

TAXONOMIC ANALYSIS OF *ENCHYTRAEUS JAPONENSIS* (OLIGOCHAETA, ANNELIDA): MORPHOLOGICAL AND BIOCHEMICAL COMPARISONS WITH *E. BIGEMINUS*.R.M.Schmelz<sup>1</sup>, R.Collado<sup>1</sup> and M.Myohara<sup>2</sup>. <sup>1</sup>Systematic Zoology, Univ. of Osnabrück, Germany, <sup>2</sup>Natl. Inst. of Sericul. and Entomol. Sci., Tsukuba, Japan.

*Enchytraeus japonensis* Nakamura 1993, a terrestrial enchytraeid, reproduces asexually by fragmentation and subsequent regeneration. Because of its extreme ability of regeneration, the species has recently been proposed as a new experimental material for regeneration study, and a method has been developed for inducing sexual reproduction in the laboratory (Myohara *et al.* 1999, *Dev. Growth Differ.* 41(5)). The inducibility of sexual reproduction allowed us for the first time to perform a taxonomic analysis of *E. japonensis* based on the morphology of the genital organs. A detailed light-microscopical investigation revealed that *E. japonensis* was morphologically almost identical to *E. bigeminus* Nielsen & Christensen 1963, another fragmenting enchytraeid. They were separable only by one character, the glandular bodies associated with the male copulatory organs that were present in *E. bigeminus* and absent in *E. japonensis*. The finding raised a question whether these species were separate or not. To address the question, protein analyses were carried out using PAGE-IEF. Contrary to the morphological similarity, protein patterns were highly divergent between the two species; 29 (55%) out of 53 distinguishable bands of general proteins and 25 (86%) out of 29 isozymes of the six enzymes examined (EST, MDH, PGM, GPI, LDH and HE) were species-specific. The results suggest that *E. japonensis* and *E. bigeminus* are closely related but different species.

SIZE DIFFERENCE AND GENETICAL RELATIONSHIP AMONG POPULATIONS OF *CLYPEASTER JAPONICUS*

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The sand dollar *Clypeaster japonicus* has been used as a material for developmental biology. In the field, no infant *C. japonicus* is found, that is, individuals are similar in size in the population. The mark-and-recapture method showed that marked individuals were constant in size and that few immigrants were observed (In other sea urchins, population is composed of individuals of various size). The size, however, varies between populations of different habitats. To know whether size difference between populations is genetical, we PCR-amplified D-loop region of mitochondrial DNA of *C. japonicus* and determined the sequence. Using the sequence data, we examined the relationship between phyletic lineage and individual size. As far as examined, no correlation between them was observed, suggesting that size difference

## GENETIC VARIATIONS OF BROWN BEARS AND ASIATIC BLACK BEARS IN JAPANESE ISLANDS AND THE EURASIAN CONTINENT.

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In Japanese islands, the distribution of the brown bear (*Ursus arctos*) and that of the Asiatic black bear (*Selenarctos thibetanus*) are separated by the Tsugaru strait, which is biogeographically called 'Blakiston's line', whereas the two species live together in the Ussuri region of the continent. In order to know their evolutionary history, we analyzed mtDNA control region sequences of the bear populations, and then investigated molecular phylogeny and geographic variations in Japanese islands and the Eurasian Continent. Our results indicate: 1) three mtDNA lineages of the brown bears are separately distributed in southern, central-northern, and eastern Hokkaido; 2) at least one part of the Hokkaido population could have immigrated into Hokkaido from Siberia/Sakhalin during the last glacial period; 3) brown bears could have rapidly colonized the wide areas of Siberia (eastern Europe to Far East); 4) the genetic diversity of Asiatic black bears in Honshu is likely smaller than that of Hokkaido brown bears.

## ANALYSIS OF REPTILIAN MITOCHONDRIAL GENOMES: STATISTICAL EVIDENCE FOR ARCHOSAURIAN AFFINITY OF TURTLES.

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Phylogenetic position of turtles among amniotes has been under intensive debate and previous molecular studies have not established a consistent and statistically well-supported conclusion on this issue. We completely sequenced mtDNAs for the green turtle, blue-tailed mole skink and Ryukyu odd-tooth snake. Phylogenetic analyses using amino acid sequences of 12 mitochondrial proteins reliably established the Archosauria (birds and crocodilians) and Lepidosauria (lizards and snakes) clades postulated from previous morphological studies. The analyses further suggested that turtles make a sister-group of the archosaurs, and this untraditional relationship was provided with strong statistical evidence by both the bootstrap and Kishino-Hasegawa tests. It is therefore likely that turtles originated from a Permian-Triassic archosauromorph ancestor with two pairs of temporal fenestrae that were subsequently lost. The traditional classification of turtles in the Anapsida may thus need to be reconsidered.

## PHYLOGENETIC RELATIONSHIPS OF THE SUBFAMILY LYGOSOMINAE (REPTILIA: SCINCIDAE) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

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Subfamily Lygosominae are mainly distributed in Asia, Australia and Africa. Phylogenetic relationships within this subfamily were inferred from 1249 base positions of mitochondrial DNA sequence of 12S and 16S rRNA genes. Results indicated the presence of five distinct lineages within this subfamily, constituting a (*Sphenomorphus* group (*Lygosoma* group (*Egernia* group (*Eugongylus*, *Mabuya* group)))) topology. Our results did not support the monophyly of the *Mabuya* group sensu lato (i.e., *Lygosoma*, *Egernia* and *Mabuya* groups), of which a number of morphological and karyological studies demonstrated a considerable similarity. Our results contradict with the hypothesis, formulated on the basis of morphological and immunological data, that argued for the sister relationship between the *Egernia* and *Eugongylus* groups, either.

## WOLBACHIA INFECTION SHARED AMONG PLANTHOPPERS AND THEIR ENDOPARASITES: A POSSIBLE INTERSPECIES HORIZONTAL TRANSMISSION OF WOLBACHIA

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Two planthopper species, *Laodelphax striatellus* and *Sogatella furcifera*, show *Wolbachia*-induced cytoplasmic incompatibility. Nucleotide sequences for each of 3 genes, *ftsZ*, *groE* and *wsp* were identical among *Wolbachia* strains infected in several local populations of both species. Diagnostic PCR for *Wolbachia* showed that a dryinid wasp, *Haplogonatopus atratus* and a strepsipteran, *Elenchus japonicus*, were infected with *Wolbachia*. Nucleotide sequences for each of the 3 genes of strepsipteran-infecting *Wolbachia* were identical with those in the two planthopper species. These results suggest that horizontal transmissions of *Wolbachia* have occurred among two planthopper species and their strepsipteran parasites.