

AN UNDESCRIBED SPECIES OF THE GENUS DACTYLOPLEUSTES (CRUSTACEA: AMPHIPODA: PLEUSTIDAE) FROM HOKKAIDO, JAPANKo Tomikawa¹, Ed Hendrycks², Keizo Yoshimura³, Shunsuke F. Mawatari¹¹Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan, ²Canadian Museum of Nature, Research Division, P. O. Box 3443, Station D, Ottawa, Canada K1P 6P4 and ³Hokkaido Hakodate Fisheries Experimental Station, Muroran, Hokkaido 051-0013, Japan

In addition to the three species of the genus *Dactylopleustes* (Crustacea: Amphipoda: Pleustidae) so far reported from the world, we have found an undescribed species in association with the urchin, *Strongylocentrotus intermedius* (Agassiz, 1863) from the coast of Hokkaido, northern Japan. The morphology of the gnathopods, pereopods 5-7, uropods, telson, mandible and maxilla 1-2 are the major characteristics that distinguish the undescribed species from its congeners. *Dactylopleustes echinoides* Bousfield and Hendrycks, 1995 from Vancouver Island, Canada is the most similar to the undescribed species, and is redescribed briefly based on holotype borrowed from Canadian Museum of Nature. An amended key to *Dactylopleustes* species is provided and their known sea urchin hosts are listed.

THE EPILACHNA ALTERNANS COMPLEX (COLEOPTERA: COCCINELLIDAE) IN INDONESIA: THEIR PHYLOGENY AND DIVERSITY IN MORPHOLOGY AND HOST PLANTSYuri Ohta¹, Norio Kobayashi², Toru Katoh¹, Susumu Nakano³, Sih Kahono⁴, Haruo Katakura¹¹Division of Biological Sciences, Graduate School of Science, Hokkaido University Sapporo, Hokkaido 060-0810, Japan, ²The Hokkaido University Museum Sapporo, Hokkaido 060-0810, Japan, ³Faculty of Human Environmental Studies, Hiroshima Shudo University, Hiroshima 731-3195, Japan and ⁴Balai Penelitian dan Pengembangan Zoologi, Puslitbang Biologi, LIPI, Jl. Raya Bogor Jakarta Km 46, Cibinong 16911, Indonesia

We conducted a comparison of genitalia and a phylogenetic analysis for 14 populations of *Epilachna alternans* and similar forms collected from Sumatra and Java, Indonesia, which included various populations that differed in their external morphology (size and elytral patterns) and/or host plants. We determined the sequences of a part of mitochondrial 16S ribosomal DNA and constructed a gene tree using Neighbor-Joining method. Seven clusters, which were characterized by their geographical locations or host plants, and fine structure of genitalia, were recognized in the tree. However the phylogenetic relationship among these clusters was not resolved. The results indicated a complicated geographic pattern of this species complex involving sympatric and allopatric genetic divergence, and demonstrated differentiation among sympatric populations that differed in their host plants. Based on the above results, we will discuss the mode of speciation in the *E. alternans* species complex.

TAXONOMIC RE-EVALUATION OF THE GENUS BATHYCOPEA (CRUSTACEA: ISOPODA: SPHAEROMATIDEA)

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The genus *Bathycopea* was known as the deep-sea living isopods from the world. I report two species belonging to the genus collected from Japan. Of these species, *Bathycopea parallela* Birstein from Otsuchi Bay was second record from Japanese coast. *Bathycopea* sp. 1 was similar to *B. typhlops* in the body shape, however, the two species are easily distinguishable by the presence of the eyes and broad uropods in *B. sp. 1*. The emended generic diagnosis was presented after the taxonomic re-evaluation based on the two species.

TWO UNDESCRIBED SPECIES OF DIPLOSOMA (ASCIDIACEA: DIDEMNIDAE) BEARING PROKARYOTIC ALGAE PROCHLORON FROM OKINAWAJIMA ISLAND, JAPANAtsushi Oka¹, Mayu Suetsugu², Euichi Hirose²¹Nagoya University Graduate School of Environmental Studies, Chikusa-ku, Nagoya 464-8601, Japan and ²Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

In tropical or subtropical waters, some didemnid ascidians are known to have symbiotic association with a prokaryotic algae Prochloron sp. Two undescribed species of Prochloron-bearing Diplosoma, *D. sp. 1* and *D. sp. 2*, were found from coral reefs in Okinawajima Island, Japan. They attached on coral lime stones or corallines at the reef edges of subtidal zone (*D. sp. 1*) or in a shallow reef lagoon (*D. sp. 2*). In these species, the colonies are thinner and the zooids are smaller than those of any other Prochloron-bearing Diplosoma species so far described. Moreover, each of these species has a unique combination of stigmal numbers: 5 stigmata in the first and third rows, 6 in the second row, and 4 in the fourth in *D. sp. 1*; 4 stigmata in the first and third rows, 5 in the second row, and 3 in the fourth in *D. sp. 2*. In both of the species, retractor muscle emerges from the zooid under the thorax.

WATER MITES OF THE GENUS HYGROBATES (ACARI: HYDRACHNIDIA: HYGROBATES) FROM JAPAN

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Among the water mites that live in aquatic environments, the genus *Hygrobates* mainly inhabits still water, i.e. lakes, ponds, and mid- and downstream parts of rivers. The genus *Hygrobates* has a global diversity of around 150 species, with 14 species so far known from Japan. An examination of three newly collected species of water mites from Shiga, Kyoto, Nara and Wakayama prefectures, Western Japan, with described *Hygrobates* species shows that the newly collected water mites are undescribed species of the subgenus *Hygrobates*. The three species share a character state in having a pair of setae on the pre-genital sclerite of the female. This character state has not been reported among other species of the genus *Hygrobates*. The three newly collected species are easily distinguished from one another by the following character states. The shape of the P-2 projection in *Hygrobates* sp. 1 is elongated, whereas in *H. sp. 2* and sp. 3 it is truncated. In the female genital field, *H. sp. 1* and sp. 2 have small genital plates, whereas *H. sp. 3* has large genital plates. In the male genital field, *H. sp. 1* and sp. 2 have a round posterior median projection, whereas *H. sp. 3* has no projection.

OVARIAN STRUCTURE AND OOGENESIS OF A SOUTH AFRICAN HEEL-WALKER, KAROOPHASMA BIEDOUWENSIS (MANTOPHASMATODEA)Tadaaki Tsutsumi¹, Koji Tojo², Toshiki Uchifune^{3,4}, Ryuichiro Machida⁴¹Faculty of Education, Fukushima University, Fukushima, Fukushima 960-1296, Japan, ²Department of Biology, Faculty of Science, Shinshu University, Matsumoto, Nagano 390-8621, Japan, ³Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan and ⁴Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano 386-2201, Japan

Ovarian structure and oogenesis of a South African heel-walker, *Karoophasma biedouwensis* were examined light and transmission electron microscopically. The ovary of the adult female consisted of five or six pairs of ovarioles, each of which connected with the lateral oviduct. The ovariole was composed of an anterior terminal filament, germarium and vitellarium. The germarium, which contained a few oogonia and remarkably flattened prefollicular cells, was very short. No intercellular bridges connecting oogonia or immature oocytes with each other were found. In the vitellarium of the full grown ovary, there were seven to 14 developing oocytes, each of which was surrounded by a follicular epithelium, arranged in a single row, and the proximal one was chorionated oocyte. No nurse cells developed, and the ovariole type of Mantophasmatodea is a typical panoistic one. The mature oocytes were characterized by a large amount of fragmented endoplasmic reticula and few Golgi complexes. We compare the ovarian structure and oogenesis of Mantophasmatodea with those of other orthopteroid orders, and discuss likely affinity of Mantophasmatodea with Grylloblattodea.

DIVERSITY OF VERMIFORM EMBRYOS IN THE PHYLUM DICYEMIDAHidetaka Furuya¹, Eric Hochberg², Kazuhiko Tsuneki¹¹Department of Biology, Graduate School of Science, Osaka University Toyonaka, Osaka 560-0043, Japan and ²Santa Barbara Museum of Natural History, Santa Barbara, California 93101, USA

Morphology, cell numbers, and cellular composition were examined in vermiform embryos of 30 species of dicyemid mesozoans that are placed in 6 of the 8 genera known in the phylum. The following genera were studied: *Conocyema*, *Dicyema*, *Dicyemeneae*, *Dicyemodoca*, *Microcyema*, and *Pseudicyema*. Vermiform embryos of *Conocyema* and *Microcyema* differ from the other genera in having a small number of peripheral cells and distinct calotte shapes. In *Conocyema* the calotte of vermiform embryos consists of 4 large cells whereas in *Microcyema* the calotte of vermiform embryos is a syncytium including 6 nuclei. Vermiform embryos typically have a constant peripheral cell number that is species-specific. Interspecific variations in total number of peripheral cells range from 10 to 40. The most frequent number of peripheral cells encountered in vermiform embryos is either 22 or 23. Differences in total number of peripheral cells are mostly due to differences in the number of trunk peripheral cells. In dicyemid species with a large number of peripheral cells interspecific variations in cell numbers occur in trunk peripheral cells.

THE DIFFERENCE OF BREEDING PERIOD AMONG COLONIES WITH DIFFERENT COLOR PATTERNS IN BOTRYLLOIDES SIMODENSIS

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Botrylloides simodensis is a colonial ascidian belonging to the family Botryllidae. This species was first reported in 1981, and one of the common species in the rocky shore around Shimoda. In this species, colonies show various colors and color patterns, and those colonies can be divided into some types with their color patterns. We observed the life histories of colonies belonging to each type in the same environment. In colonies of the one type, the period of sexual reproduction was from July to September as reported in the original description, but colonies of the other types performed sexual reproduction almost all the year round. This fact implies that the range

of the suitable water-temperature for sexual reproduction in the former type is much narrower than that in the latter types. That relationship between the color pattern and the breeding period suggests the possibility that *B. simodensis* is a polyphyletic taxon. Therefore, to clarify the taxonomic doubt much investigation is needed.

A NEW DEEP-WATER LANCELET OF THE GENUS *ASYMMETRON* COLLECTED SW OFF KAGOSHIMA PREFECTURE

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A true deep-water lancelet was firstly collected during the Hyper-Dolphin/Natsushima cruise in 2003 of the Japan Marine Science & Technology Center (JAMSTEC) off Cape Nomamisaki, southwestern end of Kagoshima Prefecture, at a depth of 229 m. This specimen was very similar to its congener, *Asymmetron lucayanum* (formerly called *Epigonichthys lucayanus*), but was easily distinguishable from it by the larger number of total myotomes (83 in the former vs. 55-72 in the latter), and therefore, should be regarded as a new species. The genus *Asymmetron* had been treated as a junior synonym of *Epigonichthys*, but was recovered as a valid genus morphologically distinct from another valid genus *Epigonichthys* (sensu stricto). The present new species may possibly give an alternative to Gibbs and Wickstead's (1996) hypothesis where the variation in myotome formula of *A. lucayanum* is regarded phenotypic, attributable to different larval durations.

AN UNDESCRIBED SPECIES OF THE GENUS *LOXOMITRA* COLLECTED FROM OKINAWA ISLAND (ENTOPROCTA: LOXOSOMATIDAE)

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An undescribed species of the genus *Loxomitra* was collected from Okinawa Island. This species resembles *Loxomitra kefersteini* (Claparede, 1867) in general shape, but the two species are different in some characters. Whereas the tentacle number is up to 14 in *L. kefersteini*, that of the species from Okinawa reach 20 in large individuals. The total size of the adult and largest bud of *L. kefersteini* (up to 1.5mm and up to 0.44mm, respectively) is considerably smaller than that of the species from Okinawa (up to 2.2mm and up to 0.7mm, respectively).

THE BEARD WORM (*OLIGOBRACHIA MASHIKOI*) ALSO PROTRUDE THE TENTACLES, WHEN HYDROGEN SULFIDE WAS SUPPLIED

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Vestimentiferan tube worms inhabit close to hydrothermal vents in deep sea. In the field, they protrude their gills from their tubes. Also under an artificial environment such as aquarium, tube worms protrude the gill, when hydrogen sulfide gas was supplied. This behavior has been so far regarded as for symbiotic bacteria, since the bacteria need the hydrogen sulfide for oxidizing substrate. In the present study, for the beard worm (*Oligobrachia mashikoi*), the same experiment as the tube worm was conducted. As a result, 9 individuals, which were supplied with only tap water into the aquarium, remained to be shrunk in the recesses of the tube. Ten individuals, however, which were supplied with the tap water containing hydrogen sulfide gas, all elongated the body to the top of the tube. Furthermore, six individuals of 10 protruded about a half length of the tentacles over the top. Remaining 4 individuals could not protrude the tentacles because of the presence of the blood clot in the tube. We would like to discuss that this behavior is not for symbiotic bacteria but for worm itself, because of shortage of the oxygen by increases in the hydrogen sulfide level.

A STUDY OF HISTOCHEMISTRY AND ULTRA-STRUCTURE OF THE TROPHOSOME IN THE BEARD WORM (*OLIGOBRACHIA MASHIKOI*)

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Beard worms lack digestive tract. This animal group develops a special structure called "trophosome" in the posterior part of the body, where chemosynthetic bacteria live and offer carbohydrates to the host cell. This type of cells is called bacteriocytes. In the trophosome, other type of cells is there, of which function is for depositing nutrients. In the present study, we made saggital sections of the trophosome using a cryostat, and conducted oil-red staining. Furthermore, we checked electron-microscopically the presence of glycogen granules in the nutrients-deposit cells. In other species, glycogen is the representative of the nutrients. As a result, few of glycogen granules were observed in the nutrients-deposit cells, although we detected much amount of triglyceride. As the lipid was not observed in the bacteriocytes, triglyceride may be synthesized from carbohydrates in the nutrients-deposit cells. Furthermore, in the light-microscope, many small granules were often observed in the periphery of the nucleus of the trophosome cells. At present, we are checking electron-microscopically whether the small granules are actually symbiotic bacteria or not.

SEGMENT AND LIMB FORMATION IN THE EMBRYO OF THE MYODOCOPID OSTRACOD *VARGULA HILGENDORFII* (CRUSTACEA)

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In many crustaceans, some are known that they hatch as nauplius larvae which bear only three pairs of anterior cephalic limbs, whereas the others undergo an "egg-nauplius" stage which is the embryo with three pairs of limb buds, as in the short germ type of insect embryogenesis. In both cases, the subsequent segments and limbs are formed posteriorly during the embryonic and/or larval stages.

The ostracods are small crustaceans enclosed their whole body with a bivalved carapace. The trunk is not divided into the thorax and abdomen and its segmental structure is generally lost. Most of them bear only seven pairs of limbs, even including the cephalic ones. Therefore, they at least morphologically could be regarded as the most oligosegmented crustaceans with obscure segmentation. In the myodocopid ostracods, the segment and limb formation should occur in a certain embryonic stage, because of hatching as the juvenile with the same in number of adult limbs. In the present study, some embryonic stages of the myodocopid *Vargula hilgendorffii* reared in laboratory have been observed with a light and a fluorescence microscope to consider the segmentation and tagmatization in the ostracods.

MORPHOLOGY OF CIRCULATORY SYSTEM IN A LARGE-SIZED SPECIES OF PYCNOGONID, *ASCORHYNCHUS JAPONICUM* (ARTHROPODA, PYCNOGONIDA)

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Ascorhynchus japonicum Ives is a large-sized species of pycnogonid mainly taken from bathyal zone of the North Pacific region. We examined the morphology of its circulatory system by paraffin sectioning and scanning electron microscopy with dissected specimens. The system consists of a longitudinal ostiated dorsal vessel in the trunk region with an anterior aorta entering the proboscis, and a horizontal septum dividing the hemocoel into dorsal and ventral sinuses in both the trunk and pedal regions. Compared with that of *Ammothella biunguiculata* (Dohrn), a small-sized pycnogonid from intertidal zone, they show a basically common architecture of the system in spite of the differences between their body size and bathymetrical range. The resemblance of the morphology of the system in both species to mandibulates would be superficial, and some possible synapomorphies with euechelicerates are confirmed. The musculature of dorsal vessel wall is very weak even in the large species, and the peristalsis of gut may more effective in hemolymph propulsion than the contraction of dorsal vessel as observed *in vivo* in *A. biunguiculata*.

OVOVIVIPAROUS ARROW WORM COLLECTED FROM TSUKUMO BAY IN NOTO PENINSULA

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Arrow worms are internally fertilizing and simultaneously hermaphroditic. The stage of the oocyte at fertilization is the first meiotic metaphase and the fertilized oocytes are arrested at this stage in the oviducts until being laid in the sea water. In July 2003, eight specimens of *Paraspadella* were collected in the coast of Tsukumo Bay in Noto Peninsula, near Noto Marine Laboratory, by towing a sledge net. The water depth was less than 5 m. The oocytes had developed in the broad oviducts and a few hatchlings stayed there. This specimen is undescribed species and the ovoviviparous arrow worm has not been reported until now. A newly-born individual of the present species was at the first feeding stage which is characterized from the developed grasping spines. They were cultured in a laboratory and compared with *Paraspadella gotoi*.

HOST SPECIFICITY AND ECOLOGY OF CRINOID-ASSOCIATED DECAPODS AROUND OKINAWA ISLAND AND ITS ADJACENT WATERS

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A survey of crinoid-decapod relationships was conducted around Okinawa Island and its adjacent waters. Twenty-four species of decapod (13 pontonines, 3 alpheid, 4 galatheids, and 4 eumedonines) were collected from 41 species of crinoid; 148 symbiotic combinations were found. Field observations revealed that the microhabitats of these decapods on their crinoid hosts differed as follows: pontonine shrimps usually hide among the pinnules or arms of crinoids; alpheid snapping shrimps always live on the oral disk; galatheid squat lobsters and eumedonine crabs usually hide between the cirri or on the bases of the arms. Host specificity of these decapods

ranged from generalism to specialization on a single host species.

AN UNDESCRIBED FALSE SPIDER CRAB OF THE GENUS *NEORHYNCHOPLAX* (CRUSTACEA: DECAPODA: BRACHYURA) FROM IRIOMOTE ISLAND, THE RYUKYU ISLANDS, JAPAN

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An undescribed false spider crab, *Neorhynchoplax* sp. (Crustacea: Decapoda: Brachyura: Hymenosomatidae) is found on the pebbly bottoms of upper part of estuarine basins and a mangrove tide pool of Iriomote Island, the Ryukyu Islands, Japan. *Neorhynchoplax* sp. is morphologically close to *N. nasalis* and *N. aspinifera*, distributed on West Bengal, India and North Queensland, Australia, respectively. *Neorhynchoplax* sp. is, however, distinguished from *N. nasalis* by the shape of the anterolateral border of the carapace, the shape of the male abdomen, and the presence of the subterminal spine on the first ambulatory dactylus. *Neorhynchoplax* sp. is also differentiated from *N. aspinifera* by the presence of the single subterminal spine on the inner margin of the ambulatory legs and the shape of the distal end of the G1. *Neorhynchoplax okinawaensis*, distributed on close geographical range with *N. sp.*, has very different characters from *N. sp.* in the absence of the posterior lateral tooth, the shape of the dorsal surface of the carapace, the shape of the male abdomen, and the shape of the G1.

DEVELOPMENT OF THE DEEP-SEA OPHIUROID, *OPHIURA SARSII SARSII* LÜTKEN, 1855

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Detailed information on the developmental pattern of deep-sea ophiuroids is needed, because previous studies concluded that most species of this taxon undergo direct development based on limited characteristics such as egg size. In the 74th annual meeting last year, we reported that *Ophiura sarsii sarsii* inhabiting a deep-sea with a constant physical environment has a seasonal reproductive cycle, and breeds between winter and spring. In this study we examined the developmental pattern of *O. sarsii sarsii* using the specimens collected in the breeding season (December - March) at a depth of 300 m off Toyama Bay. In the laboratory, we observed the development from the spawning, induced by a sudden change in seawater temperature, to the stage before metamorphosis. Fertilized eggs measuring about 120µm diameter with negative buoyancy developed into 6-armed planktotrophic ophioplutei. The larval skeleton was first formed as a pair of tetradactyl spicules. About 40 days after fertilization, larva formed a 5-lobed hydrocoel. These findings suggest that the full-grown ophiopluteus of this ophiuroid has six arms.

RECORD OF COMPLETED SPECIES BY DISTRIBUTION INVESTIGATION OF THE FAMILY MEGASCOLECIDAE (OLICOCHAETA) FROM KANAGAWA PREFECTURE, CENTRAL JAPAN

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We have investigated the distribution of earthworm (family Megascolecidae) in Kanagawa Pref. As a result, a total of 33 species including 27 species of *Amyntas*, 4 species of *Metaphire*, and 2 of species incertae sedis, which probably to *Pheretima* group, were found. In these species, 32 species that containing 2 of undescribed species were new records in Kanagawa pref. We report the morphological characteristics and the distribution of all these species found by this investigation.

FORMATION OF THE LARVAL SKELETON IN VITELLARIAE OF THE BRITTLE STAR *OPHIOPLOCUS JAPONICUS* H. L. CLARK

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Ophiuroids have 2 types of planktonic larval forms: ophioplutei and vitellariae. The former is a common feeding type with a continuous ciliary band and usually four pairs of arms supported by skeletal rods. The latter which has been reported in 10 species including *Ophioplocus japonicus* (Komatsu and Shosaku, 1993) is non-feeding and possesses transverse ciliary bands. The present study observed with a polarization microscope shows that the larval skeleton had appeared as a pair of tetradactyl spicules in the posterior corners of the blastocoel of the early vitellaria in *O. japonicus* about 36hr after fertilization. By 44hr the pair of spicules had grown, and formed a branched skeleton, however, in the 84-hr vitellaria, the larval skeleton was no longer existent among the rudiments of the adult skeletal plate. Thus, as suggested by Hendler (1982), it might be expected that the vitellaria of *O. japonicus* could retain vestiges of an ophiopluteus larval skeleton.

PHYLOGENETIC RELATIONSHIPS OF JAPANESE CUTTLEFISHES INFERRED FROM MITOCHONDRIAL 16S rDNA GENE AND CYTOCHROME C OXIDASE SUBUNIT I GENE

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We collected 10 species of cuttlefishes including 3 genera from Japanese waters and determined partial nucleotide sequences of two mitochondrial genes, mt16S rDNA gene and cytochrome c oxidase subunit I gene, to study phylogenetic relationships. Our molecular data supported the morphological classification based on the cuttlebone shape, the size and arrangement of suckers on tentacular clubs. Japanese cuttlefishes were separated into 4 groups: (1) Wide cuttlebone species with equal-sized suckers on tentacular clubs, including 2 subgenera *Platysepia* (*Septia esculenta*, *S. madokai*) and *Acanthosepion* (*S. lycidas*). (2) Narrow cuttlebone species with unequal-sized suckers on tentacular clubs, subgenus *Doratosepion* (*S. kobeensis*, *S. pertersenii*, *S. pardex*). (3) Spineless cuttlebone species, *Sepiella japonica*. (4) Rhomboidal cuttlebone species, *Metasepia tullberi*.

CUMACEAN GENUS *EUDRELLA* (CRUSTACEA, CUMACEA, LEUCONIDAE) FROM JAPANESE WATERS

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Since the establishment of the genus by Norman in 1867, about 30 species of *Eudrella* (Crustacea, Cumacea, Leuconidae) has been reported so far from Atlantic Ocean and Arctic region. Recently, R/V hakuho-Maru and R/V Tansei-Maru, the Ocean Research Institute, Tokyo University and T/V Toyoshio-Maru, Hiroshima University collected many cumacean specimens from bathyal depth. We examined these specimens. As a result, about species of *Eudrella* will be added to our fauna.

STUDY ON THE SPERMATOOZOA IN THE MALE AND FEMALE GENITAL TRACT OF THE ISOPOD, *PORCELLIONIDES PRUINOSUS*

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We have observed the ultrastructure of the spermatozoa in the male and female genital tract of the isopod, *Porcellionides pruinosus* by means of light and electron microscopy. Spermatozoa probably function in maintaining spermatozoa in bundles during their transport through the male reproductive tract. The epithelial cells of the vasa deferentia have developed microvilli at the surface portion. And the cells are characterized endoplasmic reticulum, many Golgi complex, and many secretory materials. The electron micrographs of the epithelial cells of the vasa deferentia show the cells, presumably used to form spermatozoa. Spermatozoa in the anterior vas deferens are different from those in the ejaculatory duct. The matrix material between sperm tails and heads comes progressively more electron dense at lower levels of the ejaculatory duct. In the female genital tract the spermatozoa are dissolved. The sperm tails progressively change electron dense at lower level. They seem to be absorbed. Spermatozoa appear to change the single spermatozoa without tails in the female genital tract.

IS THE JAPANESE ORIBATID MITE *EUPHTHRACARUS FOVEOLATUS* AOKI, 1980 (ACARI: EUPHTHRACARIDAE) A JUNIOR SYNONYM OF *E. CRIBRARIUS* (BERLESE, 1904)?

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Two similar species of oribatid mites, *Euphthracarus foveolatus* and *E. cribrarius*, have different distributions, with the former being endemic to Japan and the latter being a widespread Eurasian species that has not been reported from Japan. Recently the distinction between these species has become questionable, due to a mistake discovered in the original description. Also, new collections of *E. foveolatus* from Japan have shown it to be variable in morphology, and this variability overlaps in some ways with that of European populations of *E. cribrarius*. In this report, I examine the morphological variability of *E. foveolatus* and reconsider its distinctness from *E. cribrarius*, which seems to be its closest relative. Studied specimens of the latter were from Norway, the type country. Despite some overlapping characters, the Japanese and Norwegian specimens are distinguishable in two obvious ways. First is the ratio of lengths of aggenital setae (ag₁/ ag₂). Second is that *E. foveolatus* lacks three leg setae that are present in *E. cribrarius*. Thus, I feel these species are distinct and the names are not synonyms.

MOLECULAR PHYLOGENY AND BIOGEOGRAPHY OF WORLDWIDE CLAUSILIID LAND SNAILSRei Ueshima¹, Edmond Gittenberger², Dennis Uit de Weerd²¹Department of Biology, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan and ²National Museum of Natural History, PO Box 9517, NL 2300 RA Leiden, The Netherlands

Clausiliidae is one of the most speciose family of stylommatophoran land snails which includes at least 90 genera and 500 species. Extant members of Clausiliidae are now classified into 9 subfamilies based on the anatomy and conchology. However, phylogenetic relationships among the subfamilies are not yet clear. Geographic distributions of Clausiliidae are confined to three major disjunct areas, Europe, South-East Asia and Central-South America. Clausiliid subfamilies are endemic to each area but some of them show a remarkable morphological similarity despite the far remote distributions. In the present study, we examined molecular phylogeny of worldwide clausiliid taxa by using nucleotide sequences of partial 28S rDNA. Clausiliids are divided into two distinct clades characterized by the genital morphology. The result of our analysis suggests some important conchological features to be evolved convergently in clausiliids. Biogeography of clausiliid snails is discussed in the light of molecular phylogeny

EVOLUTION OF EPAXIAL/HYPAXIAL SKELETAL MUSCLE IMPLICATED BY THE EXPRESSION PATTERN OF LAMPREY MUSCLE-SPECIFIC GENES

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Gnathostome skeletal muscle consists of epaxial and hypaxial regions separated by horizontal myoseptum, and a part of the hypaxial region further undergo migration to become appendicular muscle, tongue muscle, trapezius (cucullaris) muscles. In contrast, lamprey skeletal muscles do not exhibit epaxial/hypaxial differentiation, and migratory cell lineages have not been observed to generate appendicular and other muscles. We examined the expression pattern of muscle-specific genes in the lamprey, *Lethenteron japonicum*, throughout development. One of the lamprey myosin heavy chain genes, *LjMyHC1*, is expressed only in the myotomes. Within the myotomes, *LjMyHC1* transcript is localized in the adaxial and ventrolateral portions that might be involved in interaction with surrounding tissues, such as notochord and lateral plate mesoderm. Thus, the lamprey skeletal muscle might be composed of multiple regions that are controlled by distinct gene regulatory systems, as in gnathostomes. To understand the evolutionary pathway for complex structure of vertebrate skeletal muscle, we are currently characterizing lamprey regulatory genes controlling muscle patterning, such as *Pax-3*, *Lbx1*, and *Zic*.

MORPHOMETRIC VARIATION OF HYNوبيUS NAEVIUSAtsushi Tominaga¹, Masafumi Matsui¹, Kanto Nishikawa¹, Shingo Tanabe², Shin'ichi Sato³¹Graduate School of Human and Environmental Studies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan, ²6-10 Kamikatsura-Higashi-no-Kuchi-cho, Nishikyoku-ku, Kyoto 615-8221, Japan and ³1-27 Uenogaoka, Oita 870-0835, Japan

We surveyed morphometric variation of *Hynobius naevius*. The result suggests that this species includes highly diverged two morphotypes. These two morphotypes correspond to two genetic types that have been reported and are sympatrically distributed in northern Kyushu. One of the two morphotypes (Type-A) is constituted by Chugoku and northern Kyushu populations and is not divided into further sub-groups. On the other hand, the other morphotype (Type-B) is composed of populations from Chubu-Kinki, Shikoku, and Kyushu and is subdivided into three local groups (Chubu-Kinki, Shikoku, and Kyushu) that are isolated by the Kii and Bungo Straits. A positive correlation between Mahalanobis and genetic distances is detected in the Type-B, but not in the Type-A. This result, coupled with genetic information, suggests that the morphological divergence of the Type-A was influenced more by physiographic, climatic, or ecological factors than by genetic factors, while that of the Type-B was strongly affected by genetic factors.

TAXONOMY OF MERISTOGENYS FROM A STREAM OF BORNEO (AMPHIBIA, ANURA)Tomohiko Shimada¹, Masafumi Matsui¹, Maryati Mohamed²¹Graduate School of Human and Environmental Studies, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan and ²Institute for Tropical Biology and Conservation, University Malaysia Sabah, 88999 Kota Kinabalu, Sabah, Malaysia

Torrent frog genus *Meristogenys* is endemic to Borneo and contains many unresolved taxonomic problems. We investigated adults and larvae collected from a stream at Mahua Visitor Cite in the Crocker Range National Park, Malaysia. From DNA sequences, we could recognize five types, some of which were clearly defined by larval, but not by adult morphology. Further, a larvae specimen which we could not examine DNA was morphologically completely different from the other types. From these results, it was made clear that at least six types of *Meristogenys* were revealed to coexist in a limited area, and that larvae are more useful than adults in morphologically identifying different types of frogs included in this genus.

A NEW TYPE OF RANA RUGOSA FOUND IN SADO ISLANDIkuro Miura¹, Kunio Sekiya², Hiromi Ohtani¹, Mitsuki Ogata³, Youko Ichikawa⁴¹Institute for Amphibian Biology, Graduate School of Science, Hiroshima University, Higashi-hiroshima 739-8526, Japan, ²Department of Environmental Science, Faculty of Science, Niigata University, Niigata 950-2181, Japan, ³Kanazawa Zoological Gardens, Kanagawa 236-0042, Japan and ⁴Department of Health Science, Faculty of Human Life and Environmental Science, Hiroshima Prefectural Women's University, Hiroshima 734-8558, Japan

The frog *Rana rugosa* living on Sado Island can definitely be divided into two types based on the external morphology. One type (designated white abdomen) is similar to those living on the opposite shore of the main land, while the other type is quite different. Its lower abdomen and thigh are densely yellow and the whole skin is slimy (designated yellow abdomen). In this study, we have investigated the nuclear and mitochondrial gene sequences of the yellow abdomen population to find out its genetic relationships to the other Japanese and Korean populations. We also examined whether a reproductive isolation mechanism exists. It was found that: 1) the yellow abdomen type was genetically distinct and closest to the Kanto group; 2) the hybrids were all males, of which sperm in the testes were scarce, but could be fertilized; and 3) the maternal origin of almost all the natural hybrids and backcrossed hybrids, found in the field, was of the white abdomen type. Finally, we conclude that the yellow abdomen type can be classified as a new species, because it is genetically distinct, and it will keep its genetic identity separate from the white abdomen type.

PRELIMINARY REPORT ON PHYLOGENETIC RELATIONSHIPS OF GONBIONINE FISHESYoshihide Gunji¹, Sang-Rin Jeon², Katsutoshi Watanabe³, Jun-Ichi Miyazaki¹¹University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, ²Sang Myung Women's University, Seoul 110-743, Korea and ³University of Kyoto, Kyoto, Kyoto 606-8502, Japan

The subfamily of Gobioninae (Cyprinidae, Cypriniformes) is a highly heterogeneous group. Its integrity as subfamily and phylogenetic relationships of gobionine fishes have been controversial. We determined DNA sequences (1140-bp) of the mitochondrial cytochrome b gene in order to elucidate their phylogenetic relationships. Gobionine fishes were grouped into three lineages; lineage including *Gnathopogon elongatus* and *Gnathopogon caeruleus*, lineage including *Pseudorasbora parva* and *Pungtungia herzi*, lineage including *Pseudogobio esocinus*, *Abbottina rivularis*, *Saurogobio dabryi*, *Bivia zezera*, three species of the genus *Microphysogobio*, three species of the genus *Gobiobotia*, *Hemibarbus barbatus*, *Squalidus gracilis* and five species of the genus *Gobio*. The third lineage was subdivided into two clusters, cluster including *Hemibarbus barbatus*, *Squalidus gracilis* and five species of the genus *Gobio* and cluster including the other species. These results are essentially consistent with those obtained by morphological analysis (Hosoya, 1989).

LIVING FOSSIL HORSESHOE CRABS CONTINUE TO EVOLVE

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Horseshoe crabs keep the similar shapes at least for two hundred million years. Therefore, they are called as the living fossil. I found unique horseshoe crabs in mangroves of Sundarbans (the river-mouth of the Ganges) in Bangladesh and India. There is one on the Bengal Bay in South Asia. It was concluded that the Sundarbans Horseshoe crab is a sub-species of *Carcinoscorpius rotundicauda*. But, the shapes are clearly different from the typical *C. rotundicauda* of South-East Asia. I thought that the different of mangroves of both areas induced the difference of shapes of both horseshoe crabs. Besides, I examine the difference of South China and Japan in other horseshoe crab *Tachypleus tridentatus*. As the result, the clear difference was found between ones of South China and ones of Japan. Those facts show the living fossil horseshoe crabs actively evolve even now.

THE ULTRASTRUCTURE OF SENSORY CELLS ASSOCIATED WITH A TENTACULAR TUNIC IN THE ATRIAL TENTACLES OF THE ASCIDIAN POLYANDROCARPA MISAKIENSIS

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I studied the ultrastructure of possible sensory hair cells within the epithelium surrounding the tentacular tunic, which penetrates into the atrial tentacles of

Polyandrocarpa misakiensis. The bottle-shaped sensory cells usually exist at the bottom of the tentacular tunic as a single unit, and they are supported by unspecialized epithelial cells of the descending or ascending epithelium of the siphons. The sensory cells bear apical microvilli and a cilium. There is no accumulation of matrix around the cilium, as there is in the sensory cells associated with branchial tentacles. The cytoplasm of the sensory cells is enriched by rough endoplasmic reticulum, mitochondria, lipid droplets, and dense bodies. There are many thin cell processes in the test matrix of the tentacles. Although we have not identified a basal process with the ultrastructural features of an axon, the morphology of these cells suggests that they might be primary sensory neurons, like many peripheral sensory cells of other protochordates.

ANALYSIS OF STIGMATA FORMATION USING PHARYNGEAL-GILL-RELATED GENES IN *CIONA* INTestinalis

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Pharyngeal-gill with many gill slits in lower chordates is an special organ to understand chordate body plan as well as a notochord and a dorsal nerve cord. The gill slits of ascidian named stigmata are developed after metamorphosis and associate with respiration and internal filter feeding. In order to identify useful molecules related to function and development of the ascidian stigma, expression patterns of pharyngeal-gill-related genes isolated previously were observed by means of whole-mount *in situ* hybridization in juvenile and adult specimens. These observation suggested that expression patterns of these genes were identical between juvenile and adult, and the stigma of *Ciona intestinalis* is formed by three types of cells. Culture of pharyngeal-gills dissected from adult *Ciona* was performed and measured a condition of our system by their morphology and mobility of cilia. Furthermore, formation of ectopic stigmata in the dissected pharyngeal-gill was carried out to investigate a molecular mechanism of the stigma formation. Analyses of the stigma formation using pharyngeal-gill-related genes in ascidian might be useful to understand the evolution of chordate pharynx.

CAUSE OF BODY COLOR VARIATION IN ADULTS OF THE TORTOISE BEETLE *CASSIDA RUBIGINOSA* (COLEOPTERA: CHRYSOMELIDAE)

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Adults of the tortoise beetle *Cassida rubiginosa* usually possess greenish body color. However, adults with yellowish coloration sometimes coexist with the greenish individuals. In the present study, we investigated the cause of this color variation under laboratory conditions. When field-collected adult beetles were kept in the laboratory for 22 weeks, no obvious change of the body colors between greenish and yellowish one was confirmed. On the other hand, when larvae from eggs oviposited by adults with both body colors were reared, newly emerged adults showed both of the greenish and yellowish body colors. These results indicate that the body color variation observed in adults of *C. rubiginosa* is genetic dimorphism.

POLYMORPHISM OF THE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I REGION OF MEDAKA, *ORYZIAS LATIPES*

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The mammalian Major Histocompatibility Complex (MHC) is one of the most polymorphic regions of the mammalian genome. In this study, to clarify MHC polymorphism in bony fish, we determined the complete nucleotide sequence of the MHC class I region of the HNI strain (Northern Japan population-derived inbred medaka) and compared it with the published sequence of the Hd-rR strain (Southern Japan population-derived inbred medaka) MHC class I region. The compared region (~420 kb) was divided into three segments based on degree of sequence conservation. The middle segment (~100 kb) was too divergent between HNI and Hd-rR to be aligned, whereas the other two segments were easily aligned with an average 96% nucleotide identity. Two MHC class Ia genes and the immunoproteasome subunit, PSMB8, gene were encompassed in the middle segment. This result indicates that the middle segment is evolving not only by simple nucleotide substitutions but also by extensive DNA rearrangement such as duplication, insertion and deletion within the medaka species.

ORIGIN AND EVOLUTION OF THE COMPLEMENT COMPONENT C3 GENE

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In higher vertebrates, one of the major effector mechanisms of the innate and adaptive immune responses is the complement system. Most components of the complement system show a unique combination of various domains, and are considered to have been established in Deuterostome lineage. However, the central component C3 is exceptional and does not show any characteristic domain structure. C3, two other complement components C4 and C5, serum protease inhibitor alpha-2 macroglobulin (A2M) and blood cell surface protein CD109 constitute a thioester containing protein (TEP) family whose evolutionary origin is still to be clarified. In this report, we tried to isolate and analyze TEP genes of Cnidaria and Porifera, using degenerate primers designed at the conserved region among the TEP family members. No TEP gene was found in Porifera, whereas all three members, C3, A2M, CD109, were found in Cnidaria. These results indicate that the TEP gene arose, duplicated and differentiated into C3, A2M and CD109 at the early stage of Metazoa evolution.

SURVIVAL RATES OF HYBRIDS BETWEEN *HENOSEPILOCHNA NIPONICA* AND *H. YASUTOMII* ON THE HOST PLANTS OF PARENTAL SPECIES

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Hemosepilachna niponica and *H. yasutomii* are very closely related phytophagous ladybird beetles, and are distributed in sympatry. *Hemosepilachna niponica* feeds on thistles and *H. yasutomii* dose on the blue cohosh, and almost all individuals of these two species cannot survive on the host plant of the other species. Because these two species lack post-mating isolation, they are considered to be reproductively isolated by only the difference of host plants. In this experiment, we compared survival rates of F1, F2, and back-cross (BC) hybrids between *H. niponica* and *H. yasutomii* when they were reared on the two host plants. Survival rates of F1 and F2 hybrids were similar to that of *H. yasutomii*. They grew well on blue cohosh while could not grow on thistles. On the other hand, the BC hybrids to *H. niponica* grew on blue cohosh, and some of them could grow well on thistles.

DEVELOPMENT OF THE NERVOUS SYSTEM OF THE STALKED CRINOID ECHINODERM, *METACRINUS ROTUNDUS*

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The neural development of *Metacrinus rotundus*, a member of the stalked crinoids, the most basal extant group of echinoderms, was investigated using immunohistochemistry and electron microscopy, and the results were compared with those of other echinoderm larvae. Serotonergic nerve cells and fibers appeared among the anterior epidermis of the auricularia, and they developed into an apical ganglion at the doliolaria stage. No serotonergic signals were observed along the ciliary bands, but signals of a neuron specific antibody IF9 were seen there. Observations with transmission electron microscopy revealed that axonal bundles were present along the ciliary bands and some axons were underneath the epidermis. In the doliolaria larva, an intraepithelial serotonergic diffuse nerve net was formed throughout the body, but they deteriorated prior to settlement. Doliolaria larva of the feather star *Oxycomanthus japonicus*, unstalked crinoids, also possessed an apical ganglion, but the intraepithelial nerve net was not prominent with immunohistochemistry.

THE ROLE OF THE CONCENTRATION-SENSITIVE Na⁺ CHANNEL IN THE EVOLUTION OF ANIMAL BRAIN SIZE

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Evolution of animals, especially mammals, has been dependent on the development of a larger brain. The cerebrospinal fluid (CSF) reduces practical weight of the brain for protection against mechanical injury, and the osmolality of CSF, which is determined by a major solute Na⁺, must be maintained within a small range. In 2002, we reported a new type of Na⁺ channel whose openings are dependent on changes in the extracellular Na⁺ concentration but not in the membrane potential (*Nature Neurosci.* 5:511-512), and we named it the concentration-sensitive Na⁺ channel (abbreviated to Na_C; c = concentration) (*Cur. Med. Chem. - CNS Agents* 2:59-81, 2002). In the brain, Na_C is present only in the circumventricular organs which lack the blood-brain barrier, i.e., suitable places for directly monitoring the Na⁺ concentration of CSF. In addition, Na_C has been found only in mammals. I would like to present a hypothesis to explain a factor which has enabled the evolution of a larger brain in animals; Na_C, which appears at the stage of evolutionary development of mammals, works as a sensor and a regulator of Na⁺ in CSF, and has contributed to enabling mammalian brains to become larger.

THREE-DIMENSIONAL CT ANALYSIS AND MACROSCOPIC OBSERVATIONS IN THE FORELIMB OF THE AARDVARKHideki Endo¹, Takeo Sakai², Takuya Itou², Hiroshi Koie³, Junpei Kimura⁴¹Department of Zoology, National Science Museum, Tokyo, Shinjuku-ku, Tokyo 169-0073, Japan, ²Department of Preventive Veterinary Medicine and Animal Health, College of Bioresource Sciences, Nihon University, Fujisawa-shi, Kanagawa 252-8610, Japan, ³Laboratory of Comprehensive Veterinary Clinical Studies, College of Bioresource Sciences, Nihon University, Fujisawa-shi, Kanagawa 252-8610, Japan and ⁴Department of Veterinary Anatomy, College of Bioresource Sciences, Nihon University, Fujisawa-shi, Kanagawa 252-8610, Japan

The forepaw in the aardvark was functional-morphologically examined by using three-dimensional CT and macroscopic techniques. From the 3D-reconstructed CT images, we confirmed differences of the flexing angle in metacarpophalangeal joint among digits. We suggest that the second and the third digits with large mobile angle contribute to the soil crushing, in contrast the fourth and fifth digits have no large mobility in the metacarpophalangeal joint and may act as sweeper of the crushed soil. From the macroscopic observations, the M. extensor digiti II and the M. extensor carpi radialis insert to the two medial digits-metacarpals, whereas the M. extensor digitorum lateralis and M. extensor carpi ulnaris reach the two lateral digits-metacarpals.

PHYLOGENETIC ANALYSIS OF THE JAPANESE CEPHALOPODS BASED ON PARTIAL MITOCHONDRIAL GENE SEQUENCESMikio Takumiya¹, Mari Kobayashi², Kazuhiko Tsuneki¹, Hidetaka Furuya¹¹Department of Biology, Graduate School of Science, Osaka University, Osaka, Japan and ²Seto Marine Biology Laboratory, Graduate School of Science, Kyoto University, Wakayama, Japan

We collected 33 species of cephalopod mollusks from Japanese waters and determined partial nucleotide sequences of three mitochondrial genes, mt16SrDNA gene, mt12S rDNA, and cytochrome c oxidase subunit I gene, to study phylogenetic relationships of the Japanese cephalopods. The following relationships were suggested: (1) Decapoda is separated into three groups, squids, cuttlefishes, and bobtail squids. (2) Bobtail squids are more close to squids than cuttlefishes. (3) Octopoda is separated into three groups, the Paroctopus dofleini group, Octopus vulgaris group, and Octopus minor group. Octopus minor group characterized by the long arms includes *O. ornatus* and *O. minor*. Octopus vulgaris group includes three genera, Amphioctopus, Hapalochlaena, and Octopus. In this Octopus vulgaris group, several species including Amphioctopus and Octopus form a small cluster, which is also characterized by a distinct oculus on the web. (4) Hapalochlaena is more closely related to Amphioctopus than to Octopus. (5) Argonauta argo branches out of four octopus genera, Paroctopus, Amphioctopus, Hapalochlaena, and Octopus. Close relationships did not appear between Argonauta and other genera examined.

NOTE ON THE CAECILIANS FROM THE NORTHERN BORNEOKanto Nishikawa¹, Masafumi Matsui¹, Maryati Mohamed²¹Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Nihonmatsu-Cho, Sakyo, Kyoto 606-8501, Japan and ²Institute of Tropical Biology and Conservation, University of Malaysia, Sabah, 88999 Kota Kinabalu, Sabah, Malaysia

In August and December of 2003, we collected caecilian larvae from the eastern slope of the Crocker Mountain ranges of Sabah, northern Borneo of Malaysia. They were found in two small streams on the river bank and could be split into two syntopic species. One had a pair of pale yellowish stripes on sides of the body, while the other lacked the stripe. On the basis of several morphological traits such as shape of vomero-palatine teeth series, number of splenial teeth, and ratio of width to length of body, these species were temporally identified as *Ichthyophis atricollaris* and *I. monochrous*, respectively. Occurrence of the order Gymnophiona on Borneo, especially in Sabah, is very poorly reported, and this is the first record of caecilians from the Crocker Mountain ranges. It is expected that additional species of caecilians would be found in future by our inventory studies now in progress in Sabah.

TAXONOMIC RE-EVALUATION OF BORNEAN LEPTOLALAX BASED ON ACOUSTIC AND MORPHOLOGICAL CHARACTERISTICS (AMPHIBIA, ANURA)Masafumi Matsui¹, Maryati Mohamed²¹Graduate School of Human and Environmental Studies, Kyoto University, Yoshida-Nihonmatsu-Cho, Sakyo-Ku, Kyoto 606-8501, Japan and ²Institute of Tropical Biology and Conservation, University of Malaysia, Sabah, 88999 Kota Kinabalu, Sabah, Malaysia

The pelobatid genus *Leptolalax* from Borneo was once considered to be represented only by *L. gracilis*, but is now known to include many species that are very similar in morphology, but are distinct from each other in acoustic characteristics. On the bases of morphological and acoustic traits, we could recognize eight species from the Malaysian territory, states of Sabah and Sarawak: Undescribed species 1 (Mulu, Sarawak); undescribed species 2 (Crocker, Sabah); *L. dringi* (Mulu, Sarawak and Crocker, Sabah); *L. hamidi* (Lanjak, Sarawak); *L. pictus* (Kinabalu and Crocker, Sabah); *L. arayai* (Kinabalu, Sabah); undescribed species 3 (Mulu, Sarawak and Crocker, Sabah); *L. gracilis* (Kuching, Sarawak). Another species, *L. maurus* (Kinabalu, Sabah), whose voice is unknown, is surely distinct from these species in morphology. Thus, Borneo encompasses at least nine species of *Leptolalax* and is regarded as one of the centers of diversity of this genus. Further, future surveys in wider area of the Indonesian territory, Kalimantan, would prove occurrence of many more species on this large island.

PHYLOGENETIC RELATIONSHIP OF ASIAN AND AFRICAN SPECIES OF EPILACHNINE LADYBIRD BEETLESToru Katoh¹, Norio Kobayashi², Yuri Ohta¹, Susumu Nakano³, Sih Cahono⁴, Takahide Ishida⁵, Helmt Fuersch⁶, Haruo Katakura¹¹Division of Biological Sciences, Graduate School of Science, Hokkaido University North 10 West 8, Kita-ku, Sapporo 060-0810, Japan, ²Museum, Hokkaido University North 10 West 8, Kita-ku, Sapporo 060-0810, Japan, ³Faculty of Human Environmental Studies, Hiroshima Shudo University Hiroshima 731-3195, Japan, ⁴Balai Penelitian dan Pengembangan Zoologi, Puslitbang Biologi, LIPI, Jl. Raya Bogor Jakarta Km46, Cibinong 16911, Indonesia, ⁵Graduate School of Agricultural and Life Sciences, The University of Tokyo Yayoi 1-1, Bunkyo-ku, Tokyo, Japan and ⁶Passau University Passau, Germany

The subfamily Epilachninae consists of phytophagous ladybird beetles in the family Coccinellidae. Most species in this group are classified into two genera, *Epilachna* and *Henosepilachna*. These species are distributed mainly in the tropical regions of Asia, Africa and the New World. In order to clarify the relationship of these beetles, we performed phylogenetic analyses for representatives of Asian and African species using a number of molecular data including nuclear and mitochondrial DNA. The results revealed that *Epilachna* and *Henosepilachna* in Africa might not necessarily constitute monophyletic groups with respective Asian congeners.

ENDOSYMBIOTIC BACTERIA IN THE CLOSELY RELATED HENOSEPIILACHNA LADYBIRD BEETLESKei Matsubayashi¹, Norio Kobayashi², Haruo Katakura¹¹Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan and ²The Hokkaido University Museum, Sapporo 060-0810, Japan

Phytophagous ladybird beetles belonging to the *Henosepilachna vigintioctomaculata* species complex are composed of four very closely related species, that were classified into group A (*H. vigintioctomaculata*) and group B (*H. niponica*, *H. yasutomii*, *H. pustulosa*). We determined a part of 16S rDNA of endosymbiotic bacteria obtained from 6 ladybird beetle species including all the four species of the *Henosepilachna vigintioctomaculata* species complex, and analyzed their phylogenetic relationships using Neighbor Joining method. The results showed that the phylogeny of these symbionts did not correspond to the phylogeny of their hosts. The symbionts of each of the two groups of the *Henosepilachna vigintioctomaculata* species complex belonged to different clades, and those of the three species of group B shared the same sequence.

MOLECULAR EVOLUTION OF MITOCHONDRIAL GENOMIC STRUCTURES IN RANOID FROGSAtsushi Kurabayashi¹, Chisako Usuki², Nozomi Mikami², Naomi Sano¹, Tamotsu Fujii², Masayuki Sumida¹¹Institute for Amphibian Biology, Graduate school of Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 739-8526, Japan and ²Department of Health Science, Hiroshima Prefectural Women's University, Hiroshima 734-8558, Japan

Mitochondrial genomic structures are generally conserved in vertebrates. A common gene arrangement is found through teleost fishes to mammals. However, in the members of the superfamily Ranoidea (higher anurans), the positions of several genes were found to differ from those of the typical vertebrates: 4 tRNA genes were rearranged in the family Ranidae, and the ND5 gene as well as the tRNA genes was translocated in the family Rhacophoridae. To elucidate the evolutionary pathway of the mitochondrial genomic structures in the anurans, we analyzed mtDNAs from a number of species belonging to another ranoid family Mantellidae. The gene arrangements of many mantellid mtDNAs were the same as those of rhacophorids, suggesting that the translocation of the ND5 gene occurred in a common ancestor of two families. Furthermore, the novel gene arrangements were observed in the genus *Mantella*. Especially, the mitochondrial genome of *Mantella expecta* showed unique genomic structures as follows: 1) two tRNA-Met genes were coded and 2) a tRNA-Pro-like sequence was observed at the position between tRNA-Leu(CUN) and tRNA-Phe genes in which the functional tRNA-Pro gene was found in another ranoids.

INTRA- AND INTERSPECIFIC DIFFERENTIATION IN THE BELL-RING FROG *BUERGERIA BUERGERI* AND ALLIED SPECIES INFERRED FROM mtDNA GENE SEQUENCE ANALYSIS

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The bell-ring frog, *Buergeria buergeri* of the family Rhacophoridae, is an endemic species distributed in the Honshu, Shikoku and Kyushu islands of Japan. In order to clarify the intra- and interspecific differentiation of *B. buergeri* and allied species belonging to this family, we analyzed the nucleotide sequences of mitochondrial rRNA genes using 30 individuals from 15 populations of *B. buergeri* and 5 individuals belonging to 4 species and one subspecies of genus *Rhacophorus*. The average nucleotide divergences of the 12S rRNA gene were 2.8% between populations, 5.7% between species, 21.2% between genera, while those of the 16S rRNA gene were 2.6% between populations, 3.7% between species and 12.2% between genera. The phylogenetic trees constructed by the NJ method showed that these frogs were divided into two clades, one of which was broadly divided into three groups of *B. buergeri*: the eastern, the western and the boundary. The other clade gradually diverged into several species and subspecies of genus *Rhacophorus*. These results clearly show that the 16S and 12S rRNA gene sequences are useful in clarifying the intra- and interspecific differentiation of the family Rhacophoridae.

THE EXON-INTRON STRUCTURES OF FISH HATCHING ENZYME GENES

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Full length cDNAs for hatching enzymes were amplified from RNA of *Fundulus heteroclitus* and *Takifugu rubripes* (Fugu) embryo by RT-PCR. The cDNAs highly homologous to Medaka HCE and LCE were cloned from each species. Whole mount *in situ* hybridization revealed that the hatching gland cells of both fishes differentiated from the anterior end of the hypoblast (pillow). The final location of hatching gland cells of *F. heteroclitus* was in the pharyngeal cavity, while the cells of Fugu were dispersed in the epidermis around head region. The genomic structures of hatching enzyme genes of *Oncorhynchus masou* (MsHCE1 & 2), zebrafish (ZHCE1 & 2), *F. heteroclitus* (FHCE and FLCE) and Fugu (FgHCE and FgLCE) were clarified. Structure of LCE gene comprising 8 exons and 7 introns was conserved in *F. heteroclitus*, Fugu and Medaka. However, the number of introns in HCE gene is different from species to species; five introns in ZHCE, one intron in MsHCE, and no introns in FHCE and FgHCE. In addition to the result that eel HCE has 7 introns and Medaka HCE has no introns, these results suggest that HCE genes gradually lost their introns during evolution of teleost.

CORRELATION BETWEEN THE RADIATION EVENT OF WEASELS AND MARTENS (MUSTELIDAE; CARNIVORA) INFERRED FROM MULTIPLE NUCLEAR SEQUENCES

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Phylogenetic relationships within the marten genus *Martes* have repeatedly been studied using either morphological or genetic data, but both the monophyly of the genus and the pattern of the relationship among the marten subgenera *Martes*, *Charronia*, and *Pekania* remain an issue of uncertainty and controversy. Here we present results on phylogenetic relationships among 27 species of Mustelidae (including all extant species of the genus *Martes*) as inferred from nucleotide sequences of two nuclear genes: the recombination-activating gene 1 (RAG1) and the gene encoding interphotoreceptor retinoid-binding protein (IRBP). Neighbor-joining, maximum parsimony, maximum likelihood, and bayesian phylogenetic analyses on these genes separately and combined were conducted. Although the analyses moderately to strongly supported the monophyly of the subgenus *Martes*, they resulted in a poorly resolved relationship among this subgenus, *Charronia*, *Pekania*, and the genus *Gulo*. The time of the radiation into the three marten subgenera is consistently indicated to be at 5.5 Ma by the two genes and may be correlated with the first radiation of *Mustela* lineage.

MOLECULAR PHYLOGENETIC ANALYSIS OF JAPANESE EARTHWORM (MEGASCOLECIDAE: *PHERETIMA*)

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Pheretima [Amyntas] is the most dominant genus of Japanese Megascolecidae. *Pheretima* worms collected from the Tama area of Tokyo were morphologically classified into 11 species. Ribosomal RNA genes were PCR-amplified from 50 worms, which belong to 9 species of *Pheretima* worms with several worms from each species. PCR products were directly sequenced. The analyses of 18S rRNA genes revealed high sequence identity within the genus. There was one variation for approximately 500-bps in the 5' region among 50 worms. In contrast, mitochondrial genes for 16S rRNAs have more sequence variations. To evaluate these variations, molecular phylogenetic analysis was carried out using the NJ method. 9 species could be divided into two groups; one have male pores and another do not. These results together suggest that *Pheretima* worms are genetically closely related, although they have a wide variation in morphology and thus, are currently classified into many taxa. To establish the phylogenetic relationship, it is necessary to clarify the genetic variations of these genes.

THE INCUBATION SYSTEM IN DECAPOD CRUSTACEANS: COMPARATIVE MORPHOLOGY OF THE PLEOPODS AND FINE STRUCTURE OF THE EGG ATTACHMENT SITE

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Decapod crustacean is classified into two groups: Dendrobranchiata spawning the eggs into the water directly, and Pleocyemata incubating the embryos until hatching. In Pleocyemata, fertilized eggs are attached to the pleopods arranged on the abdomen of females. Each pleopod consists of three podites, protopodite, endopodite and exopodite. Some kinds of setae are arranged on each pleopod, and the fertilized eggs are attached to the special setae, called ovigerous setae. We here compare the fine structure between ovigerous setae and other setae among decapod crustaceans with scanning electron microscopy. The structure of egg-attachment site is also examined with transmission electron microscopy. The morphology of pleopod and ovigerous or non-ovigerous setae, the number of pleopods that takes part in the incubation, and the podite of the pleopod on which the embryo are attached are common to each group (infraorder) of Pleocyemata, indicating the diversity of embryo-attachment system in decapod crustaceans.

MOLLUSCAN SHELL FORM DIVERSITY AND BMP SIGNALING

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Our interest is in reconstructing evolutionary history of the shell form diversity in mollusks, especially in linking changes of patterning mechanisms with morphological diversity of shells. We focus on the innovation of hinge in bivalves, because the hinge is the key morphological character which distinguish the shell morphology of bivalves from that of gastropods. In the present study, we asked whether the BMP signaling, which is known to be involved in the boundary formation between shell field and rest of the epidermis, is involved in the differentiation of the hinge. In other words, does the hinge differentiate as new boundary cells of the shell field? In bivalves, BMP gene and phosphorilated Smad is expressed in apical tuft, ciliary band and posttrochal none-shell field epitherium. This expression pattern suggests that function of the BMP signaling is in the differentiation of ciliated cells rather than boundary formation of the shell field. Since pSMAD expression is not detected in shell boundary of polyplacophora: chiton, we concluded that BMP signaling is secondarily recruited to shell boundary formation in gastropods.

MOLECULAR EVOLUTION OF CARTILAGE

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It is now well-accepted that the vertebrates experienced two round of genome duplications. Vertebrates have innovated several new cell types by utilizing duplicated genes. In the present study, we focus on the evolution of the chondrocytes which are responsible for production of cartilage. Transcription factors, such as Sox9 and scleraxis have been reported to have essential role in chondrocyte differentiation, and these regulate transcriptions of cartilage matrix proteins: type II collagen and

aggreacan. We have investigated these genes in protochordates which do not have cartilage, and asked what kind of molecular evolutionary processes were involved in the evolution of the new cell type: chondrocyte. We did not detect significant overlap in the expression patterns of these genes in ascidians. Thus it is likely that large scale cis-regulatory evolution was essential for chondrocyte evolution. On the other hand, protein coding region have been relatively conserved in these genes, except that exon shuffling has been involved in molecular evolution of the aggreacan gene.

REPRODUCTIVE ISOLATION BETWEEN TWO PARAPATRIC FORMS OF FLIGHTLESS *CHRYSOLINA* LEAFVEETLES

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Two forms of flightless leaf beetles belonging to the *Chrysolina angusticollis* species complex are distributed in the southern suburbs of Sapporo, Hokkaido, northern Japan. They are basically parapatric, feeding on the same kind of host plants. Previous studies conducted in laboratory revealed that there was rather strong sexual isolation between them, and there was reduction of fertility in males of F1 hybrids. However, putative natural hybrids were occasionally found in very narrow of coexistence zones formed at the boundary of their distribution. The present study was conducted to elucidate the degree of their reproductive isolation through analyzing of allozymes differences in the wild. The samples collected at the contact zone and those from parapatric populations.

GEOGRAPHIC AND SEASONAL VARIATIONS OF THE NUMBER OF B CHROMOSOMES AND EXTERNAL MORPHOLOGY IN *PSATHYROPOUS TENUIPES* (ARACHNIDA: OPILIONES)

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Psathyropus tenuipes is a harvestman that harbors B chromosomes in extremely high frequency and high numbers (mean number of Bs per individual is about 4). Geographic variations of the number of Bs and external morphology of the species and relationship between them were studied. Northward increase throughout Japanese Islands in the number of Bs was detected. Latitudinal gradients were also found in some external characters, while there were no correlations between those external morphologies and the number of Bs. Principal component analysis using eight morphological data for 21 populations revealed four geographical groups that reflect actual location of the populations. Populations along the Seto Inland Sea were characterized by lower number of Bs than those in other areas. Seasonal change was also found in a population (Yatsukami in western Honshu) in both 1994 and 1995 for the number of Bs, though the number in the same population was stable at least throughout later postembryonic stages in both 1997 and 1998. Embryos contained fewer number of Bs than adults, suggesting that females of the species tend to lay eggs with fewer number of Bs.

A THEORY ON THE EVOLUTIONARY ORIGIN OF AUTONOMIC NERVOUS SYSTEM

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Autonomic Nervous System (ANS) autonomously regulates the individual organ function and homeostasis in response to external and internal stimuli. The action of this system is characterized by the push-pull regulation of organs by Sympathetic nervous system (SNS) and Parasympathetic nervous system (PSNS). A typical way of regulation is that the heart rate is elevated by SNS and reduced by PSNS whereas digestive movements are elevated by PSNS and reduced by SNS. As a consequence, the heart rate and the digestive movements are destined to change in opposite phases to each other. How ANS appeared in metazoan evolution remains unknown. From the former results of comparative physiological studies and recent observations in Hydra, a phylum Cnidaria, we propose a possible scenario that the primitive form of the nervous system that regulates homeostasis was molded very early in metazoan evolution in Cnidaria or its ancestor.

IRIDESCENT ORGANS INVOKE MORPHOLOGICAL SEXUAL DIMORPHISM

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Ostracods possess unique morphological characteristics - all-enveloping carapace. This character indicates that physical restriction factors may affect limited parts in body. And species-specific morphological characters may relate to its habitat use or behavior. Actually, in many cases, we can find that carapace proportion reflects habitat of Ostracods. But, is that all? In this study, I present associations between light emitting organs and carapace morphology.

SPECIATION IN THE RICE FROG *FEJERVARYA LIMNOCHARIS* COMPLEX FROM BANGLADESH

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The *Fejervarya limnocharis* complex is widely distributed through Southeast Asia, and is reported to include several cryptic species. To elucidate the whole aspect of speciation of the *Fejervarya limnocharis* complex in Bangladesh, we conducted measurements of external morphology, mitochondrial DNA sequence analyses and crossing experiments using 90 individuals of 8 populations from Bangladesh. As a result, these frogs were divided into three (large, middle and small) types by the external measurements. The mitochondrial 12S and 16S rRNA gene sequence analyses revealed that the large-type greatly diverged from the other two types, and that the middle-type diverged from the small-type to some extent. Although the closed sequences to the small- and large-types were found in the DNA databank, the closed sequences to the middle-type were not found. Moreover, the large-type was reproductively isolated from the small- and middle-types by complete hybrid inviability, while there was no such isolation between the small- and middle-types. These results may suggest that the large-type is regarded as a separate species from the other two types, and that the middle-type is an undescribed taxon.

THE PHYLOGEOGRAPHY FOR CONSERVATION OF THE GENUS *LEFUA*

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The genus *Lefua* (Balitoridae, Cypriniformes) is composed of four species, *L. nikkonis*, *L. costata*, *L. echiogonia*, and *L. sp.* *Lefua sp.* is distinguished from the three species morphologically and ecologically. *Lefua echiogonia* and *L. sp.* were assigned as endangered species, and *L. nikkonis* as vulnerable species in the Red list by Ministry of the Environment of Japan. In order to establish a guideline for conservation of these threatened loaches, we investigated interspecific relationships and intraspecific variations by determining nucleotide sequences of the mitochondrial D-loop region. Each species formed a monophyletic group except for specimens of *L. sp.* from the Tokai district. The Tokai population of *L. sp.* was more closely related to one of six local populations of *L. echiogonia* rather than to the two other populations of *L. sp.* We performed RAPD analysis of mainly nuclear DNA to investigate whether the population is derived from once crossing between *L. sp.* (male) and *L. echiogonia* (female). RAPD patterns of the Tokai population were different from those of populations of *L. echiogonia* and also from those of the other populations of *L. sp.*

EVOLUTIONARY ORIGIN OF THE MEDAKA Y-CHROMOSOME

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Genetic sex determination in an XX-XY chromosome system can be realized through a locus on the Y-chromosome that makes the undifferentiated gonad to develop into a testis. Although this mechanism is widespread, only in two cases so far the corresponding master male sex determining genes have been identified. One is *Sry*. The other is *dmrt1bY* (*dmy*) from the fish medaka, *Oryzias latipes*. The mammalian Y is roughly estimated to be more than 200 Myr old. The medaka Y may be considerably younger. A comparative analysis of species from the genus *Oryzias* revealed that one sister species of the medaka has *dmrt1bY* on a homologous Y-chromosome, while in another closely related species only a non-sex linked pseudogene is present. In all other species a homologue of the medaka *dmrt1bY* could not be detected. Using mitochondrial DNA sequences the divergence time for the different species was determined. The timing was confirmed by independent calculations based on *dmrt1* sequences. We show that the medaka sex determining gene originated approximately 10 Myr ago. This makes *dmrt1bY* and the corresponding Y-chromosome the youngest male sex determining system, at least in vertebrates, known so far.

REPRODUCTIVE ISOLATION OF PHYTOPHAGOUS LADYBIRD BEETLES IN THE FIELD CAGESMasakazu Kuwajima¹, Yosiyuki Hirai², Haruo Katakura¹¹Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan and ²Graduate School of Dental Medicine, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

Interspecific matings between the synpatric phytophagous ladybird beetles, *Henosepilachna niponica* that occurs on thistles and *H. yasutomii* on the blue cohosh, are thought to be prevented by their fidelity to respective host plants. In order to test this hypothesis, we monitored and compared between-plants movement and matings of newly emerged beetles that were released into different settings of field cages. In one cage, the host plants of the two beetle species, the thistle and the blue cohosh, were planted; in another cage, the Japanese nightshade, a plants preferentially eaten by both of two ladybird species in the laboratory, was planted in addition to the thistle and the blue cohosh. The results showed that although the host fidelity acts as the reproductive barrier between the two species, interspecific matings might occur during the migration of newly emerged adults to the hibernation sites.

TERRESTRIAL ISOPODS IN TOKARA ISLANDS, SOUTHWEST JAPAN

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The senior author investigated diversity and distribution of terrestrial isopods in Toshima-Mura (Tokara Islands) in Kagoshima Prefecture Southwest Japan, and obtained two suborders ten families 13 genera 16 species from seven all islands. Species composition of terrestrial isopods of each island was different. And the habitat preference each species was different. Tokara Islands are located between Yaku-shima Island and Amami-Oshima Island, and consist of islands of the volcanic island and the upheaval coral. It is considered that the distribution of terrestrial isopods of Tokara Islands has been influenced from both the geological and climate factors. Especially, Trachelipidid and Trichoniscid isopods were limited by the habitat condition.

HEPATIC ONTOGENESIS. COMPARATIVE HISTOLOGICAL STUDY OF 200 TELEOST LIVERS IN RELATION TO PHYLOGENY

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This report presents a detailed description of hepatic architecture in 200 teleost livers by light microscopy and extensively discusses the phylogenetic viewpoint. The 200 fish livers showed a great variety of histological images, but not the same image, as in mammalian livers. The hepatocyte-sinusoidal structures of the fish livers were classified into three different types. Biliary tract structures were classified into four types. As phylogenetic advancement is graded from low to high, the parenchymal arrangement progressed from solid or tubular form to cord-like form, but the biliary tract structures were not involved. We demonstrate that this study is the first to investigate teleost livers phylogenically, and their architectural differences are shown in the route of hepatic ontogenesis. In hepatic ontogenesis, the formation of the parenchymal arrangement is acquired phylogenically, but the biliary pathway may be adapted in the ecological and behavioral patterns.

DOES THE CONTRACTILE VACUOLE COMPLEX PLAY A LEADING ROLE IN OSMOREGULATION IN *PARAMECIUM*?

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The contractile vacuole (CV) of a fresh water protozoa *Paramecium* has been regarded as an osmoregulatory organelle, since the CV expels more cytosolic fluid to the outside of the cell when the cell is subjected to a lowered osmolarity, while it stops to expel fluid when the cell is subjected to a raised external osmolarity. Recently we precisely determined the time courses of changes in both cell volume and the amount of fluid expelled from the CV after changing the external osmolarity. *Paramecium* swelled when it is subjected to a lowered osmolarity in spite of an increase in fluid expulsion from the CV, while it shrunk when subjected to a raised external osmolarity in spite of stoppage of fluid expulsion from the CV. These results imply that the CV is not an osmoregulatory organelle. We also suggested the presence of osmolyte transporters in the plasma membrane that play leading role in osmoregulation of *Paramecium* similarly to the regulatory volume control mechanisms (RVI and RVD) found in conventional animal cells. We assume that an increase in the CV activity moderates abrupt swelling of the cell immediately after lowering the external osmolarity.

MOLECULAR CLONING OF AN INTEGRAL MEMBRANE PROTEIN, IP39, OF *EUGLENA GRACILIS*

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Flagellates of the genus *Euglena* perform a characteristic movement called "euglenoid movement". Intramembrane proteins called IP39, which are regularly and densely arranged in the plasma membrane of the pellicular strip have been implicated in this movement. We carried out PCR-based cDNA cloning of IP39 from *Euglena gracilis*. As a result, we found two types of cDNA (α - and β -types). The α - and β - type cDNAs consisted of 792- and 795-base pairs, respectively. Molecular weights of both types of IP39 protein were predicted to be 29 kDa, which is much smaller than the 39 kDa estimated by SDS-PAGE. Between α - and β -type cDNAs, differences were found over the whole sequence. However, between the deduced amino acid sequences, differences were restricted to the C-terminal region, except for two residues in the middle part. Predicted secondary structures of both types of IP39 suggest that they are multipass transmembrane proteins with two or four transmembrane regions. Northern blot analysis of both types of IP39 RNA showed that about the same amount of mRNA was expressed for both types of IP39. Inhibition of the expression of IP39 was performed by RNAi technique.

APPEARANCE AND DISAPPEARANCE OF 140KD PROTEIN SPECIFIC TO THE CYST PHASE MICRONUCLEI OF THE CILIATE, *STERKIELLA CAVICOLA*Asuka Oda¹, Tadao Matsusaka²¹Systems in Natural Environment, Graduate School of Science and Technology, Kumamoto University, Kumamoto 860-8555, Japan and ²Department of Science, Faculty of Science, Kumamoto University, Kumamoto 860-8555, Japan

Our Western blot analyses and immuno-electron microscopy have revealed the specific localization of 140kD protein on the compactly packed condensed cystic micronuclear chromatins and the appearance of the antigen at the late phase of encystment (spherical cells) of the ciliate, *Sterkiella cavicola*. In the present study, we made clear, by Western blot analyses, the persistence of this antigen in the vegetative cells immediately after excystment and its disappearance in the proliferating cells. Although the just excysted cells were morphologically vegetative cells, their ultrastructural features were rather resting cyst-like; electron-dense cytoplasm, condensed granular macronuclear chromatins, compactly packed micronuclear chromatins, clustered mitochondria, contrary to the proliferating cells; rather electron-lucent cytoplasm with many empty vacuoles, string-like macronuclear chromatins, less compactly packed micronuclear chromatins, and scattered mitochondria. The micronuclei of the resting cysts were 2 types, one with compact chromatins and the other with less compact chromatins. The present observations on the just excysted cells revealed the micronuclei of the former type.

IMMUNOCYTOCHEMICAL ANALYSIS OF MASTIGONEMAL PROTEINS IN ZOOSPORIC PLANT PATHOGEN *PHYTOPHTHORA*Mikihiko Arikawa^{1,2,3}, Hardham Adrienne², Terue Harumoto¹, Toshinobu Suzuki³¹Department of Biological Science and Environment, Graduate School of Human Culture, Nara Women's University, Kitaoyanishi-machi, Nara 630-8506, Japan,²Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia and ³Department of Biology, Faculty of Science, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan

Phytophthora and other Oomycetes are known as notorious plant pathogens which cause the worldwide devastation of plant diseases. For most species of *Phytophthora*, a motile biflagellate zoospore is the main infective agent, which is responsible for rapid dissemination and initiation of infection of potential host plants. The zoospore possesses hair-like appendages called mastigonemes on the surface of an anterior flagellum, which have long been thought to play an important role in zoospore motility. There is, however, currently almost no information on the proteins that form the mastigonemes. As a first attempt to elucidate the flagellar function at molecular level, especially the role of mastigonemes in zoospore motility, we have tried to produce monoclonal antibodies by immunization of isolated and purified flagella. Consequently, we successfully obtained antibodies against various kinds of zoospore surface components. Using the mastigoneme specific antibody, immunofluorescence and immunoelectron microscopy were carried out for characterization and localization of the antigenic mastigonemal protein.

DATA ARCHIVING OF PROTIST MOVIE SERVER

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We have been working on our Protist information server as an image archive of protists cells, since 1995. Now we have started archiving the movie data of living protists. For taking both still images and movie clips of the living preparations, we have prepared our custom trinocular tube with an extra video tube, by which it