

non-GM soybean samples containing 5%, 2%, 1%, 0.5%, 0.1%, and less than 0.03% transgenic Roundup Ready® soybean. Detection limits for the 300-bp CamV 35S/*m-epsps* and the 173-bp *nos* terminator gene fragments by the multiplex PCR procedure used in this study were 0.5% and 1.0%, respectively. The three new primers designed and tested in this study can now be used for multiplex PCR detection of GM soybean containing the transgenes *CamV/EPSPS* and *nos* terminator and can be utilized in developing a commercial detection kit for such transgenes in GM soybean.

Keywords: genetically modified (GM) soybean, *Glycine max*, multiplex PCR, PCR detection limit

BIOLOGICAL SCIENCES

BSD No. 1

CHALLENGES IN THE STUDY AND CONSERVATION OF PHILIPPINE *RAFFLESIAS*

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Rafflesia (Rafflesiaceae) is a genus of plants obligately parasitic to *Tetrastigma* (Vitaceae), lianas of the lowland rainforests in tropical and subtropical Asia. To date, 21 species of *Rafflesia* have been described: one species in Thailand, eight in Indonesia (five in Sumatra and three in Java), eight in Malaysia, and four in the Philippines including the most recently described, *R. mira*, from Compostela Valley in Mindanao. Our recent explorations in Luzon have added two more new species. Three potentially new species, yet to be described, are currently known from photographic evidence only. Once formally described, they would make the Philippines the global center of *Rafflesia* with a total of at least nine species, the

highest species concentration relative to land area. The lowland rainforest, home to rafflesias, is the most threatened due to deforestation and conversion to other landuses. Because of their rarity, diversity, unique reproductive biology, and ecology, studies on Philippine *Rafflesia* have not yet gone beyond discovery, nomenclature, and mapping. Research on *Rafflesia* is currently being hampered primarily by the generally tedious and slow permitting process of the Department of Environment and Natural Resources or by the absence of competent botanists at the time of discovery. In order to make sound conservation action plans for this enigmatic genus and its host, collection and scientific studies of Philippine *Rafflesia* must be supported by all stakeholders. Furthermore, further explorations of the lowland rainforests in the Philippines should be facilitated and collaboration between Filipino and foreign parasitic plant experts should be encouraged. If the scientific studies remain to be hampered by the lack of collecting permits and the destruction and disappearance of the lowland rainforests in the Philippines is unabated, perhaps the diversity of this plant group may disappear even before all the species are discovered.

Keywords: conservation, lowland rainforests, parasite, Philippines, *Rafflesia*, *Tetrastigma*

BSD No. 2

BUTTERFLY SPECIES IN LOWLAND AND MOUNTAINOUS LOCALITIES

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Butterflies have diminishing range due to habitat degradation brought about by land developments and deforestations. Observations regarding butterflies in lowland and mountainous locality will be elucidated in this paper. This study will show the disparity of species in the said localities and the reasons for such contions.

A typical developed lowland like in Los Baños, Tarlac and Pampanga neighborhood with few surviving flora will yield the following family and species: Family Pieridae: *Eurema hecabe*, *Catopsilia pomona*, *Catopsilia pyranthe*, *Catopsilia scylla*, *Leptosia nina*, *Appias olferna*, Family Lycaenidae: --*Zizina otis oriens*, *Eucrysops cnejus*, Family Papilionidae: *Papilio demoleus*, Family Nymphalidae: *Junonia almana*, *Junonia hedonia ida*, *Hypolimnas bolina*, *Melanitis leda leda*, Family Satyridae: *Bibasis gomata*, *Amathusia phidippus* and

Erionota thrax. In contrast mountainous surroundings such as Mt. Arayat and Mt. Makiling with remaining forest covers can yield more than one hundred species. These species seldom venture outside the said mountains. The host plants land use patterns and natural enemies affect the scarcity or abundance of a given species.

Keywords: Butterflies, family, species, lowland, mountainous, localities

BSD No. 3

THE AMPHIBIAN FAUNA OF MT. MALINDANG
MINDANAO, PHILIPPINES

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The Philippines is one of the world's top 17 megadiverse countries, however, it is not an exception to the general trend of forest destruction. Mt. Malindang is one of the upland ranges where biodiversity has been severely threatened due to forest loss. To assess amphibian faunal species richness for better understanding and management of critical resources, fieldwork was conducted in Mt. Malindang, Mindanao from October 2003 until December 2004 in different vegetation types (mossy, montane, almaciga, submontane dipterocarp, lowland dipterocarp, mixed dipterocarp and plantation forest as well as agroecosystem sites) from elevation of 120 meters to over 1700 meters above sea level. Primary data were collected through opportunistic technique with the participation of Subanens (indigenous people in the area) as local researchers. Assessment revealed 26 species of amphibians, 11 of which are endemic species with seven found only in Mindanao. Nine species are in the threatened category where eight are vulnerable and one endangered. *Philautus surrufus*, a Mindanao endemic listed in the endangered category was found to be abundant in the Mt. Malindang. All endemic species captured were found to be forest dwellers. It is apparent from the results of this present study that despite habitat degradation in Mt. Malindang, species richness of amphibians is high where 26% of amphibians recorded in the Philippines and 74% recorded in Mindanao are found in Mt. Malindang, indicating the conservation importance of this mountain range.

Keywords: amphibians, endemic, species, richness, Mt. Malindang

BSD No. 4

SURVEY OF ENDEMIC, INDIGENOUS AND INTRODUCED (FOREIGN) SPECIES IN THE FRESHWATER ECOSYSTEMS OF NUEVA ECILJA

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The study was conducted to describe, identify, classify and to determine the occurrence and diversity of the endemic, indigenous and introduced (foreign) species in the freshwater ecosystems of Nueva Ecija. It also aimed to determine the sources and level of impacts of environmental degradation in the freshwater ecosystems of Nueva Ecija.

This survey was conducted in five freshwater ecosystems of Nueva Ecija, namely, Talavera River in Talavera, Pampanga River in Palayan City, Tabuating River in San Leonardo, Paitan Lake in Cuyapo and Pantabangan Dam in Pantabangan. Three sampling stations were selected in each of the five study areas and in each station ten quadrats measuring 10 m x 12 m were laid out randomly. Freshwater fishes, mollusks and macroflora were collected in the five study sites. An interview with the residents and a survey questionnaire was distributed to the 30% of the total population of fishermen in each study area. An assessment for the environmental disturbance affecting the freshwater ecosystems of Nueva Ecija was done by means of a checklist to survey the sources and level of impact of environment degradation of the freshwater ecosystems.

Twelve (12) species of indigenous and endemic species were identified and classified in the different freshwater ecosystems of Nueva Ecija which include four (4) species of freshwater fishes, two (2) species of freshwater crustaceans, one (1) species of freshwater mollusk and five (5) species of freshwater macroflora. *Leiopotherapon plumbeus* (Ayungin or Lukaok) and *Glossogobius giuris* (Biya) registered the highest percentage occurrence among the identified freshwater animal species. *Ipomoea aquatica* got the highest percentage occurrence with regards to macroflora. Pantabangan Dam registered the highest species diversity value among the freshwater ecosystems surveyed.

Sixteen (16) introduced species are described, identified and classified in the freshwater ecosystems of Nueva Ecija which include seven (7) species of freshwater fishes, five (5) freshwater mollusks and four (4) freshwater macroflora. Nile tilapia "*Tilapia nilotica*" is the most common introduced freshwater fish while *Pomacea canaliculata* also called golden snail is the most common introduced freshwater mollusk in the selected freshwater ecosystems of Nueva Ecija. Moreover, *Eichornia crassipes* also known as water lily is the most common introduced freshwater

macroflora. Paitan Lake got the highest species diversity of introduced species which include fishes, mollusks and macroflora because the people near the lake are dependent on those species as their source of food and source of income.

Based on the study, pollution such as animal wastes and solid wastes, biological pollution of introduced or alien species, recreational development, establishment of the hydroelectric power plant, toxic chemical hazards and recreational development have significantly contributed to environmental degradation of the freshwater ecosystems in Nueva Ecija.

At present, only a few number of indigenous and endemic species are present and the introduced species dominate the native species in the freshwater ecosystems of Nueva Ecija. This condition may result into the extinction of the endemic and indigenous species and to the destruction of ecological balance in the freshwater ecosystems in the upcoming years as well as loss of biodiversity.

Keywords: Endemic species, indigenous species, introduced/foreign species, lake, river, dam, biodiversity, environmental degradation, biological pollution

BSD No. 5

ASSESSMENT OF MYCORRHIZAL DIVERSITY IN ABANDONED MINE SITES IN TOLEDO, CEBU FOR BIOREMEDIATION

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A survey of mycorrhizal diversity associated with selected plant species within the 35,000-hectare Atlas Mines in Toledo, Cebu, was conducted with the goal of rehabilitating abandoned copper mines through bioremediation by utilizing indigenous plants and mycorrhizal fungi. The Atlas Mine site is generally covered by vegetation classified as a disturbed grass-shrub-agroforest plant community. Plants growing in marginal and stressed environments similar to that in mine sites usually are associated with mycorrhizal fungi. Plants were identified and rhizosphere soil (including fine roots) were collected in five sites delineated by the mining company where ten samples were randomly collected from each site. Mycorrhizal colonization was assessed after the roots were cleared with potassium hydroxide

and stained with trypan blue. Mycorrhizal spores were separated from the soil using the wet sieving and decanting procedures and counted following the grid line intersect method.

In the survey conducted, out of the 50 plants, only five (i.e. *Lycopodium*, *Saccharum spontaneum*, *Nephrolepis*, *Acacia mangium* and *Stachytarpheta jamaicensis*) collected from two sites showed colonization by vesicular-arbuscular mycorrhizal (VAM) fungi, with infection ranging from 10 – 100%. All roots of kandi-kandilaan (*Stachytarpheta jamaicensis*) were colonized solely by *Glomus* sp. Kamachile (*Pithecellobium dulce*) harboured the highest spore density (2,575 spores/plant/30g soil), consisting of *Glomus*, *Acaulospora* and *Entrophospora*. Datiles (*Muntingia calabura*), on the other hand, was the only plant associated with *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora*, but with low spore population. *Glomus* was the most prevalent among the VAM fungi. Fruit bodies of ectomycorrhizal (ECM) fungi: *Pisolithus*, *Scleroderma*, *Thelephora* and *Bolettellus* were found under *Acacia auriculiformis*, *A. mangium*, *Eucalyptus urophylla* and *E. camaldulensis*. *Pisolithus* was the most prevalent. These VAM and ECM fungi can be isolated and mass-produced as inoculants for the successful rehabilitation of mine sites. Nursery and field trials have shown that mycorrhizal fungi can enhance plant growth, promote survival in infertile and acidic soils and tolerance to heavy metals.

Keywords: mycorrhizal diversity, VAM fungi, ECM fungi, rehabilitation, mine sites

BSD No. 6

GROWTH AND HEAVY METAL ABSORPTION OF MYCORRHIZAL AND NON-MYCORRHIZAL *JATROPHA CURCAS* L. IN MARGINAL GRASSLAND AND MINE WASTE SOILS

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This study determined the effect of mycorrhizal inoculation on the growth of *Jatropha curcas* L. in 9 mine waste soils and compared it with that in marginal grassland and garden soil. Soil pH ranged from 2.4 to 6.8 and Cu concentration of 3.5 to 200 mg/kg soil. One-week old seedlings were inoculated with mycorrhizal fungus *Gigaspora margarita*. The initial height of the plants was measured a week

after transplanting and the succeeding height measurements were done once a week for ten weeks. At harvest, root collar diameter, leaf area, plant dry weight, mycorrhizal infection and Cu and P concentration and uptake of different plant parts were determined. Results showed that out of the 11 soils studied, only seven sustained growth of *Jatropha* throughout the ten-week period. Plants grown in garden soil exhibited the best growth and highest P uptake, followed by those grown in a grassland soil. The poorest growth was observed in plants grown in mine soils collected from Antamok, Benguet and Paracale, Camarines Norte. Mycorrhizal inoculation promoted better growth of *Jatropha* in all soils except in garden and grassland soils. Phosphorus and Cu concentrations and uptake were highest in the leaves and lowest in the roots which were comparable with that in the stem. This implies that mycorrhizal *Jatropha* grew better in heavy metal sites and may contribute more in the extraction of Cu and other heavy metals in grassland and mine waste soils than the non-mycorrhizal ones. Moreover, better growth of mycorrhizal plants will definitely mean more fruits where biodiesel can be extracted.

Keywords: heavy metals, mycorrhiza, *Jatropha curcas*, mine waste soil, marginal grassland, copper

BSD No. 7

MYCORRHIZAL FUNGI AND HEAVY METAL TOLERANCE OF FAST GROWING REFORESTATION TREE SPECIES

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Rehabilitation and reforestation of heavy metal contaminated sites can significantly contribute to our country's economical, agricultural and ecological stability.

Three fast growing tree species were used in the experiment namely: *Acacia mangium*, *Acacia aulacocarpa* and *Swietenia macrophylla* and inoculated with either *Gigaspora margarita*, *Glomus etunicatum*, a commercial mycorrhizal inoculant "Mykovam" and mycorrhizal fungi from mine site coded as Paracale isolates. Mykovam is a soil inoculant comprising of *G. margarita*, *G. etunicatum* and *G. macrocarpum* whereas Paracale isolates were a mixture of *Glomus*, *Entrophospora*, *Scutellospora* and *Acaulospora*.

The study was conducted to determine heavy metal tolerant fast growing reforestation tree species, and to determine the growth promoting and ameliorating effects of mycorrhiza in fast growing trees grown in mine waste soil. The experiment was one in a Randomized Complete Block Design with 10 replicates.

Inoculated *A. mangium* seedlings showed higher height and biomass than the uninoculated ones. Mycorrhizal *A. aulacocarpa*, showed higher height, biomass and phosphorus uptake than non-inoculated ones. *Swietenia macrophylla* seedlings were significantly affected in their height and biomass but the phosphorus content was higher than the non-mycorrhizal counterpart. Inoculation with mycorrhizal fungi enhanced the heavy metal tolerance of *A. mangium*, *A. aulacocarpa* and *S. macrophylla* and could be potential species for the rehabilitation and reforestation of heavy metal contaminated areas.

Keywords: *Acacia mangium*, *Acacia aulacocarpa*, mycorrhiza, copper, heavy metals, mine sites, phosphorus, *Swietenia macrophylla*.

BSD No. 8

ANALYSIS OF HEAVY METAL UPTAKE OF SOME POTENTIAL PLANTS FOR PHYTOREMEDIATION IN AN ABANDONED MINE AREA

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Phytoremediation, is offered as a “green” solution to environmental problems and may be applied *in situ* or *ex situ*, to soils, sludges, sediments, other solids, or groundwater. The study primarily aimed to investigate the tolerance mechanism of selected dominant plants as potential phytoremediators thriving in an abandoned mine area in Toledo, Cebu.

Tissue analysis of the various plant organs of silver fern, malatungaw and Japanese acacia revealed their various capacities to take up high concentrations of copper (Cu), lead (Pb), cadmium (Cd) and zinc (Zn), the usual HM contaminants in a copper and gold mining sites. Japanese acacia and silver fern showed high concentrations of Cu in the roots. Japanese acacia, as a nitrogen fixer, has rhizobial symbionts while the silver fern has mycorrhizal root association. In spite of high

concentration (435.17 mg kg⁻¹) of Cu in the roots of Japanese acacia, it was notable that the metal was not translocated in the fruits. Moreover, the levels of Cd, Pb and Zn in the fruits were very much below the allowable limits. Malatungaw, on the other hand, showed the lowest uptake of the HMs among the dominant species in the area, and thus, may be considered as a true excluder of Cu, Cd, Pb and Zn.

Key words: heavy metals, tolerance, uptake, phytoremediation, abandoned mine area

BSD No. 9

PHYTOMONITORING OF *Rhoeo discolor* AS DETERMINANT OF AIR QUALITY IN TWO DIFFERENT SITES

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Phytomonitoring is the use of plants as determinants of environmental pollution. Plants respond to pollutants in the form of changes in certain parameters such as chlorophyll level , leaf-extract pH , relative water content and stomatal density. These changes can be measured and used as a reliable indicator of exposure to airborne pollutants . Phytomonitoring as its is called can thus be used as a possible alternative and substitute to the usual physico-chemical methods and costly instruments used in air quality monitoring.

The study focuses on the potentials of *Rhoeo discolor* as biological indicator of air quality by phytomonitoring methods . Sample leaves were collected in two sites , Laguna and Manila . Four phytomonitoring methods were used : total chlorophyll content , leaf extract pH , relative water content and stomatal density.

Results showed that sample leaves from manila have lower values of total chlorophyll content , 5.11 mg/g ; leaf extract pH 5.55 ; relative water content , 93.25% ; and stomatal density , 19.82 stomata per cm² compared to those from Laguna total chlorophyll content 10.0 mg/g , leaf extract pH 6.68; relative water content 99.08% and stomatal density 29,519 stomata per cm² . Based on the results , *Rhoeo discolor* is a good biological indicator of air quality.

Keywords: Phytomonitoring , *Rhoeo discolor* , biological indicator , stomatal density

BSD No. 10

DEVELOPMENT OF A STANDARD TOXICITY TEST PROTOCOL FOR THE MARINE ENVIRONMENT USING THE BLACK TIGER SHRIMP, *PENAEUS MONODON*

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Biological assays are an essential tool for the determination of potential adverse effects of xenobiotics in the environment. However, many of the protocols for toxicity testing use temperate organisms and could not be directly applied to Philippine studies. The study explored the potential of the marine Black Tiger shrimp, *Penaeus monodon*, as a test organism for toxicity testing. The investigations focused on the following aspects: (a) standardization of laboratory conditions; (b) determination of the appropriate larval stage for use in toxicity testing; (c) selection of the appropriate reference toxicant; (d) comparison of test sensitivity between the *Penaeus monodon* test and the Standard *Artemia franciscana* toxicity test; and (e) application of the developed test to environmental monitoring.

The results showed that the post-larval stage of the shrimp is the most suitable stage to be used for toxicity testing in comparison to the protozoal and mysis stages. Moreover, among the toxicants tested (i.e. potassium dichromate, copper sulfate, and mercuric chloride), the organism exhibited the most stable response to potassium dichromate, $K_2Cr_2O_7$, which was consequently chosen as the reference toxicant. Inter-calibration tests done with the standard *Artemia* toxicity test showed that the *Penaeus* test is comparable in sensitivity with the ArTox test. With these promising results, the *Penaeus monodon* test can be a useful addition in the list of toxicity tests for the marine environment which are applicable to the Philippine conditions.

Keywords: bioassays; xenobiotics; toxicity testing; *Penaeus monodon*; *Artemia franciscana*; potassium dichromate, post larva.

BSD No. 11**GROUNDWATER QUALITY ASSESSMENT
IN PAYATAS DUMPSITE, PHILIPPINES****Glenn L. Sia Su**

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Groundwater is an important resource providing man with numerous uses. However, it has often been neglected and overly exploited. The concern over today's contamination of groundwater and surface water sources resulting from open dumpsites had been identified as a potential threat to Metro Manila's water supply. The study assessed and compared the groundwater quality of fourteen (14) selected wells continuously used in the "with" (Payatas) and "without dumpsite" (Holy Spirit) areas of Payatas estate, Philippines. Water quality monitoring and analyses of the bio-physico-chemical parameters: pH, total suspended solids (TSS), total dissolved solids (TDS), total coliform, turbidity, conductivity, salinity, nitrate-nitrogen, sulfate, color, total chromium, total lead and total cadmium were carried out for six consecutive months from April to September 2003, covering dry and wet seasons. Results showed that most of the groundwater quality parameters in both study areas were within normal water quality standards of the DENR-EMB and the DOH except for the observed high TDS (> 1000 mg/L), TSS (>50 mg/L), total coliform (MPN > 0) and low pH levels (< 6.5). No significant differences in the following parameters: nitrate-nitrogen, total cadmium, total lead, total chromium and total coliform were observed between the "with" and "without dumpsite" areas. The parameters: TDS, turbidity, conductivity, salinity and sulfate concentrations in the "with dumpsite" groundwater sources were significantly higher. The significant differences at the levels of the pollutants strongly indicate the existence of pollution at the Payatas open dumpsite. Continuous water quality monitoring is encouraged. Increasing the frequency of sampling and analyses on the study areas are needed to effectively monitor impact of dumpsites on the environment and human health.

Keywords: water quality, groundwater, dumpsite, Payatas, Philippines

BSD No. 12**ECONOMIC AND BIOTECHNOLOGICAL POTENTIAL OF MUNICIPAL SOLID WASTE: LESSONS FROM DUMPSITE SELECTION PRESSURES AND EMERGENCE OF UNIQUE MICROBIAL STRAINS**

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We focused our study on a ten-hectare, thirty four-year old dumpsite in Iligan City, where approximately 120 tons of non-segregated municipal solid waste are dumped daily. A consequence of the diverse compounds present is the selection of a unique microbial biodiversity.

Four composite sampling sites were identified representing 1.0% of the whole dumpsite area. Soil samples were obtained and processed for the isolation of facultative and aerobic bacteria and fungi. Isolates were pre-screened for their production of bioactive compounds by testing their metabolites for anti-bacterial and anti-fungal properties. Selected microorganisms were tested further for the production of DNA-binding metabolites using a two-dimensional thin layer chromatography (2D-TLC) set up.

Of the 26 bacterial isolates, four exhibit potential for an industrial application, with nine of the 73 fungal isolates also showing the same. While common microbial strains can be purchased from culture collections at a very low price (PhP 250.00 to PhP 300.00), special and industrial strains can run from PhP 10,000.00 to PhP 20,000.00 per strain, based on average international prices. Granting that the ratio we obtained from our two-step screening is the actual picture when applied to the whole dumpsite area, we calculated a total of roughly 1,300 microbial isolates with diverse capabilities waiting to be discovered in the ten-hectare site. If we place the number of industrially useful strains to be only 10% of the total, we would get 130 special strains. Translating these figures to commercial value of the microbial strains would give an estimate of PhP 1,300,000.00 to PhP 2,600,000.00 from sales of the cultures alone. This new paradigm should be considered in order to give added value to what we refer to as municipal solid waste.

Keywords: municipal solid waste, microbial biodiversity, DNA-binding, 2D-TLC

BSD No. 13

**UTILIZATION OF AN INDIGENOUS DYESTUFF FROM *Basella rubra*
(ALUGBATT) AS MICROBIOLOGICAL STAIN****Lorna T. Enerva, Winrich A. Bati, Arby B. de Jesus, Angelito T. Uldo¹
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This study was undertaken to extract anthocyanin from *Basella rubra* berries and utilize the extract as microbiological stain. It is an inexpensive, indigenous and abundant raw material.

The alugbati berries were macerated in a blender and extracted with 1% HCl in 95% methanol. The extract obtained was filtered and then concentrated. Thin layer and column chromatography methods were used to isolate and purify the anthocyanin. The samples were analyzed using infrared spectra and ultraviolet spectra. FT-IR revealed the presence of a hydroxyl group which is prominent in the structure of anthocyanin pigment at 3385cm^{-1} . The C=O stretching for aromatic ring were indicated by a peak at 1635.20 cm^{-1} , 1513.38 cm^{-1} for C=O bending, 1439.91 cm^{-1} for O-H bending, 1207.42 cm^{-1} for C-O stretching, 1151.92 cm^{-1} for alkane and 1100.77 cm^{-1} for C-C stretching. The structure of anthocyanin was further established by the λ maximum of the ultraviolet spectrum at 510.0 nm .

For the application, the crude extract was used as a stain for *Staphylococcus aureus*, a gram positive bacteria and *Escherichia coli*, a gram negative bacteria. The staining process for the microorganism used mordants like potassium alum, calcium oxide and copper sulfate for fixing the color. Only copper sulfate and lime responded positively as a mordant that gave favorable outcome in fixing the color of alugbati. The samples were screened based on the criteria of color retention and evenness. The structure of the microorganisms with respect to shape and size and certain cellular components were identified using a microscope and photomicrographs. The alugbati extract produced stain that was comparable with synthetic stains like crystal violet and safranin and can, therefore, be used as an alternative stain.

Keywords: anthocyanin, microbiological stain, mordant, dye.

BSD No. 14

**PLANT DIVERSITY AND ABOVE-GROUND CARBON BUDGET OF
BROADLEAF SPECIES ALONG NORTHEASTERN SLOPE OF MT.
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This study aims to assess the plant diversity resources and carbon budget along elevation gradient in Mt. Makiling forest. Three elevation ranges were identified along the northeastern slope of the mountain: low elevation (50-300 masl), middle elevation (301-700 masl), and high elevation (701-1114 masl). Within these ranges, vegetation samplings for broadleaf were done on tree, intermediate, and wildling layers using the Quadrat Sampling Technique. A total of 110 species, 75 genera and 35 families were identified. Family Moraceae and genus *Ficus* have the highest number of occurrence in all elevation ranges, while *Swietenia macrophylla* was the most abundant species that had occurred from low to middle elevation. Vegetation and biometrics were generally denser in low and middle elevation while partially open and low on high elevation. The ecological values also showed that density, frequency and dominance were higher on these elevations where *Parashorea malaanonan* and *Pterocymbium tinctorium* had the highest Importance Value (IV) recorded. The diversity values in all ranges were generally low to moderate. The highest diversity was observed in high elevation that exhibited a trend of increasing from low to middle elevation then decreasing from middle to high elevation. Similarly, aboveground biomass and carbon density exhibited a same trend and found highest at the middle elevations where plantations of *S. macrophylla* and Dipterocarps are located. The highest estimate for carbon density was 451.62 ± 50.07 at 400 masl while lowest at the 900 masl with 94.58 ± 24.12 . Overall, plant diversity resources and the corresponding potential carbon sequestration are important ecological values that should be considered as input in sustainable management of Mt Makiling forest.

Keywords: Plant diversity, broadleaf species, carbon budget, Mt Makiling Forest

BSD No. 15

**DEVELOPMENT AND QUALITY CONTROL OF A 10% TINCTURE OF
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Phytochemical and clinical studies have shown that the seed of *Ipomoea muricata* L. possessed promising antibacterial activities and analgesic effects. This investigation dealt with the quality control of a tincture developed from the crude ethanolic extract of the dried seeds of *Ipomoea muricata* L. for purposes of standardization in the interest of monograph preparation and drug formulation. The 10% tincture is a thin, yellow liquid turning brown to dark brown with age; with a tamarind-like and slight alcoholic odor. It has a specific gravity of about 0.9185 and a pH of 5.70. It had a mean potency of 9.89% (specification: 9-11% w/v) when assayed by a spectrophotometric method developed in the course of the research. Such method was validated for precision, accuracy and linearity.

The assay procedure may not be stability-indicating since regression analysis of accelerated stability data revealed inconsistencies of correlation data that made them unsuitable for predicting shelf life using Arrhenius equation. In view of this, a long-term stability study remains the best approach to determine stability. Based on real-time data of 12 weeks, the tincture has a predictive shelf life of 18.5 weeks.

As final output of this study, a monograph on the finished product "Tinctura *Ipomoea Muricatae* Semen" (Tonkin Seed Tincture) has been generated.

Keywords: *Ipomoea muricata* L., tincture, TLC profile, ultraviolet spectra, validation, stability, monograph

BSD No. 16

SUPERCRITICAL (SC)-CARBON DIOXIDE (CO₂) EXTRACTION AND FRACTIONATION OF BIOACTIVE METABOLITES FROM *Ganoderma applanatum*

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Traditional solvent extraction of bioactive compounds from biological materials such as the shelf fungus *Ganoderma applanatum*, usually requires an array of extraction solvents. The use of supercritical-carbon dioxide (SC-CO₂) demonstrates a time-and-cost efficient procedure for extraction of polar and non-polar components of *G. applanatum*.

Fruiting bodies of the shelf fungus were collected from a semi-forest area in Tandag, Surigao del Sur, cleaned of extraneous materials, and ground using a pre-washed laboratory mill. Extraction of the polar fraction was done by setting the temperature at 40 °C, a pressure of 300 atmospheres (atm) and a flow rate of 0.5 m³/h at normal conditions (T = 25°C, P = 1 atm). The non-polar fraction was obtained using a temperature of 40 °C, a pressure of 120 atm, and a flow rate of 0.5 m³/h. Total extraction time was four hours each, for the polar and non-polar fractions.

More than 50 ml each of the polar and non-polar fractions were obtained from separate 100 grams ground samples of *G. applanatum*, sufficient volume for use in the various bioassays. Overall, the extraction period using SC-CO₂ was much shorter (total of eight hours) compared to the traditional solvent extraction (36 to 48 h). Moreover, usually only amounts in milligrams are obtained after concentration by rotary evaporation. With SC-CO₂, concentrated amounts in solution are readily obtained. Additionally, since toxic and obnoxious chemical solvents are no longer employed, SC-CO₂ proves to be a cheaper and safer way to fractionate metabolites and extracts for bioactivity testing. In so far as literature is concerned, this is the first report on the use of SC-CO₂ extraction of metabolites from *G. applanatum*.

Keywords: Supercritical-CO₂, *Ganoderma applanatum*, bioactive, bioassay, metabolites

BSD No. 17

EVALUATION OF LEAF EPIDERMAL CHARACTERISTICS OF SOME PLUMERIA SPECIES

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Plumeria species commonly called Kalachuchi are generally planted for ornamental purposes, and are now widely cultivated throughout the country because of their resilience, robustness and versatility in different types of environments. In some countries, it is cultivated for its fragrance for perfumes, but is rarely cultivated for its medicinal properties. Plumerias are commonly found in sacred places, such as churches, monasteries, temples and even in schools. Nowadays, they are usually found in roadsides and in parking areas. It is for this reason that the main objective of this study is to look into the leaf epidermal characteristics of the plant which may be responsible for the wide adaptability of this plant in different environments. The main objective of the study is to characterize the leaf anatomy of the epidermises of the different varieties of Plumeria variants. The different cells of the epidermises, such as the hairs or trichomes, stomatal apparatus, its location, stomatal index and type were studied. Histological techniques such as leaf clearing, epidermal imprints and cross-sections of leaves using free hand, sliding and rotary microtome were used. Illustrative slides were used for anatomical characterization of different epidermal structures, observed and photomicrographed using the BH-epifluorescence and the CK-2 inverted Olympus microscopes. Results showed that both the upper and lower epidermises are cutinized. Stomata are found on both epidermises, which is amphistomatic, and stomatal type is anomocytic. Trichomes are present on both epidermises and are multicellular. Trichome length ranges from 10-20 mm in length and distributed in clusters and rarely in isolation. These structural features of the leaf epidermises may be responsible for the adaptability of Plumerias to different environments especially along dusty, open, arid roadsides.

Keywords: *stomata, trichomes, amphistomatic, anomocytic, multicellular, epidermis*

BSD No. 18

INDUCTION OF CALLUS IN LEAF EXPLANTS OF *Lagerstroemia speciosa* (L.) Pers. AND DETECTION OF COROSOLIC ACID

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Lagerstroemia speciosa (L.) Pers. (Family Lythraceae) is a deciduous tree that grows in the Philippines, India, and other subtropical and tropical countries. This species, commonly known as crape-myrtle and locally called "banaba" in the Philippines, is known to have hypoglycemic effects. Corosolic acid is among its active components. This study aimed to induce callus development in leaf explants *in vitro* and to detect presence and concentration of corosolic acid.

Leaf explants from one-year old seedlings were treated with ascorbic acid to prevent browning prior to surface sterilization with 70% ethanol (EtOH) and 20% sodium hypochlorite (NaOCl). Leaf explants were grown in Murashige and Skoog (MS) medium supplemented with naphthalene acetic acid (NAA) and kinetin (KIN) at combinations of 5 iM and 20 iM of NAA and 0 iM, 1 iM, 5 iM, and 20 iM of KIN. Ten explants were used per treatment. Data were collected on abundance of callus pinheads (calli <1mm dia.) and clumps (calli e" 1mm dia.).

Calli developed on the explants, especially along the margins, seven days after inoculation except at hormonal combination of 5 iM NAA: 0 iM KIN. Treatment with 20 iM NAA: 5 iM KIN ratio produced the highest mean number of pinheads (1.89) while that with equal concentrations of NAA and KIN (20 iM:20 iM treatment) produced the highest mean number of clumps (1.60). Fourteen days after inoculation, largest mean size of the clumps (2.46 mm) was also observed in the 20 iM NAA: 20 iM KIN treatment. After 53 days in culture, 20 iM NAA: 1 iM KIN treatment produced the largest amount of calli in terms of fresh weight (ca. 300 mg).

Approximately 50 mg of oven-dried calli per treatment were subjected to corosolic acid analysis by high performance liquid chromatography (HPLC). Percent yield of corosolic acid found in calli of all treatments ranged from 0.20 to 0.39%; the highest value was in 5 iM NAA: 0 iM KIN treatment.

Keywords: *Lagerstroemia speciosa*, callus, corosolic acid, naphthalene acetic acid (NAA), kinetin (KIN)

BSD No. 19

PREPARATION AND CRYOPRESERVATION OF CYTOPLASTS FOR THE PRODUCTION OF BOVINE AND BUBALINE CLONAL EMBRYOS

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One major factor influencing success in cloning by nuclear transfer (NT) is the recipient oocyte. The development of efficient procedure for preparation and utilization of in-vitro matured oocytes as recipients for NT is therefore essential. This study was conducted to: prepare cytoplasts with high enucleation rate and developmental competence, determine the effects of activation treatments, and utilization of vitrified bovine oocytes as recipients for intraspecies and intergeneric somatic cell NT. In study 1, vortexing cumulus-oocyte complexes in hyperosmotic media for a long period moved the first polar body and reduced enucleation rate. Removal of 20% cytoplasmic volume increased enucleation rate and showed no deleterious effect on subsequent development of reconstructed bovine embryos. In study 2, NT embryos were constructed with bovine oocytes of 3 different preparations: enucleated oocytes treated with calcium ionophore for 5 min and cycloheximide for 5 h; treated with ethanol for 7 min and cycloheximide for 2 h, and those without treatment. NT embryos constructed with ethanol-treated oocytes showed similar rates of fusion, cleavage and blastocyst formation to those of the non-treated oocytes. NT embryos constructed with ionophore-treated oocytes did not show any pronucleus-like structure and showed lower cleavage rate and no development to blastocysts. In study 3, *in-vitro* matured bovine oocytes were vitrified in microdrops. After warming, around 90% of the vitrified oocytes were morphologically normal. The enucleation rate and development to blastocysts were similar to those constructed with fresh oocytes. Intergeneric NT embryos had lower cell numbers than the intraspecies NT blasocysts. In conclusion, aspiration of the first polar body and adjacent 20% cytoplasmic volume after removing cumulus cells by vortexing for a short period using hypo-osmotic media increased enucleation rate of bovine oocytes. This study also demonstrated the efficacy of microdrop vitrification procedure and the successful production of buffalo clonal embryos using vitrified bovine oocytes.

Keywords: Activation, bovine oocytes, enucleation, intergeneric, vitrification

BSD No. 20**VITRIFICATION AND IN VITRO CULTURE OF MOUSE PREANTRAL FOLLICLES: A MODEL FOR MAXIMUM UTILIZATION OF OVARIAN FOLLICLES IN WATER BUFFALOES****Eufrocina P. Atabay*,¹ Edwin C. Atabay¹ and Libertado C. Cruz¹**

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In-vitro culture and cryopreservation of ovarian pre-antral follicles is a new tool for rescuing genetic materials in mammalian ovary for offspring production. The present study demonstrated successful production of pups from vitrified pre-antral follicles in mice which would then be applied in buffalo species. Preantral follicles mechanically isolated from ovaries of 12 day old mice were exposed to 2 mol ethylene glycol for 2 or 5 min and then to vitrification solution (VS) containing 6 mol ethylene glycol and 0.3 mol raffinose for 0.5, 1.0, or 2.0 min before vitrification. The vitrified and fresh preantral follicles were treated with collagenase, and the oocyte-granulosa cell complexes (OGCs) obtained were cultured in-vitro for 10 days in membrane inserts. Preantral follicles exposed to 2 mol ethylene glycol for 5 min then to VS for 0.5 or 1.0 min showed highest survival rates after warming. The follicular loss after warming was approximately 20%. After in-vitro culture, the proportion of viable OGCs from vitrified follicles was 10% lower than that of fresh preantral follicles. There were no differences in the rates of maturation, fertilization and development to blastocysts between the oocytes derived from vitrified follicles and those from fresh preantral follicles; however, the developmental competence of oocytes derived from both vitrified and fresh preantral follicles grown in-vitro was lower than that of oocytes grown in-vivo. One of the recipient mice that received 20 blastocysts derived from vitrified preantral follicles gave birth to six live pups. The ability to rescue preantral follicles and produce offspring is of great benefit in buffaloes because of the inherently low number of preantral follicles in their ovary compared to cattle. The vitrification and culture of preantral follicles is therefore a potentially valuable reproductive technology as this will allow maximum utilization of the limited number of ovarian follicles in water buffaloes.

Keywords: Buffaloes, preantral follicles, vitrification, mouse, ethylene glycol, in vitro culture

BSD No. 21

AN INITIAL INVESTIGATION ON THE MORPHOLOGY,
ULTRASTRUCTURE AND DEVELOPMENT OF THE ELASMOID SCALES
OF MULLET FISHES (PERCIFORMES: MUGILIDAE) WITH EMPHASIS ON
LUDONG (*CESTRAEUS PLICATILIS* VAL. 1836)

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Comparative morphologic and molecular characters of adult mullet fishes in the Philippines were investigated, with emphasis given on *ludong* (*Cestraeus plicatilis* Valenciennes 1836), a rare, catadromous species suspected to be endemic to the Cagayan River and its tributaries. *Ludong* commands a price of about Php 3000 per kilogram for its unique palatability, aroma and seasonality. Although *ludong* has been pronounced endangered by the Bureau of Fisheries and Aquatic Resources, its biology and taxonomy are, however, yet to be fully established. This study was taken as an initial investigation on *C. plicatilis* and effort was made to present its unique morphology for its proper identification, with emphasis on the histology and ultrastructure of the scale ornamentation pattern. First, five (5) mullet species were identified by morphological characterization. Secondly, the histology and ultrastructure of scale ornamentation pattern were investigated by combining ocular inspection, eosin-Y staining, light and scanning electron microscopy (SEM). Finally, the possible role of *fibroblast growth factor 8* (*fgf-8*) in scale development was investigated by genomic DNA isolation, gene amplification by polymerase chain reaction, direct DNA sequencing, sequence alignment and phylogenetic tree construction. In conclusion, mullet fishes were shown to exhibit significant variations in the sub-oral regions and in the squamation patterns, among other morphometric and meristic data. Intra-species regularity and inter-species variation of squamation pattern, scale morphology and ultrastructure were observed. Epithelial microridges were observed on the outer surface of the scale epidermis, forming a species-specific ornamentation pattern. The discreteness between morphologic and molecular data suggests the existence of both genetic cascades and epigenetic factors in scale organogenesis and scale ornamentation development. The observed scale ornamentation pattern may be employed to further analyze phylogenies and infer modes of aquatic adaptation among mullets. Descriptive data obtained in this baseline study are deemed essential for further studies on *ludong* conservation.

Keywords: *Cestraeus plicatilis*, catadromous, conservation, elasmoid scale, endemic, scale ornamentation, meristic, morphometric, SEM, *fgf-8*, ultrastructure

BSD No. 22

**THE FRESHWATER AND SEMI-TERRESTRIAL BRACHYURANS OF
SELECTED AREAS IN LUZON ISLAND, PHILIPPINES****Jimmy Tevar Masagca**

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The freshwater and semi-terrestrial brachyurans of selected areas in Luzon were studied on the basis of field collections carried out in 2004 to 2005 in Cavite, Nueva Ecija, Tarlac, Camarines Sur, Albay and Catanduanes. A total of 116 crab samples were obtained, preserved and diagnosed in the laboratory for taxonomic characters. Eight (8) taxa are reported and described here. The crabs identified belong to four families, namely: Potamidae Ottmann, 1896; Parathelphusidae Alcock, 1910 (sensu Ng, 1988); Grapsidae Mcleay, 1838; and Gegarcinidae Mcleay, 1838. Two unidentified endemic potamid crabs are tentatively described as *Ovitamon* sp. 1 from Ternate, Cavite and *Ovitamon* sp. 2 from Sulong Falls in San Miguel, Catanduanes. Although the specimens examined from Cavite showed striking similarities with *Ovitamon artifrons* Burger, 1894, the structure of male gonopodium (G_1) does not resemble that of the specimens described previously from Tagaytay by Ng & Takeda in 1992. A new endemic species *Ovitamon cavitensis* (sp. nov.?) is proposed here. Two riverine crabs belonging to the family Parathelphusidae are reported here as *Sundathelphusa* sp. 1 and 2 from Guimba, Nueva Ecija and Moncada, Tarlac, respectively. Out of the three species of the widely distributed grapsid crabs (of the genus *Varuna* H. Milne Edwards, 1830), two species are reported here as *Varuna altimana* and *V. literata*. Among the semi-terrestrial crabs, *Sesarmops* (*Sesarma*) *impressum* H. Milne Edwards, 1887 (from Lictin River, San Andres, Catanduanes Island) and *Cardisoma carnifex* Herbst, 1794 (from Balayan, Batangas) are also described in this continuing project on the "Biodiversity of Freshwater Crabs of Luzon, Philippines" at De La Salle University-Dasmariñas.

Keywords: Brachyurans, crabs, taxonomic characters

BSD No. 23

**MERISTIC AND MORPHOMETRIC CHARACTERS OF AN ENDEMIC
FRESHWATER CRAB, *OVITAMON CAVITENSIS* (SP. NOV. ?)
(BRACHYURA: MALACOSTRACA) FROM CAVITE
LUZON ISLAND, PHILIPPINES**

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Given the paramount importance of the biodiversity in freshwater systems of the country to generate more information on the crab faunal diversity and the need to conserve the remaining natural populations of these groups of crustaceans, a research project under the research program on the *Patterns of Biodiversity in Aquatic Systems* is being carried out at De La Salle University in Dasmariñas (DLSU-D). Several crab specimens which are wholly freshwater species in three families, Potamidae Ortmann, 1896, Parathelphusidae, Alcock, 1910 (both *sensu* Ng, 1988), and Grapsidae McLeay, 1838 (*partim*) were collected by handpicking and with the use of crab traps (“pukot” and “bintol”) from different riverine and lacustrine environments of southern Luzon (Cavite, Quezon, Camarines Sur, Albay, Sorsogon). These specimens are currently being diagnosed taxonomically for new records and possibly new species. The freshwater crab fauna of the Philippines is poorly known, thus more systematic studies are needed since many more species undoubtedly still await for discovery. During field collections in Cavite, specimens of an endemic wholly freshwater potamid species (tentatively identified as *Ovitamon cavitensis* (sp. nov.?) was obtained from a forest stream in Mts. Palaypalay/Mataas Na Gulod National Park (200 to 360 meters above sea level or asl). A total of sixty (30 males and 30 females) were described and subjected for meristic and morphometric analyses. Diagnostic analysis shows that carapace ovoid, branchial, cardiac, urogastric and cervical grooves very shallow; postero-lateral regions appear smooth; external orbital angle broadly triangular, epibranchial tooth low, but clearly separated from external orbital angle; antero-lateral margin convex, postero-lateral margin appears converging; male abdominal distinctly triangular, segment 7 slightly longer than segments 6 and 5, lateral margin of segment 7 straight, lateral margin of segment 6 slightly convex; third maxilliped exopod with well-developed flagellum, longer than the width of the merus; ischium with sulcus, not medial; and dorsal margins of chela not serrated, and finger slightly longer than palm. The first gonopodium (G1) stout, cylinder-shaped, curved towards the inside, and surfaces

covered with hairs. Meristically, *O. cavitensis* (sp. nov.?) in both sexes consist of 7 abdominal segments, 2 anterolateral teeth protected by the third maxilliped, numerous well-developed inner distal spines in the cheliped and maxilliped; and spinnules in the cheliped and maxilliped. Morphometric analysis includes 7 characters (carapace length, *CaL*; carapace width, *CaW*; maxilliped length, *MaL*; cheliped length, *ChL*; propodus length, *PrL*; frontal margin, *FrM*; and eye diameter, *EyD*). Results indicate an observed difference between male and female samples (as to *CaL*, *MaL* and *FrM*) of the endemic crab under study (*O. cavitensis* sp. nov.?), which belongs to the genus *Ovitamon* Ng et Takeda 1992 (Potamidae Ortmann, 1896). Further investigations are needed, particularly on the morphology (ultrastructures) of the sperm transfer tubes in the male gonopodium (G1).

Keywords: *Ovitamon cavitensis*, Cavite, lacustrine,

BSD No. 24

**MONSOONAL WINDS INFLUENCE THE ASSEMBLAGE STRUCTURE,
ABUNDANCE AND DISTRIBUTION OF NET PHYTOPLANKTON IN ILIGAN
BAY, NORTHERN MINDANAO**

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The coastal and oceanic waters of the Philippines are mainly exposed to two dominant wind patterns annually, the north easterlies during the northeast (NE) monsoon and the south westerlies during the southwest (SW) monsoon. We studied the structure, abundance and distribution of surface layer net phytoplankton assemblages in Iligan Bay during the southwest and northeast monsoons. Vertical samples were collected at 24-station sampling grid on July-August 2000 (SW monsoon) and November-December 2000 (NE monsoon). The abundance of each phytoplankton Genus was estimated following the Utermöhl method. Assemblage structure and distribution were analyzed using multivariate and univariate routines available in the PRIMER software (Warwick and Clarke 2001). Abundance was significantly higher in the NE monsoon. Although assemblages in the two monsoons have similar generic richness, cyanobacteria (*Trichodesmium*) dominated the NE monsoon assemblages while diatoms (*Chaetoceros*) in the SW monsoon. Bray-Curtis similarity dendrograms and multidimensional scaling plots revealed a bay-wide contour of assemblages that

have high, moderate, and low levels in generic diversity and relative abundance. All three levels were observed in the SW monsoon assemblages, but only two (high and low) in the NE monsoon samples. High level stations are found in the southwestern sector of the bay, the low levels near mouths of major river systems, and the moderate level in other locations. Factors including salinity, temperature, and total dissolved phosphate weakly explain the spatial variations observed, thus contrasting wind patterns and other hydrodynamic processes during the two monsoons are also important in structuring phytoplankton assemblages in Iligan Bay.

Keywords: phytoplankton ecology, *Trichodesmium*, *Chaetoceros*, northeast monsoon, southwest monsoon, multivariate analysis, Iligan Bay

BSD No. 25

MODULATION OF ANGIOGENESIS IN THE CHICK EMBRYO CHORIO-ALLANTOIC MEMBRANE (CAM) BY THE POLAR AND FRACTIONAL POLYSACCHARIDE EXTRACTS OF *Ganoderma applanatum*

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Ability to regulate angiogenesis, or *de novo* formation of blood capillaries, finds its significant role both in physiological and pathological conditions. The treatment of certain neoplastic cases with extracts of *Ganoderma* sp. has been documented in China and other Asian countries, although the specific mechanism is still not very clear.

A polar fraction and four fractional polysaccharide extracts from *Ganoderma applanatum* were tested for angiogenesis modulating effects using the chick embryo chorioallantoic membrane (CAM) assay. One-day old fertilized chicken eggs were treated with three concentrations of the *G. applanatum* extracts in three replicates. One square centimeter "windows" were aseptically created, by removing shell materials within specified areas. Two to three milliliters of albumen was removed using sterile pipettes to expose the CAM, which was then treated with 0.1 ml of the

extracts. The "windows" were sealed with 16-ply sterile gauze taped onto the shell and the eggs incubated at 37°C until the seventh day. Semi-quantitative scoring was done by removing shell materials from the periphery of the "windows" and comparing the degree of vascularization with the control.

The polar fraction exhibited a significant angiogenic stimulation whereas three out of four fractional polysaccharide extracts showed significant inhibition of angiogenesis as analyzed using Scheffe's post hoc test and t-test. These differential effects seem to demonstrate the presence of more than one type of bioactive components in *G. applanatum*. It is therefore possible that one mechanism for the anti-neoplastic effect of this shelf fungus' extract is through angiogenesis modulation. Tumors cannot thrive without abundant blood supply.

Keywords: Angiogenesis, chorioallantoic membrane, polysaccharide, *Ganoderma applanatum*

BSD No. 26

GEOMETRIC MORPHOMETRIC APPROACHES TO THE DISCRIMINATION OF STOCK POPULATIONS OF FRESHWATER COMMERCIAL FISHES FROM BALOI LAKE IN LANA DEL SUR

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The truss network in fishes has been used as a fisheries management tool effective in the description of morphological variation and identification of stocks, thus improving the biological basis of management. Traditionally, this is analyzed and compared by collecting a series of measurements between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form using a vernier caliper. In this paper, however, the truss networks in five freshwater commercial fishes are analyzed using the methods of geometric morphometrics, which includes the generation and analysis of thin-plate spline transformation grids, partial warp scores and interlandmark distances. This study aimed to present an alternative computerized approach in the collection and multivariate analysis of fish truss network morphometric data.

A total of two hundred forty-four (244) individuals were sampled. Sample sizes for each species are as follows: *Glossogobius* sp. (n = 40), *Hypseolotris agilis* (70), *Anabas testudineus* (42), *Puntius tumba* (69), and *Cyprinus carpio*

carpio (23). These fishes were scanned at 600 dpi. Then, a total of fifteen landmarks that are distinctive and homologous from specimen to specimen were selected around the outline of the fish form. The X-Y coordinate value for the positions of landmarks is digitized for each fish with the use of the *ScionImage* image digitizing and processing software. The X-Y coordinate data is then transformed into linear distances by computer using the Euclidean Distance Matrix Algorithm (EDMA). This transformed data was then used as morphometric variable for multivariate methods of statistical analysis. Such as principal component analysis, discriminant function analysis and cluster analysis.

The original coordinate data were also used to compare shape differences among the different species. Because, all samples for all populations were taken from different ages, there is a need to eliminate the size effect in the data set. This was done by subjecting the coordinate data to procrustes fitting. Then, the information contained in the procrustes-fitted coordinates were used to reconstruct the truss networks of individual fish samples using thin-plate spline transformation grids. Morphological variations within and among the different species were summarized using PCA and other multivariate methods of statistical analysis. Global and localized variations were also determined through the analysis of partial warps.

This study showed variations in the sizes and shapes of the individuals within and among the different species as evident from the thin-plate spline grids and supported by the results of the PCA and dendrogram generated through Cluster Analysis. Discriminant analysis showed that the identification accuracy was between 93% and 100%, and global identification accuracy was 98.6%. Morphological differentiation was shown to vary between the sexes in all fish species as a result of sexual dimorphism. This suggests that females and males should be treated separately in truss network analyses to remove the effect of sex from the result. The results of this study proved the usefulness of geometric morphometric approaches to the study stock populations of freshwater fishes, which are powerful in reconstructing and comparing the truss networks of fishes.

Keywords: truss network, geometric morphometrics, cluster analysis, discriminant analysis

BSD No. 27

BIOLOGY OF *Eocanthecona furcellata* (Wolff) AND ITS PREDATORY CAPACITY AGAINST CORN BORER (*Ostrinia furnacalis*)**Somkid Nuchphun and Teotimo M. Aganon***

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The biology of *Eocanthecon. furcellata* was studied under laboratory conditions. The insect oviposition period ranged from 20 – 29 days with an average of 23.4 ± 3.657 . The female laid eggs ranging from 7 to 104 ($X = 72.88 \pm 25.11$).

The egg incubation period was 5.20 ± 0.837 days. The nymph molted five times with a total nymphal stage days of 18.84 ± 2.160 broken down as: 1st instar, 2.92 ± 0.846 ; 2nd instar, 3.56 ± 0.501 ; 3rd instar, 3.46 ± 0.503 ; 4th instar, 3.50 ± 0.496 and 5th instar, 5.40 ± 0.496 . The total life cycle was 24.52 ± 1.787 days. The adult male lived within a range of 36.40 days after emergence whereas the female lived 42.49 days after emergence.

The total predatory period from the second nymphal instar to death of adult was 45.23 days.

Functional response of adults as predator on second and third larval instars of *O. furnacalis* increased with an increased in prey density. The same trend was observed on the predatory capacity of the nymphal stages. Adults predatory capacity is higher than the nymphal stages.

The results showed that *E. furcellata* was not a good predator if the corn borer density is high, whereas if the corn borer density is low, *E. furcellata* could be a good predator.

Keywords: *Eocanthecona furcellata*, corn borer, *Ostrinia furnacalis*, biological control

BSD No. 28

FIGHTING BEHAVIOR OF THE PHILIPPINE DERBY SPIDER***Neoscona punctigera* (Doleschall)****Aimee Lynn A. Barrion**

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Filipinos are game-loving people. They have managed to utilize an assortment of animals in their games and leisures like cockfighting, horse racing, dog racing, carabao fighting and spider derby. In most spider games, females of the species *Neoscona punctigera* (Doleschall) appear to be commonly used. However, the predatory nature of this spider is yet to be explored. Thus, this study provides preliminary documentation and explanations for spider fighting behavior. A wooden T-shaped rod with a catch at the bottom was used as a fighting arena. Spiders whose body sizes ranged from 13 – 15 mm were grouped as the large mature females while those whose body sizes ranged from 10 – 12.5 mm were designated as small mature females. Reproductive status was determined by observing the epigynum. Oviposited spiders were allowed to fight 2 days after oviposition. Complete sequential steps of the fights were observed, described and recorded in video. Details of the spider matches were presented in kinematic graphs. Matches involving spiders of different sizes showed that larger spiders win in all encounters that resulted in actual combat (55 of 75 matches). Only in 21 of the 75 total matches between two large female spiders actually resulted in an encounter. On the other hand oviposition status did not affect the winning chances of the spider. In view of these observations, two hypotheses on the origins of fighting behavior of derby spiders were proposed. One is that the aggressive behavior of derby spiders is due to maternal care, while the other is linked to territoriality.

Keywords: behavior, derby, epigynum, kinematic graphs, *Neoscona punctigera* (Doleschall), matches, oviposition, spider

BSD No. 29

**ALTERNATE HOSTS OF CORN EARWORM, *Helicoverpa armigera* (Hb.)
AND CUTWORM, *Spodoptera litura* (F.) IN ONION REFUGIAS**

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This study was conducted from 2002-2005 in four towns of Nueva Ecija which are the main onion growing areas in the country. It aimed to identify alternate host plants corn earworm, *Helicoverpa armigera* (Hb.) and cutworm, *Spodoptera litura* (F) are major pests of vegetables and grains in the Philippines in onion refugias of Laur, Gabaldon, Pantabangan, and Bongabon towns.

The presence of these two pests was done through ocular observation on the boundaries of onion fields where weeds abound. The results showed that the following species harbor these two pest: *Amaranthus spinosus* L., *Stachytarpheta jamaicensis* (L) Vahl., *Polygonum barbatum* L., *Cassia tora* L., *Melochia corchorifolia* L., and *Chromolaena odorata* L.

Onion farmers apply sixteen times spraying on their fields to control these pests. Knowledge of alternate host plants or where to find these pest can lessen pest management cost by directing control measures on refugias where these alternate host and these two larval pest are present. Hence, population build up of these pest that infest onions is reduced.

Keywords: refugias, corn earworm, cutworm, indicator plants, onion, larvae

BSD No. 30

**ONION MAGGOT/FLY, *Delia antiqua* MEIGEN (DIPTERA:
ANATHOMIXIDAE); A NEW PEST OF ONION IN THE PHILIPPINES**

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Since 2001 to date onion farmers in Pangasinan complained of damages in their onions described as root rot of seedlings and bulb rot. The onion leaves become brown and eventually dry up.

Field collected damaged bulbs from Amancosiling, Bayambang, Pangasinan were brought to the Plant Quarantine Support Laboratory, UP Los Baños in 2004-2005 cropping season. Each bulb with visible larva was separated and these were reared in the laboratory. Emerging adults were put in cyanide bottles. These adults were pinned and stored in standard insect boxes. Collection of adults through sweep net was also done in infested fields. The adults onion fly were separated and stored in insect boxes.

The male is large (6 – 7 mm in length), with densely grey to yellow-grey dust on thorax and abdomen. The eggs are about 1.25 mm long white and elongate-ovoid, resembling grains of rice. The larvae are typical elongate creamy-white maggots, tapering towards the head end and truncate at the posterior end where two breathing spiracles are situated. The pupae are ovoid or seed-shaped, reddish-brown. The posterior spiracles are situated on small bosses. Adults are slender yellowish-grey flies, 6 – 7 mm in length. The signs of attack are yellowing and wilting of the outer leaves. Green and apparently healthy leaves will become flaccid, and the whole plant may collapse. Later generations of larvae tunnel into the onion bulbs as well as attack the roots. This new pest of onion is prevalent in onion growing areas of Pangasinan such as Alcala, Bayambang Basista and nearby towns. It has also been found in Nueva Ecija and Nueva Viscaya onion areas.

Keywords: Onion maggot/fly, New Pest, Onion, *Delia antiqua*

BSD No. 31

**PHILIPPINE PREDATORY MITES OF THE GENUS *CHEIROSEIUS*
(ACARI: ASCIDAE)****Ireneo L. Lit, Jr.^{*1,2} and Leonila A. Corpuz-Raros^{2,3}**¹Department of Forest Biological Sciences
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A taxonomic study of Philippine mites belonging to the genus *Cheiroseius* (Acari: Ascidae) was conducted. This is a group consisting of predators of soil arthropods and other small animals like nematodes and are, therefore, potential biological control agents against soil-inhabiting pests. Habitat-wise, they can be found in soil, decaying plant litter and mosses. From only three previously reported species, namely: *Cheiroseius curtipes* (Halbert), *C. nepalensis* (Evans & Hyatt) and *C. serratus* (Halbert), there are now 11 species, including four described as new to science and four new faunal records. Two of the new species are from Mount Banahaw de Lucban, coded *Cheiroseius* spp.nov. BDL1 & BDL2, and the two others are from Polillo island, coded *Cheiroseius* spp.nov. POL1 & POL2. BDL1 is unique in having a prominent dorsal shield, so produced as to form a “humpback” effect, whereas BDL2 has very unusual ornate genital shield. POL1 has intricate reticulate patterns on the dorsal shield and POL2 has scale-like patterns on the sternal and genital shields. The four new records are *C. brownigi* (Evans & Hyatt), *C. cassiteridum* (Evans & Hyatt), *C. phalangioides* (Evans & Hyatt) and *C. politulus* Tseng. A key to Philippine species, descriptions and illustrations are provided.

Keywords: Ascidae, Acari, *Cheiroseius*, mites, predatory mites, soil-litter fauna

BSD No. 32

**THE PHILIPPINE ENDEMIC STICK-INSECT GENUS *OBRIMUS* STÅL, 1875
(PHASMATODEA: HETEROPTERYGIDAE: OBRIMINI)**

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Studies on Philippine stick insects have recently gained considerable interest from many parts of the world, especially with the recent tide of discoveries of new species and genera. Among Philippine stick insects, the tribe Obrimini stands out as being endemic at a suprageneric level. As a contribution to the systematics of this group and to the documentation of Philippine terrestrial arthropod biodiversity, a taxonomic review of the genus *Obrimus* Stål, 1875 was conducted. Six species, including two possibly new ones, are described and illustrated. They are *Obrimus bicolanus* Rehn & Rehn (Isarog), *Obrimus bufo* (Westwood), *Obrimus* sp.1 ex Northern Sierra Madre, *Obrimus* sp. 2 ex Southern Luzon, *Obrimus mesoplatus* (Westwood) and *Obrimus uichancoi* Rehn & Rehn (Greater Sipit Watershed, Mt. Makiling). The differences of these species lie in the pattern of their armature which consists mainly of spines and tubercles. All species are of narrow endemic distribution and may be possible candidates for listing as threatened or vulnerable species, considering the rapid destruction of forest habitats and their current status as among favorites of hobby collectors in Europe and other advanced countries.

Keywords: Heteropterygidae, Obrimini, *Obrimus*, Phasmatodea, Philippine endemic arthropods, stick insects, terrestrial arthropod biodiversity

BSD No. 33

BACTERIAL INHIBITION OF *Aspergillus flavus* GROWTH AND AFLATOXIN BIOSYNTHESIS: *Bacillus amyloliquifaciens* ET2004, A POTENTIAL BIOCONTROL AGENT

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Damage to crops by fungal agents like *Aspergillus flavus* and *Aspergillus parasiticus* not only lower their yield and quality but also continue to pose health hazards to consumers as a consequence of aflatoxin production. While genetic modification bestows resistance against pathogenic fungi, the use of appropriate biological control agents may also provide an equally effective alternative.

Five pre-selected bacterial isolates (*Staphylococcus epidermidis*, *Agrobacterium radiobacter*, *Bacillus megaterium*, *Enterobacter aerogenes*, and *Bacillus amyloliquifaciens* ET2004) were screened for inhibition of growth in *Aspergillus flavus* and/or inhibition of aflatoxin biosynthesis. Replicated co-cultures on potato dextrose agar (PDA) plates, which supported the growth of the fungus and bacteria, were prepared by parallel streaking. The plates were incubated at 30 °C and examined for fungal overgrowth on the bacterial streak after 48 h.

Inhibition of aflatoxin biosynthesis was determined by co-cultures in potato dextrose broth (PDB). One milliliter of 10^6 to 10^7 bacterial cells/ml and 56 ml of sterile water-suspended *A. flavus* conidia (10^7 to 10^8 /ml), were mixed in four milliliters of PDB and incubated with shaking initially at 37 °C for day one and at 30 °C for the next six days. The cultures were centrifuged at 3,000 rpm for five min. The supernatants were collected aseptically and analyzed for aflatoxin content using Competitive-Direct Enzyme Linked Immunosorbent Assay (CD-ELISA) with the VeratoxÔ System (Glenwood Technologies).

Results show significant fungal growth inhibition exhibited by *Bacillus amyloliquifaciens* ET2004, but not by the other bacterial isolates. Interestingly, no aflatoxin was detected in the latter tubes although substantive growth of *A. flavus* was evident. Growth inhibition may be attributed to the production of subtilisin by *B. amyloliquifaciens* which is known to degrade proteins extracellularly. Presence of fungal growth without the concomitant production of aflatoxin may indicate the synthesis of cell products that specifically inhibit the aflatoxin biosynthetic pathway, but not growth.

B. amyloliquifaciens is not known to exhibit pathogenicity or toxigenicity, and is therefore a very promising candidate for the biocontrol of fungal invasion of crops. Pending verification, the other pre-selected bacterial isolates may be utilized for targeting only aflatoxigenic fungi, while leaving unaffected other fungi that may play important ecological roles.

Keywords: *Aspergillus flavus*, *Bacillus amyloliquifaciens* ET2004, aflatoxin, subtilisin, biocontrol

BSD No. 34

MITOCHONDRIAL DNA ANALYSIS OF GENETIC INTROGRESSION IN SELECTED PHILIPPINE CATTLE POPULATIONS

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For the future improvement of beef cattle breeding programs in smallholder farming systems, mitochondrial DNA (mtDNA) displacement loop (D-loop) sequence variation was examined to supplement existing basic information on the genetic composition of Philippine Native cattle. To evaluate the degree of genetic introgression in native cattle populations representing the Ilocos, Batangas, Iloilo and Philippine Native types, a fragment of the hypervariable region in the mitochondrial D-loop was amplified and sequenced from a total of 100 individuals used in a previous study on autosomal microsatellite variation. Multiple sequence alignment with published D-loop sequences of foreign pure breeds and subsequent phylogenetic analysis revealed three major clades of zebu (*Bos indicus*), taurine (*B. taurus*) and banteng (*B. banteng*) maternal ancestry. Individual sequences did not cluster significantly into geographic groups, providing evidence of migration between populations though trade relationships between these regions. However, haplotypes of Native cattle were distinct from those of the foreign breeds, implying the evolution of the Philippine populations from their foreign ancestral populations. Patterns of mitochondrial DNA variation indicate that the Ilo-ilo type was of taurine ancestry with zebu genetic introgression, and the Philippine-Bali type was of zebu ancestry with banteng introgression. The hybrid zebu-taurine composition of mtDNA from the Ilocos and Batangas populations confirmed the results of previous

autosomal microsatellite analysis. Molecular characterization based on combined mitochondrial and microsatellite analysis provides a clearer perspective on the genetic composition of Philippine cattle populations and showed the potential of existing populations in the geographically-isolated islands of the Visayas for future genetic conservation and breed development programs.

Keywords: cattle, mitochondrial DNA, D-loop, genetic diversity, Philippine Native breed

BSD No. 35

**SEQUENCE ANALYSIS AND CHARACTERIZATION OF
THE MOVEMENT PROTEIN-ENCODING COMPONENT OF ABACA
BUNCHY TOP NANOVIRUS (ABTV)**

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Abaca bunchy top nanovirus (ABTV) is the most important viral pathogen of abaca. The virus causes stunting and significant reduction in yield. In this study, the DNA component of abaca bunchy top nanovirus (ABTV) coding for the movement protein was isolated and characterized. Oligonucleotide primers based on published sequence of a related virus, banana bunchy top virus (BBTV), were used for PCR amplifications. Total DNA from infected leaves was extracted from different ABTV isolates using a modified CTAB extraction method. A 380 bp fragment was amplified, cloned and sequenced. Sequence analysis showed that the movement protein gene includes regions that codes for hydrophobic protein. The nucleotide sequences were compared with published BBTV sequences and showed 94 to 96% nucleotide identities with isolates from Australia and Asian countries.

Keywords: abaca, movement protein gene, abaca bunchy top nanovirus

BSD No. 36

THE RELATIONSHIP BETWEEN ABACA BUNCHY TOP NANOVIRUS (ABTV) AND BANANA BUNCHY TOP NANOVIRUS (BBTV) BASED ON SEQUENCE ANALYSIS OF DNA-3 AND DNA-4 COMPONENTS

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Bunchy top is the most devastating disease of both abaca and banana caused by abaca bunchy top nanovirus (ABTV) and banana bunchy top nanovirus (BBTV) respectively. ABTV is assumed to be synonymous to the bunchy top virus that infects banana but this has never been confirmed. BBTV is considered to be a variable virus with strain-complexity. Previous reports on transmission experiments showed dissimilarity between these two viruses, although they exhibited strong serological relationship. In this study, a comparative analysis was made on the nucleotide sequences of DNA-3 and DNA-4 components of both BBTV and ABTV. These two DNA components contain the major ORFs encoding for the coat protein and movement protein. Primers targeting a 586 bp gene fragment of the DNA-3 component and a 380 bp gene fragment of the DNA-4 component were used for PCR amplifications. The resulting PCR products were cloned, sequenced and subjected to pairwise alignments. The mean sequence difference between BBTV and ABTV was 4 to 7% for the DNA-3 fragment and 2 to 4% for the DNA-4 fragment. Comparisons were also made for specific regions of the ORF and the encoded amino acids. The low sequence heterogeneity obtained for the two major ORFs suggests that these two viruses are strains or biotypes and not distinct Nanovirus species.

Keywords: abaca, banana, abaca bunchy top nanovirus, banana bunchy top nanovirus

BSD No. 37

SHELL SHAPE CHANGES IN NINE SPECIES OF MARINE, FRESHWATER AND LAND SNAILS USING SUPERIMPOSITION AND THIN-PLATE SPLINE ANALYSIS OF LANDMARKS IN DIGITIZED IMAGES OF SHELLS**Rizalyn T. Borra and Cesar G. Demayo**

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Morphometrics involves the quantitative study of form. Form is intuitively understood that is consisting of size and shape which are never biologically independent but are instead inextricably interrelated. In this study continuous data was obtained to assess variations in the form of the shell of the snail. This was determined by employing geometric Morphometrics, a method used to obtain detailed shape information. In general, geometric morphometric methods provide greater power than the traditional methods because the position of the landmarks can be retained and can be graphically reconstructed. Meaning, it preserves geometry of object studied and it allows visualization of shape differences between specimens and between group means in specimen shape thus was used in the study. Shell shapes of a marine snail *Marmorostoma chrysostomum*, freshwater snails namely *Pila polita*, *Belamaya angularis*, *P. pomacea*, *Pseudosuccinae peregrina* and an unknown species (designated as "unknown sp. e") and three species of land snails namely *Achatina fulica* and two unknown species (designated unknowns b and c) were used since the shell of this group is either spherical or heliciform or elongate ovate having three to five sutures with wide oval or circular aperture. It has no siphonal canal and the outer lip of the aperture is not reflected. These nine species have twenty-one (21) homologous landmarks identified thus were the bases for their use in this study. Intraspecies and between species shape variations were examined using Procrustes superimposition and thin-plate spline (TPS) analysis to examine local and global sources of variations in shapes. Results of Procrustes superimposition showed the landmark points in all the snails fall on the same location and the general shape of the shell structure was determined. Distinct differences can now be determined and variations in the deformation between the species can be visually detected. Procrustes analysis was therefore an important means of shape comparison because it removes or eliminates the differences in rotation, translation and scaling of forms. After the landmark configurations were superimposed, residuals were modeled with the Thin-Plate Spline (TPS) transforming one coordinate system into another. The parameters of the TPS transformation were used to explore patterns of shape changes between objects. Shape changes as deformation within species of snail's shell included in this group was made possible by fitting an interpolation function to the aligned

landmark coordinates of each specimen against the reference configuration, so that all homologous landmarks coincide. Variations between species were observed as the shells vary in landmark points where local and global deformations have occurred. These can easily be observed from the graphical presentation of the changes in individual shapes.

Keywords: *Superimposition, Thin-plate Spline Analysis, Geometric Morphometrics*

BSD No. 38

SIZE AND SHAPE VARIATION IN POPULATIONS OF *Achatina fulica* AND *Amphidromus maculiferus*: A MULTIVARIATE MORPHOMETRIC APPROACH

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Size and shape variation in selected populations of the land snails *Achatina fulica* and *Amphidromus maculiferus* was determined using landmark- and distance-based morphometric analysis. Three hundred thirteen *A. fulica* and ten *A. maculiferus* were sampled from Mt. Agad-agad, Iligan City and from Balangao, Zamboanga-Sibugay. All samples were scanned at 600 dpi with the snails carefully oriented with the aperture resting on the bed of the scanner. The two land snail species have obvious spiral ornamentation which allows determination of biologically meaningful landmarks, of which twenty-two points were digitized using the ScionImage software. This study used distance variables based on the twenty two landmarks with an objective of comparing the sizes of the different samples. Distances between points were calculated using the Euclidean Distance Matrix Algorithm. The measurements include the shell's total length, which measures the maximum distance parallel to the axis of coiling from the shell apex to the basal apertural edge; total width of the snail shown as the maximum distance perpendicular to the axis of coiling; height of the spire which is also the distance from the apex to the aperture-suture junction; distances between the included major spiral chords; and the angulations on the corner of the aperture. These measurements give a balanced overview of the shape of the snail's shell, with detailed representation of the whorls characteristic of each species. Principal Component Analysis (PCA) was done to determine components with defined biological meanings that account for the maximum variation of the snail samples. This multivariate method allows numerous variables to be collected, and the significant fine-scaled variation to be

extracted. Loadings for each variable in each principal component represent that variable's contribution to the variance explained by that principle component. Cluster analysis was also employed to group snails with similar sizes together. Then, test for significant differences among the snails was done using the Discriminant Function Analysis (DFA). Shape differences among the different snail samples was determined by subjecting the raw x and y coordinates of the twenty two digitized landmarks to Procrustes fitting. This procedure eliminates both rotational and size translation allowing for the analysis of snail shape variation only. The Procrusted-fitted coordinate values were also subjected to PCA, Cluster analysis and DFA.

Results showed no significant size differences among the snail samples from the two locations based on the computed interlandmark distances. A variation in shell shapes between the two species is evident from the reconstructed images of the snails. PCA of the Procrustes-fitted values returned a total of thirty-six significant components with the first component explaining ninety-eight percent (98%) of the observed variation. This variation is summarized in a scatter plot with the Zamboanga population shown to have broader apices, more expansive aperture and wider spire base. Cluster analysis of the Procrustes-fitted values separates the three populations into three well-defined groups. DFA of the data reveals that the variations observed are statistically significant. These results are further discussed in the light of ontogenetic allometry and parasite-induced changes in the morphology of the snail.

Keywords: procrustes analysis, superimposition, multivariate morphometrics

BSD No. 39 ✓

**HOMOSEXUAL ORIENTATION IN FILIPINO GAY MEN IN RELATION TO
RELATIVE LENGTH OF THE SECOND AND FOURTH FINGERS (2D:4D
RATIO), DEVELOPMENTAL INSTABILITY AND FRATERNAL BIRTH
ORDER**

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Variations between homosexual male, heterosexual male and heterosexual female populations were assessed based on relative lengths of the second and fourth fingers (2D:4D ratio), fraternal birth order and developmental instability.

Our study on homosexual and heterosexual 2D:4D ratios revealed high heterosexual male 2D:4D ratio than homosexual men. Comparison of the frequency distribution of the 2D:4D ratios also showed that for most homosexuals, the ratio is lower than of the heterosexual men. It was shown that homosexual men's ring finger lengths were shorter than heterosexual men's. The index fingers however show that homosexual men's index finger lengths did not differ from heterosexual men. It is suggested from this study that there is a correlation between homosexual orientation and the ratio of relative length of the second and fourth fingers of the hand thus confirming previous reports.

Many other studies on sexual orientation in humans also argued to be possibly influenced by levels of prenatal sex steroids which canalize neurodevelopment along sex-typical (heterosexual) or sex-atypical (homosexual) lines. We measured developmental instability by subtracting the left from the right value of the nineteen (19) bilaterally symmetrical traits (FA) among one-hundred (100) homosexual males, fifty (50) heterosexual males and fifty (5) females. These include digits on the right and left hands and feet, ears and face, lengths and width of right and left hands and feet. It is argued by many researchers that higher FA values indicate higher incidence of developmental instability. Seven (7) fluctuating asymmetry (FA) indices were used. The normality of the frequency distributions was evaluated through frequency histograms that test various aspects of distributions such as skewness, leptokurtosis and platykurtosis. Results showed variations between males, females and homosexuals based on FA values. However, homosexual males exhibited the highest FA values in all the indices used indicating high developmental instability in homosexuals than heterosexual males and females which showed low FA values.

Many earlier studies have repeatedly shown that older brothers increase the probability of homosexuality in later-born males. The collective findings suggest that birth order is perhaps the single most reliable demographic difference between homosexual and heterosexual men.

We conducted a study in 2004 and 2005 involving one-hundred seventy five (175) homosexuals and 100 heterosexual males assessing their fraternal birth order. The odds and probability of homosexuality as a function of number of older brothers and attributable fraction with zero (0) to n number of older brothers were computed. The attributable fraction, an index referring to the difference between the prevalence of homosexuality among men with one older brother and the prevalence among men with no older brothers calculates how much of the probability is attributable to fraternal birth order. The results of the study showed there is an increase in the probability of being a homosexual as the number of older brother increases. The results of the current study confirmed earlier reports that the older the brothers a boy has, the more likely he is to develop a homosexual orientation.

Keywords: fraternal birth order, 2D:4D ratio, developmental instability, fluctuating asymmetry

BSD No. 40

SCREENING FOR AMPICILLIN RESISTANCE GENES FROM *VIBRIO HARVEYI* NBRC 15634 AND PHILIPPINE *VIBRIO* ISOLATES**Prima Fe R. Franco^{1,2} and Cynthia T. Hedreyda¹**

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Vibrio harveyi, the bacterium implicated in luminous vibriosis, causes mass mortalities in hatchery-reared larvae of the black tiger shrimp *Penaeus monodon*. Antibiotics are commonly used in hatcheries and grow-out ponds as prophylactic agents for the prevention of larval bacterial infection. *V. harveyi* isolates from moribund shrimps are resistant to antibiotics. The ability of such isolates to develop resistance to antibiotics like ampicillin was explored in this study.

Preliminary results of our studies showed that a strain of *V. harveyi* becomes resistant to ampicillin when transformed with pUC18 with ampicillin resistance. Confirmation of the transformation process, however, showed that the genomic DNA of the untransformed strain contains an ampicillin resistance gene. This was done via Polymerase Chain Reaction (PCR) using primers designed based on the ampicillin resistance genes in pUC18. The presence of the gene in the reference and some Philippine *Vibrio* isolates was observed when expected amplicons approximately 700 bp were obtained. Optimized PCR conditions for 30 cycles include initial denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, elongation at 72 °C for 1.5 min and final elongation at 72 °C for 5 min. The PCR products were purified using NucleoSpin purification kit and submitted for sequencing. BLAST search results show that the obtained genes were 98% similar to the ampicillin resistance gene in pUC18.

Keywords: *Vibrio harveyi*, Philippine *Vibrio* isolates, ampicillin resistance genes

BSD No. 41

Relative Warp Analysis to Explore Scapular Shape, Mandible and Pelvic Girdle in Fruit and Insectivorous Bats

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One method that can be powerful in establishing structure-function relationships is the use of relative warp analysis of digitized images. Relative warp analysis is essentially a principal component analysis of the covariance matrix of partial warp scores produced by thin-plate splines. The technique produces the power of thin-plate splines to produce a visualization of morphospace via the transformation grids. We elucidated the functionality of this method to explore scapular, mandibular and pelvic girdle shapes in fruit- and insectivorous bats. Relative warp analysis can provide both a descriptive and quantitative description of shape. Adult specimens of bats that were accidentally caught and injured in sampling by researchers and by fruit farmers who consider the bats as pests in their farms and from the museum were processed and the images generated by digitization at 600 dpi using high resolution computer scanners. Ten (10) landmark coordinates of the mandible, eleven (11) from the scapula and twelve (12) from the pelvic girdle were identified and transported in a processing software providing translation, rotation, and resealing of landmarks with reference to a specified baseline. Mean shapes for each species were determined by averaging shape coordinates for each landmark and these means were imported to a paleontological statistics software developed by Hammer in 2002 for relative warp analysis. The mean shapes for each species were compared to the reference form to generate partial warps on the same partial warps. Shape differences for each species were then revealed by examination of associated partial warps in the transformation grids. A model for the deformation of one shape to another was then made possible by superimposition method calculating the "best fit" between the shapes. A UPGMA (unweighted pair group method using averages) cluster analysis was performed on the resulting matrix to explore patterns of shape in the scapula, mandible and pelvic girdle.

Results of relative warp analysis of the mandibles of the bats show that the evolutionary shifts in diet are reflected on the changes in mandibular shape especially in the coronoid process and angle of the jaw. Significant differences in shapes of the mandible between species, families and between the frugivorous and insectivorous group were observable in these regions. Higher coronoid process

can be found in jaws of hard object diet specialist such as frugivorous bats and are reduced in the insectivorous bats. The scapular changes within and between groups of bats were similar and were characterized by the enlargement of the teres major process, a landmark that serves as attachment of the teres major muscle which is very important in flight. This result is expected since all the bats are strong flyers. For the pelvic shape, results show major changes in the posterior interior spine, dorsal margin of the crest of the ilium, anterior margin of the ilium, ventral margin of the crest of the ilium iliopectineal eminence, both anterior and posterior margins of the obturator foramen. This result on the pelvic girdle variations reflects group status in bats. The shape differences in these landmarks also indicate the launching differences between the insectivorous and frugivorous bats. The results of this study indicate that new methods like the relative warping method in morphospace studies can be of prime importance in establishing structure function relationships in living organisms.

Keywords: *relative warps, frugivores, insectivores*

BSD No. 42

GENETIC ANALYSIS OF *Allium* ALLIINASES BY SEQUENCE ALIGNMENT AND PHYLOGRAM CONSTRUCTION

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Bioactive compounds in garlic (*Allium sativum*) and other *Allium* species originate from thiosulfinates such as allicin, which are produced by the action of the enzyme alliinase (EC 4.4.1.4) on cysteine derivatives, through cleavage of carbon-sulfur bonds. In this study, sequences of alliinases from member species of the genus *Allium* were used in order to describe phylogenetic relationships among *Allium* plants. Using BLAST (Basic Local Alignment Search Tool) searches on NCBI (the USA National Center for Biotechnology Information) databases along with searches on MapViewer and ExPASy (Expert Protein Analysis System) servers, alliinase and alliinase-related sequences within the genus *Allium* (*A. ascalonicum*, *A. cepa*, *A. chinense*, *A. fistulosum*, *A. giganteum*, *A. sativum*, *A. schoenoprasum*, *A. tuberosum*, and *A. wakegi*) were obtained and analyzed. By multiple sequence alignments using ClustalW and various distance matrix computations, cladograms and phylograms were constructed to characterize the

genetic distances among *Allium* species. Ninety nine (99) residues were found to be identical in all sequences used in the alignment. Sixty five (65) conserved substitutions and 43 semi-conserved substitutions were observed. The phylogram, a branching diagram (tree) assumed to be an estimate of a phylogeny wherein branch lengths are proportional to the amount of inferred evolutionary change, clustered the alliinase sequences from *A. fistulosum*, *A. giganteum*, *A. sativum*, *A. tuberosum* under one clade, while the *A. cepa* and *A. wakegi* alliinases were clustered separately. Molecular structures of the various alliinase sequences were also generated using Swiss-PDB Viewer or Deep View, which showed similar patterns of the arrangement of the component modules (a homodimeric structure with each monomer composed of the N-terminal domain including the EGF-like domain, followed by the central domain residues, and then the C-terminal domain). This study significantly contributes to our understanding of the genetic structure of alliinase expression among members of the genus *Allium*. Studies on the detailed structure of the conserved domains are required in order to elucidate further the evolution of the alliinase gene family.

Keywords: alliinase, *Allium*, alignment, phylogeny, genetic distance, garlic, onion, leek, chive

BSD No. 43

ISOLATION OF PLASMIDS FROM PHILIPPINE *NAEGLERIA* SP.

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Naegleria is a genus of free-living soil amoebae. One species, *Naegleria fowleri*, is opportunistically pathogenic in mammals and is the etiological agent of primary amoebic meningoencephalitis (PAM). The species is the third known eukaryotic organism with ribosomal RNA gene (rDNA) plasmid. The rDNA structure has been described in the strain of *Naegleria gruberi*; the genes carried on a 14-kilobasepair, circular, extrachromosomal DNA plasmid. Soil and water samples were obtained and tested for the presence of *Naegleria* through the enflagellation test and polymerase chain reaction (PCR). Samples were then subjected to plasmid extraction, and visualized using agarose gel electrophoresis (AGE). Bands with a

size of 14-kb were observed in all samples after AGE of the extracted plasmid. This study substantiates the presence of extrachromosomal plasmid in Philippine *Naegleria* species.

Keywords: *Naegleria*, ribosomal DNA plasmid, enflagellation test, polymerase chain reaction

BSD No. 44

**RESTRICTION ENZYME ANALYSIS OF THE 18SSU rDNA OF
PHILIPPINE *ACANTHAMOEBA* ISOLATES**

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Acanthamoeba, the most common protist found in soil, are a genus of small, free-living, and ubiquitous amoebae that exhibit a biphasic life cycle composed of vegetative trophozoite stage and a physiologically static cyst stage. They are the causative agents of *Acanthamoeba* keratitis (AK) and granulomatous amoebic encephalitis (GAE). However, despite its medical importance, the subgenus classification of *Acanthamoeba* is still problematic. Riboprinting is a promising method for the rapid identification of unknown *Acanthamoeba* isolates. It involves the use of polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) of the nuclear small subunit ribosomal RNA gene (SSU rDNA). Philippine *Acanthamoeba* isolates were collected from soil samples obtained from different geographic locations in the country. These were analyzed based on morphological characteristics as well as through the restriction enzyme analysis of 18S SSU rDNA. Majority of the isolates exhibited the group II cyst morphology. Data from riboprinting were analyzed using cluster analysis. Using this method, this study was able to prove that genetic diversity exists in Philippine *Acanthamoeba* isolates, but this diversity is independent of the location from where the isolates were obtained.

Keywords: *Acanthamoeba*, riboprinting, genetic diversity

BSD No. 45

**UTILIZATION OF PCR FINGERPRINTING FOR THE DISCRIMINATION
OF A PHILIPPINE HYPERVIRULENT *RALSTONIA SOLANACEARUM*
TOMATO STRAIN**

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Ralstonia solanacearum is the causal agent of the serious disease known as bacterial wilt affecting greater than 200 plant species. We are working on the characterization of a hypervirulent *R. solanacearum* substrain (T731) that wilted previously resistant tomato varieties compared to its wild type strain (T523). The presence of additional virulence factor might impact disease management. Different PCR- based DNA fingerprinting techniques were evaluated for differentiation of the hypervirulent mutant strain from the wild type strain. BOX and ERIC PCR were unable to differentiate the two strains while REP PCR and Pulse Field Gel Electrophoresis (PFGE) generated differential bands unique to the hypervirulent strain. Three rare cutting restriction enzymes used in generating the macrorestriction patterns in PFGE showed that strain T523 and T731 were very similar to each other except for one to three differential bands. Cloning and sequencing of a 414 bp REP PCR generated DNA fragment which was present only in the hypervirulent strain showed that the sequence is present in the megaplasmid of the sequenced *R. solanacearum* strain GMI1000, a tomato isolate from French, Guyana in three different portions. The highest homology was present at the 5' side of the transposase protein of the insertion element 13 (ISRso13). G+C content of the DNA fragment was 57%, as differentiated from the average G+C content of 67% in the rest of the megaplasmid and chromosome. *In silico* protein translation of the DNA fragment showed it was 61% identical to a hypothetical prjohnny depp otein in *Burkholderia cepacia*. The clone REP-PCR fragment might have a possible role in the hypervirulence of strain T731.

Keywords: *Ralstonia solanacearum*, DNA fingerprinting

BSD No. 46

**POTENTIAL OF *DESMODIUM CINEREA* AND MYCORRHIZAL FUNGI
FOR BIOREMEDIATION OF COPPER RICH SOILS****Joden M. Adiova¹, Nelson M. Pampolina¹ and Nelly S. Aggangan²**¹Bachelor of Science in Forestry Student
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This study was conducted to determine the effect of arbuscular mycorrhizal fungi (AMF) for the phytoremediation of copper (Cu) rich soils using *Desmodium cinerea* and to assess if this forage legume is safe for livestock grazing. Seeds of *D. cinerea* were sown in pots containing soil amended with increasing levels of Cu from nil to 400 ppm or from nil to 1600 ppm Cu in two experiments. Half of the seedlings were inoculated or uninoculated with AMF during sowing. In the third experiment, inoculated and uninoculated seedlings were planted in mine tailing soil collected in Marinduque with 82 ppm Cu. The experiments were established in a screenhouse following Randomized Complete Block Design with ten replicates. Height was monitored forth nightly while diameter, leaf area and biomass were measured at harvest (two months).

Generally, AMF inoculated plants were taller, heavier, greater leaf area and diameter than the uninoculated plants. In the first experiment, Cu concentrations of 100 to 200 ppm improved the growth of *D. cinerea* implying that this forage crop requires 100 to 200 ppm Cu for maximum normal growth. In the second experiment, highest height, diameter, leaf area and biomass were obtained from plants with no added Cu. Addition of Cu gave corresponding decreases in height, diameter, biomass and leaf area of inoculated and uninoculated plants. Cu level of 800 ppm seemed to be critical for both inoculated and uninoculated plants because beyond this level, leaf chlorosis, stunted growth and early leaf fall were observed although these were more evident in uninoculated plants. Furthermore, the roots were confined in the original potting soil. Seedling survival rate at 1600 ppm Cu was 30% in the uninoculated while 70% with inoculation. In mine tailing soil, mycorrhizal *D. cinerea* survived throughout the two months period whereas all the uninoculated ones died within one month. The results show that AMF alleviated Cu toxicity of *D. cinerea*, although it is unclear whether AMF does this by improving nutrient absorption or by binding mechanisms. Plant tissue analyses indicate that transport

of Cu within the plant is prevented by the presence of mycorrhizal fungi, thus, *D. cinerea* planted in Cu mine sites is safe for livestock.

Keywords: Arbuscular mycorrhizal fungi, *Desmodium cinerea*, copper, bioremediation

BSD No. 47

**ANTIBODY-BASED DETECTION OF MONODON
BACULOVIRUS (MBV) IN *Penaeus monodon***

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Spherical baculovirosis due to monodon baculovirus (MBV) infection is one of the crustacean diseases listed by the Office International des Epizooties (OIE) that is of social and economic importance. In the Philippines, monodon baculovirus (MBV) is a prevalent virus in the aquacultured tiger shrimp, *Penaeus monodon*. Although DNA-based techniques, such as the polymerase chain reaction (PCR), had been very useful in screening broodstock and postlarvae these techniques require sophisticated equipments and very skilled staff in very clean laboratories, which cannot be established easily in rural shrimp farm areas. On the other hand, antibody-based methods could be developed in simple but field-friendly formats with comparable sensitivity and specificity. Therefore, these experiments were undertaken to produce polyclonal antibodies that could be used as a tool in antibody-based diagnosis of MBV infection in *P. monodon*.

Polyclonal antibodies (PABs) against MBV were developed by immunization of rabbits with purified virus. Using indirect fluorescent antibody test (IFAT), intense reaction was observed in hepatopancreatic impression smears and paraffin-embedded sections of MBV-infected postlarvae (PL-15 to 20) but not in smears and/or sections infected with hepatopancreatic parvovirus (HPV) and white spot

syndrome virus (WSSV). Similarly, immunohistochemical tests on paraffin-embedded sections showed positive coloration (brownish red to rose red precipitate) in MBV-infected cells not found in sections infected with either HPV or WSSV. Indirect ELISA revealed that the antisera could detect 4 to 100 ng of purified MBV. Overall, the PABs obtained in this study have potential applications in the rapid, sensitive and simple detection of MBV provided that further purification of the antisera is undertaken to further minimize some background reactions.

Keywords: Monodon baculovirus, MBV, polyclonal antibodies, IFAT, ELISA, immunohistochemistry, *Penaeus monodon*, immunodetection assays

CHEMICAL, MATHEMATICAL, PHYSICAL SCIENCES

CHEMISTRY

CMPSD No. 1

BIMOLECULAR REACTION RATE CONSTANT OF THREE-BODY SYSTEMS: AN APPLICATION TO TRANSITION STATE THEORY

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Transition state theory (TST), also known as Activated Complex Theory (ACT), pictures elementary reaction and presumably stated that there exists an activated complex having a higher Gibbs free energy in between the reactants and product. The sole basis of the transition state theory in determining the rate constant of bimolecular reaction is on the application of statistical mechanics to reactants and activated complexes. In this study, the potential energy surface (PES) for $H + HF \rightarrow H_2 + F$ (1), $H + HCl \rightarrow H_2 + Cl$ (2), and $H + CH_4 \rightarrow H_2 + CH_3$ (3) were theoretically