National Scientist Lourdes Jansuy Cruz:

From Rice to *Conus* Research — the Journey

by Evelyn Mae Tecson-Mendoza

In the mid 1970s, Lourdes J. Cruz published a seminal paper on the venom protein of the marine snail *Conus* in *Veliger*. This was followed by a series of papers on the feeding and mating habits of the *Conus geographus* in captivity, on the assay of the toxin, on the toxin proteins of different marine snails and on the basic chemical and molecular make-up of the toxins and their effects on the nervous system. In 1985, Dr. Cruz and co-workers reported in the prestigious journal *Science* that the venom of the marine snail called *Conus geographus* from the Philippine seas contains a cocktail of many peptides with various activities that affect the ion channels and receptors of the central nervous system. There were peptides that cause muscular paralysis, peptides that cause sleepiness or drowsiness, peptides that make the test animals jump! This discovery led to a long series of researches in the laboratory of Dr. Cruz in the University of the Philippines Manila and Diliman and in the laboratory of her collaborators especially Dr. Baldomero Olivera in the University of Utah. Moreover, it attracted researchers worldwide to study this goldmine of conotoxins and their potential practical and commercial applications in neuroscience and medicine.

The researches of LJ Cruz on the conotoxins have resulted in (a) the elucidation of the biochemical and molecular structure and properties of many types of conotoxins and their mechanisms of action, (b) their uses as tool or probe to study brain activities, and (c) the study of other types of conotoxins from other cone snails by other scientists. These have been published as research articles numbering 81 in renowned journals such as *Science*, *Proceedings of the National Academy of Science*, *Journal of Biological Chemistry*, *Biochemistry*, etc. and more than 20 chapters in books and proceedings.

In 1987, Dr. Cruz was elected to the National Academy of Science and Technology (NAST). In May 2006, the NAST membership endorsed the conferment of the National Scientist rank and title on Academician Cruz to Philippine President GM Arroyo. In November 2006, Academician Lourdes J. Cruz was conferred the order of National Scientist, the highest honor bestowed by the Republic of the Philippines on a Filipino scientist for his/her outstanding achievements and contributions in science and technology.

A recipient of two international awards—the 1993 Sven

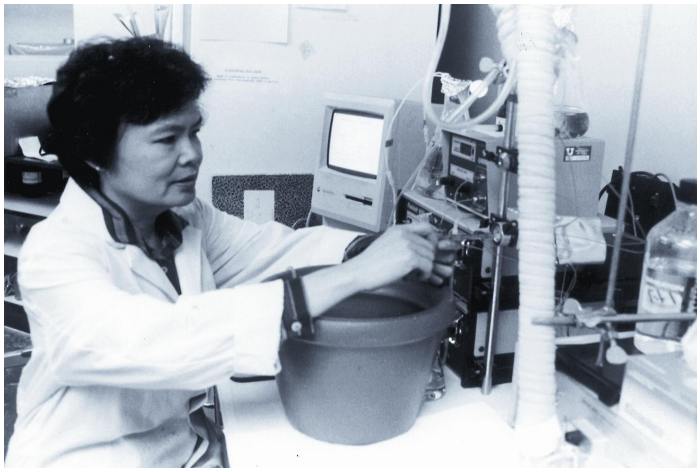
Dr. Evelyn Mae Tecson-Mendoza (EMTM) is a member and present secretary of the National Academy of Science and Technology and Research Professor and UP Scientist III of the University of the Philippines Los Baños. She has known National Scientist Lourdes J. Cruz since 1968 when they worked in the same lab in IRRI, EMTM as a research assistant and LJC as an assistant scientist. Upon completion of her PhD in Biochemistry at the University of Massachusetts, EMTM joined the UP College of Medicine's Department of Biochemistry and had the opportunity to work with LJC on *Conus magus* venom toxins. She has also worked with LJC extensively when both were officers of the Philippine Biochemical Society (now Philippine Society for Biochemistry and Molecular Biology) and from 1992 to date, as fellow members of NAST. The author acknowledges the help of retired Professor Angelita G. Reyes, Associate Professor Grace Fe B. Yu and Professor Elsie C. Jimenez, former students and mentees of LJC, in the write-up on LCJ as mentor.

Photo: LJC upon conferment of the Order of National Scientist in Malacañang.

Brohult Award from the International Foundation for Science (Sweden) and the 2001 Outstanding ASEAN Scientist and Technologist Award, National Scientist Lourdes J. Cruz has been selected as one of five women scientists worldwide to receive the 2010 L'Oréal-UNESCO Awards in the Life Sciences in ceremonies to be held in the UNESCO headquarters in Paris on March 4, 2010.

The Early Years in Research

Fresh from taking the licensure examination for chemists in late May 1962, Lourdes J. Cruz or Luly came to Los Baños to work as a research aide at the Chemistry Laboratory of the newly established International Rice Research Institute (IRRI). This neophyte in research came at a very exciting time in IRRI when scientists from all over the world were starting their research in this small town next to the region's premier agricultural University (University of the Philippines College of Agriculture) and bounded by Laguna de Bay and Mt. Makiling. The IRRI laboratories were just getting new, state-of-the-art equipment. At the Chemistry Lab, Luly was guided by research



LJC at work in the laboratory.

associates, postdoctoral fellows and the lab's head, Dr. Bienvenido O. Juliano, then, the youngest senior scientist (now a National Scientist) at IRRI, with diverse scientific backgrounds and experience who exposed her to different viewpoints and varied approaches to the conduct of research. Luly remembers that they were "all in awe" of Dr. Juliano who seemed to have a solution to any problem in the lab. Luly learned to efficiently handle hundreds of samples for proximate analyses and rice quality tests. She helped set up the simpler equipment and learned from the process. Her one-year stint resulted in co-authorship in three publications on the physicochemical properties of rice proteins [1–3]. Certainly, this was a great way to start a research career!

In September 1963, Luly pursued her MS and PhD in Biochemistry at the University of Iowa, Iowa City, with Dr. CP Berg as adviser. She worked on the metabolism of D-valine and D-leucine. Her interest in biochemistry started when she conducted her undergraduate thesis on trypsin inhibitors under the guidance of Dr. Clara Y. Lim-Sylianco (another National Scientist) at the University of the Philippines Diliman. To her, "applying chemistry principles to living organisms is very challenging."

Upon completion of her graduate studies, Luly returned to IRRI as an Assistant Scientist. Her research focused on the biochemical factors affecting the accumulation of rice in the developing grain to help understand the basis for increasing protein content of rice. This study, which was one of the first in this area, revealed that only the level of the free amino nitrogen and the capacity for amino acid incorporation of the developing grain were consistently correlated with the protein content of the grain [4]. It was important to understand this phenomenon to be able to develop rice varieties with higher protein content. However, Luly made a career change after less than two years at IRRI; she left IRRI for Kansas State University for a short postdoctoral stint and returned to Manila to start a new phase in her career.

Teaching and Research at UP Manila

Accepting an Assistant Professor position at the Department of Biochemistry of the College of Medicine, University of the Philippines in Manila in 1970, which was headed then by Dr. Solita F. Camara Besa, allowed Luly to teach, do research and be near her family. Moreover, she joined three other young biochemists, Drs. Baldomero "Toto" Olivera, Olivia Veron, and Lucy So, who had also just come back from studies abroad. Luly joined Toto's research in the metabolism of pyridine nucleotide. At that time, the lab was getting equipment like the ultracentrifuge, scintillation counter and other items from Toto's Rockefeller grant for his research on DNA synthesis and pyridine nucleotide metabolism. Soon after, they realized that it was very difficult to compete in this field with their counterparts in first-world countries because of lack of resources and the difficulty of getting supplies and reagents, even when one had the money. Toto, an avid sea shell collector, had heard from a fisherman about cone snails that cause sickness and even death to those that are stung by them. He suggested that they work on the *Conus* which are abundant in the country and not well-studied yet. Toto's excitement for science and research was very infectious. Thus was born *Conus* research in this lab.

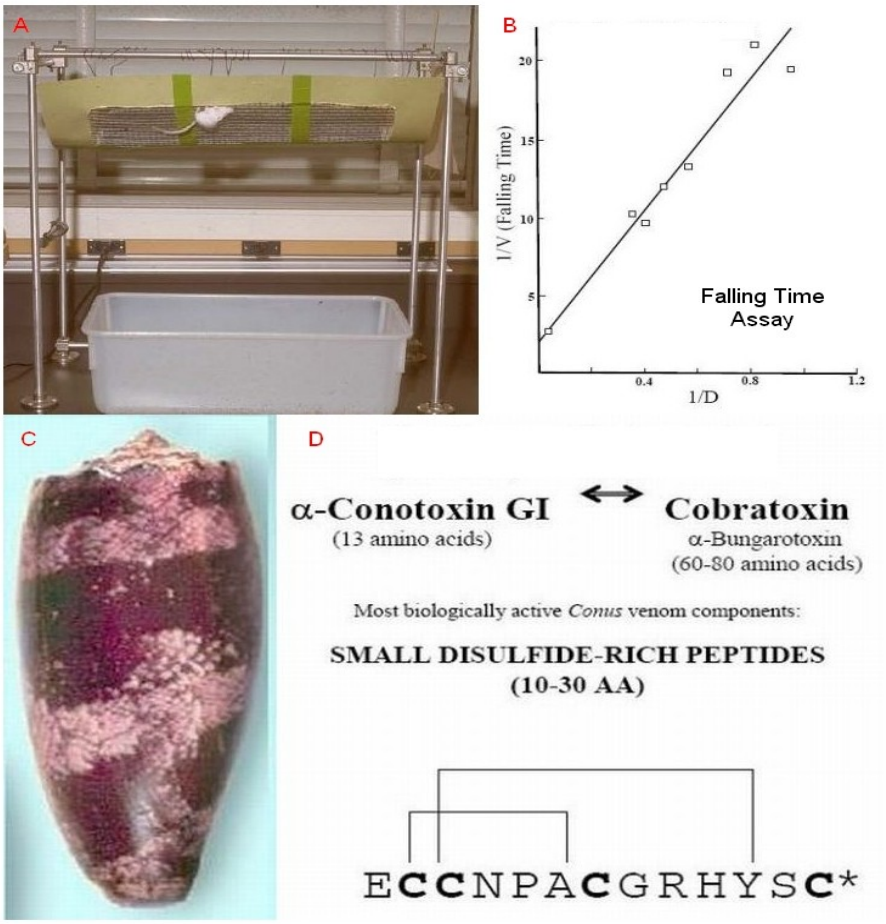
Conus snails are predators that immobilize their prey by injecting the prey with harpoon-like hollow tooth through which the venom is delivered. *Conus geographus,* considered the most venomous of all *Conus*, is a fish-hunting species from the central Indo-Pacific. There are about 500 known species of the *Conus* snails, which could therefore provide enormous resources for study.

Doing biochemistry work with the *Conus* snail started by learning how to handle it, its feeding habits, its predatory ways, and its mating. Glass tanks were set up in the lab. Trips to

Marinduque and Mindoro were made to scout for sources of the snail. When contacts had been made, the marine snails would just be delivered to the laboratory at designated times. There were false starts—such as getting specimens of the wrong species of *Conus* which meant unnecessary expense and lost time. But the excitement generated by the research more than made up for the disappointments. In 1976, the first paper on the protein toxin in the *Conus* venom was published in Veliger [5]. This was followed by a paper on the mating, spawning, development and feeding habits of *Conus geographus* in captivity [6]. This came after a daily 24-hour surveillance of the snails in the glass tanks. It was also necessary to develop an appropriate quantitative assay for the toxin's activity. And so, Luly, ingenious that she was (and still is), devised a hanging screenwire on which a mouse injected with the toxin was made to cling upside down, and the time it took the mouse to fall down was measured: the faster it took the mouse to fall, the greater the toxin's activity [7]. The various reactions of the mouse when injected with different toxins or fractions were also noted: scratching, hyperactivity, depressed activity, sleepiness, tremors, stretching, paralysis, convulsions, and death. These observations indicated the presence of different types of substances that cause such varied reactions! This, of course, was validated by the research results in the next two decades.

Shuttle Research between Manila and Utah

The research on *Conus* toxins had become hot item. To expedite the work and access to instruments and supplies not readily available locally, Luly accepted the invitation of Toto Olivera to conduct research at his laboratory in the Department of Biology, University of Utah, Salt Lake City. Luly then worked in Utah for 3 to 7 months annually, starting in 1976, first as a research associate, and from 1987 as research professor up to May 2004. Here, Luly and Toto conducted collaborative work with other experts to fully elucidate the nature of the *Conus* venom toxins. In 1978, a myotoxin was purified from *Conus geographus*; this myotoxin caused flaccid paralysis, respiratory failure and death in mice [8]. This myotoxin also turned out to be a peptide of only 13 amino acids with a disulfide linkage. In 1981, three highly toxic peptides from the same *Conus* species



(A) Screenwire setup and (B) results of the falling time assay; (C) *Conus geographus*; (D) first *Conus* peptide characterized by LJ Cruz. (photos used with permission from BM Olivera)

were found to cause their activity by inhibiting the postsynaptic terminus of the vertebrate neuromuscular injection [9]. The conotoxins consisted of 13–15 amino acids with internal disulfide linkage and were highly homologous with each other.

At the UP Manila lab and later at UP Diliman Marine Science Institute where she transferred in 1989, isolation, purification and characterization work continued on the venom toxins of different species of the *Conus* such as *C. magus*, *C. striatus*, and *C. textile*. Various enzymes and toxins were also localized in the granules of the venom. Several students did parts of their MS or PhD research with Luly at the Olivera lab in Utah.

In 1985, Luly and co-workers reported the discovery of the μ-conotoxins in *C. geographus* [10]. They reported the purification of seven μ-conotoxins consisting of 22 amino acids which were highly homologous and had hydroxyproline at similar positions. The major peptide (GIIIA) was established to block muscle sodium channels but had no detectable effect on nerve or brain sodium channels. Using planar lipid bilayers, the detailed kinetics of GIIIA action were obtained. While the kinetics of blocking by the μ-conotoxin was found to be similar to those of saxitoxin and tetrodotoxin, the specificity of the μ-conotoxin was 1000-fold greater for muscle compared to nerve sodium channels. The μ-conotoxins are therefore useful probes for investigating voltage-dependent sodium channels of excitable tissues; moreover their high tissue specificity can be used to distinguish sodium channel subtypes in different tissues.

This was immediately followed by the report on the ω-conotoxins, a class of calcium channel antagonists from *C. geographus* [11,12]. The ω-conotoxin GVIA was shown to block voltage-activated calcium channels at the frog neuromuscular junction and blocks all frog synapses tested. The ω-conotoxins also were found to be specific to a new class of target sites which affect calcium channels, different from sites bound by dihydropyridines and verapamil. The ω-conotoxin GVIA has since then been widely used in neuroscience with more than 2000 published papers using it as a pharmacological tool, mostly to inhibit synaptic transmission.

In their second review article published in Science in 1990 [13], the Cruz and Olivera group announced the great diversity of pharmacologically active small peptides present in *Conus* venoms whose targets are ion channels and receptors in the neuromuscular system. These venom peptides are highly specific and can distinguish between closely related receptor subtypes and, thus, can be used for structure-function correlations. They are also comparable to plant alkaloids and the secondary metabolites of microorganisms. Moreover, they predicted the possible pharmaceutical applications of these *Conus* peptides similar to those of the plant alkaloids and microbial fermentation products.

More than 80 publications spread over more than two decades afterwards, the number of receptors targeted specifically by conotoxins has reached nine (Na, K and calcium channels, neurotensin receptor, acetylcholine receptor, vasopressin receptor, norepinephrine transporter, nicotinic receptors and NMDA (N-methyl-D-aspartate) receptors. It is now estimated that a *Conus* species has 100–200 conotoxins or venom peptides synthesized and secreted in the venom duct. Venom peptides can be divided into two groups—the disulfide-rich conotoxins and peptides that lack multiple disulfide cross-links. The disulfide-rich conotoxins are further categorized into seven superfamilies based on the cysteine pattern and disulfide connectivity which are indicative of their pharmacological target. The non-disulfide rich conotoxins consist of five families, three (Conantokin, Contulakin and Conorfamide; take note of the Filipino-sounding words) which do not have disulfide linkage and two (Conopressin and Contryphan) which have one disulfide linkage. *Conus* peptides are small (12–30 amino acids) while polypeptide toxins from other venoms are larger with 40–80 amino acids. *Conus* peptides also exhibit an unusually diverse number of posttranslationally modified amino acids which include hydroxyproline, 0-glycosylated serine or threonine, brominated-tryptophan, γ-carboxy glutamate, D-amino acids, and sulfated-tyrosine. As of 2006, seven therapeutic products based on six different *Conus* venom peptides for pain, epilepsy and myocardial infarction, had reached phase I and preclinical trials; one (Prialt; zicontide) was approved by the United States Food and Drug Administration in December 2004, fulfilling predictions made two decades ago. Several recent review articles are available on the *Conus* venom peptides [14,15,16].

Thirteen patents covering various conotoxins or conotoxin peptides and their applications have been granted to the group of Dr. Olivera and Dr. Cruz. Luly is the first author of one patent on bromo-tryptophan conopeptides.

LJC, the Educator and Mentor

As an educator, Dr. LJ Cruz led the institution of the MS and PhD Biochemistry programs at UP Manila. She has mentored and graduated 6 PhD, 16 MS and over 40 BS students and has taught many more! Under the aegis of the Philippine Biochemical Society (now Philippine Society for Biochemistry and Molecular Biology) of which she was the second President and a long time board member, Dr. Cruz organized several biochemical education workshops for teachers all over the Philippines. Dr. Cruz also organized two very successful regional symposia of the Federation of Asian and Oceanian Biochemists and Molecular Biologists which enabled local scientists and students to attend lectures of regional and world renowned biochemists.

To her numerous advisees, LJC is more than an adviser. To them, she is a real mentor, friend and role model. LJC is cool and quiet, very hardworking, systematic, very helpful, and generous with her time and talent. She imparts her qualities of creativity, diligence, determination and perseverance to her students and staff by her actions. But she also takes time out

from her work and brings along her students and staff. On various occasions, she would take them to Los Baños, to nearby Luneta and other places just to relax, talk, eat and rest. She introduced pizza pie to students and staff and treated them with home- or lab-made pizzas or from the new pizza place nearby. Moreover, she is passionate not only about her science but also about uplifting the conditions of poor people, especially the indigenous people (see below).

Recognitions and Awards

The scientific accomplishments of Luly were first recognized by the National Academy of Science and Technology when she was named a recipient of the Outstanding Young Scientist Award for Chemistry in 1981. This was followed by the Achievement Award (Chemistry) given by the National Research Council of the Philippines in 1982. National and professional organizations have also recognized the outstanding contributions of LJC to science and technology: The Outstanding Women in the Nation's Service (TOWNS) (Science), 1986; Professional Achievement Award in Biochemistry, UP Alumni Association, 1991; Outstanding Alumnus, UP Chemistry Alumni Foundation; Emilio Aguinaldo Award, 2007, among others. The University of the Philippines recognized her scientific achievements with Gawad Chancellor Award for Research, 2002 and UP Scientist III, UP's highest award in scientific productivity.

In 1987, Dr. Lourdes J. Cruz was elected as Academician to the National Academy of Science and Technology. In May 2006, the NAST membership endorsed the nomination of Acd. LJ Cruz to Philippine President GM Arroyo for the National Scientist rank. On November 10, 2006, by virtue of Proclamation No. 1167, Acd. Lourdes J. Cruz was bestowed the rank and title of National Scientist, the highest award that the Republic of the Philippines can bestow on a Filipino scientist for his/her outstanding contributions to science and technology.

At the international level, LJC garnered the Sven Brohult Award from the International Foundation for Science (IFS) (Sweden) in 1993 for her outstanding accomplishments resulting from her IFS-funded research project on the *Conus* venom toxins. LJC was awarded the 2001 Outstanding ASEAN Scientist and Technologist Award during ceremonies in Brunei Darussalam. On March 4, 2010, National Scientist Lourdes J. Cruz will be one of five women scientists to receive the 2010 L'Oréal-UNESCO Awards in the Life Sciences in ceremonies to be held in the UNESCO headquarters in Paris.

LJC —Giving Back to Society

National Scientist Cruz's maturation as a scientist finds her tempering her passion for science with compassion for the development of poor rural communities. In 2001, with the help of volunteers and private donors, LJC established the Rural Livelihood Incubator (Rural LINC) in Morong, Bataan with the aim of harnessing S & T resources to mitigate poverty. The activities of Rural LINC include education, preservation of cultural heritage, the establishment of sustainable livelihood and improvement of the health status of the indigenous community of Aytas. Weekends find LJC with volunteers and medical missions working with the Aytas in the site. They also help the fishermen and women farmers of Morong. Her seeming shyness and quiet demeanor disappear when she has to ask for assistance for her project in Morong —asking for grants, a used vehicle, or help in training sessions.

Corollary to this, LJC also developed a course under the Civic Welfare Training Service for undergraduate students geared toward developing awareness and concern for biodiversity and conservation of watershed areas in poor rural communities.

National Scientist Cruz continues her work with the Aytas. She believes that S & T should also be used "to help raise the economic status of poor communities, particularly, in rural areas." She adds "For sustainable community development, the approach must be holistic involving not only the social aspects but also the S & T aspects. People particularly in rural areas must learn how to properly manage bioresources, how to make a living and how to become productive."

While continuing her studies of toxins and anti-TB compounds from marine organisms, LJC is now very much interested in traditional medicine as she says "this can eventually provide sustainable livelihood for the Aytas and other indigenous tribes."

Her efforts to contribute to increasing the quality of research in the country have recently revolved around the updating of research administrators and hands-on training of administrative support staff on research performance evaluation. Recently, she organized a three-day seminar and workshop on research performance evaluation sponsored by NAST, the Department of Science and Technology, the Commission on Higher Education and Elsevier Asia Pacific. Participants came from more than 70 institutions from all over the Philippines.

National Scientist Lourdes Jansuy Cruz has likewise been very active in promoting science careers among our young students and mentoring them well. To the youth, her message is "There are many research problems that one can work on and it does not matter whether one does basic or applied research as long as the science is good. While still young it is best to focus on a subject matter that you can pursue in depth. Try to find a support group with whom you can discuss science freely and explore its wonders."

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