

weight, total length and body depth. The ratio means one part of sea cucumber is mixed with one part kangkong; 1:2 ratio means one part sea cucumber is mixed with two parts of kangkong and 2:1 means two parts sea cucumber are mixed with one part kangkong. The three ratios were pelletized and given to the tilapia.

This study used the Complete Randomized Design (CRD). There were four treatments in the study. Treatment 1 is the group fed with 1:1 ratio; Treatment 2 is the group fed with 1:2 ratio; Treatment 3 is the group fed with 2:1 ratio and Treatment 4 is the group fed with commercial feed as the control group.

Results show that the group fed with 2:1 (2 parts sea cucumber mixed with 1 kangkong) had longer total length, heavier body weight and greater body depth followed by the group fed with commercial feed. The third is 1:1 ratio and the fourth is 1:2 ratio.

Results further show that there were significant differences in weight, body depth and total length in favor of the 2:1 ratio over other treatments.

Keywords: *Holothuria nigra*, *Ipomea aquatica* Turks, *Oreochromis niloticus*, aquaria

BIOLOGICAL SCIENCES

BSD No. 1 AN ASSESSMENT OF THE FOOD COMPONENTS OF FISHES THRIVING IN COASTAL WATERS NEAR A MINING SITE

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Paracale in Camarines Norte is one of the oldest mining sites in the country and is well known for its gold panning activities. Effluents from such activities may contaminate the surrounding aquatic environment and affect the biota and productivity of the waters. The feeding preferences of fishes living in the coastal waters near a mining site were assessed to see the productivity of the waters. But analysis is believed to be important in gathering information on the complexity of the habitat as shown by the diet of many fish species. Determination of food composition was done using Number and Occurrence/Frequency Method of Hynes and Rank Method of Pollard. The number method shows the percentage of the total number of individuals of a food item over the total number of individuals of all food items; the occurrence method shows the number of stomachs in

which each food type occurred and expressed as a percentage of the total number of stomachs examined; for the rank method, the different food types in each stomach were ranked in order of preponderance according to their relative volume. Nine (9) species belonging to 5 families were caught in the area, namely: Family Labridae (*C. diagrammus*, *H. trimaculetus*, *T. lunare*); Family Lethrinidae (*L. lentjan*); Family Lutjanidae (*L. lutjanus*); Family Paraperoidae (*P. polyopthalma*) and Family Serranidae (*C. argus*, *C. pachycentron*, *E. fasclatus*). Results showed they fed on a number of food organisms. Stomach content analysis revealed the presence of plant debris, microalga, fish eggs, polychaete worms, gastropods, nauplii, copepods, shrimps, crabs, and fish in varying percentages. Shrimps were the most important food component of fishes caught in the area.

Keywords: fish, feeding preferences, gut analysis, coastal waters, mining activity

**BSD No. 2 METAZOAN PARASITES FROM THE GILLS AND GUT OF
Auxis thazard (Lacepede) FROM BALAYAN BAY, BATANGAS**

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The frigate tuna, *Auxis thazard* (Lacepede), locally called tulingan, is one of six fishes that form the basis of the Philippine tuna fishing industry. The present study investigates the occurrence of metazoan parasites in the gills and gut of this fish from Balayan Bay, Batangas. Twelve specimens from the bay, obtained monthly from October 2000 to February 2001, were examined using the methods of Velasquez (1975). Mean fork length of the 60 specimens was 27.3 ± 5.0 cm. Only the copepod *Caligus* sp. was recovered from the gills; monthly prevalence of infection (mpi) ranged from 0 to 33.3%. From the gut, three species of acanthocephalans were found; *Rhadinorhynchus* sp. and *Echinorhynchus* spp. with mpi ranging from 33.3 to 100%. Also found were larval nematodes with mpi of 33.3% to 50%. Monthly values of intensity of infection for each parasite species were low (<10). Both prevalence and intensity of infection tended to be higher in bigger-sized hosts.

Keywords: *Auxis thazard*, tuna, parasites, fish, *Caligus*, *Rhadinorhynchus*, *Echinorhynchus*

BSD No. 3 THE UNIQUE MORPHOLOGICAL FEATURES OF ADULT FEMALE LAC INSECTS AND THE PHYLOGENETIC RELATIONSHIPS OF THE FAMILY KERRIIDAE WITH OTHER SCALE INSECTS (COCCOIDEA, HEMIPTERA)

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Adult female lac insects (Hemiptera: Coccoidea: Kerriidae) belonging to the genera *Austrotachardia*, *Kerria* and *Paratachardina* were examined using both scanning electron and light microscopy. Morphological features that are considered unique to the family Kerriidae like the anal tubercle, dorsal spine, post-oral lobes, brachia and brachial plates and the canellae were studied in detail. SEM images of most of these structures, previously thought as not homologous to any other character of scale insects, reveal that they are modifications and therefore homologues of lecanoid structures. Subsequent phylogenetic analyses utilizing morphological data showed the monophyly of the lecanoid families and a probable sister-group relationship between the Kerriidae and the soft scale family Coccidae. The same trend is also confirmed by similar analyses performed by colleagues overseas utilizing molecular (SSU RNA) data.

Keywords: Lac insects, Scale insects, Soft scales, Kerriidae, Coccoidae, Coccidae, morphology, phylogeny, SEM, *Austrotachardia*, *Kerria*, *Paratachardina*

BSD No. 4 STICK INSECTS (PHASMATODEA) FROM MOUNT BANAHAO DE LUCBAN AND TAYABAS, QUEZON PROVINCE: CAMOUFLAGED FEATURES OF TERRESTRIAL ARTHROPOD BIODIVERSITY

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This is the second paper in a series featuring the insect biodiversity of Mount Banahao de Lucban, Quezon Province, Philippines. Collections from the adjacent area of Mount Banahaw in Tayabas have been included. Five species of stick insects

(Phasmatodea) were identified, namely, *Aretuon echinatus* (Stal), *Eubulides igorroto* Rehn & Rehn, *Lonchodes* sp., *Orthomeria pandora* (Westwood) and *Stenobremus* sp. A previous record of *Hoploclonia fratercula* Rehn & Rehn is also reviewed. These insects, being camouflaged among the vegetation of rainforests and secondary growth, are among the least noticed creatures. The existence of at least five species collected within a short study period of only two years and in limited sites on the mountain reserve, suggest that there may be more species awaiting discovery. As such, stick insects may be good examples of terrestrial arthropods, which, in spite of being the most diverse group of organisms, are continuously disregarded in biodiversity documentation and studies. This makes the picture of biological diversity and its conservation grossly skewed towards plants and vertebrate wildlife like birds, mammals and reptiles. Stick insects reflect the fact that terrestrial arthropod biodiversity should be given more positive attitude and consideration in the Philippines and worldwide.

Keywords: Stick insects, Phasmatodea, terrestrial arthropod biodiversity, walking sticks, Mount Banahaw

BSD. No. 5 DRAGONFLIES OF CAMIGUIN ISLAND AND OF ILIGAN CITY STREAMS AND ITS ENVIRONS

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Studies on Odonatans especially the dragonflies of the Philippines are still in its infancy stage. For several decades, very few researches have dwelt in the area of dragonfly diversity. This study was therefore conducted to determine diversity within, between, and among populations of dragonflies collected in three streams and an islet in Camiguin Island and four different streams of Iligan city and its environs. Dragonflies were collected using sweep nets and stored using standard techniques of preserving and storing insects for systematics studies. Individual samples were identified based on characters of the wings, thorax and distinct structures to identify species. Results showed variability within, between, and among populations of dragonflies as shown by Shannon-Weaver Index. New genera were identified – three were endemic each for Suarez, Mimbalut, and Aya-aya and one for Dalipuga stream. Samples collected in Camiguin Island were found to be present in Iligan City indicating that geographic distance was not a major factor for the variations observed among the populations. Many individuals were suspected of belonging to new species and are still being investigated to confirm the results.

Keywords: dragonflies, Shannon-Weaver Index

**BSD. No. 6 BENTHIC INSECT FAUNA OF SUBIC BAY FOREST
RESERVE STREAMS**

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In most freshwater ecosystem, larval insects such as mayflies, trueflies (*Chironomidae* and *Chaoboridae*), caddisflies and the odonates dominate a benthic macro-invertebrate community. These organisms provide an excellent tool for biodiversity and ecological assessments. The return of the Subic Bay Naval Base to the Philippine government in 1992 paved the way towards the study of this biologically unexplored Forest Reserve. This study hopes to provide information on the freshwater benthic insects of selected streams of the present Subic Bay Forest Reserve (SBFR). Insect fauna was characterized in terms of their taxonomic richness, diversity and density.

Five streams within the SBFR were selected. In each stream, three 100 m segment were established and sampled for freshwater benthic insects during dry season (January and February 2001) and during wet season (June and July 2001). A total of 57 families of insect taxa were recorded and identified. Majorities are odonates (dragonflies and damselflies nymphs) followed by aquatic beetles (Coleoptera), blackflies (Diptera) and mayflies (Ephemeropteraus). The most diverse and abundant group were recorded in Tinalignian River whereas Ilanin River has the least number of insect taxa recorded.

SBFR streams, small as they are, is relatively diverse and rich in freshwater benthic insects. This suggests that SBFR streams include a diversity of habitats that need to be protected from any alterations/degradation that may be brought by the fast pace of industrialization in SBFR.

Keywords: Subic bay forest reserve, freshwater benthic macro-invertebrates, biodiversity

**BSD. No. 7 MORPHOMETRICS AND CYTOGENETICS OF THE MALE
ORIENTAL FRUIT FLY, BACTROCERA DORSALIS (HENDEL)**

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Length measurements of the five abdominal characters that are associated with reproduction in 50 males oriental fruit fly, *Bactrocera dorsalis* (Hendel), namely: pygofer,

subgenital plate, aedeagus, paramere and connective, were examined and statistically compared. Varying length dimensions ranged from 0.27 to 0.36 mm., majority of which exhibited 0.30 mm length in the pygofer (38%), subgenital plate (36%), aedeagus (34%) and paramere (30%). Parameter ratio was determined to express the shape of the structure. Seven male *B. dorsalis* exhibited perfect proportion (1: 1: 1: 1: 1) with respect to the five abdominal characters examined. Friedman's test on morphometrics indicated that the distribution of the length of abdominal characters was the same across 50 repeated measures.

Cytogenetic analysis of the testicular cells of *B. dorsalis* revealed that meiosis was normal in all cells observed, consisting of sequential reductional and equational divisions. The average meiotic index of male *B. dorsalis* was 51.37%. Karyotype analysis of diakinesis chromosomes revealed that the diploid chromosome number of *B. dorsalis* was $2n = 12$, consisting of five pairs of monocentric autosomes and one pair of heteromorphic sex chromosomes (XY). Average relative lengths of chromosomes ranged from 0.049 - 0.112. Analysis of variance of chromosomes' relative lengths showed insignificant difference among the individuals of sympatric local population of male *B. dorsalis*.

Keywords: morphometrics, cytogenetics, male *oriental fruit fly*, *Bactrocera dorsalis* (Hendel)

BSD. No. 8 HEMOCYTE AND ISOZYME ANALYSES OF POPULATIONS OF THE EUROPEAN HONEYBEE, *Apis mellifera* Linnaeus, REARED IN AGRICULTURAL, FORESTED AND INDUSTRIAL AREAS

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The effect on the hemocytes and isozymes in populations of European honeybees, *Apis mellifera* Linnaeus, reared in three different Laguna areas, namely: agricultural (Calauan), forested (College of Forestry and Natural Resources), and industrial (Canlubang) were determined via microscopic observations and electrophoresis, respectively. Hemocyte analysis showed that prohemocytes and granulocytes were dominant in *A. mellifera*. No significant difference was observed in the total hemocyte counts (THC's) in honeybees among different areas except in the unmanaged colonies after transport. Significant differences exist between the frequencies of prohemocyte and granulocytes (DHC's) in the control populations (before transport), however, there was an increase in prohemocytes in the experimental group (after transport) especially in the unmanaged honeybee colonies in Canlubang. On the other hand, analysis of isozymes such as alkaline phosphatase (ALPH), acid phosphatase (ACPH), and malic enzyme (ME) revealed their polymorphism in all the honeybee populations studied suggesting that the type of area can affect the

genotype and gene frequencies. Comparison of the genetic frequencies of honeybees from different areas showed that the highest genetic identity before and after transport was 1.1014 and 0.6426, respectively, both of which were the values for the colonies in Canlubang and Forestry. The lowest values obtained before and after transport was 0.4013 and 0.2251, which were both exhibited by the colonies in Canlubang and in Calauan. No significant differences were observed between the managed and unmanaged populations of honeybees. The high genetic identity values suggest that the populations of honeybees were still genetically similar despite the transports and rearings in three different areas.

Keywords: hemocytes, isozymes, prohemocytes, granulocytes, honeybees, *Apis mellifera* (Linnaeus)

BSD. No. 9 THE *IN VITRO* ACTIVITY OF AMIKACIN AND CEFUROXIME ALONE AND IN COMBINATION AGAINST EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE*

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It has been noted that clinical isolates of *E. coli* and *K. pneumoniae* are now becoming resistant to most beta-lactam drugs due to the production of extended-spectrum beta-lactamase (ESBL) enzyme. Their emergence poses a threat on the field of antibiotic therapy. As an immediate response, a preliminary experimental study on the combined effectiveness of amikacin and cefuroxime against these organisms was conducted.

Forty two clinical isolates of *E. coli* and *K. pneumoniae* suspected for ESBL-production (those resistant to any one of the 3rd generation cephalosporins or to either aztreonam or cefpodoxime) from patients admitted at Angeles University Medical Center within October - March 1999, 11 isolates (4 *E. coli* and 7 *K. pneumoniae*) were detected positive for ESBL production by double-disk synergy test. The minimum inhibitory concentrations (MIC) of amikacin and cefuroxime for these were determined by agar dilution method. Time-kill synergy test was performed to evaluate the combined killing activities of amikacin and cefuroxime at three concentrations: one dilution below, above and equivalent to the drugs' MICs. Viable counts were determined at 0, 6, 12 and 24 hr.

MIC determination showed that the organisms were susceptible to amikacin at 32-64 ug/mL and to cefuroxime at 32-128 ug/mL. For 73% of the isolates (8 out of 11) in all concentrations considered, the rate of killing increased by 2 log₁₀ after 24 hrs with the combination in comparison with the more active drug alone.

The results showed that synergy exists between amikacin and cefuroxime having greater bactericidal activity when used in combination than when used alone.

Keywords: antibiotic, resistance, beta-lactamase, time-kill, synergy

BSD. No. 10 NUTRACEUTICAL POTENTIAL OF *Collybia*

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This study explored the potential *Collybia*, a mushroom endemic to Nueva Ecija, as a nutraceutical product. It studied the effects of *Collybia* extract on some hematological properties and growth of mice.

White mice were given extract of *Collybia* as drinks for 30 days. Control group of mice were given distilled water. Hematological properties and gain in weight were assessed and compared after 30 days.

Results showed that mice given *Collybia* extract had significantly decreased bleeding time, increased hemoglobin and hematocrit and neutrophil count. Likewise, they were significantly heavier compared to the control mice. The results of the study imply that *Collybia* can enhance blood clotting, red blood cell production as well as growth. Based on these results, *Collybia* extract is a potential nutraceutical product as a vitamin and growth promoter and could be tapped as source of active ingredients for drug development.

Keywords: *Collybia*, mushroom, nutraceutical

BSD. No. 11 BLOOD CHOLESTEROL LEVEL OF MICE AS INFLUENCED BY MUSHROOM EXTRACTS

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This preliminary study determined the effect of different Philippine mushroom extracts on the blood cholesterol level of mice.

White mice were fed high fat diet and given crude mushroom extract. The extracts tested were those of *Pleurotus*, *Auricularia*, *Agaricus*, *Ganoderma*, *Volvariella* and *Lentinus*. A group of control mice was fed commercial diet and distilled water and another group with high fat diet and distilled water. The blood cholesterol level of mice was analyzed after a month of feeding.

Results revealed that mice given high fat diet had significantly higher blood cholesterol level compared to those given commercial diet. All mice fed high fat diet and given mushroom extracts, regardless of species, had significantly lower blood cholesterol

level compared to those given distilled water.

Based on the results, mushroom extracts can lower blood cholesterol level of mice.

Keywords: mushrooms, nutraceutical blood cholesterol

**BSD. No. 12 SORBITOL-COCONUT WATER AGAR MEDIUM FOR
THE INITIAL ISOLATION OF *Escherichia coli* O157:H7**

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Sorbitol-coconut water agar (SCWA) is a less expensive culture medium developed for the presumptive identification of food-borne *Escherichia coli* O157:H7, an emerging virulent infection that causes hemolytic uremic syndrome, and sometimes death to patients infected with it. Prompt diagnosis is needed for proper and immediate treatment. The developed medium harnesses the impressive amounts of major nutritional substances in coconut water necessary for the growth of microorganisms.

The performance of SCWA and the standard medium Sorbitol-MacConkey Agar (SMAC) was determined on stool specimens and diverse food samples spiked with various concentrations of *E. coli* O157:H7. Results showed that the detection limit of both SCWA and SMAC was not significant at 3-4 cfu/mL of sample. The specificity rates of SCWA and SMAC were not significant (T test) at 86.67% and 85%, respectively; the sensitivity rates were, likewise, not significant: 55% and 50% at a low inoculum level (3-4 cfu/25 ml), 68.33% and 85% at a medium level (30-40 cfu/ml), and 83.33% and 85% at a high level (300-400 cfu/ml). Results indicated that SCWA was as sensitive and as specific as SMAC. The growth rates of *E. coli* O157:H7 on SCWA and SMAC were also not significant from 30 minutes to 24 hrs.

Results indicated that despite the lack of supplementary components — peptone, sodium chloride and bile salts — the high nutritional content of coconut water would be enough to provide the substances necessary for the growth of *E. coli* O157:H7. The developed SCWA and the standard SMAC exhibited comparable performance, growth rates, sensitivity and specificity rates. Furthermore, the cost of this novel medium is 70% lower than SMAC. This study adds another important use to coconut.

Keywords: sorbitol, coconut water, *E. coli* O157:H7, Sorbitol-Coconut Water Agar, Sorbitol-MacConkey Agar, food samples, stool samples, sensitivity rate, specificity rate, growth rate

**BSD. No. 13 *IN VIVO* BIOLOGICAL ACTIVITY OF ATIS
(*Anona squamosa* Linn.) CRUDE EXTRACT ON DUCK EMBRYOS**

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Biological activities of *Anona squamosa* were observed in three-day old duck embryos showing angiogenic/ antiangiogenic and teratogenic/antiteratogenic potentials.

A.squamosa leaves were homogenized; resulting homogenate was subjected to rotary evaporation and the concentrated crude extract was lyophilized for 36 hours. Obtained crystalline powdered extract was dissolved in PBS buffer. Varying concentrations of *A. squamosa* extract solutions were introduced to the test embryos by injecting through a modified window to the air space of the egg. Negative and positive controls were made using Phosphate Buffered Saline (pH 7.2) and retinoic acid (Sigma), respectively. After a week of incubation at 37°C, the eggs were opened and morphological characters of the embryos were observed.

The 8 µg/ml extract manifested an antiteratogenic property through angiogenetic action.

Concentrations 9, 10, 20, and 30 µg/ml exhibited teratogenicity through inhibition of angiogenesis or blood vessel formation.

Keywords: *Anona squamosa*, angiogenic, anti-angiogenic, teratogenic, antiteratogenic

BSD. No. 14 ANTI-HEPATOTOXIC POTENTIAL OF *Spirulina platensis*

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Spirulina is microscopic blue-green algae, which are often found in warm alkaline volcanic lakes. The algae contain essential nutrients like beta-carotenc, phycocyanin, polysaccharide, sulfolipid, gamma linolenic acid, vitamin B complex, iron, magnesium, and trace minerals that help protect the human body by enhancing its immune system. It is also reported to reduce the hepatotoxicity in rats caused by carbontetrachloride and R- (+) pulegone administration. The effectivity of *Spirulina platensis* as an anti-hepatotoxic agent was investigated in this study by looking into the liver histology of ICR

strain mice exposed to cyclophosphamide treatment. Cyclophosphamide is a known chemotherapeutic agent. The drug is an alkylating agent and is known to cause cytotoxicity. It reacts with oxygenases and cytochrome P-450 causing formation of metabolites such as phosphoramide mustard, HN_2 and acrolein, which alkylate with DNA. The following treatment groups were made to establish *Spirulina's* anti-hepatotoxicity, namely: cyclophosphamide-treated, *Spirulina*-treated, and *Spirulina*-cyclophosphamide-treated groups. Tissues were fixed in Bouin's fluid and processed for paraffin sectioning. Light microscopy studies revealed extravasation of blood was evident in liver tissues of mice that received the cyclophosphamide drug. Mice given with *Spirulina* prior to drug treatment showed an improvement over the ones that did not receive *Spirulina* at all.

Keywords: algae, *Spirulina*, anti-hepatotoxic, mice, histology, liver

BSD. No. 15 PROPHYLACTIC AND ANTIMICROBIAL PROPERTIES OF BREAST MILK: FACT OR MYTH?

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Breast milk was once the only known milk for infants but it has largely been replaced by a great variety of commercial milk formulas, each claiming superiority over the others. More recently, the World Health Organization (WHO) has urged mothers to go back to breast-feeding. As a contribution to the Philippine government's efforts to this end, we did both laboratory experiments and surveys to obtain concrete indicators supporting the health claims concerning breast milk.

Samples of human breast milk were collected and tested for antimicrobial properties against four potentially pathogenic bacterial isolates. A replicated disc diffusion assay was used to evaluate the extent of the antimicrobial activity of the milk samples. Questionnaires with 30 carefully drafted questions were randomly distributed to 37 respondents, mothers with at least one child either breast-fed or bottle-fed for at least the first six months of life. The prophylactic values of breast milk and milk formula were compared using five commonly encountered childhood diseases as indicators. The index of prophylactic value of breast milk was also computed for each of the five diseases.

All milk samples significantly showed antimicrobial action on all test organisms. Breast milk also showed a higher prophylactic value either specifically for each disease, or generally when taken as a whole, compared to milk formula. Computed indices of prophylactic value revealed that breast milk surpassed milk formula the most in preventing the occurrence of diarrhea.

Based on our results, we conclude that the general health claims concerning breast milk can be scientifically supported. Breast milk has both desirable antimicrobial activities and prophylactic properties against a number of infections commonly affecting children. The lesser incidence of breast cancer in breast-feeding mothers should be an added incentive to all nursing women.

Keywords: breast milk, antimicrobial, prophylactic value, index of prophylactic value, diarrhea, breast cancer

BSD, No. 16 *GANODERMA* EXTRACTS: POTENTIAL SOURCES OF IMMUNE RESPONSE MODIFIERS RIGHT IN OUR BACKYARDS

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Immune response modifiers also known as immunomodulators are a group of bioactive substances that modulate the activity of the immune system. Because the immune system ultimately determines the individual's state of health or disease, global search for immunomodulators for pharmaceutical application has never been so active as today. We tested for the presence of potential immunomodulators from the extracts of *Ganoderma applanatum*, a common bracket fungus that may grow right in anyone's backyard.

Ganoderma applanatum growing on a trunk of a tree was collected from the suburb of Iligan City. Crude extract was obtained using a rotavapor apparatus with methanol as solvent. Fifteen white rats of approximately the same size were used as experimental animals which were equally and randomly divided into five groups regardless of sex. Total and differential white blood cell counts were used as immune response indicators. Treatments consisted of a combination of either azathioprine/prednisone, *Aspergillus niger*, *Klebsiella pneumoniae*, or *Staphylococcus aureus* cell suspension, plus crude *Ganoderma* extract. Blood samples were obtained three hours after administration of azathioprine/prednisone and the microbial inocula, and three hours after administration of the crude extract.

Results showed that the crude *Ganoderma* extract contains active components with potential immunomodulatory properties. Observed effects include the overriding of the immunosuppressive action of azathioprine/prednisone, and neutralization of the effects of the presence of microbial cells by the immune system of the rats. The effect of the crude extract on differential count is not very clear and is currently undergoing a thorough investigation.

Keywords: immune response modifiers, immunomodulators, cellular effectors, *Ganoderma applanatum*, azathioprine, prednisone, *Aspergillus niger*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, immunosuppressant

BSD. No. 17 ANTITERATOGENIC AND ANTIANGIOGENIC POTENTIALS OF *Hibiscus rosa sinensis* ON THE DUCK EMBRYO

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Hibiscus rosa sinensis, more commonly known as "gumamela" or "Chinarose", is well-known for its wide range of medicinal purposes such as a cure for boils and wounds, as a hair dye. It is also known to cure menstrual disorders and improve sexual charkas. This study used *H. rosa sinensis* flowers to identify the antiteratogenic as well as its antiangiogenic actions on 3-day and 7-day old duck embryos.

Flowers were homogenized, concentrated, then, lyophilized for 36 hours. Extract was dissolved in PBS buffer. *H. rosa sinensis* extract solutions were administered in varying concentrations to the test embryos by injecting to the air space of the egg. After a week of incubation at 37°C, the eggs were opened and morphological characters of the embryos were observed.

Based on the results obtained, it was found that *H. rosa sinensis* crude extracts may have an antiteratogenic activity at higher concentrations and administered at an early stage. On the other hand, it appeared that the crude extracts might be teratogenic when it was combined with retinoic acid, a known teratogen, and when administered at a later stage.

Keywords: *Hibiscus rosa sinensis*, antiangiogenic, antiteratogenic, teratogen

BSD. No. 18 STUDY OF POLYMORPHIC DNA MARKERS IN PHILIPPINE *Entamoeba histolytica* ISOLATES

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We report the application of polymorphic DNA markers used to identify and characterize isolates of *Entamoeba histolytica*. It is important that a means of identifying

isolates of *E. histolytica* be developed, since only a fraction of isolataes cause disease.

DNA taken from Philippine isolates of *E. histolytica* were collected from reference strains and cultured trophozoites, amebic liver abscess patients, and residents of the Elsie Gaches Village and the Baseco Compound. Extracted DNAs from cultured strains were screened using P11-P12 locus and subjected to PCR amplification using different polymorphic loci (SREHP, HSP 1-2 and Locus 5-6).

DNAs extracted from cysts were also screened using P11-P12 and subjected only to Locus 5-6 amplification, since only the latter adequately amplified stool derived DNA.

Trophozoite DNA patterns showed a marked diversity among isolates, with one Philippine isolate being completely different from previous strains. High prevalences of *E. histolytica* were found in both Elsie Gaches and Baseco, and we were able to amplify Locus 5-6 from stool derived DNA. Amebic liver aspirates had a 50% prevalence for *E. histolytica*, while Elsie Gaches and Baseco had high prevalences of 88.6% and 24.07%, respectively. Elsie Gaches village had more varied samples than Baseco in terms of the number of polymorphisms.

Isolates of *Entamoeba histolytica* are significantly different from each other due to polymorphisms in patterns produced by different loci, providing a means for determining intraspecific variation within isolates. These polymorphisms are widespread even within small demographic areas. Populations that have high rates of infection with the parasite are more likely to have fewer isolates. SREHP and Locus 5-6 are invaluable for studying the epidemiology of *E. histolytica* in different regions. More polymorphic DNA markers are needed to fully differentiate isolates of *E. histolytica*.

Keywords: *Entamoeba histolytica*, SREHP, Locus 5-6, polymorphism, pathogenicity, Philippine isolates.

**BSD. No. 19 ASSESSMENT OF GENETIC DIVERSITY AMONG
FILIPINOS FROM DIFFERENT REGIONS OF THE PHILIPPINES
USING SDS-PAGE PROTEIN PROFILE**

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SDS-PAGE of 107 adult Filipinos from different regions of the Philippines was done to assess their genetic diversity and identify a possible biochemical marker for Filipinos in a region. No significant differences were noted in the amount of protein among Filipinos from different regions. A total of 14 major bands and 36 protein band patterns were resolved. There was no variation in major protein bands found in positions

3, 4, 6, 7, 8, 9, 10 and 11 which exhibited 100% occurrence, although differences were noted in protein band position 1, 2, 5, 12, 13, and 14 in addition to the observed differences in relative intensity of staining. The total protein band patterns revealed similarities and distinct differences among individuals from different geographic origin. The data show an existence of protein band pattern specific to an individual in a region or exclusive to a group of individuals in a particular location. BP 2, 9 and 31 were observed only in Laguna and absent in other groups. Likewise, BP 17 and 18 were identified only among individuals in Quezon Province.

A dendrogram of the different total protein band patterns shows the extent of differences of relatedness among them. The variations observed in the SDS protein band patterns possibly indicate substantial differences in amino acid composition and differences in the genetic make-up of individuals in the populations.

Keywords: SDS-PAGE, dendrogram, genetic diversity

BSD. No. 20 THE GENETIC BASIS OF ABNORMAL LEVELS OF SERUM CREATININE AMONG FILIPINOS IN LOS BAÑOS, LAGUNA

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The epidemiology, inheritance pattern and enzyme variabilities of Filipinos from Los Baños, Laguna with abnormal levels of serum creatinine were determined. Sex ratio of 1.4 males: 1.0 female indicates that males are more predisposed to the abnormality. The 40-50 age group had the highest frequency of cases, followed by the 60 years old group, thus, the abnormality is age-dependent. The number of cases increased from 1997-2001. Pedigree analysis did not fit to any one inheritance pattern but a heterogenous group. Serum from 80 normal and abnormal individuals, nine years old and above, subjected to starch-gel electrophoresis showed a total of 11 presumptive loci present for the three enzymes (acid phosphatase, esterase and malate dehydrogenase). All loci were controlled by two alleles and majority exhibited slow and fast moving bands, except for ACPH-1, EST-3 and MDH-2 which also showed moderate moving bands. All loci of those with normal serum creatinine levels, were polymorphic (100%). Those with abnormally high levels of serum creatinine were polymorphic for most loci (81.82%) ACPH-4 and EST-4, were monomorphic for the S and F alleles, respectively. All loci and their corresponding genotypes were significantly different between normal and abnormal individuals for the three enzymes tested except ACPH-1, F/F genotype of ACPH-3, MDH-2 and F/F genotype of MDH-3. The average number of alleles for the three enzymes tested was 2 alleles, and heterozygosity was higher in the abnormal group (36.06%) as compared to the normal individuals (21.51%).

Keywords: serum creatinine, epidemiology, enzymes, starch gel electrophoresis isozymes, creatinine, heterozygous, dehydrogenases

**BSD. No. 21 CYTOGENETICS OF *VANDA LAMELLATA* LINDL.
VAR. CALAYANA**

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Vanda lamellata Lindl. is an orchid species native to the Philippines which is a potential parent in breeding for exotic hybrids. Unknown yet about the species is its cytogenetics, thus, this study. Chromosome number, morphology and behavior in Carnoy's fixed anther meiocytes and root tip cells of *V. lamellata* Lindl. var. Calayana were determined using acetocarmine squash technique. All cells at diakinesis have 11 bivalents with each univalent slightly far apart from each other. The different stages of meiosis showed normal chromosome behavior except in Anaphase I with a low frequency (0.22%) of the total cells exhibiting bridge and laggards. Pollen fertility was high (97.07%). Meristematic cells from the root tips also manifested normal mitosis with an average mitotic index of 75.5% Karyotype of pro-metaphase cells established a diploid chromosome number of $2n = 38$. Because of the uniform small sizes of the chromosomes whose mean relative length range from 0.036 to 0.072, the karyotype is classified as symmetrical consisting of S type chromosomes.

Keywords: cytogenetics, chromosome number, karyotype, chromosome behavior, meiocytes, mitosis, *Vanda lamellata* Lindl. var. Calayana

**BSD. No. 22 APPLICATION OF RAPD MARKERS IN THE STUDY
OF FAMILY ZINGIBERACEAE**

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Taxonomic delineation in family Zingiberaceae is difficult because of their short-lived flowers compounded by the lack of helpful vegetative characters. Isozyme studies have revealed variations that can possibly be used to differentiate taxa up to the species level. In this study, the use of random amplified polymorphic DNA (RAPD) markers was investigated to determine if they can be utilized to distinguish between samples belonging to the same species, same genus, or different genera. Six arbitrary decamers were used to amplify DNA from seven samples of Zingiberaceae spp. RAPD profiles were analyzed using cluster analysis with UPGMA as the clustering method and Jaccard's Coefficient as

the binary measure. The resulting dendrogram groups the two *Zingiber officinale* accessions together, with a *Zingiber* species joining at a greater distance. Likewise, the two *Curcuma* samples together formed a separate clade to which a suspected *Curcuma* accession clustered farther on. *Z. purpureum*, however, which was expected to group with the other *Zingiber* samples clustered last. These results suggest that interspecific and -generic differences exhibited through the RAPD profiles can separate samples belonging to different species as well as those from different genera. Also, there appears to be sufficient interspecific RAPD variation to differentiate conspecific samples, and at the same time, enough similarities to group them together – similarities that can potentially be used as markers for species level identification.

Keywords: genetic variation, RAPD, Zingiberaceae

**BSD. No. 23 POPULATION GENETIC ANALYSIS OF *ARIUS*
MANILENSIS FROM LAGUNA DE BAY USING PCR-RFLP
MITOCHONDRIAL DNA NADH 5/6 REGION**

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The kanduli (*Arius manilensis*) is one of four native *Arius* species inhabiting Laguna de Bay. The kanduli is an important commercial fish in the region and is being targeted for farming by SEAFDEC. Despite its economic importance there is very limited biological data on the kanduli and no information regarding its stock structure. In this study, analysis of the mitochondrial DNA (mtDNA) polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) on three populations of kanduli in Laguna de Bay were investigated. Twenty samples each from the Western bay, Central bay and Southern bay were collected for analysis. The mtDNA NADH 5/6 (ND 5/6) region was PCR amplified and analyzed with 10 restriction enzymes. Three endonucleases (*Hap II*, *Hinf I* and *Hha I*) had restriction sites in the ND 5/6 region. Polymorphism was observed with the RFLP pattern generated by *Hinf I*. Only two different haplotypes were observed, which probably indicates the decline in the genetic diversity of the population. This finding correlates with the observation that the kanduli population in the lake is diminishing.

Keywords: PCR-RFLP, Mitochondrial DNA, Population Genetics, *Arius Manilensis*

**BSD. No. 24 DETECTING MORPHOLOGICALLY ABNORMAL
HATCHERY-BRED MILKFISH (*Chanos chanos* Forsskal) WITH DIRECTLY
AMPLIFIED LENGTH POLYMORPHISMS IN DNA**

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Milkfish (*Chanos chanos* Forsskal) is an important aquaculture commodity in the Philippines. Efforts are being made to develop hatchery-breeding techniques, but high incidences of morphological abnormalities observed in hatchery-bred fish have hampered these efforts. A new PCR technique, called Directly Amplified Length Polymorphisms (DALP), was used to develop genetic markers to screen against abnormal individuals. Two DALP primers, DALP 2-21 and 2-31 (both modifications of the M13 sequencing primer), were used to screen three sample populations: hatchery-bred normal, hatchery-bred abnormal, and wild type. Preliminary results show that the DALP 2-21 primer is able to amplify two polymorphisms differentiating abnormal from normal populations. The first polymorphism is approximately 500kb in size and found only in abnormal individuals, while the second is 400kb and found only in normal populations. These initial results seem to indicate the potential of using this technique in population and genetic studies of milkfish.

Keywords: milkfish, PCR, DALP, genetic markers, primers

**BSD. No. 25 ASSESSING DIFFERENT PROTOCOLS OF DNA
EXTRACTION FROM *Bacillus* ISOLATES FOR PCR AND HYBRIDIZATION**

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Efficient DNA extraction is crucial for the development of a rapid procedure for screening of bacterial isolates that possess commercially important enzymes. In this study, different protocols for the extraction of *Bacillus subtilis* and *Bacillus pumilus* DNA namely modified Li *et al.*, ROSE, lysozyme digestion, Nucleospin Tissue Kit, boiling, cryofreeze-heart shock, and zinc-induced DNA sedimentation were performed. The suitability of the extracted DNA for PCR and DNA hybridization-based detection of protease genes was tested. All protocols could generate DNA template from *Bacillus* useful for PCR except the ROSE method. However, the ROSE procedure was found to be the most rapid and cost-effective among the protocols suitable for DNA hybridization.

Keywords: DNA extraction, bacterial isolates, *Bacillus subtilis*, *Bacillus pumilus*, ROSE method

**BSD. No. 26 ELECTROSTATIC ATTRACTION PROMOTES THE
FORMATION OF A RECOMBINANT BISPECIFIC ANTIBODY**

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A typical antibody molecule is a homodimer stabilized by disulfide bonds between the two heavy chains. In order to produce a bispecific antibody (bsAb) with the Fc region, a heterodimer must form. Past strategies towards the construction of BsAbs include chemical crosslinking and use of polypeptide linkers. The latest reported innovation involves the use of knobs-into-holes engineering where a large amino acid is designed to fit into a smaller one within the antibody's Fc region. We constructed a humanized bispecific antitumor antibody such that oppositely charged amino acids were introduced in the CH3 region of each of the 2 different monomers. Each monomer targets a different tumor antigen that is associated with a wide range of cancer types. Molecular biology techniques were employed to introduce the antibody into a baculovirus expression vector. SF9 insect cells were transfected with the final gene construct and the expressed products analyzed using ELISA and western blot. For controls, transfection was also done using a non-engineered counterpart of the bispecific construct as well as those encoding for monospecific antibodies. Results show that heterodimerization of the antibody is enhanced by electrostatic attraction between chains. This bispecific heterodimer, besides being a potential anticancer molecule, may serve as a model for the generation of other multispecific antibodies against human cancers and other infectious diseases.

Keywords: bispecific antibody, electrostatic attraction, cancer, baculovirus expression system

**BSD. No. 27 THE ACONITASE MECHANISM OF GENE REGULATION:
A COMMON THEME IN DIVERSE BIOCHEMICAL PATHWAYS**

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The aconitase family consists of proteins with highly conserved cysteine residues required for the formation of an iron-sulfur cluster. This [4Fe-4S] cluster forms a cubane structure which dictates the catalytic function of aconitases. Protein sequence alignment

of several diverse enzymes revealed the presence of these conserved amino acid residues.

We have recently cloned and sequenced the first homoaconitase gene from *Penicillium chrysogenum* by complementation. The gene sequence was then compared with other homologues in world-wide databases using the tools of bioinformatics and a powerful software in molecular biology called DNASTAR. Aside from having the iron-sulfur cluster characteristic of the aconitase family of proteins, the homoaconitase structure surprisingly showed the presence of putative RNA-binding domains which are found in other aconitases, the first to be reported. The protein sequence of the homoaconitase encoded by our cloned gene was also checked using the sequence of the iron-responsive element-binding protein (IRE-BP), one of the aconitases exhibiting an RNA-binding capacity and serving as a model for post-transcriptional regulation. Although involved in diverse biochemical pathways, the catalytic mechanism shown by aconitases is strikingly similar involving a two-step stereospecific isomerization.

We have previously shown through gene regulation studies that homoaconitase regulates at least two other genes in the α -aminoadipate pathway of lysine biosynthesis. We now propose a novel regulatory mechanism for homoaconitase through RNA-binding similar to the IRE-BP regulation in addition to its biosynthetic function in lysine biosynthesis. This proposal is strongly supported by evidence obtained through studies in functional genomics.

We consider these results to be highly significant because an important generalization regarding gene regulation mediated by the various aconitase proteins is emerging for the first time. Though structurally similar, these proteins catalyze metabolically diverse and vital biochemical pathways necessary for the survival of the various organisms in which they function.

Keywords: aconitase, iron-sulfur cluster, bioinformatics, complementation, aconitase, homoaconitase, α -aminoadipate, *Penicillium chrysogenum*, RNA-binding, IRE-BP, functional genomics

**BSD. No. 28 CLONING AND PARTIAL CHARACTERIZATION
OF PUTATIVE ACYL-ACP THIOESTERASE GENE
IN COCONUT (*Cocos nucifera* L.)**

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Three cloning strategies were employed to identify the putative acyl-ACP thioesterase gene specific for medium chain fatty acids in coconut. The RT-PCR strategy was able to generate a 1.59 kb DNA fragment that contains a highly conserved amino acid region, DRFPDW which is also present in the other plant thioesterase. This 1.59 kb PCR

fragment (obtained using THIO 1 and THIO 2 as gene specific primers for PCR) is not the full length cDNA and the strategy to extend the 5' and 3' end of the partial cDNA was to employ 5' RACE and 3' RACE, respectively. A 460 bp PCR fragment was generated by 3' RACE but no PCR fragment was generated at the 5' end due to technical difficulties. With cDNA library, the full-length thioesterase gene was isolated by a modified colony PCR screening method using gene specific primers. Based on PCR screening of the coconut cDNA library, a similar 460 bp fragment was also produced from plasmids 47 and 48 which suggests that these two plasmids contain the full-length sequence. Results of this study set the groundwork for the identification of genes playing important roles in the fatty acid synthesis of coconut.

Keywords: cDNA, RACE, RT-PCR, THIO, acyl-ACP

**BSD. No. 29 ISOLATION AND CHARACTERIZATION OF
PHENOLOXIDASE FROM RHINOCEROS BEETLE (*Oryctes rhinoceros*)**

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The rhinoceros beetle (*Oryctes rhinoceros*) is the most destructive pest of coconut (*Cocos nucifera*), an important agricultural commodity. This common insect pest synthesizes phenoloxidase, an enzyme known to play a major role in insect immunity particularly in defense against pathogens, wound healing, cuticular tanning and sclerotization. Thus, a better understanding of phenoloxidase and its inhibitions may aid in the development of strategies to control insect infestation.

Since there has no report yet on phenoloxidase in coconut rhinoceros beetle, we first characterized the optimum assay conditions for this enzyme using different substrates and activators at various pH, incubation time, and temperature. Phenoloxidase was also purified using various chromatographic techniques. Homogeneous preparation of phenoloxidase was subjected to SDS-PAGE, blotted and PVDF membrane and was submitted for N-terminal amino acid sequence analysis.

Keywords: phenoloxidase, *Oryctes rhinoceros*, chromatography