the taxa studied, multiple independent data sets be considered (including sufficient number of base pairs). Molecular data from a single gene region reveal only one facet of evolution, and may be misleading. It is also known that certain positions in the Cytochrome b sequences are more conserved than the other. Therefore, resolution of the branching order would require the collection of more molecular characters and employment of genes evolving at varying rates.

**The Unbearable Lightness of Being Monophyletic: Clade Stability and the Addition of Data: A Case Study from Erigonine Spiders (Araneae: Linyphiidae, Erigoninae).** Jeremy Miller\* and Gustavo Hormiga. Department of Systematic Biology–Entomology, Smithsonian Institution, National Museum of Natural History, Washington, DC, USA; Department of Biological Sciences, The George Washington University, Washington, DC, USA.

We have added taxa and morphological characters to a previous analysis of erigonine relationships. Parsimony analysis of the expanded matrix results in a single most parsimonious tree. Our phylogeny differs markedly from the previous hypothesis of erigonine relationships.

We investigate how the addition of characters and taxa (alone and together) has altered the original phylogeny. We conclude that topological changes from the previous study to the current one are largely the result of adding and modifying characters, not adding taxa. We also evaluate progress toward a stable phylogeny using Continuous Jackknife Function (CJF) analysis. CJF analysis uses character removal and a reference hypothesis to evaluate the stability of the hypothesis under test. The results are presented as a graph of the number of clades recovered after character removal and reanalysis against the percent probability of character removal. Stable phylogenies are expected to take the form of a decreasing asymptotic curve with a high rate of clade recovery.

**BOGEN: A faster parsimony program for computing larger phylogenetic trees.** Nobuhiro Minaka\*<sup>1</sup>, Takashi Suemura<sup>2</sup>, Takahiro Asano<sup>3</sup>, Haruo Yamamoto<sup>3</sup> and Kouki Machii<sup>2</sup>. <sup>1</sup>National Institute for Agro-Environmental Sciences; <sup>2</sup>Turaltech; <sup>3</sup>Bogenpfeil.

BOGEN is a new, more efficient program for calculating most parsimonious trees from molecular sequence data. The principal characteristic of our program is that it incorporates a new heuristic search strategy for building optimal initial trees using simultaneous subtree-connections. The upper limit of the size of data matrix is 10 000 OTUs/50 000 base pairs for the current version of BOGEN. From our benchmark test, the computing time on a Pentium 4 (2.26 GHz) Windows PC is as follows: for 500 OTUs/1000 bp, 50 s (initial tree) and 37 min (branch-swapping); for 1000 OTUs/1000 bp, 220 s (initial tree); for 5000 OTUs/1000 bp, 1 h (initial tree). Calculating a 10 000 OTU tree requires, on the average, at least 30 h for the optimal initial tree reconstruction. As far as we can compare the optimal trees from BOGEN with those from PAUP\* 4.0, BOGEN trees have consistently shorter lengths than PAUP\* trees even without any branch-swapping operation. We are now developing a branch-swapping program for larger trees. Several examples are presented for comparing BOGEN with other parsimony programs.

**The DIALIGN Multiple Alignment Program: Recent Developments and Applications.** Burkhard Morgenstern. University of Göttingen, Institute of Microbiology and Genetics, Göttingen, Germany.

DIALIGN is a widely used software program for pair-wise and multiple alignment of DNA and protein sequences. It combines local and global alignment features by assembling alignments from pair-wise local sequence similarities, so-called “fragments” or “fragment alignments”. Most recently, the program has been applied by a number of research groups to analyze large genomic sequences. The talk gives an overview about the algorithmic strategy behind DIALIGN and reports

on some recent applications. Some recent improvements of the program and new developments are outlined.

**The Road to Pathogenesis: “Illumination” of Animal–Bacterial Interactions Through the Sepiolid Squid–*Vibrio* Mutualism.** Michele K. Nishiguchi. Department of Biology, New Mexico State University, Las Cruces, NM, USA

Understanding the evolution of animal and bacterial associations has been an underlying theme in establishing the development and specificity of symbiotic relationships. The mutualistic association between sepiolid squids (Mollusca: Cephalopoda) and their *Vibrio* symbionts provides a versatile and experimentally tractable model system to study speciation, virulence transmission, and gene expression between bacterial species and their diverse squid hosts. Since the symbiotic bacteria are environmentally transmitted to new hosts with every generation, this system is ideal for the study of specificity amongst the wide variety of bacteria that reside in the water column. Moreover, it provides a system to resolve whether the ecology of the free-living symbiont is as important as the interactions of the mutualism in the architecture of bacterial–host associations. In particular, we are interested both in proximal factors (such as strain recognition and specificity among virulent and avirulent populations) that influence the establishment of specific interactions, and in the ultimate causes in terms of specific regions in the *Vibrio* genome that confer the ability to colonize hosts and engage in free-living, mutualistic, or pathogenic interactions. Our approach to address these questions is to combine both empirical and theoretical studies in an integrated way such that the information from the laboratory is directly relevant for developing models. The models can then focus on subsequent empiric studies for specific regions of potential functional importance and predicting patterns of virulence evolution. The interplay between the empiric and theoretical components of this research is important to its conception and, we hope, to its ultimate success. In this way we expect this unique combination to enhance not only our understanding of an important host–bacterial association, but also the ability of functional bioinformatics to identify important structural determinants that are related to the evolution of pathogenesis.

**The Encino Project Is Described.** Kevin C. Nixon, Cornell University, Ithaca, NY, USA.

It represents a comprehensive solution to the problem of cladistic data management. In terms of legacy data (Winclada, NONA, Hennig86 and NEXUS), data sets can be combined into virtual data sets and automatically merged upon loading, with current support for up to 256 simultaneously loaded data sets. These supermatrices can then be maintained and

modified separately, and merged simply by loading the supermatrix definition. Any data set (independent or virtual) can also be managed directly with commercial or public domain standard SQL database engines, such as Microsoft Access, Oracle, or MySQL, using the Encino front end. The Encino model extends to Web-based collaborations, using XML data structures to transfer cladistic matrices, diagnostic matrices, classifications and documenting images to the DOL (DiversityofLife) website, where additional features are available through secure password-protected interfaces. The DOL structure is a complete MySQL relational database with associated server-side programs (in C, C++, and Perl) for archiving of images, cladistic data, cladograms, specimen data and bibliographic citations. The DOL site provides tools for management of monographic, floristic, faunistic, and classification projects. The DOL interface also provides illustrated interactive diagnostic keys that are accessible through any browser over the Web, and as standalone applications on Windows PCs and handheld devices. Finally, the DOL site provides JAVA-based tree viewers that allow rapid downloading and manipulation of large cladograms (to several thousand taxa; the largest currently on the site is > 1500 taxa), with automatic linkages from cladograms to images, diagnostic keys, and external metadata. The DOL model is extensible, and can be propagated to multiple interlinked servers, so that individual workers or groups can maintain their own nodes.

**The Use of Optimality Criteria in DNA Sequence Alignment and Its Application in A New Computer Program.** Kevin C. Nixon and Damon P. Little\*. Cornell University, Ithaca, NY, USA.

There are two basic types of alignment procedures for molecular data: (1) those in which the sequence data are aligned during tree construction and search and (2) those in which the alignment is conducted prior to the construction of the tree. The first type will not be discussed here. Alignment of DNA sequences is of critical importance to the final result, but is often given very little attention. Different sequence alignments can, and often do, result in different phylogenetic trees. Artifacts produced by available alignment algorithms frequently require workers to manually adjust the alignment “by eye”. Unfortunately this makes it difficult, if not impossible, to exactly reconstruct published analyses and makes adding new sequence data difficult. Commonly used alignment algorithms (e.g. Clustal) begin by calculating a guide tree based upon pair-wise distance estimates. Each sequence is then aligned to every other sequence in a pair-wise manner following an input order determined by the guide tree. This approach is not guaranteed to find the optimal pair-wise alignment, nor are multiple optimal solutions considered.

Arbitrary choices among equally optimal paths are made independently for each clade of the guide tree, and once made each pair-wise alignment is fixed. These arbitrary decisions coupled with other errors and superfluous gaps are propagated throughout the alignment. We will present a new method for calculating an overall alignment cost (and therefore global optimality). This allows alternative alignments (and alignment methods) to be compared unambiguously. In addition we will demonstrate a new global multiple alignment procedure that does not use a guide tree, considers multiple optimal solutions, and is computationally much faster than existing alignment algorithms.

**Independence of Alignment and Phylogenetic Reconstruction and Their Optimality Criteria.** Helga Ochoterena. Instituto de Biología, UNAM, Mexico City, Mexico.

Different length DNA sequences can be treated for the establishment of primary homology hypotheses in two main ways. They can be aligned first to establish homology at the level of nucleotide base pair positions prior to phylogenetic analysis (multiple alignment procedures) or both steps can be combined in a single analysis, discarding alignment prior to phylogenetic analysis (direct optimization procedures). Most of the commonly used software for multiple alignment do not revise, prior to phylogenetic analyses, the obtained alignments to globally minimize putative events at two levels: indels for each terminal and events for each position. Currently, this is a laborious procedure that needs to be manually and visually done. Criteria to define minimum putative events are here recapitulated. In the case of direct optimization, fragments of DNA sequences are considered as the comparable (homologous) units. This option implicitly results in the creation of complex characters, which can be problematic. Implied alignments obtained from direct optimization procedures show that this method does not guaranty optimum alignments in terms of minimizing postulated events. If alignments are globally optimized to minimize the added number of putative events per terminal and position, a greater number of potentially informative characters can result. The consequence of this is that the most parsimonious trees obtained after the alignment is globally optimized can be longer than those obtained when the multiple alignment is not reevaluated. A simple example shows how to use the cladogram lengths as an optimality criterion to prefer an alignment over another can be misguided. Until algorithms that incorporate criteria to globally optimize multiple alignments are implemented in DNA sequence alignment software, producing DNA phylogenies that aim for primary homology hypotheses that minimize the number of putative events will require time investment and in some cases subjective judgments.

**The Paleoptera Problem: Sense and Sensitivity.** T. Heath Ogden\* and Michael F. Whiting. Brigham Young University, Salt Lake City, UT, USA.

While the monophyly of winged insects (Pterygota) is well supported, phylogenetic relationships among the most basal extant pterygote lineages are problematic. A robust phylogeny for basal pterygote lineages is required to elucidate the evolution of the subimago, the origin of flight, and other important evolutionary innovations. The three competing hypotheses are: (1) Ephemeroptera as the most basal pterygote lineage, sister group to Odonata + Neoptera; (2) Odonata as the most basal pterygote lineage, sister group to Ephemeroptera + Neoptera; and (3) Ephemeroptera + Odonata (Paleoptera) sister group to Neoptera. A recent molecular analysis concluded that Paleoptera (Odonata + Ephemeroptera) is monophyletic (Hovmöller et al., 2002). However, we demonstrate that this result is only supported under a narrow range of alignment parameters. We have further tested the monophyly of Paleoptera using additional sequence data from 18SrDNA, 28S rDNA, Histone 3, and morphology for a broader selection of taxa, and a wider range of analytical methodologies. Our results suggest that the current suite of molecular data ambiguously resolve the three basal insect lineages. However, when morphological evidence is included, the total evidence analysis supports Odonata + Neoptera. Issues of topological sensitivity, in relation to parameter perturbations and analytical methodologies, are discussed.

**Cladistics of Communities and the History of the Effect of Forest Fragmentation.** R. Pellens<sup>1</sup>, P. Grandcolas<sup>2</sup>, E. Guilbert<sup>2</sup>. <sup>1</sup>Universidade Federal do Rio de Janeiro, CCS, Ilha do Fundão, Rio de Janeiro, Brazil; <sup>2</sup>FRE 2695 CNRS, Departement Systematique et Evolution, Muséum national d'Histoire naturelle, Paris, France.

Cladistics has been used in several contexts to relate different kinds of objects, when reconstructions of historical patterns were needed, in phylogenetics, in biogeography (PAE), in linguistics, and in textual analyses. Until now, cladistics was very rarely used in ecology and only for providing classifications of communities in synecological studies, regardless of species patterns. We propose to extend this use to the reconstruction of “evolutionary” patterns for species in communities, species “inheritance” vs. colonization or extinction. This use is immune to the criticisms made of PAE since the communities and their species can be reasonably assumed independent when they are studied at a local, not a regional level. Carrying out these analyses in the case of the effect of forest fragmentation can be especially interesting. Forest fragmentation has been shown to cause species loss, a conclusion mainly drawn both from the positive correlation between fragment size and specific richness and from the

nestedness of communities (poor communities nested in rich ones). This conclusion can be powerfully tested using the species patterns inferred from a cladistic analysis of communities of both the nuclear area and the fragments. Re-analyzing two ecological case studies reveals undescribed species patterns even if these communities appear classically nested. Contrary to the expectation of a simple trend of species loss in smallest fragments, variable colonizations events were inferred together with simultaneous species loss. Also, specific richness was found to be originally low in some places, and not explained by a postfragmentation species loss. These results emphasize the need for using cladistics in historical studies of ecological communities to test a priori historical assumptions.

**The Ribosomal Small Subunit Genes of Malaria Parasites: Mode and Tempo of Evolution in a Multigene Family.** Susan L. Perkins\*. Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA.

Malaria parasites (*Plasmodium*) have highly unusual ribosomal RNA (rRNA) genes. In virtually all other eukaryotic organisms, these genes are arranged in loci consisting of the ribosomal subunits (18S, 5.8S, and 28S) separated by external and internal transcribed spacers. The loci are arranged in tandem arrays within one or a few arrays per genome. In stark contrast, *Plasmodium* parasites have very few (4–8) single-copy loci that are scattered amongst their chromosomes. That is although the basic structure of the locus is like other eukaryotes, the loci are not repeated and each locus may exist on a separate chromosome. Furthermore, different rRNA genes are expressed at different times in the parasite's life cycle: some genes are transcribed only when the parasites are asexually dividing in the blood of the vertebrate host; other genes are transcribed only in the insect vector. These genes do not appear to evolve in a concerted fashion, like the rRNA genes of most other organisms. Only a few species of *Plasmodium* have been well studied at the molecular and genomic level. Here, I present results of a comparative study of the 18S genes from several species of *Plasmodium* that infect mammals, birds, and lizards as well as several *Haemoproteus* and *Hepatozoon* parasites. DNA was extracted from these parasites and a large portion (~1100 bp) was amplified using oligonucleotide primers that are general to all parasite 18S gene types. PCR products were cloned into *E. coli* and the products were screened using restriction enzyme digests. A sample of all variant restriction types was sequenced. Sequences were analyzed phylogenetically, both in a large dataset that included other apicomplexan 18S sequences and also using reconciled tree analysis against a malaria parasite species tree generated with cytochrome b data. The results show that there have been numerous rRNA gene

duplications and losses throughout the evolution and diversification of these lineages, and, as other studies have shown, no evidence for concerted evolution in these genes. The pattern of cladogenesis of these genes is not consistent with a single switch to the unusual differentiated expression, but rather several transitions. Continued broad comparative work such as this will be necessary to uncover the origin, pattern, and function of these truly bizarre genes.

**Bacterial Endosymbionts of Blood-Feeding Leeches.**

Susan L. Perkins\* and Mark E. Siddall. Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, USA; American Museum of Natural History, New York, NY, USA.

Three groups of blood-feeding leeches have been found to have endosymbiotic bacteria that are housed in structures known as mycetomes. The morphology of these mycetome structures differs from group to group and DNA sequence data from the bacterial ribosomal small subunit (16S) gene have shown differences in the phylogenetic history of the endosymbiont inhabitants as well. The Placobdellid leeches of North America have symbiotic bacteria that belong to the alpha proteobacterial clade and are closely related to various symbiotic nitrogen-fixing bacteria *Haementeria*, from South America, and *Placobdelloides*, from Africa, have symbionts that are more closely related to gamma proteobacteria and other endosymbionts of invertebrates, such as those found in tse tse flies. Fluorescent *in situ* hybridization (FISH) has confirmed the presence of only alpha proteobacteria in the mycetomes of *Placobdella*.

**Do Bayesian Support Values Reflect Probability of the Truth?**

Kurt M. Pickett\*<sup>1</sup>, Mark P. Simmons<sup>2</sup> and Chris P. Randle<sup>3</sup>. <sup>1</sup>Department of Entomology, Ohio State University, Fort Collins, CO; <sup>2</sup>Department of Biology, Colorado State University, Columbus, OH; <sup>3</sup>Department of Evolution, Ecology and Organismal Biology, Ohio State University, OH, USA.

Bayesian support values have been promoted as posterior probabilities of clades. Like early interpretations of the bootstrap, we are told these values represent the probability of truth. We present evidence from two separate lines of reasoning that challenge this view. First, Bayesian support values and Jackknife values are compared to an idealized support index for a reference phylogeny. Statistical treatment of the distributions of Bayesian values and Jackknife values show that Bayesian values depart from ideal significantly more than Jackknife values. In a second study, we show that the use of flat topological priors does not imply flat clade priors. Specifically, under flat tree priors, clade priors differ depending on the number of taxa in a clade and the number of taxa in the analysis. Bayesian support

values for clades are significantly correlated with these prior values, whereas bootstrap values are not.

**The Tangled History of the Widespread Colonization Island.** P. J. Planet\*, R. DeSalle and D. H. Figurski. Columbia University, New York, NY, USA; American Museum of Natural History, New York, NY, USA.

Genomic islands are clusters of genes with a common history that is distinct and divergent from the histories of the organisms in which they reside. Because genomic islands encode complex and potentially advantageous traits that may be acquired by horizontal transfer, they are major contributors to the evolution and diversification of microbial life. Although the most intensively studied genomic islands are pathogenicity islands, which carry genes that enhance virulence or disease lifecycles, other genomic islands are involved in symbiosis, colonization of an ecologic niche, metabolic pathways, etc. One putative genomic island, The Widespread Colonization Island (WCI), encompasses the tight adherence (*tad*) locus of genes in the human pathogen *Actinobacillus actinomycetemcomitans*. The *tad* locus is required for colonization of surfaces and tenacious biofilm formation in *A. actinomycetemcomitans*, and recent work has showed that the genes are required for colonization of the oral cavity and progression to periodontal disease. Similar loci are found in a broad range of pathogenic and non-pathogenic prokaryotes suggesting a role for these genes in disease and in the environment. Mobile genes can be considered to be parasites or symbionts that have histories that are independent of their host organisms. Therefore, phylogenetic tools for inferring the coevolution of host parasite assemblages can be directly applied to reconstruct the series of events that led to the present day distribution of these genes. We used tree reconciliation and tests of phylogenetic incongruence to rigorously test the hypothesis that the WCI is a genomic island, and to infer the evolutionary history of the locus. Our analysis strongly supports a complex history of gene shuffling by recombination, and multiple horizontal transfers, duplications, and losses of the entire region. One important possible horizontal transfer event may have led to the recruitment of WCI-encoded traits for disease.

**DNA Taxonomy of Tiger Beetles.** Joan Pons, Matthew Hale, Daniel P. Duran, Anabela C. Diogo and Alfried P. Vogler\*, The Natural History Museum and Imperial College London, London, UK.

What should be the role of DNA sequences in taxonomy and species descriptions? The proposition of “barcoding” all species on Earth has triggered an intense debate about the possibility of DNA sequences to represent “species”. Here we will investigate the problems and potential of DNA taxonomy, using empiric examples from tiger beetles (genus *Cicindela*).

**Burrowing Scorpion Phylogeny and the Evolution of Paleodrainage Systems in Southern Africa.** Lorenzo Prendini, L.<sup>1\*</sup>, Tim M. Crowe<sup>2</sup> and Ward C. Wheeler<sup>1</sup>. <sup>1</sup>Division of Invertebrate Zoology, American Museum of Natural History, New York, NY, USA; <sup>2</sup>Percy FitzPatrick Institute, University of Cape Town, Rondebosch 7700, South Africa.

The Orange River is the largest crossing the arid interior and western coastline of southern Africa. Geological evidence suggests that it underwent major drainage system changes during late Cretaceous-Cainozoic times, including alternations in use of the 28° (modern Orange River) and 31°S (modern Olifants River) exit points and radical reorganizations of internal drainage geometry. During the late Cretaceous, the 28° exit was used, and the middle Orange River followed a course up to 150 km south of its modern channel. Two subsequent alterations in exit point occurred in the late Cretaceous-early Tertiary (31°S) and late Oligocene-early Miocene (28° exit). Other internal reorganizations coincided with paleoclimatic oscillations between the late Miocene and Pleistocene, until the present course was achieved. The burrowing scorpion (Scorpionidae: *Opisthophthalmus*) faunas occurring north and south of this river are distinctly different. Only five species occur on both sides. The remaining 75 are endemic either to the region north (Namibia) or south (South Africa). A spatial dataset of *c.* 8000 point locality records for *Opisthophthalmus* species is used to plot their distributions in relation to the present and paleodrainage channels. Areas of present occurrence are optimized on a cladogram for *Opisthophthalmus* species, derived from a simultaneous analysis of 262 morphological characters and 1.8 kilobases of DNA sequence. Not only the five transriverine distributions, but also the evolution of the largest clade of *Opisthophthalmus* species, are inferred as consequences of dispersal and vicariance associated with drainage shifts that coincided with the development of increasingly arid conditions in southwestern Africa.

**Molecular Phylogeny of Haplosporidia and Characterization of Unknown Parasites.** Kimberly S. Reece\*<sup>1</sup>, Mark E. Siddall<sup>2</sup>, Nancy A. Stokes<sup>1</sup>, Susan M. Bower<sup>3</sup> and Eugene M. Bureson<sup>1</sup>. <sup>1</sup>Virginia Institute of Marine Science, The College of William and Mary, Gloucester Point, VA, USA; <sup>2</sup>American Museum of Natural History, New York, NY, USA; <sup>3</sup>Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC, Canada.

The unique morphology of parasites in the phylum Haplosporidia has confounded determination of the phylogenetic position of this group. In addition, relationships among haplosporidian taxa are sometimes difficult to resolve. We collected DNA sequence data for small subunit ribosomal (SSU) rRNA and actin

genes of several haplosporidians and other protists for conducting molecular phylogenetic analyses to help elucidate relationships of taxa within the phylum, as well as placement of this phylum among Eukaryota. Analyses were conducted using DNA sequence data from more than 100 eukaryotic taxa. In almost all analyses using various combinations of the datasets, Haplosporidia grouped with Cercozoa. The genera *Minchinia* and *Urosporidium* were found to be monophyletic while Haplosporidium was paraphyletic. “Microcell” parasites, *Bonamia* spp. and *Mikrocytos roughleyi*, were sister to *Minchinia*, the most derived genus, with Haplosporidium falling between the “microcells” and the more basal *Urosporidium*. Two recently discovered parasites, one from abalone in New Zealand and another from spot prawns in British Columbia, fell at the base of the Haplosporidia with very strong support indicating a taxonomic affinity to this phylum. Initial histological and ultrastructural studies on the spot prawn parasite had suggested a relationship to parasitic dinoflagellates. Phylogenetic analyses, however, with both genes grouped this parasite with Haplosporidia, not with dinoflagellates. Four sets of PCR primers and two different DNA probes for *in situ* hybridizations were designed to target unique regions of the parasite SSU rRNA sequence. Both probes hybridized specifically to parasite cells in sections of prawn tissue infected with the unknown parasite and the PCR primers specifically amplified only DNA from infected hosts confirming that the sequence was derived from the spot prawn parasite, not a contaminating haplosporidian.

**From Polka Dots to Zig-Zags: Color Patterning and the Phylogeny of Pleasing Fungus Beetles (Coleoptera: Cucujoidea: Erotylidae).** James A. Robertson<sup>\*1</sup>, Joseph V. McHugh<sup>2</sup> and Michael F. Whiting<sup>1</sup>. <sup>1</sup>Brigham Young University, Salt Lake City, UT, USA; <sup>2</sup>University of Georgia, Athens, GA, USA.

Phylogenetic relationships of Erotylidae (Pleasing Fungus Beetles) were inferred based on DNA sequence data. Relationships of clades within Erotylidae were examined as was the relationship of the entire family to Languriidae (Lizard Beetles). 18S and 28S ribosomal DNA were sequenced for 61 taxa representing major erotylid lineages and outgroups. Phylogenetic analyses under varying parameter settings using standard parsimony and likelihood techniques were performed. These data indicate: (1) a paraphyletic Erotylidae and Languriidae, (2) Encaustinae (including *Coptengis*), Megalodacninae, and Erotylinae are supported as monophyletic, while Dacninae and Tritominae are paraphyletic, (3) there are no recognizable phylogenetic trends in coloration across higher-level erotylid lineages. Other taxonomic and biological implications are discussed in light of this phylogeny.

**Evolution of Acoustic Devices in Eneopterinae Crickets: A Phylogenetic Test of Historical Patterns for Stridulum Transformations.** Tony Robillard\* and Laure Desutter-Grandcolas. FRE 2695 CNRS, Département Systematique et Evolution, Muséum national d'Histoire naturelle, Paris, France.

Acoustic communication is at the basis of reproductive behaviors in many vertebrates (birds, frogs) and insects. Calling with a tegminal stridulatory apparatus is widespread in crickets, which song is produced by a file-plectrum mechanism and radiated by a mechanical resonator, mainly constituted of the harp. Previous models for the evolution of stridulatory devices in crickets were based only on prephylogenetic studies and considered few cricket species. They assumed that the stridulum was homogeneous and perfect from the start; its subsequent evolution was hypothesized only as multiple, progressive losses; associated evolutionary patterns would have involved many intermediate steps leading from a complete stridulum to a partial or complete loss of the structure. Cricket venation is however, far more complex and diverse than previously thought. Recent developments in comparative biology and the use of explicit phylogenetic methods allow now to study significant ingroups for characters of interest defined on precise homology statements. Few studies have been made in crickets using this approach; they concluded that the stridulum may be labile and homoplastic, and may evolve through several types of regressive evolution. We focus here on the whole Eneopterinae subfamily, remarkable for its acoustic diversity, to study the evolution of the stridulum. For the first time, a cladistic analysis is done on exemplars of almost every genus of this subfamily, and evolutionary patterns of venation characters are analyzed. At the scale of the subfamily, stridulum is lost several times independently, totally or partially. This involves multiple and complex evolutionary patterns which result in contrasted morphologies, arising directly at different levels of the tree, or through progressive changes of forewing structure. Some of these modifications in stridulum architecture may be responsible for large increases in fundamental frequencies of calling songs and may thus be related to changes in calling behaviors, habitats and/or population structures.

**Morphology, Phylogeny and Reproduction and the Problem of the Annelid Root.** Greg Rouse. South Australian Museum, Adelaide, South Australia, Australia.

The first broad scale morphological cladistic analysis of annelids was only published in 1997, with some new characters in subsequent analyses resulting in largely congruent results. These are outlined with

respect to their implication for the transformation of the diverse reproductive modes found in annelids. This has some interesting implications for popular hypotheses of the evolution of reproduction in Metazoa in general. The anatomy of annelid larvae, in particular that of the trochophore larva has had a surprisingly influential role that requires reassessment. However, before this can be undertaken with confidence a recent review of polychaete anatomy suggests a new synthesis may now be needed. Also, there appear to be fundamental problems in the root placement of earlier broad scale analyses. Molecular sequence data has been of little help thus far in resolving this issue of the annelid root; largely due to limited taxon sampling. More robust estimates of relationships among annelid taxa will be forthcoming, combining morphological and molecular data.

**Molecular Phylogeny of the Annelida.** Vincent Rousset<sup>1\*</sup>, Mark E. Siddall,<sup>2</sup> Greg W. Rouse,<sup>3</sup> Fredrik Pleijel<sup>1</sup> and Christer Erséus.<sup>4</sup> <sup>1</sup>Muséum National d'Histoire Naturelle, Département Systématique et Evolution, CNRS UMR 7138, Systématique, Adaptation, Evolution, Paris, France; <sup>2</sup>American Museum of Natural History, Division of Invertebrate Zoology, New York, NY, USA; <sup>3</sup>South Australian Museum Nth Terrace, Adelaide, SA, Australia and Environmental Biology, The University of Adelaide, SA, Australia; <sup>4</sup>Swedish Museum of Natural History, Department of Invertebrate Zoology, Stockholm, Sweden.

The inter-relationships between the major clades within the Annelida remain unresolved, although these issues have been addressed earlier both in molecular and morphological studies. One classical dispute relates to whether clitellates (leeches, earthworms and allies) and polychaetes (marine bristle-worms) are sister-groups, or if clitellates are nested within polychaetes. Based on sequences of three ribosomal genes, two nuclear (18S rRNA and the D1 region of 28S rRNA) and one mitochondrial (16S rRNA) we analyze the relationships and position of clitellates. Parsimony and Bayesian analyses unequivocally indicate a monophyletic Clitellata. Although less well supported under parsimony (most parsimonious solution, but without bootstrap support), the clitellates have a sister-group relationship within the polychaetes, consisting of a subgroup of a polyphyletic Scolecida. However, a delineation problem with the polychaetes is demonstrated by the situation that several of the outgroups are nested among them. In order to address this issue, we also present (very) preliminary results on a much more extensive selection of terminals and molecular data including sequences of the nuclear protein-coding gene (histone H3). This second study aims to resolve the problem with the delineation of the whole of Annelida, and the currently controversial position of the root of that group.

**Parsimony-Based Tree-Fitting: A New Method to Infer Dispersal and Vicariance Patterns in Southern Hemisphere Biogeography.** Isabel Sanmartín. Department of Systematic Zoology, Evolutionary Biology Center, Uppsala University, Uppsala, Sweden.

*Dispersal* and *vicariance* are often considered competing hypotheses in historical biogeography. Disjunct distribution patterns can be explained either by fragmentation of widespread ancestors by vicariance or by dispersal across a pre-existing barrier. However, current biogeographic methods are based on vicariance because dispersal hypotheses are difficult to falsify, and because dispersal is thought to be a *random* process that cannot give rise to congruent patterns. The biogeography of the *Southern Hemisphere* is the textbook example of the vicariance scenario: *trans*-Pacific disjunct distributions are typically explained by the sequential break-up of the southern supercontinent Gondwana, causing vicariant division of an ancestral biota. However, recent studies based on molecular clocks indicate that dispersal has been more important than traditionally assumed. A new method is proposed here to examine the relative role played by dispersal in shaping Southern Hemisphere biotas. Based on a large data set of 54 animal and 18 plant phylogenies (1257 terminals), we used *parsimony-based tree-fitting* to test to what extent Southern Hemisphere biogeography departs from the break-up sequence of Gondwana, by fitting each phylogeny to a geological area cladogram derived from paleocontinental reconstructions. Phylogenetically constrained (“concordant”) dispersal patterns were identified by calculating the frequency of dispersal events remaining after geologically predicted events, such as vicariance, have been removed. The statistical significance of the patterns was assessed by permutation tests. Results show that dispersal was important in the development of Southern Hemisphere continental biotas, but in partly different ways in plants and animals: *trans*-Antarctic dispersal (Australia-S. South America) was the most significant dispersal event in animals, whereas *trans*-Tasman dispersal (New Zealand-Australia) is the dominant pattern in plants. Our results confirm the “hybrid” origin of the South American biota, with surprisingly little biotic exchange between the northern tropical and the southern temperate regions of South America.

**Phylogenetic Analysis of Developmental Sequences.** Susanne Schulmeister\* and Ward Wheeler. American Museum of Natural History, New York, NY, USA.

Event pairing had been proposed by Smith (1996, *Am. Zool.* 36: 70–79) and Velhagen (1997, *Syst. Biol.* 46: 204–210) for the optimization of developmental sequences on a given cladogram, in order to derive scenarios of developmental sequence heterochrony. Here, we show that event pairing is faulty, leading to the optimization of impossible hypothetical ancestors,

the underestimation of the lengths of the developmental sequences on the tree, and the proposition of synapomorphies that are not supported by the data. When used for phylogenetic analysis, event-pairing can even produce phylogenies that are inconsistent with the data. These errors are caused by the fact that event-pairing treats dependent events as if they were independent. We present a new method for comparative and phylogenetic analysis of developmental sequences that does not exhibit these errors. Our method applies Search-Based character optimization (Wheeler, in press, *Cladistics*) and treats the entire developmental sequence as a single character which is then analyzed via dynamic-programming (Sankoff and Rousseau, 1975, *Math. Progr.* 9: 240–246). In other words, the developmental sequence is directly optimized on the tree.

**On the Cladistic Analysis of Microarray Data.** Mark Siddall\*, Paul Planet, Neil Sarkar and Robert DeSalle. American Museum of Natural History, New York, NY, USA; Columbia University, New York, NY, USA.

A predilection toward the evaluation of microarray data with a variety of overall similarity clustering methods and the application of multivariate centroidal routines for evaluation of diagnostic or prognostic test cases in medicine caused us to consider whether greater detail and analytical power could be obtained through a more cladistic approach to the problem. Insofar as microarray data arrive in continuous data form, first we explored variations on discretization of those data. Once discretized to “on/off” states, microarray patterns can be analyzed cladistically with common parsimony programs thereby affording an opportunity to avoid solutions that are not globally optimal for these notoriously very large and complex datasets. Whether or not a transformational view of the resulting cladograms can be justified (as in cases of immunological cell line maturation and lymphomas) or not, the resulting trees do offer more specific diagnostic insights for clades of interest that phenetic approaches allow. By way of extension we have developed a Characteristic Attribute Organization System (CAOS) method for developing diagnostic sets and evaluation of test cases that proves to be more discriminating and more powerful than current alternatives.

**Nucleotide vs. Amino Acid Characters: Relative Character-State Space, Amount of Potential Phylogenetic Information, and Heterogeneity.** Mark P. Simmons\*, Timothy G. Carr and Kevin O’Neill. Department of Biology, Colorado State University, Fort Collins, CO, USA; Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, NY, USA; Computer Science Department, Cornell University, Ithaca, NY, USA.

We quantified three of the six factors that determine whether nucleotide or amino acid characters should be

preferred for phylogenetic inference based on our broad selection of matrices of protein-coding loci from a diverse array of clades and genomes. First, we quantified the difference in character-state space between nucleotide and amino acid characters. Second, we quantified the loss of potential phylogenetic signal from silent substitutions when amino acid characters are used. Third, we quantified the relative heterogeneity of nucleotide and amino acid characters, and determined how common convergent (rather than unique) shifts in nucleotide and amino acid composition are. The advantage of greater potential phylogenetic signal for nucleotide characters, and the greater character-state space and lower heterogeneity of amino acid characters, was confirmed. Although the greater potential phylogenetic signal for nucleotide characters was found to be enormous (average increase of 4.4 times), the greater character-state space for amino acid characters was considerably less impressive (average increase of 50.4%). Given the ability of increased terminal sampling to better-utilize the greater potential phylogenetic signal of nucleotide characters and decrease the potential for artifacts to occur caused by heterogeneous nucleotide composition among terminals, we suggest that increased taxon sampling be performed whenever possible rather than only analyzing the amino acid characters.

**Efficiently Resolving the Basal Clades of a Phylogenetic Tree Using Bayesian and Parsimony Approaches: A Case Study Using Mitogenomic Data from 100 Higher Teleost Fishes.** Mark P. Simmons\* and Masaki Miya. Department of Biology, Colorado State University, Fort Collins, CO, USA; Department of Zoology, Natural History Museum and Institute, Chiba, Chuoku, Chiba, Japan.

Many phylogenetic analyses that include numerous terminals but few genes show high resolution and branch support for relatively recently diverged clades, but lack of resolution and/or support for “basal” clades of the tree. The various benefits of increased taxon and character sampling have been widely discussed in the literature, albeit primarily based on simulations rather than empiric data. In this study, we used a well-sampled gene-tree analysis (based on 100 mitochondrial genomes of higher teleost fishes) to test empirically the efficiency of different methods of data sampling and phylogenetic inference to “correctly” resolve the basal clades of a tree (based on congruence with the reference tree constructed using all 100 taxa and 7990 characters). By itself, increased character sampling was an inefficient method by which to decrease the likelihood of “incorrect” resolution (i.e. incongruence with the reference tree) for parsimony analyses. Although increased taxon sampling was a powerful approach to alleviate “incorrect” resolution for parsimony analyses, it had the general effect

of increasing the number of, and support for, “incorrectly” resolved clades in the Bayesian analyses. For both the parsimony and Bayesian analyses, increased taxon sampling, by itself, was insufficient to help resolve the basal clades, making this sampling strategy ineffective for that purpose. For this empirical study, the most efficient of the seven approaches considered to resolve the basal clades when adding nucleotides to a dataset that consists of a single gene sampled for a small, but representative, number of taxa, is to increase character sampling and analyze the characters using the Bayesian method.

**The Origin of Madagascar’s Freshwater Fishes.** W. Leo Smith\* and John S. Sparks. American Museum of Natural History, New York, NY, USA.

Paleontologists have argued that the origins of Madagascar’s extant vertebrate fauna remain one of the greatest unsolved mysteries of natural history. Given a nearly complete absence of fossil evidence, these scientists continue to argue that Madagascar’s extant and recently extinct vertebrate faunas (including freshwater fishes) owe their origins to Cenozoic colonization via oceanic dispersal. Here we show, using phylogenetic and biogeographical patterns, evidence congruent with the temporal sequence of Gondwanan fragmentation for the dominant clades of Madagascar’s extant freshwater fishes: rainbowfishes (Bedotiidae), killifishes (Pachypanchax), and cichlids (Cichlidae). Although predicted under an oceanic dispersal scenario, no sister-group relationships for these clades are recovered between Malagasy and African lineages (isolated for *c.* 165 Ma by at most 430 km). On the contrary, the results of our molecular analyses suggest that the closest relatives to these Malagasy fish clades, representing ~70% of Madagascar’s freshwater fishes, are present on Gondwanan landmasses separated from Madagascar by thousands of kilometers of open ocean since the Early Cenozoic.

**Where Did the Desert Locust Come from?—Insight from a Phylogenetic Study.** Hojun Song. Department of Entomology, Ohio State University, Columbus, OH, USA.

The locust genus *Schistocerca* has an interesting disjunct distribution. Out of about 50 species, only one species occurs in the Old World and the rest occur in the New World. Old World species is the desert locust, *S. gregaria*, known for the enormous swarming capacity. Researchers in the past debated on how this kind of disjunction arose, which resulted in various speculations. Here I present the first comprehensive phylogeny of *Schistocerca* based on morphology and attempt to explain the current disjunct distribution. The tentative results suggest that the desert locust invaded Africa rather recently, after the genus diver-

sified in the New World. The invasion route is suggested to be the eastward transatlantic dispersal by a large swarm.

**Phylogenetic Relationships of the Tribe Cheilosini (Diptera: Syrphidae).** Gunilla Ståhls and Jyrki Muona, Finnish Museum of Natural History, Entomology Department, University of Helsinki, Helsinki, Finland.

The phylogenetic relationships of the tribe Cheilosini (Diptera: Syrphidae) were investigated using 1183 nucleotide characters of the mitochondrial gene cytochrome *c* oxidase subunit I in combination with *c.* 600 characters of the D2-3 domain of the 28S rRNA gene and characters of adult morphology. All 10 presently recognized genera of the predominantly Holarctic tribe Cheilosini were represented by at least one species. The most speciose genus of the tribe is *Cheilosia*, with about 600 recognized species currently. The analyses included > 50 species of this genus, representing nearly all of the described subgeneric groupings. The aim of this study was to resolve the phylogenetic relationships of the tribe and of the genus *Cheilosia*. Of special interest was the placement of the Nearctic taxon *Hiatomyia* and the Oriental taxon *Endoiasimyia*, described as genera of Cheilosini but later also treated as subgenera of the genus *Cheilosia*. Parsimony analyses used both manual alignment and dynamic alignment, using programs Nona and POY, respectively.

**Phylogeny of Mantodea Based on Molecular Data: Evolution of a Charismatic Predator.** Gavin J. Svenson and Michael F. Whiting. Department of Integrative Biology, Brigham Young University, Provo, UT, USA.

Phylogenetic relationships of Mantodea (praying mantids) are previously unknown and were inferred from DNA sequence data. Five genes (16S rDNA, 18S rDNA, 28S rDNA, Cytochrome Oxidase II, and Histone 3) were sequenced for 63 taxa representing major mantid lineages and outgroups. The monophyly of mantid families and subfamilies were tested under varying parameter settings using parsimony and Bayesian analyses. These analyses support the paraphyly of Hymenopodidae, Iridopterygidae, Mantidae, and Thespididae and the monophyly of Amorphoscelidae. All represented subfamilies of Iridopterygidae and Mantidae are paraphyletic. Mantoididae is the most basal mantid, sister to the rest of Mantodea. Lineages congruent with current subfamilial taxonomy include Paraoxypilinae, Hoplocoryphinae, Hymenopodinae, Acromantinae, and Oligonicinae. Mantid hunting strategy is defined as either generalist, cursorial, or ambush predators. The ancestral predatory condition was generalist hunting, with three independent shifts to cursorial hunting and one shift to ambush hunting, which gave rise to the largest radiation of mantid species.

**A Phylogeny of Robber Flies (Diptera: Asilidae) at the Subfamilial Level: Molecular Evidence.** S. D. Taylor\*, S. M. Bybee, C. R. Nelson and M. F. Whiting. Brigham Young University, Salt Lake City, UT, USA.

We present the first formal analysis of phylogenetic relationships among the Asilidae, based on four genes: 16S rDNA, 18S rDNA, 28S rDNA and cytochrome oxidase II. Twenty-six ingroup taxa representing 11 of 12 described subfamilies were selected to produce a phylogenetic estimate of asilid subfamilial relationships via optimization alignment, parsimony, and maximum likelihood techniques. Phylogenetic analyses support the monophyly of Asilidae with Leptogastrinae as the most basal robber fly lineage. Apocleinae + (Asilinae + Ommatiinae) is supported as monophyletic. The laphriinae-group (Laphriinae + Laphystiinae) and the dasypogoninae-group (Dasypogoninae + Stenopogoninae + Stichopogoninae + Trigonimiminae) are paraphyletic. These results suggest that current subfamilial classification only partially reflects robber fly phylogeny, indicating the need for further phylogenetic investigation of this group.

**The Step Matrix Approach Applied to SE Asian Biogeography.** Hubert Turner<sup>1,2</sup> and Peter C. van Welzen<sup>1</sup>. <sup>1</sup>Nationaal Herbarium Nederland, Leiden Department, The Netherlands; <sup>2</sup>Institute of Biology, Leiden University, the Netherlands.

The Step Matrix Approach to biogeography (proposed by Turner at the XVI meeting of the Willi Hennig Society [Turner, 1997]) is applied to a dataset constructed for an analysis of SE Asian biogeography (Turner et al., 2001). One of the advantages of SMA is that an Incongruence Length Difference (partition homogeneity) test can be performed on the dataset to check whether different signals are present, without randomly breaking up the different characters pertaining to the same cladogram, as will happen in BPA. Step Matrix Approach, no outgroup, but all-zero ancestor assumed for all characters. Tree building by 100 random additions with TBR, steepest descent enforced. If many trees were found, the analysis was broken off and a check was made to see if any trees were left that were not compatible with the strict consensus. If no further trees were found, then a search was made for as many trees as possible that were compatible with the strict consensus. For the Incongruence Length Difference test trees were built by simple addition and NNI, steepest descent enforced, keeping only one MP tree. All characters are apparently compatible: partitioning the characters a priori according to the taxon-area cladograms into an East to West pattern (basal area Australia or New Guinea, or clade not occurring West of the Wallace line) vs. a West to East pattern (basal area continental Asian or on the Sunda shelf, or clade not occurring East of the Wallace line) does not result in significantly shorter trees

than random partitioning (ILD test,  $P = 0.55$ ). Moreover, the spread in lengths is not large (1076.3–1119.3). The partitions gave partly resolved majority-rule consensus trees (EW pattern completely resolved E of Wallace line, unresolved W of Wallace line,  $l = 506.2$ ; WE pattern almost completely resolved, but less clearly divided along the Wallace line,  $l = 554.1$ ). All characters together gave a quite well resolved result (6 trees,  $l = 1104.3$ ).

**Phylogenetic Diversity of Amazonian Elateroids: Does It Reflect the History of Different Forest Types?** Varpu Vahtera. Finnish Museum of Natural History, University of Helsinki, Finland.

A new method to study the diversity and age of two Peruvian Amazonian forest types was used. In addition to number of species, it takes into account the number of higher taxonomic levels (genera, tribes, subfamilies). The general idea is that an area with higher phylogenetic diversity can be assumed to be older than an area where the diversity is lower. False-click beetles (Eucnemidae) and click beetles (Elateridae) were chosen to be the study organisms because of their long life history and tight association with trees. Two terra firme forest types were chosen, one growing on clay soil and the other on white-sand soil. The former is a common soil type in Peruvian Amazon and the latter a patchily distributed one. It is assumed that white-sand forests are older than clay soil forests and our aim was to resolve this by comparing the phylogenetic diversity of these two forests. The results show that more specimens, species, genera, tribes and subfamilies were collected there than from clay soils. Thus our findings support the hypothesis of the older age of white-sand forests. Despite the relatively dry and nutrient poor environment of white-sand forests, elaterids seem to survive there well. One explanation may be the thick root and humus layer of the soil type, which also contains ectomycorizas. It provides a humid and nutrition-rich environment for elaterids to develop.

**Evolutionary Speculations Regarding Spiral Cleavage and Segmentation, Based on Studies of Glossiphoniid Leech Embryos.** David A. Weisblat\*, Foster C. Gonsalves, Françoise Z. Huang, Dongmin Kang<sup>1</sup> and Mi Hye Song<sup>2</sup>. University of California, Berkeley, CA, USA; <sup>1</sup>Stanford University, Palo Alto, CA, USA; <sup>2</sup>University of Michigan, Ann Arbor, MI, USA.

Leeches, including the experimentally accessible glossiphoniid species *Helobdella robusta*, develop via a highly modified version of unequal spiral cleavage. Uniquely identifiable AB and CD blastomeres arise at first cleavage, with CD specified to generate the D quadrant lineages. Despite the distinct identities of cells AB and CD, we have found stochastic expression (i.e. either cell AB or cell CD) of a WNT-class intercellular signaling molecule during the early part of the two-cell stage of

development. Evidence for the stochastic activation of two other intercellular signaling pathways (Notch/Delta and MAPK) is seen at this stage as well. We speculate that these phenomena may be a reminiscence of lateral interactions among blastomeres in equally cleaving spiralian ancestors. By the end of cleavage, the D quadrant has generated five pairs of teloblasts, which generate columns of segmental founder cells in anterior-to-posterior progression. Studies of the expression and function of engrailed-, hedgehog-, even-skipped- and hes-class genes provide little support for the idea that annelids and arthropods share a common segmented ancestor, consistent with the interpretation that segmentation evolved independently in Ecdysozoa and Lophotrochozoa.

**The Whole Genome Is Not Enough.** John W. Wenzel<sup>1</sup>, John V. Freudenstein<sup>2</sup>, Mark P. Simmons<sup>3</sup> and Kurt M. Pickett<sup>1</sup>. <sup>1</sup>Department of Entomology, and <sup>2</sup>The Herbarium, Ohio State University, Columbus, OH, USA, and <sup>3</sup>Department of Biology, Colorado State University, Fort Collins, CO, USA.

Whole genomes are becoming available for phylogenetic analysis, suggesting to some workers that other data will soon be obsolete. Yet, not all character information is encoded in the sequence of nucleotide bases. We discuss characters that retain historical information for use in phylogenetic analysis, beginning at DNA sequence data (the lowest, most reduced level), through phenotypes derived from genetic sequences, and ending at characters that are biologically transmitted but not genetically encoded. Using different levels of characters can improve analyses when higher-level characters provide evidence of adjacency among the states of the lower-level characters. This amounts to finding homology statements that plot to different nodes of a cladogram. A hazard of using different levels together is that redundant information may inadvertently lead to differential weights among characters, and it is advisable to guard against logical redundancy.

**New Species of Leeches (Salifidae) from Madagascar and South Africa: Implications for Understanding the Phylogeny of Erpobdelliformes.** S. Westergren\* and M. Siddall. University of Nebraska, Lincoln, NE, USA; American Museum of Natural History, New York, NY, USA.

Recent AMNH expeditions have discovered (or possibly rediscovered) new species of predatory leeches from each of Madagascar and South Africa. The former is the first described non-terrestrial leech from that continent, though a *nomen nudem* suggests Malagasy freshwater leeches were previously known. As well, two new species of *Barbronia* from the vicinity of Kruger National Park may well be those that Jan Oosthuizen intended to describe prior to his untimely death.

Morphological characterization of these species, as well as nuclear and mitochondrial sequence DNA data, allow for their inclusion in an expanded treatment of the suborder Erpobdelliformes.

**Optimization, Alignment, and Homology.** Ward Wheeler. American Museum of Natural History, New York, NY, USA.

Cladograms are the goal of systematic analysis. Multiple sequence alignment is a process we may, or may not; use to find desirable (usually parsimonious) cladograms. The parsimonious optimization of sequence data requires dynamic (i.e. topology specific) homology. The static, global homology derived from multiple sequence alignment is incompatible with this, yielding non-optimal, less informative cladograms.

**Q Factor Analysis as a Tool for Morphological Data Evaluation.** Bernhard Wiesemüller\* and Hartmut Rothe. Universitaet Goettingen Institut fuer Zoologie und Anthropologie, Ethologische Station, Gleichen, Germany.

Q factor analysis is a variant of factor analysis (principal components analysis, PCA) using a transposed data matrix, i.e. a matrix with reversed columns and rows. In contrast to a common factor analysis, the resulting factors of the Q variant do not group characters, but instead individuals in a typological way. Unlike in the case of cluster analysis, the resulting clusters of individuals are not necessarily organized hierarchically. The inclusion of an outgroup in the analysis permits an interpretation of similarities as apomorphic or plesiomorphic, respectively. Therefore, this statistical procedure can also be applied to morphological studies in phylogenetic systematics *sensu* Hennig. So far, Q factor analysis has never been used for morphological data. In this study, we tested it as a technique for cladistics, using skull measurements of New World monkeys (Platyrrhini). As a result, the procedure yielded excellent taxonomic results on certain levels of the phylogenetic hierarchy, whereas no clear distinctions could be made on very high or very low levels, respectively. Although the necessary data preparations, calculations, and the morphological interpretation of the extracted factors take up a great deal of time, the tool is recommendable for lower level systematics, when taxa morphologically only differ in shape and certain body proportions. In this presentation, we will discuss the main steps of this procedure and present some exemplary results.

**What do Bootstrap Values Tell?** Bernhard Wiesemüller\* and Hartmut Rothe. Universitaet Goettingen Institut fuer Zoologie und Anthropologie, Ethologische Station, Gleichen, Germany.

Bootstrap Analysis is a common tool in cladistics, and consequently many authors tend to believe that it could

be close to a test of monophyly. In fact, it is only a procedure to calculate the redundancy of a certain character pattern among taxa. To demonstrate this, we set up a study with questionable data: Four skulls of great apes and humans were digitally photographed, and the pixels' brightness values were simply transformed to a one-zero-matrix, which was then used to calculate a Wagner tree with Phylip. As a rule, the higher the resolution of the photos is, the higher are the bootstrap values of supported taxa (and the lower are the bootstrap values of non-supported taxa). Redundancy of intertaxic information might indeed be an indicator of phylogenetic relationship, but can also be due to other reasons, like functional-adaptive needs in morphology, or semantic needs in a DNA-code. As a result, we tend to believe that high bootstrap values are actually less important than low ones. It is safer, based on a low bootstrap value, to claim that a certain taxon is not well supported by certain data. Therefore, we recommend discussions of low bootstrap values in future publications.

**Review of Generic Limits and Biogeographic Patterns in the New World Tiger Moth Genera *Holomelina* and *Virbia* (Arctiidae: Arctiinae: Arctiini).** Jennifer M. Zaspel\* and Susan J. Weller. Department of Entomology, University of Minnesota, MN, USA.

The moth genera *Virbia* Walker and *Holomelina* H-S (Arctiidae: Arctiinae: Arctiini) are brightly colored tiger moths whose generic limits may be defined more by geography and color pattern. Species of *Holomelina* occur from Canada to Guatemala, while species of *Virbia* occur from Mexico to Brazil (Watson and Goodger 1996). Both genera include widespread species with highly variable and confusing phenotypes, complicating their taxonomic status. In 1985, Ferguson listed various characters that would unite genera in what he called the *Holomelina* group. Although Ferguson did not include *Virbia* species in his paper, he did suggest that the two genera might not be distinct. We undertook a generic review to address generic limits and examine biogeographical implications of the phylogeny. Taxon sampling included males and females of 15 *Virbia* and 13 *Holomelina* species and an additional

13 species of *Virbia* and 8 of *Holomelina* that were examined for males only. Of the 74 species (and subspecies) described for these genera, 44 (60%) were obtained and complete male and female pairs were used in the analysis. Seven outgroup genera were chosen from the Arctiini tribe and included: *Phragmatobia fuliginosa* (L.), *Spilosoma virginica* (F.), *Hypercompe permaculata* Packard, *Paraclis fusca* Walker, *Arachnis picta* Packard, *Pyrrarctia isabella* (J. E. Smith), and *Heliactinidia bimaculata* Walker. These taxa were selected as outgroups based on prior work suggesting that they are more closely related to *Virbia* and *Holomelina* than other Arctiini (Jacobson and Weller, 2001, Watson and Goodger, 1996, Ferguson, 1985). Specimen preparation followed Winter (2000). Forty morphological characters (2–13 states) were described from the head and appendages (3 characters, 13 states), wing venation (3 characters, 7 states), male (24 characters, 135 states) and female genitalia (10 characters, 37 states). A total of 10 binary and 24 multistate characters were scored. Multistate characters were treated as nonadditive. Phylogenetic trees were constructed using maximum parsimony (MP) with PAUP\* (Swofford, 2000). In all searches, 100 replicates of random-taxa additions were performed to uncover hidden topology islands (Maddison et al., 1984). Outgroups were jack-knifed (Lanyon, 1981) to determine if ingroup relationships were sensitive to inclusion/exclusion of taxa. Area cladograms were constructed to examine geographic structure (Humphries and Parenti, 1999), and a weighted ancestral area analysis was used to estimate ancestral areas for the genera (Hausdorf, 1998). In our initial results, a strict consensus of 180 trees revealed that *Holomelina* and *Virbia* were not reciprocally monophyletic genera. This analysis uncovered 3 topology islands with a tree length of 289; consistency and retention indices were 51 and 62, respectively. Placement of *Holomelina* as junior synonym of *Virbia* is supported, although a subgenus may be warranted. Biogeographical patterns suggest that the North American fauna is a composite of at least two unrelated lineages, and that multiple exchanges between Central and South American fauna have occurred.