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# Experiences with the marsupial frogs: Reminiscences of a developmental biologist

EUGENIA M. DEL PINO

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EUGENIA M. DEL PINO

Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, Quito 170517, Ecuador

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**Abbreviations:** AAAS, American Academy of Arts and Sciences; AAUW, American Association of University Women; ACE, Academy of Sciences of Ecuador; ACAL, Latin American Academy of Sciences; CDF, Charles Darwin Foundation for the Galápagos Islands; DKFZ, German Cancer Research Center; LASDB, Latin American Society for Developmental Biology; LASPAU, Latin American Scholarship Program of American Universities; NAS, USA National Academy of Sciences; PUCE, Pontifical Catholic University of Ecuador; SENESCYT, Secretariat of Higher Education, Science, Technology and Innovation of Ecuador; TWAS, World Academy of Sciences for the Advancement of Science in Developing Countries

**Address correspondence to**: Eugenia M. del Pino, Pontificia Universidad Católica del Ecuador, Escuela de Ciencias Biológicas, Apartado 17–01–2184, Avenida 12 de Octubre 1076 y Roca, Quito 170517, ECUADOR.

Telephone: +593 2 299 1700 extension 1280

e–mail address: edelpino@puce.edu.ec

eugeniamdelpino@yahoo.com

**ABSTRACT**

**This article provides a brief account of the career of Eugenia M. del Pino. Casual events and serendipity played important roles in modeling her career as developmental biologist. In collaboration with colleagues and students, she analyzed the biology and development of the marsupial frog *Gastrotheca riobambae* (family: Hemiphractidae) in comparison with *Xenopus laevis* and tropical frogs. The emphasis was placed on oogenesis and the early stages of development. Topics include the mono- and multi-nucleated modes of oogenesis. She described two modes of gastrulation in frogs, gastrulation modes one and two according to the timing of notochord elongation. She was able to establish a pioneer laboratory for the comparative analysis of frog development in Ibero America at the Pontifical Catholic University of Ecuador, in Quito. Her contributions to society include her influence in the establishment of the National Academy of Sciences of Ecuador, and efforts toward the conservation of the Galápagos Archipelago. She is part of a pioneer group of professors that placed Biology as an academic discipline in Ecuador. The experiences of her career reveal that we all face difficulties in our jobs. However, nothing is impossible when we follow a passion. Her work reveals that the key to success is to turn obstacles into opportunities.**

## Introduction

Each day we make choices that may have unpredictable consequences in our lives. A choice that changed my life was my decision, in 1963, to enter the School of Education of the Pontifical Catholic University of Ecuador (PUCE) in Quito. Without professional orientation, I was unsure about choosing a career. However, one of my brothers said that parents do not live forever and that it was important for me to have a career. For this reason, I entered the PUCE School of Education to be trained as high school teacher. I did not know then, that my studies at PUCE were the first steps into my becoming a developmental biologist.

When I began to study at PUCE, a cooperation program with the University of Saint Louis, Saint Louis, Missouri, United States of America (USA) (1963–1969) had just started. This program strengthened the education programs at PUCE with faculty and teaching resources. Biology was taught for the first time, and the availability of new laboratory equipment was a novelty for me. The cooperation with the University of Saint Louis was sponsored by the Alliance for Progress Program of the USA. Dr. Cándida Toro Acosta, from Puerto Rico, was one of the foreign professors of Biology. She encouraged students to seek graduate training in the USA so that by the end of the cooperative program they would replace the foreing faculty.

Following the advice of Dr. Acosta, I applied for a scholarship from the Latin American Scholarship Program of American Universities (LASPAU). LASPAU selected me as one of its scholarship recipients and I travelled to the USA for advanced Biology training in 1967.

In addition, the American Association of University Women (AAUW) awarded me an international fellowship for my last year of graduate studies in the USA. Thus, I would like to formally acknowledge the support of both LASPAU and the AAUW and their impact on my professional development. In 1967, the PUCE awarded me the "Licenciatura", the equivalent of a Bachellor of Arts Degree. In 1969, Vassar College (Poughkeepsie, NY, USA) awarded me the Magister of Science Degree, and in 1972, Emory University (Atlanta, GA, USA) granted me the PhD degree. I was considered to be the happiest Emory University PhD–graduate of the year 1972 due to the joyful picture of myself taken during the Commencement ceremony (Fig. 1).

I returned to Quito, Ecuador immediately after my graduation from Emory University, and obtained a teaching position at PUCE in Quito. I was a Professor of Biology at PUCE, for nearly 41 years, until my retirement in 2013. Although Developmental Biology was not a reseach priority in Ecuador, the University gave me the academic freedom to pursue my academic interests. Some years ago, PUCE began an annual program of research grants to support faculty research. My research group benefited from the PUCE research grants. I acknowledge the PUCE support with gratitude.

It was at the PUCE headquarters where I established a pioneer laboratory of Developmental Biology in Ibero America for comparative studies of frog development (del Pino, 2003). The expertise I had acquired was put to work as I introduced a course in Developmental Biology at PUCE as a core requirement for biology students (del Pino, 2003). I consider that the study of Developmental Biology is very important because it allows the integration of concepts derived from various branches of biology and evolution. Moreover, the highly conserved molecular mechanisms of development provide

extraordinary examples of the unity of biology (del Pino, 2003). In collaboration with international colleagues and PUCE students, we published nearly 100 articles on the development of marsupial frogs and their comparison with *Xenopus laevis* and other tropical frogs. Among these, I would like to highlight the invitation from Scientific American to write an article about marsupial frogs (del Pino, 1989a). Translations of this article into ten different languages were printed in the international editions of Scientific American. Thus, Scientific American contributed to our outreach efforts in disseminating our research to a wide audience. Our work received extensive recognition, as evidenced by the invitations that I received to deliver plenary lectures in Ecuador, Europe, Ibero America, and North America.

Colleagues from the Emory Department of Biological Sciences provided support for my activities in Quito. At the start of my career, my mentor, Asa Alan Humphries, Jr., provided scientific advice and key reagents for my studies. In addition, Dr. Charles Ray visited PUCE and taught Population Genetics to my students. His visit was possible through a retirement prize from Emory University. Many years later, and following the example of Dr. Ray, Dr. Darrell Stokes visited us after his retirement. He taught Animal Physiology to our students. Darrell Stokes, Victoria (Vicky) Finnerty and Steven W. L'Hernault gave me guest access to the online Emory University library for many years (Fig. 2). Emory University not only provided funds and intellectual resources but in addition, publicly recognized my research efforts. In 2003, I received "The Sheth Distinguished International Emory Alumni Award". Another high honor for me was the invitation of Emory University to deliver the 2014 Rhodes Lecture in Biology. I acknowledge these recognitions and the support provided by members of the Biology Faculty of Emory University with gratitude.

My elections into several academies of science represent great international recognitions, as well as honors bestowed by local and international organizations. The Latin American Academy of Sciences (ACAL) with headquarters in Caracas, Venezuela elected me as Fellow in 1987. The World Academy of Sciences for the Advancement of Science in Developing Countries (TWAS), Trieste, Italy, elected me to its membership in 1989. I served as Council Member of TWAS from 2004–2009. In 1996, I was elected as Honorary Foreign Member of the American Society of Ichthyologists and Herpetologists. The USA American Academy of Arts and Sciences (AAAS), (Cambridge, Massachussetts, USA) elected me to its membership in 2006. In the same year, the USA National Academy of Sciences (NAS) (Washington DC, USA) elected me as Foreign Associate, the name given to international fellows. I have been the first citizen of Ecuador to be elected to these academies. In 2012, I received "The Eugenio Espejo Prize in the Sciences" (Fig. 3). The Eugenio Espejo Prize is the highest award given by the Government of Ecuador.

The Latin American Society for Developmental Biology (LASDB) awarded me "The 2019 LASDB Prize in Developmental Biology" during the 10$^{th}$ Congress of this society in Buenos Aires, Argentina in October 2019. I was invited to deliver one of the keynote lectures of the congress. My lecture was preceded by introductions about my career and scientific contributions by Drs. Edward M. (Eddy) De Robertis and Oscar D. Pérez, a former student of mine. The President of the LASDB, Dr. Pablo Wappner, explained that the LASDB Prize 2019 was awarded as recognition to my scientific work and the training of Ecuadorian students (Fig. 4). The award ceremony was moving and I enjoyed the messages of congratulation sent from different parts of the world by my former students, and diligently delivered by Oscar D. Pérez (Fig. 4). As a message to young investigators, I

explained that we all face difficulties in our jobs. I explained that nothing is impossible when we follow a passion, and that the key to success is to turn obstacles into opportunities. I acknowledged the 2019 LASDB Prize with gratitude.

In the year 2000, I was awarded "The L'Oréal/UNESCO Prize for Women in Science" for Latin America at the UNESCO Headquarters in Paris, France. Some years later, a former student of my laboratory, Michael Venegas-Ferrín, who was doing graduate studies in the Netherlands, went to Paris, France for a weekend. As he walked along the Champs–Elysées he admired a photo retrospective organized by the L'Oréal Foundation to celebrate the 15th anniversary of the L'Oréal-UNESCO Awards (Fig. 5A). To his surprise, he found my photo in the retrospective. Michael sent me a photo of him standing by my image (Fig. 5B) with the following message: "…This weekend I went to Paris and found a surprise that fills me with pride as an Ecuadorian, as student, and former member of your laboratory. In the attached image you can see what I am talking about (02 April 2013)" (Fig. 5B). The words of Michael Venegas-Ferrín disclose the feelings of pride and honor that my students and collaborators shared each time that I received a recognition. Our publications, prizes and recognitions represent the stimuli not only for my collaborators and myself but also for the advancement of scientific research at PUCE, Ecuador, and Ibero America.

**Becoming a developmental biologist**

At Emory University, Dr. Asa Alan Humphries, Jr., accepted me as one of his graduate students (Fig. 1). When I started in his laboratory, I had a vague idea of his research with newt and frog oocytes, lampbrush chromosomes, and frog fertilization. Working in his

laboratory was not easy because of my lack of research experience. However, I soon learned about the questions of Experimental Embryology, and Dr. Humphries helped me to develop the analytical skills required to approach scientific problems. My dissertation dealt with the role of the egg–jelly in the fertilization of the Sub–Saharan African clawed frog, *Xenopus laevis* (Pipidae) (del Pino, 1973).

I have clear recollections of the midmorning coffee breaks with Dr. Humphries and other students at Cox Hall, the Emory University cafeteria. More than once, Dr. Humphries talked about Theodor H. Boveri (1862-1915), and Marcella O'Grady Boveri (1863-1950) and their contributions to Experimental Embryology (Fankhauser, 1972; McKusick, 1985; Wright, 1997). Boveri is considered one of the founders of Experimental Embryology. He understood the role of chromosomes in heredity, development and cancer (Fankhauser, 1972; McKusick, 1985). Marcella O'Grady, a USA citizen, and a professor at Vassar College, took a sabbatical leave and went to Boveri's laboratory in Würzburg, Germany. Marcella O'Grady was the founder of the Vassar Department of Biology after the Department of Natural Sciences split into the Departments of Biology and Geology/Mineralogy (Wright, 1997). She never returned to Vassar College because of her marriage to Theodor H. Boveri (Wright, 1997).

When I began working in his laboratory, I did not know that Dr. Humphries was a direct scientific descendant of Theodor H. Boveri and Marcella O'Grady Boveri. His eminent scientific lineage is now mine. Humphries studied at Princeton University, Princeton, NJ, USA under the guidance of Gerhard Fankhauser. Fankhauser studied in Berne, Switzerland under the direction of Fritz R. Baltzer. In turn, Fritz R. Baltzer was a student of Boveri. Another student of Boveri was Hans Spemann, a well known

developmental biologist because of his discovery of the frog organizer; work done in collaboration with his student Hilde Mangold (Spemann and Mangold, 1924). Spemann was awarded the Nobel Prize in Medicine in 1935. One of Dr. Humphries beliefs was that the basic questions, posed by the fathers of Embryology, were revisited during every generation, as new technologies develop and become applied to older questions.

**The biology of marsupial frogs**

I returned to Ecuador upon completion of my PhD and applied for a teaching position at PUCE, my Alma Mater. After a few months, I was hired as Full Professor of Biology. The PUCE does not have a doctoral program and only offers a "Licenciatura" in Biological Sciences. My obligations were teaching, directing undergraduate theses, and administration. I was head of the PUCE Department of Biological Sciences for two years. These obligations interfered with my desire to continue an academic career. More problematic was the fact that I had no research funds to purchase *X. laevis* specimens. Thus, without access to this model organism, I searched for local frogs. I wanted an easily accessible frog to continue with the analysis of the frog egg–jelly and fertilization. However, I did not come back to this problem because I found an unsual frog, a marsupial frog (Fig.6). These frogs became the focus of my studies as I worked out different aspects of marsupial frog biology in comparison with other frogs.

Colleagues from the PUCE Department of Biological Sciences helped me with the identification of frogs. There were only two frog species in the PUCE gardens: the frog without tadpoles, *Pristimantis unistrigatus* (Terraranae: Strabomantidae), previously known

as *Eleutherodactylus unistrigatus* (Heinicke *et al.*, 2018; Nina and del Pino, 1977) and the marsupial frog, *Gastrotheca riobambae* (Hemiphractidae) (Duellman, 2015). *G. riobambae* no longer occurs in the PUCE campus due to urban growth and other reasons that have not been well investigated. Frogs of the family Hemiphractidae are commonly known as hemiphractid frogs, and were previously classified in the family Hylidae. At that time, I did not know that hemiphractid frogs inhabit wet and humid lowland forests of South America, Costa Rica, Panama and Trinidad and Tobago and are seldom collected (Schmid *et al.*, 2012). The exceptions are the species that inhabit the high environments of the Andes, such as *G. riobambae* (Duellman, 2015; Schmid *et al.*, 2012). Thus, I was very lucky to find a marsupial frog at the doorstep of my laboratory.

My first encounter with the marsupial frog *G. riobambae* occurred in 1972 in the gardens of PUCE (Fig. 6). The *G. riobambae* female that I saw looked extraordinary to me because this frog incubated her embryos in a dorsal pouch. Of course, this vertebrate looked like a frog, but the incubation of embryos in the body of the mother resembled that of mammals. From the very beginning, the biology of marsupial frogs was appealing. However, I knew that I was in front of a reseach problem greater than my abilities and resources. I wrote a letter to Dr. Humphries, and I asked him to search in the Emory University library for information on the development of marsupial frogs. He wrote that the development of marsupial frogs was totally unknown, and that I had an important problem in front of me. In addition, he wrote that I could dedicate my scientific career to study the biology and development of marsupial frogs. When he explained my work to Dr. Gerhard Fankhauser, Fankhauser only wished that he would still be teaching his Comparative

Embryology Course. He would have very much liked to include the marsupial frogs in his lectures. In conclusion, I found an important scientific problem.

In the following years, we made an outline of the biology of the *G. riobambae* female and the embryogenesis of this frog (del Pino *et al.*, 1975; del Pino and Escobar, 1981; del Pino and Loor-Vela, 1990). We found that the incubation of embryos by the mother is associated with hormonal control of reproduction, as in mammals (de Albuja *et al.*, 1983; del Pino, 1983; del Pino and Escobar, 1981). Moreover, only in hemiphractid frogs, the embryos develop disk–shaped gills, called bell–shaped gills (Noble, 1931). I performed comparative studies using preserved specimens of marsupial frogs due to the difficulty of obtaining living frogs (del Pino, 1980; del Pino and Escobar, 1981; del Pino and Humphries, 1978). William E. Duellman invited me to study the world largest collection of hemiphractid frogs, at the Museum of Natural History of the University of Kansas, Lawrence, KS, USA. In addition, he provided living frogs of several species for my studies. I also visited four additional museums: Dr. Raymond F. Laurent of the Miguel Lillo Foundation, Tucumán, República Argentina, invited me to study the herpetological collection of this institution. Likewise, Dr. Pedro M. Ruiz–Carranza of Instituto de Ciencias Naturales, Museum of Natural History of Universidad Nacional de Colombia in Bogotá, invited me to study the frog collection of this museum. Further, I studied the specimens of the California Academy of Sciences in San Francisco, CA, USA thanks to the invitation of John Simmons. Dr. Paolo E. Vanzolini invited me to study brasilian species of Hemiphractidae at the Museu de Zoologia, Universidade de São Paulo, Brasil. In my career, I had the contribution of several students that conducted their undergraduate

"Licenciatura" theses under my guidance, and of international collaborators, including my former advisor, Dr. Asa Alan Humphries Jr. I acknowledge their help with gratitude.

I wanted to apply molecular methods to the analysis of *G. riobambae* development. For this reason, I attended an International Course of Molecular Biology organized by Dr. Jorge E. Allende at the University of Chile, Santiago de Chile (1977). One of the invited lecturers was Dr. Dieter Söll, Yale University, New Haven, CT, USA. Thanks to his invitation, I spent a few months in his laboratory at Yale University learning Molecular Biology methods. Back in Quito, I could not implement molecular methodologies due to my lack of expertise, time, personnel and funds. Dr. Igor B. Dawid (National Institutes of Health, Bethesda, MD, USA) once said that a key to success is the use of available resources in an optimal way (Fig. 7). He added that scientists do not have all of the resources that they may wish to have, even those scientists that work in affluent laboratories. His comments allowed me to recognize my advantages and limitations. My extraordinary advantage was the marsupial frog. I focused on feasible methods: the morphological analyses of embryos and the whole–mount immune detection of proteins of developmental interest.

We found that the early embryos of *G. riobambae* maintain an extraordinary planar orientation on the surface of a large egg. The flat orientation allows the identification of the prominent cranial neural crest cell–streams, branchial arches, and bell gill primordia (del Pino and Medina, 1998). We found that the expression patterns of neural genes is similar with other vertebrates, indicating that *G. riobambae* neural development was conserved in evolution (del Pino and Medina, 1998). We analyzed several genes at the protein expression level in early embryos and in the gastrula of *G. riobambae* and other frogs. We detected that the genes are conserved, however, the timing of expression varies between

frogs probably associated with their reproductive strategies (Benítez and del Pino, 2002; del Pino, 1996; del Pino *et al.*, 2007; Moya *et al.*, 2007; Pérez *et al.*, 2007; Sudou *et al.*, 2016; Venegas-Ferrín *et al.*, 2010).

**The discovery of multinucleated oocytes**

Dr. Humphries did a sabbatical visit to the laboratory of Prof. Herbert C. Macgregor in Leicester, UK, an important laboratory in the study of lampbrush chromosomes. In the Macgregor laboratory, Dr. Humphries met Dr. James Kezer and saw the eight–nucleated oocytes of the tailed frog of North America, *Ascaphus truei* (Macgregor and Kezer, 1970). Back at Emory University, Humphries gave a seminar about the extraordinary oocytes of *A. truei* with eight nuclei instead of just one nucleus. I became interested in frog oocytes with eight nuclei. For this reason, back in Quito, I analyzed the nuclear status of oocytes in all available frogs (del Pino and Humphries, 1978; Schmid *et al.*, 2018). I found that in the majority of frogs, oocytes contained a single nucleus, or germinal vesicle, as in other vertebrates (del Pino, 2018; Schmid *et al.*, 2018). Surprisingly, in some species of hemiphractid frogs, we detected many nuclei in each oocyte (del Pino and Humphries, 1978). We called these multinucleated oocytes, thus separating them from the mononucleated oocytes, the oocytes with a single nucleus (del Pino and Humphries 1978).

At one point, I opened up a freshly excised oocyte of the marsupial frog from Venezuela, *Flectonotus pygmaeus*, under a dissecting microscope and saw thousands of spherical structures released into the culture medium. Dr. Humphries came to Quito to observe for himself these extraordinary oocytes. The spherical structures were actually

meiotic nuclei, surrounded by a nuclear envelope, and were active in RNA synthesis. The largest nuclei had lampbrush chromosomes (del Pino and Humphries, 1978). Some years later, I visited the laboratory of Prof. Herbert C. Macgregor to analyze the ribosomal gene amplification of the multinucleated oocytes of *F. pygmaeus* (Macgregor and del Pino, 1982). We were able to demonstrate that, in this species, the previtellogenic oocytes contained between 2000–3000 meiotic nuclei instead of the single nucleus that characterize frog and vertebrate oocytes. During oogenesis, the number of nuclei decreases until a single nucleus remains in the fully grown oocyte (del Pino and Humphries, 1978; Macgregor and del Pino, 1982). The multinucleated oocytes of *F. pygmaeus* are a source of important developmental questions that need further analysis (del Pino, 2018). However, the study of multinucleated oogenesis was impossible because frogs with this type of oogenesis are rarely collected. Due to this difficulty, I changed my research interests to the study of the mononucleated oocytes of *G. riobambae*.

In 1984–1985, I took a sabbatical leave from PUCE to do research in Germany, under the sponsorship of the Alexander von Humboldt Foundation. I visited the laboratory of Prof. Dr. Michael Trendelenburg at the German Cancer Research Center (DKFZ) in Heidelberg, Germany to analyze *G. riobambae* oocytes (del Pino *et al.*, 1986). The laboratory of Prof. Dr. Trendelenburg was associated with the Department of Prof. Dr. Werner W. Franke at the DKFZ. In addition, Profs. Drs. Peter Hausen and Christine Dreyer of the Max Planck Institute for Developmental Biology in Tübingen, Germany provided additional facilities to analyse the oocytes of *G. riobambae.*

I took another sabbatical leave from PUCE in 1990. Dr. Joseph G. Gall of the Carnegie Institution for Science, Department of Embryology in Baltimore, MD, USA

invited me to visit his laboratory (Fig. 7). The Carnegie Institution for Science, and the Fulbright Comission sponsored this visit. The Gall laboratory is the world centre for the study of lampbrush chromosomes. Dr. Donald D. Brown from the same department gave me advice and gene probes for the analysis of the 5S ribosomal RNA of *G. riobambae* oocytes (del Pino *et al.*, 1992).

During these sabbatical visits, I used some of the emerging technologies of molecular biology that were applied to developmental problems. The results of our analyses showed that the mononucleated oocytes of *G. riobambae* have no obvious similarity with the multinucleated oocytes of *F. pygmaeus*. Instead, the *G. riobambae* oocytes resemble the oocytes of *X. laevis* (del Pino, 1989b; del Pino *et al.*, 1986; del Pino *et al.*, 1992; del Pino *et al.*, 2002; Schmid *et al.*, 2012).

**The embryonic disk and the comparison of gastrulation**

A major problem for the analysis of *G. riobambae* early development was the lack of an appropriate culture medium (Elinson *et al.*, 1990). The problem was solved by the addition of urea to a physiological saline solution (del Pino *et al.*, 1994). The culture medium was devised as an outcome of the study of nitrogen waste excretion in *G. riobambae* (Alcocer *et al.*, 1992; del Pino *et al.*, 1994). The study of the mode of nitrogen waste excretion received the collaboration of PUCE students, of Drs. Karl-Heinz Thierauch, Herbert Steinbeisser, and of Prof. Dr. Horst Grunz, who was then at the University of Essen, Germany (Alcocer *et al.*, 1992; del Pino *et al.*, 1994). Dr. Thierauch taught at PUCE for two years sponsored by the "Deutsche Gesellschaft für Internationale Zusammenarbeit". Dr. Steinbeisser visited

my laboratory with the sponsorship of a Feodor Lynen Fellowship from the Alexander von Humboldt Foundation (De Robertis and Niehrs, 2014). We learned that urea is the main excretory product of embryos, free–living tadpoles and adults of *G. riobambae* (Alcocer *et al.*, 1992; del Pino *et al.*, 1994). We reasoned that the maternal pouch of *G. riobambae* represents a condition of water stress. Therefore, the accumulation of urea in the capsular fluid of embryos may play an osmoregulatory role for the conservation of water, needed for embryonic development (Alcocer *et al.*, 1992; del Pino, 2018; del Pino *et al.*, 1994). With the urea–containing solution, we cultured *in vitro* some embryos that have been removed from the maternal pouch. Thus, we were able to analyze the early development and gastrulation of *G. riobambae* (del Pino *et al.*, 1994).

One day, I received the visit of Dr. Richard P. Elinson, then at the University of Toronto, Canada. This visit was the beginning of our collaborative efforts on the analysis of *G. riobambae* gastrulation. Living *G. riobambae* embryos are uniformly pale yellow, and it is difficult to visualize embryonic structures. Dr. Elinson sent me the protocol of silver impregnation, used in the study of fish embryos. An area of small cells, an embryonic disk, was observed on the surface of the *G. riobambae* gastrula after silver impregnation. The *G. riobambae* embryo develops from the embryonic disk in a fashion that resembles the development of the chick (del Pino and Elinson, 1983). *G. riobambae* gastrulation is the most divergent mode of frog gastrulation, and elongation of the body begins after completion of gastrulation (del Pino and Elinson, 1983; Elinson and del Pino, 1985). In contrast, in *X. laevis*, body elongation begins during gastrulation (del Pino and Elinson, 1983; Elinson and del Pino, 2012; Moya *et al.*, 2007).

We undertook a comparative analysis of frog early development and gastrulation to gain an understanding of the embryonic disk of *G. riobambae* (Benítez and del Pino, 2002; del Pino *et al.*, 2004; del Pino *et al.*, 2007; Hervas *et al.*, 2015; Romero-Carvajal *et al.*, 2009; Sáenz-Ponce, Santillana-Ortiz, *et al.*, 2012; Sáenz-Ponce, Mitgutsch, *et al.*, 2012; Salazar-Nicholls and del Pino, 2015). Unfortunately, we did not detect an embryonic disk in embryos of other frogs. However, the analysis revealed two modes of gastrulation according to the onset of body elongation. Gastrulation mode 1 is exemplified by *X. laevis* and occurs in frogs with small eggs and aquatic reproductive modes. In these frogs body elongation begins during gastrulation, suggesting that accelerated elongation of the body may be important for the rapid development of the tadpole. Gastrulation mode 2 is exemplified by *G. riobambae* and occurs in frogs with larger eggs and terrestrial reproductive modes (del Pino, 2018; del Pino *et al.*, 2007; Vargas and del Pino, 2017) suggesting that rapid body elongation is not essential in frogs with terrestrial reproductive modes. The comparison indicated that body elongation and gastrulation are two separate developmental processes. These processes are independent of each other in frogs with gastrulation mode 2. We concluded that each frog species represents a successful natural experiment in the diversification of frog reproduction and development (del Pino, 2019). In conclusion, in Quito, I was able to develop further my critical thinking in the analysis of the biology and development of marsupial frogs (Fig. 8).

**Society, teaching and research**

Ecuador was one of the few countries in Ibero America that did not have an Academy of Sciences. For this reason, I invited several colleagues to discuss the possibility of establishing the national Academy of Sciences of Ecuador (a.k.a. ACE because of its Spanish acronym). We undertook the task of drafting the statutes and of obtaining the legal recognition from the Government of Ecuador. In 2013, the Secretariat of Science and Technology of Ecuador (SENESCYT) recognized the ACE. The six of us became the Founding Members of ACE. I served as Vice President of ACE from 2013 to 2016. The ACE membership grew in the following years, and at present has more than 50 Fellows. ACE provides recognition to its fellows, allows communication between Ecuadorian scientists, and seeks exchanges with institutions at home and abroad.

In 1972 shortly after I started to work at PUCE, Dr. Peter Kramer visited me because he had established a fellowship program for Ecuadorian students in the Galápagos Islands. Dr. Kramer was then the Director of the Research Station of the Charles Darwin Foundation for the Galápagos Islands (CDF) in Isla Santa Cruz, Galápagos. The students were invited to conduct research for their undergraduate theses in the Galápagos Islands under the supervision of CDF visiting scientists. For many years, I recommended PUCE students for those fellowships. Many of the former Galápagos students were later conservation specialists in Ecuador and Ibero America. I visited the Galápagos Archipelago to monitor the research of several students. Those visits were priviledges for me. The association with the CDF allowed me to learn about the Galápagos Islands, and I became involved with Galápagos education and conservation issues for about 25 years. In addition, I served as CDF Vice President for Ecuador from 1991 to 1996. Eventually, I had to withdraw from Galápagos conservation activities due to time limitations.

I needed current scientific articles for my seminar courses (del Pino, 2003). However, access to the literature was extremely limited during most of my career. I became aware of publication called "Current Contents in the Life Sciences", once it became available at PUCE. I selected articles from Current Contents pages, and directly requested reprints from authors. During my visits abroad, I photocopied numerous articles. Moreover, I bought personal subscriptions to journals such as Development and Cell. International colleagues were always generous and provided me with photocopies of articles. Later, and for a number of years, I received guest–access to the online services of the Emory University library, as previously explained. With Internet tools, it became easier to keep up with the current literature and students learned to do their own searches in the Internet.

In Quito, I was isolated from the scientific community. Isolation is an advantage because it allows the analysis of a scientific problem without distractions. However, interactions with colleagues are necessary as they contribute to the refinement of ideas. I discussed my research ideas with PUCE undergraduate students. I hope that the discussions helped the students in the development of their own critical thinking. My discussions with colleagues at scientific meetings were particularly important for the analysis of *G. riobambae* development. For many years, I participated in the *Xenopus* International Conferences, and I met many colleagues (Fig. 7). We had important discussions. In addition, I visited several colleagues at their institutions for the purpose of scientific exchange and discussion. Several colleagues provided me with literature and reagents. For teaching purposes, I received donations of textbooks. I am most grateful to my international colleagues for their help.

When I started to work at PUCE, I never expected that I would be part of a group of pioneers that established Biology as an academic discipline in Ecuador. Today, the PUCE School of Biological Sciences is the main centre in Ecuador for the study of Biological Sciences. Moreover, the majority of Biology professors working at other universities and institutions in Ecuador received at least part of their training at PUCE. In conclusion, my academic life in Ecuador has been extremely rich. I am grateful.

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**FIGURES**

**Figure 1.** Doctoral hooding of Eugenia M. del Pino, Emory University Commencement 1972. From left to right, Eugenia M. del Pino, her mentor, Dr. Asa Alan Humphries, Jr., and the Dean of the Graduate School, Dr. Charles T. Lester (seen from the back).

**Figure 2.** Emory University Professors during a visit to the laboratory of Eugenia M. del Pino in Quito, 2013. Front row from left to right: Santiago Espinosa, a PUCE student, the Dean of the PUCE School of Exact and Natural Sciences, Dr. Hugo Navarrete, Eugenia M del Pino, and the PUCE students Andrés Melo, Alexandra Vargas and Francisca Hervas. Back row from left to right: Emory University professors: Jacobus (Jaap) De Roode, Darrell Stockes, and Steven W. L' Hernault.

**Figure 3.** The Jesuit priest, Dr. Manuel Corrales Pascual, S. J., then Rector of PUCE, and Eugenia M. del Pino, Palace of Carondelet in Quito (Government Palace of Ecuador) during the reception following the Award of the Eugenio Espejo Prizes 2012. Photo: Iliana Alcocer.

**Figure 4.** Award of the 2019 Prize of the Latin American Society for Developmental Biology (LASDB) to Eugenia M. del Pino (at the 10th LASDB Congress). From left to right: Oscar D. Pérez, Edward (Eddy) M. De Robertis, Eugenia M. del Pino and Pablo Wappner at the auditorium of the UADE Foundation in Buenos Aires, Argentina (2019). Photo: Andrés Romero-Carvajal.


**Figure 5. (A)**. Photo retrospective to celebrate the 15th anniversary of the L'Oréal-UNESCO Awards for Women in Science. The exhibit run along the Champs-Elysées between the Rond-Point des Champs-Elysées and the Théatre de Marigny. March 25th to April 14th, 2013. Photo: L'Oréal–Unesco for Women in Science. **(B)** Michael Venegas-Ferrín standing next to the picture of Eugenia M. del Pino at the Champs-Elysées photo exhibit.

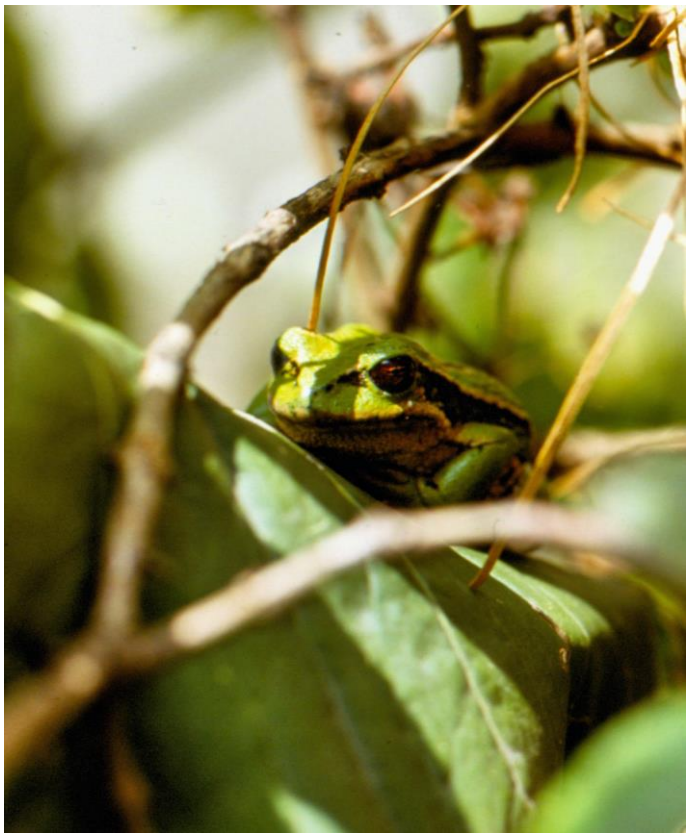

**Figure 6.** A brooding female of the marsupial frog, *Gastrotheca riobambae* basking in the sun. Marsupial frogs were maintained in an enclosed garden of my house in Quito, and the frogs reproduced spontaneously (around 1980). Photo: Eugenia M. del Pino

**Figure 7.** The 13th International *Xenopus* Conference, Chateau Lake Louise, Banff National Park, Alberta, Canada. From left to right: Drs. Joseph (Joe) Gall, Eugenia M. del Pino, Igor B. Dawid, and Diane M. Dwyer during a break of the meeting. September 2010.


**Figure 8.** Eugenia M. del Pino at work in her laboratory, PUCE, 2007. Photo: Micheline Pelletier L'Oréal–Unesco for Women in Science.