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BIOLOGICAL SCIENCES

42. ASSESSMENT OF SELECTED MANGROVE SITES IN HOOK BAY, POLILLO ISLAND FOR THE ESTABLISHMENT OF AN EFFECTIVE COMMUNITY-BASED RESOURCE MANAGEMENT PROGRAM

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Mangrove swamps play a significant role in ecological stability and in providing a source of livelihood to coastal communities. In coordination with the Community-Based Coastal Resource Management Program of the Institute of Social Order, a baseline ecological study was conducted in selected mangrove sites in Hook Bay, Polillo Island towards developing resource management program. Four mangrove sites in the said area were selected for assessment based on the following criteria: occurrence of reforestation, proximity to human settlement, and possible effects of upland agricultural runoff from nearby tributaries. Three 10 m x 10 m plots were established in each site, and the following activities were conducted from August to November 2000: (a) mangrove inventory (i.e., species identification, saplings and seedlings count, and girth at breast height (GBH) and height measurements); (b) soil sampling and analysis (i.e., particle size, pH, salinity, total nitrogen, available phosphorus, potassium, total cation exchange capacity (CEC), and exchangeable bases such as calcium and magnesium); and (c) water quality monitoring (i.e., salinity, temperature, pH, conductivity, turbidity, and dissolved oxygen (DO). Eighteen mangrove species and their associates were identified, with the most dominant species being *Rhizophora apiculata*, *Sonneratia caseolaris*, and *Ceriops tagal*. Initial laboratory results show high soil phosphorus levels (at 42 to 74.5 ppm) and average soil salinity (27.3 to 39.7 ppt). The pH of the water is moderately basic (pH = 8.09 to 8.52), and salinity generally decreased between September to November (13.5 to 21.6 pp). The results of both field and laboratory

investigations will be integrated with the demographic and socio-economic data as basis for a management plan that the community can serve as a tool for sustainable utilization of the mangrove resources in their area.

Key words: mangrove, bay, physico-chemical analysis, soil analysis, water analysis, species inventory, environmental management, sustainability, Polillo Island

43. THE POPULATION DENSITIES OF CIGUATERA-ASSOCIATED DINOFLAGELLATES IN LINGSAT REEF, LA UNION, PHILIPPINES

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The mouse lethality assays for ciguatoxin done on the viscera of the herbivore, *Siganus spinus* from Lingsat Reef (16°39'N, 120°18"E) in La Union, Philippines (Pocsidio and Cabrera, 1999) had suggested that the human intoxication that took place in the locality in 1996 could have been caused by ciguatera. In the present study, a survey on the population densities of ciguatera-causing epiphytic dinoflagellates was conducted. In 1999, only possible red algal substrates and two species of brown algae were collected for the population density assay. In the following year, all other algae and sea grasses found in the area were added to the collection. This paper reports the population densities of *Gambierdiscus* sp., *Ostreopsis* spp. and *Prorocentrum* spp. In April, June, and August 1999 comprising 12 species of algae and monthly collections in the year 2000 except in January, July, and November. In the year 2000, the population of the dinoflagellates was low. The highest densities reached were for *Ostreopsis* spp. 43.1 cells/g alga in April, *Prorocentrum* spp. 43.1 cells/g alga in April, both in *Dictyota linearis* and *Gambierdiscus* sp. 6.01 cells/g alga in February in *Dictyota dentate*. In the previous year, though, in August, *Ostreopsis* spp. Reached 186.7 cells/g alga in *Galaxaura elongata, Prorocentrum* spp., 91.9 cells/g alga in *Gelidiella ocerosa*. There was no *Gambierdiscus* sp. seen at that time. Based on the data, the ciguatera fish poisoning that happened in 1996 could have been caused by *Ostreopsis* sp. and *Prorocentrum* spp. Which had high levels at that time of poisoning.

Key words: ciguatera, dinoflagellates, *Gambierdiscus, Ostreopsis, Prorocentrum*, fish food poisoning

44. HABITAT AS A DETERMINANT OF GEOGRAPHICAL DISTRIBUTION OF DIVERSITY IN PHILIPPINE *Conus*

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Cone snails (*Conus*) comprise the most diverse coral reef gastropod taxon. The Philippines has the most diverse cone snail fauna in the world. The biogeography of these snails can give new insights on the nature and distribution of marine biological diversity in the Philippines. Using Professor Alan Kohn's published data on ecology and life history and the primary material deposited with the Philippine National Museum (PNM), habitat, life, history parameters and cone snail geographic distribution were explored to predict the distribution of cone snail diversity in the Philippines. Geographical occurrence of 27 shallow water *Conus* species whose life and habitat characteristics are known was plotted. A stepwise multiple regression of *Conus* occurrences in 15 geographical regions in tbe Philippines on habitat characteristics of the sites where the PNM collected the snails was done. Initial findings suggest that distribution of predominantly sandy habitats is a greater determinant of cone snail diversity than planktonic precompetency as a life history strategy and other habitat factors. This supports the ecological determinism hypothesis of coral reef species diversity.

45. ORGANOGENESIS IN HE SUPERMALE YY, GENETICALLY MALE (GMT) AND MIXED SEX TILAPI (MST)

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The genetic manipulation of tilapia sex by the University of Sansea, UK and the Central Luzon State University, Muñoz, Nueva Ecija has produced an all-male population of tilapia: the supermale YY and the genetically manipulated (GMT) XY tilapia. To determine if the lack of an X chromosome which is fatal in humans has an effect on the development of organs in the supermale, organogenesis was

compared up to 10 days postfertilization in YY, GMT, and MST. No abnormalities were observed in the organogenesis of the digestive, excretory and respiratory structures, although the general trend was that towards the free-swimming larva stage, differences in measurements taken of the size of the organs, layers, tubules and lining cells became statistically significant giving the YY developmental superiority.

Key words: supermale UU, Genetically Male Tilapia (GMT), mixed-sex tilapia (MST), *Oreochromis niloticus*

46. STUDIES ON THE EXISTENCE OF *Dynophysis* sp. AND *Gambierdiscus* sp. IN PHILIPPINE COASTAL WATERS

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Diarrhetic Shellfish Poisoning (DSP) and Ciguatera Fish Poisoning (CFP) are types of human illnesses associated with red tide that has been reported in the Philippines and extensively in other parts of Asia. In the Philippines, however, there are still very little documentations about DSP and CFP. The present study aimed to determine the presence of some of the causal organisms of DSP (*Dynophysis* sp.) and CFP (*Gambierdiscus* sp.) in the Philippines. This was carried out by isolation and examination of the toxins [dinophysistoxins (DTX) & okadaic acid for DSP and ciguatoxins (CTX) for CFP] associated with these organisms. Samples from suspected red tide areas in the country, i.e., shellfish and fish from both Masbate and Zambales, were gathered for analysis. Toxin extraction was done using the modified method of Lewis *et al.* (1998). Fish viscera and shellfish meat were cooked in AR grade acetone at 56°C and allowed to stand overnight. A portion of the acetone layer was used in mouse bioassay. The solution was then decanted and later evaporated in a rotary evaporator. Further cleanup was carried out by separatory funnel extraction method using a 1:1 petroleum ether and methanol: water (4:1) solution. The methanol: water layer was extracted twice with petroleum ether and finally evaporated to dryness in a rotavap at 60-70°C at 25 rpm. Samples for High Performance Liquid Chromatography (HPLC) analysis were redissolved in 5 ml of 0.1% heptanesulfonic acid prior to injection. Certain fractions of these injected samples were used for HPLC-mass spectrometer (MS) analysis. Based on earlier studies elsewhere, the retention times for DTX-1 and okadaic acid were 24 and 28 min, respectively. The chromatograms of the Zambales' shellfish samples showed peaks at 24.9 and

28.4 min with 0.02% and 0.07% of total area, respectively, which may indicate that both toxins were present. The bioassay further proved the presence of toxins from these samples as indicated by the death and paralysis of two and one mice, respectively. Results of the MS revealed that DTX-1 (819.5 m/z) was most likely the toxin present in the Zambales shellfish samples because the observed peak had a value of 819.2 m/z. P-CTX-1, on the other hand, was the most likely toxin present in the Masbate fish samples. Mass spectra of the samples gave two peaks with 1111.1 and 1111.9 m/z, while previous studies set the value of P-CTX-1 at 1110.3291 m/z. Overall, there are strong indications that red tide organisms, in this study, namely *Gambierdiscus* sp. and *Dinophysis* sp. are present in selected Philippine coastal waters.

Key words: red tide, toxins, diarrhetic, ciguatera, poisoning, mouse bioassay, mass spectra, ciguatoxin, Dinophysis toxin, HPLC, mass spectrometry

47. A COMPARATIVE STUDY OF THE TOXICITY OF THREE COMMERCIALLY AVAILABLE DETERGENTS ON TILAPIA *(Oreochromis niloticus)*

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Although there have been studies conducted locally on the toxicity of surfactants on aquatic organisms, little is known about the impacts of the detergents themselves. Studies have shown that such complex chemical mixtures can be significantly influenced by factors like temperature, pH, water hardness, antagonism, synergism, and length of exposure, which altogether can affect their toxicity to the text organism. Thus, it is important that data on the toxicity of detergents on a local fish species be established.

Both 96-hr range-finding and definitive toxicity tests were conducted on the following commercial detergents using tilapia (*Oreochromis niloticus*) as test organism: Brand B (approx. 10% national market share by volume), Brand T (approx. 17% national market share volume) and Brand V (<1% national market share volume). Test set-ups were monitored daily for significant water parameters such as dissolved oxygen and temperature, as well as fish mortality. Seventy percent of the test solutions were renewed daily. Reference tests were also performed simultaneously using copper sulfate ($CuSO_4.5H_2O$; Ajax Chemicals, Analytical Grade). Tests were considered valid if the controls exhibited a 90% survival.

The results of the first set of definitive tests indicate that the mean LC_{50}s of the three detergents belong to the same order of magnitude. Their rank order of

toxicity is as follows: Brand B (mean 96-hr LC_{50} = 10.92 ± 1.29 mg/ or 9.16 Toxic Units) Brand T (mean 96-hr LC_{50} = 24.47 ± 0.8 mg/L or 4.09 Toxic Units) > Brand V (mean 96-hr LC_{50} = 33.19 ± 2.84 mg/L or 3.01 Toxic Units). The data indicate that Brand B is two times more toxic than Brand T, and three times more toxic than Brand V. Since no specific guidelines for toxicity classification has been provided by the DENR, the following classification proposed by Persoone *et al.* (1993) was applied in this study. The data shows that the resulting LC_{50} values (expressed in Toxic Units) fall under the category of TOXIC substances. Given the fact that these three detergents comprise nearly 28% of the total volume consumed by the market, their potential adverse impact on our aquatic ecosystems must be thoroughly investigated.

Key words: fish toxicity tests, LC_{50}, detergents, copper sulfate, Tilapia, water quality

48. REPRODUCTIVE ANATOMY, HISTOLOGY AND GESTATION OF THE PHILIPPINE SEAHORSE *Hippocampus barbouri*

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Male and female seahorses obtained from the waters of Santiago Island, Bolinao, Pangasinan were compared morphologically and histologically for structural differences. Paraffin sections were prepared to examine the histological profile of gonads involved in selected stages of reproduction. Morpho-histological results confirmed the presence of paired tubular testes and ovaries which converge to form into a common spermatic duct and oviduct in male and female seahorses, respectively. Mature gametes are released through the anal pore. During fertilization, females deposit eggs into the brood pouch opening of the male. At the time of oviposition, sperm are ejaculated immediately which traverse quickly from the anal pore into the brood pouch opening via a minute connecting canal. Fertilization follows shortly inside the male brood pouch cavity. During gestation, histo-physiological results confirmed the presence of gradually thickening epithelial linings to support and protect the developing embryos. Likewise, tiny crevices where the embryos lie in quiescent were prominent from mid to late pregnancy. Paraffin sections showed that signs of active spermatogenesis were observed during the course of gestation.

49. CAGE CULTURE OF JUVENILE SEAHORSES, *Hippocampus kuda*, IN THE PHILIPPINES

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There is very little scientific information on the basic biology of seahorses. A limited amount of literature has concentrated on raising seahorses intensively in tank cultures fed with live prey organisms, in the hopes of establishing breeding populations that can likely lessen pressure on natural populations. While these investigations show promise of improving our knowledge of the culture requirements of seahorse, it is costly for large-scale production, especially for developing nations. This study concentrated on an alternative technique for raising juvenile seahorses, *Hippocampus kuda*, in the field. A caging protocol with high and low stocking densities was conducted across two habitat types (seagrass bed and mangrove) for the period of five weeks. Seahorse growth and survival was monitored along with physico-chemical data (sea water temperature, salinity, turbidity, dissolved oxygen content, including total suspended solids and total organic matter). This study observed that juvenile seahorses grew to 37.9 ± 0.7 mm in length with survival of 57.6 ± 11.8% after 5 weeks at the seagrass site. The growth rate obtained from this study was four times higher than aquarium raised seahorse juveniles. *H. kuda* juveniles at the mangrove site, however, suffered 100% mortality after 2 weeks in captivity. This was probably due to fouling and associated factors. No stocking density effects were detected for growth and survival in the seagrass site. This benchmark investigation indicates that extensive culture of seahorses is possible and may serve as a low technology and less costly alternative for seahorse culture, highly relevant for sustenance fishers in developing countries.

Key words: caging, seahorses, *Hippocampus kuda*, stocking density, habitat type, physico-chemical parameters, growth rate, mortality, extensive culture, intensive culture

50. MORPHOLOGICAL, PHYSIOLOGICAL, AND MOLECULAR CHARACTERIZATION OF PHILIPPINE *Naegleria* ISOLATES

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Naegleria is an amoeba with a transient flagellate stage and a resitant cyst in a life cycle. At least two members of the genus are known to cause primary amoebic meningoencephalitis (PAM) in humans. Eight environmental isolates of *Naegleria* were obtained from Calamba, Laguna (Cal A and Cal B), UP Diliman (Mal A and Mal B), Marikina (Mari B), Mt. Arayat (MA-B), and Taal Island (Taal A and Taal B), after which they were subsequently cloned. Together with a cloned local clinical isolate (RITM) previously from the Department of Parasitology, Kurume University of Medicine, Japan, these isolates were characterized using morphological, physiological, and molecular parameters. Cyst morphology demonstrated that the Taal isolates had the largest cyst diameter while Cal A had the smallest. The number of pores varied within isolate, but only *N. fowleri* ,(IT9611) had no rim or lip on its pores. Rate of enyctinent of individual cells on non-nutrient agar indicated that Cal B, MA-B, *N. fowleri*, and RITM took the longest time to encyst with 4 hours on the average. Enflagellation of individual cells in distilled water also revealed that the RITM and *N. fowleri*, had the longest time of enflagellation with 3.0-4.0 hours and 4.0 hours, respectively. Cal B took 1.25 hours while the rest enflagellated in 0.5-0.75 hours. Thermal tolerance at 45°C demonstrated that only MA-B, Mal A, Mal B, and *N. fowleri*, were thermophilic. *Naegleria fowleri* persisted as trophozoites at 45°C while the other three thermophilic isolates encysted. Sequence analysis of the 5.8 S rDNA gene and internal transcribed spacers (ITS) of the isolates showed that the 5.8 S rDNA region was highly conserved for the Philippine isolates. Polymorphism was exhibited in the two ITS regions that flank the gene due to base substitutions, insertions, or deletions of several sites, most notably in the ITS 2. Cluster analysis of the 5.8 S rDNA gene and ITS sequence was done on the isolates in this study and the known *Naegleria* species with available sequence using UPGMA algorithm and Euclidean measure. Of the 4 major clusters formed in the dendrogram, 3 contained the local isolates and *N. fowleri*. Cluster 1 included Cal A, Cal B, Mari B, and RITM. Cal A was closely related to *N. australiensis* while Cal B was most similar to *N. jamiesoni* and *N. andersoni*. Mari B and the clinical isolate RITM were branched together with the *N. gruberi* AUD1 isolate. Cluster 2 included the three thermophilic local isolates and the Taal isolates. MA-B, Mal A, and Mal B formed one group that was independent of the thermophilic *N. fowleri* and *N. lovaniensis* while the Taal isolates were most similar to *N. clarki*. *N. fowleri*

(IT9611) used in this study and all the other *N. fowleri* isolates formed the *N. fowleri* cluster. The study concludes that the local *Naegleria* isolates do not form a homogenous group.

Key words: *Naegleria*, encystment, enflagellation, thermal tolerance, DNA sequencing, 5.8 S rDNA gene, ITS, cluster analysis

51. GENETIC VARIATION IN ABNORMAL AND NORMAL HATCHERY-BRED MILKFISH (*Chanos chanos* FORSKÅL)

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Milkfish (*Chanos chanos* Forskål) is one of the most important fish commodities in the Philippines. Significant advances have already been achieved in its effective hatchery breeding, yet public acceptance has been hindered by mutations resulting in morphological abnormalities. To assess these abnormalities, PCR-RAPD was applied to both the normal and abnormal hatchery-bred milkfish juveniles. PCR amplification using UBC decamer primers 235, 267, and 268 produced 100 bp., 300 bp, and 150 bp fragments, respectively from normal samples only. Statistical analysis using the chi-square test showed the dependence of these possible markers on the type of sample used. Restriction site analysis of the 12s mt DNA was also performed. PCR products were digested with Alu I, Msp I, Hap II, Hha II, Hinf I, Rsa I, and Taq I. Digestion with Alu I and Hinf I provided significant differences in banding patterns from agarose gel electrophoresis that can differentiate normal samples from abnormal ones. Sequencing and computer restriction site analysis show several unique restriction sites in abnormal milkfish samples.

Key words: *Chanos chanos*, PCR-RAPD, 12s mt DNA

52. THE RATE OF GROWTH OF *Siganus guttatus* FED WITH BROWN ALGA (*Sargassum polycystum*) AND GREEN ALGA (*Ulva lactuca*) IN CAGES IN THE MARINE WATERS OF SAN FRANCISCO, CEBU

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Sargassum polycystum and *Ulva lactuca* are two kinds of algae abundantly found at the coast of Northern Poblacion, San Francisco, Cebu. They are just used by fish vendors to cover their fishes during marketing time and just drifted by the current ashore, dried up and decomposed. Thus, these two kinds of algae were tested as feed for cultured siganids *(Siganus guttatus)* to maximize their utilization.

These study used the Randomized Complete Block Design (RCBD) with three treatments and in each treatment has two replicate cages. Treatments 1, the cages fed with brown alga; Treatment 2, fed with green alga and Treatment 3, no feed given as control.

Each cage was stocked with 52 siganids fingerlings and fed daily with the two kinds of algae based on 30% of the mean body weight of the stock for five months using body weight, total length and body depth as indicators for growth. The whole plant in each species of alga were utilized as feed in the study.

Results of the study show that the siganid fed with *Ulva lactuca* (green alga) had heavier body weight, longer total length, greater body depth and high survival rate than the siganids fed with *Sargassum polycystum* and the control.

Based on the analysis of variance (ANOVA), it shows that there is significant difference in terms of body weight, total length and body depth among the treatmentst.

Key words: *Siganus guttatus, Ulva lactuca, Sargassum polycystum,* Cebu

53. OVARIAN DEVELOPMENT OF *Atherinomorus endrachtensis* FROM TAAL LAKE, BATANGAS AND *Decapterus macrosoma* FROM QUEZON PROVINCE

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The silverside, *Atherinomorus endrachtensis* (Quoy and Gaimard), locally known as guno, is one of the economically important fish species found in Taal Lake, Batangas province. About twenty female specimens of guno were collected monthly from the lake for nine months (November 1999 to July 2000) to study its ovarian development.

Fifteen to twenty specimens of *Decapterus macrosoma* (Bleeker), commonly known as round scad, were obtained monthly from July 1999 to February 2000 from the coastal waters of Lucena, Quezon.

Histological sections of the ovaries of the two fishes showed the presence of oogonia, chromatin nucleolar stage, perinucleolar stage, yolk vesicle stage, vitellogenic and ripe oocytes. These were seen occurring in the ovaries simultaneously. However, ripe oocytes were only observed during the month of August in *D. macrosoma*. The monthly mean values of the Gonadosomatic Index (GSI) of *A. endrachtensis* were highest in the months of February and July. On the other hand, the GSI values of *D. macrosoma* peaked during the month of August. Present findings indicate that *A. endrachtensis* and *D. macrosoma* are multiple spawners.

54. OCCURRENCE OF *Caligus pelamydis*, A PARASITIC COPEPOD, ON LOCAL TERAPONID FISHES

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Among the parasitic crustaceans that have been reported to cause mortality of fish hosts are species of *Caligus*. Caligid copepods are mainly parasitic on marine fishes; with increased aquaculture of marine fishes, the economic impact of these parasites will also increase. Teraponid fishes found in the coastal waters of the Philippines include *Pelates quadrilineatus* (Bloch) and *Terapon jarbua* (Forskål) locally called *bagaong* or *babansi*. They enter brackishwaters and freshwaters. Because of their migratory habit and the present practice of culturing fish in coastal areas, it is possible for terapons to enter fishponds or cages and for

their parasites to infect cultured fish. Examination of *P. quadrilineatus* and *T. jarbua* obtained monthly from fishermen and fish vendors in La Union, Metro Manila, and South Cotabato resulted in the recovery of *Caligus pelamydis* from the gills and buccal cavity. Prevalence of infection in *T. jarbua* ranged from 19.6% (Divisoria Market) to 37.5% (La Union). In *P. quadrilineatus*, only those from La Union were infected. Parasite burden in *T. jarbua* ranged from 1-13; for *P. quadrilineatus*, 1.

55. CHROMOSOMES OF GOBIES FROM TAAL LAKE, LUZON

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Despite the great diversity of freshwater forms in the Philippines, the Cytogenetics of riverine and lacustrine populations of fishes is still unexplored. This on-going study on the Cytogenetics of freshwater teleosts in the CALABARZON Area, Luzon Island focuses on the chromosomal complements of naturally occurring gobies (including eleotrids) to benchmark future studies in fish genetics and chromosomal evolution.

Fish specimens of an eleotrid, snakehead gudgeon (*Ophieleotris aporos*) and two species of gobiids, tank goby (*Glossogobius giurus*) and rock goby (*Glossogobius celebricus*) were obtained from Taal Lake and rivers of Cavite for this investigation. A routine solid tissue technique with conventional staining was used in preparing metaphase cells from head kidneys of fish specimens. Different concentrations of colchicines and sodium nitrate for hypotonization were tried to get the appropriate amount in arresting cells at metaphase stage.

Initial findings indicate that *O. aporos* and *G. giurus* both showed diploid chromosome number of 2n = 46 similar with the eleotrids: *Oxyeleotris marmoratus*, *Eleotris acanthopomus* and *Dorminator maculatus*. The snakehead gudgeon, *O. aporos* has a tentative *Nomen Fondemental* (NF) of 48 (2 bi-armed chromosomes and 44 mono-armed chromosomes), while *G. giurus* has NF of 46, confirming the previous works. The other goby (*G. celebricus*) inclusive in the present study, has a tentative diploid chromosome number of 2n = 44.

Key words: fish chromosomes, gobies, Taal lake, Cytogenetics, teleosts, eleotrids, diploid chromosomes

56. GENETIC DIVERSITY AMONG NATURAL POPULATIONS OF GIANT HONEYBEE (*Apis dorsata* F.) IN THE PHILIPPINES

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SDS-PAGE and morphometric analysis was done to determine the diversity between two populations of the giant honeybee from Jubileeville, Bay, Laguna and Forestry Campus, UPLB, College, Laguna, Philippines. A total of five protein bands of high molecular weight were present in both populations. Band 1, 2, and 3 were present in both populations at 100%. Band 4 was present in both the Jubileeville bee population, and Forestry bee populations at 100% and 85%, respectively. Band 5 had the lowest frequency of occurrence of 20 and 40% in the Jubileeville and Forestry giant honeybee populations, respectively. A total of three protein band patterns (BP) were observed. These were BP-A, with bands 1, 2, 3, 4 and 5; BP-B with bands 1, 2, 3, and 4; and BP-C with bands 1, 2, and 3. BP-A and BP-B was common to the two populations while BP-C was observed only in the Forestry population. The Jubileeville population had a high similarity index (SI) of 80-100% while the Forestry samples gave a SI of 60-100% The two populations exhibited an average SI of 71%. Morphological measurements showed that the two populations were separate from each other by clustering into two separate groups based on location. The only body part that can possibly distinguish the two populations from each other was the distance of the wax mirror. It may be concluded that the populations are highly similar to each other both morphologically and biochemically based on protein composition.

Key words: honeybee, *Apis dorsata*, genetic diversity, protein profile, SDS-PAGE, electrophoresis, similar index, morphometric analysis, principal component analysis

57. GENETIC PLASTICITY OF LEPIDOPTERA

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Lepidoptera, the second largest order of insects, consists of 140,000 butterflies and moths uniquely distinguished through their overlapping scales on wings, legs and most body parts. Their colorful appearance and elegant beauty earned popular

appeal to collectors and hobbyists, however, their larvae are serious pests of agricultural crops. Ecological success of butterflies and moths is attributed to their genetic plasticity facilitated by specialized modes of reproduction and genetic systems. Lepidopterans' modes of reproduction include sexual reproduction and regressed sexuality = parthenogenesis, telytokous parthenogenesis which can be either automatic or apomictic. Genetic systems of lepidopterans include Mendelian inheritance; halokinetic chromosomes with modal haploid number (n) of 31 capable of Robertsonian fusions and fissions; ZW >ýC?ý : ZZ >ýX?ý sex-determining system; achiasmatic meiosis and somatic polyploidy. The variations and adaptations of lepidopterans are products of evolutionary mechanisms such as genetic drift (e.g. bottlenecks and Founder's effects), mutations, migrations, selections, and effects of environmental factors. The summative product is genetic plasticity of Lepidoptera.

Key words: lepidoptera, butterflies, moths, insect genetics, chromosomes, reproduction, parthenogenesis, holokinetic, evolution, genetic plasticity

58. OSMOTIC STRESS INCREASED IN PLANT REGENERATION OF OLD RICE CALLUS

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Increase in rice production will entail the continued development and use of high yielding inbreds, hybrids and new plant type lines. In support of this strategy, genetic engineering for improved pest resistance will also focus on these genotypes. Optimization of conditions to increase plant regeneration in these genotypes was conducted as a prerequisite for successful genetic engineering. Tissue culture factors such as the type of explant, genotype, selection conditions, and artificial culture media were studied. However, plant regeneration is greatly affected by co-cultivation with *A. tumefaciens*. Old, unregenerable cells of three cultivars were subjected to different osmotic conditions that include physical and chemical osmoticants. Addition of sorbitol, mannitol, and exposure to drying conditions of the laminar flow hood increased the plant regeneration of unregenerable transgenic cells by three-fold. This finding will be very useful in succeeding attempts to regenerate transgenic plants with economically-important characteristics.

Key words: *Oryza sativa*, genetic engineering, *Agrobacterium tumefaciens*, sorbitol, mannitol, plant regeneration

59. MAPPING SALINITY TOLERANCE GENES IN RICE (*Oryza sativa* L.) USING RFLP AND SSLP ANALYSES

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A molecular map of rice chromosome 1, consisting of 11 restriction fragment length polymorphisms (RFLP) and eight simple-sequence length polymorphisms (SSLPs), was constructed using a population of F_8 recombinant inbred lines (RILs) of rice to map the major gene and quantitative trait loci involved in salt tolerance. The mapping population originally consisted of 80 RILs from the extreme tails of a population of 276 RILs developed via single seed descent from an intercross between the genetically divergent parents Pokkali and IR29. Pokkali is salt tolerant while IR28 is salt susceptible. The RFLP and SSLP markers were in the same order as in the published reference maps, thereby implying the reliability of the constructed map based on this particular RIL population. The integrated map of RFLP and SSLP markers had a total of 129.9 cM, with an average interval size of 6.8 cM. Two RFLP markers, C52903S and C1733S, with 10.1 and 22.6 cM distance, respectively, flanked the major gene, Saltol. Two microsatellite markers RM23 and RM140 flanked the Saltol gene with 16.4 and 10.1 cM distance, respectively. PLABQTL for quantitative trait loci analysis was used to detect quantitative trail loci (QTL) associated with salinity tolerance (low Na^+ absorption, high K^+ absorption, and low Na^+/K^+ absorption ratio) in chromosome 1. A common QTL for these three quantitative traits was observed within a 50 to 65 cM segment of the integrated map with a peak Log of Odds (LOD) score greater than 6.7. RM140, a microsatellite marker, and C52903S, a RFLP marker, flanked the QTL peak within 1.9 cM. Using basic information derived from this study, further fine mapping using BAC libraries in a large backcross population (BC_3F_4) will be done.

60. HYBRIDS OF *Allium cepa* L. *x Allium fistulosum* L. ANALYZED USING RANDOM AMPLIFIED POLYMORPHIC DNAS (RAPDS)

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Random amplified polymorphic DNA (RAPDS) was used to verify the interspecific hybrids of *Allium cepa* L. and *Allium fistulosum* L. Polymorphic RAPD markers were identified. Forty primers were initially used, from which thirty-one generated scorable bands. Of these, only thirteen primers showed polymorphism among the parents of the five crosses. These were then used to analyze and verify the progenies of each cross.

Hybrids of Cross CF 54 had a total of twenty-six bands, fifteen from the female and nine from the male parent. Two bands did not seem to come from either parent. Results of FC 45 cross had sixty total bands, twenty four from the female and thirty six from the male parent. Hybrids of CF 19 cross had fifty-one total bands, eighteen from the female and twenty eight from the male parent. Five bands did not seem to come from either parent. CF 1 progenies had sixty two bands, twenty four from the female and thirty three from the male parent. Again, five bands did not seem to have come from either parent. Results of CF 16 showed twenty bands, ten from each parent. The details of these crosses are hereby presented.

Results proved that RAPDS is a suitable method in verifying interspecific hybridity between the two *Allium* species.

Key words: RAPDS, *Allium cepa*, *Allium fistulosum*, polymorphic bands, interspecific hybrids, *Allium*, hybrids, onion

61. DETECTION OF PUTATIVE TUNGRO RESISTANCE GENES IN RICE THROUGH mRNA DIFFERENTIAL DISPLAY

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Tungro continues to be the most devastating rice disease, affecting many of the modern varieties including the widely popular IR64. This study aims to identify and clone the genes for resistance against rice tungro spherical virus (RTSV), the primary causal viral agent, to be used in the genetic engineering of tungro resistance (R) in the modern varieties. We have identified potential R-related genes from examining the differentially displayed messages between two near-isogenic lines namely, TN1 (susceptible) and TI-11-8 (resistant). TI-11-8 contains the resistance gene(s) from an Indian landrace ARC11554. At 21 days after sowing, the R and S plants were inoculated with viruliferous green leafhopper for three days under mylar cage. At 20 days after inoculation, the R and S plants were phenotyped by ELISA method. At 21 days after inoculation, RNA was extracted and subsequent cDNA synthesis, PCR amplifications and gel electrophoresis were performed. Together with oligo(dT)G, four out of 20 arbitrary primers showed differential PCR products between the TI-11-8 (R) and the TN1 (S) lines on the agarose and polyacrylamide gels. The R-specific bands may represent R-related genes and are now under intensive cloning efforts.

Key words: rice tungro spherical resistance, PCR, ELISA, ARC11554, TN1

62. A BOTANICAL STUDY OF SOME UNDEREXPLOITED MEDICINAL PLANTS

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Four underexploited species of plants with medicinal value namely, *Centella asiatica* (Linn.) Urban. *Heliotropium indicum* Linn., *Phyllanthus niruri* Linn. and *Stachytarpheta jamaicensis* (Linn.) Vahl were studied. These plants are common weeds found in waste lands.

Ethnobotanical information, particulary from inhabitants of Capiz and Isabela, revealed that these plants are extensively utilized in rural areas for the treatment of numerous skin diseases (*C asiatica*), respiratory diseases (*H. indicum*), liver diseases (*P. niruri*) and digestive system disorders (*S. jamaicensis*). Morphological and biochemical data deemed valuable in the medical and pharmaceutical fields

for scientific study and identification of active components were obtained. Histochemical tests showed that vegetative as well as reproductive parts of all the species were positive for alkaloids, tannins, glucosides and saponins. Protein profiles of the different plant organs and isozyme banding patterns of leaf proteins were generated using polyacrylamide gel electrophoresis (PAGE).

Key words: Polyacrylamide gel electrophoresis, alkaloids, tannins, saponins, glucosides, ethnobntanical. isozyme

63. MICROSPOROGENESIS AND ICROGAMETOGENESIS IN *Pittosporum resiniferum* Hemsl. (PETROLEUM NUT PLANT)

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This study aimed to trace the series of events that takes place during male sporogenesis and gametogenesis and to classify the type of development in *Pittosporum resiniferum*. The differentiation of the male reproductive structure was also studied. For the process of microsporogenesis and microgametogenesis, smears of pollen from the anther was used and prepared. The modified paraffin technique was followed in the study of the development of the microsporangium.

Results showed that the young anther had a homogenous mass of meristematic cells bounded by an epidermis. As the anther primordium became four-lobed, a 3-cell wide hypodermal archesporium became differentiated in each of the four lobes and the cells showed dense cytoplasm and conspicuous nuclei. The archesporial cells divided periclinally forming a parietal layer of cells towards the outside and the primary sporogenous layer of cells towards the inside. The primary parietal cells divided periclinally and anticlinally giving rise to the endothecium, middle layer and tapetum. The primary sporogenous cells underwent a few mitotic divisions, enlarged and differentiated to form the microspore mother cells. The microspore mother cells underwent meiosis to form the microspore tetrads, which were tetrahedral in arrangement. Cytokinesis is by furrowing and is of the simultaneous type. Each of the microspore tetrads separated, enlarged, and finally differentiated. Each microspore underwent nuclear divisions. The first nuclear division gave rise to a large vegetative cell and a small generative cell. The second division, which involves only the generative cell, gave rise to two sperm cells. The pollen grain was shed in a three-nucleate stage. The pollen grains are tricolpate with smooth exine and on inner intine. Abnormal pollen grains, which were shriveled in shape, were also observed.

Results from the study can be used as a tool in determining sterility/fertility in pollen grains, which can be used as baseline data in genetic engineering methods. It can also provide baseline data for use in research on pollen gene expression in the isolation and characterization of genes involved in pollen development.

Key words: archesporial cells, endothecium, exine, gametogenesis, generative cell, intine, microspores, sporogenesis, tapetum, tricolpate, vegetative cell

64. FREQUENCY OF A MITOCHONDRIAL DNA 9-bp DELETION PHENOTYPE IN PHILIPPINE ETHNOLINGUISTIC GROUPS

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The advent of DNA analysis has provided anthropologists, historians, and geneticists an objective method of assessing variation among peoples, superseding many classical anthropological, linguistical, and even biological methods. In human genome diversity research, length changes in human mitochondrial DNA (mtDNA) serve as potentially useful markers for inferring the evolutionary history of populations. A 9-bp mtDNA deletion located in the intergenic region between the COII gene and the lysine tRNA gene has been used as a genetic marker to trace the descent of various Asian populations. Using PCR technology, the presence and frequency of the 9-bp mtDNA deletion phenotype was determined in two major linguistic populations in the Philippines, Tagalog (f=38%) and Cebuano (f=24%), and an Ivatan ethnic community (f=56%). While these data fall within the observed ranges for other Philippine populations, it is interesting to note that these differ from those previously reported for other Asia-Pacific populations. The anthropological implications may therefore be further studied by including other ethnolinguistic populations in the Philippines and contributing the data to global genetic matrices.

Key words: mitochondrial DNA, Philippine ethnolinguistic groups

65. DEVELOPMENT OF DNA EXTRACTION PROTOCOLS FOR FORENSIC APPLICATIONS

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Whole blood and body fluids such as saliva and urine are the samples of choice in routine human DNA testing. In forensic casework however, samples may be hard material e.g. bones and teeth, or archival samples e.g. paraffinized or formalin-fixed tissues. This work involved the development and optimization of DNA extraction protocols that may be used when processing these alternative materials. Using simulated casework, a protocol consisting of (1) decalcification in EDTA for bones and teeth or deparaffinization in xylene for embedded material; (2) proteinase K and lysozyme treatment; (3) organic solvent extraction; and (4) salting-out and isopropanol precipitation, was optimized to give high-quality DNA extracts that are amenable to PCR-based typing and RFLP analysis. Typically, the whole procedure can be completed in 2 days and cost of extraction is estimated at less than P20/sample. This procedure is a feasible and efficient alternative to expensive kit-based extraction methods.

Key words: DNA extraction, forensic science, human DNA testing, molecular genetics

66. EXPRESSION DYNAMICS OF GENES IMPLICATED IN LIMB DEVELOPMENT

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The vertebrate limb is an outgrowth of the embryonic body wall, consisting of the mesenchyme derived from the somites and the somatic portion of the lateral plate mesoderm, surrounded by an extodermal jacket. The formation of the limb is controlled by a complex set of molecules such as those belonging to the Transforming Growth factor ² (TGF²) superfamily, Fibroblast Growth Factors (FGFs), Homeobox (Hox), retinoic acid and Chondromodulin-I (ChM-I), that interactively promote axis formation, stimulate growth, and pattern the individual

skeletal elements. In order to study the expression dynamics of some of these genes, we performed wholemount ribonucleic acid (RNA) *in situ* hybridization analyses on different stages of mouse embryos and excised limb buds. The procedure consists of cloning the complementary DNAs (cDNAs) of the genes encoding for bone morphogenetic protein-4 *(BMP4), Wnt, Shh* (Sonic hedgehog), *N-myc* and *Chm-1* into a plasmid vector with flanking T3 and T7 RNA Polymerase binding sites and utilizing these sites to transcribe *in* vitro digoxigenin-labeled sense and antisense RNA probes for hybridization to target messenger RNAs (MRNAs). Our results show that these various genes exhibit a spatio-temporal pattern of expression in the developing mouse limb bud. For instance, *N-myc* expression is detected early in the limb bud mesenchyme in an increasing proximo-distal gradient with peak expression levels at embryonic days 9.5-10 after which its expression is rapidly down-regulated. On the other hand, *Wnt* mRNA expression is confined to the ectoderm while that *BMP-4* is found in the anterior and posterior regions of the limb bud encompassing the antero-posterior organizer center, the zone of polarizing activity or ZPA. *Chm-1* is the latest gene to be expressed and its mRNA is confined mainly to regions of presumptive digits where cartilage condensations are confined. The expression dynamics of these genes have been correlated with their roles in either promoting chondrogenesis or in controlling the fates of various cell types in the vertebrate limb.

Key words: vertebrate limb development, mRNA *in situ* hybridization, bone morphogenetic protein, chondromodulin, transforming growth factor b, sonic hedgehog

67. THE Y-CHROMOSOME STR SYSTEM AND FORENSIC DNA ANALYSIS IN THE PHILIPPINES

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Conventional procedures for mix strain analysis incorporate differential lysis to separate male and female DNA for effective profiling. Various protocols for differential lysis were previously validated by the laboratory and were found to be effective in identifying female and male DNA in mixed samples using the autosomal STR system. But in cases of trace evidence, more DNA is lost when differential lysis methods are used. To circumvent this problem, the Y-chromosome Short Tandem Repeat (STE) system was developed and validated.

Using in-house laboratory validated protocols, a Y-chromosome database in the DYS19, DYS390, DYS393, DYS385 Y-STR loci was constructed and the

system used to analyze the reference and mixed samples. Results show the Y-STR system was successful in first, determining the presence of male DNA and second, in identifying the male source of the DNA relatively high probability in all mixed samples tested. In tandem with the autosomal STR system, a higher power of discrimination was achieved thus demonstrating the effectiveness of the Y-STR database for forensic cases. Overall, this shows a new system that can compliment the already existing autosomal database of the Philippine population.

Key words: forensic, Y-chromosome, short tandem repeat, DNA typing, DYS19, DYS390, DYS393, DYS385

68. PHILIPPINE POPULATION DATABASE AT STR LOCUS FGA FOR FORENSIC APPLICATIONS

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A Filipino population database has previously been established at eight short tandem repeat (STR) loci. In the United States and in Europe, STR locus FGA has been widely used in forensic DNA typing due to its high degree of polymorphism and amenability to PCR amplification. The allele frequency distribution for a Filipino population from the National Capital Region (NCR, N = 107) was determined for STR locus FGA. DNA was extracted and amplified using standard procedures and analyzed using an automated DNA sequencer (ALFexpress, AP Biotech). A total of 13 alleles were found in the population, ranging from allele 17 to allele 27 and including rare variants 21.2 and 22.2. The most common allele found was 23 (f=0.21). Statistical analysis showed that the population conformed to Hardy-Weinberge rules (p=0.7810); therefore the allelic frequencies may be used for forensic calculations. FGA had an average power of paternity exclusion of 0.7185 and an index power of discrimination of 0.9001. FGA was found to be in linkage equilibrium with the eight other STR loci currently being used in the laboratory, namely: F13A01, FES/FPS, vWA, FOLP23, D8S306, CSF1PO, TH01 and TPOX; therefore cumulative values for APE and PD were calculated. The addition of FGA brought the average power of paternity exclusion of the nine loci to 0.9984 and the combined power of discrimination of 0.9999999965. The data obtained in this study has therefore increased the power of DNA typing system for use in forensic testing.

Key words: FGA, short tandem repeat 8, Philippines, DNA typing, population database, forensic, paternity testing

69. TRANSCRIPTS AND CLONE CONTIG MAPPING WITHIN 13q32, A SUSCEPTIBILITY REGION FOR BIPOLAR DISORDER AND SCHIZOPHRENIA

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Recently, independent reports highlight the importance of chromosome 13q32 as possible location for genes that may underlie bipolar disorder and schizophrenia, indicating a possible overlap of susceptibility locus. To obtain a fine resolution of loci mapping in this region and determine positional candidate genes, a high-resolution physical map was assembled using bacterial artificial chromosome (BAC) cloning system. Unique transcripts (expressed sequence tags, ESTs) and linked markers D13S1252 and D13S1271 found within the region were used as primers in isolating by PCR 25 BAC clones which were eventually used to assemble a clone contig within the ~2 cM interval stSG9874-D13S1267. The BAC contig reduced the physical distance of the interval from 2.4 Mb to ~600-800 kb. *Not*I digestion of BAC DNA released inserts and revealed 12 *Not*I sites reportedly associated with CpG islands marking location of multiple active transcribing units. The terminal ends of selected BAC clones were sequenced to obtain 19 new end sequences, 14 of which were found to be novel and five showed homologies in the databases. The sequences are nearly 100% homologous with random sequences in high throughout genome sequences (HTGS) in Genbank. New sequence tagged sites (STSs) generated become new landmarks that increase loci resolution and serve as template for further dissection of the region.

Considered as first positional candidates were the transcripts and genes that were localized nearest the linked markers. The presence of *EB12* (Epstein Barr virus induced gene 2), a G protein-coupled receptor involved in phospatidylinositol (PI) pathway, supports *IMPA2*, one of the key enzymes of this signaling pathway, as a strong candidate gene. One of the candidate ESTs in NIB529, a novel EST expressed in the brain that elicited a low homology to a microtobule-associated protein (MAP). NIB529 was used to initially extend cDNA towards the 5' end as an attempt to obtain the full-length cDNA sequence using random amplification of cDNA ends (RACE)-PCR coupled with database search. The refined physical map showing positional candidates is a valuable resource for facilitating the precise

localization and identification of candidate genes for these diseases and for other diseases linked to these regions.

Key words: bipolar disorder, schizophrenia, ESTs, BAC, 13q32

70. LIGHT-MEDIATED RESPONSE OF *Anabaena* sp. STRAIN BATG-01 TO SALT STRESS*

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Cyanobacteria or the blue-green algae are important constituents of tropical agricultural fields. They are mostly capable of nitrogen fixation. They are also reported to exhibit considerable tolerance to salt and osmotic stress. Salinity, as a consequence of organic and industrial pollution, is a critical deterrent to agriculture since it reduces crop yield. Salt-tolerant strains of cyanobacteria have been used for the reclamation of saline soils, particularly rice paddy fields. Light has also been postulated to play an important role in the adaptation of cyanobacteria to different environmental stresses (Leukart and Hanelt 1995).

The main objective of this study is to determine whether varying light periods would affect the response of a cyanobacterial isolate from Batangas, *Anabaena* sp. strain Batg-01. Cultures of the isolate were grown under four different light regimes; Set I (24 h light: 0 b dark), Set II (16 h light: 8 h dark), Set III (12 h light: 12 h dark), and Set IV (8 h light: 16 h dark). The growth rate and generation time was computed for each set-up. DMRT analysis showed that set-Up III had the highest growth rate and subsequently the shortest generation time. The cultures were then treated with 240 mM NaCl (the maximum concentration that allows growth of the cyanobacterium based on previous studies) upon reaching the mid-log phase. After 0, 4 and 8 hours of salt-treatment, proteins were extracted, quantified and visualized for the presence or absence of salt stress proteins (SSP). Results showed that light influenced the synthesis of SSPs that are produced as a response to salt stress such that more proteins were synthesized in cultures exposed to longer light periods.

Key words: cyanobacteria, blue-green algae, salt stress, NaCl, light, salinity, growth rate, generation time, DMRT analysis, salt-stress proteins

71. AN ASSESSMENT OF THE MORPHOLOGICAL SYSTEM OF CLASSIFICATION OF PHILIPPINE *Acanthamoeba* ISOLATES BY RIBOPRINTING

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Acanthamoeba spp. is a group of free-living, ubiquitous amoebae that are the causative agents of keratitis and granulomatous amebic encephalitis. Due to problems encountered in the identification of *Acanthamoeba* isolates based on the current morphological system of classification, other methods have been employed in determining the correct species designations of *Acanthamoeba* isolates. Riboprinting (PCR/RFLP) is one of the most recent methods which involves restriction digestion of PCR products after amplification of the mitochondrial and nuclear small subunit (SSU) rRNA gene. Eight *Acanthamoeba* environmental isolates were obtained from Baguio (Bag), Mt. Arayat (MA), Tanauan, Batangas (MB), Misamis Oriental (MO), Puerto Princesa (PP), Sierra Mader (SM), Tuguegarao (TS) and Novaliches (W4). These were studied based on morphological characteristics, isoenzyme analysis, and Riboprinting. These were also identified through PCR using genus- and species-specific primers. All isolates exhibited the characteristics of morphological Group II (polygonal cysts). Data from isoenzyme analysis and Riboprinting were analyzed using cluster analysis. Grouping based on cyst morphology correlated well with isoenzyme analysis and Riboprinting. MA, MB,MO, and PP could belong to a different species based on morphology, 18S Riboprinting, isoenzyme analysis, and PCR identification using species-specific primers. W4 was found to be very similar to the reference strains *A. castellani* (Ma) and *A. polyphaga* (Jones). SM could belong to another species that is related to the Castellani group of *Acanthamoeba* isolates. It is different from the other isolates based on morphology, isoenzyme analysis, and PCR identification using species-specific primers. However, further studies would have to be done on Bag and TS for species identification.

In determining the correct species designation of asexually reproducing organisms such as *Acanthamoeba*, both morphological and molecular data should be incorporated. A classification scheme based on these data would be provide information regarding the diversity of various *Acanthamoeba* isolates.

Key words: *Acanthamoeba*, keratitis, Riboprinting, RFLP, small subunit (SSU) rRNA gene, isoenzyme analysis, cluster analysis, cyst morphology

72. CLONING AND SEQUENCING OF THE LYS3 GENE ENCODING HOMOACONITASE IN *Penicillium chrysogenum*

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Two completely different biosynthetic pathways for L-lysine exist in nature. The diaminopimelic acid pathway is observed in green plants, bacteria and some phycomycetes. Yeasts and filamentous fungi synthesize L-lysine through the a aminoadipic acid (α-AAA) pathway. While much has been known about the latter pathway in yeasts, so little is known about it in filamentous fungi in terms of the genes involved and its regulation. In *Penicillium*, only two genes in the pathway have been cloned so far. It is therefore imperative that more studies on the molecular genetics of the α-AAA pathway be undertaken for a clearer picture of this unique biosynthetic pathway. We have cloned the *lys*3 gene from *Penicillium chrysogenum* by complementation of a lysine-requiring strain of *P. chrysogenum* called the L2 mutant with a clone from a genomic library. This clone carries a 4.3 kilobase pairs (kbp) of DNA fragment constructed on the plasmid vector pAMPF9L. Complementation was confirmed by plasmid rescue and re-transformation of the L2 mutant. A restriction map of the complementing fragment was prepared and sub-clones were constructed using pBluescript KS+/SK+ in two orientations for sequencing. Computer-aided assembly of contigs generated a 3.412 kbp sequence with an open reading frame (ORF) size of 2.406 kbp. Comparison with homologues in DNA databases world-wide revealed that the cloned *lys*3 gene encodes for homoaconitase, the enzyme that functions in the second and third steps in the α-AAA pathway. The gene contains one intron and several putative regulatory sequences. Results are highly significant not only because the *lys*3 gene encoding homoaconitase is the first to be cloned in *Penicillium* but also because of the existence of putative functional domains in the gene based on sequence analysis.

Key words: L-lysine, alpha-aminoadipic acid, *Penicillium chrysogenum*, *lys*3 gene, cloning, complementation, genomic library, homoaconitase, restriction map, domains

73. ACETYLCHOLINERGIC RECEPTORS IN THE CA1 REGION OF THE RAT: AN OPTICALS RECORDING STUDY

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Optical responses were recorded from the rat hippocampal slices (450 μm), stained with a voltage sensitive dye, RH 482 (0.01%), using a real-time optical recording system to study the cholinergic influence on hippocampal activity. The recording system consists of a camera head with a 128 x 128-photodiode array with a high time resolution of 0.6 ms. Electrical stimulation was applied through a bipolar electrode placed in the fornix to stimulate the cholinergic pathway.

Electrical stimulus evoked a response that propagated both to the dentate gyrus and CA1 region. Latencies of response varied among slices with a median value of 13 ms. In the presence of acetylcholine (Ach) or carbachol (CCh) the optical response was diminished specially in the CA1 region. This effect was nearly abolished by atropine. Furthermore, GABA at low concentration also attenuated the response and with the addition of Ach/CCh further inhibition was seen. Application of Ach and CCh had little effect on optical signals around the stimulating electrode where direct activation of nearby cells or non-synaptic response is mostly responsible for the recorded optical responses.

We conclude that the inhibitory effect of acetylcholine and its agonist can be brought by activating the muscarinic receptors in the GABAergic inhibitory interneurones. This activation increases the excitability of the interneurones and that synaptically released Ach increases interneural activity. The partial effect of muscarinic antagonist aatropine suggests that not only muscarinic but also nicotinic receptors are activated as demonstrated by the effect of d-tubocurarine.

74. PARTIAL SEQUENCES OF THE MITOCHONDRIAL 16s rRNA AND CYTOCHROME B GENES OF *Loriculus philippensis* (PHILIPPINE HANGING PARROT) FROM DIFFERENT LOCATIONS IN THE PHILIPPINES

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Molecular data on wildlife species in the country could be used in understanding their taxonomic relationships and thus could also be valuable in evaluating the conservation status of the species. The research was aimed at developing a DNA-based procedure to study the Philippine Hanging Parrot or Colasisi (*Loriculus philippensis*), an avian species endemic to the Philippines. One to three parrots were obtained from Laguna, Negros, Davao, Leyte, and Cebu. Extraction of total DNA from avian blood samples was optimized based on reported procedures of Seutin, Kirby, Wang and their co-workers. Extracted DNA was successfully used for restriction enzyme digestion and Polymerase chain reaction. Parameters for the optimized PCR amplification of mitochondrial 16s rRNA and cytochrome b of the samples used were determined. Amplified products of about 600 and 350 bp for the mitochondrial *16s rRNA* and *cyt b* gene, respectively, were cloned into the pUC18 or pUC19 plasmid vectors for DNA sequencing. Partial sequences of the mitochondrial genes were obtained and sequence analyses were performed including homology searches, DNA sequence alignment, and construction of genetic distance tree using Phylip ver 3.573. This paper reports the partial DNA sequences for Colasisi obtained from Laguna, Negros, Davao, Leyte, and Cebu. Although avian blood samples were limited to just one to three birds from each location, partial DNA sequences of the 16s rRNA and cytochrome be genes from these birds were determined and suggest greater than 90% homology among Colasisi from different places in the country. There is a need, however, for obtaining complete sequences for genes studied and to get phenotypic and geographical data in order to fully assess their phylogenetic relationships. More importantly, this study has shown that the molecular-based characterization of avian species is feasible and that the procedure can be performed in the country.

Key words: *Loriculus philippensis*, PCR, cytochrome b gene, 16s rRNA gene, genetic distance tree

75. AN ASSESSMENT OF THE HYPOGLYCEMIC PROPERTY OF *Syzygium cumini* LINN. and *Musa paradisiaca* LINN.

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Syzygium cumini L. and *Musa paradisiaca* L. are used in traditional medicine in lowering blood glucose levels. Although these plants have been reported to have hypoglycemic properties, their effects on blood glucose levels should be studied in detail to be able to use them judiciously even at crude state.

The juice from the ripe fruits of *S. cumini* L. was freeze-dried while the unripe fruits of *M. paradisiaca* L. was extracted using ethanol as solvent. Each crude fruit extract dissolved in water was given orally at a dose of 1.25 g/kg BW to nondiabetic and diabetic Swiss mice at different prandial states: fasting and postprandial. Blood was collected at different time intervals through the ocular vein. Concentration of glucose in the blood was determined by glucose-oxidase method. The hypoglycemic activity was expressed in terms of % reduction in the blood glucose level.

Results showed that in nondiabetic mice, *S. cumini* L. and *M. paradisiaca* L. had significant effect in fasting state ($p<0.02$) and in postprandial state when each extract was fed simultaneously with glucose solution ($p<7x10^{-5}$). In diabetic mice, none of the two extracts showed any effect in the fasting state. However, *S. cumini* L. opposed the rise in postprandial blood glucose level when extract was given thirty minutes before glucose load (percentage reduction of blood glucose level: -117+/-35 in the control vs. –10+/-2, $p<0.002$) *M. paradisiaca* L. showed a tendency to raise (-337+/-56) the postprandial blood glucose levels 30 minutes after it was administered orally together with glucose solution. Reduction of –56+/-20 followed at 75 minutes. The results indicated that these two fruits have interesting possibilities as source of oral hypoglycemia agents.

Key words: *Syzygium cumini*, *Musa paradisiaca*, hypoglycemia, diabetes mellitus

76. CYTOGENETIC EFFECTS OF SAMBONG (*Blumea balsamifera* L.) TABLETS ON HUMAN LEUKOCYTES CULTURED *In Vitro*

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Cytogenetic effects of sambong (*Blumea balsamifera* L.) tablets, a diuretic medicinal drug were determined on human leukocytes cultured *in vitro*. Four concentrations (%) (0, 0.5, 1.0, and 1.5) were tested on 20 year-old male blood donors while concentrations (%) 0, 2.0, 3.0, and 5.0 were tested on 40 years old and above donors. The concentrations tested on young donors did not significantly affect mitotic index (0.140, 0.125, 0.111, and 0.104 for control, 0.5, 1.0 and 1.5, respectively). Mean frequency of cells with chromosomal breaks, gaps, loose sister chromatids and condensed chromosomes was low. Mean frequency of cells with gap was 0.019 for 0.5% and 0.039 for 1.5% with break was 0.005 for control and 0.039 for 1.5% and with loose sister chromatids was 0.034 for control while 0.074 or 1.5%. Concentrations tested on older donors significantly decreased the mitotic index; 0.122, 0.079, 0.072 and 0.041 for control, 2, 3, and 5%, respectively. No significant differences on mean frequency of cells with chromosomal aberrations were noted. Mean frequency of cells with gap was 0.019 for control and 0.021 for 5%. The number of cells with break remained low (0.014 for control and 0.019 for 5%). The same observation was noted for cells with condensed chromosomes (0.089 for control and 0.116 for 5%). Results indicate that *B. balsamifera* is not a mutagen since it exhibited no chromosome-damaging effect on human leukocytes.

Key words: Cytogenetics, mitotic index, chromosomal aberrations, sambong, *Blumea balsamifera*, loose sister chromatids, breaks, gaps, condensed chromosomes, mutagen