FAUNAL STUDY ON TERRESTRIAL NEMATODES NEAR THE GREAT WALL STATION (CHINA) IN KING GEORGE ISLAND, ANTARCTICA.

K. Kito[1] and Y. Ohyama[2].
[1]Dept. of Biol., Sch. of Med., Sapporo Medical Univ., Sapporo and [2]Nat. Inst. Polar Res., Tokyo.

We investigated the fauna of terrestrial nematodes in King George Island (KGI) of the South Shetland Islands, Antarctica. Eleven species belonging to 9 genera were found from the samples of algae, mosses and soils collected near the Great Wall Station of China. As the 7 of them are new to the island, there are at least 12 species in KGI, including 5 previously known species (Tsalolikhin, 1981). This nematode fauna consists of 1 cosmopolitan, 1 bipolar and 10 Antarctic species. This high proportion of endemism corresponds to the characteristic of the Antarctic nematode fauna pointed out in Maslen (1979). The number of species in KGI is similar to 13 in Deception Island (DI) but less than a half of 29 in Elephant Island (EI), though they are in the same islands. Considering scarcity of common species between KGI and DI in comparison with its numbers between KGI/DI and EI, it is probable that there are some more unknown species common to both islands. Further faunal investigation on terrestrial nematodes in KGI and DI is essential in confirming that the diversity of nematodes in the maritime Antarctic zone increases with decrease in latitude (Maslen, 1979).

GEOGRAPHICAL DISTRIBUTION OF SIX STREAM-DWELLING SPECIES OF FRESHWATER PLANARIANS IN THE FAR EAST.

M.Kawakatsu[1], I.Oki[2], S.Tamura[3], M.Takai[4] and O.A. Timoshkin[5]. [1]Fuji Women's Coll., Sapporo; [2]ECELI, Ōsaka; [3]Nara Women's Univ., Nara; [4]Saga Med. School, Saga; [5]Center for Ecol. Res., Kyoto Univ., Ōtsu (per. ad.: Limnol. Inst. - RAS, Irkutsk, Russia).

The following 6 stream-dwelling species of freshwater planarians are distributed widely in certain areas in the Far East: Dugesia japonica Ichikawa et Kawakatsu, 1964; D. ryukyuensis Kawakatsu, 1976; Phagocata vivida (Ijima et Kaburaki, 1916); Polycelis (Polycelis) sapporo (Ijima et Kaburaki, 1916); Seidlia auriculata (Ijima et Kaburaki, 1916); S. schmidti (Zabusov, 1916).

Their geographical distribution ranges now became very clear: D. japonica (Japan, Taiwan, Korea, China, and Primorskiy in Russia); D. ryukyuensis (the Southwest Islands of Japan and the areas on the East China Sea in Kyûshû); Ph. vivida (Japan, Korea, E. China, and Primorskiy); Pol. (P.) sapporo (Aomori Pref. in Honshû and Hokkaidô in N. Japan; S. Sakhalin in Russia); S. auriculata (central part of Honshû to Hokkaidô in Japan); S. schmidti (the north and east of Hokkaidô; Sakhalin, Primorskiy, the Chukotskiy Peninsula, and the Kamchatka Peninsula in Russia). These 6 species show charateristic vertical distribution.

IMMUNOLOGICAL CHARACTERISTICS OF THE CYSTS REFLECT THE PHYLOGENETIC RELATIONSHIPS OF THE STICHOTRICH CILIATES.

M. Kikukawa and T. Matsusaka. Dept. Biol. Sci., Fac. Sci., Kumamoto Univ., Kumamoto

Many ciliate species have been known to form cysts. Cystic characters, however, have been neglected for the consideration of ciliate phylogeny, because the cysts have been regarded as an adaptive form against adverse environmental conditions. We reported in 1987 and 1988 that ultrastructure of the cysts and encystment morphogenesis seemed to reflect phylogenetic relationships of the stichotrich ciliates. In the present study, we examined immuno-electron microscopically the cysts of 11 ciliate species, including 9 stichotrich ciliates (6 Oxytrichidae, 3 Urostylidae) and 2 Euplotidae. A polyclonal antibody against endocyst of the stichotrich ciliate *Histriculus cavicola* recognized endocyst of all species of stichotrich ciliates examined. Another polyclonal antibody, which recognizes cystic micronuclear chromatins of *H. cavicola* decorated cystic micronuclear chromatins of all 6 species of Oxytrichidae and 1 species of Urostylidae. A monoclonal antibody against endocyst of *H. cavicola* detected endocyst of 5 species of Oxytrichidae. The cysts of 2 Euplotine species are negative to all 3 antibodies. The present results together with our earlier data indicate the usefulness of the immunological characteristics and the ultrastructure of the cysts and encystment morphogenesis for the consideration of ciliate phylogeny.

PLACOZOA, TRICHOPLAX ADHAERENS OCCUR IN OKINAWAN REEF.

T.[1] Yamasu, M. [2]Chinen and T. [2]Uehara. [1]Kano, Yamaguchi, [2]Dept. of Chem., Biol.,and Mar. Sci., Coll. of Sci., Univ., Ryukyus Nishihara, Okinawa.

We found many living individuals of Placozoa, *Trichoplax adhaerens* from the reef edge in Sunabe, Chatan, Okinawajima Island on the fall of August 1995. Average size of the 83 animals was 75.000μm^2.The largest individual was 928.000μm^2 and the smallest was 900μm^2. As reported by V. B. Pearse, T. Uehara and R. L. Miller in 1994, all individuals, so far as examined, displayed globular birifringent granules in a ring in the peripheral margin by polarized light. Number of the granules in a fairy large individual (46.000 mm^2) were counted 43 while the smallest one (900 mm^2) had only three granules. Sometimes two or three granules seemed to be contained in single cell. Solubilities of granules were tested using hydrochloric acid and sodium hydroxyde.Acid disolved granules but alkaline did not.Electron microscopic X ray analizer tests on the specimens after removing of soft part of the specimens revealed that the part including granules showed a sharp peak of calcium ion suggesting of existence of $CaCO_3$ intensively, while the other parts without granules responded negative.

TAXONOMY OF DICYEMID MESOZOANS FROM JAPANESE CEPHALOPODS AND THE FECUNDITY OF DICYEMIDS.

H. Furuya, K. Tsuneki and Y. Koshida.
Dept. of Biol., Grad. Sch. of Sci., Osaka Univ., Osaka.

We examined renal sacs of eight species of Japanese cephalopods, namely *Octopus vulgaris, Octopus fangsiao, Octopus minor, Octopus hongkongensis, Octopus dofleini, Sepia esculenta, Sepia lycidas,* and *Sepioteuthis lessoniana* and we found twenty dicyemid species. These dicyemids were included in the four genera, *Dicyema, Pseudicyema, Dicyemennea,* and *Dicyemodeca,* respectively. The genus *Dicymodeca* is the first report from Japanese cephalopods. Among these twenty dicyemid species, six were previously described species. The large cephalopod species had large dicyemid species such as those belonging to the genus *Dicyemennea*. Two or three dicyemid species were usually found in the single cephalopod individual, namely in *O. vulgaris, O. fangsiao, O. minor, O. hongkongensis, O. dofleini, S. esculenta,* and *S. lycidas*. Only one species was found in *Sepioteuthis lessoniana*. A narrow host specificity of dicyemid species was seen in *O. minor, O. hongkongensis, O. dofleini,* and *Sepioteuthis lessoniana*. In addition, we examined the number of the hermaphroditic gonads and eggs per single individual in various dicyemid species, and estimated their fecundity.

FINDING OF GNATHOSTOMULIDA

H. Minegishi, Biol. Lab., Nihon Daigaku.
Omiya-shi. Saitama-ken

Gnathostomulida resemble turbellarian flatworms, but their feeding apparatus of chitinous jaws with basal plate and other features

The worms are found as interstitial organism in poorly oxygenated mudy marine sediments at Awa Kominato Chiba Prefecture from 1995 to 1996

Sea-ice method by 165μm nylon nesh is most effective

Habitats are black mudy sand of rocky cracks near intertidal zone, where lives many Annelida, Nematoda and large type Ciliata.

From 100 ml, sediments the wormes are extracted about 10~20 individuals here, but very weak in rich oxygenated sea water after 6~12 hours almost die.

Tow species can be recognized from their characters

AN UNDESCRIBED GENUS OF MONSTRILLOID COPEPODS (CRUSTACEA) WITH ANTERIORLY POINTING GENITAL SPINES AND SUBTHORACIC EGG BROODING.

M.J.Grygier[1] and S.Ohtsuka[2]. [1]Trop.Biosphere Res.Cent., Univ. Ryukyus, Okinawa and [2]Fisheries Lab., Hiroshima Univ., Takehara.

Monstrilla turgida Scott, M. longipes Scott, and at least five undescribed species of monstrilloid copepods from coral reef plankton in the Ryukyu Islands comprise a well defined genus-level group, known only from females, with the following characteristics: pair of anteriorly pointing genital spines arising from genital double somite and serving to hold egg mass subthoracically between the legs (these spines trail posteriorly in all other monstrilloids); swimming legs of each pair far apart, connected by low and wide intercoxal sclerite. Most species with somewhat bulbous cephalothorax and distinctly protruding oral papilla. All except M. turgida with reticulated cephalothorax, no inner coxal seta on legs 1-4, uniramous leg 5 with two setae, and ventral protrusion of genital double somite; two species with unusual lobes at outer base of leg coxae. SEM photographs of specifically distinctive cuticular ornamentation (denticles, pores, ridges) are presented for M. turgida and two undescribed species. The leg separation, low intercoxal sclerite, and missing coxal setae are seen as adaptations to subthoracic brooding, recorded here for the first time among planktonic copepods; brooding habits in non-planktonic copepods are briefly reviewed. The nauplius of one species, hatched from brooded eggs, generally resembles that described by the authors for Monstrilla hamatapex, at least at the light microscope level.

FORMATION OF PROCTODAEUM AND ABDOMINAL METAMERISM IN A MAYFLY EPHEMERA JAPONICA MCLACHLAN (INSECTA, EPHEMEROPTERA)

K. Tojo[1] and R. Machida[2]
[1]Inst. of Biol. Sci., Univ. of Tsukuba, Tsukuba
[2]Sugadaira Montane Res. Ctr., Univ. of Tsukuba, Nagano

We examined the abdominal development in a primitive pterygotan mayfly Ephemera japonica McLachlan, with special reference to the proctodaeum formation and abdominal metamerism.

In the longest-embryo stage, the abdomen is folded and divided into four regions (regions I to IV). In the regions I and II are formed the 1st to 5th and the 6th to 11th abdominal segments, respectively. Any attributes suggestive of segmentation are not found in the regions III and IV, and they fuse with each other to form the proctodaeum. A terminal structure called caudal filament develops at the apex of IV, so that the caudal filament has its origin much away from the 11th abdominal segment, with the regions III and IV interposing between. This may reject the traditional interpretation that the caudal filament should be the elongated tergum of 11th abdominal segment.

COMPARISON OF N-TERMINAL AMINO ACID SEQUENCES OF HEMOCYANIN SUBUNITS IN MYGALOMORPH SPIDERS

T. KUWADA and H. SUGITA. Inst. of Biol. Sci. , Univ. of Tsukuba, Tsukuba.

Mygalomorph spider hemocyanins are composed of 2-8 subunits. Therefore, molecular evolution of these subunits would be studied by comparing their amino acid sequences within and between species, and species evolution of these primitive spiders by comparing orthologous subunits between species. In order to select the orthologous subunits in Mygalomorphae, N-terminal amino acid sequences of their hemocyanin subunits were analyzed. There are sequence similarities of more than 80% among subunits LT2(*Latouchia typica*), UF1(*Ummidia fragaria*), MSP1 (*Macrothele* sp.) and Ec-a(*Eurypelma californicum*). It seems that LT2, UF1, MSP1 and Ec-a are orthologous subunits. Therefore, we would analyze the divergence pattern and divergence time of spiders having these subunits. Subunits AR4(*Antrodiaetus roretozi*) and AY4(*Antrodiaetus yesoensis*) are similar to each other in N-terminal amino acid sequences, but no subunit similar to them is found in other family than Antrodiaetidae. Therefore, in spider lineage it is thought that hemocyanin subunits tend to be deleted or that hemocyanin subunits have high rate of molecular evolution.

GENETIC DIFFERENTIATION OF *LITTORINA SITKANA* PHILIPPI IN HOKKAIDO.

M. Nohara. Graduate School of Human Informatics, Nagoya University, Nagoya.

L. sitkana (Mollusca, Gastropoda) commonly lives on intertidal rocky shores in Hokkaido. This species has three different forms in terms of shell sculpture, and their relative abundance varies geographically there(Ohgaki, 1982). As *L. sitkana* is a direct developer, the level of gene flow can be regarded low among local populations, which are therefore expected to be genetically differentiated.

To test this hypothesis, samples were collected from Akkeshi, Konbumori, Abashiri, Bikuni, Shikabe and Minamikayabe in Hokkaido. They were analyzed using horizontal starch gel electrophoresis. Allozymic data of 7 polymorphic loci were statistically examined, and these 6 local populations were clustered using UPGMA.

Allele frequencies at each locus were proved different significantly among the populations. Moreover, the 6 populations could be clustered into 3 groups on the basis of genetic similarity. This genetic differentiation was almost consistent with the geographical variation in shell sculpture.

This geographical variation in genetic structure and shell sculpture may be attributable to the founder effect.

HEAD MORPHOGENESIS IN LEPIDOCAMPA WEBERI OUDEMANS (HEXAPODA, DIPLURA)

Y. Ikeda[1] and R. Machida[2]. [1]Inst. Biol. Sci., Univ.Tsukuba, Ibaraki and [2]Sugadaira Montane Res. Ctr., Nagano.

The Diplura is one of the most primitive hexapod groups and belongs to the Entognatha which is characterized by entognathous mouth parts. Here we observed the external features of entognathous mouth parts formation in a dipluran, L. weberi. As the gnathal appendages develop, the intercalary, mandibular and maxillary terga extend ventrally to form the mouth folds. Finally the mandibles and maxillae except for their tips come to be surrounded anteriorly with labrum, laterally mouth folds and posteriorly labium, and the entognathy completes. Unipue morphogenetical processes such as the rotation of labial anlagen and formation of admentum are found to be involved in the entognathy formation of L. weberi, the same as in the other diplurans. These prosseses may be considered to be autapomorphic for Diplura.

GENETIC VARIABILITY OF mtDNA IN *Halocyntia roretzi*

- difference between the coast of Japan sea and Pacific ocean-

T.Kakuda and A. Hino
Department of Biological Sciences, Kanagawa Univ. Hiratsuka.

Halocynthia roretzi distributes around the coast of Hokkaido, Honsyuu island and Korean Peninsula. On account of difference of the breeding season and the start time of spawning, *H.roretzi* is classified into three types on A, B, C. Only type C distributes all around Honsyuu island. Totally over 150 individuals of 10 populations of *H.roretzi* that collected from shore of Hokkaido and Honsyuu island, were studied by the RFLP and restriction site analysis of mtDNA using 13 restriction enzymes. From these analyses, we found some individuals which showed polymorphic pattern after digestion by *Hin*cII, *Hpa*I, *Ban*II, *Eco*T22I and *Xba*I, and finally defined 17 haplotypes. SUGASHIMA(Ise Gulf) population had no polymorphism. In this area, oyster farming has begun for 10 years. It seems that a small population of *H.roretzi* influx with juvenile oyster, and spread their habitat to SUGASHIMA area. Even if, we calculate without this population, the frequencies of polymorphism of the Japan-sea populations were higher than that of the Pacific ocean populations.

DIVERSITY OF THE MITOCHONDRIAL DNA IN JAPANESE WILD POPULAITONS OF THE MEDAKA *ORYZIAS LATIPES*

M. Matsuda[1], S. Hamaguchi[2], and M. Sakaizumi[2]. [1]Grad. Sch. of Sci. & Tech., Niigata Univ., Niigata, [2]Dept. of Environ. Sci., Fac. of Sci., Niigata Univ., Niigata.

To examine the evolutionary history of Japanese wild populations of medaka, we analyzed mitochondrial DNA (mtDNA) restriction fragment length polymorphisms. We identified 63 haplotypes using six restriction endonucleases. Phylogenetic analysis of haplotypes revealed three clusters; two major (cluster A and B) and one minor cluster (cluster C). Estimated sequence divergence among the clusters ranged from 7.3 to 12.4%. The distribution of seven haplotypes of the cluster A, coincided with that of the Northern Population recognized by allozymic analysis. Other two clusters, cluster B and C contain 55 and one haplotype(s), respectively. The distribution of these haplotypes coincided with that of the Southern Population. We assume that a haplotype of the cluster C represents an ancestral polymorphism. A large number of haplotypes was found in the eastern Seto Inland Sea and the Kanto districts. In other areas a smaller number of haplotypes was noted. The distribution pattern of haplotypes may reflect the history of the dispersal of medaka in Japan.

A NEW SYNONYM OF *DROSOPHILA POLYCHAETA*, WITH THE RESULTS OF CROSS-EXPERIMENTS.

H. Watabe and N. Toribe
Biological Laboratory, Sapporo College, Hokkaido University of Education, Sapporo 002.

Drosophila polychaeta Patterson and Wheeler, 1942 is a domestic species with a wide range of distribution, and its closely related species, *D. asper* Lin and Tseng, 1971 is a riparian species having been recorded from natural forests in Taiwan. These two species belong to the *D. polychaeta* species group established by Sturtevant (1942). We have comparatively examined the external morphology, genitalial structure, karyotype and sexual behavior of these species.

Only slight differences were observed in the external morphology and genitalia: 1) color patterns on abdominal tergites, 2) the number of tiny thorn-like spines on the flap of surstylus, 3) size of aedeagus. In the interspecific cross, however, F_1 flies were completely fertile for both sexes and produced much offspring. Further, both species have the same number of chromosomes, 2n=12 (2R+2J+1V+1D).

Therefore, we have concluded that *D. asper* is a junior synonym of *D. polychaeta*. The present results also suggest that "*polychaeta*" and "*asper*" might be regarded as "eco-species" with different habitat characteristics.

INTRASPECIFIC VARIATION OF A JAPANESE STREEM-BREEDING FROG, *BUERGERIA BUERGERI*.

H. Ueda, Y. Hasegawa and J. Marunouchi. Lab. for Amphibian Biol., Fac of Sci., Hiroshima Univ., Higashihiroshima.

Morphometric characters and age-structure of a Japanese stream-breeding frog *Buergeria buergeri*, were compared among 38 females and 68 males collected from the Ohta River in Hiroshima Prefecture and among 13 females and 28 males collected from the Iwaki River in Aomori Prefecture. Our results showed that the mean snout-vent length of the females and males of the Hiroshima population was 67.4 mm and 42.9 mm, respectively, whereas that of the Aomori population was 49.1 mm and 37.2 mm, respectively. However, the number of growth linens (LAG) observed on the cross-sections of phalanges from each frogs suggested that both the females and males of the Aomori population begin to breed one year later than those of the Hiroshima population. Principal component analysis and discriminant analysis based on the measurements of 15 body sites of the adult frogs and 6 body sites of the tadpoles at stage 13 demonstrated complete or almost complete discrimination of the Hiroshima population from the Aomori population.

Though reciplocal interpopulational hybrids between these two populations using 5 females and 3 males of the Hiroshima population and 3 females and 3 males of the Aomori population showed the same cleavage rates of 99 - 100% as those of the controls, many hybrid embryos became abnormal before and after hatching and died. Thus, 23.0% of the total 1359 hybrid eggs metamorphosed, while 62.7% of the total 1093 control eggs metamorphosed.

EVOLUTION OF THE *RANA ADENOPLEURA* GROUP.

M. Matsui. Grad. Sch. Hum. and Env. Stud., Kyoto Univ., Kyoto.

Rana adenopleura from Taiwan and eastern China breeds in a normal manner in open waters, but its relatives *R. psaltes* from Yaeyama and *R. daunchina* from western China make a special breeding hole. In advertisement calls, however, *R. daunchina* is more similar to *R. adenopleura* than to *R. psaltes*. From the recent discovery of *R. psaltes* from Taiwan, the following hypothesis is proposed for the evolution of these frogs. In continental China, a *daunchina*-like population with a specialized breeding habit arose allopatrically from *adenopleura*-like ancestral form with a normal breeding habit. When these two populations met, their sympatric distribution was made possible through the enforcement of slight differences in advertisement calls in the *daunchina*-like population. The ancestor of *R. psaltes* with the *daunchina*-like, specialized breeding habit, but with a unique advertisement call may be thus produced in the periphery of eastern China.

PHYLOGENETIC RELATIONSHIPS WITHIN THE 24-CHROMOSOME SPECIES OF BROWN FROGS FROM JAPAN AND THE NEIGHBORING COUNTRIES .

T. Tanaka[1], M. Matsui[1] and O. Takenaka[2]. [1]Grad. Sch. Hum. Env. Stud., Kyoto Univ., Kyoto and [2]Primate Res. Inst., Kyoto Univ., Inuyama.

The brown frogs of the *Rana temporaria* group can be grouped into two types by the number of diploid chromosomes (2N=24 or 26). We reported previously that, in all species of the Japanese brown frogs, the 24-chromosome species (*R. pirica, R. dybowskii and R. ornativentris*) were closer to each other than the 26-chromosome species. Although only *R. dybowskii* is not endemic to Japan, distributed widely in continent. Variations among local populations was not elucidated very much. In addition, there is a brown frog *R. sp.*, whose phylogenetic position have not been decided yet, with 2N=24 chromosomes in Sakhalin. The 24-chromosome species have these phylogenetically problems. We analyzed cytchrome b sequences as to the brown frogs of the 24-chromosome species in Japan and the adjacent areas (Korea and Russia) to clarify the intra and/or inter relationships. This study obtained the following results. 1) Sequences between Tsushima and Korean populations of *R. dybowskii* were same. However, *R. dybowskii* of Lazo population (Russia) was quite different genetically from that of those populations, but close to *R. pirica*. Consequently, within *R. dybowskii*, local populations seem to have genetically well differentiated. 2) First *R. ornativentris* diverged from the others, and then *R. sp.* from Sakhalin diverged. *R. dybowskii* and *R. pirica* formed a cluster. Genetic differentiation of *R. sp.* from Sakhalin was similar to that of the other brown frogs.

KARYOTYPES OF *RANA SAKURAII* AND *RANA TAGOI* DISTRIBUTED IN NISHITAMA DISTRICT IN TOKYO.

M. Ryuzaki[1], N. Takizawa[2] and M. Ito[3]. Depts. of [1]Biol. and [2]Physiol. ,School of Med. , [3]Dept. of Biol. Sci., Kitasato Univ., Sagamihara.

Karyotypes of *R. sakuraii* from Musashiitsukaichi Machi and *R. tagoi* from Hinohara Mura in Nishitama district were analyzed by conventional Giemsa staining, C-banding and late replication (LR)-banding on 20 frogs, ten frogs of each type, consisting of 5 females and 5 males. Chromosome numbers were 2n=26 in all cases. Thirteen pairs of chro-mosomes (Nos.1-13) were arranged in order of relative length (RL) which was expressed as percentage of genome length. Chromosome 10 had a secondary constriction with the long arm. Twelve pairs other than chromosome pair 8 showed the same sex features in all frogs. For chromosome 8 pair, sex-specific changes were noted in all cases. All frogs had chromosome pair No. 8, the XY type sex chromosomes. In *R. sakuraii*, the X chromosome was metacentric and whose numerical value of the centromere position (NVC) was 45.2±0.71 and 48.3±0.61. The Y chromosome was submetacentric and NVC was 27.7±0.73. In *R. agoi*, the X chromosome was submetacentric with NVC from 36.1±0.84 and 29.1±0.72. The Y chromosome was submetacentric with the NVC of 27.4±0.71. C-banding analysis indicated that, in *R. sakuraii*, neither X nor Y chromosomes C-band except for centromere, while in *R. tagoi* the X chromosome possessed a C-band with a long arm and the Y chromosome to have no C-band possessing both a long and short arm. LR-banding analysis demonstrated the X and Y chromosomes to possess a LR-band with a short arm and two LR-bands each with a long arm, respectively and the banding for *R. sakuraii* and *R. tagoi* to be essentially same.

COMPARISON AMONG GROEL HOMOLOGS FROM INTRACELLULAR SYMBIONTS OF CLOSELY INTERRELATED SPECIES OF APHID.

K. Komaki, S. Sato, and H. Ishikawa. Department of Biological Sciences, Graduate School of Science, University of Tokyo.

Buchnera, an intracellular symbiont harbored by aphid selectively synthesizes symbionin, a stress protein homologous to E. coli GroEL. Like many other GroEL homologs, symbionin functions as molecular chaperone in vivo and in vitro. Through studies on the pea aphid, Acyrthosiphon pisum, it has been demonstrated that symbionin functions not only as molecular chaperone, but also as an enzyme that transfers phosphate group from ATP to substrates through its autophosphorylation at His-133. In this study we analyzed the symbionin genes of two species of aphid, A. kondoi and A. solani that are closely related to A. pisum. It turned out that the two symbionins were more than 99% identical with symbionin of A. pisum at the amino acid sequence level, although the His-133 was not conserved in the two symbionins. Instead the 133rd amino acid was Asn in these two symbionins. Apparently, the codon for the 133rd amino acid of symbionin is highly susceptible to variation. From these results, it was suggested that amino acid substitutions at the position 133 were positively selected, through which two GroEL homologs of symbionts have acquired a unique function such as phosphotransferase activity.

HISTOCHEMICAL DETECTION AND MOLECULAR PHYLOGENY OF YEAST-LIKE SYMBIONTS OF APHIDOIDEA, COCCOIDEA AND FULGOROIDEA.

T. Fukatsu, K. Watanabe and H. Kuriyama.
National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Higashi 1-1, Tsukuba, Ibaraki 305, JAPAN.

The tribe Cerataphidini is an aphid group which embraces more than 10 genera, 60 species found mainly from south-eastern Asia. We have demonstrated that in a lineage of the Cerataphidini prokaryotic intracellular symbiont was replaced by a yeast-like microorganism. Molecular phylogenetic analysis revealed that the yeast-like symbiont of a cerataphidine aphid, *Hamiltonpahis styraci*, belongs to the class Pyrenomycetes, the suborder Ascomycotina.

In Homoptera, in addition to the Cerataphidini, yeast-like symbionts have been reported from many species of coccids and fulgorids. Thus it is conceivable that in Homoptera yeast-like symbionts have evolved many times independently. In order to identify the origins of yeast-like symbionts in Homoptera, we determined almost entire length of 18S rDNA sequences of yeast-like symbionts of cerataphidines, coccids and fulgorids in which yeast symbionts were detected histochemically.

PHYLOGENY AND BIOGEOGRAPHY OF 23 SPECIES OF THE GENUS *ISOBACTRUS* (ACARI: HALACARIDAE).

H. Abé. Dept. of Mar. Sci. and Technol., Sch. of Engineer. Hokkaido Tokai Univ., Sapporo.

The phylogenetic relationships of 23 species of the genus *Isobactrus* (Acari: Halacaridae) were inferred on the basis of some external morphological characters by using a cladistic approach. A strict consensus tree was generated from a large number of equally parsimonious cladograms. The consensus tree showed several monophyletic groups in the genus. The branching pattern of the consensus tree and the geographical distributions suggested the following order of divergence: 1) the mid-Pacific species, 2) the Antarctic species, 3) the North Atlantic plus North Pacific species. The mid-Pacific species and the North Atlantic plus North Pacific species are monophyletic respectively, whereas the Antarctic species is paraphyletic.

GENETIC POLYMORPHISM IN RDNA SPACER REGION OF VELVET SHRIMPS (DECAPODA).

S. Shirai[1], T. Kobayashi[2], and J. Ohtomi[3]. [1]Seikai Natn. Fish. Res. Inst., Nagasaki, [2]Natn. Res. Inst. Aquaculture, Nansei (Mie Pref.), [3]Fac. Fish., Kagoshima Univ., Kagoshima.

Metapenaeopsis, the penaeid shrimp genus consisting about 60 species, includes considerable confusion in taxonomy and morphological diversity within some species. The internal transcribed spacer (ITS) of the rDNA tandem repeat should be one of the ideal candidate to resolve such problems and to clarify the interrelationships of these species. The ITS shows considerable differences in size among 11 species around JAPAN; the size of the ITS-1 (the spacer between 18S and 5.8S regions) is about 1.0 kbp in *M. dalei*, while over 2.0 kbp in *M. sinica*. Although the size and sequence of the ITS are more or less conservative within each species, we found some insertions and deletions, which should be informative to recognize polymorphism among local forms.

ISOLATION OF PHARYNGEAL GILL-SPECIFIC GENES IN THE ASCIDIAN *Halocynthia roretzi*

K. J. Tanaka[1], M. Ogasawara[2], K. W. Makabe[2], N. Satoh[2]
1.Depart. of Biol., Fac. of Sci., Konan Univ., Kobe and 2.Depart. of Zool., Grad. Sch. of Sci., Kyoto Univ., Kyoto

Pharyngeal gill-slit, endostyle and notochord are key structures to understaning mechanisms underlying the origin and evolution of chordate. We made an ascidian phalyngeal gill cDNA libraly and isolated cDNA clones for two phalyngeal gill-specific genes (*HrPhG1* and *HrPhG2*) by differential screening of the library.

Transcripts of these genes were detected in phalyngeal gill wall a few days after metamorphosis. This expression pattern was retained to adult stage. These genes may serve as useful probes for further analysis of molecular mechanisms involved in the formation of the phalyngeal gill in chordates.

EXPRESSION OF ENDOSTYLE-SPECIFIC GENES IN THE ASCIDIAN *Halocynthia roretzi*

M. Ogasawara[1], K. J. Tanaka[2], K. W. Makabe[1], N. Satoh[1]
[1]Depart. of Zool., Grad. Sch. of Sci., Kyoto Univ., Kyoto, and [2]Depart. of Biol., Fac. of Sci., Konan Univ., Kobe

Notochord, nerve cord, pharyngeal gill and endostyle are structures key to understanding of the molecular mechanisms underlying the origin and evolution of chordates. We made an ascidian endostyle cDNA library and isolated two endostyle-specific genes, *HrEnds1* and *HrEnds2* by differential screening of the library. Predicted amino acid sequences of these genes suggested that both genes encode novel secreted proteins. Transcripts of these genes were detected in the endostyle of 1-month young adult under our *in situ* hybridization condition. These genes may serve as probes for further analyses of molecular mechanisms involved in formation of the endostyle.

PHYLOGENY OF HELICOIDEAN LAND SNAIL FAMILIES INFERRED FROM MITOCHONDRIAL GENOMIC STRUCTURE.

R. Ueshima[1], Y. Noguchi[2] and A. Kurabayashi[2]. [1] Dept. of Biol. Sci., Graduate School of Sci., Univ. of Tokyo, Tokyo and [2] Inst. of Biol. Sci., Univ. of Tsukuba, Ibaraki.

Superfamily Helicoidea is one of the largest superfamily in pulmonate land snails and is composed of more than 10 families. Current taxonomic system of Helicoidea is based on the morphology of genitalia. However, phylogeny of the helicoidean families is still controversial, because some important genital features are suggested to be evolved convergently. We found that some mitochondrial gene orders are drastically different among some helicoidean families. Thus, we have determined partial mitochondrial gene orders of many helicoidean and outgroup taxa. Based on some shared apomorphic gene orders, we could reconstruct the phylogeny of some helicoidean families. Our results are not consistent with the current taxonomic system. Possible convergent evolution of some genital features in helicoideans and phylogenetic significance of mitochondrial genomic rearrangements will be discussed.

INTRA- AND INTERPOPULATIONAL GENETIC VARIATIONS OF ANDERSON'S RED-BACKED VOLES

M. Iwasa[1], H. Suzuki[1], E. Kitahara[2], Y. Kimura[3] and Y. Obara[4]. [1] Graduate School of Env. Earth Sci., Hokkaido Univ., Sapporo, [2] Kansai Res. Cen., Forestry and Forest Prod. Res. Inst., Kyoto, [3] Fac. of Educ., Fukushima Univ., Fukushima, [4] Fac. of Sci., Hirosaki Univ., Hirosaki.

Anderson's red-backed voles (*Eothenomys andersoni*) inhabit the eastern part of Honshu and the Kii Peninsula, being sometimes classified into two species, *E. andersoni* and *E. imaizumii* respectively. Sequencing analysis in 402 bp of the gene for the mitochondrial cytochrome *b* showed presence of two distinct types of mitochondrial DNA in the population of the Kii Peninsula, one was homologous to those observed in the eastern populations and the other was specific to the Kii Peninsula. Substantial difference within the population of the Kii Peninsula was also observed in sequences of approximately 370 bp of a DNA fragment from the Y-linked, sex determining locus, *Sry*. These data suggested that the population of the Kii Peninsula has been generated through complex evolutional processes such as occasional genetic interchange with other populations of Anderson's red-backed voles.

ORIGIN OF NINE SPECIES OF JAPANESE SMALL RODENTS

H. Suzuki[1], M. Iwasa[1], K. Tsuchiya[2], S. Sakaizumi[3]
[1]Grad. Sch. Env. Earth Sci., Hokkaido Univ, Sapporo, [2]Miyazaki Med. College, Miyazaki, [3]Fac. Sci., Niigata Univ., Niigata.

In order to know the origin of small wild rodents native to Japan, comparative analysis with several DNA markers was performed between nine native species, namely *Apodemus speciosus*, *A. argenteus*, *Clethrionomys rufocanus*, *C. rex*, *C. rutilus*, *Eothenomys andersoni*, *E. smithii*, *Diplothrix legata*, *Tokudaia osimensis*, and their related species or populations distributed in the Continent. All species showed large sequence divergences, more than 3% , from their related or the same species of the Continent in the mitochondrial cytochrome *b* gene. The results suggested that the ancestral populations of these species came from the continent to Japan at the Early Pleistocene period or before. Two species, *C. rufocanus* and *C. rutilus*, showed no considerable genetic differentiation from the continental populations in some nuclear DNA markers, suggesting the presence of subsequent genetic interchanges between the Japanese and continental populations during the Ice Age.

LOW GENETIC DIVERSITY OF THE IRIOMOTE CAT POPULATION, INFERRED FROM MICROSATELLITE ANALYSIS

R. Masuda and M.C. Yoshida. Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo.

We have investigated molecular phylogeny of the Iriomote cat and then reported that this endangered wildcat is a geographically isolated population of the Asian leopard cat, *Felis bengalensis*. The genetically estimated divergence time (about 200,000 years before present) between the Iriomote cat and the continental leopard cat was in agreement with the formation date of the Ryukyu Arc including Iriomote Island. The population of the Iriomote cat on Iriomote Island has been estimated at about 100 and portected as an endangered animal. For studying evolution of animal populations isolated on islands as well as for conservation of endangered populations, it is quite necessary to investigate the genetic diversity in the Iriomote cat population. Therefore, we examined polymorphic status of several microsatellite loci in the Iriomote cat population, compared with that in the continental population of the leopard cat. As a result, some alleles were observed at each locus examined in the continental population. By contrast, the Iriomote cat population showed much lower heterozygosity at the same loci. The result suggests that the reduction of genetic diversity in the Iriomote cat population has been due to possible bottleneck and random genetic drift after geographic isolation in Iriomote Island.

HARPACTICOID COPEPODS AS THE BIOINDICATOR IN RIVER HOZAKI, IBARAKI PREFECTURE

Y. Kikuchi
Itako Hydrobiological Station, Ibaraki University, Ohu, Itako-machi, Ibaraki

River Hozaki, very small stream, located at the east-southeast of Mt. Tsukuba. I have monthly collected harpacticoid copepods at 4 stations,A-D. A site is contaminate comparatively because the polluted water from human activity flows into this river. On the contrast, C and D were the clearn water as mountainous stream. The results as the following:

A:absent, B:*Bryocamptus zschokkei*, *Bryocamptus nivalis*, *Attheyella* sp.,
C:*Bryocamptus nivalis*, *Canthocamptus prominulus*, *Parastenocaris* sp.,
D:*Canthocamptus prominulus*

Canthocamptus prominulus is living in clean water as mountainous trickle and *Parastenocaris* sp. is also in non-polluted condition, especially in interstitial water.

This present study reveals that harpacticoid copepods as bioindicator are able to judge whether the stream water is polluted or not.

SYMBIOTIC CHLORELLA ENHANCES TOLERANCE TO THE HEAT AND THE HIGH CONCENTRATION OF K^+ IN PARAMECIUM BURSARIA.

M.Nishidoi, M.Sano and K.Iwatsuki. Dept. of Biology, Fac. of Education, Kagawa Univ. Takamatsu, Kagawa.

We studied the role of symbiotic chlorella on the tolerance to heat and to high concentration of K^+ in *Paramecium bursaria*. In order to know the effects of chlorella, we used *P. bursaria* with (normal organisms; Green) and without (White) chlorella. Organisms of Green and White were transferred from adaptation medium (K^+1mM,Ca^{2+}1mM) to test solution (K^+100mM,Ca^{2+}1mM). White could live in 100mM K^+ solution for less than 15 min, while Green could live more than 20 min. Green and White were transferred from 20°C adaptation medium to 40°C medium. White died within 200 s, while Green tolerated more than 300 s. However, these tolerances of Green which were in the medium with inhibitor of photosynthesis (DCMU) was same as those of White. Similarly the degree of the tolerances of Green which were not given light was same as those of White. These facts suggest that photosynthesis of symbiotic chlorella are deeply related to these two tolerances in *P.bursaria*.

MOLECULAR PHYLOGENY OF ASIAN AND NORTH AMERICAN SQUIRREL SPECIES INFERRED FROM MITOCHONDRIAL DNA SEQUENCES.

T.Oshida, R.Masuda, and M.C.Yoshida. Chromosome Res. Unit, Fac. of Sci., Hokkaido Univ., Sapporo

In order to investigate phylogenetic relationships between flying squirrels and non-flying squirrels, 12S rRNA and cytochrome b sequences were analyzed for three flying squirrel genera (*Petaurista*, *Pteromys*, and *Glaucomys*), four tree squirrel genera (*Sciurus*, *Callosciurus*, *Tamiasciurus*, and *Funambulus*), and one semi-arboreal squirrels genus (*Tamias*), all which are distributed in Asia and North America. Phylogenetic trees of mitochondrial DNA sequences indicated that the flying squirrels were clustered together and separated from the non-flying squirrels. The result suggests monophyletic evolution of the flying squirrels.

GREGARINES FROM JAPANESE TENEBRIONIDAE

K. Hoshide Biol. Lab. Fac. of Educ. Yamaguchi Univ.,Yamaguchi

Twenty-one species of eugregarines have been reported from 16 species of Japanese Tenebrionidae. They belonged to 6 genus, Gregarina, Hirmocystis, Stylocephalus, Stylocephaloides, Steinina and Asteropho-ra. Gonocephalum japanum Motschulsky was collected at Hikari and Obatake in Yamaguchi Prefecture. Stylo-ceaphalus japonicus Tsugawa was parasitized in the digestive tract of G. japanum. The gamont of S. japonicus is solitary. The measurement of TL is 1100 µm, LP 60 µm, WP 80µm, WD 120 µm. The nucleus is ovoid or ellipsoid. The gametocyst is spherical and 450 µm in diameter. The oocyst is hemisphrical or helmet-shaped and brown in color. It is dehisced by simple rupture and extruded in chains. Several hours after adding the extracts of the digestive tract the oocyst cracks at the side wall and thread-like sporozoites excyst through the cracks. Levine classified S. japonicus as a synonym of S. bahli Misra from Indian Tenebrionidae, Gonocephalum helopioides. According to the comparison of the characters of both species, S. japonicus shows some resemblances with S. bahli, but the former shows significant difference from the latter in several characters. Both species may belong to different species.

THE NATIVE AND ALIEN CRAYFISH SITUATION IN EASTERN HOKKAIDO, JAPAN (*CAMBAROIDES JAPONICUS, PACIFASTACUS LENIUSCULUS*)

Sh. Hiruta[1] and K. Saito[2]. [1]Biol. Lab. Hokkaido Univ. of Educ. at Kushiro, Kushiro and [2]Grad. Sch. Environ. Earth Sci. Hokkaido Univ. Sapporo.

There are two species of crayfish living in Eastern Hokkaido: *Cambaroides japonicus* and *Pacifastacus leniusculus*. The former is a native species and the latter was introduced from the Colombia River area of the United States in 1930. It has been discovered that *P. leniusculus* is now widely distributed in the rivers and lakes of the Kushiro Shitsugen Marsh and its adjacent areas. Although the distribution of the native crayfish is extensive in Hokkaido, its population has declined due to changes in water quality, habiatat modification, and probable invasion of *P. leinusculus* in Eastern Hokkaido. It is now necessary to study both species distribution in detail and consider countermeasures for the conservation of the native species as soon as possible. Included is a report of native and alien crayfish situation in Britain, where a similar problem exists, this is presented as a reference example.

PHYLOGENETIC RELATIONSHIPS OF THE *EPILACHNA VIGINTIOCTOMACULATA* COMPLEX. II.

N. Kobayashi[1 2], M.Kumagai[2], K. Tamura.[2], T. Aotsuka.[2] and H. Katakura[1]
[1]Div. of Biol. Sci., Grad. Sch. of Sci., Hokkaido Univ., Sapporo . [2]Dept. of Biol.. Tokyo Metropolitan Univ., Hachiouji.

The *Epilachna vigintioctomaculata* complex consists of four species of phytophagous ladybird beetles. These species are classified into two groups (group A; *E. Vigintioctomaculata*, and group B; *E. pustulosa*, *E.niponica* and *E. yasutomii*). In group B, all the species are almost the same morphologically but can be recognized by differences in host plant preference and in geographic distributions. Because this complex is a useful material for studying animal speciation process including changes in host-utilization, many ecological studies have been done. However, the phylogenetic relationships among these species have not been clarified yet. In this study, we determined the nucleotide sequences of mitochondrial cytochrome c oxidase I gene (1kb) for 64 individuals from 13 populations and constructed a phylogenetic tree by neighbor-joining method. In the tree, members of the complex were divided into two major clusters corresponding to the two groups by the morphological classification. In group A, apparent geographic divergences were observed. In group B, the magnitude of the inter-specific divergences did not exceed the intra-specific level.

MULTIREGIONAL MITOCHONDRIAL DNA INTROGRESSION IN THE GOBIID FISHES, TRIDENTIGER OBSCURUS AND T.BREVISPINIS

T.Mukai[1], K.Naruse[2], T.Sato[1], A.Shima[2] and M.Morisawa[1]
[1]Misaki Marine Biological Station, Univ. of Tokyo, Kanagawa. [2]Dept. of Biol. Sci., Univ. of Tokyo, Tokyo.

We investigated the allozyme polymorphisms and mitochondrial DNA (mtDNA) sequence variations in the gobiid fishes, the genus *Tridentiger* and obtained the results which suggest that the mtDNA introgression occurs in two species, *T. obscurus* and *T. brevispinis*.

In this study, we determined the partial sequences of cytochorome b gene (402bp) in mtDNA of *T. obscurus* and *T. brevispinis* obtained from geographically distant populations and compared the sequence variations in the species. From phylogenetic analysis, the mtDNA types from geographically near populations of the two species were clustered together and every mtDNA types were very similar. These results suggest that multiregional mtDNA introgression occurs and the mtDNA of the one species may possibly displace anothers.

LARVAL DEVELOPMENT OF *GONIOPUGETTIA SAGAMIENSIS* GORDON (CRUSTACEA, BRACHYURA) UNDER LABORATORY CONDITIONS

Hajime Taishaku [1] and Kooichi Konishi [2]. [1] Natl. Res. Inst. of Aquaculture, Mie, 516-01 Japan, and [2] Toba Aquarium, Mie, 517 Japan

Larval stages of the spider crab, *Goniopugettia sagamiensis* (Gordon) (subfamily Acanthonychinae) were documented based on laboratory reared material. Larval development of *G. sagamiensis* consists of two zoeal and one megalopa stages as typical in majid crabs. It took 31-35 days from hatching to first crab at 14.1-18.5 °C. The diameter of eggs of the present species is relatively large (1.09-1.20 mm), and the larvae have a mass of yolk granules throughout the larval stages. The larvae metamorphosed to the first crab stage without feeding. Such a lecithotrophic development as found in the present species is only known in *Chlorinoides longispinus* (De Haan) (subfamily Majinae) among the majid species of Japan. General morphology of the zoeas of *G. sagamiensis* is similar to those of *C. longispinus* : e.g., rudiment of mandiblular palps in the zoea 1 stage.

CLADISTIC ANALYSIS OF THE GENUS *ANTOCHA* RECORDED FROM JAPAN AND ITS ADJACENT AREA (INSECTA: DIPTERA: TIPULIDAE)

T. Torii. Dept. Syst. Zool., Fac. Sci., Tokyo Metropol. Univ., Hachioji.

Antocha is a crane fly genus with its size being small to medium. Typical members have the following wing features: anal angle prominent; Sc close to R, Sc2 not evident; basal section of Rs usually straight, diverging from R1. They spend their immature stages in rapidly flowing streams; adults are found abundantly along the streams during summer, and often collected at light. Until present, 18 species of the genus *Antocha* were recorded from Japan, Korea Peninsula, and far east Russia.

In this study, a phylogenetic relationship of the 18 species is discussed on the basis of the cladistic analysis of morphological characters, which are obtained from adults. In total 25 characters—mainly from male and female terminalia, wing venation, legs, and thoracic pleura—are evaluated. A computer software is applied; the results of the analysis are the following:

1) Sixteen species are arranged in one clade showing dichotomous branchings; the relationship among the clade and the other two species is left trichotomous;
2) Subgenus *Proantocha* is monophyletic, while subgenus *Antocha* is paraphyletic.

Phylogenic status of chaetognaths, as deduced from actin sequences.

E. Yasuda[1], N. Sato[2] and T. Goto[1]. [1]Dept. of Biol., Fac. of Educ., Univ. of Mie, Tsu and [2]Dept. of Zool., Grad. Sci. of Sci., Univ. of Kyoto., Kyoto.

Chaetognath (arrow worm) is conventionally classified into deuterostomes despite that their morphological features are different from those of other deuterostomes.

Therefore, phylogenic status of chaetognaths has been debated for a long time.

Actin genes are conserved well in various animals. We have made an attempt to search relationship between arrow worm and other animals, using amino acid sequences of actins. For this purpose we isolated by RT-PCR three different of cDNAs from a benthos arrow worm *Paraspadella gotoi*. Comparison between amino acid sequences deduced from the nucleotide sequences of the chaetognath actin genes and those of other animals showed that one resembled human β-cytoplasmic actin. However, two others were less similar to actins of any other animals.

Adaptation of Bacteria to Aphid Gut and Relevant Adhesion Factors: An Implication for Origin of Intracellular Symbiont

Hosami Harada[1] and Hajime Ishikawa[2]

[1]Institute of Medical Science, [2]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

Pea aphid, *Acyrthosiphon pisum*, harbors prokaryotic intracellular symbionts in the mycetocytes, huge cells in the abdomen, which were differentiated specifically to accommodate the symbionts In view of the location and nutritional role of these symbionts in insects, it is generally believed that they originated from gut microbes that are descendants of free-living bacteria taken in with diet by the host insect in the evolutionary past.

In the previous study, we demonstrated based on the molecular phylogenetic analyses, that pea aphid harbors at least three groups of bacteria which were members of the family Enterobacteriaceae, and sharing the closest common ancestor with the intracellular symbiont of aphid.

In this study, in order to investigate the aphid-bacteria interaction, the synthetic diets mixed with bacteria from log phase cultures, were given to aphids deprived of their gut microbes. It turned out that bacterium X which is predominant species in aphid gut, and bacterium T which is identified as *Erwinia herbicola*, have the ability to infect aphids. It was observed that both the infectious bacteria produce, in common, extracellular polysaccharides, and form mucoid colonies when grown in the medium which mimics the constituents of aphid gut and body cavity. In spite of these similarities between bacteria X and T, the former had stronger infectiousness than the latter, and was supposed to be more adapted to aphid gut environment. When bacterium X was exposed to the sugars that cause the mucoid growth, 16.9 and 17.2 KDa proteins were specifically induced on its surface.

CHROMOSOMAL EVOLUTION IN THE EAST ASIAN *CROCIDURA* (MAMMALIA: SORICIDAE)

M. Motokawa[1], M. Harada[2], L.-K. Lin[3], K. Koyasu[4], and T. Hikida[1]. [1]Dep. of Zool., Fac. of Sci., Kyoto Univ., Kyoto, [2]Exp. Anim. Center, Osaka City Univ. Med. School, Osaka, [3]Lab. of Wildlife Ecol., Tunghai Univ., Taiwan, R.O.C., and [4]Second Dep. Anat., School of Dent., Aichi-Gakuin Univ., Nagoya

We investigated conventional karyotypes of several East Asian *Crocidura*: *C. l. lasiura* (2n=40, FN=56), *C. attenuata tanakae* (2n=40, FN=56), *C. dsinezumi chisai* (2n=40, FN=56), *C. d. umbrina* (2n=40, FN=56), *C. suaveolens shantungensis* (2n=40, FN=50), *C. russula hosletti* (2n=40, FN=50), *C. horsfieldii kurodai* (2n=40, FN=50), and *C. watasei* (2n=26, FN=52). Karyotype of Taiwanese *hosletti*, which have been classified as *C. russula* or *C. dsinezumi*, is differentiated from those of these two species and that of Taiwanese *kurodai* is distinct from previously known karyotype of *C. horsfieldii* from Mysore. On the basis of chromosomal comparisons among three different types observed in East Asian *Crocidura* (2n=40, FN=56; 2n=40, FN=50; and 2n=26, FN=52), by using G-band stained karyotypes of *C. a. tanakae*, *C. r. hosletti*, and *C. watasei*, it was suggested that 2n=26, FN=52 type had been derived from 2n=40, FN=56 type, not from 2n=40, FN=50 one.

SEASONAL AND GEOGRAPHIC VARIATIONS OF THE NUMBER OF B-CHROMOSOMES IN *METAGAGRELLA TENUIPES* (ARACHNIDA: OPILIONES).

N. Tsurusaki and T. Shimada. Dept. of Biol., Fac. of Educ., Tottori Univ., Tottori.

A Japanese harvestman, *Metagagrella tenuipes* (L. Koch) (Phalangiidae, Gagrellinae) is a maritime species with a univoltine life cycle. The species usually retains as many as 4-5 supernumerary B-chromosomes per cell, and the fluctuation of the number causes both interpopulational and intrapopulational variations of the chromosome number in the species (2n=18-36). As a result of a survey on the seasonal change of the number of the B-chromosomes in a population (Yatsukami, Tottori), we found that the average number in the spermatogonial metaphase plates steadily increases from ca. 3 to 6 through the adult stage extending from July to October. On the other hand, the number of B's in developing embryos was estimated to be 1.4. This fact suggests that the B's sparingly transmitted from their parents continue to increase the number through the cell cycles throughout the life of their bearer, probably due to non-disjunction at mitosis. The number of B's also varies geographically. The number is low in three populations facing the Seto Inland Sea (means: 1.2-2.2), whereas it amounts to at least 4-5 in the other populations, when compared at the stage of full maturation in September. No correlation could be elucidated between the number of B's and external morphologies, though the latter also exhibit enormous geographical variation.

PECULIAR GONADAL STRUCTURE IN THE SIMPLE STYELID ASCIDIAN SERIOCARPA SP. FIRSTLY COLLECTED FROM JAPANESE WATERS.

T. Nishikawa, Grad. School of Human Informa., Nagoya Univ., Nagoya.

In the Ascidiacea, the tunic is usually separable more or less easily from the mantle except in siphonal areas. Rarely, however, a small part of tunic is mingled complicatedly with adjoining area of mantle (sometimes involving gonads) to form a complex, as is known in the genus Seriocarpa. I made an intimate observation of serially thin-sectioned or whole specimens clearly assignable to this genus, collected from sandy bottoms of Omura Bay and the Seto Inland Sea, ca. 25 m deep. In the specimens, up to 14 mm long, a small patch of tunic was protruded inwards to enclose thinly and almost completely ca. a dozen of globular hermaphoroditic gonads individually, situated in two rows beneath the endostyle. Each gonad was projected from the mantle body outwards with enveloping thin mantle epidermis, and opened into the peribranchial cavity with short ducts. In the gonadal structure, the present specimens were rather similar to S. rhizoides Diehl, 1969 from the North Atlantic and the Banda Sea, of the known 3 congeners.

PIGMENT GRANULES OF *BLEPHARISMA* AS DEFENSIVE EXTRUSOMES: FURTHER EVIDENCE.

T.Harumoto, N.Ishikawa, R.Sugibayashi. Dept. Biol., Sci., Nara Women's Univ., Nara.

Pigment granules of *Blepharisma* are extrusomal organelles having the function of defense against predatory ciliates including *Dileptus margaritifer*. We observed interaction between *B. japonicum* and *D. margaritifer* with a video microscope and a scanning electron microscope (SEM).

Dileptus attacks prey by hitting it with a long toxicysts-bearing proboscis. When a blepharisma was hit by a dileptus proboscis, a red cloudy mass surrounded the proboscis. The dileptus retreated and the blepharisma escaped.

Observations with SEM showed that a hit by the proboscis inflicted a local cut on the surface of *Blepharisma*. If observed at this moment, many spherical granules 0.2-0.6 µm in diameter were seen on and just above the surface of *Blepharisma* at the site of the cut and at the area surrounding the cut. We interpreted this as the scene in which pigment granules of *Blepharisma* are being discharged responding the attack by *Dileptus*. We take this results as strong evidence for the extrusomal nature of pigment granules and for their defensive function.

COMPARISON OF MITOCHONDRIAL DNA STRUCTURES AMONG THE FISHES IN *SCORPAENIDAE*.

T. Ishii[1], N. Ebitani[2], T. Kubo[1]. [1]Life Sci. Inst., Sophia Univ., Tokyo, [2]Biol. Lab., Shohoku Coll., Atsugi, Kanagawa.

In this study, 595 base pairs DNA fragments of mitochondrial gene (cytochrome *c* oxydase subunit I) from *Sebastes scythrop, S. inermis, Sebastiscus marmoratus, Scorpaena neglecta miostoma* and *Helicolenus hilgendorfi* were cloned with PCR methods and sequenced. Comparing the percentage of nucleotide sequence homology among five species, the highest value was obtained between *S. inermis* and *S. scythropus*. The lowest values were obtained between *S. neglecta miostoma* and the other four species. *S. marmoratus* and *H. hilgendorfi* were laid between *S. neglecta miostoma* and the other two species.

SPECIES DIFFERENCE APPEARS IN TIMING OF MEDUSA LIBERATION IN TWO CLOSELY RELATED SPECIES

S. Kubota. Seto Mar. Biol. Lab., Kyoto Univ., Shirahama, Wakayama

Two species of hydrozoans which show similar mode of life differ in timing of medusa liberation. Materials examined were commensal hydrozoans with bivalves (Leptomedusae; Eirenidae) and four forms have been known in Japan. They are rarely sympatrically distributed. At Tsushima Island, where sympatry occurs, the timing of medusa liberation was studied for a week under near-natural conditions. The diurnal periodicity was detected in *Eugymnanthea japonica* Kubota and the *intermedia* form of *Eutima japonica* Uchida, both are commensal with an introduced blue mussel *Mytilus edulis galloprovincialis* Lamarck collected from the intertidal region. From the mantle cavity of this host the medusae were released for several hours a day in the evening, but the peak time was apparently different between the two species: *Eutima japonica* were released earlier in the day than *Eugymnanthea japonica* irrespective of weather conditions. Extrinsic and intrinsic factors synchronizing the release of medusae were analyzed and the release was strongly correalted with sunset in *Eugymnanthea japonica* in the Tsushima population at the entrance of Sea of Japan. Such a correlation was confirmed in the Shirahama population, facing the Pacific, where *Eutima japonica* was absent.

ROTIFERA FROM THE JINZU RIVER BASIN CONTAMINATED BY CADMIUM, *ETC.*

M.Sudzuki.Biol.Lab.,Nihon Daigaku,Univ.,Omiya

For ca.50 yrs.rice paddies were contaminated by waste waters containing Cd.discharged from a mine. Recently these paddies have cleaned up one by one and the rice crop has increased.To analyse the degree of recovery based on Rotifer biology, 11 samples were collected on 14/V'95 under the guidance of Y.Takagi(Itai-Itai disease countermeasure conference).The samples include 3 each from paddy fields:one cleaned up already(Rc),another from some not yet recovered(No),3 from the Jinzu river (J) and 2 from irrigation canals(I),all located in the Fuchu-Machi area,where the victims of Itai Itai disease caused by Cd.have appeared in concentration.Preliminary investigations have revealed: 1) Rotifera were very scanty in both numbers of species and individuals,compared with those of other places;the number of species detected is 0-5 in No(Leading Species or LS=*Cephalodella xenica Monostyla sylvatica,Colurella colurus,Lepadella acuminata,Proales provida)*,5-15 in Rc(LS=*Monostyla closterocerca,Euchlanis dilatata lucksina,C. xenica,Lecane* spp.,*Monommata* sp.,*Cephalodella* spp & bdelloids).5-7 in J,3-5 in I.The reasons for this are (1)a large amount of paddy soil has been exchanged for new soil,and (2) colonization f Rotifera in various habitats has just started.

MYSTIDES (POLYCHAETA, PHYLLODOCIDAE) FROM JAPAN

T. Kato and S. F. Mawatari. Div. of Biol. Sci., Grad. Sch. Sci., Hokkaido Univ., Sapporo.

The polychaete genus *Mystides* of the family Phyllodocidae contains eleven species worldwide. Only one species *M. triangulifera* (Augener, 1913) has previously been reported but not described from Japan (Uchida, 1988). During a survey of phyllodocids from the Japanese coast, we found three species of *Mystides*: *M. triangulifera*, *M. borealis* and *Mystides* sp., the last of which is probably new to science.

One specimen of *M. borealis* was collected from Usujiri, Hokkaido. This specimen closely fits the original description by Théel, 1879, in having a pair of eye spots, a rounded prostomium and bottle-shaped tentacular cirri; but differs in possessing a dorsal, longitudinal, black band. Three specimens of *M. triangulifera* from Sado are characterized by the presence of an arrowhead-shaped dark spot on the prostomium instead of eyes. Two specimens of *Mystides* sp. collected from Amakusa, Kyushu, are easily distinguished from all other species of the genus *Mystides* by the character combination of eyelessness and the unique setal shaft with a strongly developed rostrum. The most similar described species, *Mystides rarica*, has the same kind of setae, but has longer prostomium with a pair of eyes.

ORIGIN OF "HAWAIIAN *DROSOPHILA*" INFERRED FROM ALCOHOL DEHYDROGENASE GENE SEQUENCES

T. Katoh, S. Chiba, C. Tanaka, J. Park, K. Tamura, and T. Aotsuka
Dept. of Biol., Tokyo Metropolitan Univ., Hachioji, Tokyo.

In Hawaiian islands, there are numerous endemic drosophilid species known as "Hawaiian *Drosophila*". For the origin of this group, Throckmorton's hypothesis, that this group originated in the *immigrans-Hirtodrosophila* radiation, has been widely accepted by many evolutionary biologists. However, on the basis of alcohol dehydrogenase (*Adh*) gene sequences, Tamura et al (1996) suggested that this group is more closely related to the groups of the *virilis-repleta* lineage than to that of the *immigrans-Hirtodrosophila* lineage. To asses further the origin of Hawaiian *Drosophila*, we determined *Adh* gene sequences for several species of the genus *Drosophila*, and analyzed phylogenetic relationship among them together with sequences published for 53 species. Our results suggested that Hawaiian *Drosophila* is a group which diverged at early stage in the *virilis-repleta* radiation.

PHYLOGENY OF PAPILIONIDAE BUTTERFLIES INFERRED FROM MITOCHONDRIAL ND5 GENE SEQUENCES.

H. Makita[1,2], T. Shinkawa[2], K. Ohta[1,2] and T. Nakazawa[2]
[1]Dept. Biol., Fac. Sci., Toho Univ., Funabashi, [2]Biol. Lab., The Univ. of the Air, Chiba.

The phylogenetic relationships between the species of the family Papilionidae were analyzed by comparing nucleotide sequences in the mitochondrial gene coding for NADH dehydrogenase subunit 5 (ND5). In this study, the partial sequences of ND5 gene of *Luehdorfia*, *Atrophaneura*, *Graphium* and *Papilio* were amplified by polymerase chain reaction then sequenced by dideoxy termination method. ND5 gene is effective for the study on the phylogeny of Papilionidae.

DIFFERENTIATION IN THE PYURID ASCIDIAN, *Halocynthia hispida* (Herdman) AS VIEWED FROM MITOCHONDRIAL DNA.

T. Kakuda[1], A. Hino[1], T. Numakunai[2] and T. Nishikawa[3]
[1]Dept. of Biological Sciences, Kanagawa Univ. Hiratsuka. [2]Mar. Biol. St., Fac, of Sci., Tohoku Univ.Asamushi. [3]Grad. Sch. Human informatics, Nagoya Univ., Nagoya

In the Japanese population of *Halocynthia hispida*, two forms are recognizable based on the difference in the density of tunic spines. Although these forms have been referred only to an individual variation, our present results strongly suggest their genetic differentiation. We extracted and purified mtDNA from both forms which were collected at Shimokita Peninsula, and digested by 8 restriction endonucleases. After the frgment pattern analysis, we estimated the genetic divergence d (Nei & Li '79) between two forms to be 4.2×10^{-2}. This value is far larger than the value within reproductively isolated three types of *H.roretzi* , but almost equal to the value between *H.roretzi* and *H.aurantium* (Kakuda etal, '95 ZS).

THE HOMOLOGOUS SEQUENCES TO THE SM50 GENE OF THE SEA URCHIN (*Hemicentrotus pulcherrimus*) AMONG ECHINODERMS.

M. Mizuno[1], A. Hino[1] and T. Kitajima[1,2]
[1]Department of Biological Sciences, Kanagawa Univ. Hiratsuka
[2]Department of Biology, Tokyo Metropolitan Univ.

To compare the sequences homologous to sea urchin SM50(spicule matrix protein), we extracted genomic DNA from some echinoderms and subjected to Southern hybridization using the SM50 gene(HSM41) from *Hemicentrotus pulcherrimus* as a probe. To obtain enough signals, we amplified the genomic DNA with primers designed to amplify the nucleotide sequence encoding the repeat region of the HSM41 protein. Although this region is detected as the fragment of 611bp in the HSM41 gene, it was about 450bp and 520bp in *Pseudocentrotus depressus* and *Anthocidaris crassispina* respectively. The former sequence showed higher reactivity with SM50 than the latter. Significant signals have not yet been detected in other echinoderms such as *Asthenosoma*, *Asterina*, *Ophioplocus* and *Holothuria*, by the Southern hybridization, although PCR amplified bands were observed.

KARYOTYPES OF TWO ACONTIATE SEA ANEMONES.

Yoko Fukui. Biol.Lab.,School of Nursing,Tokyo Women's Medical College, Tokyo.

Karyotypes of two acontiate sea anemones, Haliplanella lineata and Aiptasiomorpha sp. were studied in early embryos by the Giemsa staining and banding methods.

The genera Haliplanella and Aiptasiomorpha belong to the subtribe Acontiaria. Although the taxonomic treatment differs slightly among authors, these two genera are often included with Diadumene as members of family Diadumenidae. But the relationaship of the three genera in the family are not definitely known at present. It will be of interest to clarify the phylogenetical relationship between these sea anemones.

With respect to the general features of the chromosomes, H. lineata and Aiptasiomorpha sp. have the same chromosome number but very different chromosome morphology. Namely the two large pairs in Aiptasiomorpha sp. are metacentric, but the six pairs of larger chromosomes in H. lineata consist of one metacentric and five telocentric pairs.

JAPANESE FRESHWATER SPONGES

Y. Masuda. Dept. of Biol., Kawasaki Med. Sch., Okayama

From Japan, 11 genera and 26 species of freshwater sponges have been recorded up to the present. Except for *Spongilla inarmata*, they are reviewed and their status is revised. All materials of the survey were observed by scanning electron microscopy (SEM). As a result of these observations, they are classified into at least 11 genera and 25 species, that is, *Spongilla lacustris, S. shikaribensis, S. alba, S. inarmata, Stratospongilla clementis, S. akanensis, Eunapius fragilis, E. coniferus, E. sinensis, E. ryuensis, Radiospongilla cerebellata, R. sendai, R. crateriformis, R.hozawai, Ephydatia fluviatilis, E. muelleri, E. fortis, Trochospongilla phillottiana, T. latouchiana, T. sp., Heteromeyenia stepanowii, Pectispongilla subspinosa, Umborotula bogorensis, Heterorotula multidentata, Sanidastra yokotonensis. Ephydatia japonica* and *Eunapius shojiensis* of previous reports are synonyms of other species. *E. shojiensis* is a synonym of *E. fragilis* because the structure of the gemmule coat of the former resembles that of *E. fragilis. E. japonica* is a synonym of *E. muelleri* because many samples which were collected in recent surveys showed variable intermediate characteristics of the megasclere. The main new findings with SEM are observed in the structure of the gemmule coat. The pneumatic coats of Japanese freshwater sponges are classified into 3 types: 1)spherical air space (*Ephydatia Type*), 2)polygonal air space (*Eunapius Type*), and 3)reticular fibers (*Pectispongilla Type*).

PHYLOGENETIC EXAMINATION OF THE SURFACE STRUCTURE AND ARRANGEMENT IN LEPIDOPTERAN SCALE(LEPIDOPTERA)

T.Shinkawa,T.Nakazawa
Biological Laboratory,The University of the Air, Mihama-Ku
Wakaba,Chiba, Japan

Almost whole body of Lepidoptera is covered with scales differentiated into various patterns ranging from sensory hairs to ordinary scales.We carried out a comparative study using SEM on the surface structure of scales of various genera classified as Lepidoptera, which are distributed widely throughout the world. The relationship among the surface structures, the arrangement and color- patterns of scales was also examined. The surface structure of scales can be classified phylogenetically into several types. The arrangement of scales is closely related with these properties of scale.The stable color-patterns might be organized. This phylogenetic classification is applicable to every species of the order Lepidoptera.The surface structures of scales appears to be common in every species of each genus. Different structures of scales between each genus,however,suggest the phylogenetic development of scale and the specific evolution of cover scales causes a wide variety of color-patterns. The arrangement of scales also varies from a random arrangement to a concentric circular arrangement around the thorax. In conclusion there are five characteristics in these morphological development of scales.

A SYSTEMATIC STUDY OF THE SUBORDER FILIFERA (HYDROZOA) FROM JAPAN

H. Namikawa, Dept. of Zool., Sci. Mus., Tsukuba

The cnidome is a taxonomic character of hydrozoans. In order to elucidate the phylogenetic relationship between filiferan hydroids, the composition of the polyp cnidomes was compared across 23 species of five families: 2 species of Clavidae, 3 species of Eudendriidae, 3 species of Bougainviliidae, one species of Pandeidae, 3 species of Cytaeidae, and 11 species of Hydractiniidae.

Cnidome composition was stable within each hydrozoan family as follows: one type of microbasic eurytele (a penetrant nematocyst) and/or one type of macrobasic eurytele in the hydroids of Eudendriidae; one type of desmoneme (a volvent nematocyst), one type of haploneme (a penetrant nematocyst) and two types (large and small) of microbasic euryteles in those of Hydractiniidae; and one type of desmoneme and one type of microbasic eurytele in those of the other four families. Species of Hydactiniidae are distinguished from those of other families by the possession of large microbasic euryteles and haplonemes. The polyps of hydractiniids, armed with abundant, large, microbasic eulyteles, were observed to play a primary role in defence. This suggests that this type of nematocyst is specialised in the hydroids of the Hydractiniidae.

SYSTEMATICS OF EIGHT *CELLEPORINA* SPECIES (BRYOZOA, CHEILOSTOMATA) FROM HOKKAIDO AND OKINAWA, JAPAN WITH NOTES ON EARLY ASTOGENY.

H. Ikezawa[1] and S. F. Mawatari[2],
[1] Ibaraki Nat. Mus., Iwai, Ibaraki, [2]Div. of Biol. Sci., Grad. Sch. of Sci, Hokkaido Univ., Sapporo.

Adult morphology, ancestrula form, and budding pattern were compared in the following eight species of the genus *Celleporina*: *C. porosissima*, *C. umbonata*, *C. fusiformis*, *C.* sp. 1, *C.* sp. 2 and *C.* sp. 3 from Hokkaido; and *C.* sp. 4 and *C.* sp. 5 from Okinawa. The eight species can be divided into the following four tentative groups on the basis of the ancestrula and early astogenic traits. Group 1 (consisting of *C.* sp. 5) has a modified tatiform ancestrula with spines around the orifice. Group 2 (*C. porosissima*) has schizoporelloid ancestrulae with oral spines and a budding zone around the distal half, from which a single daughter zooid buds. Group 3 (*C. fusiformis*, sp. 2, and sp. 4) has schizoporelloid ancestrulae with oral spines and a pair of small lateral projections, from which the first pair of daughter zooids bud simultaneously. Group 4 (*C. umbonata*, sp. 1 and sp. 3) has schizoporelloid ancestrulae without spines, but with paired lateral buds. Assuming that such characters as schizoporelloid ancestrulae, the presence of paired zooid buds, and the absence of oral spines are apomorphic, it is suggested that *C.* sp. 5 from Okinawa is the most primitive, *C. porosissima* the second most primitive, and the remaining six species the most derived of the eight species.