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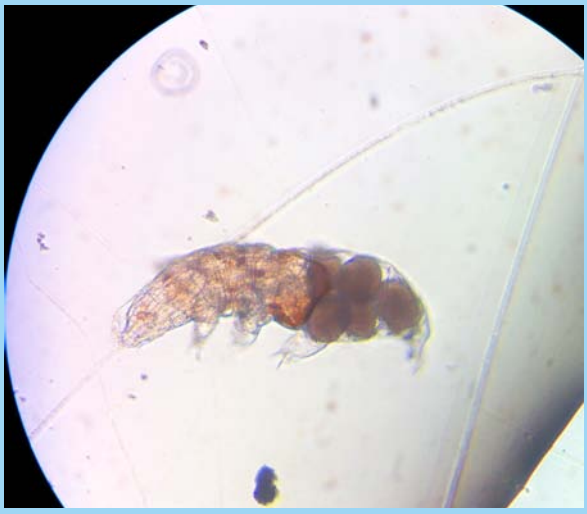

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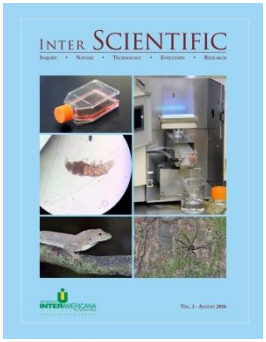

Nuestra portada combina imágenes de los temas abordados en los artículos de este ejemplar.
Diseño:
Roberto Robles Miranda
Fotografía:
Rafael Colón González
Fotos del opilión y lagartijo:
Dr. Alberto Puente-Rolón
Foto de tardígrado:
Alvin Vega, estudiante

Illustration of the diversity of topics covered in this issue.
Design:
Roberto Robles Miranda
Photography:
Rafael Colón González
Picture of the harvestmen and lizard:
Dr. Alberto Puente-Rolón
Picture of tardigrade:
Alvin Vega, student

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MENSAJE DEL RECTOR
DR. RAFAEL RAMÍREZ RIVERA

Esta edición evidencia la diversidad de áreas para desarrollar proyectos de investigación. Provee el espacio para la divulgación de nuevo conocimiento generado por la facultad. Además, ofrece oportunidad de presentar los trabajos de estudiantes bajo mentoría de la facultad. Exhortamos a utilizar este medio de divulgación para la presentación de los diferentes proyectos de investigación.

MESSAGE FROM THE CHANCELLOR
DR. RAFAEL RAMÍREZ RIVERA

This edition highlights the diversity of areas to develop research projects. It provides space for the disssemination of new knowledge generated by the faculty. It also offers an opportunity to present the work of students under faculty mentorship. We urge you to use this means of dissemination for the presentation of the different research projects.

MENSAJE DE LA
DECANA DE ASUNTOS ACADÉMICOS
DRA. ANNETTE VEGA

La relevancia de investigaciones multidisciplinarias en la academia es excelentemente representada en esta edición. La variedad de temas presentados refleja el interés de abonar nueva información en una amplia gama de campos. Continuamos con el compromiso de apoyar la investigación en la comunidad universitaria.

MESSAGE FROM THE DEAN OF ACADEMIC AFFAIRS
DR. ANNETTE VEGA

The relevance of multidisciplinary research in the academia is excellently represented in this edition. The variety of topics presented reflects the interest to provide new information in a wide variety of fields. We continue with the commitment to support research in the university community.

DESDE EL ESCRITORIO DE LA EDITORA
DRA. LIZBETH ROMERO-PÉREZ

El campo de las ciencias se nutre en gran parte de investigaciones multidisciplinarias, es por ello que con mucho orgullo presentamos el tercer volumen de nuestra revista Inter Scientific en el cual encontrará artículos que reflejan esa tendencia.

En la sección de Artículos de Investigación, encontrará varios artículos de investigaciones realizadas por estudiantes del Departamento de Ciencias y Tecnología bajo la mentoría de la Profesora Arlyn Pérez. Entre ellos, un estudio sobre detección de metales pesados y la presencia de polimorfismos utilizando fragmentos de ADN amplificados al azar en muestras de salmón y camarones. El artículo *Optimización de un método para extraer ADN genómico y amplificar los genes 16S y ND1 para establecer la filogenia entre especies de Opiliones* busca identificar especies de Opiliones encontrados en Puerto Rico. Uno de los organismos considerados mas raros es el tardígrado; en este volumen se presenta el estudio de la supervivencia a altas temperaturas y luego de exposición a radiación ultravioleta del *Milnesium tardigradum*. Estudiantes del bachillerato en Biología identificaron lugares de descanso de un reptil y presentan sus hallazgos en el artículo *Uso de perchas para dormir por Anolis cristatellus en la Universidad Interamericana de Puerto Rico, Recinto de Arecibo.* Por último, en la incesante búsqueda de nuevos tratamientos contra el cáncer estudiantes evaluaron el efecto de un antioxidante natural y sus resultados se presentan en el artículo *Efecto de resveratrol en la viabilidad y actividad del proteosoma de células de mieloma múltiple y cáncer pancreático.*

FROM THE EDITOR'S DESK
DR. LIZBETH ROMERO-PÉREZ

The field of Science thrives largely on multidisciplinary research, which is why we proudly present the third volume of Inter Scientific that includes articles reflecting that tendency.

In the Research Articles section, you will find various articles of research by students of the Science and Technology Department under the mentorship of Professor Arlyn Pérez. Among them, a study of heavy metal detection and the presence of polymorphisms using random amplified polymorphic DNA in salmon and shrimp is presented. The article *Optimization of gDNA extraction and amplification of 16S and ND1 genes to establish phylogeny between species of harvestment* approaches ways to identify Opiliones species found in Puerto Rico. Tardigrades are organisms considered rare; in this volume the survival at high temperatures and after exposure to ultraviolet light of *Milnesium tardigradum* is evaluated. Students of the biology degree identify places where a particular reptile rests and they present their findings in the article "*Sleeping sites" used by Anolis cristatellus at the Inter American University of Puerto Rico, Arecibo Campus*. Finally, in the tireless effort to find new treatments for cancer, students evaluated the effect of a natural antioxidant and present their results in *Effect of resveratrol on the viability and proteasome activity of multiple myeloma and pancreatic cancer cell lines.*

Detection of cadmium, chromium, nickel and copper in commercial frozen shrimp and salmon by flame atomic absorption spectroscopy and polymorphism in salmon by random amplified polymorphic DNA (RAPD)

Detección de cadmio, cromo, níquel y cobre en camarones y salmón congelado por espectro de absorción atómica en flama y polimorfismos en salmón utilizando fragmentos de ADN polimórficos amplificados al azar (RAPD)

Barrios, L., Betancourt, I., Rivera, A., Rivera F., Rosado, C., and Pérez Samot, A.

Science and Technology Department, Inter American University of Puerto Rico, Arecibo Campus

ABSTRACT

Pollution of heavy metals in aquatic ecosystems is growing at an alarming rate and has become an important worldwide problem. Heavy metals can increase in concentration throughout the aquatic food chain. They can accumulate within the tissues of the organism and this is a concern to both animals and humans. RAPD assay allows the detection of genetic alterations, after contamination with pollutants in aquatic organisms, as well as detection of polymorphism between species, subspecies and among related organisms. This study was aimed to determine the concentration of cadmium (Cd), Chromium (Cr), Copper (Cu) and Nickel (Ni) in commercial frozen farm shrimp, wild shrimp and wild salmon using Flame Atomic Absorption Spectroscopy (FAAS). Farm shrimp showed the highest concentrations of Cr and Cd. The highest levels of Cu and Ni were detected in wild shrimp. RAPD- PCR was used to determine genetic variation between two species of commercial frozen salmon. Different RAPD fragment patterns were observed for the two species.

RESUMEN

La contaminación por metales pesados en ecosistemas acuáticos esta creciendo a niveles alarmantes y se ha convertido en un problema a nivel mundial. La concentración de metales pesados puede aumentar a través de la cadena alimenticia. Estos pueden acumularse en los tejidos del organismo tenidendo así un efecto en animales y en humanos. El ensayo RAPD permite detectar alteraciones genéticas y polimorfismos en especies, subespecies y organismos relacionados, luego de exposición a contaminantes. Este estudio tiene como objetivo determinar la concentración de cadmio (Cd), cromo (Cr), cobre (Cu) y níquel (Ni) en productos congelados incluyendo: camarones y salmón capturados en estado salvaje y camarones cultivados, por espectroscopía de absorción atómica en flama (FAAS). Los camarones cultivados presentaron las concentraciones mas altas de cr y cd. Los niveles mas altos de Cu y Ni se detectaron en camarones capturados en estado salvaje. RAPD se utilizó para determinar variación genética en las muestras de salmón congelado.

KEYWORDS heavy metal, salmon, shrimp, FAAS, RAPD, Polymorphism

PALABRAS CLAVE metal pesado, salmón, camarón, FAAS, RAPD, polimorfismo

INTRODUCTION

Over the past few decades, heavy metal contamination in aquatic systems has attracted the attention of investigators in the developed and developing countries of the world. The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment make these toxicants harmful to the aquatic environment and consequently to humans who depend on aquatic products as sources of food (Farombi, 2007). Many of the sediments in our rivers, lakes, and oceans have been contaminated by pollutants. Some of these pollutants are directly discharged by industrial plants and municipal sewage treatment plants; others come from polluted runoff in urban and agricultural areas (Begum, 2009). Pollution of heavy metals in aquatic ecosystems is growing at an alarming rate and has become an important worldwide problem (Malik, Biswas and Qureshi, 2009). Fishes are one of the most important organisms in the aquatic food chain, which are

sensitive to heavy metal contamination (Akan, Mohmoud, Yikala and Ogugbuaja, 2012). Heavy metals can accumulate in their tissues, and as such, tissue concentrations of heavy metals can be of public health concern (Farombi, 2007). Metals, such as iron, copper, zinc and manganese, are essential since they play important roles in biological systems, whereas mercury, lead and cadmium are toxic, even in trace amounts (Alturiqi and Albedair, 2012). The absorption of metals takes place in humans mostly via the intake of food. Crustaceans and fish are present in our diet; they are great bioaccumulators of metals even if they originate from sites in which the levels of contaminants are considered low. (Ismahene and El Hadi, 2012).

Random Amplified Polymorphic DNA (RAPD) involves the amplification of random segments of genomic DNA and no prior knowledge of the genome under investigation is required. Arbitrarily chosen short primers (generally a 10 bp sequence) are used to amplify multiple segments from the DNA (Hadrys, Balick and Schierwater, 1992; Bardakci, 2001).

RAPD assay allows the detection of genetic alterations, after contamination with pollutants in aquatic organisms. The RAPD technique is used in ecotoxicology studies by quantification of alleles (bands) and analysis of their addition/loss due to mutation, inversion, deletion or chromosomal rearrangement (Banci, Pinheiro, de Oliveira, Paganelli and Pereira, 2009). More recently, the RAPD methodology has been applied in detecting the genotoxic potential of some chemicals and metals in fish (Salem et al., 2014). Banci et al., 2009, evaluated the concentrations of Cd, Pb, Cu, Cr and Hg in *U. cordatus (crab)* collected on three Brazilian mangroves. The study showed, using RAPD, that toxic compounds can result in the reduction of genetic variability. The technique has also been used to study genetic variation in several fish, mosquito, parasitic protozoa and species of *Aspergillus* (Bardakci, 2001). Bardakci and Skibinski (1993) used RAPD markers to differentiate between commercially important species, subspecies and strains of tilapia. Thirteen random 10-mer primers were used to assay polymorphisms within and between populations. Different RAPD fragment patterns were observed for different species, although not always for different subspecies.

The present study aims to determine the concentration of chromium, cadmium, nickel and copper in commercial frozen salmon and shrimp consumed in Puerto Rico by flame atomic absorption spectroscopy (FAAS). Also it aims to detect genetic variation between two species of commercial salmon from Chile and China using Random Amplification Polymorphic DNA (RAPD).

MATERIALS AND METHODS

Wet digestion

Commercial frozen shrimp and salmon were dried at 80°C for 48 hours and grinded with a mortar and piston. For the initial pre-digestion step, 2 g of the grinded material were transferred to an Erlenmeyer flask with concentrated nitric acid overnight. Samples were heated, cooled and heated again after adding perchloric acid 70% to digest residues. Hydrochloric acid 6 N and lanthanum chloride solution 1% were added to samples at room temperature to complete the digestion process. Samples were filtered using a polypropylene membrane (0.45 µm).

Flame Atomic Absorption Spectroscopy (FAAS)

Dilutions of known concentration of chromium, cadmium, copper and nickel standards were prepared using lanthanum 1%. Calibration curves of the standards and concentrations of Cr, Cd, Cu and Ni in the samples were determined by FAAS (SHIMADZU).

Genomic extraction

Salmon muscle was washed with 15% Clorox and rinsed with ultrapure water. Tissue was weighed and cut into small pieces. Genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen), according to the manufacturer's instructions. RNase (100 ng/mL) was added to the samples. All genomic samples extracted were analyzed in a Nanodrop Lite spectrophotometer (Thermo Scientific) to determine concentration and purity (A260/280). A 1% agarose gel electrophoresis was done to confirm the presence of gDNA. The samples were stored at -20^{o}C.

RAPD PCR

Random Amplification of Polymorphic DNA PCR was done using gDNA with a final concentration of 25 ng and Go Taq Green Master Mix (Taq polymerase, 3 mM $MgCl_2$, 400 µM dNTPs, 2X Buffer pH 8.5) (Promega). Primers were RAPD 1, RAPD 2, RAPD 5 and RAPD 6 (Table 1) and had a final concentration of 0.5 µM. The total reaction volume used was 25 µL. The parameters used for PCR were: 95^{o}C for 2 minutes, 40 cycles of 95^{o}C for 1 minute, 36^{o}C for 2 minutes, 72^{o}C for 2 minutes and a final extension at 72^{o}C for 4 minutes. A negative control was used to verify external DNA contamination. RAPD PCR products were detected by running a 1% agarose gel electrophoresis with ethidium bromide.

Table 1. Sequence of primers used for RAPD PCR.

Primer	Sequence (5'-3')
RAPD 1	GGTGCGGGAA
RAPD 2	GTTTCGCTCC
RAPD 5	AACGCGCAAC
RAPD 6	CCCGTCAGCA

RESULTS

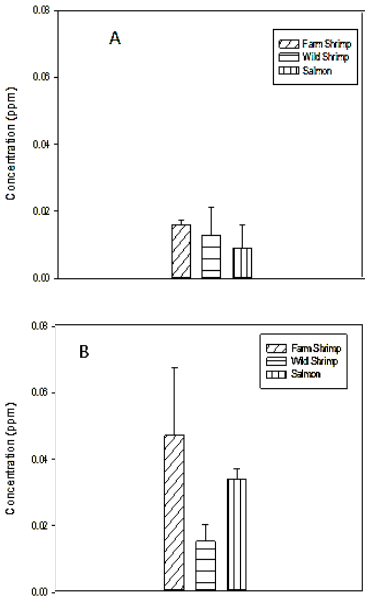

Figure 1. Levels of cadmium in frozen shrimp and salmon (A). There was not a significant difference in Cd content between samples. The highest concentration of cadmium was found in farm shrimp samples. Chromium in frozen shrimp and salmon (B). There was a significant difference in Cr content between farm and wild shrimp samples (p = 0.049). The highest concentration of chromium was found in farm shrimp samples.

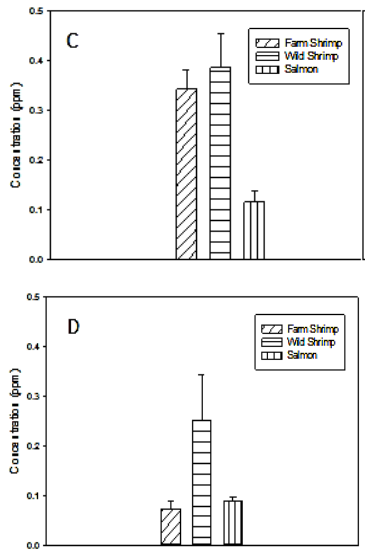

Figure 2. Levels of copper in frozen shrimp and salmon (C). There was a statistically significant difference in Cu content between farm shrimp vs salmon and wild shrimp vs. salmon samples (p < 0.001). The highest concentration of copper was found in wild shrimp. Levels of nickel in frozen shrimp and salmon (D). There was a statistically significant difference in Ni content between farm shrimp vs. salmon and farm shrimp vs. wild shrimp samples (p = 0.014). The highest concentration of nickel was found in wild shrimp samples.

Table 2. Concentration and purity (A260/280) of gDNA extracted from frozen salmon from China.

Sample	Weight tissue (mg)	Concentration (ng/µL)	A 260/280
1	12.87	1576.7	2.07
2	19.79	704.6	1.99
3	10.63	180.9	1.78
4	12.47	465.0	1.81
5	10.70	341.0	1.78
6	10.23	289.6	1.80

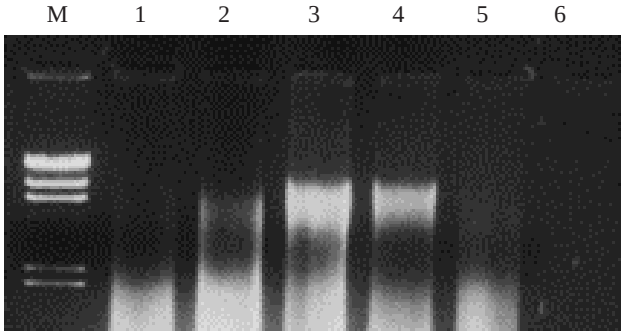

Figure 3. Gel electrophoresis (1% agarose) to confirm the presence of gDNA extracted from the muscle of frozen salmon from China. Marker Lambda DNA/HindIII (lane M) and gDNA (lanes 1-5).

Table 3. Concentration and purity (A260/280) of gDNA extracted from salmon from Chile.

Sample	Weight tissue (mg)	Concentration (ng/µL)	A 260/280
1	10.25	92.6	1.79
2	10.68	63.4	1.76
3	10.97	142.3	1.78
4	10.30	146.1	1.77
5	10.02	107.6	1.84
6	10.93	176.7	1.80

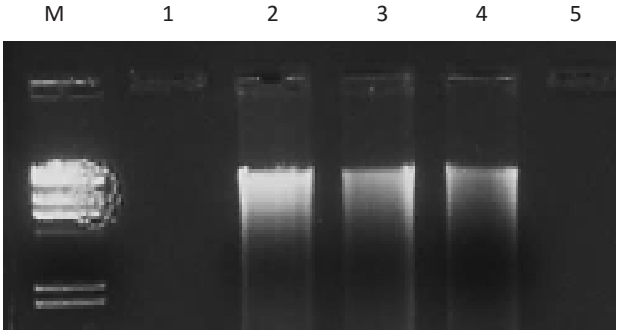

Figure 4. Gel electrophoresis (1% agarose) to confirm the presence of gDNA extracted from salmon muscle from Chile. Marker Lambda DNA/HindIII (lane M) and gDNA (lanes 2-4).

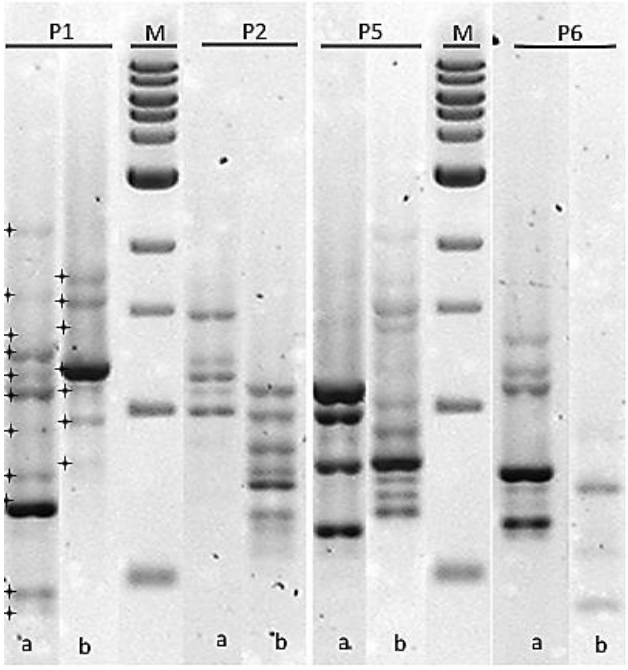

Figure 5. RAPD-PCR patterns from genomic DNA of two species of commercial salmon from Chile (a) and China (b). RAPD profile was generated using random primers P1, P2, P5 and P6. Amplification products were observed in a 1% agarose gel. Lanes M contain the Lambda/HindIII marker.

Table 4. Number of bands obtained from the amplification of gDNA by RAPD of two species of salmon (from Chile and from China).

Commercial salmon	Number of bands generated by primer				
	P1	P2	P5	P6	Total
Chile (a)	11	6	6	8	31
China (b)	7	8	11	4	30

DISCUSSION

Heavy metals like cadmium and chromium are more toxic to the body since they are not needed for biological processes and are metabolized slower. Chronic exposure to high levels of cadmium can cause kidney, lung and liver damage (Bernard, 2008). High levels of chromium can cause renal damage and cancer.

The highest concentration of cadmium was detected in frozen farm shrimp samples (0.016 ppm) and the lowest in wild salmon (0.009 ppm) (Figure 1 A). There was not a significant difference in Cd content between all samples studied. According to the Food and Drug Administration (FDA), the maximum concentration of Cd allowed for human consumption in fish and shrimp is 2 ppm. Our results show that cadmium concentrations in frozen commercial shrimp and salmon did not exceed the permissible levels.

The highest concentration of chromium was detected in farm shrimp samples (0.0445 ppm) and the lowest in wild shrimp (0.0111 ppm) (Figure 1 B). There was a significant difference in Cr content between farm and wild shrimp samples (p = 0.049). According to FDA, the maximum permitted concentration of Cr in fish and shrimp is 1 ppm. Our results show that Cr concentrations in frozen commercial shrimp and salmon did not exceed the acceptable levels.

Although copper and nickel are essential trace metals, high concentrations can cause liver and kidney problems (Alturiqui and Abedair, 2012). The highest concentration of copper was detected in wild shrimp (0.3846 ppm) and the lowest in wild salmon (0.1145 ppm) (Figure 2 C). There was a statistically significant difference in Cu content between farm shrimp vs. salmon and wild shrimp vs. salmon samples (p <0.001).

The highest concentration of nickel was found in wild shrimp samples (0.2498 ppm) and the lowest in farm shrimp (0.0729 ppm) (Figure 2 D). There is a statistically significant difference in Ni content between farm shrimp vs. salmon and farm shrimp vs. wild shrimp samples (p = 0.014).

In this study, the concentration of heavy metals was Cu>Ni>Cr>Cd. Farm shrimp had higher concentrations of cadmium and chromium when compared to wild caught. This high concentration in farm shrimp can be due to high production of the external contaminants by industries that travel with the air or wastewater and can be deposited in farm tanks.

RAPD markers have been used for the detection of genetic variation in several fish species. Previous studies by Bardakci and Skibinski (1993) evidenced polymorphisms between commercially important tilapia species, subspecies and strains of tilapia. In our study, four random 10mer primers (RAPD 1, 2, 5 and 6) were used to assay polymorphisms between two species of salmon from Chile and China. Different RAPD fragment patterns and number of bands were detected (Figure 5 and Table 4). In order to optimize the RAPD technique, different concentrations of template DNA, number of cycles of the PCR reaction and annealing temperatures were tested. The optimal concentration of genomic DNA concentration used was 25 ng and the best number of cycles was 40. An annealing temperature of 36^{o}C for 2 minutes allowed the observation of clear band patterns.

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Optimization of gDNA extraction and amplification of *16S* and *ND1* genes to establish phylogeny between species of harvestmen

Optimización de un método para extraer ADN genómico y amplificar los genes *16S* y *ND1* para establecer la filogenia entre especies de Opiliones

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ABSTRACT

One of the oldest and third-largest order of arachnids is Opiliones, commonly known as harvestmen. Most harvestmen live in damp and shadowed areas, are dark-colored, and are active mainly at night. In terms of general morphology, harvestmen are typical arachnids. They have an omnivorous diet that includes carrion, plants and fungi. In this research, some known species of harvestmen in Puerto Rico were studied: *Heterolacurbs perezassoi, Neocynortoides dorsalis, Paecilaema luquillense, Neocynortoides spp*, and an unknown species. There are several phylogenetic problems that fail to resolve the placement of harvestmen. Optimization of gDNA extraction from one appendage of a harvestman is important to preserve the animal. The detection of *16S rRNA* and *MT-ND1* genes was carried out by PCR and Sanger sequencing was used to establish phylogenetic relationship among species of Opiliones. The optimized genomic extraction protocol, primer selection and PCR protocol were important to successfully amplify *16S rRNA* and *MT-ND1* in *H. perezassoi*, *N. dorsalis* and *N.spp.*

RESUMEN

Uno de los órdernes de arácnidos más antiguos y el tercero más grande es Opiliones. La mayoría de estos viven en zones húmedas y sombreadas, son de color oscuro y activos pricipalmente durante la noche. En términos generales su morfología es la de un arácnido típico. Los Opiliones son omnívoros y su dieta incluye carroña, plantas y hongos. En esta investigación se estudiaron algunas especies de Opiliones en Puerto Rico: *Heterolacurbs perezassoi*, *Neocynortoides dorsalis, Paecilaema luquillense, Neocynortoides spp*, y otras especies desconocidas. Existen problemas filogenéticos por lo que no se ha logrado clasificar del todo a los Opiliones. La optimización de un protocolo para extraer ADN genómico de una sola pata de opilión es esencial para poder preservar el especimen. La detección de los genes *16 S rRNA* y *MT-ND1* se llevó a cabo por PCR para ser secuenciados y poder establecer las relaciones filogenéticas entre especies de Opiliones. La optimización del método de extracción, selección de cebadores y protocolo de PCR fueron importantes para amplificar exitosamente los genes *16S rDNA* y *MT-ND1* en *H. perezassoi*, *N. dorsalis* and *N.spp.*

KEYWORDS Harvestmen, gDNA, PCR, DNA sequencing, phylogenetics

PALABRAS CLAVE Opiliones, ADN genómico, PCR, secuenciación de ADN, filogenética

INTRODUCTION

One of the oldest and third-largest order of arachnids is Opiliones, commonly known as harvestmen (Wachter et al., 2015). Harvestmen are considered one of the most primitive forms of arachnids that are possibly closely related to scorpions, pseudoscorpions, and solifuges. Most species of Opiliones have secretive habitats, live in damp and shadowed areas, are dark-colored, and are active mainly at night (Pinto da Rocha, Machado and Giribet, 2007). Also, they can be found in disturbed habitats, as well as in forests, under stones, in caves, on the trunks of trees, on the soil, in forest litter, in soil crevices, and sometimes rather deep in the soil (Giribet et al., 1999). In terms of general morphology, harvestmen are typical arachnids. They have two basic body regions, a cephalotorax (pro-soma), which carries all the appendages, and a limbless abdomen (opisthosoma), which has the spiracles (breathing hole) and the genital opening, often covered by an operculum. The junction between both body regions is not constricted, giving them the appearance of "waistless" spiders. Some unique characteristics of Opiliones are paired trachea, the penis, the ovipositor, and the opening of the scent glands (Pinto da Rocha et al., 2007). These species are not dangerous because they don't have venom glands like some spiders. Another difference is that Opiliones do not have silk glands; therefore, they do not build webs (Lucio and Chame, 2005). Because of these characteristics, harvestmen are

good models for studies of biogeography, ecology, sexual selection, among others (Hedin, Starett, Akhter, Schonhofer and Shultz, 2012).

Harvestmen species have an omnivorous diet that includes small, soft skinned arthropods and other invertebrates, as well as carrion, plants and fungi (Pinto da Rocha et al., 2007); they also feed upon worms (oligochaetes), insects, other harvestmen, and occasionally vegetation, particularly flowers and fruits (Burns, Hunter and Townsend, 2006).

According to Alegre and Armas (2012), *Heterolacurbs perezassoi* constitutes a new distributional record of the subfamily Stenostygninae and it is found in Puerto Rico. With the complicated geological history of the Antilles and favorable conditions for harvestmen (tropical forests, mountainous territories and high humidity), it is expected to find new members of this subfamily in this area. Twelve other species have been described in Puerto Rico which are: *Cynortoides roeweri, Kimula elongate* (Goodnight and Goodnight, 1942*), Mirda insulana* (Silhavy, 1973), *Neocynortoides obscura, Neocynortoides dorsalis, Paecilaema luquillense, Paraconomma ovale, Paraconomma spinooculorum, Pseudomitraceras minutus* (Goodnight and Goodnight, 1942), *Stygnomma spinulata, Vampyrostenus kratochvili* and *Yunquenus portoricanus* (Silhavy, 1973).

Harvestmen are divided into 4 suborders that contain 45 recognized families, about 1,500 genera (Pinto da Rocha et al., 2007), and more than 6,500 species described (Hedin et al., 2012; Resende, Pinto da Rocha and Bragagnolo, 2012). There are several deep-level phylogenetic problems in harvestmen that are still unresolved to varying degrees and most recent phylogenetic and phylogenomic analyses generally fail to resolve the placement of harvestmen (Hedin et al., 2012). In order to understand the evolution and origin of the order Opiliones, its phylogenetic placement within the arachnid tree of life needs to be considered. However, the phylogenetic position of Opiliones has remained one of the most contentious issues in arachnid systematics and requires extensive discussion. Also, because of the dramatic increase in environmental disturbance around the world, especially in tropical regions, many species of harvestmen may have been driven to extinction even before their formal description by taxonomists (Pinto da Rocha et al., 2007). Fortunately, progress has been made in recent times, in part because of the use of sound phylogenetic methodologies applied to the analysis of morphological data (Giribet and Kury, 2007).

Genomic analysis is a useful tool in science because it gives direct access to the genomic diversity, history and evolution of the harvestmen. It helps in the identification of species (Mega and Revers, 2011). In order to perform biological studies including molecular identification, phylogenetic interference, genetics, and genomics, an efficient genomic DNA extraction is required (Chen, Rangasamy, Tan, Wang and Siegfried, 2010).

The past three decades have seen rapid advances in the field of molecular biology, and DNA sequence data now play a key role in defining species and in phylogenetic reconstructions (Wachter et al., 2015). Molecular phylogenetics has proved to be a powerful tool for research in systematics because sequencing of DNA can very quickly yield large quantities of data for phylogenetic analysis (Giribet et al., 1999). According to Hedin et al., (Hedin, Derkarabetian, McCormack, Richart and Shultz, 2010) it is needed to develop additional rapidly-evolving, informative nuclear genes for harvestmen that will allow more accurate reconstructions of population and species' history, providing the necessary framework for addressing "shallow" systematic questions that abound in the diverse group that is Opiliones. Generally, a phylogenic analysis is performed after DNA extraction, PCR (Polymerase Chain reaction) and sequencing of the PCR product.

This research is aimed to optimize gDNA extraction protocols so they would be applied to only one appendage of a harvestman to preserve the rest of the organism. Another objective of this study is the detection of *16S rRNA* and *MT-ND1* genes in *Heterolacurbs perezassoi*, *Neocynortoides, spp*. and *Neocynortoides dorsalis* by Polymerase Chain reaction (PCR). And, lastly, to sequence the PCR products to establish phylogenetic relationship among related species of Opiliones.

MATERIALS AND METHODS

DNA extraction

Opiliones were provided by Dr. Alberto Puente from the Inter American University, Arecibo campus. Samples included *Neocynortoides dorsalis, Heterolacurbs perezassoi, Paecilaema luquillense*, *Neocynortoides spp*. and an unknown specie (Figure 1). The specimen was initially preserved in 95% ethanol until used for DNA extraction. Tissues were washed with sterile water, weighed and cut into smaller pieces with a sterile razor blade and placed into a microtube. The total genomic DNA was extracted by two methods, each one with one or two legs of the specimen.

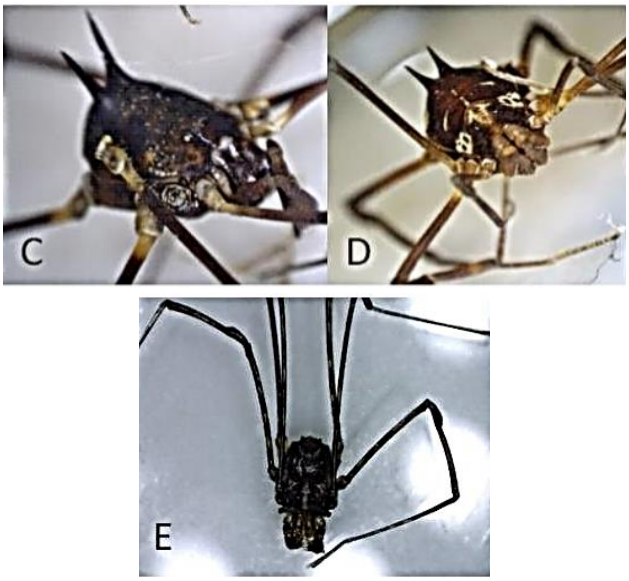

Figure 1. Dorsal view of carapace of *Neocynortoides dorsalis* (A), *Heterolacurbs perezassoi* (B), *Paecilaema luquillense* (C), *Neocynortoides spp*. (D), unknown specie (E).

The first method was maceration with lysis buffer I (50 mM Tris pH 8, 20 mM EDTA pH 8, 1.1 M NaCl, 1% CTAB, 1% PVP40, 0.5% Tween 20, 0.2% β-mercaptoetanol), lysis buffer II (10 mM Tris pH 8, 100 mM EDTA pH 8, 2% SDS) or lysis buffer III (0.01 M Tris pH 8, 0.1 M NaCl, 0.01 M EDTA, 0.5% SDS). Proteinase K and RNAse were used to remove proteins and RNA from samples.

Samples were incubated for 24 hours at 56ºC or 1 hour at 65ºC. NaCl 5 M solution and cold isopropanol were added to each sample and incubated for 30 to 60 minutes at -20ºC. DNA pellets were washed with cold 70% ethanol. After the washing step, the samples were centrifuged at 14,000 rpm at room temperature and excess ethanol was removed with a micropipette. DNA pellets were resuspended in ultrapure water.

The second method used to extract gDNA from Opiliones was the DNeasy Blood and Tissue Kit (QIAGEN), according to the manufacturer's instructions. The protocol was run with and without RNAse. All genomic samples extracted were analyzed in a Nano drop Lite spectrophotometer (Thermo Scientific) to determine concentration and purity (A260/280). Genomic DNA samples were stored at -20 ºC.

Polymerase Chain Reaction (PCR)

A PCR reaction was done using 2X Go Taq Green Master Mix (Taq polimerasa, 3 mM $MgCl_2$, 400 µM dNTPs, 2X Buffer pH 8.5) (Promega) and the Thermal Cycler (Applied Biosystems). The primers for the amplification were: *16S rDNA* and *MT ND1* (table 1), each with a final concentration of 0.3µM. The cycling parameters were: 94°C for 5 min, 35 cycles at 94°C for 30 s, 55°C or 56°C for 30s and 72°C for 1 min, and a final extension step at 72°C for 10 min. A negative control with ultrapure water was used to verify external DNA contamination. The PCR products were verified by a 1% agarose gel electrophoresis with ethidum bromide. Two markers, 100 bp (NEB) and 123 bp (Sigma), were used to determine the size of the PCR products.

Table 1. Sequence of the primers used for the PCR amplification.

Primer	Sequence (5'- 3')
ND1 FW	CCR GAR ACA AGY TCA GAC TC
ND1 RV	GGG TAT ATT CAA ATT CGA AAA GG
16S FW	CCT TTT CGA ATT TGA ATA TAC CC
16SRV	TGA CCT CGA TGT TGA ATT AA

PCR purification

The purification of the PCR products was performed using the Wizard SV Gel and PCR Clean-Up System (Promega), according to the manufacturer's instructions. The purified products were sequenced by GENEWIZ (New Jersey, USA).

RESULTS

Table 2. Method used for DNA extraction and concentration and purity (A 260/280) of the extracted gDNA from harvestmen.

Species	Method	Concentration (ng/µL)	A260/280
Neocynortoides dorsalis	CTAB	4.3	1.87
	SDS	4.4	1.79
	DNeasy	4.2	1.90
		17.8	1.84
		225.5	1.69
Heterolacurbs perezassoi	DNeasy	3.0	1.73
	SDS	15.1	1.91
Paecilaema luquillense	SDS	11.6	1.94
		116	1.74
	DNeasy	59.6	1.81
Neocynortoides spp.	DNeasy	52.6	1.79
		29.8	1.80
		49.9	1.98
Unknown	SDS/Proteinase K	5.8	1.91
		17.9	1.89

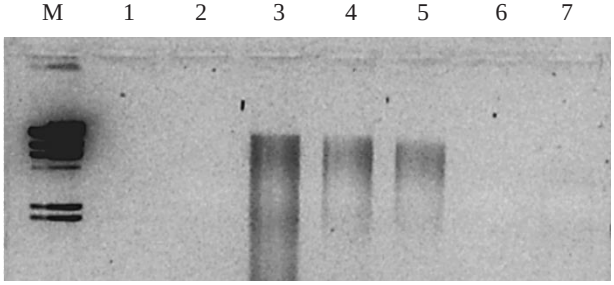

Figure 2. Electrophoresis of extracted genomic DNA (agarose 1%). Marker Lambda/Hind III (lane M). *N. dorsalis* (lanes 2 and 3), *N. spp* (lanes 4 and 5), and *H. perezassoi* (lanes 6 and7).

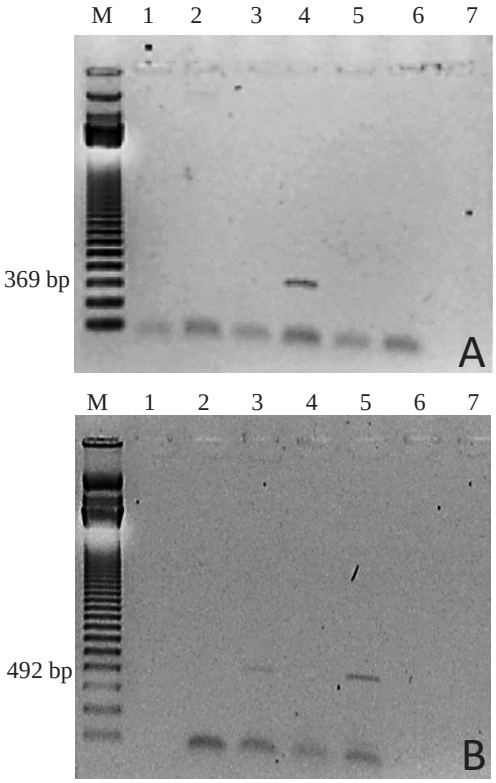

Figure 3. PCR products using primers 16S and ND1 at an annealing temperature of 55ºC. Marker was 123 bp (lanes M). Amplification product obtained for *H. perezazzoi* using 16S primers (A, lane 4). Amplification products obtained for *H. perezazzoi* (B, lane 3) and *N. spp*. (B, lane 5) using ND1 primers. Agarose 1% electrophoresis.

Table 3. Concentration and purity (A260/280) of PCR products purified using the Wizard SV Gel and PCR Clean-Up System kit**.**

Species	Primer	Concentration (ng/µL)	A260/280
Neocynortoides dorsalis	ND1	10.0	1.55
Heterolacurbs perezassoi	ND1	10.0	1.77
		14.1	1.67
Heterolacurbs perezassoi	16S	5.8	1.59
		10.8	1.86
Neocynortoides spp.	ND1	10.7	1.57
		10.9	1.79

DISCUSSION

The most efficient methods to extract genomic DNA were DNeasy Blood and Tissue kit and the traditional method using lysis buffer II with SDS. The highest concentrations of gDNA were from *N. dorsalis, P. luquillense* and *N.,* spp (Figure 2, Table 2). All genomic samples obtained had a A 260/280 ratio between 1.7-1.9 (Table 2). The appendages of the harvestmen were lysed effectively using incubation temperatures of 56^{o}C and 65^{o}C.

In order to have a good PCR product, high quality gDNA is important. In our study, the optimal concentration of genomic DNA for amplification was 15 ng. Another important factor to have a good product is the annealing temperature (T_A) used in the PCR reaction. The best T_A for the primers tested was 55^{o}C. For *H.perezazzoi* a PCR product of 369 bp was amplified using 16S primers and a product of 492 bp using ND1 primers. For *N. spp* a product of 492 bp was also obtained using ND1 primers (Figure 3). By adjusting the concentration of gDNA and the annealing temperature these products were obtained. Further tests are needed to optimize the reaction and be able to obtain product for other harvestmen.

The primers were used in a previous study by Burns, Hedin and Shultz (2012) analyzing Opiliones in North America (Table 1). Other genes such as *12S rDNA, 28S rDNA* and *EF-1α* were also used to study phylogenetic relationships allowing *Leiobunum vittatum, Hadrobunus maculosus,* and *Eumesosoma roeweri* to be placed in a phylogenetic tree. In other studies, 16S was used by Wachter et al. (2015) to study three species of harvestmen in the Alps of Austria. *Megabunus coelodonta, Megabunus cryptobergomas,* and *Megabunus lentipes* were added to a phylogenetic tree based on their analyses.

Molecular studies of harvestmen in Puerto Rico have not been reported. In our study, the optimized genomic DNA extraction protocol, primer selection and PCR protocol were important to successfully amplify *16S rRNA* and *MT-ND1* in *H. perezassoi*, *N. dorsalis* and *N. spp*. However, based on the mitochondrial and ribosomal RNA genome data sequence, a phylogenetic relationship with other species of Opiliones was not established.

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Extreme temperature survival of *Milnesium tardigradum* and *RAD 51* gene expression after exposure to ultraviolet radiation

Estudio de la supervivencia de *Milnesium tardigradum* a temperaturas extremas y expresión del gen *RAD51* luego de exposición a radiación ultravioleta

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ABSTRACT

Tardigrades are a group of invertebrates that belong to the phylum tardigrada. They have the ability to enter a latent reversible state known as cryptobiosis in extreme environmental conditions, such as low temperatures, desiccation, high salt concentration and insufficient oxygen. In this state they form a tun, a barrel-shaped, dormant tardigrade. Their metabolism is extremely low and shows apparently no visible signs of life. Tardigrades are known to tolerate ionizing and UV radiation in both the hydrated and the dehydrated state. DNA breaks can be caused by radiation. RAD51 protein is important for DNA repair. The objective of this study was to determine the survival rate of thirty nine *Milnesium tardigradum* to low temperatures of -80°C from 3 to 14 days. We examined the tolerance of three groups of thirty individuals of *Milnesium tardigradum* to high temperatures using heating to 32°C, 64°C and 96°C while active and in tun state. *M. tardigradum* had high survival rates at low temperature. The tun tardigrade was able to tolerate temperatures of 32° and 64°C. There was a dramatic reduction in survival at high temperatures of 96°C in both stages of the animal. Gene expression of *RAD 51* could not be achieved due to inadequate primers. New primers for *the RAD51* must be designed to determine effectively the expression of the gene.

RESUMEN

Los tardígrados son un grupo de invertebrados que pertenece al filo tardígrada. Poseen la capacidad de adoptar un estado latente y reversible llamado criptobiosis cuando están expuestos a condiciones extremas como temperaturas bajas, disecación, altas concentraciones de sal e insuficiencia de oxígeno. A este estado latente se le llama tun. El tardígrado adquiere forma que asemeja un barril, su metabolismo se mantiene muy lento y no muestra signos de vida. Estudios previos demuestran que los tardígrados pueden tolerar radiación ionizante y ultravioleta tanto en condiciones de hidratación como de deshidratación. La radiación puede causar rompimiento del ADN. RAD 51 es una proteína importante para la reparación del ADN. Este estudio tiene como objetivo determinar la tasa de supervivencia de *Milnesium tardigradum* expuesto a una temperatura de -80 ºC por 3 a 14 días. También, examinamos la tolerancia de *M. tardigradum* a altas temperaturas incluyendo 32, 64 y 96 ºC en estado activo y de tun. *M. tardigradum* tuvo una alta tasa de supervivencia a bajas temperaturas, mientras que a una temperatura de 96 ºC se observó una dramática reducción. Los tardígrados en tun pudieron tolerar las temperaturas de 32 y 64 ºC. La expression de *RAD51* no se pudo determinar efectivamente debido a los cebadores utilizados. Estudios futuros son necesarios para diseñar y evaluar otros cebadores y poder estudiar de forma efectiva la expresión del gen.

KEYWORDS tardigrades, cryptobiosis, tun, extreme temperatures, UV radiation

PALABRAS CLAVE tardígrados, criptobiosis, temperaturas extremas, radiación UV

INTRODUCTION

Tardigrades are microscopic animals whose body length ranges from 0.1–1.2 mm. They are aquatic or semi-aquatic invertebrates comprising the phylum *Tardigrada*. (Wang, Grohme, Mali, Schill and Frome, 2014). They comprise two main clases: *Heterotardigrada and Eutardigrada*. *Heterotardigrada* includes marine species with legs with 4 – 6 digits (toes) that have complex claws and/or sucking disks. It also includes terrestrial species with an armored cuticle and freshwater unarmored species with legs bearing up to 13 claws. The class *Eutardigrada* includes the unarmored terrestrial species and freshwater and marine with legs that terminate in claws without digits (Nelson, 2002). Recent molecular studies have indicated that tardigrades have a sister group relationship with *Onychophora* and *Arthropoda* (Wang, et al., 2014). They were first described by

the German zoologist Goeze in 1773, who named them 'water bears' because of their strong resemblance to these animals. The current name, tardigrades from Latin tardigradus, (slow-moving) was given by the Italian natural scientist Spallanzani. Tardigrades are exceptional in their adaptations to the most extreme environments. Tardigrades have a hemocoel-type of fluid-filled body cavity that functions in circulation and respiration, a complete digestive tract, and a lobed dorsal brain with a ventral nerve cord (Nelson, 2002). They can be found almost everywhere on earth from the top of the Himalaya to the bottom of the oceans (Neuman, 2006). Although all individuals require water to be active, the environments in which tardigrades live are generally divided into marine and estuarine, freshwater, and terrestrial habitats. (Nelson, 2002). They are able to withstand extreme environmental conditions that range from sub-zero temperatures to high heat or drought (Hengherr, Worland, Reuner, Brummer and Schill, 2009).

Recent studies have showed that tardigrades are also resistant to ultraviolet radiation (Altiero, Guidetti, Caselli, Cesari and Rebecchi, 2011). They are capable of reversible suspension of their metabolism and entering of cryptobiosis (latent life) (Förster, 2009) which is the collective name for a state of life used by some organisms to overcome periods of unfavorable environmental conditions. (Jönsson, 2003).

Cryptobiotic organisms are known from both the plant and animal kingdom, but in animals it occurs mainly among invertebrates. The four main factors inducing cryptobiosis are: anhydrobiosis (desiccation), cryobiosis (freezing), osmobiosis (elevated salt concentrations), and anoxybiosis (low oxygen levels) (Horikawa et al., 2006) Though most tardigrades are herbivorous, some species can be carnivorous like in the case of *Milnesium tardigradum*. This species has been found to eat nematodes and rotifers, as part of their diet (Förster, et al., 2009). Tardigrade reproduction may differ between species. Some species have separate sexes (male or female) while others are parthenogenetic (without a male). The eggs of the tardigrades can be laid on a substrate or placed inside their own cuticle during molting for protection (Altiero, et al., 2011).

Tardigrades are known for being resistant to extreme conditions, including tolerance to ionizing and UV radiation in both the hydrated and the dehydrated state. DNA breaks can be caused by radiation. RAD51 protein is highly conserved and is used in homologous recombination for DNA repair (Jönsson, 2003). RAD51 protein binds to DNA at the site of the break and allows the repair of double strand breaks or single strand gaps.

The aim of this research is to study the survival rate of a population of *M. tardigradum* after exposure to extreme temperatures. Also it aims to detect the expression of *RAD51* gene in *Milnesium tardigradum* after exposure to ultraviolet radiation.

MATERIALS AND METHODS

Sample collection and observation

Tardigrades were collected from lichens of palm trees located on the Arecibo campus. The lichen was scraped from the palm tree with a spatula and placed in a beaker. Deionized water was added and mixed to release the tardigrades from the lichens. The specimens were placed in a petri dish to observe morphology, locomotion, feeding and molting with a stereo microscope (Nikon SMZ-1) or an inverted microscope (Olympus CK-X31, magnification 100X and 200X). Tardigrades were maintained in small petri dishes with lichens and deionized water at RT.

Exposure to extreme low temperature

Thirty nine tardigrades were collected and separated into 2 groups of 15 and 1 group of 9, each in a 1.5 ml microtube. The water was removed to induce the specimens into a dehydrated state (tun). After induction the tubes were placed in the freezer at -80ºC for a period of 3, 7 and 14 days. Once this period was achieved the tardigrades were re-hydrated and observed under a microscope to determine their survival rate.

Exposure to extreme high temperatures

Sixty tardigrades were collected and separated in 6 groups of 10 each. Three groups were induced into a dehydrated state (tun) and three groups were maintained in active state. Each group was exposed to 32ºC, 64ºC and 96ºC for 15 minutes. Tardigrades were transferred to a small petri dish with 5 ml of fresh deionized water. Survival rate was determined 1 and 24 hours after exposure using a stereo microscope (Nikon).

Exposure to ultraviolet tradiation

A total of 135 tardigrades were separated in 9 groups of 15 each in a petri dish with DEPC water; three groups were used as a control (fully active, stage I), six groups were induced into a dehydrated state and exposed to UV light (312 nm) in a PCR Workstation (Captair Bio, Erlab) for 90 minutes. The plates with the specimens were placed 30 cm from the UV lamp and without the cover. For the RNA extraction, three groups were kept dehydrated (tun, stage II) while the other 3 groups were re-hydrated for one hour (partially active, stage III).

RNA extraction

The RNA from tardigrades was extracted using TRIzol Reagent (Sigma). Chloroform was added to remove proteins and centrifuged for 15 minutes at 12,000 *x g* at 4ºC. Isopropyl alcohol and 75% ethanol/DEPC were used to precipitate RNA. Centrifugation was done for 20 minutes at 12,000 *x g* at 4ºC followed by centrifugation for 5 minutes at 7,500 *x g* at 4ºC. The

pellet was resuspended in RNase free water. Genomic DNA was removed using the Ambion DNA free DNase Treatment and Removal Reagents (Applied Biosystems). RNA concentration and purity (A260/280 ratio) was determined using the NanoDrop Lite spectrophotometer (Thermo Scientific).

cDNA synthesis

Genomic DNA was removed and cDNA was synthesized from the RNA using the Quanti Tect Reverse Transcription Kit (QIA GEN) (Quantiscript Reverse Transcriptase, RT Buffer 5X, Mg, dNTPs and RT Primer Mix). cDNA concentration and purity (A260/280 ratio) was determined using the NanoDrop Lite spectrophotometer (Thermo Scientific). All cDNA samples were stored at -20°C.

qt RT PCR

The final concentration of the primers to amplify RAD51 and β-ACT used for the reaction was 30 µM (table 1).The cDNA samples were diluted to 50 ng/µl and 2 µg of the template were added to the master mix. Reactions were performed in triplicates in 48-well plates (Applied Biosystems) with a final volume of 25 µl. Amplification was determined with Fast SYBR Green Master Mix and the Step One Real-Time PCR System (Applied Biosystems). PCR was performed with a 15 min pre-incubation of 95°C, subsequently, 40 cycles of amplification, 15 s at 95°C, 30 s at 64°C, 30 s at 72°C. The melting curve parameters used were: 15 s at 95°C, 1 min at 60°C and 15 s at 95°C with increments of 0.3°C. A negative control with ultrapure water was used to determine external DNA contamination.

Table 1. Sequence of the primers used.

Primer	Sequence (5'- 3')
RAD51 FW	CGATGATGTTACCGCCGATCG
RAD51 RV	CCTCATTAGCGATCGCGAACA
β-ACT FW	ATGAAGATCAAGATCATCGCTCC
B-ACT RV	GAGATCCACATCTGCGCTGGAAG

RESULTS

Table 2. Survival rates of *M. tardigradum* after exposure to -80°C.

Number of tardigrades before exposure	Time (days)	Number of viable tardigrades after exposure	Rate of survival (%)
15	3	13	86.7
15	7	14	93.3
9	14	9	100

Table 3. Number of viable *M. tardigradum* individuals after exposure to heat.

	Temperature (°C)	Number of tardigrades before exposure	Number of tardigrades after exposure		
			Time(hrs)		
			T_0	T_1	T_{24}
Active Stage	32	10	10	7	7
	64	10	0	0	0
	96	10	0	0	0
Tun Stage	32	10		10	0
	*64	10		7	7
	96	10		0	0

*3 animals were lost when transferring from the microtube to the petri dish

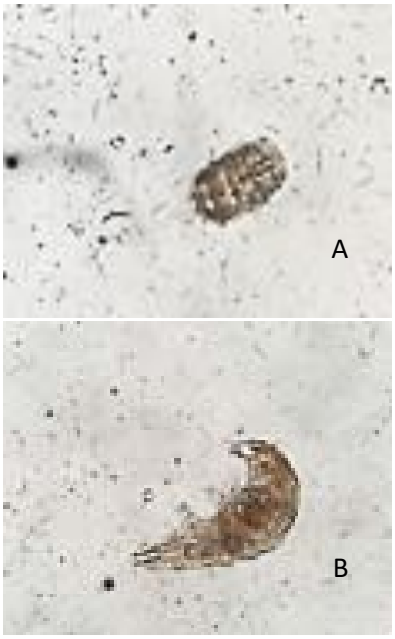

Figure 1. Tardigrade in tun state after dehydration (A). Tardigrade re-hydrated after exposure to -80°C after 3 days (B). Pinctures taken at a total magnification of 400X using the inverted microscope CK - X 31 Olympus.

Table 4. Survival rates of tardigrades after exposure to heat.

	Temperature (°C)	Rate of Survival (%)	
		T_1	T_{24}
Active Stage	32	100	70
	64	0	0
	96	0	0
Tun Stage	32	100	0
	64	*100	*100
	96	0	0

*Since 3 animals were lost when transferring, the survival rate

was determined considering the remaining 7 individuals.

Table 4. Concentration and purity (A260/280 ratio) of RNA extracted from tardigrades in each stage.

Phase	RNA Concentration (ng/µL)	A260/280
1	848.6	2.17
(Active)	670.7	2.05
	638.3	1.98
2	12.2	2.30
(Tun)	32.7	1.70
	27.1	1.72
3	180.6	1.70
(Partially active)	216.9	1.73
	102.6	1.66

Table 5. Concentration and purity (A260/280) of cDNA in each phase.

Phase	cDNA Concentration (ng/µL)	A260/280 Ratio
1	1481.8	1.85
(Active)	1399.9	1.87
	1247.0	1.84
2	1368.8	1.86
(Tun)	1281.9	1.86
	952.0	1.83
3	1586.6	1.84
(Partially	1486.1	1.85
active)	1340.5	1.86

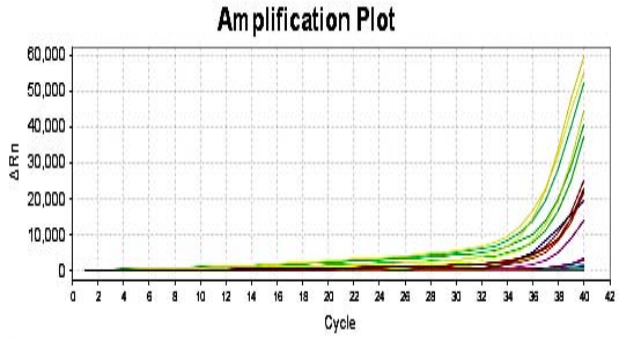

Figure 2. Amplification plot of the target gene *RAD51* and reference gene *β ACT* of *M. tardigradum* in Stage I, II and III. This amplification plot showed that all samples entered an exponential amplification at a high Ct value of 34.

DISCUSSION

The objective of this study was to determine the survival rate of thirty nine *Milnesium tardigradum* after exposure to a low temperature of -80 °C from 3 to 14 days (table 2, figure 1). *M. tardigradum* had high survival rates at low temperatures. Previous studies by Hengherr et al. (2009) demonstrated freeze tolerance in tardigrades. The temperatures they tested were 25 to -30°C at a cooling rate of 1°, 3°, 5° and 9°C per hour (°Ch^{-1}). A high survival rate of 95.0±4.1% at a cooling rate of 9°Ch^{-1} and a lower survival rate of 71.3±20.2% at 3°Ch^{-1} for *M. tardigradum* was observed. Also, they showed that tardigrades species can tolerate fast cooling and fast ice formation within their bodies. Trehalose was not detected in the active or anhydrobiotic state of *M. tardigradum*. The survival rate at slow cooling rates down to 1°Ch^{-1} indicated that the animals may synthesize ice active proteins or cryoprotective compounds to increase their survival ability.

We examined the tolerance of thirty individuals of *Milnesium tardigradum* to high temperatures: 32°C, 64°C and 96°C while active and in tun state (table 3 and 4). This study showed that *M. tardigradum* in active stage has low tolerance to temperatures above 32ºC. In tun form, they were able to withstand a temperature of 64º C. However survival above that temperature was lethal. This study concurs with previous studies that high temperatures and relative humidity have negative effects on both survival and time to recover after re-hydration (Glime, 2013).

Tardigrades are known for being resistant to extreme conditions, including tolerance to ionizing and UV radiation in both the hydrated and the dehydrated state. DNA breaks can be caused by radiation. Recombinase (RAD51) protein is important for DNA repair. It is highly conserved and is used in homologous recombination (Jönsson, 2003) to repair damaged DNA. RAD51 protein binds to DNA at the site of the break and allows the repair of double strand breaks or single strand gaps.

Based on purity and concentration, RNA was successfully extracted from *M. tardigradum* (table 4 and 5), but detection of the expression of *RAD51* in Stage I, II, and III of *M. tardigradum* was not achieved (figure 2). New primers for *RAD51* and *β-ACT* will be designed to determine effectively the expression of both genes in *M. tardigradum.*

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"Sleeping sites" used by *Anolis cristatellus* at the Inter American University of Puerto Rico, Arecibo Campus

Uso de perchas para dormir por *Anolis cristatellus* en la Universidad Interamericana de Puerto Rico, Recinto de Arecibo

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ABSTRACT

To better describe the ecological behavior and sleeping biology in urban areas, we studied anoles classified as tree-trunk in the north coast of Puerto Rico. It is recognized that behavior can strongly vary depending on the habitat in which anoles are located. Thus, we observe how perching behavior changes in the Interamerican University, and what may be forcing these changes. Fifteen percent (15%) of anoles preferentially selected non-vegetative substrate to perch while 67% demonstrated fidelity to sleeping site. Moreover, 69% of lizards were facing away from the leaf base. Also, a possible thermal relation to perching was found. Sex and age of the lizard was not determined in our study.

RESUMEN

Para describir mejor el comportamiento ecológico y la biología de dormir en las zonas urbanas, se estudió al lagartijo crestado clasificado como tronco de árbol en la costa norte de Puerto Rico. Se reconoce que el comportamiento puede variar fuertemente en función del hábitat en el que se encuentran los lagartijos. Por lo tanto, en la Universidad Interamericana, observamos que 15% de los lagartijos crestados seleccionan sustratos no vegetativos como percha y el 67% demostraron fidelidad al sitio para dormir. Por otra parte, el 69% de los lagartos se acomodan lejos de la base de la hoja. Además, se encontró una posible relación térmica en el uso de perchas. El sexo y la edad de los lagartijos no se determinó en nuestro estudio.

KEYWORDS *Anolis cristatellus*, sleeping behavior, anoles, substrate fidelity

PALABRAS CLAVE *Anolis cristatellus,* comportamiento de sueño, anolis, fidelidad de sustrato

INTRODUCTION

The study of where and how animals sleep has a huge impact in their behavior, spatial and temporal ecology. (Singhal, Johnson, and Ladner, 2007). However, studies on sleeping sites selection are important because is the period where the animal is immobilized and it is exposed to predators (Singhal et al., 2007).

One group that has not received a lot of attention in terms of its sleeping biology is the lizards group of the genus *Anolis.* These groups of lizards tend to spend one third to half of their lives asleep. In their natural habitat they use plant branches, leaves, and stems. One benefit of this strategy is the ability to detect potential predators by the vibrations of the leaves and branches that allow anoles to escape (Losos, 2011).

Anoles, in general are being studied by evolutionary biologist due to its ability to adapt rapidly to new environments. (Matthew and de Quieroz, 2004). Urban areas provide impervious surfaces, nonnative and managed vegetation, continuously changing micro climates, and patches of natural remnants. One species that is commonly seen in these urban areas is the Puerto Rican crested anole (*Anolis cristatellus*). Ongoing research on the species in urban environments has shown that the species is adapting to urban areas (Winchel, Reynolds, Prado-Irwin, Puente-Rolón and Revel, 2016).

To observe the factors that can affect the sleeping biology of lizard species in urban areas, we studied the *Anolis cristatellus* at the Inter American University of Puerto Rico in Arecibo. These lizards are diurnal and use perching for warding off other males, attracting females, and for food seraching. They perch in walls and fences because they can have a better view of their predators. They are territorial, and tend to be located in the same areas during the day and night (Singhal et al.,2007). In Puerto Rico, crested anoles are preyed by birds, mongoose, snakes, frogs, other lizards and cats (McDaniel, 2012). In this study we observed the sleeping behavior of the *Anolis cristatellus* in an

urban environment.

MATERIALS AND METHODS

Study site

We conducted the study at the Inter American University of Puerto Rico, Arecibo Campus located at the northern coast of Puerto Rico (18°28'29.60"N, 66°45'33.75"W). This site has small areas with vegetation and is crowded with human activity until 22:00 hrs, when the institution closes. It is located adjacent to main roads, which means that the level of vehicle flow fluctuates within our timeframe.

Sampling

Nocturnal visits were conducted from March 5th and April 13 of 2015 to observe the nocturnal habitat used by *A. cristatellus*. The time of search was from the 19:00 hrs to 24:00 hrs, two or three times a week. For each located lizard, we pinpointed the exact location on a map using "Google Earth". Height of the perch site used by the lizard was quantified in meters using a measuring tape. If the height was beyond our measuring tape, the altitude of the perch was estimated by eye trying to be as accurate as possible. Despite the fact that perch visibility at great heights is very difficult, we did not limit ourselves by searching only close to the ground.

For every sleeping site, the temperature was recorded and an average of the environmental temperature was documented with a laser thermometer (Metris Instruments). Additionally, the orientation and angle in which the anole was found sleeping or perching was noted. In addition, the substrate used by the anole was documented. Finally, we used "liquid paper" or "White-Out" to mark the anoles to determine if there was a site of fidelity of the lizards to their perching site. We conducted statistical tests such as: Standard Deviation, One way ANOVA and T-test to further observe and compare the results.

RESULTS

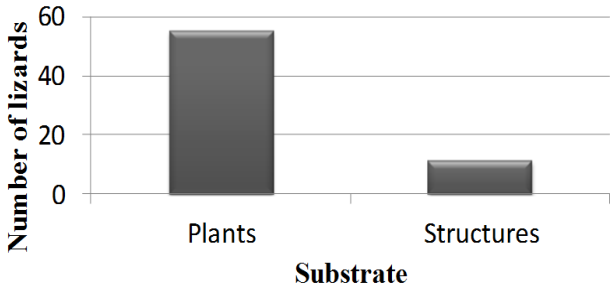

Figure 1. Difference between the substrate the lizards chose as their sleeping site. Plants indicate that the substrate was organic material, and structures are manmade substrates. The result for our T-test from this data was t=3.17, n=7, p=0.0194.

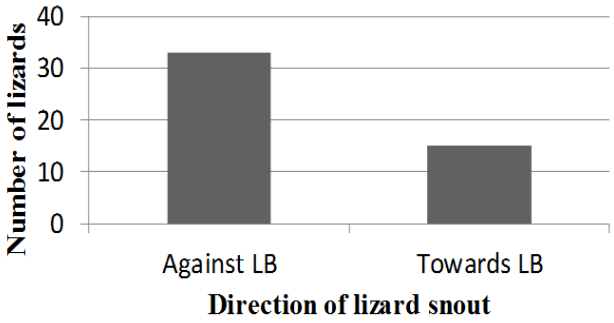

Figure 2. Difference between directions of the lizard's snout, regarding the base of the leaf. Against LB indicate a direction that is opposite to the leaf base, and Towards LB indicate a direction that is facing the leaf base. The result for our T-test was t=-5.53, n=7, p=0.0017.

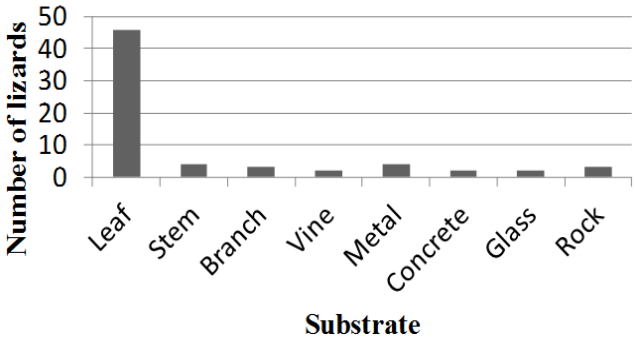

Figure 3. Quantity of lizards found on each specific type of substrate. The result for the One way ANOVA test we did from this data is F= 9.71, n=7, p=0.001.

Figure 4. Aerial photograph showing the percentage of lizards per area and the temperature at each area.

DISCUSSION

During the night survey, we were able to find 66 *Anolis cristatellus*. Males tend to sleep on the top area of the trees, while females and juveniles tend to sleep on lower areas (McDaniel, 2012). Based on the data we collected, *Anolis cristatellus* have an average perching height of 1.1 m, similar to other lizards in their natural habitat (Genet, 2002). In terms of substrate, anoles use more plants than manmade structures for sleeping (Figure 1.). Observations in the study show that the surfaces in which we found the lizards were very unstable upon motion (thin leaves, branches and others). This observation may suggest that the sensitivity to movement with the lizard's vision may provide protection from an upcoming predator when sleeping (Singhal et al., 2007), meaning that lizards have greater opportunities to escape from an upcoming predator. Also, lizards tend to select sites where they can obtain food, defend territory and may help them gain easy access to females for reproduction upon wakening.

Additionally, a significant difference was found in the orientation of the lizard sleeping on the lamina of the leaf [Towards the Leaf Base (TLB) or Against Leaf Base (ALB)] (Figure 2.). At the campus anoles tend to sleep Against LB (Figure 2). Further analyses are needed on order to determine if the intensity of predation on the campus is not the same as if they were in their natural habitat. Cats, birds, and maybe dogs that are found in the area may be possible predators for anoles. Furthermore, human activity during diurnal and nocturnal hours may also be a factor for lizards to sleep Against LB.

The quantity of anoles found in natural substrates was greater than that on none vegetative structures (Figure 1 and 3). Despite the fact that *A. cristatellus* is the type of lizard that normally spend a great amount of time perching fences, walls, rocks and other surfaces here in Puerto Rico, they prefer perching in plant substrates during the nocturnal hours. Plant substrates provide lizards a natural camouflage that will guarantee their safety from predators at night. However, the manmade substrates such as a wall, fences and others, will put in disadvantage the lizard against predators due to the vulnerability of being exposed and visible.

The analysis carried out suggests that the anoles that were found in manmade substrate were not found randomly. These lizards went through a process where they decided to perch on that site. One possible answer may be that the quantity of lizards in the sleeping sites may be too high; therefore the few plants in each site were already taken. Factors that influence the selection of sleeping sites are body temperature during the night, body temperature at emergence from the overnight period of inactivity, or voluntary hypothermia for conservation of energy (Cabrera and Reynoso, 2012).

Furthermore, the sites that provided appropriate environment for the lizards to sleep where aggregations of plants in some spots within the campus. Also, the distance that a lizard would have to travel thought open space in order to reach another vegetative aggregation could be approximately 8-12m (Figure. 4).

Four out of the six marked lizards were found sleeping in the same area where they were found several consecutive nights. Also, we could observe that the lizards used the same nocturnal area during the diurnal hours. Studies suggest that the lizards tend to sleep in the same sites to guarantee their refuge against predators and other natural resources (Singhal et al., 2007). Two out of the six lizards were never found again. A possible explanation to this is that bigger lizards (male) tend to have a larger area of territory. Thus, they can sleep in different areas within their territory range at night. However, other studies suggest that the lizards' nocturnal territory range is smaller than their diurnal range. Since females tend to have smaller home range, they can demonstrate a high level of fidelity to their sleeping sites. However, we did not verify the sex for all anoles in our study.

A notable relation between temperature and perch was found during the investigation. The majority of lizards were found in an area with the highest temperature (Figure. 4). Studies suggest that lizards select perches with higher temperature to help them prevent hypothermia and regulate their body temperature without spending large amounts of energy. The cost of raising body temperature in the University may be lower than in forests due to the high density of buildings and the open canopy areas. Consequently, lizards tend to perch closer to buildings and main roads in order to absorb heat during the night (Cabrera, 2012).

In general, *A. cristatellus* sleeping site is toughly influenced by the vegetation disposition and natural resources. Also, limited home range may consequently force sleep site fidelity. Despite the fact that lizards at the campus tended to sleep in more horizontal leaves, shows that anoles' behaviour has changed to suit their needs in the urban area. Smaller perching areas may imply that smaller territory range may reduce also; therefore, increasing the competition and making the habitat more challenging for the lizards existence/survival.

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Effect of resveratrol on the viability and proteasome activity of multiple myeloma and pancreatic cancer cell lines

Efecto de resveratrol en la viabilidad y actividad del proteosoma de células de mieloma múltiple y cáncer pancreático

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ABSTRACT

Pancreatic Cancer (PaCa), is a highly lethal cancer that remains the fourth leading cause of cancer deaths in both men and women. Multiple myeloma is the second most prevalent hematologic cancer after non-Hodgkin lymphoma; it accounts for 10% of all hematologic cancers and approximately 2% of all cancer deaths. The Proteasome is a multi-catalytic enzyme complex that degrades most intracellular proteins by a targeted and controlled mechanism. In many cancer types the proteasome is overexpressed promoting cell survival by degrading tumor suppressors. For that reason, proteasome inhibitors are considered a treatment being Bortezomib the first proteasome inhibitor approved to treat multiple myeloma. Resveratrol is a natural antioxidant that exhibits a role inhibiting the proteasome. The objective of our research is to evaluate the viability and proteasome activity of multiple myeloma and pancreatic cancer cells treated with different doses of resveratrol for 24 hours. Our results show that resveratrol reduced the viability of PANC-1 cells at levels between 5 to 22 %. An increase in viability was observed for multiple myeloma cells. For both cell types proteasome activity was reduced. More studies are required to evaluate different exposure times and concentrations of resveratrol.

RESUMEN

El cáncer pancreático es un cáncer altamente letal que continua siendo la cuarta causa de muerte por cáncer tanto en hombres como en mujeres. El mieloma múltiple es el segundo cáncer hematológico mas prevaleciente, luego del linfoma no Hodgkin; este es responsable del 10% de los cánceres hematológicos y 2% de las muertes por cáncer. El proteosoma es un complejo enzimático multi catalítico que degrada proteínas intracelulares de forma altamente regulada y dirigida. En muchos tipos de cáncer se ha observado sobre expresión del proteosoma promoviendo así la superviviencia de las células cancerosas al degradar los supresores de tumor. Por esa razón, los inhidores del proteosoma se han vuelto un blanco para la creación de nuevos tratamientos anti cáncer, siendo Bortezomib el primer inhibidor del proteosoma aprobado para tratar el mieloma múltiple. Resveratrol es un antioxidante natural que exhibe actividad anti proteosoma. Es por esto que el objetivo de nuestra investigación es evaluar la viabilidad y actividad del proteosoma en células de mieloma múltiple y cáncer pancreático expuestas a diferentes dosis de resvetarol por 24 horas. Nuestros resultados muestran que resveratrol redujo la viabilidad de las células de cáncer pancreático un 5 a 22%. En el caso de mieloma múltiple, se observó un aumento en viabilidad. Para ambos tipos de células, se redujo la actividad del proteosoma. Más estudios son necesarios para evaluar otras dosis y tiempos de exposición a resveratrol.

KEYWORDS multiple myeloma, pancreatic cancer, proteasome, resveratrol

PALABRAS CLAVE mieloma múltiple, cáncer pancreático, proteosoma, resveratrol

INTRODUCTION

Over 32.6 million people are estimated to be living with cancer worldwide (Bundela, Sharma and Bisen, 2015). Among the more lethal forms of cancer is pancreatic cancer (PaCa). It remains the fourth leading cause of cancer deaths in both men and women. In the US the incidence of PaCa has risen with nearly 38,000 cases diagnosed per year, and fewer than 5% of patients surviving beyond 5 years. In addition, PaCa is highly resistant to chemotherapy (Awasthi, Schwarz and Schwarz, 2009) (Spratlin et al., 2011).

Multiple Myeloma (MM) or plasma cell myeloma, is a malignant neoplasm chracaterized by latent accumulation of secretory

plasma cells with a low proliferative index and an extended life span in the bone marrow (Wada et al., 2015) (Sagawa et al., 2015). It is the second most prevalent hematologic cancer after Non-Hodgkin lymphoma and it accounts for 10% of all hematologic cancers and approximately 2% of all cancer deaths (Bhardwaj et al., 2007).

The proteasome is a multicatalytic and multisubunit protease of 2.0-2.5 MDa that degrades cytosolic, endoplasmic reticulum and nuclear proteins. It is responsible for keeping a balance of intracellular proteins by eliminating misfolded or nonfunctional proteins through a selective ubiquitin tagging mechanism. Ubiquitinated proteins can serve as substrate for the 26S proteasome. It is formed by a 20S cylindrical core with the proteolytic active site, and two regulatory complex 19S that unfold ubiquitinated proteins and guide them to the 20S.

It has been found that the proteasome is overexpressed and/or highly active in many cancer cell types. This hyperactivation is associated with the targeting of tumor suppressants including p53, cyclins, cyclin dependent kinase inhibitor and the inhibitor of NF-kB, IkB. Proteasomes perform a critical role in the degradation of key signaling molecules that promote cell cycle progression, cellular adhesion, proliferation, and as a result anti-apoptosis(Almond and Cohen, 2002). Any damage, disruption or inhibition of the proteasome can affect important signaling cascades and ultimately can lead to apoptosis Therefore, due to these effects, the proteasome has emerged as an attractive target for cancer therapy (Adams, 2003; Awasthi et al., 2009), specifically the evaluation of proteasome inhibitors.

Bortezomib was the first proteasome inhibitor to enter clinical trials and receive approval from FDA for treatment of multiple myeloma. Even though much progress has been made in therapies to improve survival in multiple myeloma patients (Kumar et al., 2008), many experience recurrent relapse and become intolerant to the treatment including bortezomib (Stewart, 2012). That fact has motivated scientists to look for natural ways to enhance current treatments and increase survival rates.

Resveratrol is a stilbene substrate accumulated as a phytoalexin in several plant species including grapes. Resveratrol is not a new discovery, it has been used as a dietary supplement since the 80s. It is well known for its health benefits including antiaging, anti-inflammatory, and cardio protectant. It has antioxidant properties, which reduce the formation of reactive oxygen species that lead to genetic damage and successive tumor formation (Lang et al., 2015). Numerous studies have demonstrated the beneficial effects of resveratrol and the potential anti tumor properties (Kotha et al., 2006). Using relatively high concentrations, resveratrol has been shown to induce an artificial checkpoint at the G1-S transition phase via induction of p21WAF1 and down-modulation of G1-S specific cyclins/cyclin-dependent kinases, therefore imposing G1 arrest (Jazirehi and Bonavida, 2004). Resveratrol also suppressed the NFkB activation and expression of cyclin D1, COX-2, ICAM-1, MMP-9 and surviving in tumor tissues. Treatment with 30 μM and 100 μM resveratrol showed a statistically significant loss of cell viability at 48 hours (Lang et al., 2015). Furthermore, it was demonstrated that resveratrol induced apoptosis by activating capase-3/7 and inhibiting the expression of Bcl-2 and XIAP in human pancreatic cancer cells (Roy, Chen, Fu, Shankar and Srivastava, 2011). Also, resveratrol has been proposed to be a proteasome inhibitor (Qureshi, 2012).

The objective of our research is to evaluate the effect of various doses of resveratrol on the viability of pancreatic cancer cell line PANC-1 and Multiple Myeloma cell line NCI-H929 via the MTT assay and measure proteasome activity after exposure to resveratrol for 24 hours.

MATERIALS AND METHODS

Cell lines

Cell lines used were NCI-H929 of multiple myeloma (ATCC CRL-9028) (Figure 1) and PANC-1 of pancreatic carcinoma (ECACC) (Figure 2). Cells were grown on RPMI 1640,10% FBS + Pen 10 U/ Strept or DMEM:F-12, respectively at 37º C and 5% CO_2.

Resveratrol

Resveratrol was obtained from Sigma-Aldrich (R5010). It was diluted with DMSO at 1 uM, 10 uM, 25 uM, 50 uM, 100 uM, and 150 uM concentrations.

Viability assay

For the MTT assay, Roche Cell Proliferation Kit I (MTT), was used. Briefly, 10^3, 10^4 or 10^5 cells were added to a 96 well plate. Resveratrol was added to each well and the plates were incubated for 24 hours at 37 ºC, 5% CO_2. Samples with media only or DMSO were used as controls. After incubation, 10 µL of labelling reagent were added to each well followed by 4 hours of incubation. Finally, 100 µL of solubilization solution were added to each well and plate incubated overnight. After incubation, plate was read at 550-600 nm. All samples were tested in triplicate.

Proteasome activity

1 x 10^6 cells were added to a 24 well plate. Resveratrol was added to each well and the plates incubated for 24 hours at 37 ºC, 5% CO_2. After exposure to resveratrol, cells were centrifuged at a speed of 1000 rpm for 5 minutes at 40 ºC. Cells were rinsed with PBS Ca, Mg Free 1X. Lysis buffer (CelLytic MT, Sigma-

Aldrich) with protease inhibitors was added to each well for 15 minutes. For PANC-1 cells, media was removed and cells rinsed with PBS Ca, Mg Free 1X, followed by addition of lysis buffer with protease inhibitors and incubation for 5 minutes. Cells were centrifuged at 10,000 *xg* for 10 minutes at 4 ºC. Supernatants were collected and proteasome activity was measured using the Pierce Colorimetric Protease Assay Kit (Thermo Fisher Scientific). Samples were tested in triplicate.

RESULTS

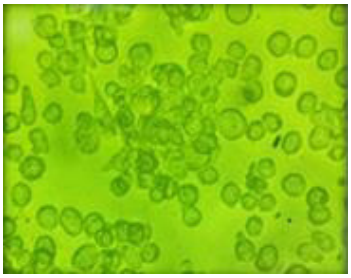

Figure 1. Multiple myeloma cancer cell line NCI-H929 grown on RPMI-1640 media. 4X magnification on a Phase Contrast Microscope.

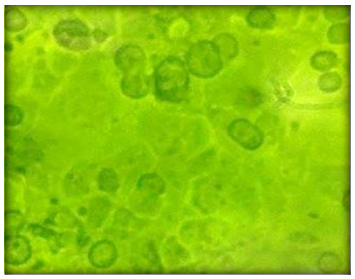

Figure 2. Pancreatic cell line PANC-1 grown in DMEM F:12 media. Cells treated with trypsin. 4X magnification on a Phase Contrast Microscope.

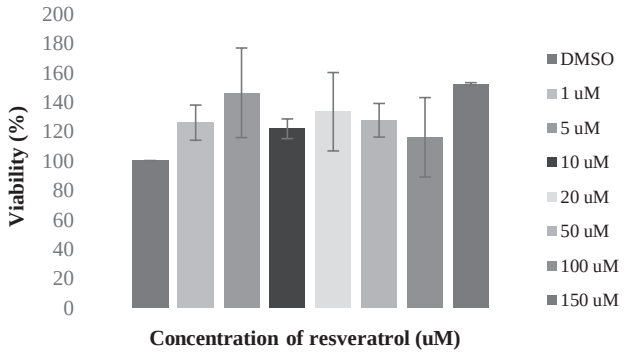

Figure 3. MTT assay. Effect of resveratrol on the viability of multiple myeloma cancer cell line NCI-H929. Cells were exposed to various doses of resveratrol for 24 hours. DMSO is control.

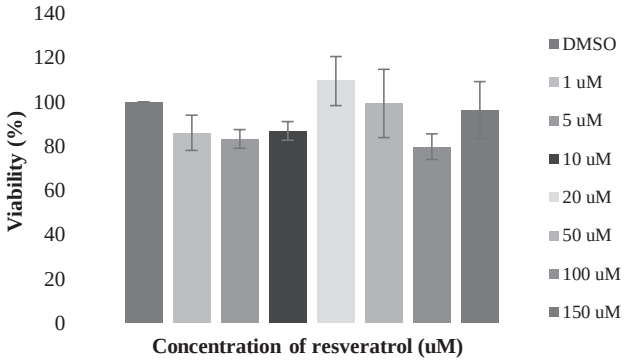

Figure 4. MTT assay. Effect of resveratrol on the viability of pancreatic cancer cell line PANC-1. Cells were exposed to various doses of resveratrol for 24 hours. DMSO is control.

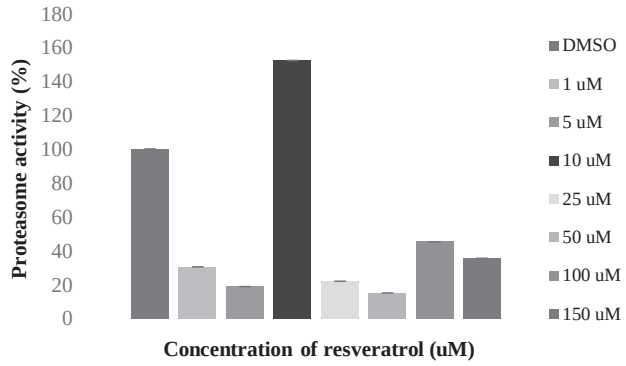

Figure 5. Proteasome activity of multiple myeloma cancer cell line NCI-H929. Cells were exposed to resveratrol for 24 hours. DMSO is control.

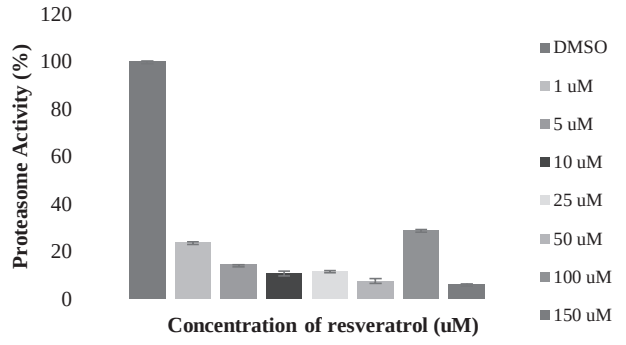

Figure 6. Proteasome activity in pancreatic cancer cell line PANC-1. Cells were exposed to resveratrol for 24 hours. DMSO is control.

DISCUSSION

It has been shown that resveratrol at a concentration of 50 μM suppresses the proliferation of multiple myeloma cancer cell lines such as U266, MM.1R cells, RPMI-Doxo6 822 cells and RPMI 8226-LR5 cells (Bhardwaj et al, 2007). In a study by Roy in 2011 he showed that resveratrol inhibits cell viability of PANC-1 cells at various concentrations: at 5 μM it decreased viability by 10%, 10 μM decreased 15%, 15 μM decreased 30%, 20 μM decreased 40%, and 25 μM decreased more than 50%. For NCI-H929 cell line, the MTT assay showed that resveratrol did not cause a decrease in viability (Figure 3). For cell line PANC-1 decreased in viability ranging from 5 to 22% was observed. At a concentration of resveratrol of 1 μM viability decreased by 12%, at 5 μM decreased 15%, at 10 μM decreased 5%, at 50 μM decreased 13% and at 100 μM decreased 22% (Figure 4).

For cell line NCI-H929 there was a reduction in the activity of the proteasome at all concentrations except 10 μM. At 1 μM activity decreased 70%; at 5 μM, 81%; at 25 μM, 78%; at 50 μM decreased 85%; at 100 μM, 55% and at 150 μM decreased by 64% (Figure 5). In the cell line PANC-1 there was a reduction in the activity of the proteasome at all concentrations. At 1 μM decreased by 76%; at 5 μM, 86%; 10 μM, 89%; 25 μM, 88%; 50 μM, 93%; 100 μM, 71%; and at 150 μM decreased by 94% (Figure 6).

In our study no inhibiton in the viability of NCI-H929 cells was observed. Previous studies showed inhibiton of multiple myeloma cells by resveratrol but using different cell lines (Wand, Galson, Roodman and Ouyang, 2011). More studies are required to test the effect comparing various cells lines. For PANC-1 the highest reduction in viability was 22 %; that is consistent with a study by Roy et al., 2011. The effect they observe was stronger, but also the exposure time was doubled. Our results on the inhibiton of proteasome activity are consistent with findings by Qureshi et al., 2012, that propose resveratrol as a potent proteasome inhibitor. Further studies are required to evaluate more doses of resveratrol and exposure times.

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LA INVESTIGACIÓN EN EL CAMPUS/ RESEARCH ON CAMPUS

Resumen de las investigaciones realizadas por nuestros estudiantes en el Recinto durante el año académico 2015-2016 en los campos de bioquímica y biología molecular, genética, biotecnología de plantas, ecología, microbiología y química.

Summary of research conducted on campus by our students during the academic year 2015-2016 in the fields of genetics, ecology, microbiology and chemistry.

GENÉTICA/ GENETICS

1-A Development and optimization of molecular protocols for harvestmen species identification
Arnaldo Román, Patricia Romero, Ignailiz Moldován, Jorge Domena and Lizbeth Romero-Pérez

From the phylum Arthropods and under the Arachnid class we find the order Opiliones, which are organisms similar to spiders. This is a cosmopolitan group commonly known as Harvestmen that includes approximately 5000-7000 species worldwide. Opiliones are often found in disturbed habitats as well as in forests, under stones, in caves, on the trunk of trees, on the soil, in forest litter, in soil crevices and sometimes rather deep in the soil. They are divided in four suborders: Cyphophtalmi, Dyspnoi, Eupnoi and Laniatores. Not much is known about harvestmen and their classification has been object of multiple disputes. In Puerto Rico, only 10 species have been identified. The objective of our research is to optimize a DNA extraction method that will allow the isolation of DNA from one harvestman leg in order to be able to perform a genetic analysis for the identification of species. Harvestmen samples were collected and saved in 95% ethanol. DNA was extracted using lysis buffer, salt buffer and precipitation with isopropanol. Our results show that a simple method of extraction with a 24 hours lysis step was successful in allowing the isolation of pure DNA and in high concentration. The purified DNA was used for PCR analysis using primers for the 16SrDNA, 28SrDNA and NADH genes. More studies are required for the optimization of the PCR reaction using other primer sequences.

ECOLOGÍA/ ECOLOGY

2-A Detection of heavy metals and identification of genotoxicity in fishes and land crab
Kimber Rodríguez, Valerie Rodríguez and Lizbeth Romero-Pérez

Heavy metals are toxic and their density is five times the density of water. They can be introduced into aquatic systems by industrial activities, agriculture and mining. Fish are the most important organisms in the aquatic food chain and are highly sensitive to heavy metal contamination. Crabs can serve for biomonitoring studies because thay can tolerate contaminated environments and reflect pollution levels due to accumulation of metals in their tissues. The objectives of this research are: evaluate the presence of copper, cadmium and nickel in fish and crutaceans collected in Arecibo, Camuy and Quebradillas, and investigate the potential genotoxic effect of metal pollution using RAPD-PCR. Our results show that *Lutjanus vivanus* had the highest concentration of cadmium in the liver and the lowest concentration of copper. *Cichla monoculus* had the lowest concentration of cadmium and was the only specie with nickel. The highest concentration of copper was detected in *Cardisoma guanhumi*. No direct relation was established between genomic stability and the levels of heavy metals. Further studies are required to explore more fish and crab samples.

2-B Diet of the invasive *Boa constrictor* in Puerto Rico

Melanie Cubano, Kevin Soto, Michael Rivera and Alberto R. Puente-Rolón

Invasive species can impact negatively by causing ecological disturbances extirpating native species from their habitat, and competing with other native species. The *Boa constrictor* is an example of an invasive species well established, caused mainly by the illegal traffic of exotic animals in Florida, Aruba, Cozumel and recently in Puerto Rico. The diet of this species on its natural distribution (Central and South America), consists mostly of mammals (bats, rats, porcupines, mongoose, rabbits, deer), reptiles (iguanas) and birds. The objective of our study was to determine the diet of the invasive *B. constrictor*. A total of 90 individuals (44 females and 42 males) were captured by the personnel of the Department of Natural Resources and Environment. Each individual was dissected to evaluate the stomach and gut content. Also, stables isotopes were used to compare the muscle signatures of *B. constrictor* with the muscle signal of the Puerto Rican Boa (*Epicrates inornatus*). Prey items documented include rat, mice, chickens, iguanas, mongoose and cats among others. Rat and mice (90%) were the prey items most commonly consumed by the *B. constrictor.* No difference in isotopic signal was detected when we compare muscle samples of *B. constrictor* and *E. inornatus.* Previous research in Guam, Aruba, Cozumel and Florida, show that invasive snakes are responsible of population declines of mammals, birds and reptiles. Therefore, long term monitoring of the diet of this new invasive species is important to identify potential threats to our biodiversity.

2-C Anti-depredatory Strategy: Flight Initiation Distance of *Anolis cristatellus*

Z. Barreto-Ayende, J.M. Cabán-Pérez and R. Jirau-Cruz

Predation risk may be considered as one of the most influential selective forces. In Puerto Rico, there is a variety of daytime predators such as anoles. The most common are *Anolis cristatellus*. The most important anti-predatory strategy in anoles is flight. That is why this study was based on flight initiation, and the understanding of flight conduct of this species. It has been proposed to study the escape response; there is an optimum distance of flight between predator and prey. The researcher acts as a predator and simulates an attack on the lizard and when the anole initiates the flight, the distance between the researcher and lizard was measured. After weeks of research, we concluded that *Anolis critatellus* young males vs. females responded mostly to the same distance flight.

2-D Behavior of the (*Anolis cristatellus*) in the Interamerican University of Puerto Rico, Arecibo Campus

S. Vega-Otero, M.T. Burgos-Cruz and A.M. Millet-Valle

For years, there have been studies about the lizards, their behavior, feeding, evolution and many other things. Few data about the common lizard of Puerto Rico, *Anolis cristatellus*, in urban areas was available. We used the Interamerican University of Puerto Rico, Arecibo Campus as our study site, to observe and evaluate the behavior of *A. cristatellus* in urban areas. During two months approximately, we studied the behavior of the *Anolis cristatellus* at different hours during the day. Our results show that 60% of the population was inactive. Moreover, the activities they invested less time were combat and mating, both with the same percent of 0.03%. Other aspects we studied include time they invested in dewlap deployment and/or “push-ups”, search for food and sunbathing.

2-E Fungal Bioremediation of organic compounds
Carlos Betancourt, Juan Abreu and Richard Giles

Contamination by manufacturing processes and toxic spills have negatively impacted flora, fauna and human habitats. Bioremediation is a technology that uses biological elements, in the majority of the cases these elements are microorganisms, that eliminate pollutants from soil or water. These microorganisms can degrade wastes to less toxic ones, concentrate and immobilize toxic substances and heavy metals or rehabilitate affected areas with multiple pollutants. Fungi are multicellular organisms that are distinguished for their ability to grow in extreme places. Basidiomycota, commonly known as polypores or "bracket fungi" are wood decay experts. These fungi have enzymes that could be used to degrade aromatic compunds. The objective of this research was to examine novel fungi isolated from Puerto Rico for their potential to be used in the bioremediation of contaminated soil and water. Eight different fungi were cultured on plates with toluene, chlorobenzene, methylene blue or malaquite green. Growth was measured for a period of 28 days for toluene and cholorobenzene. For the dyes, degradation was measured by absorbance. Of the eight isolated fungi, only one was able to grow on cholorbenzene. All the eight fungi grew on toluene. Six of the fungi could not degrade fully methylene blue, but only one for malaquite green. Our results show that the isolated fungi can degrade organic compounds. Our investigation was successful in finding multiple fungal isolates that can be considered for bioremediation purposes. Further studies are needed to characterize fungi in Puerto Rico suitable for biotechnology.

MICROBIOLOGÍA/ MICROBIOLOGY

5-A Total bacteria count in raw milk from dairy farms in the Northern Region of Puerto Rico
Julio Román, Willianne Cruz, Yolfren Báez and Lizbeth Romero-Pérez

Milk is an essential component of the diet worldwide that provides nutrients required for proper growth. Every year, 549 million tons of milk are produced. The chemical composition of milk becomes an excellent substrate for the growth of a wide variety of microorganisms. The Northern Region of Puerto Rico has more than 50% of the dairy farms on the island, for that reason the objective of our research is to establish a profile of the bacteria commonly found in raw milk collected in Arecibo, Hatillo and Camuy. Samples were analyzed by total plate count, gram stain and biochemical tests. Our results show that none of the farms exceeded the parameters of bacteria count in raw milk. The most common bacteria found were Gram negative rods. Only gram positive cocci and gram negative rods were tested biochemically. The identified bacteria were *Staphylococcus*, E. coli and *Sphing. paucimobilis*. Additional studies are required to test gram positive rods, increase the number of analyzed samples and explore genetic tests to develop a faster identification method.

CHEMISTRY/ CHEMISTRY

6-A Determinación teórica de calores de formación y energías de disociación de enlace en piridina halogenada
Enid Samot Feliciano y Víctor D. Vásquez Moll

El uso de reacciones isodésmicas permite predecir calores de formación en fase gaseosa a 298 [K], utilizando el programa de Química Computacional Gaussian 09 y Gaussian View. Las reacciones estudiadas tienen la forma general: piridina + fluoruro de hidrógeno → X-P + hidrógeno molecular, donde

X- P representa piridina halogenada [X = F, Cl, Br o I]. Utilizando el método de cálculo PM3, encontrado en Gaussian, se obtuvieron los resultados siguientes para el calor de formación, ΔH_f^0: para F-CP se obtuvo un valor de -121 kcal/mol, para Cl-CP de 41 kcal/mol, para Br-CP fue 54 kcal/mol y para I-CP fue de 73 kcal/mol, donde F-C representa el enlace entre Flúor y Carbono. Por otra parte, utilizando el mismo método de cálculo anterior, se obtuvieron las curvas de energía potencial en función de la longitud de enlace y realizando regresiones no lineales con la función potencial de Morse, se obtuvieron los resultados siguientes para la energía de disociación de enlace, D_e: para F-CP fue 172 kcal/mol, Cl-CP 75 kcal/mol, Br-CP 156 kcal/mol y para I-CP fue 156 kcal/mol. Finalmente, mediante el programa computacional Scigress, se realizaron regresiones lineales y múltiples de los resultados anteriores para observar el efecto de distintos descriptores físico-químicos, tanto en el calor de formación como en la energía de disociación de enlace.

6-B Cálculo teórico de calores de formación y energías de disociación de enlace en acido barbitúrico halogenado

Arnaldo Rivera Román y Víctor D. Vásquez Moll

Aprovechando las bondades de Software en Química Computacional, específicamente Gaussian View 5 y Gaussian 09, se pudo predecir los calores de formación y las energías de disociación en la halogenación de la molécula de ácido barbitúrico. En primer término, para determinar el calor de formación de la molécula de ácido barbitúrico halogenado, se usó el esquema de reacción isodésmico, donde todos los componentes de la reacción se encuentran en fase gaseosa y a 298 [K]. El método de cálculo propuesto en Gaussian es el método semiempírico PM3, Las reacciones estudiadas tienen la forma general: ácido barbitúrico + fluoruro de hicrógeno ↔ X-AB + hidrógeno molecular, donde donde X-AB representa el ácido barbitúrico halogenado [X = F, Cl, Br o I]. Los resultados que se obtuvieron para el calor de formación, ΔH_f^0 son: para F-NAB fue 187 kcal/mol, Cl-NAB 183 kcal/mol, Br-NAB 173 kcal/mol, I-NAB 185 kcal/mol, F-CAB 141 kcal/mol, Cl-CAB 181 kcal/mol, Br-CAB 105 kcal/mol y para I-CAB 79 kcal/mol, donde F-N representa el enlace entre Flúor y Nitrógeno y F-C representa el enlace entre Flúor y Carbono. En una segunda parte, utilizando el mismo método de cálculo anterior, se obtuvieron las curvas de energía potencial como función de la longitud de enlace y realizando regresiones no lineales con la función potencial de Morse, se obtuvieron los resultados siguientes para la energía de disociación de enlace, D_e: para F-CAB 141 kcal/mol, Cl-CAB 209 kcal/mol, Br-CAB 121 kcal/mol y para I-CAB 64 kcal/mol. Finalmente, mediante el programa computacional Scigress, se realizaron regresiones lineales y múltiples de los resultados anteriores para observar el efecto de distintos descriptores físico-químicos, tanto en el calor de formación como en la energía de disociación de enlace.

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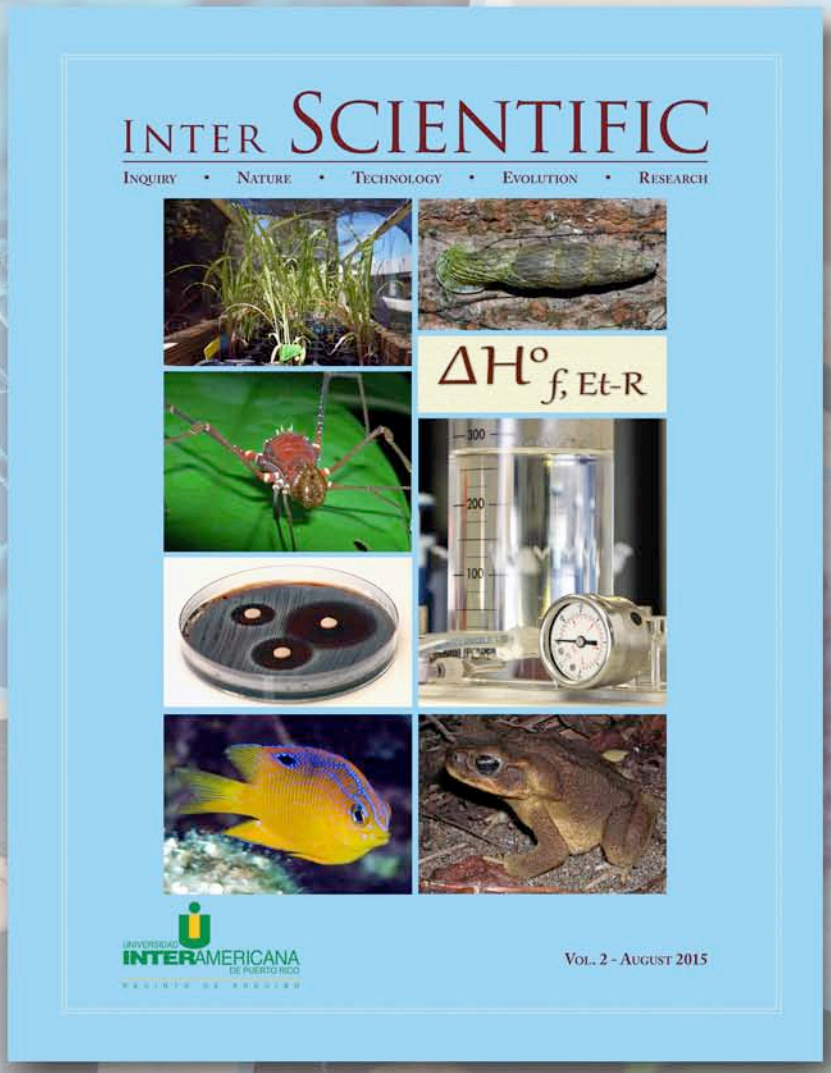

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