International Summer Research Experiences in Urban Environmental & Ecological Sciences at

Alabama A&M University

and

Nanjing Forestry University

Proceedings of the Student Research & Trip Reports May-July 2015

Center for Forest Ecosystem Assessment
Department of Biological and Environmental Sciences
College of Agricultural, Life, and Natural Sciences

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Research Experiences for Undergraduates and Graduates in China

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Alabama A&M University, Tuskegee University, and

Nanjing Forestry University

2015

Students and mentors, from left to right; top row: Lisa Gardner, Michelle Phillips, Hollis Dahn, La'Teasha Watkins, Dr. Abreeotta Williams and, John Fleurimond; from left to right, bottom row: Jeremy Isabelle, Jessica Williams, Eboney Stallworth, and Dr. Kozma Naka, in front of the Pearl Radio and T.V. Tower in Shanghai, China, on our first day in the country after a long flight from the U.S., June 2015.

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Preface

Background

Through the efforts of the Center of Forest Ecosystem Assessment (funded by the National Science Foundation's CREST program, Grant number HRD-1036600), Alabama A&M University (AAMU) was awarded a three-year grant by the USDA-National Institute of Food and Agriculture (NIFA) International Science and Education Program (ISE) in 2009 (Grant number: 2009-51160-05462) to develop an international exchange program with China. The program was designed to strengthen AAMU's ability to develop globally competent students and faculty through collaborative partnerships with higher education institutions and research organizations in China. The program focuses on the fields of agricultural and environmental sciences. This program also aims to enhance courses with international contexts to prepare students for international opportunities in agricultural and environmental sciences, as well as to add new dimensions to scientific research and teaching capabilities of AAMU faculty via exposure to international resources and technologies. Our partner institution in China for this program is Nanjing Forestry University (NFU), a comprehensive university with a tradition of forestry programs. The establishment of this program led to a three-year international Research Experiences for Undergraduates (REU) program in China, funded by the National Science Foundation (NSF) in 2011 (Grant number: DBI-106310). The primary goal of the NSF REU program is to expose undergraduate students **with an interest in pursuing a graduate research degree in science to hands-on research experiences**. In 2013, we received additional funding through the National Institute of Food and Agriculture's Capacity Grant (No. 2013-38821-21250) to further enhance our program through collaboration and involvement of students and faculty from Tuskegee University (TU), another minority institution in Alabama. This multi-institutional and multi-agency joint project has created opportunities for many faculty and students, particularly those from minority serving institutions with limited access to research oriented international programs. Participants in this program have gain valuable research experience under a culturally challenging, but ultimately enjoyable, international setting. Prior to the NSF REU funding, twenty-five students and faculty from AAMU travelled to China during the summers of 2010 and 2011, participating in an exploratory program to vet different higher learning institutions in China, including NFU, for compatibility. From these visits, we established our partnership with NFU. Since the summer of 2012, a total of 45 students from xx institutions across the United States have participated in the program through 2015 (**Table 1**).

Table 1. Number of participants across years for the REU program at Alabama A&M Univesity and Nanjing Forestry University, 2012-2015. Some faculty and students participated in the program in China across years.

REU CHINA PARTICIPANTS 2012-2015

	2012	2013	2014	2015	Total
Undergraduate Students (n=32)	8	10	8	6	31*
Graduate Student Mentors (n=8)	4	4	3	2	11*
Faculty/Post Doc/ Staff Mentors (n=9)	7	8	7	4	13*

* Duplicate participants: Hollis Dahn (2014-2015); Jonjala Jackson (2012-2013); Khairy Soliman (2012-2014); Kozma Naka (2014-2015); Jianqiang Li (2012-2013); Wubishet Tadesse (2013-2014); Ramble Ankumah (2012, 2014); across all years: Yong Wang, Elica Moss, Lisa Gardner.

This Year's Program

In the summer of 2015, six undergraduate students from four different institutions across the U.S. (including AAMU), four AAMU graduate students, and four faculty/staff from two different institutions participated in the REUG program **(Table 2).** After a three day orientation program at AAMU, students and faculty engaged in a variety of courses including Chinese language, culture, and history classes taught by NFU faculty, as well as scientific writing, statistics, and how to create PowerPoint presentations and posters by AAMU faculty and graduate students. All students were paired with a primary mentor in the U.S. prior to travel. Mentors included faculty and staff from both AAMU, Columbia Community College, and TU. Upon arrival at NFU, students were paired with a second mentor from NFU, with similar research interests. Students met with mentors to devise and conduct a research project. Research topics included a diversity of subjects such as "*Songbird Species in China: Evaluation of DNA Extraction Kits, Primers, and Feather Age*", "*Occurrence of Typical Antibiotics in Huai River and Hongze Lake, Eastern China,*" "*Bat Forage and Insect Communities in Three Habitat Types in Nanjing, China*," and "*The Adsorption Behavior of Black Carbon in Urban Forest and Traffic District Soils Toward Heavy Metal Ions (Cu, Zn),*" "*Establishment of a Riparian Buffer Strip for Alleviating Lake Eutrophication,*" among others. Students learned how to design a research project, collect data, operate research equipment, analyze data, and communicate their research results to their peers.

Table 2. Participants in the REU program at Alabama A&M Univesity and Nanjing Forestry University for 2015.

REUG CHINA PARTICIPANTS 2015			
Name	**Institution**	**Title/grade level**	**Research area**
Hollis Dahn	Central Florida Univ.	Super senior	Phylogenetics / herpetology
Jeremy Isabelle	Alabama A&M Univ.	Junior	Agroforestry
Chrystal Li	Southern California Univ.	Sophomore	Urban wildlife ecology
Michelle Phillips	Tuskegee University	Sophomore	Urban environmental science
La'Teasha Watkins	Alabama A&M Univ.	Freshman	Evironmental science
Jessica Williams	Alabama A&M Univ.	Sophomore	Environmental science
John Fleurimond	Alabama A&M Univ.	Postgraduate (MS)	Agroforestry
Eboney Stallworth	Alabama A&M Univ.	Postgraduate (MS)	Plant genetics
Janine Antalffy	Alabama A&M Univ.	Postgraduate (MS)	Biogeography
Kevin Messenger	Alabama A&M Univ.	Postgraduate (PhD)	Conservation biology
Lisa Gardner	Alabama A&M Univ.	Program coordinator and mentor	Wildlife biology
Abreeotta Williams	Columbia State Community College	Professor and mentor	Genetics
Kozma Naka	Alabama A&M Univ.	Professor and mentor	Forest mensuration and operations
Yong Wang	Alabama A&M Univ.	Professor and mentor	Statistics, multivariate analysis, wildlife ecology

The team took two extended educational/cultural trips in Jiangsu Province during the program. The first trip (2 days) brought them to Wuxi, Yixing, and the Tai Lake (*Tài Hú*) area. This trip was designed to help students and faculty understand bamboo ecology, the applications of bamboo in daily life, and bamboo as a biomass potential. During this trip, they visited a bamboo experimental forest and a bamboo processing plant; while at these sites, they were able to interact with researchers and workers. They also had opportunities to explore local culture and history: they watched a Chinese ballet ("*The Red Detachment of Women,*" 红色娘子军), performed by the National Ballet of China at the Wuxi Grand Theater, visited the Huishan Clay Figurines Museum (惠山泥人博物馆) and enjoyed tea along the streets of Zhongshan Road. The

second trip (3 days) took the team to Sheyang in northern Jiangsu Province. This trip was designed to help students and faculty to understand forest ecology, applications of forest research, the value of popular forests and their impact on local economies and ecosystems, and other conservation programs in the region. During this trip, they visited several poplar tree plantations and industry based in Sheyang County. In the early 1980s, faculty from NFU helped to introduce a hybrid poplar (genus *Populus*, hybridized in part from eastern cottonwood originating in Mississippi), to Sheyang. Once one of the poorest counties in China because of its high human population density, shortage of natural resources, and frequent flooding, Sheyang's economy has been transformed, and the poplar tree hybrid and the related agroforestry industry have played a major role in its economic growth. During this trip we also visited the Yancheng Dafeng National Milu Reserve (*Yánchéng Dàfēng mílù guójiā jízìránbǎohùqū,* 盐城大丰国家级自然保护区), which was established in 1983 to start a breeding program for the Père David's deer (*Elaphurus davidianus*), known simply as *mílù* in China, which is extinct in the wild in China. In addition, the team got to view the endangered red-crowned crane (*Grus japonensis*), also in a breeding program at the reserve.

While completing their research and collaborative lab or field work at Nanjing, the AAMU team experienced the culture, people, and a whole host of foods that they never in their lives dreamed they would eat! They explored the city by foot, bus, taxi, and subway and became intimately familiar with the city in a way that tourists almost never experience when visiting a foreign land. Students climbed Purple Mountain; visited Dr. Sun Yat-sen's Mausoleum; paid tribute to the fallen victims of the massacre by the Japanese during World War II, as commemorated in the Nanjing Massacre Memorial Hall; posed with enormous hand-carved mythical figures at the Xiaoling Mausoleum of the Ming Dynasty; celebrated the Chinese traditional Dragon Boat Festival with a big crowd of locals and foreign visitors; and visited a wide variety of stunningly beautiful gardens.

The team had the opportunity to visit other major cities such as Shanghai, Beijing, and Yangzhou and witnessed the effects of dramatic economic development during last 30 years. In Shanghai, we took a boat tour of the Bund on the Huangpu River and viewed the City from the top floor of the Oriental Pearl Radio and TV Tower (东方明珠塔). From Nanjing, we travelled to Beijing on a high-speed train at over 125 miles per hour. There, we climbed the Great Wall at the Mutianyu (**慕田峪**长城), walked through the halls of the (once) Forbidden City (**紫禁城**), maneuvered through a crowded Tiananmen Square (**天安**门广场) and observed the mile-long line of people waiting to see Chairman Mao's body, ate a wonderful homemade meal in the Lingdang Hutong district, and finished off our stay with a final Peking Duck meal that was phenomenally delicious.

We maintained our recent change of completing the program in China instead of returning to AAMU before heading home. Students completed in full all of their work in China, including a research report, a cultural report, a PowerPoint presentation, and a scientific poster. Students presented their research to a full audience at NFU prior to leaving for Beijing.

The documents in this proceeding consist of the students' cultural reports and their scientific research papers. Students and mentors worked together to complete these documents.

Acknowledgements

On behalf of all of the Research Experiences for Undergraduates and Graduates (REUG) program coordinators at Alabama A&M University (AAMU), we would like to thank the Alabama A&M University administration, the Nanjing Forestry University (NFU) administration, and most importantly, the National Institute of Food and Agriculture for their support in the implementation of this program. This program was a great success, and it absolutely would not have transpired without the support and understanding of the aforementioned. Thank you to NFU for allowing the REUG program participants into your labs, your office spaces, and being so forgiving of our social faux pas. The students and faculty had a wonderful, once-in-a-lifetime educational and cultural experience in China, and you all contributed significantly to this occurring.

In addition to PI and Co-PI, the AAMU and TU professors, Drs. Tyesha Farmer, Kozma Naka, William Stone, Eunice Bonsi (Tuskegee University), and Guohao He (Tuskegee University), chose these eight undergraduate students and three graduate students. These students were chosen because of their academic strengths, their interest in the areas to be researched, and their compatibility with the project. Additionally, Zhiyun Cheng from the University of British Columbia, joined our group at NFU. He participated in all activities and completed all work, including delivering a presentation at the end of the program. We are proud of each of these students and all that they accomplished at Nanjing Forestry University and Alabama A&M University during this time.

Thank you!

Yong Wang, PI and travel coordinator, AAMU REUG China
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Department of Biological and Environmental Sciences
College of Agricultural, Life, and Natural Sciences

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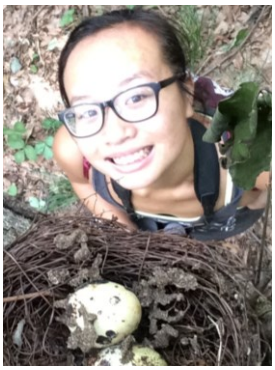
REUG Participating Students

Undergraduate Students

Hollis Anne Dahn: Ms. Dahn is a double senior majoring in biology at the University of Central Florida in Orlando, Florida. She was mentored by Dr. Yong Wang (AAMU) and Dr. Ding Yulong (NFU). She partnered with Kevin Messenger (PhD candicate) to continue the research they began in 2014. Her research examined the phylogenetics of two closely related frog populations to determine whether or not they are evolutionarily distinct. The title of her research paper is "*A New Species of the Genus* Megophrys *Gunther (Amphibia: Anura: Megophryidae) from Mount Wuyi, China.*"

Jeremy Isabelle: Mr. Isabelle is rising senior majoring in Forestry Science at Alabama A&M University in Normal, Alabama. He was mentored by Dr. Kozma Naka (AAMU) and Dr. Guibin Wang (NFU). His research focused on optimal light intensity for maximum camptothecin production in the plant Camptotheca acuminata. Camptothecin produces anticancer enzymes. The title of his research paper is "*Effect of light intensity on primary and secondary metabolism, phytohormones and enzyme activities in* Camptotheca acuminata*.*"



Chrystal Li: Ms. Li is a rising junior majoring in Environmental Sciences at University in Southern California, Los Angeles, CA. She was mentored by Dr. Yong Wang and Lisa Gardner (AAMU) and Dr. Changhu Lu (NFU). Her research focused on the effects of urbanization on songbirds of Nanjing. The title of her research paper is "*Artificial Nest Predation in Urban Areas in Nanjing, Jiangsu Province, China.*"

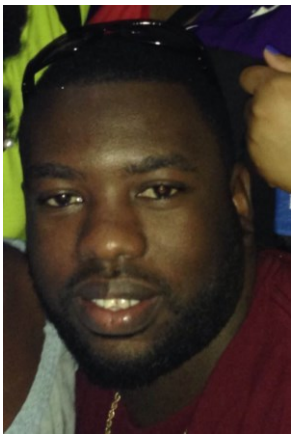
Michelle Phillips: Ms. Phillips is a rising junior majoring in Biochemistry at Tuskegee University in Tuskegee, Alabama. She was mentored by Drs. Ramble Ankumah and Conrad Bonsi (TU), Dr. Abreeotta Williams (CSCC), and Dr. Yuanchun Yu (NFU). The title of her research paper is "*Identification and dispersal analysis of polycyclic aromatic hydrocarbons (PAHs) present in urban forest soils of Nanjing, China.*"

La'Teasha Watkins: Ms. Watkins is a rising junior majoring in Environmental Science at Alabama A&M University in Normal, Alabama. She was mentored by Dr. Abreeotta Williams (AAMU) and Dr. Li Wei (NFU). She examined the presence and sources of antibiotics in a lake and a river in the Jiangsu province of China. The title of her research paper is "*Occurrence of Typical Antibiotics in Huai River and Hongze Lake, Eastern China*."

Jessica Williams: Ms. Williams is a rising junior majoring in Environmental Science at Alabama A&M University in Normal, Alabama. She was mentored by Lisa Gardner (AAMU), Dr. Abreeotta Williams (Columbia State Community College) and Dr. (NFU). Her research examined the effectiveness of varying buffer strip widths in alleviating lake eutrophication. The title of his research paper is "*Establishment of a Riparian Buffer Strip for Alleviating Lake Eutrophication.*"

AAMU Graduate Students

Janine Antalffy: Ms. Antalffy is an MS candidate in the Department of Biology and Environmental Sciences at Alabama A&M University. Under the guidance of Dr. Yong Wang, she has traveled to China to work in collaboration with Dr. Longying Wen at LeShan Normal University in Sichuan Province, China. Her research is focused on the effects of the Sichuan Basin in the distribution and genetic structure of the White-browed Laughingthrush, *Garrulx sannio*, in the region. She is examining how the Sichuan Basin impacts the phylogenetics of *G. sannio*, using GIS and morphometrics in conjunction with DNA analyses. Through these methods, she hopes to illustrate the historical biogeographical influence of the Sichuan Basin on *G. sannio*. The title of her research for this program is, "



John Fleurimond: Mr. Fleurimond is an MS candidate of biology with a concentration in microbiology at Alabama A&M University in Normal, Alabama, under the guidance of Dr. Florence Okafor. He traveled to China as a research participant in the REUG-China program at Nanjing Forestry University. He assisted and was mentored by Dr. Kozma Naka (AAMU) and Zhuge Qiang (Nanjing Forestry University). His research focus was to verify the presence of cry-genes (cry1Ah1) that were inserted into the transgenic *Populus deltoides* x *P. euramerican* "Nanlin895" using the soil bacteria *Agrobacterium tumefacien* (updated scientific name *Rhizobium radiobacter*) infection method. The title of Mr. Fleurimond's research paper is "*The Verification of cry-genes (cry1Ah1) Incorporated into the Genome of Select Transgenic* Populus Deltoides *x* P. euramerican *"Nanlin895.*"

Eboney Stallworth: Ms. Stallworth is an MS candidate in the field of genetics at Alabama A&M University in Normal, Alabama, under the guidance of Dr. Stylianos Fakas. Ms. Stallworth traveled to China as a research participant in the REUG-China program at Nanjing Forestry University. She was mentored by Dr. Williams (CSCC), and Dr. Lian Xu and graduate student Jianwen Wang (NFU). Her research focused on isolating and identifying a gene from the Chinese Tamarisk tree. The title of her research paper is "*Isolation and Identification of a Salt Tolerant Gene in* Tamarix chinensis*, a Gene that Closely Matches Dehydrin.*"

Kevin Messenger: Mr. Messenger is a PhD candidate under the guidance of Dr. Yong Wang. His research focuses on the conservation and natural history of herpetofauna in the heavily populated areas of southern China. Mr. Messenger partnered with Hollis Dahn to continue their work from 2014, focusing this year on the phylogenetic aspect of a potential split of a single toad species into two species in China. Mr. Messenger recently defended his dissertation and is expected to receive his PhD this year.

Participating REU Mentors

Alabama A&M University Mentors

Yong Wang, PhD: Dr. Wang is professor of biometry and wildlife ecology at AAMU. His research interests are behavioral ecology of avian migration; wildlife and habitat relationships and conservation; and natural resource modeling with statistics, Geographic Information System (GIS), and remotely acquired data. His recent research efforts include (1) stopover ecology of migratory birds at the southern Cumberland Plateau of northern Alabama; (2) wildlife (amphibians, reptiles and birds) response to anthropogenic habitat and landscape alternations such as forest management practices and urbanization; (3) breeding distribution and biology of Cerulean Warblers; (4) classification of land types for forest management based on GIS, remotely sensed data and statistical models; and (5) avian biology and conservation in China. Dr. Wang's research has been supported by organizations or agencies such as NSF, USDA, Forest Service, EPA, State of Alabama, TNC, and private landowners. Thus far, Dr. Wang has mentored seven undergraduate students across all three years of the program, with the assistance of coordinator Lisa Gardner (2014-2015), his PhD candidate Kevin Messenger (2012-2014), and Postdoctoral fellow Dr. Jianqiang Li (2012). In 2012, Dr. Wang mentored David Farris and Iwo Gross; in 2013, he mentored Christopher Griffith, Jacob Drucker, and Justin Waraniak; and in 2014 he mentored Hollis Dahn and Junqiao Wang; in 2015 he mentored his graduate student, Janine Antalffy (MS). **(Dr. Changhu Lu, Dr. Zhen Wang, and Dr. Ding Yulong)**

Elica Moss, PhD: Dr. Moss is assistant professor of environmental microbiology. Her research interests are in identifying microbes that contribute to the environment and subsequently effect human health. Her recent research efforts include: arsenite oxidizing/resistance genes in soils; presence and abundance of pathogenic fecal bacteria in water systems; and biogeochemical nutrient cycling in a disturbed forest ecosystem. She was instrumental in the accreditation of the Environmental Health Science program at Alabama A&M University after one year from its inception; rendering it the only accredited Environmental Health Science Program in the state of Alabama. She is heavily involved in undergraduate research, which is evident in her involvement in the REU and URM programs. Additionally she oversees a program that takes undergraduates to scientific meetings to present their research. Dr. Moss's research has been supported by organizations or agencies such as NSF, USDA, and the State of Alabama. Thus far, Dr. Moss has mentored four undergraduate students and one graduate student across all three years of the program. The graduate student, Jonjala Jackson also assisted with mentorship of the undergraduate students (MS candidate, 2012-2013). In 2012, Dr. Moss mentored Rakeyta Scales and Nara McCray; in 2013, she mentored Antionette Fowlkes; and in 2014, she mentored Linzi Thompson. **(Dr. Shengzuo Fang and Dr. Tian Ye)**

Kozma Naka, PhD: Dr. Naka is associate professor of forest mensuration and forest operations at AAMU. He also teaches biometric statistics. His research interests are focused on biomass harvesting, forest products, and forest management. Dr. Naka is new to the program as of 2014, and mentored two undergraduate students, Andrew Lawhorn and Michael Kennedy.

Additionally, he taught statistics to the students and co-mentored Rosie Long with Dr. Stone. **(Dr. Yulong Ding and Dr. Yongbo Wu)**

Abreeotta Williams, PhD: Dr. Williams is an assistant professor of genetics at Columbia State Community College in Summerville, Tennessee. Her research interests are primarily in the effects of atmospheric pollutants on environmental systems (i.e. humans, animals), and determining the effects of agricultural emissions on ambient air quality. Dr. Williams co-mentored Eboney Stallworth, La'Teasha Watkins, and Jessica Williams in 2015 with Lisa Gardner. **(Dr. Yinlang Zhang and Dr. Yulong Ding)**

Lisa Gardner, MS: Ms. Gardner is coordinator of this program and technical manager of the Center for Forest Ecosystem Assessment in the Department of Biological and Environmental Sciences at AAMU. Her research background is in wildlife biology. Her research interests are stopover ecology of migrating songbirds and urbanizing effects on urban wildlife. Ms. Gardner co-mentored La'Teasha Watkins and Jessica Williams with Dr. Abreeotta Williams in 2015.

China Mentors

This year, most students had a faculty mentor from the College of Forest Resources and Environment at NFU. One student was mentored by a faculty from LeShan Normal University. More information on NFU faculty can be found at http://eng.njfu.edu.cn/info.php?id=125

Yulong Ding, PhD: Dr. Ding is former dean of the College of International Education and professor of forestry ecology of Nanjing Forestry University, and is currently the Director and professor of Bamboo Institure at NFU. Dr. Ding is well-known bamboo ecologist. He has been instrumental in getting this Program established at NFU. Dr. Ding has mentored students across all years. This year, he mentored Kevin Messenger and Hollis Dahn.

Jiangang Han, PhD: Dr. Han is a wildlife biologist in the College of Biology and Environment of Nanjing Forestry University. His research is currently on He worked with Lisa Gardner to mentor Jessica Williams on her research examining …

Qiang Zhuge, PhD: Dr. Zhuge is a professor of molecular biology and biotechnology in the College of Forestry and Environmental Sciences of Nanjing Forestry University. Dr. Zhuge worked with Dr. Kozma Naka to mentor John Fleurimond on his research which looked to verify the presence of cry-genes (cry1Ah1) inserted into the transgenic *Populus deltoides* x *P. euramerican* "Nanlin895" using the soil bacteria *Agrobacterium tumefacien* (updated scientific name *Rhizobium radiobacter*) infection method.

Wei Li, PhD: Dr. Li is a professor of environmental science of the College of Biology and Environment of Nanjing Forestry University. Her research is currently focused on antibiotic pollution. She worked with Dr. Abreeotta Williams to mentor La'Teasha Watkins, whose research focused on … China.

Guibin Wang, PhD: Dr. Wang is …. and professor of the College of Forestry and Environmental Sciences at of Nanjing Forestry University. He worked with Dr. Kozma Naka to mentor Jeremy Isabelle, in his research project … in Nanjing, China.

Yuanchun Yu, PhD: Dr. Yu is a professor of soil biology and chemistry of the College of Biology and Environment of Nanjing Forestry Universitywith an interest in soil carbon sequestration. He assisted Dr. He Guohao with mentoring Ms. Michelle Phillips on her research identifying polycyclic aromatic hydrocarbons in urban forest soils around Nanjing. His student, Yang Jingyu, also helped with the process.

Changhu Lu, PhD: Dr. Lu is a professor of wildlife ecology of the College of Forestry and Environmental Sciences at of Nanjing Forestry University. His research interests are on plant-animal interactions, and bird biodiversity and behavior. He worked with Dr. Wang and his student Chrystal Li, who investigated the effect of urbanization bird nest predation.

Longying Wen, PhD: Dr. Wen is professor of genetics at LeShan Normal University in Sichuan Province. She is graduate advisor to Ms. Janine Antalffy, MS student at AAMU, who is studying the White-browed Laughingthrush, *Garrulx sannio*, in the region. She is examining how the Sichuan Basin impacts the phylogenetics of *G. sannio*, using GIS and morphometrics in conjunction with DNA analyses.

Lian Xu, PhD: Dr. Xu is professor of forest genetics and breeding and is Director of the Department of Forest Genetics and Gene Engineering at the College of Forest Resources and Environment at NFU. His research focus is on the population genetics and genomics of forest trees, and improvement of forest trees and ornamental plants. He helped to mentor Eboney Stallworth, an MS candidate at AAMU, on her project isolating and identifying a salt-tolerant gene in *Tamarix chinensis*.

Other Participants and Institutions

From Alabama A&M University

Lisa Gardner: Ms. Gardner was program coordinator and assisted Drs. Wang and Moss in coordinating the REUG program on the AAMU side. She helped set up the website, created documents, organized the three-day pre-travel orientation and the two-week post-China program. She traveled to China to assist students and faculty with their needs and to act as liaison between NFU staff and faculty and AAMU staff and faculty when Dr. Wang was not available. She created the REU Proceedings, and assisted in creating and submitting the REU Annual Report.

From Columbia State Community College

Abreeotta Williams, PhD: Dr. Williams is a professor of genetics and biology at Columbia State Community College in Taft, Tennessee. She is a former participating graduate student (2013) of the REUG Program, and this year she participated again as a mentor. She mentored Eboney Stallworth (*Isolation and Identification of a salt tolerant gene in* Tamarix chinensis*, a Gene that Closely Matches Dehydrin*);

From Nanjing Forestry University

Jiangang Zhu, PhD: Dr. Zhu is the dean of the College of International Education of the Nanjing Forestry University. He helped to arrange REU program and provided logistic support.

Shengzuo Fang, PhD: Director, Office of International Cooperation and Exchange, and Professor of Silviculture, Department of Forestry and Environmental Science. Dr. Fang's research is focused on the effects of silvicultural regimes on the biomass production, carbon sequestration, and wood quality. He is also interested in the physiological and ecological performance of trees.

Chaonian Feng, PhD: Dr. Feng is the Chairman of Nanjing Forestry University. He is in charge of the operation of the university, and provided leadership role for helping AAMU REU-China program. He visited AAMU in August 2012 to promote the strengthening and expansion of collaborative efforts.

Guofen Li, PhD: Dr. Li is professor of civil engineering of Nanjing Forestry University. In August 2012, she visited AAMU to explore opportunities for further collaboration.

Pingping Li, PhD: Dr. Li is the Vice President of Nanjing Forestry University and a professor of Agronomy. She provided guidance and support for the operation of AAMU REU program at NFU.

Xueqin Liu, PhD: Dr. Liu is an associate professor of the Department of Chinese Language and Literature of Nanjing Forestry University. She trained REU students for Chinese language.

Jianren Ye, PhD: Dr. Ye is the Dean of the College of Forest Resources and Environment and a professor of forestry pathology. As the dean, he supervised his staff and faculty to collaborate with AAMU REU-China team. In August 2012, he visited AAMU to explore opportunities for further collaboration.

Dongrong Shi, PhD: Dr. Shi is the new Director of the Office of International Cooperation at Nanjing Forestry University. He is in charge of the operation of the international program and assisted the development and implement of the AAMU REU-China program. He is now co-Dean of the AAMU Confucius Institute, along with Dr. Yong Wang, and currently lives in Huntsville, Alabama.

Ms. Qingyu Wang: Ms.Wang is former director of the Office of International Cooperation at Nanjing Forestry University. She helped to develop AAMU REU-China program, and provides logistic support for the AAMU Confucius Institute, where she periodically resides in Huntsville, Alabama to assist the Institute's programs.

Other Participating Institutions in China

Hainan Normal University

Wuyishan Nature Reserve

Shennongjia Nature Reserve

LeShan Normal University

Forestry Bureau

Dafeng National Wildlife ReserveShenyang

Research

A NEW SPECIES OF THE GENUS *MEGOPHRYS* GUNTHER, 1864 (AMPHIBIA: ANURA: MEGOPHRYIDAE) FROM MOUNT WUYI, CHINA

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Dr. Yong Wang
&
Kevin Messenger
Alabama A&M University
Normal, Alabama 35762

Dr. Ding Yulong
Nanjing Forestry University
Nanjing, China

Abstract

A new species of horned toad, *Megophrys ombrophilia* **sp. nov.**, is described based on specimens found from Guadun village from Mount Wuyi in northwestern Fujian Province, China. The species is distinguished from two sympatric species of *Megophrys* by morphology, bioacoustics, and molecular data of the 12S and 16S mitochondrial gene. The species is characterized by its rotund stature, head length approximately equal to head width, canthus rostralis well developed, tympanum large and distinct, vomerine teeth absent, margin of tongue smooth, not notched from behind, heels of the feet not meeting when femurs are held at 90° to the axis of the body and tibias are depressed against the femur, toes weakly webbed at base, dorsal skin mostly smooth with scattered granules and ridges, usually the presence of two discontinuous dorsolateral ridges, supratympanic fold distinct and well-developed, and with females averaging a length of 34.2 mm snout-to-vent length, and males measuring 30.2 mm SVL. In the Mount Wuyi area, the species is sympatric with *M. boettgeri* and *M. kuatunensis* and is a sister species to *M. obesa* from southwest Guangdong province.

Keywords: *Megophrys, horned toad, phylogenetics, China*

In publication now.

Introduction

The horned toads of the genus *Megophrys* Gunther, 1864, represent a highly diverse and cryptic group of frogs found throughout central and southern Asia, from as far west as India, to as far east as Fujian Province, China, and as far south as the Philippines. Interestingly, the genus is lacking from both Taiwan (adjacent to Fujian) and Hainan Island (adjacent to Guangdong, 20 km gap). It is due to their cryptic nature and hidden diversity that up until recently (2012), only three species were known to Eastern China, i.e., *M. boettgeri* (Boulenger 1899), *M. kuatunensis* (Pope 1929), and *M. huangshanensis* (Fei & Ye 2005), and it was proposed that eastern China is likely underestimated (Wang et al. 2012). From 2012 to 2014, an additional five new species of *Megophrys* were found in southeastern China (Wang et al. 2012; Li et al. 2014; Wang et al. 2014), bringing the total known species to the genus to 58. In China, 37 species are recognized (Li et al. 2014). Of these 37, eight species that measure less than 50 mm in both males are females are found in southeast China (Li et al. 2014).

During herpetological surveys from 2013 to 2015, mostly during the second half of May, June, and July, at Wuyishan National Nature Reserve (N27.74 E117.69) (Figure 1), on the Fujian Province side of the reserve, China, we found an unknown frog that fit into the genus *Megophrys* but did not match either of the two known species of the genus to the area, i.e., *M. boettgeri* and *M. kuatunensis*, as well as other recognized congeners of the genus. In this paper, we describe it as a new species based on morphological, bioacoustic, and molecular data.

Prior to this work, the Wuyishan area, and more specifically, the village of Guadun, has been a significant area for new species to Chinese herpetology for over 100 years. It was first made known to science by Armand David, who visited the site in 1873. The tiny, remote village

has been the type locality for at least a dozen species of reptiles or amphibians. David's visit and collections inspired a subsequent visit by Irish ornithologist, John D. La Touché in 1896 and 1898. La Touché visited Guadun village, spelled "Kuatun" until recent times, and made a collection of reptiles and amphibians and sent them back to George A. Boulenger, who then described four new snakes: *Hebius (=Amphiesma) craspedogaster, Opisthotropis latouchii, Plagiopholis styani, Sinonatrix percarinata;* 1 lizard: *Dopasia (=Ophisaurus) harti;* and three frogs: *Amolops ricketti, Hylarana latouchii,* and *Megophrys boettgeri*. In the 1920's and 1930's, Clifford H. Pope visited Guadun village, and described another species of *Megophrys* from the village: *M. kuatunensis*. Additionally, from Guadun, he also described the type specimens for *Amolops chunganensis, Opisthotropis kuatunensis,* and *Sinomicrurus kelloggii*. It is an interesting note of history and coincidence that now a third species of *Megophrys* shares the same type locality as the two sympatric species after nearly 100 years since the last species was described from the site.

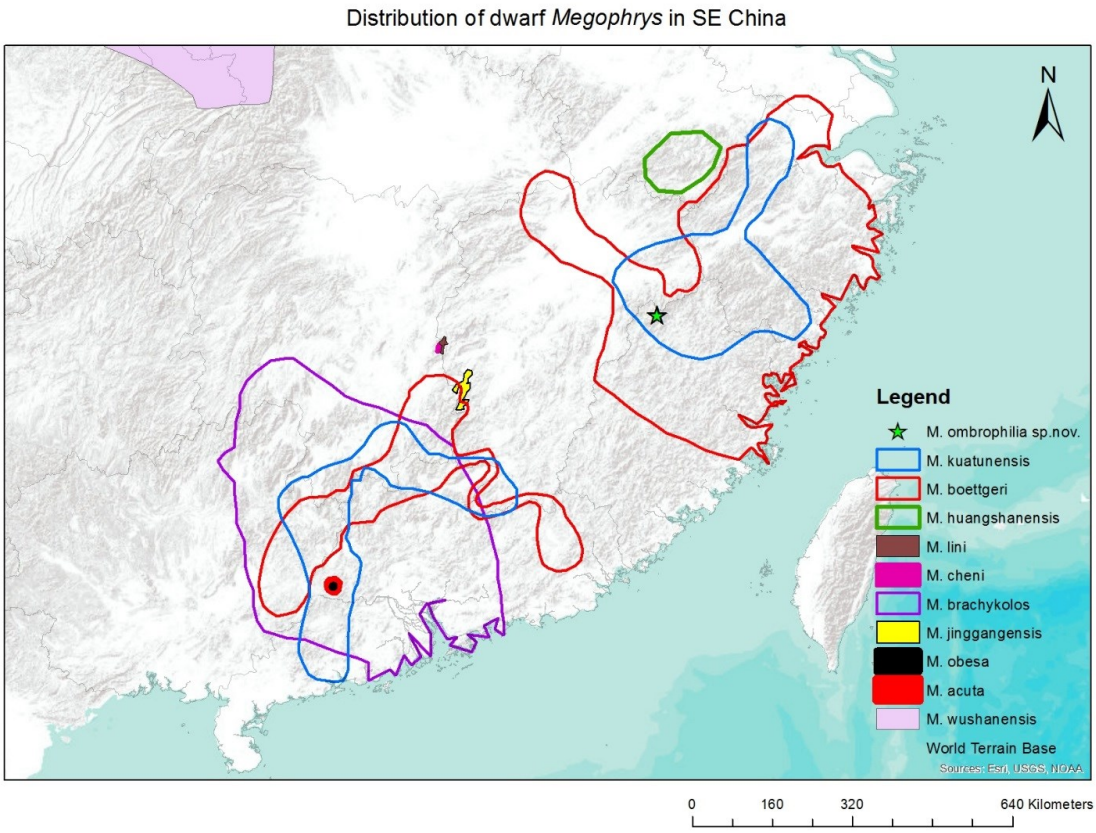

FIGURE 1. Distribution of dwarf (<50 mm) megophryid species in southeastern China as of 2015.

Methods

Ethics Statement

Permission to visit the study sites were issued by the management administration of the Wuyishan National Nature Reserve. We obtained permissions for specimen and tissue collection from the Fujian Provincial Forestry Bureau. This study did not involve protected species. All the animal operations were approved by Institutional Animal Care and Use Committee of Alabama A & M University (Permit #XXXXX), and strictly complied with the ethical conditions by the

Chinese Animal Welfare Act. Specimens were knocked out and subsequently euthanized with 20% benzocaine via cutaneous absorption on the venter.

Taxon sampling: Samples used for molecular analysis included *M. boettgeri* and *M. kuatunensis* from Mount Wuyi, Guadun (= Kuatun) Village, Fujian Province; *M. obesa* from Heishiding Nature Reserve, Guangdong Province. From GenBank, sequences from 24 available congeners, *Atympanophrys shapingensis*, and *Brachytarsophrys carinense* were also used.

All specimens examined from Wuyishan were deposited at the Biology Museum at Nanjing Forestry University, Nanjing, Jiangsu Province, China (NFU), and the Wuyishan National Nature Reserve headquarters, Wuyi, Fujian Province, China (WUY). The *M. obesa* specimens came from The Museum of Biology, Sun Yat-sen University (SYS), Guangzhou, Guangdong Province, China.

Four of the collected specimens were fixed in 10% buffered formalin after preserving liver and muscle tissue in 95% ethanol, and later transferred to 75% ethanol, the rest were placed in 95% ethanol and then later transferred to 75% ethanol. Permitting issues stipulated a set number of specimens for preservation (n = 10).

DNA Extraction, amplification, and sequencing: Total genomic DNA was extracted from tissue using TIANamp Genomic DNA Kit (TIANGEN).The quality of the DNA extraction was assessed via gel electrophoresis on a 2% agarose gel. Amplification of the mitochondrial loci 16S rRNA (16S), and 12S rRNA (12S) was attempted for all tissue samples using the primers and standard PCR conditions listed (Table 2). Amplified fragments were again visibly assessed via gel electrophoresis on a 2% agarose gel before sequencing in both directions at Shanghai Invitrogen Biotechnology Co., Ltd.

Table 2. Locus and primer information for loci utilized in phylogenetic analyses.

Locus	Forward Primer	Reverse Primers	Temp (°C)	MgCl (mM)	Size (bp)	Model	Reference
16S	FPhe40L 5'-AAA GCA CAG CAC TGA AGA YGC-3'	12S600Ha 5'-TTA TCG ATT ATA GAA CAG GCT CCT CT-3'	48	1.0	541	GTR + I + G	Zhang *et al*. (2013)
12S	L3975 5'-CGC CTG TTT ACC AAA AAC AT-3'	H4551 5'-CCG GTC TGA ACT CAG ATC ACG T-3'	50	1.0	576	GTR + I + G	Simon *et al*. (1994)

Sequence alignment and phylogenetic analyses: Sequences were checked for quality and edited based on chromatograms in Sequencher 5.1 (Gene Codes Corp.). Appropriate IUPAC ambiguity codes were input at ambiguous sites. Sequence alignments were assembled and aligned using the MUSCLE alignment algorithm (Edgar, 2004) on default settings in the program MEGA 6.06 (Tamura et al., 2013). Genetic distances between and within individual *Megophrys* species were calculated in MEGA 6.06 (Tamura et al., 2013) using the Tamura-Nei (TrN) model (Tamura & Nei, 1993) (Table 3). The Bayesian Information Criterion (BIC) and the greedy search scheme in PartitionFinder (Lanfear et al., 2012) were used to determine the appropriate model of evolution for use in all phylogenetic analysis programs utilized in this study (Table 2). The loci were not partitioned by a codon, and the tRNA region of 16S was excluded from phylogenetic analyses. Concatenated phylogenies of 16S and 12S was recovered via the programs MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and RAxML 8.0.20 (Stamatakis, 2014), using Bayesian Inference (BI) and Maximum Likelihood (ML) analyses respectively (Figure 2).

Table 3. Taijma-Nei group mean genetic distance for the mitochondrial locus 12S.

		1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
1.	*A. shapingensis*													
2.	*B. carinense*	0.067												
3.	*M. boettgeri*	0.087	0.111											
4.	*M. brachykolos*	0.105	0.134	0.073										
5.	*M. cheni*	0.077	0.096	0.050	0.060									
6.	*M. jinggangensis*	0.075	0.103	0.050	0.058	0.028								
7.	*M. kuatunensis*	0.084	0.107	0.052	0.058	0.028	0.034							
8.	*M. lini*	0.092	0.113	0.063	0.070	0.041	0.052	0.045						
9.	*M. minor*	0.091	0.119	0.073	0.084	0.064	0.063	0.065	0.079					
10.	*M. nasuta*	0.132	0.141	0.161	0.166	0.144	0.158	0.159	0.160	0.150				
11.	*M. obesa*	0.085	0.107	0.045	0.066	0.035	0.044	0.038	0.052	0.077	0.165			
12.	*M. omeimontis*	0.096	0.119	0.067	0.084	0.073	0.066	0.071	0.079	0.067	0.147	0.079		
13.	*M. ombrophilia* **sp. nov.**	0.088	0.111	0.056	0.065	0.039	0.051	0.051	0.057	0.077	0.164	0.027	0.084	

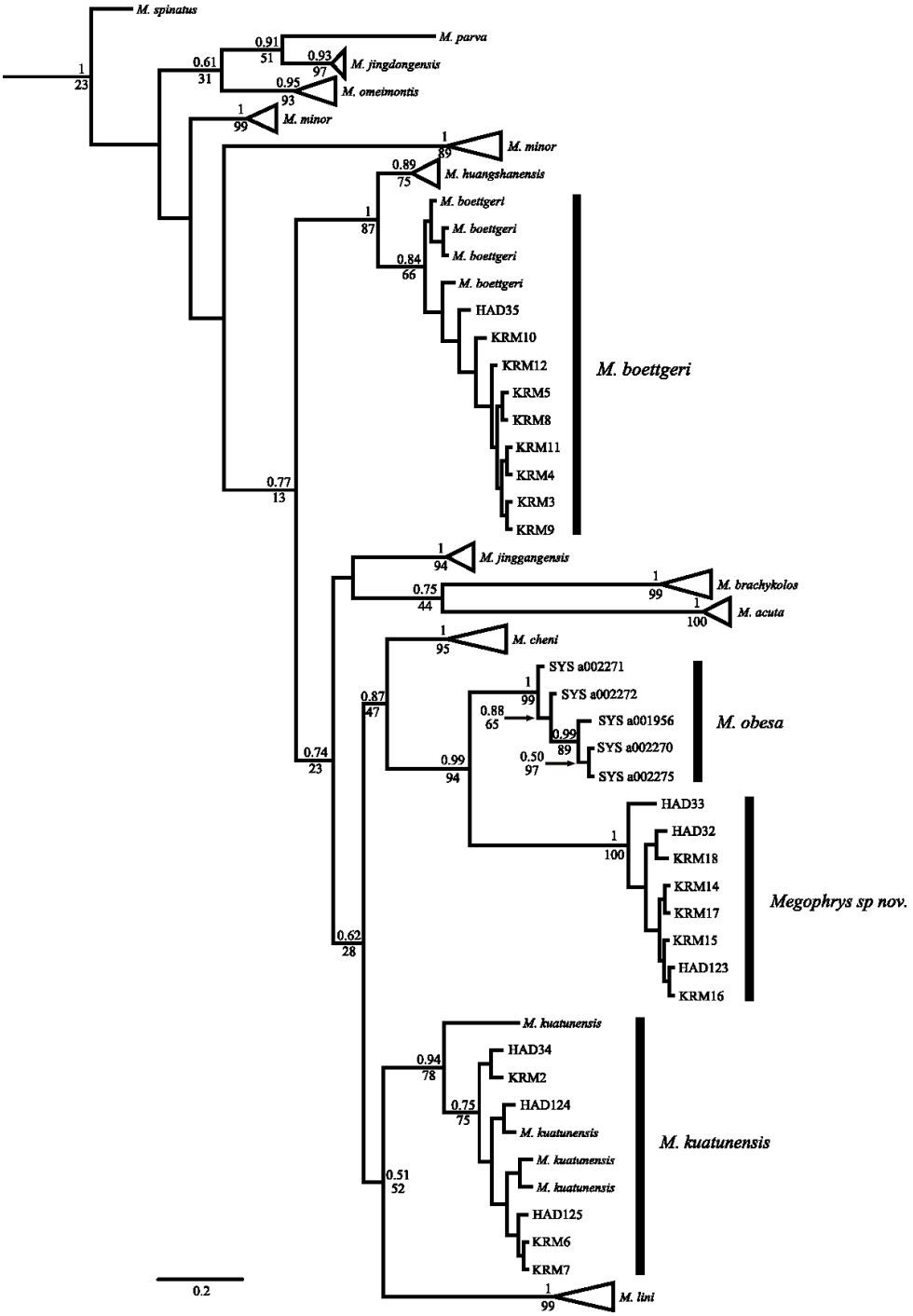

Figure 2. Concatenated phylogenetic estimate of taxa representing a 50% majority-rule consensus. Nodal values of supported nodes list posterior probabilities above and bootstrap support values below. Outgroup taxa placed outside of the clade displayed are *A. shapingensis*, *M. aceras*, *M. baluensis*, *M. dringi*, *M. kobayashii*, *M. lateralis*, *M. lekaguli*, *M. longipes*, *M. majora*, *M. mangshanensis*, *M. maosonensis*, *M. nankiangensis*, and *M. nasuta*. Sequences acquired from GenBank are labeled as their listed species.

In MrBayes, the analysis was run twice simultaneously with 1 cold and 3 hot chains for 7 million generations, sampling one of every 1000 trees. Burn-in was set to discard the first 20,000 trees. We used Tracer 1.6.0 (Rambaut, Suchard & Drummond, 2013) to ensure stationarity was reached by plotting log-likelihood scores with generation number. ML analyses were conducted in RAxML 8.2.0 (Stamatakis, Ludwig & Meier, 2005; Stamatakis, 2006) with 950 bootstrap replicates under MRE-based bootstrapping criterion. Topologies were visualized using the program FigTree 1.4.0 (Rambaut & Drummond, 2012). Nodes are considered supported when a posterior probability (PP) greater than or equal to 0.95 and bootstrap support greater than or equal to 75% are recovered in BI and ML respectively.

Morphological characters: Character measurements followed that of Li et al. (2014). Measurements were made with digital calipers to the nearest 0.1 mm. We measured snout-to-vent (SVL), head length (HDL), from tip of the snout to articulation of the jaw, head width (HDW) via distance between left and right articulations of jaw, snout length (SNT) from tip of snout to the anterior corner of the eye, eye diameter (EYE) from the anterior to the posterior corner of the eye, internasal distance (IND), interorbital distance (IOD), tympanum diameter (TMP), tympanum-eye distance (TEY) from anterior edge of tympanum to posterior corner of eye, hand length (HND) from distal end of radioulna to tip of 3rd distal phalanx, radioulna length (RAD), foot length (FTL) from distal end of tibia to tip of 3rd distal phalanx, and tibia length (TIB). We compared differences between these parameters to the two sympatric species and to the closest genetically similar species (*M. obesa*) via Mann-Whitney U test in IMB SPSS Statistics 20.

Bioacoustic analyses: We recorded male advertisement calls from 10 each of the sympatric species of *Megophrys,* and two advertisement calls from *M. ombrophilia* **sp. nov.** (one call from the field, one call from a captive animal responding to playback). The wild call was recorded in the field using a Sony ICD-MX20 Digital Voice Recorder, held within ca. 2-3 m of the calling individual next to a mountain stream. The sound file was imported into the program Audacity (Audacity, http://web.audacityteam.org/) at 22 kHz. Due to stream noise, the file was resampled to 11 kHz to further isolate the frog call. This value was chosen because the call itself was well below 11 kHz and eliminating values between 11 to 22 kHz resulted in much cleaner audio. To remove low stream frequencies below that of the frog call, we ran a high pass filter and defined a cutoff frequency of 2 kHz, meaning that only values below 2 kHz would be affected. A value of 2 kHz was chosen because the call itself was above 2 kHz. A rolloff value of 6 decibels (dB) was chosen. The final procedure to eliminate stream noise and further isolate the frog call was to highlight the noise profile of the stream sound between calls. Once a sample was highlighted and "captured" by the program, we ran a noise removal filter with the following default presets: noise reduction 24 dB, sensitivity 0 dB, frequency smoothing 150 Hz, attack/ decay time 0.15 seconds, noise: remove. We define a call as a continuous vocalization with pauses no greater than 1-second, and we define the smallest non-split syllable as a note. Spectrum analyses were generated by Audacity software. Waveforms and sonograms were generated by Raven Lite (Cornell Lab of Ornithology). Frequency parameters such as notes per call and per second, notes per 10-sec, note duration, inter-note interval, high and low frequency, carrier signal (peak frequency), and frequency band-width were all measured from the waveforms, sonograms, and spectrum analyses. The category of "notes per 10 sec" was created due to the fluctuation of the inter-note duration of *M. ombrophilia* and is not simply extrapolated

from the value of "notes per second." Differences between the three sympatric species of *Megophrys* were tested with a one-way ANOVA and Kruskal-Wallis test in IBM SPSS Statistics 20.

Results

Megophrys ombrophilia **sp. nov.** Messenger & Dahn

Holotype. Adult male, WUYXXX1, collected by Kevin R. Messenger and Hollis A. Dahn (hereafter KRM and HAD, respectively) on 16 June 2014, from a bamboo plantation outside Guadun village, Wuyishan National Nature Reserve (N27.735980 E117.640810, 1242 m a.s.l.), Wuyi City, Fujian Province, China (Fig. 3, A-H).

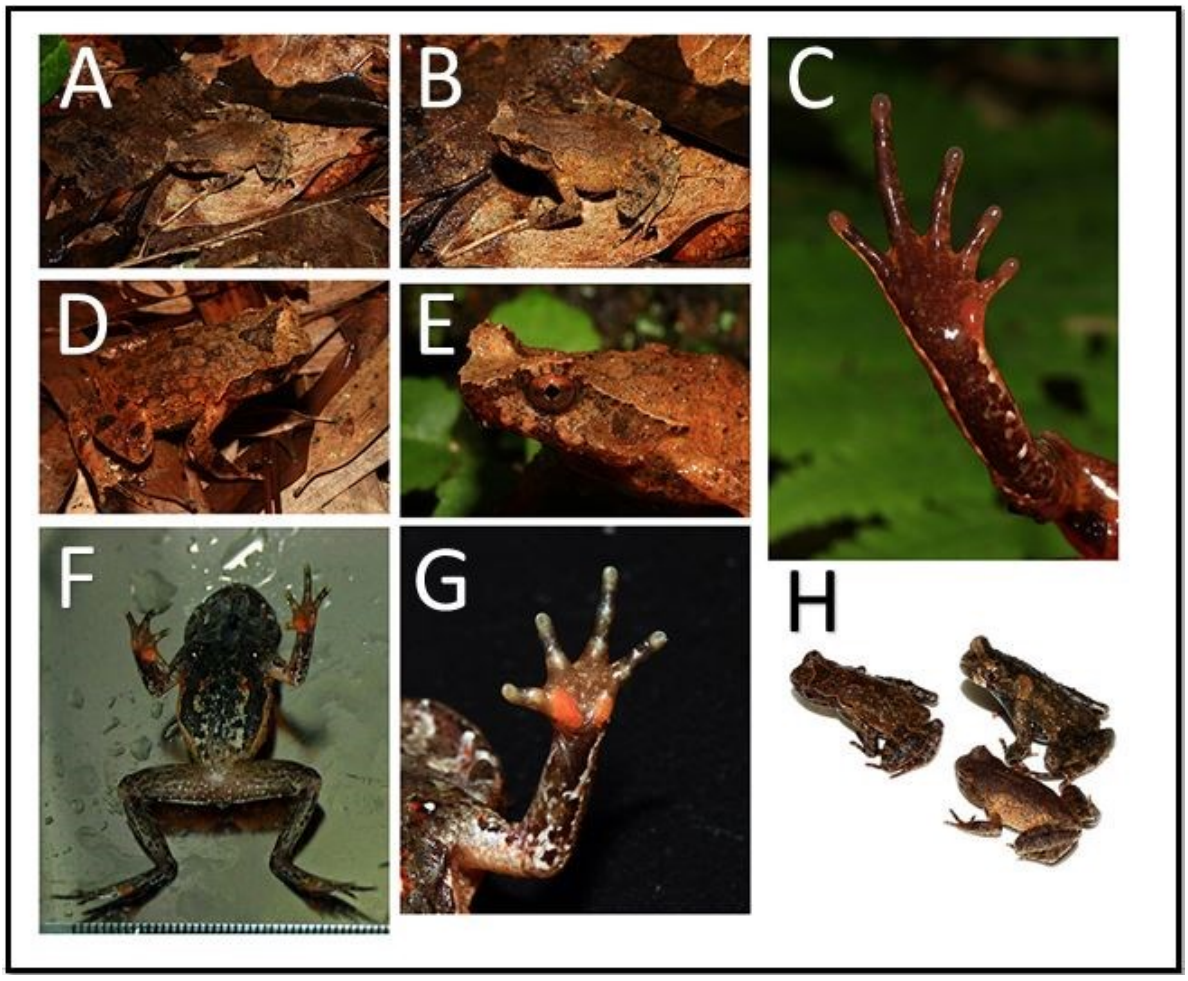

FIGURE 3. A. View of the adult male holotype WUYI0001 of *Megophrys ombrophilia* **sp. nov.** in life, among leaf litter. **B:**.Dorsolateral view of the holotype in life. **C:** Foot of the holotype. **D:** Phenotypic variation of the species. **E:** Close up view of supraocular tubercles and supratympanic fold. **F:** Ventral view of the holotype. **G:** Hand of the holotype. **H:** Dorsolateral view of *M. kuatunensis* in upper left, *M. boettgeri* in upper right, and *M. ombrophilia* **sp. nov.** in lower right.

Paratypes. Nine adults (4 males, 4 females, 1 unknown): Two adult males, WUYXXX2 and WUYXXXX3, collected from the same locality as the holotype at 1257 to 1302 m a.s.l. by KRM and HAD on 16-17 June 2014; an adult male, NFUXXX1, collected by KRM on 27 May 2015 in tea plantation, about 50 m from bamboo forest edge; an adult male, NFUXX3, collected by KRM on 28 May 2015, found on rocky path/ ephemeral stream in secondary bamboo forest; an adult female, WUYXXXX4, collected by KRM and HAD, from an abandoned tea plantation near (0.6 km linear distance) the type locality at 1262 m a.s.l. on 17 June 2014, 27.7317 117.6366; an adult female, NFUXXX4, collected by KRM on 30 May 2015 on footpath between tea plantation and bamboo forest; an adult female, NFUXXX5, collected by KRM on 1 June 2015 north and west of Guadun village in bamboo plantation, representing the northern and western most find as well as the highest elevational record at 1350 m a.s.l.; 1 adult female, WUYXXXX5, collected by KRM and HAD on 23 June 2015, found after a rainstorm; 1 specimen of unknown sex, NFUXXXX2, collected by KRM on 27 May 2015, was killed on the road that leads into Guadun village at 27.734 117.646, which also represents the easternmost and lowest elevation find at 1125 m a.s.l.

Diagnosis. . The specimen matched the genus *Megophrys* based on the following characters: head broad and depressed, tympanum distinct, tubercles above the eye, tubercles on the chest, vertical pupil, supratympanic fold, no mid-dorsal fold, and a lack of black spines on the dorsum (Li & Wang 2008). *Megophrys ombrophilia* **sp. nov.** is characterized by the combination of the following characters: (1) a small-sized species with 27.4.0-34.5.0 mm SVL in adult males and 32.8-35.0 mm SVL in adult females; (2) head length approximately equal to head width (HDL/HDW ratio 0.9); (3) snout rounded in dorsal view, slightly protruding, sloping backward to mouth in lateral view, protruding beyond the mandible; (4) canthus rostralis

developed; (5) tympanum large and distinct, TMP/EYE ratio 0.7-0.8; (6) vomerine teeth absent; (7) margin of tongue smooth, not notched behind; (8) heels of the feet not meeting when femurs are held at 90° to the axis of the body and tibias are depressed against the femur; (9) tibio-tarsal articulation reaching forward to the posterior corner of the eye; (10) tibia relatively short, TIB/SVL ratio 0.41; (11) relative finger length I < II ≤ IV < III; (12) toes weakly webbed at base; (13) distinct subarticular tubercles at the base of each finger; (14) dorsal skin mostly smooth with scattered granules and ridges; (15) triangular ridge with granules between the eyes; (16) a Y-shaped dorsal ridge with granules; (17) usually the presence of two discontinuous dorso-lateral ridges; (18) horn-like tubercle on posterior edge of eyelid, distinct but small; (19) supratympanic fold distinct and well-developed, extending to scapula; (20) dorsal surface usually orange to light brown, with a complete or incomplete dark interorbital triangle; (21) ventral surface usually orange; (22) throat possessing a single short streak down the midline, darker in color than surrounding tissue.

Comparisons, physically. Here we compare *Megophrys ombrophilia* **sp. nov.** with the other 37 species of *Megophrys* known to China.

Megophrys ombrophilia **sp. nov.** can be readily distinguished from the two sympatric species, *M. boettgeri* and *M. kuatunensis*. It is distinguished from both species by being more rotund and stout (vs. slender). The relative eye size is larger in *M. ombrophilia* **sp. nov.** than either *M. boettgeri* or *M. kuatunensis.* The heels do not meet in *M. ombrophilia* (vs overlap in *M. boettgeri* and *M. kuatunensis*) (Fig. 4).

It is distinguishable from *M. boettgeri* by being smaller in length (range: 28-35 mm vs 34.5-42.5 mm SVL) and distinguishable from *M. kuatunensis* by being heavier bodied and by having slightly less pronounced horn-like tubercles on the eyelids. *M. ombrophilia* **sp. nov.** is

usually patterned with a "Y" with a square positioned at the intersection of the arms of the "Y" (Fig. 5). Sometimes the "Y" or the square are very small to absent, but usually present. When specimens are in their dark coloration, seeing a pattern can be very difficult. Comparatively, *M. kuatunensis* has a very ornate dorsum with a myriad of lines, frequently ending in an "X" or a variation of an "X." *Megophrys boettgeri* has a very broad dorsal blotch, taking up the majority of the dorsum, often leaving just the shoulders "exposed" which is where it gets its common name: Pale-shouldered horned toad.

FIGURE 4. From left to right: *M. boettgeri, M. kuatunensis, M. ombrophilia* **sp. nov**.

Megophrys ombrophilia **sp. nov.** is sister to *M. obesa* in our phylogenetic tree, and is distinguished by having a significantly shorter SVL (27.4-35.0 vs 35.6-41.2 mm; U=0.00 p=0.017); significantly shorter head length (9.4-12.3 vs 12.6-14.6 mm; U=0.00 p=0.017), a significantly narrower head (10.8-13.6 vs 13.8-15.5 mm; U=0.00 p=0.017), significantly shorter snout (2.5-4.2 vs 4.2-4.8 mm; U=1.0 p=0.025), typically a smaller eye (3.2-4.6 vs 4.1-4.9 mm), shorter tympanum-to-eye length (1.6-2.0 vs 2.1-2.6 mm), significantly smaller hand length (5.8-8.2 vs 8.2-9.8 mm; U=1.5 p=0.042), significantly smaller radioulna length (5.4-7.1 vs 8.8-10.8 mm; U=0.0 p=0.017), a significantly shorter tibia (10.1-14.1 vs 15.7-17.6; U=0.0 p=0.008), a

significantly shorter foot (16.3-20.3 vs 20.9-25 mm; U=0.0 p=0.008), a larger IOD/HDW ratio (0.33 vs 0.27-0.30), a smaller TEY/TMP ratio (0.66 vs 0.78-1.09), a shorter RAD/SVL ratio (0.20 vs 0.25-0.28), a shorter TIB/SVL ratio (0.37 vs 0.41-0.47), a shorter FTL/SVL ratio (0.57 vs 0.59-0.66).

FIGURE 5. Examples of variation in the dorsal pattern, which usually resembles a "Y" or asymmetrical "X."

Megophrys ombrophilia is a fairly small species, with a maximum SVL of 35.0 mm in adults and can be distinguished from 24 congeners with a minimum SVL >40 mm: *M. aceras, M. ancrae, M. auralensis, M. baolongensis, M. binlingensis, M. caudoprocta, M. damrei, M. dingi,*

M. gigantica, M. glandulosa, M. jingdongensis, M. lekaguli, M. longipes, M. major, M. mangshanensis, M. medogensis, M. megacephala, M. nankiangensis, M. omeimontis, M. robusta, M. sangzhiensis, M. shapingensis, M. spinata, and *M. takensis*.

Of the remaining 24 relatively small sized congeners (maximum SVL < 40 mm), *M. ombrophilia* can be distinguished by the following combinations of characteristics: Heels not meeting when femurs are held at right angles to the body axis and tibias depressed vs. heels meeting or overlapping in: *M. binchuanensis, M. boettgeri, M. cheni, M. daweimontis, M. kuatunensis, M. palpebralespinosa, M. parva, M. tuberogranulatus, M. vergrandis, M. wawuensis, M. wuliangshanensiss, M. wushanensis, M. zhangi*; absence of vomerine teeth vs presence in: *M. ancrae, M. jinggangensis, M. daweimontis, M. oropedion, M. pachyproctus, M. palpabralespona, M. paralletal, M. parva, M. serchhipii, M. zhangi,* and *M. zunhebotoensis*; by the presence of a supraorbital horn-like tubercle vs lacking in: *M. bichuanensis, M. pachyproctus, M. wawuensis, M. wuliangshanensis,* and *M. zhangi*; margin of tongue not notched vs notched in: *M. boettgeri, M. cheni, M. huangshanensis, M. oropedion, M. pachyproctus, M. vegrandis, M. wawuensis,* and *M. zhangi;* TIB/SVL ratio 0.33-0.41 vs 0.38-0.45 in *M. acuta,* 0.5-0.54 in *M. cheni*, 0.54 in *M. daweimontis*, 0.47-0.5 in *M. jinggangensis*, 0.46-0.53 in *M. lini*, 0.49 in *M. pachyproctus* and *M. zhangi*, 0.55 in *M. palpebralespinosa,* 0.48-0.53 in *M. parva*, 0.45-0.51 in *M. tuberogranulatus,* 0.49-0.54 in *M. vegrandis,* 0.51-0.59 in *M. wawuensis*, 0.5-0.51 in *M. wuliangshanensis*, 0.47-0.48 in *M. wushanensis*, 0.46-0.5 in *M. zunhebotoensis*; tympanum distinct, tympanum diameter (TMP) to eye diameter ratio, TMP/EYE 0.53-0.69 vs 0.29 in *M. pachproctus,* 0.33 in *M. wawuensis,* 0.33-0.5 in *M. binchuanensis*, 0.39 in *M. serchhipii,* 0.4 in *M. parva,* 0.41-0.54 in *M. cheni,* 0.44-0.56 in *M. vegrandis,* <0.5 in *M. huangshanensis,* 0.5 in *M. tuberogranulatus, M. wushanensis, M. wuliangshanensis,* and *M.*

zhangi, 0.57-0.71 in *M. acuta,* "tympanum small" in *M. palpebralespinosa*; tibio-tarsal articulation reaches the posterior corner of the eye when hind limbs are stretched anteriorly vs reaching between the tympanum and shoulder in *M. brachykolos*, reaches the middle of the eye in *M. zunhebotoensis*, reaches the anterior corner of the eye in *M. lini, M. pachyproctus,* and *M. serchhipii*, reaches the tip of the snout in *M. daweimontis* (Wang et al. 2012, Li et al. 2014, Wang et al. 2014).

Comparisons, acoustically. A single wild call was recorded from a male on 18 May, calling on a rock between a mountain stream and the embankment. The individual was calling amongst *M. kuatunensis.* Calls tend to be single. Notes are between 2.7 and 5.1 kHz. In the field, the most distinguishing feature of the call would be the inter-note duration. In a 10-sec window, there are about 6 to 7 notes. A single note is much longer in duration than either sympatric species, lasting about 0.3 s. There is a carrier signal (peak frequency) at 3486 Hz. *M. boettgeri* has an extremely fast paced call, with over 4 notes delivered in a single second, *M. kuatunensis* has a slower pace than *M. boettgeri,* but it is still much faster than *M. ombrophilia. M. kuatunensis* calls with a pace of about half that of *M. boettgeri* (about 15 calls in a 10 sec window compared to about 40 of *M. boettgeri*). See figures 5-9.

Megophrys ombrophilia **sp. nov.** differs significantly from the calls of *M. boettgeri.* In note length duration, *M. ombrophilia* was significantly longer (M=0.305s, SD=0.051) than *M. boettgeri* (M=0.128s, SD=0.043; $t(39)=11.9$, $p<0.01$). In all other measured criteria (notes per second, notes per 10 sec, inter-note interval, low frequency, high frequency, and frequency range), *M. boettgeri* differed significantly than *M. ombrophilia* (Kruskal-Wallis test, each parameter $p<0.001$). A single call from *M. boettgeri* can last well over 10 seconds (up to about 2 min in our observations). During a 10-second span, there are approximately 30-40 notes

(M=43.2, SD=1.8), or about 4.3 notes per second (M=4.3, SD=0.47), each one between 0.7 to 6.3 kHz. The inter-note duration is on average 0.12 seconds (SD=0.03). The carrier signal (peak frequency) is at 3476 Hz.

By contrast, calls from *M. kuatunensis* are more similar to *M. ombrophilia* **sp. nov.,** at least to the human ear with regard to pitch, but not so similar in other aspects. *M. ombrophilia* note length duration (M=0.305s, SD=0.051) is significantly longer than *M. kuatunensis* (M=0.212s, SD=0.016; $t(39)=7.8$, $p<0.01$). In a 10-second span, there are an average 15.3 notes (SD=1.3), with each note being between 1.5 and 6.7 kHz. The inter-note duration is on average 0.44 s (SD=0.07). There is a carrier signal at 3698 Hz. There was significant difference between *M. ombrophilia* and *M. kuatunensis* in regard to notes per second, notes in 10 sec, inter-note interval, low frequency, high frequency, and frequency range (Kruskal-Wallis test, each parameter, $p<0.01$).

The call of *M. ombrophilia* **sp. nov.** is primarily singular (1 note = 1 call), and occasionally in pairs (two notes with less than 1 second duration between the notes), and during a 10-second span, produces an average 6.9 notes (SD=0.44). The most striking difference between *M. ombrophilia* and its two sympatric species is the inter-note duration, which on average is 1.34 s (SD=0.6), compared to the inter-note duration of *M. boettgeri* (0.11 s) and *M. kuatunensis* (0.44 s). The call has a narrower range than either conspecific*,* each call is between 2.7 and 5.1 kHz, with an average bandwidth (frequency range) of 1.7 kHz (SD=0.26), compared to an average bandwidth of 3.89 kHz (SD=1.0) for *M. boettgeri* and 4.3 kHz (SD=0.3) for *M. kuatunensis*. There is a carrier signal at 3477 Hz (Fig. 5).

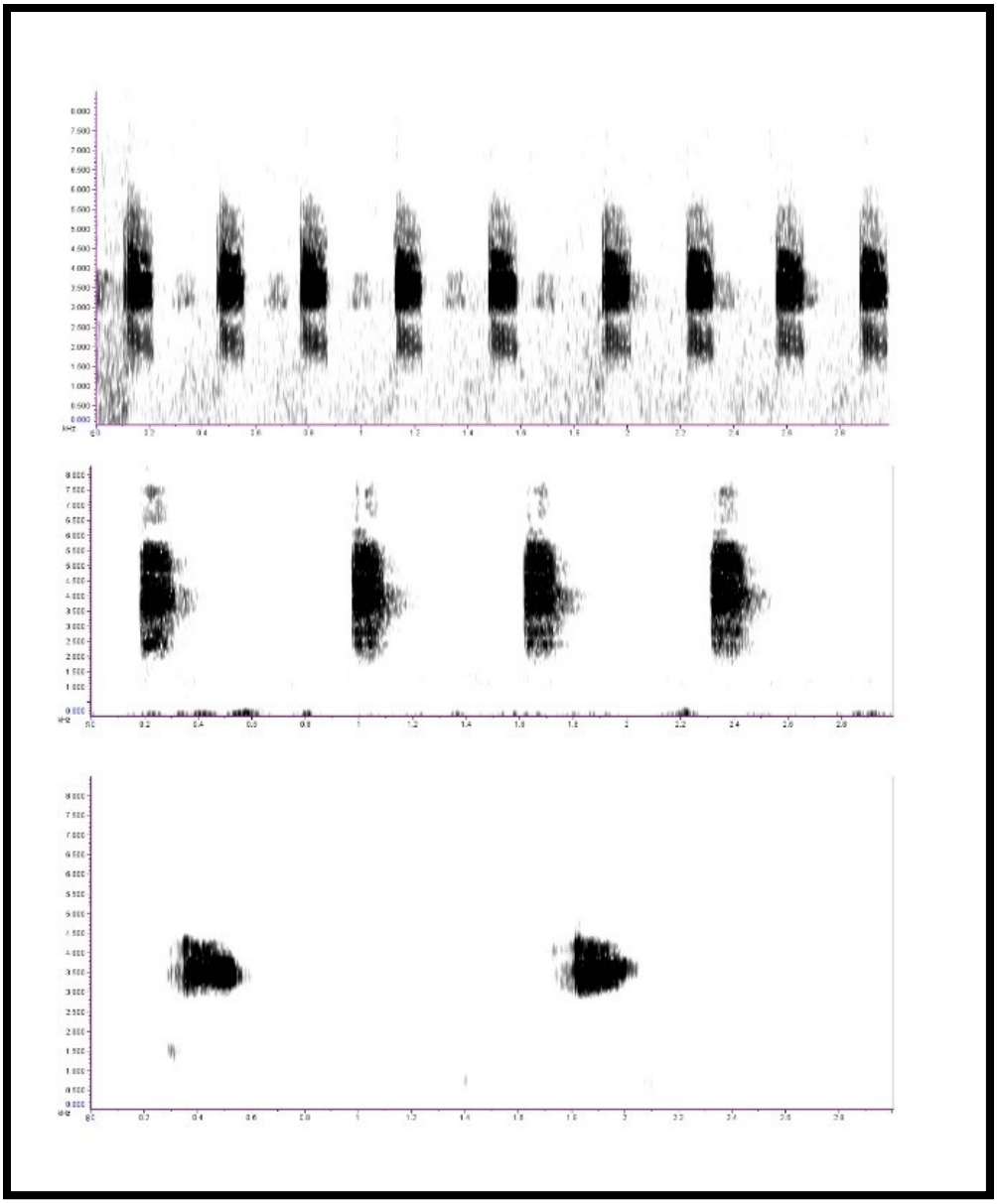

FIGURE 5. Sonogram of a 3-second clip: *M. boettgeri* on top, *M. kuatunensis* in the middle, *M. ombrophilia* on bottom.

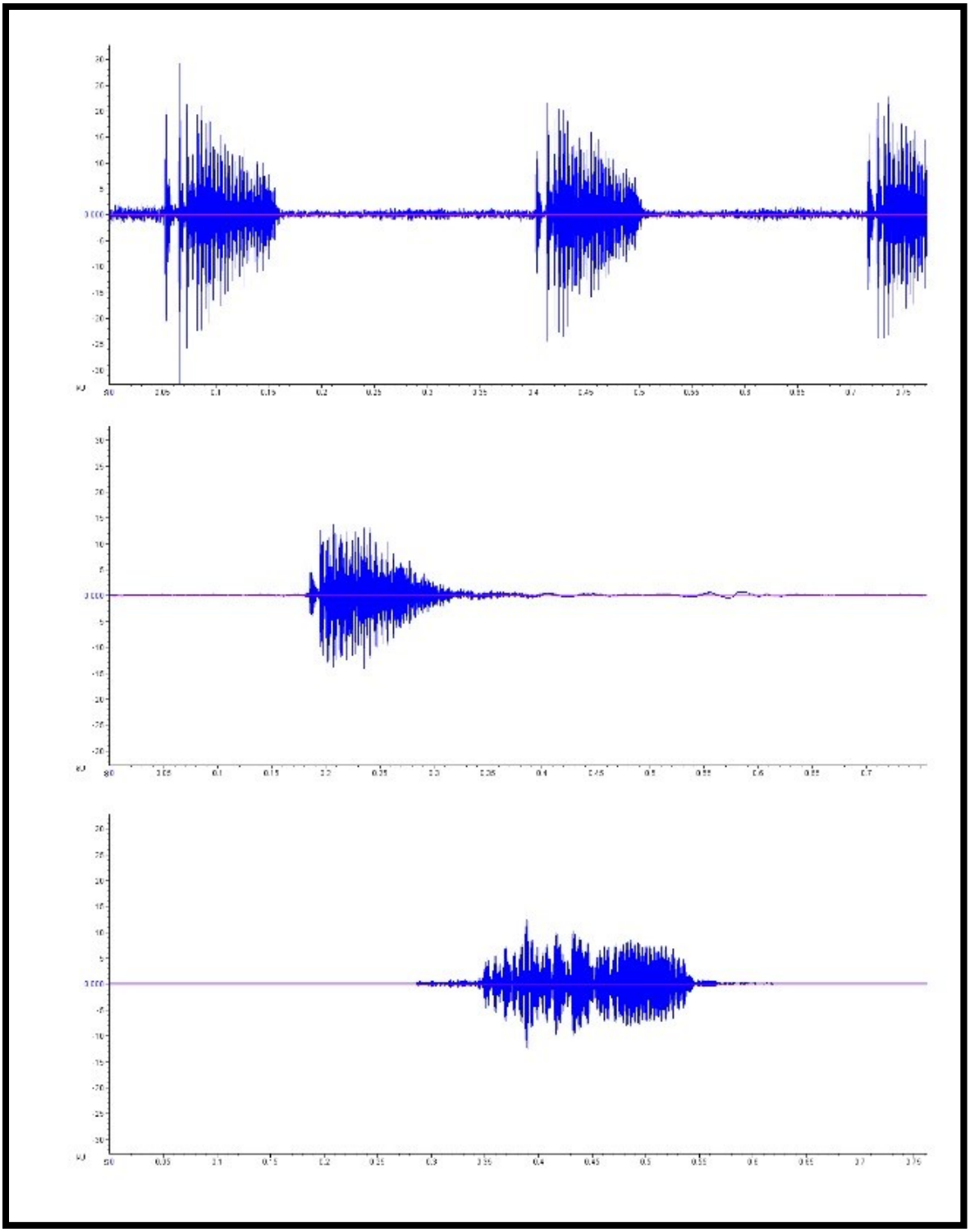

FIGURE 6. One second waveform clip of each species: *M. boettgeri* top, *M. kuatunensis* middle, *M. ombrophilia* **sp. nov.** bottom.

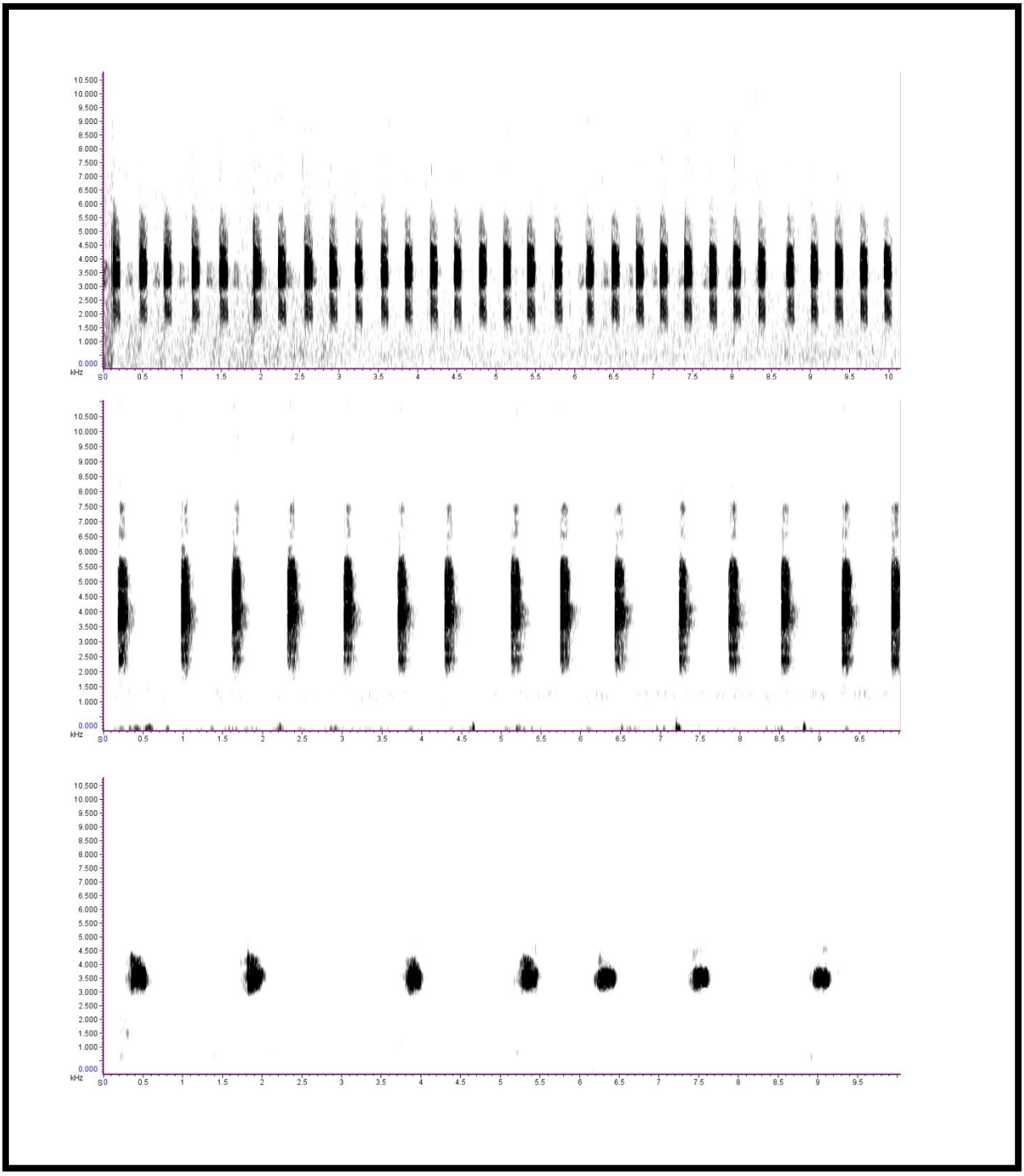

FIGURE 7. 10-second sonogram of each species: *M. boettgeri* top, *M. kuatunensis* middle, *M. ombrophilia* **sp. nov.** bottom.

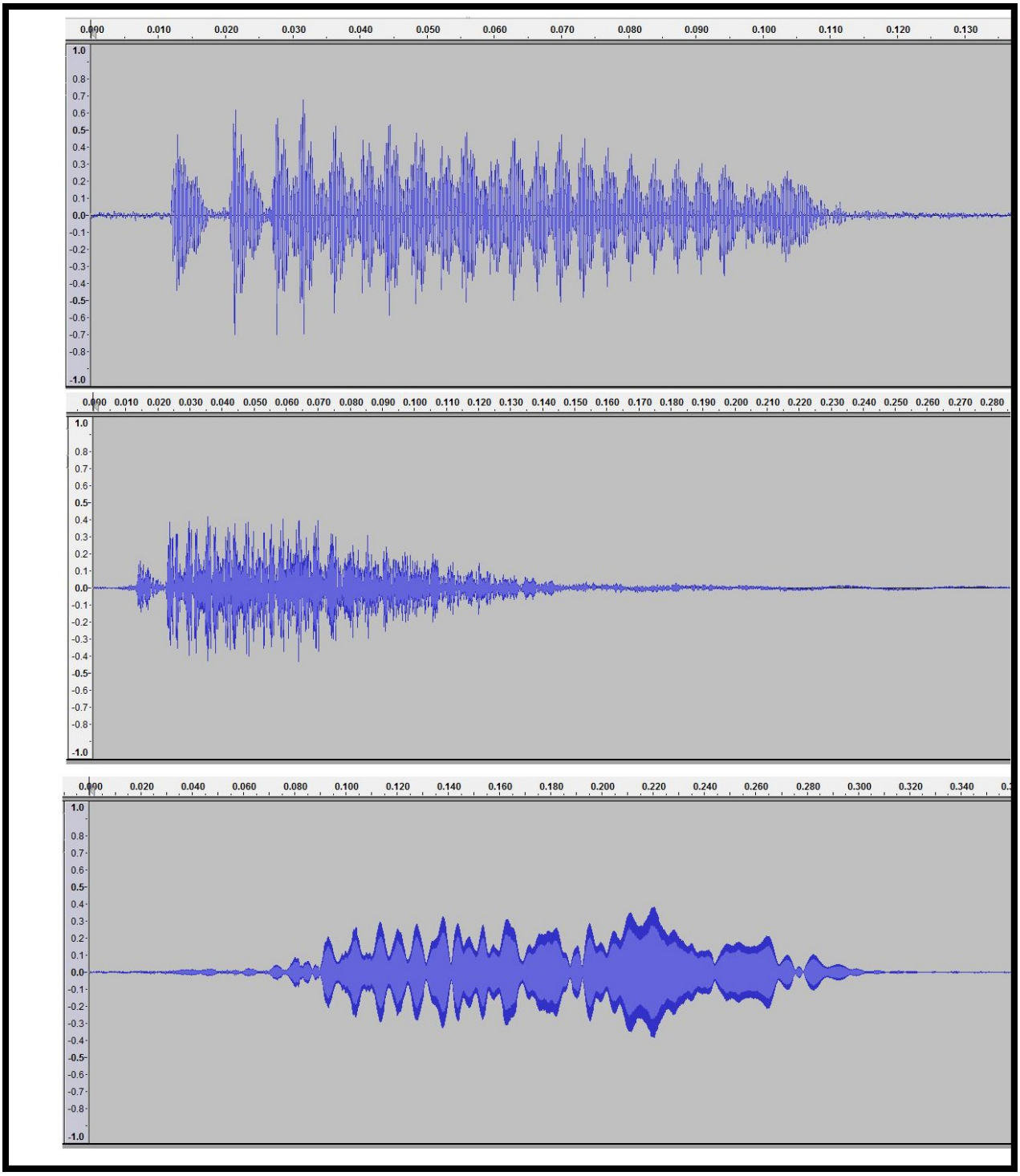

FIGURE 8. Close examination of a single note of each sympatric species; *M. boettgeri* top, *M. kuatunensis* middle, *M. ombrophilia* **sp. nov.** bottom.

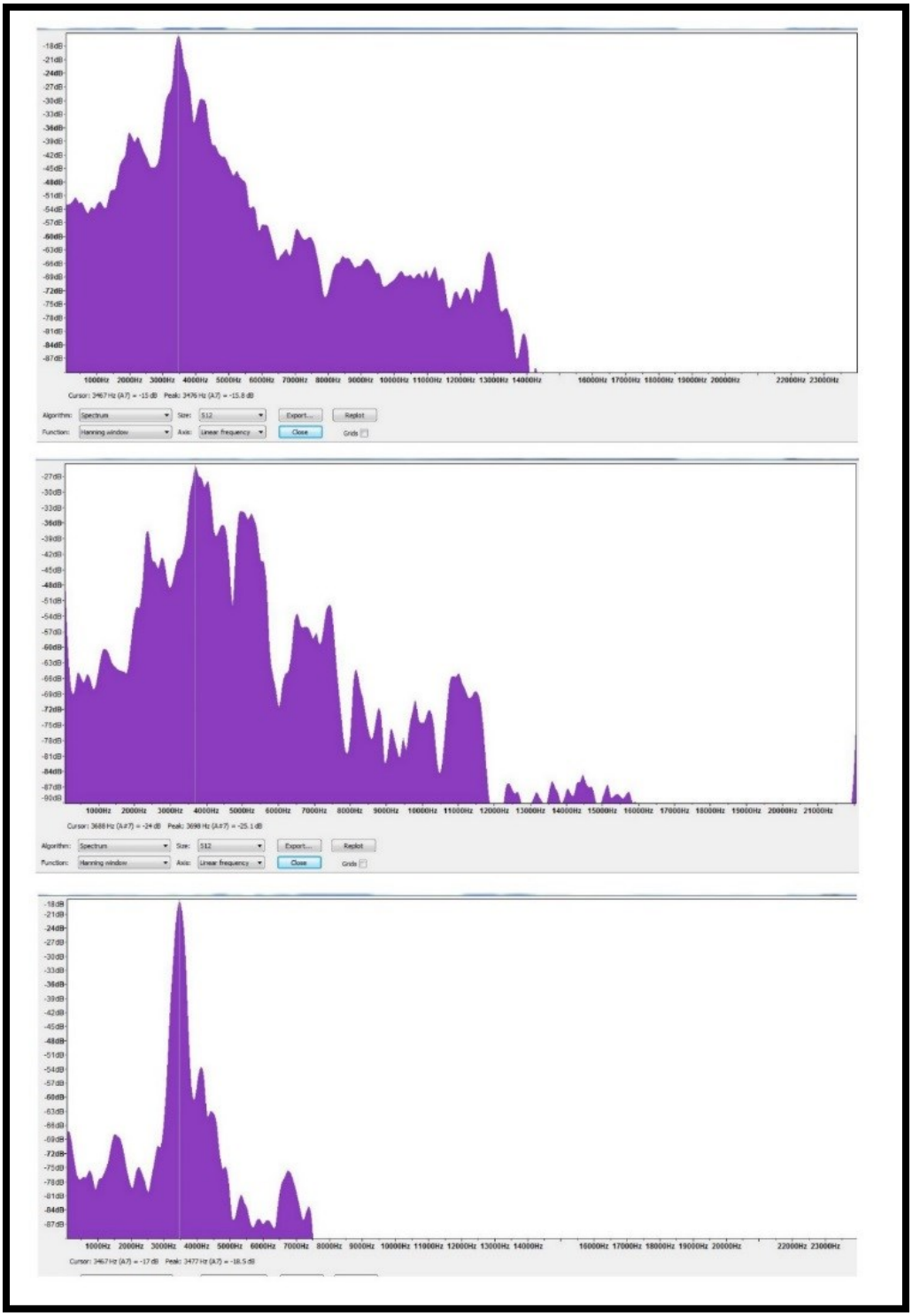

FIGURE 9. Frequency analysis of *M. boettgeri, M. kuatunensis,* and *M. ombrophilia*, illustrating the peak (carrier) frequency of each species.

Description of holotype. An adult male individual. Body very stout and short with 28 mm SVL; head length slightly longer than wide, HDW/HDL ratio 1.2; snout rounded in dorsal view, slightly protruding; in lateral view, snout protruding beyond the mandible, sloping backward to mouth; top of the head flat

Measurements of holotype (in mm). SVL 27.4, HDL 9.4, HDW 10.8, SNT 2.8, IND 3.5, IOD 2.7, EYE 3.2, TMP 1.9, TEY 1.9, HND 5.8, RAD 5.4, FTL 16.3, TIB 10.1.

Coloration of holotype in life. Dorsal color light brown with dark brown "Y"-shape. The fork of the "Y" comprised of a raised ridge. Lateral to the ridges of the "Y" are dorso-lateral ridges running lengthwise. Dark brown interorbital triangle, two corners over each eye and third corner facing caudal. Center of triangle is slightly lighter brown in color. As you move ventrally, the light brown turns to tan to orange. Venter is mostly light tan and orange with dark brown stripes running parallel to the body axis. A prominent stripe running down the center of the throat to the chest. Pectoral glands white.

Coloration of holotype in preservation. Light tan fades to white on dorsal surface. The dark brown patterning turns very dark, near black. On the ventral surface, the groin is white, chest is mostly grey with black stripes running parallel to body axis.

Variation. See Table 1 for a list of variations in measurements of the type series. There is much variation in color. Majority of specimens were orange or yellow-orange in color, but other colors include slate grey, light brown, and dark brown. The dark brown coloration is usually temporary (likely temperature induced) as these dark brown individuals lighten in captivity.

TABLE 1. Secondary sexual characteristics. Single vocal sac in males. Measurements in mm except for weight (in grams).

specimen #	SVL **SVL**	HDL **head length**	HDW **head width**	SNT **snout length**	EYE **eye diameter**	IND **internasal distance**	IOD **interorbital distance**	TMP **tympanum**	TEY **tympanum to eye**	HND **hand length**	RAD **radioulna length**	FTL **foot length**	TIB **tibia length**	WT **weight (g)**
1	27.4	9.4	10.8	2.8	3.2	3.5	2.7	2.7	1.9	5.8	5.4	16.3	10.1	1.2
2	28.8	10.0	11.1	3.1	3.2	3.7	3.1	3.1	1.8	6.5	5.5	16.5	10.4	1.2
3	30.0	10.1	11.4	2.5	4.0	3.6	3.2	3.2	1.6	6.5	6.2	18.1	12.2	1.3
4	32.8	11.0	12.5	3.0	4.2	4.0	3.5	3.5	1.8	6.6	6.7	17.9	12.9	1.4
7	30.4	11.3	11.2	3.1	4.1	4.0	3.2	3.2	2.4	7.9	6.7	17.8	11.4	2.0
9	34.9	12.1	13.2	3.3	4.4	4.3	4.2	4.2	1.8	7.9	7.0	19.3	14.1	4.2
10	34.5	11.6	12.5	3.2	4.5	4.1	3.8	3.8	2.7	8.0	6.4	18.1	11.3	2.6
14	34.9	11.3	13.2	4.0	4.4	4.4	4.0	4.0	1.9	7.9	7.0	19.3	12.5	2.5
15	34.2	11.2	13.2	3.4	4.2	4.3	4.1	4.1	1.8	8.0	6.9	19.5	11.9	3.5
23	35.0	12.3	13.6	4.2	4.1	4.6	3.9	3.9	2.0	8.2	7.1	20.3	13.0	3.4
Average	32.3	11.0	12.3	3.3	4.0	4.1	3.6	3.6	2.0	7.3	6.5	18.3	12.0	2.3
SD	2.9	0.9	1.1	0.5	0.4	0.4	0.5	0.5	0.3	0.9	0.6	1.3	1.2	1.1

Etymology. *Megophrys ombrophilia*: Greek: *ombros* (rainstorm), Greek: *philia* (loving, fondness), in reference to the species' propensity for being especially active during and after heavy rainstorms. The species has such a tendency for rainstorms, that it was never encountered beyond two days post rain. For an English standard name, we are recommending the name Rainy Horned Toad. The recommended Chinese name is Xia Yu Jiao Chan.

Distribution and habits. Currently *M. ombrophilia* **sp. nov.** is only known from the outskirts of Guadun village. Five specimens (three collected; WUYXX1, WUYXX2, WUYXX3) were found in a bamboo plantation near the village. During surveys that yielded *M. ombrophilia*, the species was strongly associated with: *Megophrys boettgeri, Theloderma rhododiscus,* and *Leptobrachium liui*. We never found the species syntopic with *M. kuatunensis*, though Jianhuan Yang (JHY) did observe an individual (a calling male) syntopically with *M. kuatunensis* in mid-May. A sixth individual (WUYXX4), also on the outskirts of Guadun village, was found in an abandoned tea plantation surrounded by bamboo plantation and secondary forest (distance to bamboo forest habitat <50m). A seventh specimen (not collected; observed by JHY), was found next to the stream adjacent to this abandoned tea plantation, but further upstream where *M. kuatunensis* starts to occupy the microhabitat. An eighth specimen (NFUXXX2) was found dead on the road (ran over by a car) at the edge of a bamboo plantation, this find represented the lowest elevational find at 1125 m. A ninth specimen (NFUXXX5) was found north and west of Guadun village, uphill from a tea plantation, just inside a bamboo plantation. This find represented the highest elevational find at 1350 m. In general, the species was most commonly found on edge habitat, between bamboo forest (including bamboo plantations) and mixed broad leafed evergreen forest. Common vegetation and tree species include Moso bamboo (*Phyllostachys edalis*), Long-ear Cane bamboo (*Indocalamus longiauritus*), Masson Pine (*Pinus*

massoniana), Chinese fir (*Cunninghamia lanceolate*), Tea tree (*Camellia sinensis*) and Chinese cedar (*Cryptomeria fortunei*).

Several individuals (n=4, 1 collected: NFU0001) were found in an active tea plantation, but in all cases, the tea plantation was small ($\leq$1 ha) and surrounded by bamboo forest. Specimens were never more than 70 m from bamboo edge. A fourteenth specimen (not collected) was found in secondary forest that would not qualify as bamboo or tea plantation or edge habitat. This forest was mixed broad leafed evergreen forest primarily made up of Masson Pine, Chinese fir, Long-eared Bamboo and Moso bamboo mixed with smaller hardwoods. Three specimens (1 collected; NFUXXX3) were found in secondary bamboo forest (non-plantation) that was comprised mostly of Long-eared Cane Bamboo, Moso Bamboo, and sparse hardwoods. Eight specimens (2 collected; NFUXXX4, WUYXXX5) were found on the edge habitat between a Long-eared Bamboo Forest and tea plantation, specimens were emerging from the bamboo side of the edge. Based on these observations, it would appear as if the species prefers slightly disturbed habitat, as edge habitat tended to have the highest densities. Observations within bamboo plantations, or secondary forest were always singularly observed (as opposed to seeing multiple specimens within a few meters of each other as on edge habitat). However, the species seems to avoid heavy disturbance as no specimens were found within the "city limits" of the village of Guadun. And only a single observation of a specimen on a road was made.

Compared to *M. boettgeri* and *M. kuatunensis*, *M. ombrophilia* seems to be more terrestrial. Based on our observations, *M. kuatunensis* is heavily associated with streams, as we never encountered individuals more than 10 m from a stream or river. By contrast, *M. boettgeri* is much more of a generalist as several individuals were found both adjacent to rivers and streams as well as in upland habitats far from water. Compared to both of these species, *M.*

ombrophilia was frequently found in upland habitat, often times far from water. In such cases, the closest sources of water were usually very small and it is unclear whether or not they use these small streams for breeding efforts.

All specimens were encountered by active searching, as opposed to listening for calling males. Elevations ranged from 1125 to 1350 m, with an average of 1242 m (SD ± 39.7 m; n=23). JHY found the one and only calling male (not collected) on 18 May. The species seems to be tied closely to rain events. In May 2015, during nights of rain events, each night yielded 5-7 specimens. One day post rain would yield 2-3 specimens, and any day beyond 2 days post rain would not yield any specimens. In our experience (n=24), the rain should be enough to leave standing water on the ground by nightfall, not simply a small rain in the morning which was soaked up by sunset. The species is very predictable in this regard. Nighttime temperatures of finds averaged 20.5 (SD ± 0.8, range 19.2–22.3°C, n=23), with an average humidity of 92.3 (SD ± 4.7, range 86.3–98.9, n=23), and barometric pressure averaged 25.8 (SD ± 0.1, range 25.57–25.93, n=23).

Surveys in July failed to turn up any specimens. As of yet, no surveys outside of these months have been conducted by the authors in the study area searching for this species. We cannot confirm the mating season. Currently, it is suspected that the breeding season may be during early spring, i.e., March, April and early May, as we have thoroughly sampled during the second half of May, all of June, and July without hearing advertisement calls.

The type locality (Guadun village) has an annual mean temperature of 13.4°C and annual mean precipitation of 2143 mm. During the months of May and June, seemingly the most active period of the species as of yet, the mean temperatures are 17.0°C and 20.1°C, respectively, with a mean precipitation of 343.0 mm and 339.9 mm, respectively. The month of April, currently

suspected to be part of the breeding season, has a mean temperature of 13.4°C and mean precipitation of 264 mm.

With regard to activity, one specimen was found in the day time (15:47 on 14 June 2013, a rainy day) and 24 specimens were found at night. Nocturnal animals were found as soon as 49 minutes after sunset, to as late as 4 hours 2 minutes after sunset. A higher number of specimens was found immediately after heavy rains, and only a few specimens were actually found during the heavy rains.

Remarks. Under preservation, most specimens retained the lighter coloration, making dorsal patterns more visible. Initially we thought the specimen calling on 18 May was likely calling toward the beginning of the breeding season, and efforts were made to find calling males during the second half of May in 2014 and 2015, but as none were found, despite finding several individuals each night, and using play back of the species to elicit males to call, we now suspect that 18 May is around the end of the breeding season for this species. This would seem to make more sense as the species has a very similar call to *M. kuatunensis* and since they occupy the same area, the best way to avoid interspecific competition would be to space themselves out temporally if spatially is not possible.

It is our recommendation that for future surveys for this species, especially for parties interested in the breeding ecology that surveys for breeding males begin around March and conducted to mid-May.

Discussion

Recent years have illustrated a need for re-examining the amphibian species diversity of southeast China, especially in regards to megophryid diversity, and perhaps re-examining other regions of China as well. As early as 2010, only three species were known to southeastern China, one of those three having been described only five years earlier, *M. huangshanensis* (Fei & Ye 2005), prior to that, only two species were known for about 70 years. In the last four years, six new species have been identified in the region, and not simply from cryptic species (species which are visually identical but genetically distinct). These species have been either overlooked or misidentified for decades possibly due to the still young state of Chinese herpetology, i.e., presence of unexplored regions, wildlife officials having a very basic level of species identification skills. To the casual observer, it is reasonable for this group of anurans to be overlooked and misidentified because many are small, highly variable in color and pattern, and some are extremely secretive.

The finds have illustrated a high degree of endemism and the significance of isolated mountain ranges, which is where many of these forms are popping up. The region requires significant work to determine how widespread and connected these species and populations are between one another as there are still several more isolated mountain ranges that could hold some of the "endemics" or could hold yet more new species. Currently, many of these species are restricted to small geographic ranges, and further still, often restricted to narrow elevational ranges which speaks to their delicate nature by comparison to their more widespread congeners and the need for their protection.

Megophrys ombrophilia does not currently face too many threats. It is not of economic concern, there is no worry about the species being used for consumption, medicine, or a high

demand for the pet trade. Within the Wuyishan nature reserve, the biggest threat is that of habitat destruction. The species is semi-tolerant of disturbance, seemingly to prefer mildly disturbed areas to less disturbed. Mild disturbance in this context is referring to the creation of a trail through the woods, or a small (<1 ha) tea plantation. The species has yet to be found within the city limits of the small town it surrounds.

Due to the long history of the Wuyishan area, it is hard to say exactly what the original habitat of the region was like before the settlement of humans. Currently, a vast percentage of the reserve is made of up tea and bamboo plantations (approximately half of the protected area is composed of human-made and maintained plantations). What makes the discovery of this species fairly interesting is the fact that it had gone unnoticed to science for so long in such a heavily studied area. Its type locality was intensely surveyed in the late 1800's and early 1900's, and due to so many species being found during those times, the site became even more popular throughout the 20th century. Specialists in multiple fields of study came to Wuyishan, and continue to do so, on a regular basis. Until more populations are found outside of the type locality (<10 km^2), in accordance with IUCN Red List Categories and Criteria (Version 3.1), we consider this species to meet criteria "B 1 a" for Critically Endangered. Theoretically, from an elevational and habitat point of view, the species should have approximately 200 km^2 of habitat within the nature reserve, but this should be verified.

In this study, we characterized a third species of megophryid in the northern Wuyishan mountain range. The species is readily distinguished from its two sympatric species through morphology, bioacoustics, and molecular genetics. Though its genetic distinction is based only on two mitochondrial genes, it is of comparable genetic difference to other known *Megophrys*. Ecologically, it is most commonly sympatric with *M. boettgeri*, and occasionally sympatric with

M. kuatunensis. The calls between *M. boettgeri* and *M. kuatunensis* are significantly different from one another, and as such, there is no issue with these two species advertising and overlapping in breeding season, which they do from at least May to July. Interestingly, the calls of *M. ombrophilia* sound strikingly similar to *M. kuatunensis*, and perhaps it is for this reason that we never found male *M. ombrophilia* displaying advertisement calls. The species may be isolating themselves temporally since they are not isolated spatially from *M. kuatunensis*.

Acknowledgments

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Literature Cited

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic acids research*, 32(5), 1792-1797.

Guayasamin, J.M., Krynak, T., Krynak, K., Culebras, J., Hutter, C.R. 2015. Phenotypic plasticity raises questions for taxonomically important traits: a remarkable new Andean rainfrog (*Pristimantis*) with the ability to change skin texture. Zoological Journal of the Linnean Society, Vol. 173(4):913-928.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8), 754-755.

Fei, L., Ye, C. (1992) Two new species of the genus Megophrys, Pelobatidae (Amphibia: Anura) from China. Zoological Research. 13 (1), 5−12.

Fei, L., Ye, C., Jiang, J. (2012) Colored Atlas of Chinese Amphibians and Their Distributions. Sichuan Publishing House of Science & Technology, Chengdu, 619 pp.

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular biology and evolution*, 29(6), 1695-1701.

Li, Y., Jin, M., Zhao, J., Liu, Z., Wang, Y., Pang, H. (2014) Description of two new species of the genus Megophrys (Amphibia: Anura: Megophryidae) from Heishiding Nature Reserve, Fengkai, Guangdong, China, based on molecular and morphological data. Zootaxa. 3795(4): 449-471.

Li, C., Wang, Y. (2008) Taxonomic review of Megophrys and Xenophrys, and a proposal for Chinese species (Megophryidae, Anura). Acta Zootaxonomica Sinica. 33: 104−106.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, 30(12), 2725-2729.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, 10(3), 512-526.

Pyron, A., Wiens, J. (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogeny and Evolution. 61: 543-583.

Rambaut, A., & Drummond, A. (2012). FigTree: Tree figure drawing tool, v1. 4.2. *Institute of Evolutionary Biology, University of Edinburgh.*

Rambaut, A., Suchard, M., & Drummond, A. J. (2013). Tracer v1. 6.0. *WWW document URL http://beast. bio. ed. ac. uk/Tracer [accessed 30 July 2014]*.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19(12), 1572-1574.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312-1313.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22(21), 2688-2690.

Stamatakis, A., Ludwig, T., & Meier, H. (2005). RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics*, 21(4), 456-463.

Wang, Y., Zhang, T., Zhao, J., Sung, Y., Yang, J., Pang, H., Zhang, Z. (2012) Description of a new species of the genus Xenophrys Günther, 1864 (Amphibia: Anura: Megophryidae) from Mount Jinggang, China, based on molecular and morphological data. Zootaxa. 3546: 53-67.

Wang, Y., Zhao, J., Yang, J., Zhou, Z., Chen, G., Liu, Y. (2014) Morphology, Molecular Genetics, and Bioacoustics Support Two New Sympatric Xenophrys Toads

(Amphibia: Anura: Megophryidae) in Southeast China. PLOSone. 9: e93075.

EFFECT OF LIGHT INTENSITY ON PRIMARY AND SECONDARY METABOLISM, PHYTOHORMONES AND ENZYME ACTIVITIES IN *CAMPTOTHECA ACUMINATA*

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Abstract

The reactions of primary and secondary plant growth due to different light intensities are useful measurements to conclude the proper environment for the cultivation of medicinal plants. Camptotheca acuminata is a deciduous hardwood tree native to southern China that has the capability of producing high contents of camptothecins. Camptothecin is a natural based anticancer substance that produces enzymes that fight against antitumor activities, has capabilities to cooperate with the DNA of topoisomerase 1 and is used to black retroviruses such as the human immunodeficiency virus and the equine infectious anemia. Knowing that light is the primary source of energy and is one of the most important environmental factors that regulate plant growth and development, we wanted to determine how the intensity of light would effect the production of camptothecins in this species. The objective of this study was to assess the effect of light intensity on primary and secondary metabolism, phytohormones and enzyme activities in Camptotheca acuminata seedlings. To examine these components, three light intensities were applied to the seedlings: (i) 300 (PPF), (ii) 500 (PPF) and (iii) 800 (PPF). We used the soluble sugar and soluble protein curve determine if the optical density of them both would be adjacent to the contents. This was concluded by measuring the total leaf, root and stem biomass, camptothecin content, enzyme activities and hormones. My hypothesis is that lower light levels may contribute to the accumulation of secondary metablites.

Keywords: *Campothecin,* Camptotheca acuminata*, light intensity, metabolism, phytohormones, enzymes, anthrone, anthrone colorimetry, sucrose, optical density, photosynthetic Photon Flux*

Introduction

Camptotheca acuminate Decne, which belongs to the Nyssaceae family, is a deciduous hardwood native to southern China where it's known as "Xi Shu" or "Happy tree" (Avemann, K 1988). Lately *C. acuminata* has become progressively more important because of the high content of camptothecins (CPT). Camptothecin is a natural product based anticancer substance separated from an organic extract of the bark of *C. acuminate (*Pommier, 1994*)*. Campothecins are cytotoxic quinoline alkaloids that produce enzymes that are vital to its remarkable antitumor activities, capability to cooperate with the DNA of topoisomerase 1 and because of its inhibition of retroviruses such as the human immunodeficiency virus and the equine infectious anemia (Lodish H, 2000).

In June 1996, two of the most distinguished CPT components topotecan (Hycamtin) and irinotecan (campto) were accepted by the U.S Food and Drug Administration for the use of medical healing of colon (Camptosar, Pharmacia and Unjohn, Inc) and ovarian cancer (Hycamtin, SmithKline Beecham Pharmaceuticals) (Wiseman and Markham, 1996). Colon cancer is created when the uncontrollable cell growth comes in contact with cell in the large intestine.

Most colon cancers derive from small noncancerous tumors called adenomatous polyps, which grow on the inner walls of the large intestine (Pommier, 2012). These polyps have the ability to grow into colon cancers over time if they aren't removed during colonoscopy. Ovarian cancer happens when abnormal cells grow in one or both of your ovaries (Wiseman, L.R., Markham, A., 1996). Both of these enzyme work as an inhibitor which is used to slow down the action of topoisomerase 1 during the chemotherapy treatment process (Kepler et. Al 1996).

Topoisomerases l are enzymes with the ability to separate one of the two fibers of double-stranded DNA, calm the fiber and reassemble them back together.

As a primary source of energy, light is one of the most important environmental factors for plant growth (Naoya et al., 2008). Light is the factor that impels photosynthesis, regulates plant growth and development and has been shown to have important effects on secondary metabolites biosynthesis (Award et al., 2011). It has been stated that stress such as light and drought before leaf harvest could be the main cause of increase CPT concentration in *C. acuminata* leaves, but will stunt the growth and inhibit the accumulation of biomass in the leaves (Lie et al., 1997, 2015; Liu, 2000). Knowing that this species can be life changing, it's imperative to understand the most suitable environment for better growth of *C. acuminata* and high leaf biomass. The objective of this research is focused on the effect on the inter-relationship of light intensity on primary and secondary metabolism and phytohormones in *Campotheca acuminata* seedlings, to clarify how the enzymatic activity responds. The information generated from this study is expected to show under which environment the effectiveness of light can change the composition of *C. acuminata* DNA to be more effective during medical treatment such as chemotherapy.

Materials and Methods

Seedlings

In June 2015, a pot experiment was established to examine the effects of different light intensities on the physiological and biochemical changes in *C. acuminata* seedlings. The experiment was conducted with different thickness shading nets in a room with a controlled environment at Nanjing Forestry University in Nanjing, China. The *C. acuminate* seedlings were

transferred into one hundred and fifty plastic pots (16.5cm inner diameter, 18cm height) at 3 seedlings a pot and filled with a mixture of organic nutrient and high humus nutrient soil. We irrigated the pots twice a week to keep the plants well-watered. After sixty days of growth, we moved each pot from the lab to the climate chamber, taking 1 week for the seedlings to adapt to the new environment.

We then set the climate chamber light intensity to three hundred PPF (Photosynthetic Photon Flux). We programmed the room temperature to 2,000 ℃ during the day and 20,000 ℃ during the night. The lights were programmed to turn on everyday at 5:30 am and off every night at 6:30 pm. On June 19 while the seedlings were all under the same light intensity of 300 ppf, we selected thirty of the seedlings at random and tagged them as sample seedlings, six seedlings were selected at random to be measured for biomass, measured the ground height with a 2 cm ruler to and a vernier caliper scale to measure the diameter (mm) **(Fig. 1).** We picked four leaflets randomly from the seedlings (not the sample seedlings) resulting in twenty bags to measure the enzyme activity and their phytohormones.

The seedlings were placed into a 5cm bag and immediately into a 1 ml liquid nitrogen container to be frozen and stored at 70℃ for further experiments. After collecting all the data, we then separated the seedlings into three groups of light intensities using a light shield. The first group represented the seedlings that would be under the ppf of 300, the second group 500 ppf and the last group 800 ppf. One week later on June 26 the same process was repeated (group by group). The height and diameter of the seedlings were measured, selecting six seedlings for biomass and four leaflets at random (not the sample seedlings), resulting in twenty bags to measure the enzyme activity and their phytohormones, for each group. On the third of July, the exact same process was conducted.

Soluble Sugar

The Anthrone colorimetry method was used to determine the content of soluble sugars, using five test tubes. A measurement of 50 mg of sucrose was poured into a 500 ml flask of distilled water, resulting in a volume of 500 ml. We placed the flask into an ultrasonic cleaner to dissolve the sugar. We mixed 1 g of anthrone into 50 ml with 50 ml of Ethyl Acetate, using a 50 ml beaker. Then we obtained five test tubes, numbered them and used a pipette to extract the liquids. For test tube 1, we extracted 0.2 ml of sucrose, 1.8 ml of water, 0.5 ml of anthrone and 5 ml of sulfuric acid, test tube 2, 0.4 ml of sucrose, 1.6 ml of water, 0.5 ml of anthrone and 5 ml of sulfuric acid, test tube 3, 0.6 ml of sucrose, 1.4 ml of water, 0.5 ml of anthrone and 5 ml of sulfuric acid, test tube 4, 0.8 ml of sucrose, 1.2 ml of water, 0.5 ml of anthrone and 5ml of sulfuric acid and for test tube 5, extract 1.0 ml of sucrose, 1.0 ml of water, 0.5 ml of anthrone and 5 ml of sulfuric acid (See figure 1.). The test tubes should all have the same amount of liquid and slight differences in color. The test tubes were then placed one by one into a ultraviolet spectrophotometer to detect the optical density to understand the contraction of soluble sugars.

Standard curve for soluble protein

The standard curve was measured by extracting .01 g of Albumin from bovine serum. After the extraction is weighed, it was mixed with 100 ml of water to create the standard solution. Using six test tubes, in test tube (1) 0.2 ml of Albumin Bovine, 1.0 ml of distilled water and 5 ml of Coomassie G-250 was inserted. In test tube we inserted (2) 0.2 g of Albumin Bovine, 0.8 ml of distilled water, 20 ml of soluble protein content and 5 ml of Coomassie G-250. Test tube 3 contained 0.4 ml of Albumin Bovine, 0.6 ml of distilled water, 40 ml of soluble protein content and 5 ml of Coomassie G-250. In test tube 4 we added 0.6 ml of Albumin Bovine, 0.4 ml of distilled water, 60 ml of soluble protein content and 5 ml of Coomassie G-250. We added

0.8 ml of Albumin Bovine, 0.2 ml of distilled water, 80 ml of soluble protein content and 5 ml of Coomassie G-250 to test tube 5. Test tube 6 had 1.0 ml of Albumin Bovine, 100 ml of soluble protein content and 5 ml of Coomassie G-250. Each test tube then was taken to be measured using the ultraviolet spectrophotometer, to detect the optical density. (Figure 1)

Soluble Protein

To determine the soluble protein in the leaves we started off by measuring 0.25 g of the leaf and grinding it using water 5 ml of water along with 0.1 g of quartz sand. We used a total of twenty one centrifuge tubes to put the grinded leaves into. The reason we used twenty one centrifuge tubes is because we had three replicas from only two weeks of data (due to time). 0.1 ml of solution was extracted from the protein into twenty one test tubes. Then we added 50 ml of coomissie blue G 250 to the test tubes. (Figure 2)

Biomass measurements

DUE TO TIME RESTRAINTS, THE BIOMASS MEASURES WERE NOT RECORED

(Figures 3, 4, 5)

Camptothecin extract solution

To create the camptothecin extraction solution, the leaves of the seedlings were dried at and measured at 0.5 g and ground with 60% ethonal and 40% water. After leaves are well decomposed, we then poured the mix into a 10 ml test tube. Twenty one centifuge tubes were filled by extracting 1 ml of the solution. 1.5 ml was put into the centrifuge machine to be spun. (Figure 6)

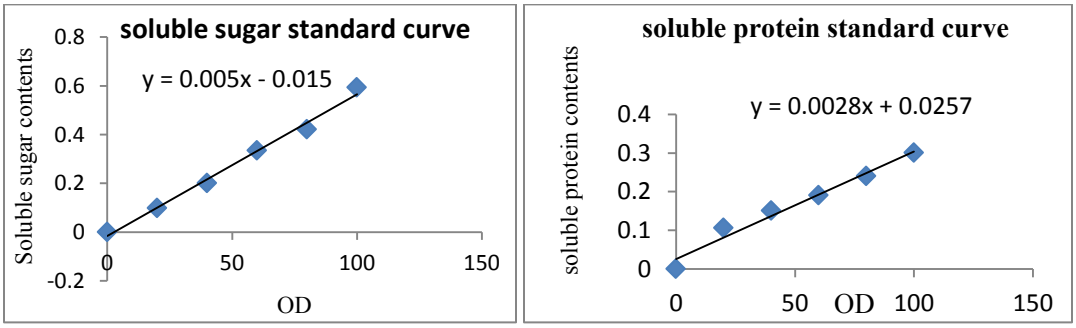
Statistical analyses

The Statistical analyses were conducted using Statistical Product and Service Solutions for Windows, version 7. The data were analyzed using analysis of variance (ANOVA), and the

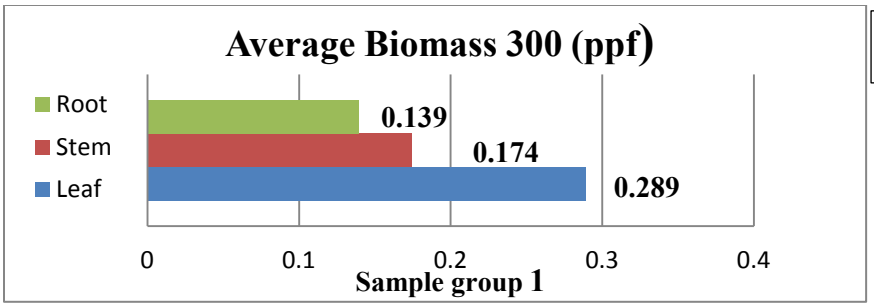
differences between the means were tested using the Tukey T test ($P < 0.05$). This method was used to determine if the soluble protein content and high and diameter of the seedlings hypothesis failed to reject or rejected the test. (Figure 7 8,)

Results

As I looked into the differences in the height and diameter of the seedlings under all three light intensities, there was a small variation in the diameter but the height showed a significant amount of growth. The variations between group L1 - L2 and L2-L3 both were slim to none, but as we found the difference between L1-L3 there was a difference. The difference is simply due to the amount of light the seedlings are receiving from 300 ppf vs 800 ppf. The higher intensity may be stressing the seedlings and causing them to stunt their growth and production.



Figures 1-2. These two curves are only a representation of the positive relationship between the optical density and the contents available. Once the sugar and protein contained the leaves of the *C. acuminate* are measured and graphed, the results should indicate the same relationship as the graphs above.



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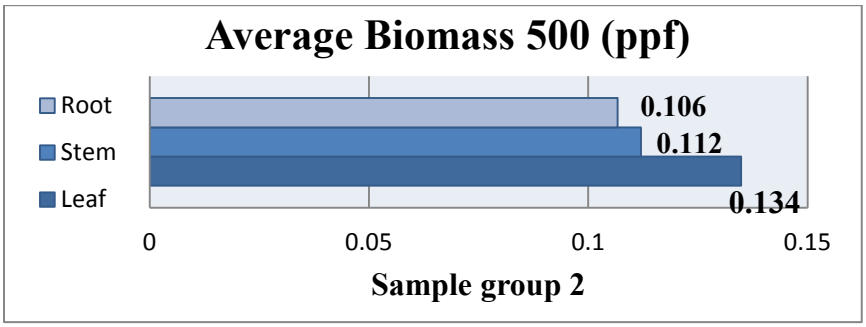

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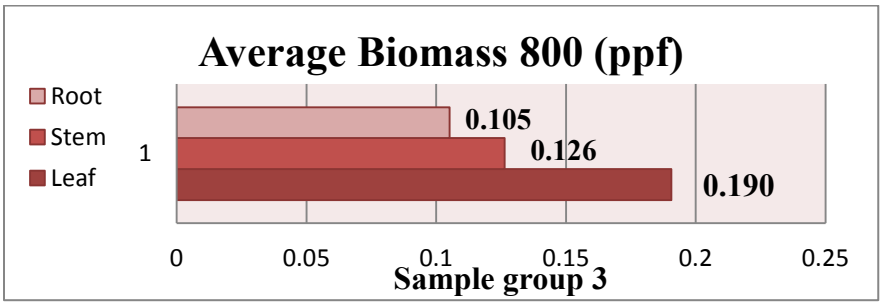

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Figures 3-5. On June 26 and July 3, as the seedlings were under the different light intensities the leaves, roots and stems were randomly collected.) The graphs below indicate the amount of biomass that was measured in all three light intensities.

Taking a look at the three graphs represented, it appears that the lower light intensity has the most production of biomass present. There are a few differences between the 500 and 800 group. The root biomass of the 500 is slightly higher than the root biomass of the 800.

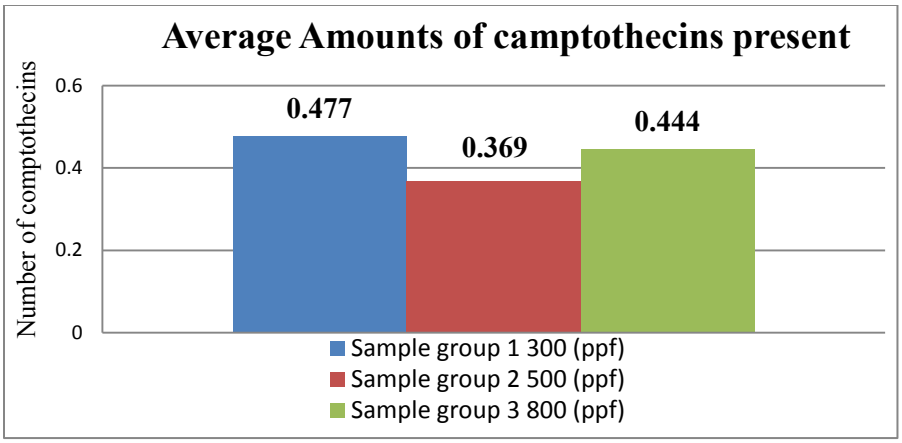

Figure 6. The effects of light on plants are varied and extremely important to the long-term health of any species. According to my results, my data indicates that the higher the light intensity is, the fewer amounts of camptothecins are produced.

Statistics

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
L1 300 ppf	10	167.6	16.76	11.39822
L2 500 ppf	10	152.1	15.21	1.969889
L3 800 ppf	10	136.8	13.68	1.275111

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	47.43267	2	23.71633	4.858835	0.015761	3.354131
Within Groups	131.789	27	4.881074			
Total	179.2217	29				Fail to reject

Figure 7. The height of the seedlings on July 3, 2015.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
L1	10	21	2.1	0.066311
L2	10	22.59	2.259	0.095477
L3	10	21.99	2.199	0.103854

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.12894	2	0.06447	0.728085	0.492072	3.354131
Within Groups	2.39078	27	0.088547			
Total	2.51972	29			Reject	

Figure 8. The diameter of the seedlings on July 3, 2015.

Conclusion

The results from this study may advance the understanding of the effect of light intensity on the primary and secondary metabolism, phytohormones and enzyme activities in this species to optimize plant growth. There wasn't enough time to complete some of the measurements but from the data we do have, you can clearly see that the effect of higher intensities of light on these seedlings produces less growth in height, diameter and comptothecins. The protein and soluble sugars should be measured in the future to complete this study.

Acknowledgements

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Literature Cited

Arabidopsis. (n.d.). *WordNet 3.0, Farlex clipart collection*. (2003-2008). Retrieved June 15 2015 from http://www.thefreedictionary.com/Arabidopsis

Auxins. (n.d.). *Dictionary of Unfamiliar Words by Diagram Group*. (2008). Retrieved June 15 2015 from http://www.thefreedictionary.com/auxins

Avemann K, Knippers R, Koller T, et al. Camptothecin, a specific inhibitor of type I DNA topoisomerase, induces DNA breakage at replication forks.*Mol Cell Biol.* 1988;**8**:3026–3034

Avemann K, Knippers R, Koller T, et al. Camptothecin, a specific inhibitor of type I DNA topoisomerase, induces DNA breakage at replication forks.*Mol Cell Biol.* 1988

block the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome. Introduction of these breaks subsequently leads to apoptosis and cell death.

Chabot GG: Clinical pharmacokinetics of irinotecan. Clin Pharmacokinet. 1997 Oct; 33(4):245-59. Pubmed

D.L. Comins, H. Hao, K.S. Jayanta, J. Gao J. Org. Chem., 59 (1994), p. 5120 Kepler et al., 1969 J.A. Kepler, M.C. Wani, J.N. McNaull, M.E. Wall, S.G. Levine

Ecologically relevant UV-B dose combined with high PAR intensity distinctly affect plant growth and accumulation of secondary metabolites in leaves of Centella asiatica L. Urban. *Müller V, Albert A, Barbro Winkler J, Lankes C, Noga G, Hunsche M J Photochem Photobiol B. 2013 Oct 5; 127():161-9.* [PubMed] [Ref list]

Gibberellins. (n.d.) *Collins English Dictionary – Complete and Unabridged*. (1991, 1994, 1998, 2000, 2003). Retrieved June 15 2015 fromhttp://www.thefreedictionary.com/Gibberellins

Gottschalk K. W. (1994). Shade, leaf growth and crown development of *Quercus rubra, Quercus velutina, Prunus serotina* and *Acer rubrum* seedlings. Tree Physiol. 14, 735–749. 10.1093/treephys/14.7-8-9.735 [PubMed] [Cross Ref]

Gupta M, Abdel-Megeed M, Hoki Y, et al. Eukaryotic DNA topoisomerases mediated DNA cleavage induced by a new inhibitor: NSC 665517. *Mol Pharmacol*. 1995;**48**:658–665.

Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramirez J, Rudin CM, Vokes EE, Ratain MJ: Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol. 2004 Apr 15;22(8):1382-8. Epub 2004 Mar 8. Pubmed

J. Org. Chem., 34 (1969), p. 3853

jasmonate vapour increases the developmentally controlled synthesis

Li S., Yi Y., Song L.L., Yu W. W., Hu Y. Y., Ma X. H., Wu J. S., et al. (2015). Light quality modifies camptothecin production and gene expression of biosynthesis in *Camptotheca acuminate* Dene seedlings. Ind. Crop Prod. 66, 137-143 10.1016/j.indcrop.2014.12.046 [Cross Ref]

Liu LF, Desai SD, Li TK, et al Mechanism of action of camptothecin. *Ann NY Acad Sci.* 2000;**922**:1–10

Liu Z. J. (2000). Drought-induced in vivo synthesis of camptothecin in *Camptotheca acumin*ata seedlings. Physiol. Plant. 110, 483-488 10.111/j.1399-3054.2000.11000.1100409. x [Cross Ref]

Liu Z. J., Carpenter S. B., Constantin R.J. (1997). Camptothecin production in *Camptothecin acuminate* seedlings in response to shading and flooding. Can. J. Bot. 75, 368-373 10.1139/b97-039 [Cross Ref]

Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 12.3, The Role of Topoisomerases in DNA Replication. Available from: http://www.ncbi.nlm.nih.gov/books/NBK21703/

Masuda et al., 1992 N. Masuda, M. Fukuoka, M. Takada, Y. Kusunoki, S. Negoro, K. Matsui, S. Kudoh, N. Takifuji, K. Nakagawa, S. Kishimoto CPT-11 a new derivative of camptothecin for the treatment of refractory or relapsed small-cell lung cancer J. Clin. Oncol., 10 (1992), pp. 1225–1229.

Moudi, Maryam et al. "Vinca Alkaloids." *International Journal of Preventive Medicine* 4.11 (2013): 1231–1235. Print.

O'Dwyer PJ, Catalano RB: Uridine diphosphate glucuronosyltransferase (UGT) 1A1 and irinotecan: practical pharmacogenomics arrives in cancer therapy. J Clin Oncol. 2006 Oct 1;24(28):4534-8. Pubmed of alkaloids in *Catharanthus* and *Cinchona* seedlings, Plant J. 5 (1994) 635–643.

Pommier Y, Leteurtre F, Fesen MR, et al. Cellular determinants of sensitivity and resistance to DNA topoisomerase inhibitors. *Cancer Invest.*1994; **12**:530–542.

Pommier, Yves. *DNA Topoisomerases and Cancer*. New York: Humana, 2012. Print.

Priel, E., Showalter, S.D., Blair, D.G., 1991a. Inhibition of human immunodeficiency

R.J. Aerts, A. Schäfer, M. Hesse, T.W. Baumann, A. Slusarenko,

R.J. Aerts, D. Gisi, E. De Carolis,V. De Luca, T.W. Baumann, Methyl

Ramesh M, Ahlawat P, Srinivas NR: Irinotecan and its active metabolite, SN-38: review of bioanalytical methods and recent update from clinical pharmacology perspectives. Biomed Chromatogr. 2010 Jan;24(1):104-23. Pubmed *roseus* seedlings, Phytochemistry 42 (1996) 417–422.

Rubin E, Pantazis P, Bharti A, et al. Identification of a mutant human topoisomerase I with intact catalytic activity and resistance to 9-nitro-camptothecin. *J Biol Chem.* 1994; **269**:2433–2439.

Signalling molecules and the synthesis of alkaloids in *Catharanthus*

topoisomerase inhibitor. AIDS Res. Hum. Retroviruses 7, 65–72.

virus (HIV-1) replication in vitro by non-cytotoxic doses of camptothecin, a

Wall, Monroe E. "Discovery of Camptothecin and Taxol - National Historic Chemical Landmark - American Chemical Society." N.p., Apr. 2003. Web. 16 June 2015.

Wiseman, L.R., Markham, A., 1996. Irinotecan: a review of its pharmacological properties and clinical efficacy in the management of advanced colorectal cancer. Drugs 52, 606–623.

ARTIFICIAL NEST PREDATION ON A NON-URBAN TO URBAN SCALE

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Abstract

Nest predation is a major influence on the nesting and population success of songbird species. Investigating whether or not nest predation rates change from non-urban to urban areas may provide insight on the effect of urbanization on songbird biodiversity. This is especially interesting in the context of rapidly urbanizing China, where certain outcomes of such rushed development have yet to manifest or be understood. Predation rates on artificial nests in Nanjing, China were monitored across urban, semi-urban, and forested habitats to determine the potential impact of urbanization. The study yielded limited preliminary data

($n \leq 6$; $\chi^2 = 1.31$; d.f. = 2; $p = 0.51$) nonetheless indicative of a decrease in nest predation rates from the forested to urban habitat, in particular a decrease in avian predation. A comparison of study results with a previous bird survey of the sampling sites introduced a potential correlation between nest predation rates and avian predator biodiversity. It is believed that an effect is present, although continued surveys will be needed to substantiate this belief.

Keywords: *Urban ecology . Urban gradient . Nest predation . Artificial nests*

Introduction

Urbanization dramatically transforms landscapes, with great effect on existing ecosystems and the population structures within. This is especially true in areas that have experienced rapid development, such as many cities in China. Certain changes wrought by rapid urbanization, such as air and water pollution, have direct effects on people and invoke public calls for responsible action. Impacts on surrounding ecosystems, however, are not always as apparent, but still equally important to understand.

One area that receives significant attention in urban ecology is songbird biodiversity. The ability of different species to adapt and survive in urban environments is influenced by a variety of factors. Prior studies have investigated the effects of changes in predator assemblages (Major et al., 1996), predation rates (Jokimaki & Huhta, 2000; Gering et al., 1999), vegetation cover (Borgmann & Rodewald, 2004) and competition with new assemblages of species, native and introduced (Major et al., 1996).

Many of these studies, and others outside of the focus on urbanization, agree that nest predation is an important influence on the nesting and population success (Lack 1954, Martin 1992) of songbirds. As a fundamental mechanism in the structure of natural communities (Shochat et al. 2006), predation exerts top-down control on population sizes of prey species with a cascading catalog of related factors (Fischer et al. 2012). Predation on eggs and nestlings of passerines limits potential nesting success and has been shown to influence nest-site suitability and selection (Martin 1988a,), and subsequently, avian population density (Fretwell 1972), community structure (Lahti, 2001), and life history traits (Martin 1988b, Martin 1995).

A common hypothesis on predation rates over changing landscapes is the “edge effect” hypothesis. The hypothesis purports that proximity to habitat edges has an effect on nest

predation rates due to prior "edge effects" on bird nesting density and the idea that nest predation is nesting density dependent (Lahti, 2001). While it is commonly acknowledged that the "edge effect" is not very well understood due to contradictory implications by different studies (Lahti, 2001; Hartley & Hunter, 1998), a salient possibility presented by existing research is that predation rates increase at habitat edges, particularly at edges where landscapes are fragmented or deforested (Hartley & Hunter, 1998; Matthews, Dickman & Major, 1999).

The "edge effect" hypothesis suggests an impact due to the presence of a habitat edge, but in many cases attention is not given to the type of habitats at edges. The smaller subset of studies investigating forest-to-urban edge effects presents a different array of evidence suggesting alternately increases (Jokimaki & Huhta, 2000; Borgmann & Rodewald, 2004; Lopez et al., 2009) or decreases (Gering & Blair, 1999; Tomialojc, 1982) in nest predation pressure along an urban gradient. The hypothesis supporting decreasing predation pressure is in line with a larger overall theory of "urban predation relaxation" (Fischer et al., 2012). Possible reasons for this could be that the urban habitat is less suitable for predators, that predator diets may be subsidized by anthropogenic sources such as garbage or animal feed, and that changes in prey assemblages may cause different predation patterns.

This is important as these studies focus on the urban habitat and the influence of the urban ecosystem rather than or in addition to the natural habitat edge as factors affecting nest predation. It is this information that informs this study's hypothesis that predation pressure will decrease in increasingly urban areas.

A popular method used to measure nest predation has been the artificial nest method, where predation rates are measured not by monitoring actual nests but using artificial nests put out by scientists, typically with quail eggs. This is the method chosen to investigate nest

predation rates on small songbirds in Nanjing, China in this study. While it has its drawbacks and does not reflect real predation rates, studies done with both artificial and actual nests have found the method to be a good indicator of nest predation rates, and so the method remains popular for its relative effectiveness, ease, and controllability (Wilson et al., 1998; Zanette, 2002).

Materials and Methods

Study area

The study was conducted in the city of Nanjing in late June-July of 2015. Nanjing has a population of over 8 million, an area of 6598 km2 and is located in the Yangtze River Delta, east of the river and the Ningzhen mountain range. The city has a humid subtropical climate and experiences the East Asian tropical monsoon, with hot, cloudy weather and frequent rain for the duration of the study. As a historical capital of China and a cultural and industrial hub today, Nanjing is a very well established urban area.

The urban gradient

Three habitat locations were selected to represent urban, semi-urban, and forested areas on an urban to non-urban gradient. Purple Mountain, Nanjing Forestry University's campus, and Xinjiekou, a shopping district, were selected as the forested, semi-urban, and forested areas respectively. Selection was based on the relative densities of built cover and vegetative cover, with consideration of local anthropogenic effects such as density of human population, foot traffic, noise and light pollution on an observational basis.

To ensure our chosen locations fit our classifications, a basic habitat assessment was conducted to characterize the areas. The percentage of built and vegetative cover within a 50 m radius at each sampling site was calculated using satellite images based on the method used by Lopez-Flores et al., 2009. In addition, canopy cover in the immediate vicinity (within 2-3 m of

where nests were placed) was measured at each site using a spherical densiometer. At semi-urban and urban sites, the distance to the nearest building and road were estimated as well. Figures were then averaged for all habitats for overall comparison.

Artificial nests

In each habitat, nests were placed at six sample sites roughly 100 m apart to ensure that predation events were isolated. Distance was approximated using footsteps as well as GPS coordinates. Two nests were placed at each site, one on the ground (below 1 m) and one in a tree (between 2 and 3 m). Care was taken, particularly in the urban area, to place nests where it was considered actual birds might nest, such as under thick but reasonably accessible shrub and leaf cover. Two quail eggs were placed in each nest using gloves to reduce a human smell that might deter potential predators.

Nests were left out for 15 days, similar in time to a nesting period, and checked every 3 to 5 days, weather permitting. In addition, two motion-activated cameras were used to monitor one tree nest and one ground nest on Purple Mountain, where the most predation was expected. A nest was considered predated if the eggs had been disturbed in any way, including removal from the nest.

Results

3.1 Habitat assessment

As expected, some habitat characteristics varied between environments (Table 1). There was a clear decrease in vegetative cover from the forested to urban area with a corresponding increase in man-made, built cover. Canopy cover was relatively consistent, staying within a 16% range, though it pertained more specifically to the nest placement sites than the area as a whole. For example, measurements taken in the urban area would be representative of the canopy cover

within small, isolated areas designated for plants in the city ideal for nest placement (10-15 m^2 in area at best), rather than the area as a whole. Within these measurements, the forested area had the most cover, while the semi-urban area had the least. These measurements, being site-specific, may reflect a certain bias as to what was deemed a suitable nest site. Between the semi-urban and urban areas, the average distance of the nesting sites to the nearest building or road did not differ much, with the semi-urban sites having slightly more distance than the urban sites.

Table 1.

	Type of Cover			Avg. Distance to Nearest	
	Vegetative	**Built**	**Canopy Cover**	**Road**	**Building**
Urban	13.1%	86.9%	87%	8.0 m	15.2 m
Semi-urban	48.2%	51.8%	78%	8.3 m	18.5 m
Forested	95.9%	4.1%	94%	N/A	N/A

Nest Predation

Relatively low predation rates on the small sample size produced limited data, so that the figures used in analysis were low and comparison difficult due to the large factor of chance in any differences between habitats. However, it is believed that our figures are sufficient as preliminary data that may guide further investigation.

Our results showed that nest predation rates differed between forested and other areas (Chart 1). Twice as many nests were depredated in the forested habitat than in the semi-urban and urban areas respectively, which showed no difference. This observation supports the

hypothesis that predation pressure is decreased in urban areas. There was no indication as to why semi-urban and urban predation rates did not differ.

However, these are solely observations as data was not statistically significant (Table 2, $\chi^2 = 1.31$; d.f. = 2; $p = 0.51$). As noted, due to the small figures used ($n \leq 6$ for disturbed nests) there is a large possibility for the role of chance in our results and that our observations may not indicate a true effect.

Chart 1. Nest Predation Rates

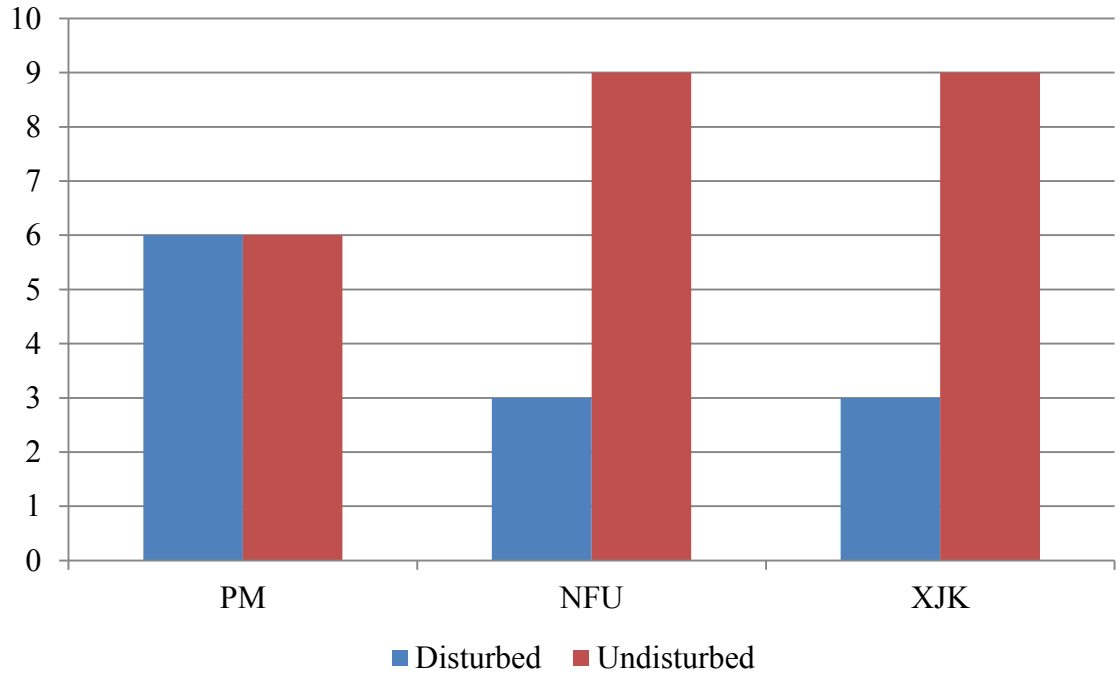


Table 2.

	PM	NFU	XJK
Disturbed	6	3	3
Undisturbed	6	9	9

Survivorship	0.50	0.25	0.25

A comparison of predation on ground and tree nests shows that ground nests were depredated in all habitats in approximately equal numbers (Chart 2, Table 3). One ground nest in the urban area showed evidence of disturbance by a large animal such as a dog, with the nest turned over and surrounding shrub trampled and broken. One in the urban area and one in the semi-urban area had eggs that were removed from the nest, but not taken as they were located within one meter of the nest. These were counted as predation events as the nest had been discovered and disturbed by a potential predator. All other events on ground nests had both eggs taken from the nest, with no broken eggshells or other evidence.

Predated tree nests showed a distinct decrease from the forested to urban habitat, with none occurring in the urban area, suggesting a decrease in avian predation in urban areas. All tree nest predation events bar one were cases of both eggs being taken from the nest, with no eggshells found, and with the exception of one nest having only one egg taken. Two predated nests were found disturbed from the original placement, although this could be attributed to heavy wind and rain.

Due to the extremely limited sample size (all $n \leq 3$), statistical analysis was foregone for this data. Again, the suggestion of decreased predation pressure in urban areas should be taken only as a note due to the lack of supporting data.

Chart 2. Number of predated nests

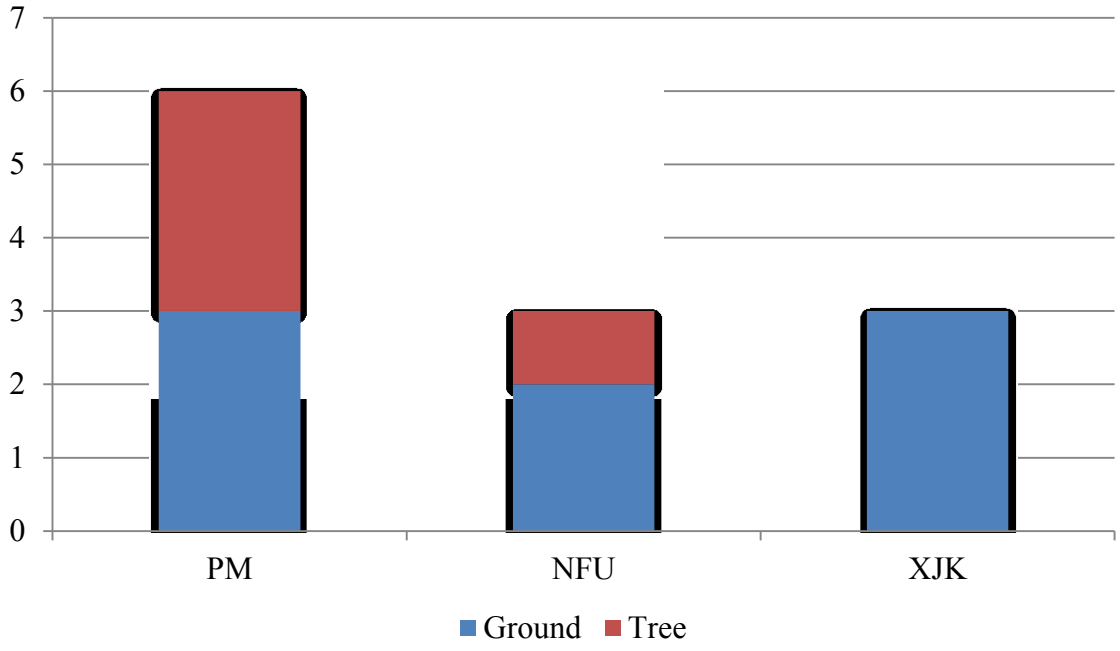
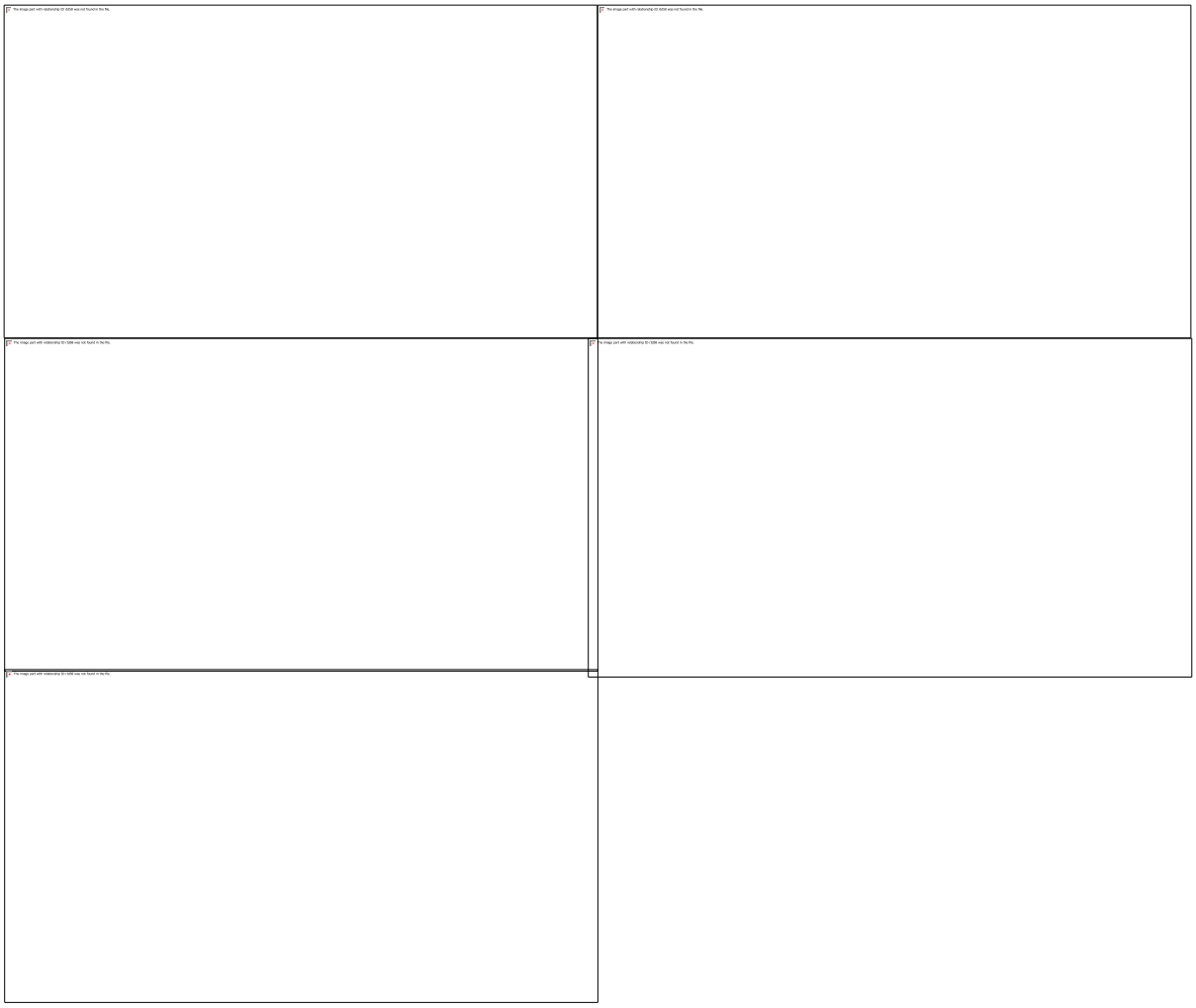


Table 3.

	PM	NFU	XJK
Ground	3	2	3
Tree	3	1	0

Additionally, one predation event on Purple Mountain was captured by the motion activated camera. Images showed the taking of both eggs by one bird as well as curious investigation of the nest by two birds before the event and one after (select images shown below in chronological order). These images confirm the presence of avian predators.



Discussion

As mentioned, the small sample size was a strong limiting factor in this study and results yielded very little conclusive evidence. However, the data is enough to prompt speculation for potential trends that may be more thoroughly investigated in the future. These speculations include:

- A decrease in predation pressure from forested to urban areas, as originally hypothesized and indicated by preliminary data
- A decrease in avian predation from forested to urban areas

- Any possible effect on ground-level nest predation that went unnoticed

A study on the sample sites conducted in 2013 (Drucker, J. & Griffith, C., unpublished data) was used to identify potential avian predators in each habitat (Table 4). The study surveyed bird species in a number of habitats around Nanjing, of which “urban”, “park”, and “forest” habitats were chosen to represent the urban, semi-urban, and forested habitats used in this study. Medium-sized, tree-nesting birds were chosen from the species list as potential predators. In addition to birds, other potential predators seen while monitoring the nests include cats, dogs, rats, squirrels, and humans.

Table 4.

Species	**Forest**	**Park**	**Urban**
Black-billed Magpie	44	17	44
White-cheeked Starling	14	5	27
Myna	7	14	14
Masked Laughingthrush	14	12	7
Azure- winged magpie	6	2	13
Great Tit	20	3	2
Indian Cuckoo	0	0	0
Common Koel	0	3	7
Ashy Drongo	7	0	0
Common Pheasant	8	0	0
Red-billed Blue Magpie	2	1	1
Long-tailed Shrike	2	0	0
Black Bulbul	2	1	1
Swinhoe's Minivet	3	2	0
Eurasian Cuckoo	3	0	0
Hwamei	2	0	0
Hair-crested Drongo	0	0	1
Total	**134**	**60**	**117**
Species present	**14**	**10**	**9**

The survey showed a decrease in predator populations from the forested to semi-urban area, as expected based off our hypothesis of decreased predation rates, but also showed that the

urban area had nearly as many predators as the forested area. However, a species count shows higher biodiversity in the forested area with roughly equivalent biodiversity in the semi-urban and urban areas, correlating more closely to predation data. This may suggest that the abundance or type of predator species present plays a larger role than overall predator population.

Other factors that may have influenced results must also be discussed. The period during which the study was conducted was not the regular nesting period of songbirds, so predators may not have been looking for nests. Additionally, it was the rainy season, and Nanjing experienced heavy rain for 9 out of 15 days that the study was conducted, which may have affected predation rates. For avian predators that rely heavily on sight to find nests, the artificial nests had no parents flying in and out of nests for potential predators to look for. And, as previously mentioned, there may also have been a deterrent human smell on the eggs, although this is unlikely due to the rain, especially for nests located in urban and semi-urban areas.

There is also some debate over the effectiveness of quail eggs in measuring predation rates. Studies have shown that quail eggs, which are larger than the eggs of certain passerines, do not show predation by smaller predators such as squirrels for whom the eggs are too large to eat (Haskell, 1995a; Maier and DeGraaf, 2000). There is counter-evidence for these, however, by researchers who have shown that small predators will break or take away eggs that are too large, or alternately, that even if they are too large to be eaten, the eggs will still attract predators which can be detected using clay eggs or cameras (Craig, 1998; Haskell, 1995b). Despite the defense, this potential shortcoming of the method is useful to keep in mind when evaluating results.

Conclusions

Our data recommends further research into the hypothesis that urban areas correlate with a decrease in nest predation pressure. A continuing study would benefit from a larger sample size

at least twice what was used in this study. In addition, a survey on the biodiversity and population sizes of potential predators as they correlate to nest predation rates would be an interesting supplement. A change in the methodology that might help this idea would be to use clay eggs, which can help distinguish by marks left on predated eggs.

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Literature Cited

Borgmann, K. & Rodewald, A. (2004) Nest predation in an urbanizing landscape: the role of exotic shrubs. Ecological Applications, 14(6): 1757-1765.

Craig, D. P. (1998) Chipmunks Use Leverage to Eat Oversized Eggs: Support for the Use of Quail Eggs in Artificial Nest Studies. The Auk, 115(2): 486-489.

Fischer, J. D., Cleeton, S. H., Lyons, T. P., & Miller, J. R. (2012) Urbanization and the Predation Paradox: The Role of Trophic Dynamics in Structuring Vertebrate Communities. Bioscience, 62(9): 809-818.

Fretwell, S.D. (1972) Populations in a Seasonal Environment. Princeton University Press, Princeton, NJ.

Gering, J. C. & Blair, R. B. (1999) Predation on artificial bird nests along an urban gradient: predatory risk or relaxation in urban environments? Ecography, 22: 532-541.

Hartley, M., & Hunter, M. (1998) A Meta-Analysis of Forest Cover, Edge Effects, and Artificial Nest Predation Rates. Conservation Biology, 12(2): 465-469.

Haskell, D. (1995a) Forest Fragmentation and Nest Predation: Are Experiments with Japanese Quail Eggs Misleading? The Auk, 112(3): 767-770.

Haskell, D. (1995b) A Reevaluation of the Effects of Forest Fragmentation on Rates of Bird-Nest Predation. Conservation Biology, 9(5): 1316-1318.

Jokimäki, J. & Huhta , E. (2000) Artificial nest predation and abundance of birds along an urban gradient. The Condor, 102(4): 838-847.

Lack, D. (1954) The Natural Regulation of Animal Numbers. Clarendon Press, Oxford.

Lahti, D.C. (2001) The “edge effect on nest predation” hypothesis after twenty years. Biological Conservation, 99: 365-374.

López-Flores, V. et al. (2009) Artificial nest predation along a Neotropical urban gradient, Landscape and Urban Planning, 92: 90–95.

Maier, T. J., & DeGraaf, R. M. (2000) Predation on Japanese Quail v. House Sparrow eggs in artificial nests: small eggs reveal small predators. The Condor, 102(2): 325-332.

Major, R. E. et al. (1996) Nest predation in Australian urban environments and the role of the pied currawong, Strepera graculina. Australian Journal of Ecology, 21: 399-409.

Martin, T. E. (1988a) Habitat and area effects on forest bird assemblages: is nest predation an influence? Ecology, 69; 74-84.

Martin, T. E. (1988b) Processes organizing open-nesting bird assemblages: competition or nest predation? Evolutionary Ecology, 2; 37-50.

Martin, T. E. (1992) Breeding productivity considerations: what are the appropriate habitat features for management? In: Hagan, J. M. I., Johnston, D. W. (Eds.) Ecology and Conservation of Neotropical Migrant Landbirds. Smithsonian Institution Press, Washington, D. C. 455-473.

Martin, T. E. (1995) Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monographs, 65: 101-127.

Matthews, A., Dickman, C. R., & Major, R. E. (1999) The influence of fragment size and edge on nest predation in urban bushland. Ecography, 22: 349-356.

Shochat et al. (2006) From patterns to emerging processes in mechanistic urban ecology. Trends in Ecology and Evolution, 21(4): 186-191.

Wilson, G. R. et al. (1998) How well do artificial nests estimate success of real nests? The Condor, 100: 357-364.

Zanette, L. (2002) What do artificial nests tells us about nest predation? Biological Conservation, 103: 323–329.

IDENTIFICATION AND DISPERSAL ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) PRESENT IN URBAN FOREST SOILS OF NANJING, CHINA

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Abstract

Identification and analysis of four major polycyclic aromatic hydrocarbons (PAHs) dispersed throughout the soils of major urban areas within Nanjing, China. Data was collected from 180 soil samples from four major land use zones. The zones displaying the greatest average concentration of ∑PAHs decreased in value as follows: University campus zone (258.75 μg·kg-1)>Traffic green belt zone (240.71 μg·kg-1)>Forest park zone (226.3 μg·kg-1)>Residential areas (218.45μg·kg-1). All zones were classified as weakly contaminated based upon the Maliszewska-Kordibach method for classification of contaminated soil. The finding of PAHs in Nanjing's urban soils presents a significant source of pollutants being input into the environment. The findings of the research conducted within this experiment can be applied to the field of human and environmental health.

Keywords: *Polycyclic aromatic hydrocarbons, Nanjing, soil pollution, urban soil*

Introduction

Polycyclic aromatic hydrocarbons are aromatic hydrocarbons with two or more fused benzene rings that occur as colorless, white/pale yellow solids; they are characterized by low solubility in water, high melting and boiling points and low vapor pressure. [5] Polycyclic aromatic hydrocarbons (PAHs) are formed during the thermal decomposition of organic molecules and their subsequent recombination. Incomplete combustion of organic molecules at high temperatures (500-800C) or subjection of organic material at low temperature (100-300C) for extended periods of time result in the formation of PAHs and their subsequent release into the atmosphere. The sources of PAHs are both natural and anthropogenic. Natural sources include forest and rangeland fires, oil seeps, volcanic eruptions and exudates from trees. Anthropogenic sources of PAH are the burning of fossil fuels, coal, tar, wood, garbage, refuse, used oil municipal solid waste incineration and petroleum spill and discharge.[5] PAHs also increase when organic materials like coal, diesel, wood and vegetation are burned and the burning process is not complete. [7]

Industrialization

Population growth and rapid economic growth in recent decades led to a rapid increase in energy consumption in China. As a result, China became the largest coal consumer and is among the largest petroleum users in the world. Annual consumption of coal and petroleum in China reached 1660 million and 287 million tons in 2003, respectively. In addition, biomass burning is a traditional practice in rural China, and it was estimated that approximately 537 million tons of biomass were burned either as fuel or in the field in 2003. [10] The economy of Nanjing is mainly based on the “five pillar industries” including electronics, cars, petrochemicals, iron and steel, and power. Of these industries, the coking industry, also known as metallurgical coal

production, is of significant importance. Global steel production is dependent on coal. Seventy percent of the steel produced today uses coal. Metallurgical coal is a vital ingredient in the steel making process. The process involves carbonization of coal to high temperatures (1,100°C) in an oxygen deficient atmosphere in order to concentrate the carbon utilized in the process. Metallurgical coal is produced by the destructive distillation of coal in coke ovens. Prepared coal is heated in an oxygen-free atmosphere until most volatile components in the coal are removed. The material remaining is a carbon mass called coke. Coke is used in iron and steel industry processes to reduce iron ore to iron. Over 90 percent of the total coke production is dedicated to blast furnace operations. Most coke plants are collocated with iron and steel production facilities, and the demand for coke generally corresponds with the production of iron and steel.

The concerns surrounding the output of PAHs increase as China progresses in its current path of development and industrial expansion. Emission of 16 polycyclic aromatic hydrocarbons (PAHs) listed as United States Environmental Protection Agency (USEPA) priority pollutants from major sources in China were compiled. Geographical distribution and temporal change of the PAH emission, as well as emission profiles, are discussed. It was estimated that the total PAH emission in China was 25 300 tons in 2003. The emission profile featured a relatively higher portion of high molecular weight (HMW) species with carcinogenic potential due to large contributions of domestic coal and coking industry. Among various sources, biomass burning, domestic coal combustion, and coking industry contributed 60%, 20%, and 16% of the total emission, respectively. Total emission, emission density, emission intensity, and emission per capita showed geographical variations. In general, the southeastern provinces were characterized by higher emission density while those in western and northern China featured higher emission intensity and population-normalized emission. Although energy consumption in China went up

continuously during the past two decades, annual emission of PAHs fluctuated depending on the amount of domestic coal consumption, coke production, and the efficiency of energy utilization. [10] It is necessary to understand the full range of effects PAH pollution presents to the environmental system. [3]

Urbanization

As a result of widespread application in practice, PAHs are globally distributed and the highest concentrations generally occur within close proximity to urban centers. With an increase in industrial activity within China, there has been a major increase in amounts of commuter traffic. Although traffic is mainly dependent on railway in China, highways are being built to meet the needs of economic development. As the economy expands, the need for readily accessible and reliable mobility grows. Highway development has become one of the key projects of infrastructure development. Analyses of soil samples collected near highways and further investigations show that traffic, most specifically on highways, is the major pollution source in urban and industrial areas. [3] The exhaust of motor vehicles used for transportation release emissions of PAHs into roadside environments. Due to their low vapor pressure, most of the PAHs do not rise, but immediately condense and are absorbed by other particulates which then settle on earth as deposits of the soil or even directly absorb into the soil. [3]

Soil Pollution/Environment

PAHs infiltrate the soil as micropollutants which are resistant to environmental degradation largely due to their highly hydrophobic nature. Certain PAHs move through the soil to contaminate underground water resources. [8] PAHs in soils can be dispersed by surface runoff and dust production; soils can therefore be considered as one of the pollution sources for PAH contamination in air and sediments. Soil types and properties such as organic carbon play

the most important role in the adsorption of PAHs in soils. [9] PAHs are pollutants which present a formidable threat to soil health in characteristics such as resistance towards biodegradation in natural conditions, the tendency to remain present in the environment for long periods of time, and the potential to bio-accumulate [7] Though they are the chief pollutants of air, soil acts as the ultimate depository of these chemicals. Their fate in the environment includes volatilization, photo-oxidation, chemical oxidation, adsorption on soil particles, leaching and microbial degradation. [5] Because individual PAHs vary in behavior it can be difficult to combat their presence in sediment. [7] Concerns over their present and potential long term effects have resulted in studies centered in remediation methods to combat PAH soil contamination. [4]

Human Health

Due to potentially harmful effects to human health, the occurrence and distribution of PAHs is important. [1] Soil contains measurable amounts of PAHs, primarily from airborne fallout. Documented levels of PAHs in soil near oil refineries have been reported as high as 200,000 micrograms per kilogram (μg/kg) of dried soil. Levels in soil samples obtained near cities and areas with heavy traffic were typically less than 2,000 μg/kg. [8] Further research on the toxicity of PAHs has resulted in classification as having toxic, mutagenic and carcinogenic properties. [5] Benzo[a]pyrene is recognized as a priority pollutant by the US Environmental Protection Agency as this compound is known to be one of the most potently carcinogenic of all known PAHs. [1] Exposure to soil contaminated with PAH can occur through direct and indirect contact. The major route of exposure is dermal in both occupational and non-occupational settings as highlighted by a study of 12 coke-oven workers. [8] An estimated 75% of the total absorbed amount of PAHs entered the body through the skin, highlighting this as a major exposure route of PAHs. The rapid absorption of PAHs by humans results in a high potential for

biomagnification in the food chain. [1] Consumption of food grown in contaminated soil, soil, or dust particles that contain PAHs are routes for these chemicals to enter your body, though absorption is generally slower when PAHs through ingestion. [8] PAHs have been shown to cause tumors in laboratory animals through food, contaminated air, and dermally. When pregnant mice consumed high doses of a PAH (benzo(a)pyrene) they experienced reproductive problems. In addition, the offspring of the pregnant mice showed birth defects and a decrease in body weight. Other effects include damage to the skin, body fluids, and the immune system. However, these effects have not been seen in humans. [7]

Past Studies and the Context of this Study

A past study conducted in Beijing found anthropogenic activities to be the main sources of PAHs, the levels of PAHs in urban soils are approximately a factor of 2–10 higher than those in rural areas. The study included an extensive and systematic survey has been undertaken to evaluate the contamination with PAHs of urban soils in Beijing, China. Soil samples were collected from campuses of universities, schools and kindergartens, public squares, fallow land and roadsides, and were analyzed for 16 PAHs by GC–MS. There was a high variability in the total PAHs (∑PAHs) concentrations, ranging from less than 366 to 27,825 ng g− 1. The highest ∑PAHs concentrations were found at roadsides and industrial sites. Soil organic carbon is an important factors that can influence the concentrations of PAHs in soils. The ∑PAHs were found to be significantly correlated to the soil organic carbon. To trace the sources of PAHs, the ratios of phenanthrene to anthracene and fluoranthene to pyrene were used to identify pyrogenic and petrogenic sources, respectively. In most cases, PAHs in soils in urban areas of Beijing were pyrogenic. These sources included motor vehicle exhausts, industrial activities and coal burning. [9]

The objective of this study is to investigate and quantify the pollution of PAHs in soil sediments along four key topographic areas in Nanjing, China. Four functional urban soil sample zones were examined including (1) park area, (2) residential area, (3) traffic area, and (4) campus area. The central hypothesis of the study expected to find the highest amount in urban forest soils located within the traffic district area due to the large impacts that urban activity has been found to cause in soils. It is expected that the campus, park, and residential areas will each contain decreasing quantities of PAHs present. Using these sample zones, the study seeks to identify PAHs and obtain quantifiable data reflecting PAH concentrations in Nanjing urban soils that is to be used to determine the distribution of PAHs across the various locations and identify PAH sources and assess the threat to environmental and human health.

Methods

Area of Study

Nanjing, China is the capital city of the Jiangsu province about 560 miles (900km) southeast of Beijing, the national capital. The total area of Nanjing is 6,582 km^2 and it is located on the coast of the Yangtze River in east-central China. As of 2010, the total urban population averages 6.5 million. As an important industrial, manufacturing, and transportation center, Nanjing faces not only exponential increases in productivity, but also large increases in pollution rates. For 2011, Nanjing's figure for coal consumption totaled 2.8 x 10^7 tons, which accounted for 80.5% of the city's total energy consumption. Demands upon transportation have caused the local vehicular fleet to increase by an annual rate of more than 20% at an average of 1.4 million. According to the Ministry of Environmental Protection's findings for the year 2013, Nanjing was ranked number 24 in Chinese cities categorized by levels of PM2.5 air pollution. Annually, Nanjing's average PM2.5 level in micrograms per cubic meter was 75.3. The average of the

maximum daily PM2.5 level in micrograms per cubic meter was 312. The national standard for the average annual PM2.5 air pollution concentration is 35 micrograms per cubic meter. This data reveals that Nanjing fails to reach the standard. 92% of the 74 Chinese cities from which data was collected also failed the standard. [11]

Description of four functional urban soil sample zones

Sample zone (1) Park Area

Samples were collected from a local recreational park in Nanjing. The land use of this area is generally maintained for foot traffic.

Sample zone (2) Residential Area

Samples were collected from the neighborhood communities.

Sample zone (3) Traffic Area

Samples were collected from the traffic green belt of one of Nanjing's major urban areas, which is exposed to high amounts of commuter travel daily.

Sample zone (4) Campus Area

Samples were collected from a site located on the Nanjing Forestry University campus where general land use is to contribute to campus aesthetics.

Soil Sampling.

Collection took place over a total area of 222 km^2 within Nanjing's main city region. The total area was divided into 180 cells (Dimensions: 1 km x 1 km) from which between 3 to 6 vertical topsoil samples were taken between the depths of 0 to 10 cm. The soil samples taken were placed in paper bags for storage and transport back to the university laboratory where they were dried at room temperature (20 °C) for 3 days. To prepare for analysis, soils were sieved to remove debris and eliminate interference with data collection.

Laboratory Procedure. Of the 16 US EPA standard solutions (200 ug/mL) for PAH, 4 of the most common PAHs were used in the duration of this study. These included anthracene (Ant), pyrene (Pyr), chrysene (Chr), and benzo[a]pyrene (BaP).

The Fan et al. (2008) and Sun et al. (2012) protocols for the extraction, purification and determination of PAHs in soil were followed. The steps taken within the context of this experiment are as follows: 1) Each 5.0 g soil sample was mixed into solution with 20 ml of dichloromethane. 2) A 1 hour extraction by sonication in an ultrasonic bath (-40) was then performed. 3) The solution is dehydrated with anhydrous Na_2SO_4. 4) Using a rotary evaporator it is then reduced to a concentration of 2ml. 5) It is concentrated further using a purified nitrogen stream as a drying method. 6) Finally it is dissolved in 2ml methanol.

A Water Model e2615 high performance liquid chromatograph and a Waters Model 2998 wavelength 254 nm PDA detector were used to analyze PAH values. The specifications of the stationary phase used were a Waters issued symmetric C18 column. A solution of 85 to 15 methanol and water with a flow rate of 1 ml/min was used as the mobile phase at an injection volume of 20 ul. Quality control measures were taken as follows: analyses of sample blanks, use of reference materials and utilization of spiked samples**.** The correlation coefficients for calibration curves of PAHs were all greater than 0.9999. The range for the recovery of the PAHs in the soil was between 76% and 100% of the certified values. The range for the recovery in the solid phase was between 75% and 100%. A matrix spike (200 $ng \cdot g^{-}1$) per PAH, laboratory control methods, and duplicate samples were tested alongside the procedure of determination's main experimental pool to ensure quality control and assurance. For each PAH, the limit of detection (LOD) was calculated as the standard deviation of the six concentrations of uncontaminated, duplicate sample.

PAH Data Analysis. The software utilized in the description of variables and experimental principal component analysis was Windows version 19.0 IBM SPSS Statistics. Visual displays such as graphs were created using Microsoft Excel 2007.

Results

The original hypothesis of this experiment stating that the largest total concentration of PAHs would be found in the traffic district was rejected. The hypothesis also projected that the campus zone, forest park zone, and the residential areas zone would each decrease in relation to one another by the order in which they are named. This was found to be partially correct. Both the forest park and residential areas were placed within the position stated by the hypothesis. It is supposed that the residential areas held the least amount of total PAH concentration due to increased construction and restorative initiatives currently taking place in the area studied.

Table 1 represents the concentrations of each of the key PAHs tested for and the total summation of concentrations (∑PAH) for four different urban areas in Nanjing. Of the 180 samples studied, 47 were collected from the forest park area, 20 were taken from residential areas, 103 were taken from the traffic green belt, and 10 were taken from the university campus area. The mean, range, standard deviation and frequency of detection of each compound is reported. The ∑PAH of the means decreased by zone as follows: university campus zone, traffic green belt zone, forest park zone, residential areas zone. Figure 1shows the differences in the total average concentrations for each compound and the zone from which it was collected. Figure 2 shows the total average concentrations for all four of the compounds and a comparison of these totals according to the zone from which it was collected.

Maliszewska-Kordibach published a set of criteria to evaluate the level of soil contamination in the agricultural soils of Poland. [13] The system classifies soils into levels of contamination using a range of values for the PAH concentrations. Values <200 are considered not contaminated, values between the range of 200-600 are weakly contaminated, values between the range of 600-1000 are contaminated, and values >1000 μg·kg are heavily contaminated. The method of classification when applied to the findings of this experiment show that each of the sample zones within Nanjing's main urban area contained soils that were weakly contaminated.

Table 1. Concentrations ($\mu g \cdot kg^{-1}$) of individual PAHs in different soil sample zones in urban Nanjing, China.

Zones	Individual PAH					
Forest Park (n=47)		**Ant**	**Pyr**	**Chr**	**BaP**	**∑PAHs**
	Mean	28.04	71.42	27.55	99.29	226.3
	Range	ND-79.02	16.67-347.40	4.37-83.19	13.74-344.12	34.78-853.73
	SD*	21.28	56.60	19.68	77.46	175.02
	FD**	33	43	43	43	
Residential Areas (n=20)	Mean	23.91	74.54	42.54	77.46	218.45
	Range	ND-58.05	20.24-174.73	7.95-445.34	29.88-174.25	58.07-852.37
	SD*	15.78	44.36	95.4	43.9	199.44
	FD**	12	20	20	20	
Traffic Green Belt (n=103)	Mean	17.77	92.68	28.83	101.43	240.71
	Range	ND-77.70	15.00-723.68	5.99-158.36	18.72-608.84	39.71-1568.58
	SD*	16.76	116.4	25.16	94.76	253.05
	FD**	79	103	103	103	
University Campus (n=10)	Mean	14.47	71.9	40.46	131.92	258.75
	Range	ND-44.82	34.24-184.47	3.79-216.07	17.66-742.78	55.69-1188.14
	SD*	14.86	49.08	63.32	217.06	344.32
	FD**	9	10	10	10	

* Standard Deviation **Frequency of Detection.

The study of contamination of PAHs in urban soils in Beijing, China provided the following results. The highest pollution levels were observed for soil samples in roadside showing heavy traffic. PAHs concentrations near some petrol stations were also high. These stations are always jammed with motor vehicles and close to industrial activities, which explained the relatively high PAHs concentrations at these sites. PAHs in soils collected in the study included compounds each of the 16 compounds with a wide range of molecular weights, from naphthalene to benzo(ghi)perylene. The study found the variation in percentages of PAH compounds with different ring numbers in soils. The sampling sites located in the main traffic areas, and the major organic pollutants are mostly dominated by PAHs with 3–5 rings, such as pyrene. Sampling sites that had much less anthropogenic activities, resulted in the dominance of PAHs with 2 rings. Generally speaking, the concentrations of PAHs with 6 rings were relatively low in most soil samples. [9]

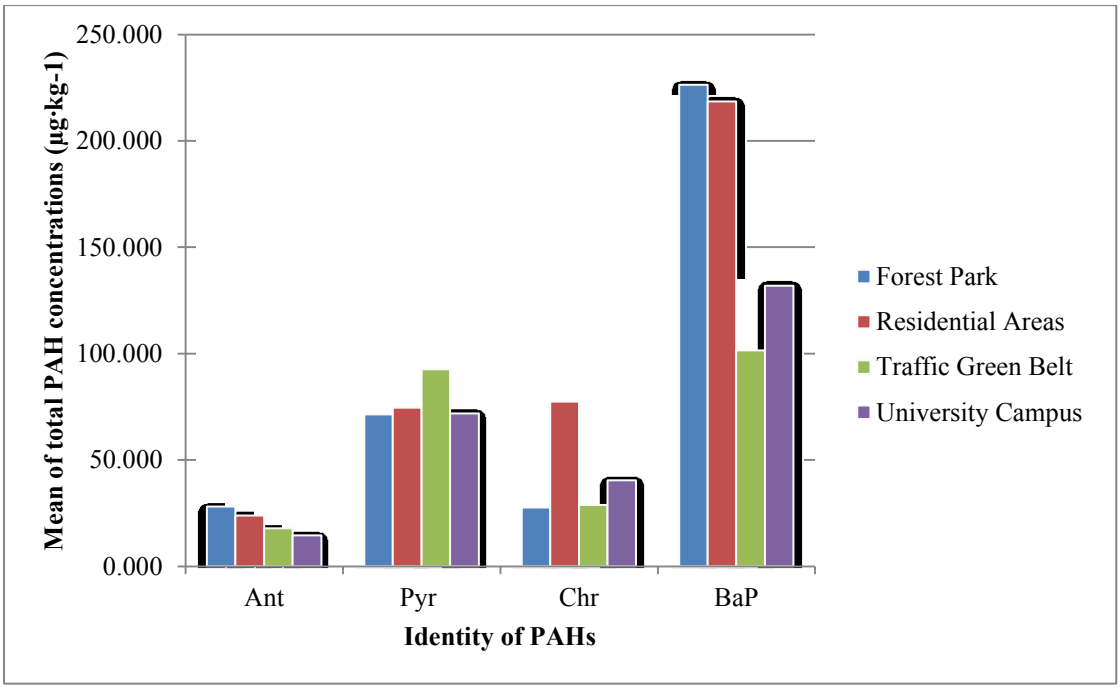

Figure 1. Comparison of average concentrations of PAHs found in four individual urban soil sample zones.

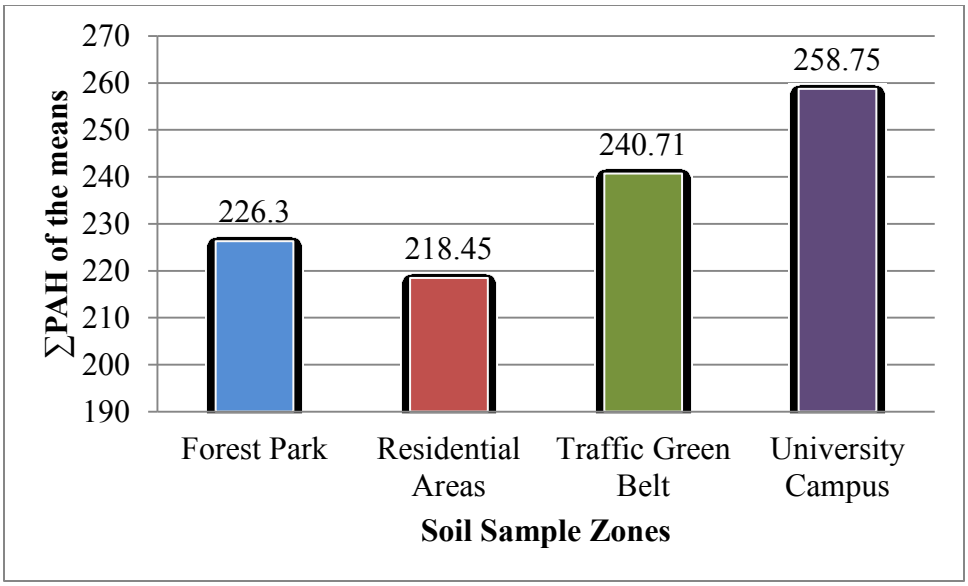

Figure 2. Average concentrations ($\mu g \cdot kg^{-1}$) for the ∑PAHs in different soil sample zones in urban Nanjing, China.

Conclusion

The findings of the research conducted within this experiment can be applied to the field of human and environmental health. The finding of PAHs in Nanjing's urban soils presents a significant source of pollutants being input into the environment. Due to the pollution, the soil health has been depleted and could pose dangers of exposure to workers and others who come into contact. The soil should not be utilized for agricultural purposes because of the danger of phyto-accumulation in crops.

The United States Environmental Protection Agency compiled a list of 16 polycyclic aromatic hydrocarbons that are released into the environment as pollutants. [7] Of the 16 listed, this study focused on 4 of the most known and potentially harmful compounds. In the event of further study, the presence and quantity of the remaining 12 compounds in the soil samples should be investigated and compiled with the data collected within this experiment.

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Literature Cited

Bamforth, S.M., and I. Singleton. 2005. Review Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. Journal of Chemical Technology and Biotechnology 80: 723–736Available at http://users.rowan.edu/~jahan/site_remediation/example paper.pdf (verified 2015).

Chiou, C.T., S.E. Mcgroddy, and D.E. Kile. 1998. Partition Characteristics of Polycyclic Aromatic Hydrocarbons on Soils and Sediments. Environmental Science & Technology Environ. Sci. Technol. 32(2): 264–269 Available at http://www.researchgate.net/profile/cary_chiou/publication/231290781_partition_characteristics_of_polycyclic_aromatic_hydrocarbons_on_soils_and_sediments/links/0c960524a0c862d6b2000000.pdf (verified 2015).

Chu, S.-G., H. Liu, L.-L. Ma, and X.-B. Xu. 2003. Polycyclic Aromatic Hydrocarbons in Soil Adjacent to Highways in Beijing, People's Republic of China. Bulletin of Environmental Contamination and Toxicology 70: 972–977Available at http://download.springer.com/static/pdf/695/art%3a10.1007%2fs00128-003-0077-2.pdf?originurl=http://link.springer.com/article/10.1007/s00128-003-0077-2&token2=exp=1434471106~acl=/static/pdf/695/art%253a10.1007%252fs00128-003-007 (verified 2015).

Gan, S., E.V. Lau, and H.K. Ng. 2009. Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). Journal of Hazardous Materials 172(2-3): 532–549Available at http://www.sciencedirect.com/science/article/pii/s0304389409012473 (verified 2015).

Haritash, A.K., and C.P. Kaushik. 2009. Biodegradation Aspects Of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Journal of Hazardous Materials 169(1-3): 1–15Available at http://www.researchgate.net/profile/ak_haritash/publication/24427524_biodegradation_aspects_of_polycyclic_aromatic_hydrocarbons_(pahs)_a_review/links/0912f513ad2cd7a51e000000.pdf (verified 2015).

Miguel, A.H., T.W. Kirchstetter, R.A. Harley, and S.V. Hering. 1998. On-Road Emissions Of Particulate Polycyclic Aromatic Hydrocarbons And Black Carbon From Gasoline And Diesel Vehicles. Environmental Science & Technology 32(4): 450–455Available at http://www.researchgate.net/profile/thomas_kirchstetter/publication/231290756_on-road_emissions_of_particulate_polycyclic_aromatic_hydrocarbons_and_black_carbon_from_gasoline_and_diesel_vehicles/links/00b4953177137509b7000000.pdf (verified 2015).

Polycyclic Aromatic Hydrocarbons (PAHs). 2008. Epa.govAvailable at http://www.epa.gov/osw/hazard/wastemin/minimize/factshts/pahs.pdf (verified 2015).

Polycyclic Aromatic Hydrocarbons (PAHs) ToxFAQs. 1996. atsdr.cdc.govAvailable at http://www.atsdr.cdc.gov/toxfaqs/tfacts69.pdf (verified 2015).

Tang, L., X.-Y. Tang, Z.-G. Zhu, M.-H. Zheng, and Q.-L. Miao. 2008. Contamination of polycyclic aromatic hydrocarbons (PAHs) in urban soils in Beijing, China. Environmental International 31(6): 822–828Available at http://www.sciencedirect.com/science/article/pii/s0160412005001017 (verified 2015).

Zhang, Y., S. Tao, J. Cao, and R.M. Coveney. 2007. Emission of Polycyclic Aromatic Hydrocarbons in China by County. Environmental Science & Technology Environ. Sci. Technol. 40(3): 683–687Available at http://www.researchgate.net/profile/raymond_coveney_jr/publication/6478725_emission_of_polycyclic_aromatic_hydrocarbons_in_china_by_county/links/02e7e5296277d7b6a5000000.pdf (verified 2015).

Tan, M. 2014. Bad to worse: ranking 74 Chinese cities by air pollution. Greenpeace East AsiaAvailable at http://www.greenpeace.org/eastasia/news/blog/bad-to-worse-ranking-74-chinese-cities-by-air/blog/48181/ (verified 2015).

Polycyclic Aromatic Hydrocarbons (PAHs). 2015. Cancer in Illinois: Risk Factors Available at http://www.idph.state.il.us/cancer/factsheets/polycyclicaromatichydrocarbons.htm (verified 2015).

B. Maliszewka-Kordybach, Polycyclic aromatic hydrocarbons in agricultural soils in Poland: preliminary proposals for criteria to evaluate the level of soil contamination, Appl. Geochem., 1996, 11, 121–127.

AN ANALYSIS OF CARBON ADDITION ON SOIL NITROGEN TRANSFORMATION IN JIANGSU PROVINCE, CHINA

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Abstract

To better understand the effects of carbon addition on soil nitrogen, a study of carbon addition on nitrogen transformation was conducted in Nanjing, China at Nanjing Forestry University. We studied soil rhizospheres of three different tree species; the Liriodendron chinense Sarg. (Chinese tulip tree), Lithocarpus glaber Thunb. (Japanese oak), and Cupressus funebris Endl. (Chinese weeping cypress). This experiment had three replications and nine dupilcations. This experiment consisted of four treatments: control (H20), glucose, citric acid, and glucose + citric acid. We measured the moisture content, soil pH, and inorganic nitrogen of the soil samples prior to and following the incubation. This incubation experiment occurred over a 7 day period. We hypothesized that carbon addition would stimulate more microbial activity and increase the availability of inorganic nitrogen. Instead, we found that the control treatment, H20, had the highest nitrification rate. This study also suggests that carbon addition could have potentially hindered the microbial processes such as nitrification and mineralization. Conclusively, this study should be continued. Future studies could possibly lead to agricultural and pharmaceutical breakthroughs.

Keywords: *Soil, Carbon, Nitrogen, Nitrogen Transformation, Trees, Microbes*

Introduction

Soil is a limited natural resource that blankets much of the Earth's land surface and is produced through a process known as erosion (Bezdicek et al., 1996). Erosion is the decay of rocks over time due to heat, wind, water, geological movement, freezing, and thawing (Scottish Natural Living Landscape, 2015). Within soil, there is a mixture of inorganic matter, such as weathered rock, air, and minerals, and organic matter, such as leaves, decomposing plants, bacteria, fungi, and air (Enhart, 2015). There are three types of soil; clay, silt, and sand (Scottlands Natural Heritage, 2015). Soil acts as a natural filter for water, and regulates the Earth's temperature (Scottlands Natural Heritage, 2015). Soil is also the primary growing medium for most plants (Enhart, 2015). And thus core provider of our food, antibiotics, and fossil fuel (Enhart, 2015). Soil also contributes to biodiversity for it is the home to millions of microorganisms (Roesch et al., 2007). Of the many biogeochemical cycles that occur in soil- such as the hydrologic cycle, the rock cycle, and the phosphorous cycle- the two most significant to this study are the carbon cycle and the nitrogen cycle.

The carbon cycle is the exchange of carbon between the atmosphere, biosphere, lithosphere, geosphere, and hydrosphere (Trudinger, 1979). A major part of the carbon cycle takes place in the soil system through photosynthesis, decomposition, and respiration (Ontl and Schulte, 2012). Soil contains 2500 GT of carbon making soil the second largest pool of carbon, globally. Of that 2500 GT, 1500 is organic and 950 is inorganic (Ontl and Schulte, 2012). Carbon is found abundantly in soil organic matter (Ontl and Schulte, 2012). Soil organic matter is composed of the soil microbial population, such as fungi and bacteria, and decaying materials, such as animal tissue and fecal matter (Ontl and Schulte, 2012). Soil organic carbon is the foundation of soil fertility and it contributes to plant growth (Ontl and Schulte, 2012).

Nitrogen gas accounts for approximately 80% of the Earth's atmosphere and is an essential element necessary for the survival of all organisms (Bernhard, 2010). The nitrogen cycle is a process in which atmospheric nitrogen is converted to nitrate (NH3-) or ammonium (NH4+). Atmospheric nitrogen exists in the soil system and is transformed as a result of several important biological processes carried out by the microbial community such as fixation, nitrification, and mineralization (Lamb, 2014). Nitrogen fixation occurs as bacteria transform, or "fix", nitrogen into NH3 or NH4+, a form that is usable by other organisms (Bernhard, 2010). Once the nitrogen is fixed it cannot revert to its previous state. After nitrogen has been fixed, oxidization occurs (the bacterium furthers the process) (Bernhard, 2010). Oxidation provides energy for this process to occur and this process is known as nitrification (Bernhard, 2010). During mineralization, a plant or animal dies and as they decompose, the organic nitrogen in their bodies is converted to plant-available inorganic forms (NH4+ and NO3-) (Bernhard, 2010). As mineralization occurs, the nitrogen is converted back to ammonium (Bernhard, 2010).

Nitrogen is an unreactive element and must often be transformed into other forms to be utilized by microbes and plants (Ontl and Schulte, 2012). The addition of carbon gives microbes the energy to transform nitrogen into usable forms (Bernhard, 2010). Microbes need nitrogen to form proteins, nucleic acids, and other cellular components (Robertson et al., 2007). Plants are able to use nitrogen, in the form of ammonium or nitrate, to develop DNA, enzymes, and proteins (Robertson et al., 2007). Studies indicate that nitrogen is so significant in soil, microbials and plants compete for it (Kaye and Hart, 1997).

This study will investigate the effects of carbon addition on soil nitrogen transformation in three forest types. We hypothesize that the addition of carbon will stimulate microbial activity and increase inorganic nitrogen.

Materials & Methods

Nanjing Forestry University's Plantation.

The significance level is 0.05. Soil was collected at Nanjing Forestry University's tree plantation on the main campus, from the rhizosphere of three tree species: *Liriodendron chinense* Sarg. (Chinese tulip tree), *Lithocarpus glaber* Thunb.(Japanese oak), and *Cupressus funebris* Endl.(Chinese weeping cypress). Soil samples were taken three times for each tree species. For each sample, moisture content, inorganic nitrogen concentration, and pH were determined. All nine samples were sieved, at 2 mm, and stored at 0 Celsius. The sieved soil was divided into 100 gram increments. Low molecular carbon (0.1 grams carbon per kilogram soil every 30 days) was added to soil containing one of three treatments: citric acid, glucose, and a mixture of citric acid and glucose (2:1). The incubation lasted 7 days. Soil was thoroughly mixed to ensure homogeny.

Moisture Content.

Moisture content was determined using a dry oven. The soil was weighed. Both the sample and the container were placed in the dry oven at 70° C. The samples were placed in a box with a water absorber until the soil cooled. The sample was again weighed.

Moisture of soil = (A-B)/(B-C)×100%

pH.

To determine pH, 8 grams per soil and mixed with a glass rod for 60 seconds in a measuring beaker. The samples were then placed on a table for 30 minutes to allow the solution to settle. A digital pH meter, model PHS-25, (China) was used to determine the pH of the soil samples.

Inorganic Nitrogen.

To determine inorganic nitrogen content, an Auto-Analysis 3 (Germany) was used to test the soil. Potassium chloride (25 ml) was added to 5 grams of soil from each soil sample. The samples were then placed into a. shaker with 180 rpm for 1 hour. Inorganic nitrogen was extracted by adding 2 moles of KCL.

Results

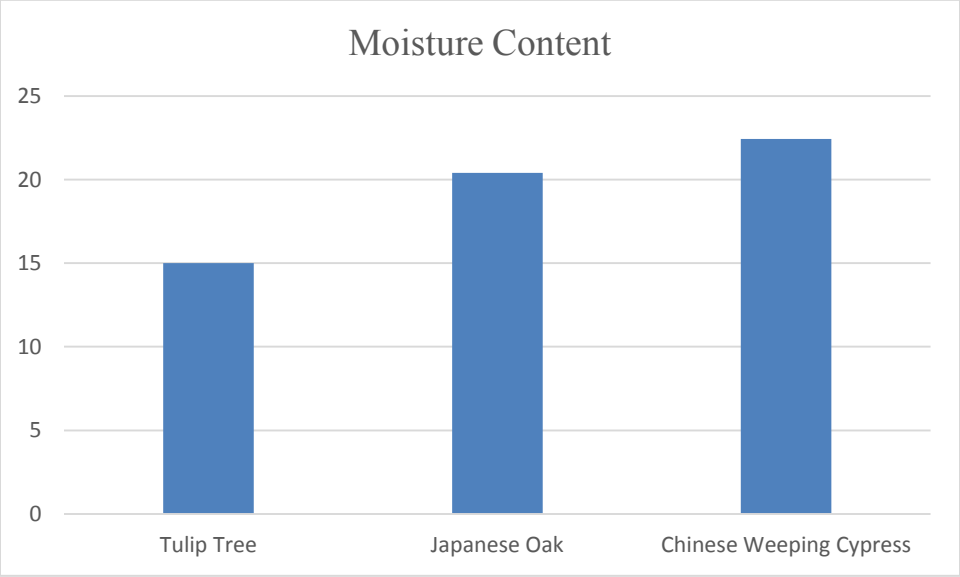

Figure 1. The moisture content of the soil in the Tulip Tree (*Liriodendron chinese*), Japanese Oak (*Lithocarpus glaber*), and Chinese Weeping Cypress (*Cupressus funerbris*).

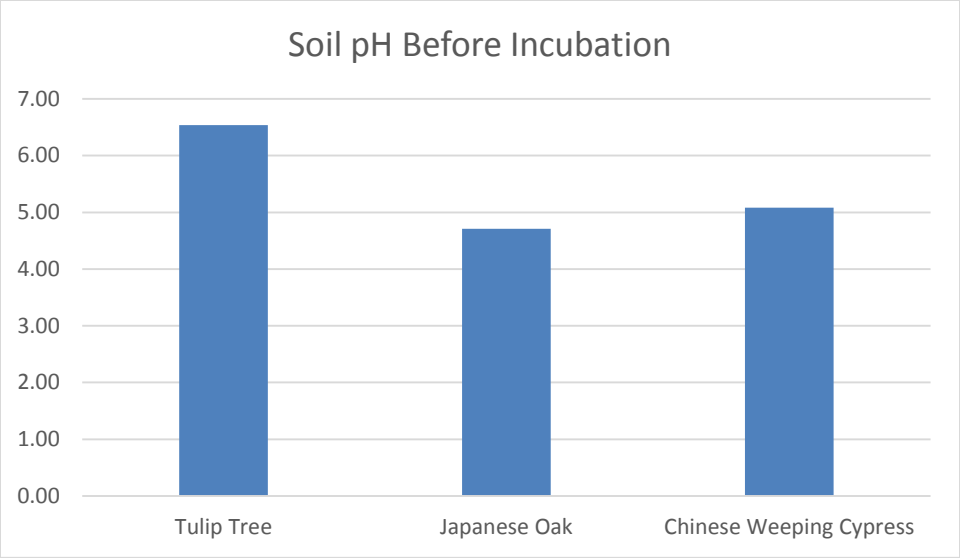

Figure 2. The pH of the soils in the Tulip Tree (*Liriodendron chinese*), Japanese Oak (*Lithocarpus glaber*), and Chinese Weeping Cypress (*Cupressus funerbris*), before the carbon treatments and incubation.

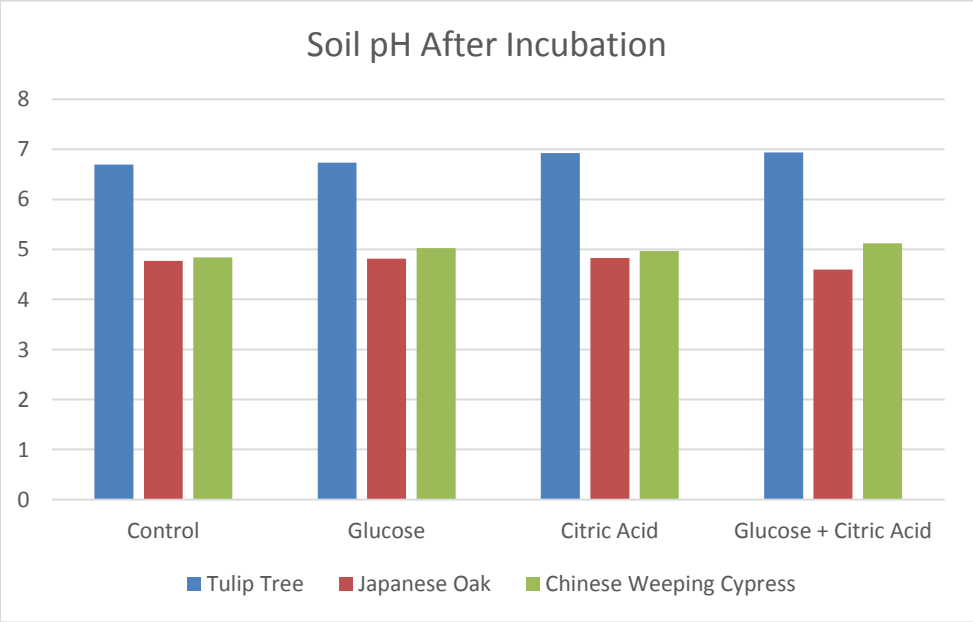

Figure 3. The soil pH and carbon treatments of the Tulip Tree (*Liriodendron chinese*), Japanese Oak (*Lithocarpus glaber*), and Chinese Weeping Cypress (*Cupressus funerbris*) after incubation.

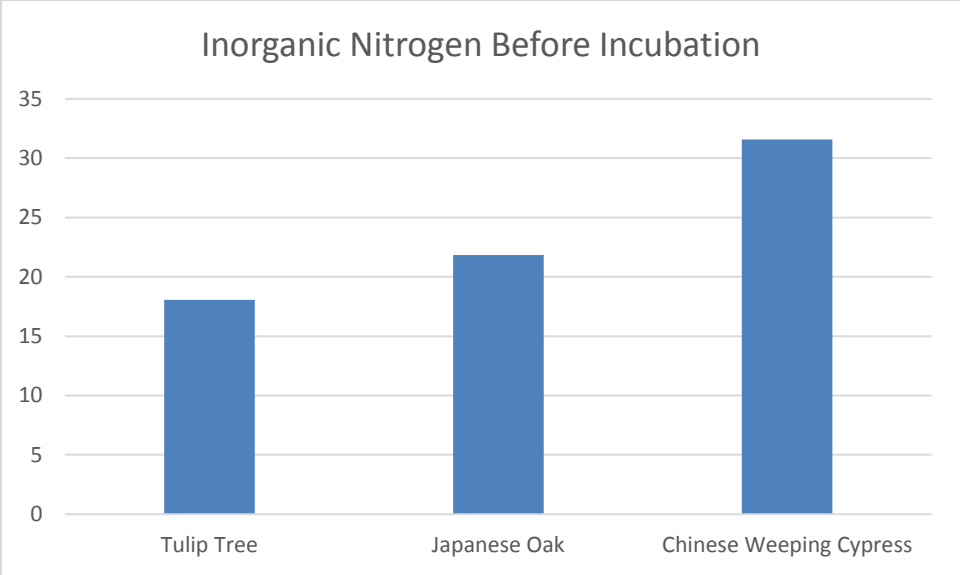

Figure 4. The soil nitrogen, in the Tulip Tree (*Liriodendron chinese*), Japanese Oak (*Lithocarpus glaber*), and Chinese Weeping Cypress (*Cupressus funerbris*), before the carbon addition and incubation.

Tests of Between-Subjects Effects

Dependent Variable:TotalInorgN_mean

Source	Type III Sum of Squares	df	Mean Square	F	P
Corrected Model	291.093[a]	2	145.546	4.283	.070
	5109.982	1	5109.982	150.366	.000
treespecies	291.093	2	145.546	4.283	.070
Error	203.902	6	33.984		
Total	5604.977	9			
Corrected Total	494.995	8			

a. R Squared = .588 (Adjusted R Squared = .451)

Figure 5. Analysis of Variance Single Factor of initial inorganic nitrogen.

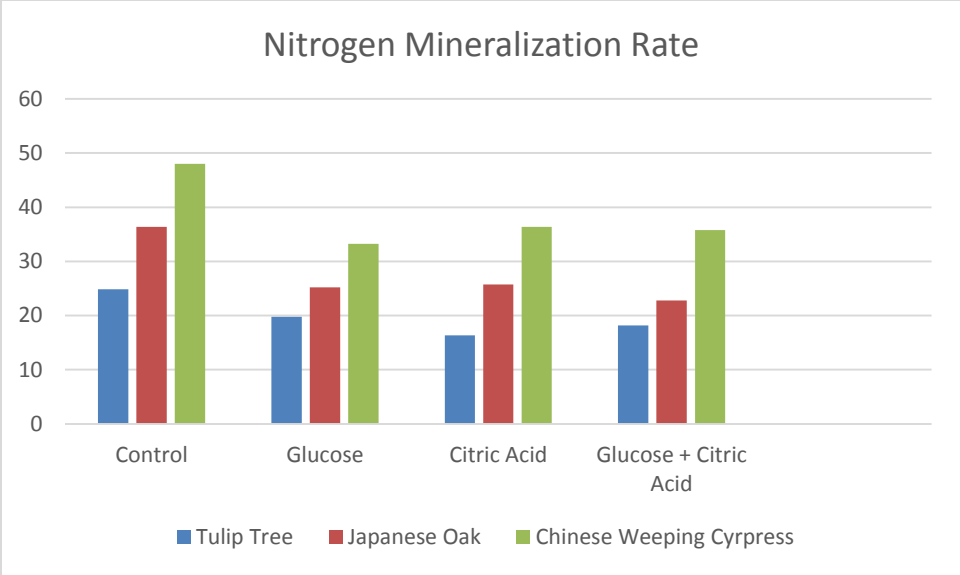

Figure 6. The soil inorganic nitrogen changes, in the Tulip Tree (*Liriodendron chinese*), Japanese Oak (*Lithocarpus glaber*), and Chinese Weeping Cypress (*Cupressus funerbris*), after the carbon addition and incubation process.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Species	2088.544	2	1044.272	23.89512	1.95E-06	3.402826
Carbon	743.3739	3	247.7913	5.669981	0.004405	3.008787
Interaction	95.49075	6	15.91512	0.364171	0.894328	2.508189
Within	1048.855	24	43.70231			
Total	3976.264	35				

Figure 7. Analysis of Variance Single Factor of nitrogen mineralization rate.

Discussion

The moisture content was used to calculate the dry soil mass we used for the extraction of inorganic nitrogen. The other reason is that, we imaged if the moisture contents of soils were different among three tree species, the microbial composition might also be different due to the

distinct adaptability of different microbes to soil moisture condition. Therefore, the changes of microbial properties might have influenced on N transformation.

Because soil pH generally affects the nitrification process (nitrifying bacteria prefer a neutral environment), we decided to examine the changes in pH before incubation and after incubation. As we studied the effects of carbon addition on soil, we found no significant difference in pH. The pH was nearly the same for each soil prior to and following incubation. Although the nitrifying bacteria prefer a neutral environment, sometimes the nitrification will cause a decrease in pH. In this study there was no decrease in pH.

After analyzing the nitrogen mineralization rate, we discovered a rapid increase in concentration of inorganic nitrogen. The highest increase in soil inorganic nitrogen was in the control group (H20). The second highest increase in soil inorganic nitrogen occurred in the soils with the citric acid treatment. The third highest increase in soil inorganic nitrogen occurred in the soils with the citric acid and glucose treatment. Last, the glucose had the lowest increase in soil inorganic nitrogen. Although the studies suggest that carbon addition did affect the soil nitrogen transformation, the results indicate that the control group had the strongest affect.

Conclusions.

Due to time constraints and malfunctioning machinery, we were not able to record the data for the amount of microbial mass in the soils. In future studies, soil microbial mass should be considered in the experimental design. The soil microbes drive the nitrogen transformations so an increase or decrease in microbial mass, after carbon addition, should be examined. Increases in the amount of tree species could also be an area of interest for future experimentation.

Although our findings did reveal significant results, we cannot say, for sure, if the carbon addition had a positive effect on the soil nitrogen transformation. In fact, our study indicates that the carbon addition hindered the microbial community from performing soil nitrogen transformations. Because the control had the highest nitrogen mineralization rate, we are now puzzled. There are many factors that could have affected our experiment which is why this study should be continued. Because neither of the carbon additions had the highest nitrogen mineralization rate, we are rejecting our hypothesis. We believe that carbon addition on soil nitrogen transformation is still a valid idea that should be considered for further study. The addition of carbon is a positive step towards the increase in nitrogen availability among plants and microbes. By adding carbon to soil, microbes gain energy to carry out necessary transformations, such as nitrogen fixation and mineralization. With an increase in inorganic nitrogen, microbes can build nucleic acids, proteins, and other cellular components. As microbes continue to transform organic nitrogen into inorganic nitrogen, plants are able to form proteins, DNA, and other cellular components. Increases in soil nitrogen could potentially result in the reduction of synthetic fertilizer usage; reducing the amount of chemical run off into nearby waterways. Last, if this study is continued, it could particularly benefit farmers, sea life, land animals, and health care professionals.

Acknowledgements

Thank you to my mentors, Dr. Ye Tian, Dr. Elica Moss, Mrs. Lisa Gardner, Ms. Abreeotta Williams, as well as Alabama A&M University and Nanjing Forestry University for making this research possible. Also thanks to the National Science Foundation “REU Site in China: Ecological and Environmental Research” Grant #1063101 and the USDA National Institute of Food and Agriculture for this research opportunity.

Literature Cited

Bassham, James A., Berry, Joseph A., Bishop, Norman I., Buchanan, Bob B., Buetow, Dennis E., Chollet, Raymond, Christy, A. L., Dowtown, John S., Drews, Gerhart, Geiger, Donald R., Giaquinta, Robert T., Gifford, Roger M., Govindjee, Hesketh, John D., Jenkins, Colin L., Kamen, Martin D., Kluge, Manfred, Ogren, William L., Ohad, Itshak, Peters, Doyle B., . Portis Jr., Clark A, Saunders, Venietia A., Alan, J. S, Wim , Vermass F. J., and Wolley, Joseph T. "Photosynthesis V2, Development, Carbon Matabolism, and Plant Productivity." Hacourt Brace Jovanovich, 2 Dec. 2012. Web. 25 June 2015.

Bernhard, Anne. "The Nitrogen Cycle: Processes, Players, and Human Impact." Nature Education Knowledge. Nature, 2010. Web. 25 June 2015.

Bezdicek, David F., Papendick, Robert I., and Lal, Rattan. "Introduction: Importance of soil quality to health and sustainable land management." Methods of assessing soil quality. SSSA Spec. Publ 49 (1996): 1-18.

Erhart, H. "Why Is Soil Important?" *Soil Science* 82.5 (1956): 437. Soil Science Society of America. Web. 22 June 2015.

Kaye, Jason P., and Hart, Stephen C. “Competition for nitrogen between plants and soil microorganisms.” Trends in Ecology and Evolution 1997 : 139-143. Print.

Lamb, John, Fernandez, Fabian, and Kaiser, Daniel. "Understanding Nitrogen in Soils." : Nitrogen : Nutrient Management : Agriculture : University of Minnesota Extension. University of Minnesota, 2014. Web. 23 June 2015.

Ontl, Todd A., and Schulte, Lisa A. "Soil Carbon Storage." Nature.com. Nature Education Knowledge, 2012. Web. 22 June 2015.

Robertson, G.P. and Groffman, P. M. 2007. Pages 341-364 in E.A. Paul, ed. Soil Microbiology, Nitrogen transformation. Biochemistry, and Ecology. Springer, New York, New York, USA.

Roesch, Luiz F. W., Fulthorpe, Roberta R., Riva, Alberto, Casella, George, Hadwin, Allison K. M., Kent, Angela D., Daroub, Samira H., Camargo, Flavio A. O., Farmerie, William G., and Tripplet, Eric W. "Pyrosequencing Enumerates and Contrasts Soil Microbial Diversity." Nature.com. Nature Publishing Group, 5 July 2007. Web. 20 June 2015.

Scottlands Living Landscape. “Soils: Why is soil Important?” Soils. Scottish Natural Heritage. Web 23 June 2015

Trudinger, P. A. "Biogeochemical Cycling of Mineral-Forming Elements." Elsevier Scientific Publishing Company, Jan. 1979. Web. 25 June 2015.

THE EFFECTS OF BIOCHAR ON SPENT GREENHOUSE SOIL

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Abstract

Biochar is a soil enhancer that has growing interest in the scientific world. Soil that is spent may have been over exploited or polluted in some instance, usually making the soil unusable for common agricultural practices. Biochar has the ability to amend physiochemical properties of soil. Conductivity and pH are important to the soil, as far as, for plants to access the nutrients in the soil that are key for healthy plant growth and development. The objective of this study is to assess the effects of biochar on biomass of the plant and pH, conductivity, and phosphatase enzyme activity of the soil. These parameters above were determined and revealed that biomass was not affect by biochar, pH and conductivity were improved, and phosphatase enzyme had a negative response to the increase in biochar concentration. Speculation insists that biochar is the possible answer to construct spent soils enabling them to be reusable, reducing soil pollution, and allowing plants to be grown in place unfit for agricultural practices.

Keywords: Tamarix chinensis*, Salt tolerance, PCR, Peroxidase, Bioinformatics, Phylogenetic tree*

Introduction

Interest in the study of biochar, sometimes known as black carbon, for agriculture and environmental management applications is increasing. The uses of biochar are still being explored as a source of renewable energy like bio-oils and synthesis gas (Alburquerqur et. Al, 2013). This provides new opportunities for adding value to organic wastes through waste management processes such as pyrolysis: thermochemical decomposition in an atmosphere with low oxygen content (Albuquerque et al., 2013). Biochar is commonly used as an alternative to mulches, cover crops, and manure additions that have limited duration and it helps improve soil quality (Lehmann and Rondon, 2006)..

Soil that has been over exploited or polluted are usually derived of physiochemical properties that are essential for healthy plant growth and development; the pollutants can be released by vehicle emission, chemical industry, coal combustion, municipal solid waste, the sedimentation of dust and suspended substances in the atmosphere (Xia et al., 2010). In China approximately 20% of arable soil, 66 million acres of land, had been contaminated by heavy metals and industrial waste water (Pei, 2014).When biochar is added to soil it alters its physiochemical properties (Song et. Al, 2013). Biochar is applied to the soil as a conditioner, where it remains in an essentially permanent form and leads to net removal of carbon from the atmosphere (Graber et al.). Biochar has been present in the Amazon from 500 to 2500 years ago (Rondon, 2006). The beneficial effects of biochar are determined by some of its properties: high porosity, its high water retention capacity; high cation exchange capacity, that favors the retention of nutrients and prevent their loss; direct nutrient supply dependent upon the type of biochar; and the capacity of being a habitat for beneficial microorganisms, which can promote the release and uptake of nutrients by plants (Atkinson et al. 2010; Sohi et al.2010).

Some of the physiochemical properties that can be altered by include pH, conductivity, Nitrogen, and Phosphorous (Song et. Al, 2013). The pH of the soil is very important for plants to access the macronutrients and the micronutrients present in soil. If the pH is too alkaline or acidic the plant will not be able to absorb the nutrients that are needed to support itself. Conductivity is also important because it shows the level of the ability of soil water to carry and electric current. The electric current reveals the presences of macronutrients and micronutrients in the soil. It also expresses the ability of the soil to resists outside forces and the ability of particles to bind together. Phosphatase is a catalytic enzyme used in a process of the Phosphorous cycle.

The main focus of this study is to assess the effects of biochar addition on key variables that cause differences in growth such as the biomass of leaves and roots of the plant, measurement of pH and conductivity, as also on the influence of biochar on phosphatase enzyme activity. This is done by examining the impact of rice hull derived biochar on nutrient derived soil and Lettuce (Latuca sativa) development in a greenhouse, providing an opportunity to evaluate specific factors potentially responsible for plant development effecting the biomass.

Materials and Methods

The biochar was obtained from the raw material of rice hull. It was created with Pyrolysis technology, meaning it was burned with low oxygen. The lettuce plant was used for this experiment. The seedlings are from Nanjing Academy of Agricultural Sciences. The soil used in this experiment was greenhouse soil obtained from Nanjing Forestry University Experimental Base of Baima, which is also the study site for this experiment. The testing of the pH, conductivity and alkaline phosphatase was done in the College of forestry, Lab 1108-2.

Biochar concentration and planting lettuce seedlings

Nine treatments were performed in triplicates with varied levels of biochar for every 6kg of soil. The concentration of biochar for each treatment are can be found in table1. Lettuce seedlings were planted in the soil after the bio-char was added. The soil was collected when seedlings were planted on April 12th, on April 27th when the seeds sprouted, on May 12th when the lettuce was maturing, and on May 26th when the lettuce was harvested. The crown and roots of the each lettuce were collected and weighed, giving the biomass.

Table 1. The treatment number of the soil sample and its concentration of biochar.

Treatment Number	Treatment concentration (g/kg)
0	0
1	4
2	8
3	10
4	15
5	20
6	30
7	40
8	80

Measuring pH and conductivity

Fresh soil samples were air-dried and passed through a sieve of 1.0mm, 10g of each of the air-dried soil sample was placed into 100ml centrifuge tube, and then there was 50ml of de-ionized water added to the centrifuge tube. This was repeated 3 times for 11 soil

sample. The soil suspension was centrifuged for 8 minutes. The supernatant was filtered through a Millipore filter in a drying conical flask. Soil leachate between 30-40ml was absorbed in a 50 ml beakers, then the conductivity meter was used to test the liquid conductivity and a pH meter was used to measure the pH. The readings were recorded for each sample.

Alkaline Phosphatase Determination

A soil sample was treated by allowing soil samples to air dry and then 40ml pH8.4 Tris- HCl (hydrochloric acid) buffer solution was added. Next 1ml of a 5mol/L PNPP (para-Nitrophenylphosphatase) and 1ml of 0.5mol/L $CaCl_2$ (Calcium Chloride) were added and shaken, after samples were put in a water bath for one hour but control samples did not go into the bath. After finishing the water bath, the glasses were taken out and 4ml of 0.5mol/L NaOH (Sodium hydroxide) immediately and shaken. Then miscible liquids from the samples were filtrated with funnels in the Erlenmeyer flasks. This was repeated 3 times for each sample.

In color comparison tubes 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 5, 10, 15, and 20mL p-NP were absorbed, then ultrapure water was added until it was 25mL and set aside for the standard curve. Next 5mL of 0-1000 μ mol/L p-NP was placed in 12 color comparison tubes. Then 4ml of 0.5mol/L NaOH and 2ml of 2mol/L Tris was added to each tube one by one. Afterwards ultrapure water was added to the solution until it reached 25ml in the tube and was shaken. The solution was set aside for 10 minutes, then tested with the UV-1200 Ultraviolet visible spectrometer at the wavelength of 400nm. 0 p-NP mol/L solution was used as a reference in the concentration of 0, 10, 20, 40, 60, 80, 100, 200,400,600,800, 1000 μ mol/L p-NP.

Statistical Analysis

Conductivity and pH data were subjected to a Univariate analysis of variance (UNINOVA) with type III sums of square considering the biochar concentration and the time, while biomass and the Phosphatase enzyme data were subjected to a Univariate analysis of variance (UNIANOVA) with type III sums of squares, considering biochar concentration. The time was accounted for by measurements taken before and after the addiction of biochar.

Results

Effects of biochar on pH and Conductivity

As expected, the status of the pH and the conductivity improved due to biochar additions to the spent greenhouse soil. Measurements taken of the pH before and after showed that the lower concentration of biochar additions improved significantly, while higher concentration did improve but did not have a notable difference on the pH (Figure1). Measurements of conductivity before and after showed that conductivity did improve significantly (Figure 2). Before biochar additions all measurements of conductivity were relatively closed in measurements. After measurements of conductivity varied as concentration increased but still had improved the overall conductivity of the soil. This displays a positive relationship between biochar and the two factor. As one increases so does the other.

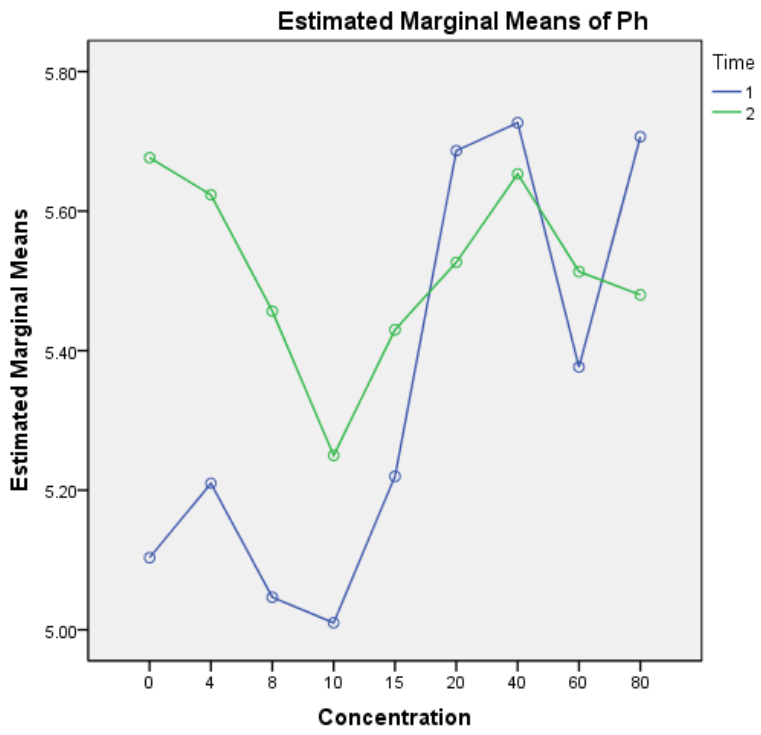

Figure1. The comparison of before (1) and after (2) biochar additions on the pH of the green house soil.

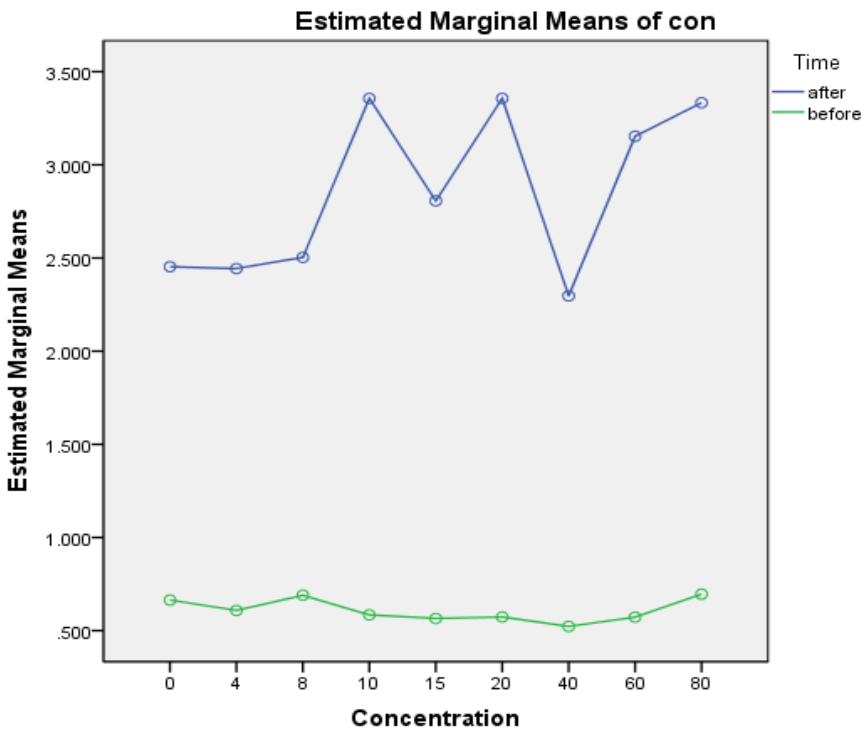

Figure 2. The comparison of before and after biochar additions on conductivity of the greenhouse.

This suggest that biochar addition for pH and conductivity enhancer is it primary use and concern although it does alter other physiochemical properties in the soil. Biochar addition to soil should be monitored as some studies show that the alterations may have negative effects under the right circumstances while others show that they have a positive effect.

Effects of biochar on Biomass

There was not a significant difference of the biomass of the lettuce (*L. sativa*) plant due to biochar additions. The data displayed too many variations in biomass (Figure 3). This suggests that further testing should be conducted. If it is at all possible that biochar can affect the biomass then it may be possible to see what parameter directly affect plant growth and development.

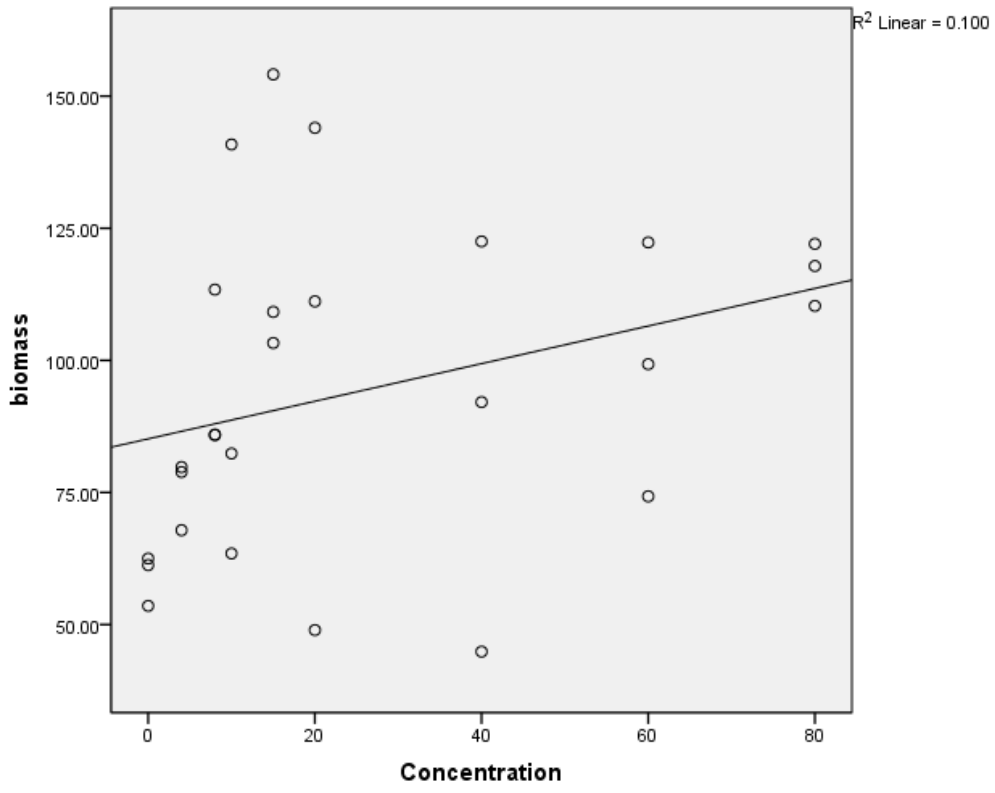

Figure 3. The biomass of each plant is represented by the open points on the graph. As the concentration of biochar increases, there is no direct correlation to the effects of it on biomass.

Biochar may not have a direct effect on the biomass of the plant. A study on biochar with fertilizers at different levels revealed that biomass was higher in the plants with the biochar than the ones with out. The data had too many variations deferring conclusions on the effects of biochar on the biomass of the plant.

Effects on Phosphatase enzyme

As expected, the Phosphatase enzyme decreased due to higher concentration of biochar (Figure 4). This may suggest that although biochar has positive effect for many factor in soil it may have negative impact on crucial enzyme used for the phosphorous cycle. A previous study shows that monooxygenase is a catalyst for the first step in ammonia oxidation but was

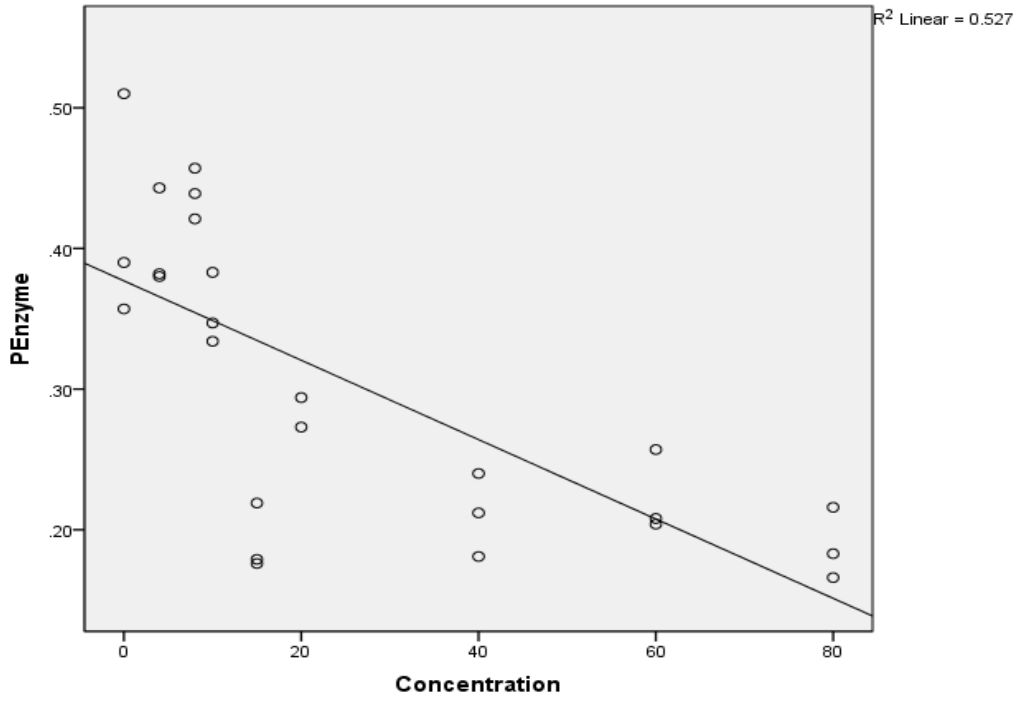

Figure 4. The phosphatase enzyme activity is represented by the open points on the graph. As the concentration of biochar increases, the activity decreases.

decreased due to addition of biochar (Song et al.). Enzyme activity may have decreased because the phosphatase enzyme may be produced by microorganisms in the soil. If the biochar disrupted

their ability to function decreasing the enzyme activity in the soil. The speculation call for further testing.

Discussion

Overall, biochar has a direct correlation to the improvement of pH and conductivity, and decrease in phosphatase enzyme activity. Conductivity and pH have a positive relationship between each other. Biochar additions triggered a negative response on phosphatase enzyme activity. From the data, biomass has no relation to biochar concentration or additions. Further testing should be conducted to understand what parameters effect the biomass and then determine how biochar effects those parameters. Also, further testing should be conducted to understand the effect of biochar on any enzyme activity in the soil, and how that relates to the microorganisms present in the soil. Biochar does have beneficial properties that can be used to amend spent soils as an alternative to other practices. Mulches, cover crops, manure, and fertilizers have limitations, while biochar is semi-permanent in soil once presented. Also biochar helps prevent the leaching of nutrients, which if occurs often causing other environmental issues. Biochar may allow high valued cash crops to be grown in places usually considered unsuitable for agricultural purposes. This could be beneficial for farmers or food producers who have access to poor soils, countries that may be in a food deficit due to being unable to use the soil, and bringing and saving money for farmers who depend on the yield and quality of crops being grown.

Literature Cited

Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 33:1–18.

Alburquerque, José Antonio et al. 2013. "Enhanced Wheat Yield by Biochar Addition under Different Mineral Fertilization Levels." *Agronomy for Sustainable Development* 33(3): 475–84.

"Biochar Addition Affected the Dynamics of Ammonia Oxidizers and Nitrification in Microcosms.pdf."

Graber, Ellen R et al. 2010. "Biochar Impact on Development and Productivity of Pepper and Tomato Grown in Fertigated Soilless Media." : 481–96.

Hu, Yuanan, and Hefa Cheng. 2013. "Water Pollution during China ' S Industrial Transition." *Environmental Development* 8: 57–73. http://dx.doi.org/10.1016/j.envdev.2013.06.001.

Lehmann, Johannes, and Marco Rondon. 2002. "Bio-Char Soil Management on Highly Weathered Soils in the Humid Tropics." *Biological approaches to sustainable soil systems*: 517–30.

Luo, Xiao-san, Shen Yu, Yong-guan Zhu, and Xiang-dong Li. 2012. "Science of the Total Environment Trace Metal Contamination in Urban Soils of China." *Science of the Total Environment, The* 421-422: 17–30. http://dx.doi.org/10.1016/j.scitotenv.2011.04.020.

Pei, By Minxin. 2014. "China's Soil Pollution: It's Much Worse than You Think." : 2011–12.

Sohi SP, Krull E, López-Capel E, Bol R (2010) A review of biochar and its use and function in soil. Adv Agron 105:47–82.

Wang, Quanying et al. 2009. "European Journal of Soil Biology Indication of Soil Heavy Metal Pollution with Earthworms and Soil Microbial Biomass Carbon in the Vicinity of an Abandoned Copper Mine in Eastern." *European Journal of Soil Biology* 45(3): 229–34. http://dx.doi.org/10.1016/j.ejsobi.2008.12.002.

Windeatt, Jayne H et al. 2014. "Characteristics of Biochars from Crop Residues : Potential for Carbon Sequestration and Soil Amendment." 146: 189–97.

Xia, Xinghui, Xi Chen, Ruimin Liu, and Hong Liu. 2011. "Heavy Metals in Urban Soils with Various Types of Land Use in Beijing , China." *Journal of Hazardous Materials* 186(2-3): 2043–50. http://dx.doi.org/10.1016/j.jhazmat.2010.12.104.

Xu, Chi et al. 2007. "The Spatiotemporal Dynamics of Rapid Urban Growth in the Nanjing Metropolitan Region of China." : 925–37.

VERIFICATION OF CRY-GENES (CRY1AH1) *POPULUS DELTOIDES* × *P. EURAMERICANA* ('NANLIN 895') HYBRID FEMALE CLONES

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Abstract

The cry gene cry1Ah1 was cloned from *Bacilus thuringiensis* strain; this gene encoded a protein which possesses strong insecticidal activity against Hyphantria cuneawe. For the genetic transformation we cloned *Populus deltoides* x *P. euramericana* 'Nanlin895' from a poplar hybrid female. The transformation was carried out by using *Agrobacterium tumefaciens* infection method. Transgenic seedlings were grown up in the MS medium contained hygromycin. PCR and Southern Blot analysis were performed with the hygromycin resistance transgenic plants to confirm the presence of the cry gene cry1Ah1 into the genome of the *Populus deltoides* x *P. euramericana* 'Nanlin895' was successful. The expression of high-copy number of transgenes in plants may be more or less when comparing with a single copy of transgene which could be good for molecular analysis and genetic engineering (Ali Movahedi, Zhuge et al., 2014). The DNA extraction had a concentration of 301 µl/nl, it is good enough to detect the gene and the protein. The PCR showed good result, the expected fragment was there; all the bands were about 561p.b. We used the Dig High Prime DNA Labeling and Detection Starter kit to perform the Southern Blot analysis. The process allows the detection of specific molecule among a mixture separated by gel electrophoresis. The protein couldn't be detected by the Southern Blot analysis because the process was contaminated. We need to do the Southern Blot analysis again to make sure the protein has been successfully encoded. The PCR result shows proof of the gene present, therefore the Southern Blot should give us a good result. However, if the Southern Blot analysis shows the protein is absent, we will conclude that the PCR result is a false positive. Our DNA concentration was good, therefore we will use the same DNA extracted for this research. At this point, we can't deduce that the cry gene (cry1ah1) has been inserted successfully into the genome of the *Populus deltoides* x *P. euramericana*.

Keywords: *Cry1Ah1 gene,* Populus deltoides *x* P. euramericana *'Nanlin895', PCR detection, Southern Blot analysis*

Introduction

Poplar is a versatile tree species that is highly amenable to vegetative propagation (Jiaxing et al. 2004), it has a rapid growth rate which is a good model system for the transformation of woody plant species (Ali-Movahedi et al.2014). These trees are highly beneficial to our environment; their oxygen plays a vital role in the protection of soil erosion in eastern China. They are used as habitat for wildlife, soil carbon sequestration, and phytoremediation. They are able to grow in lowland and highland forestation (Bernd et al., 2004), which makes them very important to the pulp, paper, and cosmetic industries (Ferreira et al., 2009). The P. deltoides is one of the fastest growing trees in North America (Haverbeke et al., 2000). They are the largest North American hardwood tree (Steve Nix, et al., 2001).

Poplar plantation plays an important role in eastern China agriculture industries. In 2002, the Republic of China launched the world's largest tree planting project, within the same year their State Forestry Administration approved genetically modified poplars for commercial planting. China has over one million insect genetically modified crops that have been planted (Chris Lang, 2004). Furthermore, they have over 7.0 million hm2 poplars planted, which makes them one of the countries with the largest poplar plantations.

As many countries, The Chinese government are highly interested in poplar plantations for a stronger social-economic, environmental improvement, and substantial development (Ying Yong, et al., 2008). Poplars have a lot of great characteristics, their fast growth makes them more desirable for biofuels than many other woody crops, They have the ability to produce a significant amount of biomass, high cellulose, and low lignin contents in a short period of time (Brunner et al., 2009).

Bamboo woods have been very dominant in China for centuries. Today, poplars are well known in China's industries, they replace a lot of materials usually made of bamboos such as chopsticks, desks and chairs. The wood materials from these trees are found in almost everything in many cities in China. The protection of poplar plays a vital and integral role in the modern-day industry, and our environment. In eastern China, these plants are used in building materials to control erosion of the soil through the process of photosynthesis as a waste product (Zhuge et al., 2014). Poplars are genetically diverse, our project was conducted with the use of the poplar hybrid female clones (populus deltoides x P.euramericana 'Nanlin895') for the genetic transformation. It is important to consider how different clones respond to different climatic factors to optimize biomass production levels (Zalesny et al., 2009). The energy industries watch closely their poplar plantations agricultural conditions to assure a great amount of biomass production from poplars.

The ever-increasing demands of poplar urge countries to intensify farmer routine, and help scientists conducting research on ways to protect plantations against organisms such as fungi, bacteria, and insects (Constantin, A., et al., 2010). Indeed their great characteristics, poplars encounter many challenges with these insects that are ruining many plantations. Once the plants are affected the removal of the infected trees could be removed to prevent further spread, but often an accumulation of a large quantities of plants already been affected before the early detection of fetal abnormalities. When the pathogen defense gene encoding in the poplar is damaged, the pathogen can evade detection and suppresses early host responses (Manoela Miranda, et al., 2010).

Scientists are working vigorously to produce different types of cry genes to fight the infestation of poplar plantations. They have found different type of cry genes that have positive

effects on these transgenic poplars. These cry genes cloned from Bacillus thuringiensis strain, and are categorized against the class of insect they're restraining (Crickmore, et al., 2011). The Bt strain carries different class of cry genes meaning the overall toxicity profile of the isolate depend on the expression regulation of individual cry genes (Soberon, M, et al., 2011). The Bacillus thuringiensis is a gram positive, spore forming bacterium that produces a variety of insecticidal crystal proteins toxic (Gore et al., 2001). These genes are well known in the modern-day agricultural production, they are considered as one of the best method used in the resistance of transgenic plants against these insects. They help keep in control the ongoing infestations of plants around the world. These researches are very important to our society, the insecticidal properties using different cry genes is an ongoing laboratory and field studies.

The Bt gene produces insecticidal crystal proteins (ICPs), also called d-endotoxins, consisting of one or more crystal (Cry) proteins and Cytolitic (Cyt) toxins (Bravo, et al., 2007). There are scientific studies who argues the suppression of insects resistance to Bt cry toxins could be achieved by modifying the cry gene in such a way that they can kill the insect without binding to the receptor (Soberon et al. 2007, Pardo-Lopez et al. 2010). Most of the commercial Bt formulations used for the control pests contain toxins of Cry1A family, especially Cry1Aa, Cry1Ab, and Cry1Ac proteins (Stobdan et al., 2004).

Our study is using the cry gene (cryAh1) cloned in laboratory from Bacilus thurigiensis strain, this gene encoded a protein that possesses strong insecticidal activity against Hyphantria cuneave. Poplar hybrid female clones (Populus deltoides x P. euramericana 'Nanlin895') were used for the genetic transformation. The transformation was carried out by using the Agrobacterium tumefaciens infection method. This method is the most frequently used for plant engineering; it enables the T plasmid to transfer the gene of interest into the plant cells. To

shorten the time required for studies of transgenic poplar, efforts have been made to optimize transformation using the Agrobacterium tumefaciens method (Ali Movahedi, Zhuge et al., 2014).

The Transgenic seedlings for our study were grown in a MS medium contained hygromycin, an antibiotic that kills organisms and helps the growth of our seedlings in the dark. The genetically modified transformation was done with the Agrobacterium mixture of the plants cells to allow the transfer of DNA into the plant chromosomes. After the co cultivation and regeneration of the poplar transforming in the MS selection medium, the elongation medium shooting, and the MS rooting medium. PCR and Southern blot analysis will perform with the hygromycin resistance to confirm the presence of the transgene.

Materials and Methods

The poplar hybrid clone "Nanlin895" (*Populus deltoides* × *P. euramericana* "Nanlin895") was cultured in a MS medium for the genetic transformation. The transformation was carried out by using *Agrobacterium tumefaciens* infection method. This method enables the insertion of the transgene into the T-plasmid after the isolation of the genes of interest and introduces the T-DNA containing plasmid into the Agrobacterium. We mixed the *Agrobacterium* infection with the plants cells to transfer the gene of interest into the plants chromosome. The PCR and Southern blot was performed to trace the gene in the host plant.

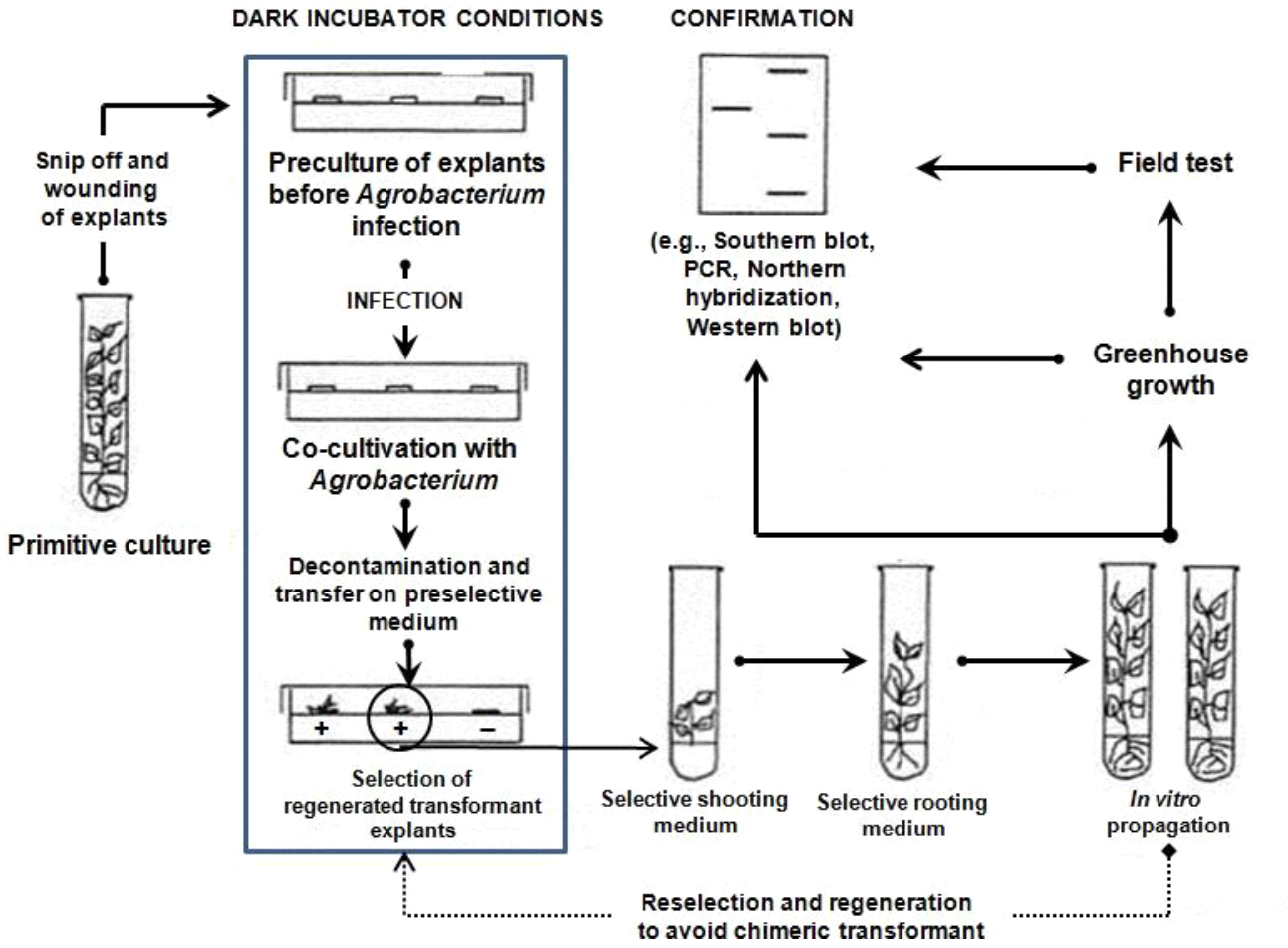


Figure 1. An overview the steps for *Agrobacterium*-mediated transformation in *Populus* species by Ali Movahedi and Zhuge Qiang.

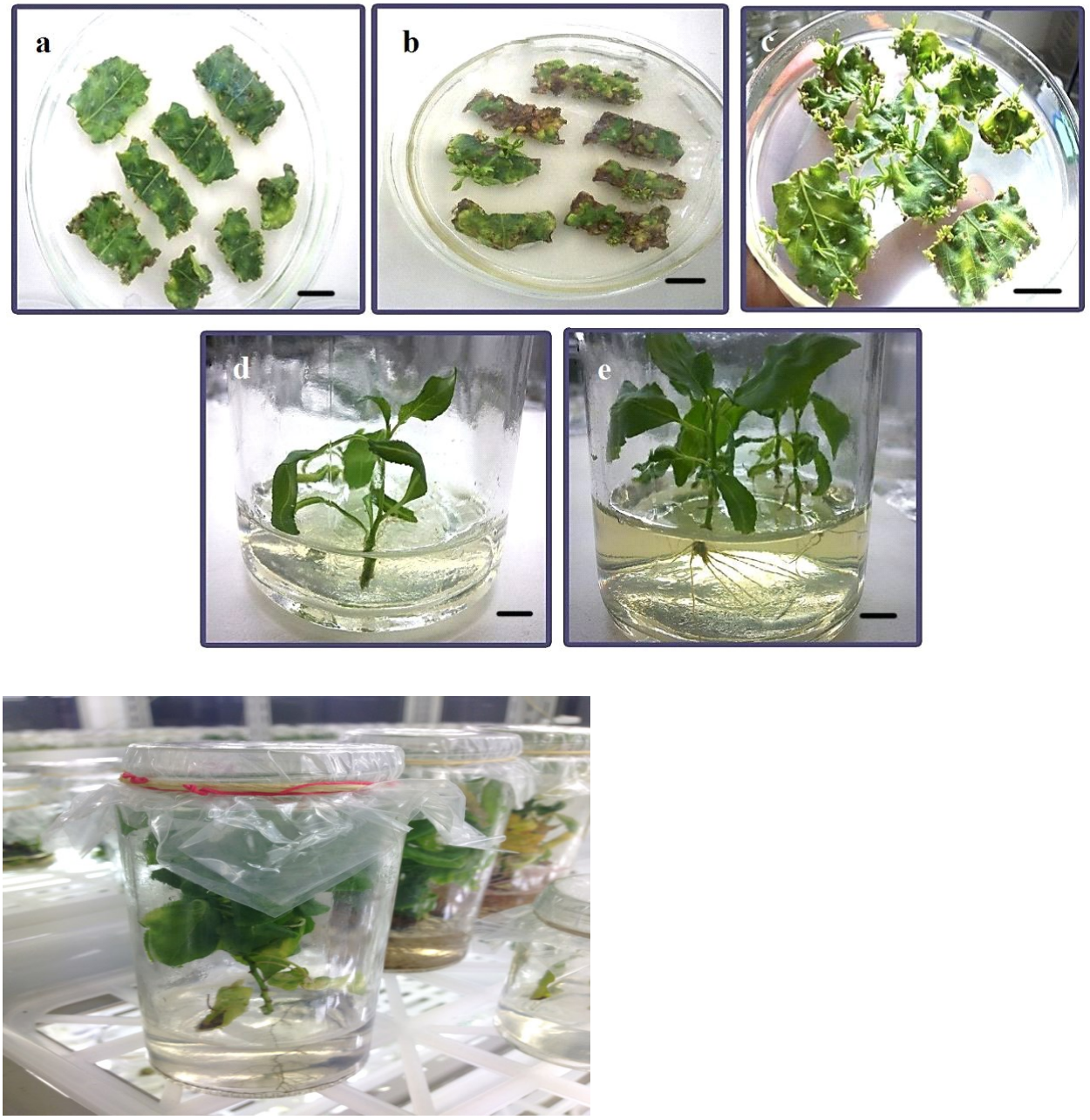


Figure 2. Poplar hybrid female clones (Populus deltoides × P. euramericana 'Nanlin895') were used for genetic transformation. Nanlin895' poplar leaves were excised (discs of 1-2 cm2) and pre-cultured for 3 days on MS1 medium (MS plus 0.5 mg/L 6-BA and 0.002 mg/L TDZ).After cocultivation on MS1 in the dark for 4 days, the discs were washed with sterile water containing 100 mg/L cefotaxime and transferred to MS2 medium (MS plus 0.2 mg/L 6-BA, 0.001 mg/L TDZ, 100 mg/L cefotaxime, and 20 mg/L kanamycin). Regenerated shoots were cultured with 20 mg/L kanamycin in 0.5 × MS. PCR and Southern blot analyses were performed with the 1st generation transgenic (T0) plants to confirm the presence of the transgene. The transgenic plants and CK plants were grown in the culture room with 16 h cool white fluorescent light (~200 µmol m-2 s-1).

DNA Extraction

We extract the DNA from the transgenic poplar leaves using the Cetyl Trymethylammonium Bromide method (CTAB), we ended up with four DNA samples (1-4,1-6), and our previous DNA that was extracted by Dr Sun (1-1,4). There were two subgroups and two samples which came from the same transgenic poplar. After the DNA extraction each sample was analyzed using the BioDrop followed by the gel electrophoresis.

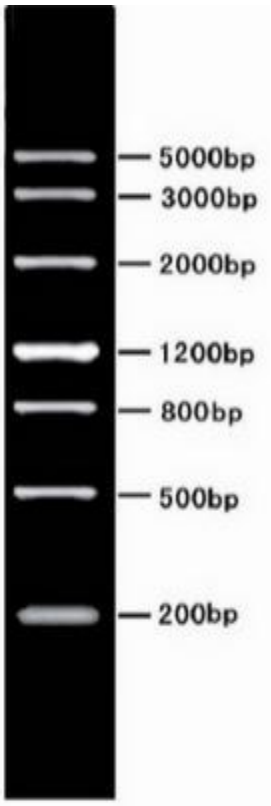
Sample Name	DNA Concentration
1-4	129.1 µl
1-6	114.67µl
1-1	307.4 µl
4	301.8 µl

Figure 2. The DNA concentration results of the 4 samples; 1-4, 1-6, 1-1, 4.

PCR Detection

The PCR detection was performed with these primers in order to detect if the gene was present.

Primer: zg1-F: GTTTCGTGTTGGGACTTATT
zg1-R: CGAGCACAGTAAGAGTAAGT



The Expected fragment should be 561 bp.

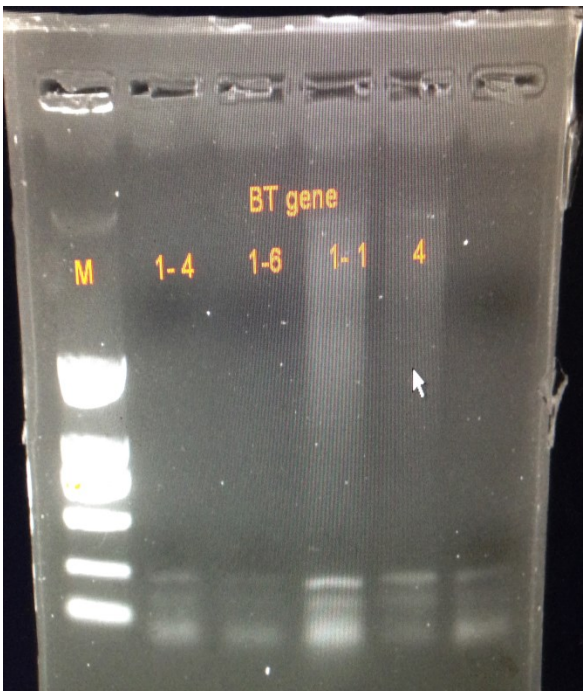


Figure 3. The agarose-gel electrophoresis result shows the bands that were expected are present.

Southern Blot Analysis

For our southern blot analysis we used the Dig High Prime DNA Labeling and Detection Starter kit, the DNA showing on the nitrocellulose paper contaminated in the bag (Fig. 4.) is not able to confirm the presence of the protein.

Figure 4. The Southern Blot analysis didn't show any result on the nitrocellulose paper.

Results and Discussion

Because of the time constraint we couldn't prove our hypothesis. The gene was found in our PCR detection, But the Southern Blot analysis came out negative, it looks like our result was contaminated during the DNA transfer to the nitrocellulose paper. This research would be analyzed by other scientists at Nanjing Forestry University. There will be another Southern Blot analysis using the same Dig High Prime DNA Labeling and Detection Starter kit to confirm the

presence of the protein. If the protein is not found or isn't eluded during the purification, we will conclude that the PCR detection gave us a false result. If the Protein is found, we will conduct further studies to see the efficiency of the protein. The transgenic poplar *P. deltoides* x *P. euramericana* 'Nanlin895' would be tested against the moths.

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Literature Cited

Bravo, A., Gill, S. S., & Soberon, M. (2007). Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. Toxicon, 49, 423–435.

Vachol toxin Cry1Aa. Biochemistry 41, 6178–6184.n, V., Préfontaine, G., Coux, F., Rang, C., Marceau, L., Masson, M., Brousseau, R.,Frutos, R., Schwartz, J.L., Laprade, R., 2002. Role of helix 3 in pore formation bythe Bacillus thuringiensis insecticidal toxin

Estruch, J.J., Warren, G.W., Mullins, M.A., Nye, G.J., Craig, J.A., Koziel, M.G., 1996.

Vip3A, a novel Bacillus thuringiensis vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proc. Natl. Acad. Sci. USA 93,

Confalonieri, M.; Balestrazzi, A.; Bisoffi, S. Genetic transformation of *Populus nigra* by *Agrobacterium tumefaciens*. *Plant Cell Rep.* **1994**, *13*, 256–261.

Ferreira, S.; Batista, D.; Serrazina, S.; Pais, M.S. Morphogenesis induction and organogenic nodule differentiation in *Populus euphratica* Oliv. leaf explants. *Plant Cell Tissue Organ Cult.* **2009**,

Gozukirmizi, N.; Bajrovic, K.; Ipekqi, Z. Genotype differences in direct plant regeneration from stem explants of *Populus tremula* in Turkey. *J. For. Res.* **1998**, *3*, 123–126.

Han, X.; Ma, S.; Kong, X.; Takano, T.; Liu, S. Efficient *Agrobacterium*-mediated transformation of hybrid poplar *Populus davidiana* Dode × *Populus bollena* Lauche. *Int. J. Mol. Sci.* **2013**, *14*, 2515–2528.

Int. J. Mol. Sci. **2014**, *15* **10792**

Kang, B.; Osburn, L.; Kopsell, D.; Tuskan, G.A.; Cheng, Z.M. Micropropagation of *Populus trichocarpa* "Nisqually-1": The genotype deriving the *Populus* reference genome. *Plant Cell Tissue Organ Cult.* **2009**, *99*, 251–257.

Yadav, R.; Arora, P.; Kumar, D.; Katyal, D.; Dilbaghi, N.; Chaudhury, A. High frequency direct plant regeneration from leaf, internode, and root segments of Eastern Cottonwood (*Populus deltoides*). *Plant Biotechnol. Rep.* **2009**, *3*, 175–182.

Horsch, R.B.; Fry, J.E.; Hoffmann, N.L.; Eichholtz, D.; Rogers, S.G.; Fraley, R.T. A simple and general method for transferring genes into plants. *Science* **1985**, *227*, 1229–1231.

Han, K.H.; Meilan, R.; Ma, C.; Strauss, S.H. An *Agrobacterium tumefaciens* transformation protocol effective on a variety of cottonwood hybrids (genus *Populus*). *Plant Cell Rep.* **2000**, *19*, 315–320.

Meilan, R.; Ma, C. Poplar (*Populus* ssp.). In *Methods in Molecular Biology*, *Agrobacterium Protocols*, 2nd ed.; Wang, K., Ed.; Humana Press: Totowa, NJ, USA, 2006; pp. 143–151.

Ma, C.P.; Strauss, S.H.; Meilan, R. *Agrobacterium*-mediated transformation of the genome sequenced poplar clone, Nisqually-1 (*Populus trichocarpa*). *Plant Mol. Biol. Rep.* **2004**, *22*, 1–9.

Yevtushenko, D.P.; Santosh, M. Efficient *Agrobacterium*-mediated transformation of commercial hybrid poplar *Populus nigra* L. × *P. maximowiczii* A. Henry. *Plant Cell Rep.* **2010**, *29*, 211–221.

Husaini, A.M. Pre- and post-agroinfection strategies for efficient leaf disk transformation and regeneration of transgenic strawberry plants. *Plant Cell Rep.* **2010**, *29*, 97–110.

Bartlett, J.G.; Alves, S.C.; Smedley, M.; Snape, J.W.; Harwood, W.A. High-throughput *Agrobacterium*-mediated barley transformation. *Plant Methods* **2008**, *4*, 1–12.

Kondou, Y.; Higuchi, M.; Takahashi, S. Systematic approaches to using the FOX hunting system to identify useful rice genes. *Plant J.* **2009**, *57*, 883–894.

Nakamura, H.; Hakata, M.; Amano, K.; Miyao, A. A genome-wide gain-of-function analysis of rice genes using the FOX-Hunting system. *Plant Mol. Biol.* **2007**, *65*, 357–371.

Fowler, S.; Thomashow, M.F. Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cel* **2002**, *14*, 1675–1690.

Nakashima, K.; Ito, Y.; Yamaguchi-Shinozaki, K. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiol.* **2009**, *149*, 88–95.

Nakashima, K.; Takasaki, H.; Mizoi, J.; Shinozaki, K.; Yamaguchi-Shinozaki, K. NAC transcription factors in plant abiotic stress responses. *Biochim. Biophys. Acta (BBA) Gene Regul. Mech.* **2012**, *1819*, 97–103.

Rabbani, M.A.; Maruyama, K.; Abe, H.; Khan, M.A.; Katsura, K.; Ito, Y.; Yoshiwara, K.; Seki, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.* **2003**, *133*, 1755–1767.

Yamaguchi-Shinozaki, K.; Shinozaki, K. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* **2006**, *57*, 781–803.

Holsters, M.; Waele, D.; Depicker, A.; Messens, E.; van Montagu, M.; Schell, J. Transfection and transformation of *Agrobacterium tumefaciens*. *Mol. Gen. Genet.* **1978**, *163*, 181–187.

Int. J. Mol. Sci. **2014**, *15* **10793**

Tzfira, T.; Frankmen, L.; Vaidya, M.; Citovsky, V. Site-specific integration of *Agrobacterium* T-DNA via double-stranded intermediates. *Plant Physiol.* **2003**, *133*, 1011–1023.

Thakur, D.K.; Srivastava, D.K. High-efficiency plant regeneration from leaf explants of male Himalayan poplar (*Populus ciliata* Wall). *In Vitro Cell Dev. Biol. Plant* **2006**, *42*, 144–147.

Zhan, X.C.; Kawai, S.; Katayama, Y.; Morohoshi, N. A new approach based on the leaf disc method for *Agrobacterium* mediated transformation and regeneration of aspen. *Plant Sci.* **1997**, *123*, 105–112.

Herrera-Estrella, L.; Simpson, J.; Martinez-Trujillo, M. Transgenic plants: An historical perspective. *Methods Mol. Biol.* **2005**, *286*, 3–32.

Hui, P.; Cheng, H.Y.; Chen, C.; Yu, X.W.; Yang, J.N.; Gao, W.R.; Shi, Q.H.; Zhang, H.; Li, J.G.; Ma, H. A NAC transcription factor gene of chickpea (*Cicer arietinum*), *CarNAC3*, is involved in drought stress response and various developmental processes. *J. Plant Physiol.* **2009**, *166*, 1934–1945.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant Physiol*. **1962**, *15*, 473–497.

Horsch, R.B.; Fry, J.; Hoffmann, N.; Neidermeyer, J.; Rogers, S.G.; Fraley, R.T. Leaf disc transformation. In *Plant Molecular Biology Manual*; Gelvin, S.B., Schilperoort, R.A., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988; pp. 1–9.

Satchel, S.E.; Messens, E.; van Montagu, M.; Zambryski, P. Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. *Nature* **1985**, *318*, 624–629.

Doyle, J.J.; Dickson, E.E. Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* **1987**, *36*, 715–722.

Porebski, L.; Bailey, L.; Baum, B. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.* **1997**, *15*, 8–15.

Bravo, A., Gill, S. S., & Soberon, M. (2007). Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. Toxicon, 49, 423–435.

Crickmore, N. (2011). http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/index.html

Bravo, A., Likitvivatanauong, S., Gill, S. S., & Soberon, M. (2011). Bacillus thuringiensis: A story of successful bioinsecticide. Insect Biochemistry and Molecular Biology, 41(7), 423–431.

Tiewsiri, K., & Wang, P. (2011). Differential alteration of two aminopeptidases N associated with resistance to Bacillus thuringiensis toxin Cry1ac in cabbage looper. PNAS, 108(34), 14037–14042. Heckel, D. G., Gahan, L. J.,

Baxter, S. W., Zhao, J. Z., Shelton, A. M., Gould, F., et al. (2007). The diversity of Bt resistance gene in species of Lepidoptera. Journal of Invertebrate Pathology, 95, 192–197.

Gomez, I., Arenas, I., Benitez, I., Miranda-Rios, J., Becerril, B., Grande, R., et al. (2006). Specific epitopes of domains II and III of Bacillus thuringensis cry1Ab toxin involved in the sequential interaction with cadherin and aminopeptidase-N receptors in Manduca sexta. The Journal of Biological Chemistry, 281(45), 34032–34039.

Ferre, J., & Van Rie, J. (2002). Biochemistry and genetics of insect resistance to Bacillus thuringiensis. Annual Review of Entomology, 47, 501–533.

Morin, S., Biggs, R. W., Sisterson, M. S., Shriver, L., Ellers-Kirk, C., Higginson, D., et al. (2003). Three cadherin alleles associated with resistance to Bacillus thuringiensis in pink bollworm. Proceedings of the National Academy of Sciences USA, 100, 5004–5009.

Xu, X., Yu, L., & Wu, Y. (2005). Disruption of a cadharin gene associated with resistance to cry1Ab d-endotoxin of Bacillus thuringensis in Helicoverpa armigera. Applied and Environmental Microbiology, 71, 948–954.

Soberon, M., Pardo-Lopez, L., Lopez, I., Gomez, I., Tabashnik, B. E., & Bravo, A. (2007). Engineering modified Bt toxins to counter insect resistance. Science, 318, 1640–1642.

Pardo-Lopez, L., Tabashnik, B. E., Soberon-Chavez, M., & Bravo-De, L. M. (2010). Suppression of resistance in insects to Bacillus thurigensis cry toxins, using toxins that do not require the Cadherin Receptor. Patent application publication US 01

Abro, G. H., Syed, T. S., Tunio, G. M., & Khuhro, M. A. (2004). Performance of transgenic Bt cotton against insect pest infestation. Biotechnology, 3, 75–81.

Adamczyk, J.J., Adams, L. C., & Hardee, D.D. (2000). Quantification of Cry1Ac delta-endotoxin in transgenic Bt cotton: correlating insect survival to different protein levels among plant parts and varieties. In: Proceedings Beltwide

Cotton Conference, San Antonio, USA.4-8 January 2000,Vol.2, (pp.929-932).

Arshad, M. A., Suhail, M. J., Arif, & Khan, M. A. (2009). Transgenic Bt and non-transgenic cotton effects on survival and growth of Helicoverpa armigera. International Journal of Agricultural Biology, 11, 473–476.

Ashfaq, M., Young, S. Y., & Mcnew, R. W. (2000). Development of Spodoptera exigua and Helicoverpa zea (Lepidoptera: Noctuidae) on transgenic cotton containing Cry1Ac insecticidal protein. Journal of Entomological Science, 35, 360–372.

ISOLATION AND IDENTIFICATION OF A SALT-TOLERANT GENE IN TAMARIX CHINENSIS, A GENE THAT CLOSELY MATCHES DEHYDRIN

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Abstract

In this study, the TcPOD1 gene was identified and isolated from the *Tamarix chinensis* plant. *Tamarix chinensis* is a halophytic plant native to China that is prolific in many different soil environments, including soils of high salinity. However, the salt tolerance mechanisms within this species and its relatives are still poorly understood. In previous studies, it has been shown that enzymes involved in phenylalanine metabolic pathway are up regulated under salt stress conditions. This gene is thought to be responsible for salt tolerance, because transcriptome RNA sequencing revealed that this gene is expressed in higher levels when exposed to high salt solutions. There is an ever-increasing need to study these types of genes, because pollution has caused an influx of free salts to exist inside of soil used for agricultural purposes. This free salt causes death of vegetation and desertification. It is hypothesized that the use of transgenic plants containing a gene responsible for salt tolerance could aid in this phenomenon. BLASTP also results from the database showed that this gene is regulated along the phenylalanine pathway. Also, in another BLASTP search, there was a 97% identity match between this gene and the dehydrin gene of *Tamarix hispidia*. This is groundbreaking, because the dehydrin family genes are most active during drought response. Furthermore, the exposure to salt triggers some of the exact same pathways as those responsible for drought response.

Keywords: *Halophylic,* Tamarix chinensis*, dehydrin, Salt Tolerant, Drought Response*

Literature Review

"Rapid industrialization, increased anthropogenic activities, modern agricultural practices and faulty waste disposal methods have increased concentrations of elemental pollutants in the environment, which cause toxicity to all living organisms" (Eapen, et. al, 2005). The world population is steadily increasing, while available land and resources are becoming scarcer. Scientists and world leaders are now faced with various issues concerning available agricultural land, as well as the level of pollution associated with urbanization and land use. The composition of soil is changing due to the introduction of some pollutants into the environment. These pollutants have many side effects, but the one of concern in this study is the change in soil salinity. Soil salinity levels are increasing, so there is a need for further study of plants that have evolved to withstand this effect and maintain homeostatic balance in the presence of high concentrations of salt. One major downfall of increased soil salinity is the inverse relationship between salinity and crop yields. This has major implications for the food industry regarding food availability and prices. Increases in soil salinity can be caused from irrigation, land clearing, and natural occurrence among other things. Identifying and isolating the peroxidase gene, or salt tolerance gene of *Tamarix chinensis* could shed much needed light on the behavior and characteristics of this type of gene and the many mechanisms involved when this gene is expressed.

Tamarix chinensis tends to grow near shorelines and beaches where the soil is sandy and contains high amounts of salt, and has origins in arid regions in Eurasia and North Africa. The *Tamarix* genus belongs to the Tamaricaceae family, and China alone has 16 species of *Tamarix* distributed throughout the mainland. *Tamarix chinensis* is native to the Anuhui, Hebei, Jiangsu, Liaoning, and Shandong provinces (Yang and Gaskin, 2007). Although *Tamarix* is considered a

noxious invasive species in the American Southwest, *T. chinensis* is harmless to its native environment in China, and has many useful applications such as erosion control, windbreak, and honey production, and ornamental purposes (Jiang et al., 2011). The wood can also be cultivated as used for firewood and lumber (Everitt, 1980). The dried twig of *T. chinensis* is also used in Chinese herbal medicine to treat measles without adequate eruption, rheumatoid arthritis, and neuralgic pain in joints. (Liu et al., 2010; Cui et al., 2010). Since some plants and animals have evolved glands that regulate salt balance, identifying, isolating and studying the gene responsible for this characteristic, should provide valuable information about how the salt tolerance gene is regulated (Berry, 1970).

Although plants of the *Tamarix* are native to drier areas, they have been successful at survival in other environments such as semi-arid areas, mountains, wetlands, and beaches (Jiang et al., 2011). Knowledge obtained in this gene study has major implications for a food scientist and environmentalist, because due to pollution, there has been a gradual increase in the accumulation of salt in soil where there had not been before. The accumulation of salt in soil could drastically impact agriculture, because the environment could shift in favor of arid conditions. Potentially, the information from this study could be used to create transgenic plants that persist and thrive in high salt conditions.

Tamarix chinensis belongs to a family of plants termed halophytes. They possess the innate ability to actively excrete excess salt using glands in the leaves. This excess salt would accumulate to toxic levels in non-halophytes (Wilkinson, 1966; Storey and Thomson, 1994). The development of salt-tolerant crops with truly halophytic responses to salinity, in which the consumable part is botanically a fruit, such as grain or berries or pomes is needed. In these plants, Na+ would accumulate mainly in their leaves and, because the water transport to the

fruits and seeds is mainly symplastic, much of the salt would be screened from these organs (Epstein, 1983).

Materials and Methods

Plant of Interest: *Tamarix chinensis*

Image 1. *Tamarix chinensis.*

Sample Preparation

0.1-0.2 grams of *T. chinensis* leaves were cut away from the plant and ground in a sterile mortar and pestle while regularly adding liquid nitrogen to flash freeze the samples and to aid in grinding. This process was done for three samples from the same clonal population.

Image 2. Sterilization of mortar and pestle using fire induced by alcohol.

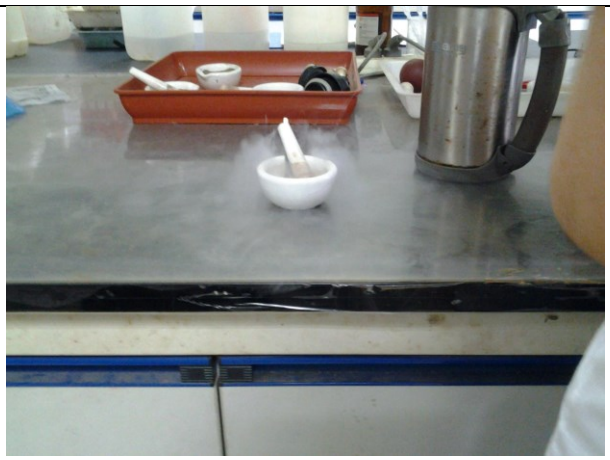

Image 3. Grinding sample leaves.

Salt Response Gene Detection

The three prepared samples were submerged in a salt solution of 2% NaCL for 0.5 and for 1.5 hours. As a control, some roots were submerged in water for 1.5 hours. Following treatment, these roots were flash-frozen in liquid nitrogen and stored at -80°C for future use. A sample from each of the three groups of roots were sent to Novogene for Illumina transcriptome RNA sequencing to detect the level of gene expression in response to increased time submerged in the 2% NaCl solution. Putative salt tolerance genes were detected based on their differential expression.

Total RNA Extraction and Purification

Total RNA was extracted from the leaf powder using the Qiagen RNeasy Plant Mini Kit. DNase I was used to digest any residual DNA, subsequently purifying the RNA. This was followed by phenol chloroform extraction and isopropanol precipitation of the total RNA. The pellets were dissolved in 20µl RNase free water and RNA concentration was determined with the

NanoDrop 2000c Spectrophotometer. Quality and size of the RNA was checked via electrophoresis in a 1% agarose TAE gel.

Primer Design

This Illumina sequence obtained during salt response gene detection was the basis for primer design and subsequent gene sequence alignment.

Primer Name	**5'-3' Sequence (Takara Kit)**
Outer Primer	TACCGTCGTTCCACTAGTGATTT
Inner Primer	CGCGGATCCTCCACTAGTGATTTCACTATAGG

Table 1. Primers.

3' RLM-RACE Nested PCR

Rapid amplification of cDNA ends (5'-RACE) is a polymerase chain reaction-based technique developed to facilitate the cloning of sequence from the 5'-ends of messages. Benefits include this kit's ability to select full-length RNA (no rRNA or degraded mRNA). Full-length mRNAs were selected by treating total or poly(A) RNA with calf intestinal phosphatase (CIP) to remove the 5'-phosphate from all molecules which contain free 5'-phosphates (ribosomal RNA, fragmented mRNA, tRNA, and contaminating genomic DNA). Full-length mRNAs were unaffected. The RNA was then treated with tobacco acid pyrophosphatase (TAP) to remove the cap structure from the full-length mRNA leaving a 5'-monophosphate. A synthetic RNA adapter was ligated to the RNA population; only molecules containing a 5'-phosphate, the uncapped, full-length mRNAs, accepted the adapter. Random-primed, reverse transcription reactions and nested PCR were then performed to amplify the 5'-end of the specific transcript.

Gene Cloning

The purified 3' RACE PCR product was ligated into a pMD19-T plasmid. The plasmid was then transformed into Top Ten E. coli cells via heat shock. The cells were then grown on an LB plate with ampicillin to enable the growth of only cells that contain the antibiotic resistant plasmid that was constructed. However, the cells did not grow as supposed. Where cells were to be, there was no microbial growth on the plates. Multiple colonies from each plate were supposed to be selected and cultured in liquid LB with ampicillin. A PCR of the liquid bacterial cultures was then supposed to be run and the sizes of the bands determined on a TAE 1% agarose gel. Liquid cultures possessing the correct bands were then supposed to be sent for plasmid extraction sequencing (Bejing Genomics Institute, China) to confirm that the correct gene was amplified.

Bioinformatic Analysis

Content	**Software**
1. cDNA blast	http://www.ncbi.nlm.nih.gov/
	http://www.ncbi.nlm.nih.gov/
2. Protein primary structure analysis	http://us.expasy.org/tools/protparam.html
	http://web.expasy.org/protscale
3. Secondary structure prediction	https://www.predictprotein.org
4. Tertiary structure prediction	http://swissmodel.expasy.org/
	TASSER Serve
5. Transmembrane signal analysis	http://www.ch.embnet.org/software/TMPRED_form.html

Table 2. Bioinformatic Analysis Programs.

Results

Salt Response Gene Detection

Using transcriptome RNA sequencing, the TcPOD1 gene was found to have higher expression during high salt conditions and it was found to exist along the Phenylalanine metabolic pathway.

Total RNA Extraction and Purification

Below, two bands can be observed at 18S and 28S in the lane next to the DNA ladder. These two bands are indicative of the correct extraction and purification of total RNA.

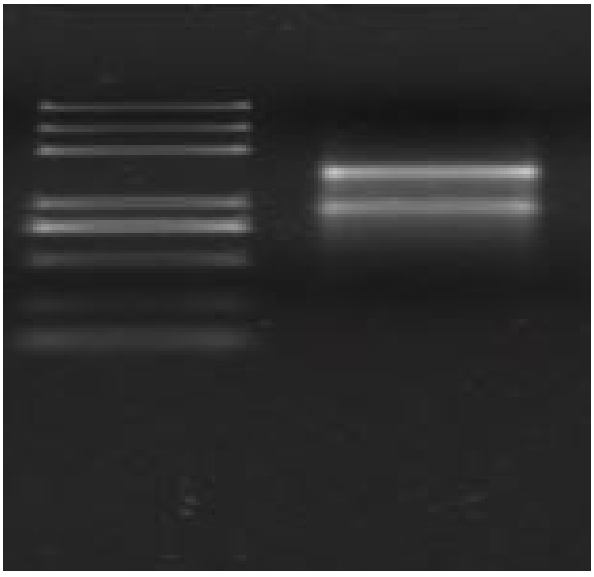

Image 4. 1% TAE Gel.

3' RLM-RACE Nested PCR

Multiple samples were run on the same gel; however, the only one that is usable for the next step is the bright band in lane 1. This band was cut out and used for the next step.The band of the successfully amplified gene cDNA sequence was cut out of the gel and recovered using a Generay Biotech DNA Gel Cleanup Kit.

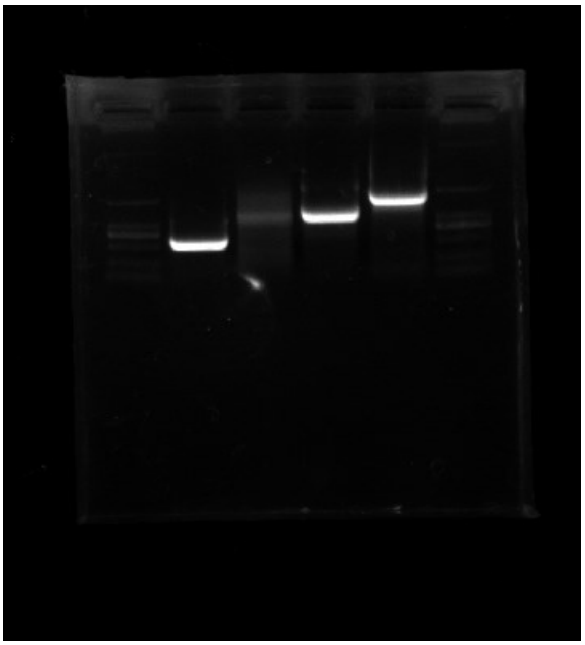

Image 5. cDNA sequence of interest in lane 1.

Gene Cloning

The annealing of the DNA containing the gene of interest with a Ti plasmid was successful. However, upon transforming the vector into *E. coli*, there was no growth observed on the LB Agar plates. This step needs to be repeated, because the *E. coli* may have been preserved too long or the medium may not have been fresh enough to provide proper conditions for microbial growth.

Bioinformatic Analysis

BLASTP: cDNA (CDS) sequence

>lcl|Sequence 1 ORF:268..1008 Frame +1

ATGGCAGACGAGCAAAACTGCCAGCACCACCAAGCTGAGAAGATGAAAACTGAGGA
GCCTGCTGCCCCGCCTATGGAGAGCACCGATCGCGGTCTTTTTGATTTTGGGAGGAA
AAAAGAAGAAAGTAAGCCTCAAGAGGAGGTAATTGTTACCGACTTCGAGAAAGTTC
AAATCTCTGAAGCCGAGGAGAAGAAGCATGGTATCGGTGGTGGTGTCTTAGAGAAA

CTCCACCGAGCTGATAGTTCATCATCTAGTTCTTCAAGTGATGAGGAAGGAGCTGAC
GAAGAAAAGATGAGGAGGAGAAAGGAAAGGAAGGAAAAGAAAGGCATTAAAGAA
AAGCTGAAGGAAAGAATCCCAGGACACAAGAAAGAGGGCGAAGAAAAGAAGCACG
AAGAAGACACAGCTGTGCCCATTGAGAAATGTGAGGAAGAAAAGGAGAAAATCCAT
GTTGAAGAAGCAGTCTATTCAGAGCCTTCTCACCCAGAAGAAAAGAAAGGAATCCT
GGGCAAGATCAAGGACAAACTCCCTGGTCATCAAAAGAAAACGGAGGATGTTCCAT
CCCCGGCCGCTGCTCCTGTACCACCCGCTGATCTGGCCCAGGCACCGGAGTGTGAGG
CAACCCAGGCCGACGCTGAGAAGAAGGGTTTCTTGGACAAAATTAAGGACAAAATT
TCTGGGTTCAGCTCCAAGGGTGACGAGGAGAAGAAGGAAAAGGAGAAGGAGTGTGC
ATCTACTACCTAA

Protein sequence:

>lcl|Sequence 1 ORF:268..1008 Frame +1

MADEQNCQHHQAEKMKTEEPAAPPMESTDRGLFDFGRKKEESKPQEEVIVTDFEKVQIS
EAEEKKHGIGGGVLEKLHRADSSSSSSSSDEEGADEEKMRRRKERKEKKGIKEKLKERIP
GHKKEGEEKKHEEDTAVPIEKCEEEKEKIHVEEAVYSEPSHPEEKKGILGKIKDKLPGHQ
KKTEDVPSPAAAPVPPADLAQAPECEATQADAEKKGFLDKIKDKISGFSSKGDEEKKEKE
KECASTT*

BLAST for Homology

BLASTP showed only one match, which was a **97%** identity match with the *Tamarix hispida* dehydrin family. This is significant, because substances in the dehydrin family are triggered during drought response. It will be interesting to see what similarities lie between plant hormone signaling via the phenylalanine pathway and the pathways associated with dehydrin drought response. This connection leaves many unanswered questions.

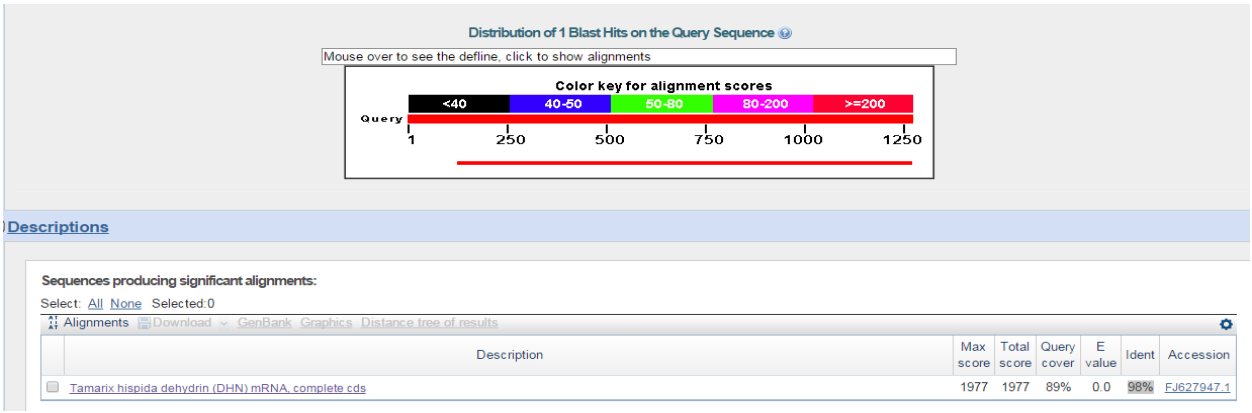

Figure 1. BLASTP Result showing Homology.

Primary Protein Structural Analysis

Extinction coefficients:

Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.

Ext. coefficient 54610
Abs 0.1% (=1 g/l) 0.908, assuming all pairs of Cys residues form cystines

Ext. coefficient 53860
Abs 0.1% (=1 g/l) 0.895, assuming all Cys residues are reduced

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 36.53
This classifies the protein as stable.

Aliphatic index: 99.57

Grand average of hydropathicity (GRAVY): 0.261

Secondary Protein Structural Analysis

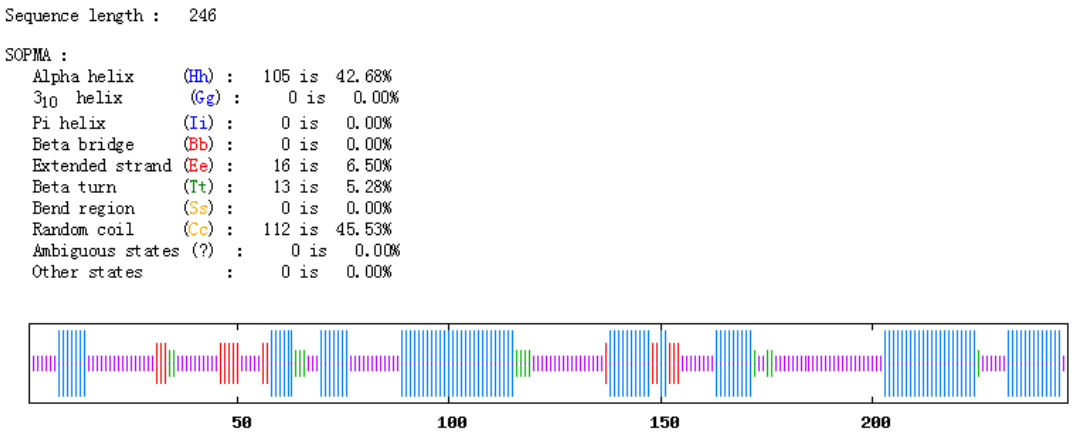

Image 6. 2' Protein Structure.

Tertiary Protein Structure Prediction

The Swiss-model software had no result for the tertiary structure prediction. There was no match in the database for this particular protein.

Transmembrane Prediction and Signal Peptide

These results were inconclusive, because the database did not have any prerequisites to match the protein sequence to. It can be suggested that the protein is not a transmembrane protein. In addition, there was no signal peptide match in the database to the protein sequence as well. This could mean that the protein is an intracellularly binding protein.

Discussion

In conclusion, this process was successful aside from some setbacks. Parts of this experiment need to be repeated to rule out human error as the source of discretions in any of the data. The Oligo 6 software was instrumental in designing the experiment. Also, the experimental

design should be scrutinized more adamantly to insure that no contamination is occuring in any of the protocols, which could result in false positives in the gel electrophoresis.

More needs to be done to see if there is a connection between the dehydrin pathway and the peroxidase pathway (phenylalanine), and more work needs to be done to understand the pathway responsible for plant stress and drought response. The overall identity of this gene sequence was unknown, because no explicit matches were found on the online databases. Eventually the gene cloning will transition to the use of these salt response genes for the purposes of transgenic plants.

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Literature Cited

Berry W (1970) Characteristics of Salts Secreted by Tamarix aphylla. American Journal of Botany 57(10): 1226-1230.

Cui BS, Yang QC, Zhang KJ, Zhao XS, You ZY (2010) Responses of saltcedar (Tamarix chinensis) to water table depth and soil salinity in the Yellow River Delta, China. Plant Ecol 209:279–290.

Eapen S., D'Souza S.F., *Prospects of Genetic Engineering of Plants for Phytoremediation of Toxic Metals*, Biotechnology Advances 23 (2005) 97-114.

Epstein E: Crops tolerant to salinity and other mineral stresses. In Better Crops for Food, Ciba Foundation Symposium. Edited by Nugent J, O'Connor M. London: Pitman; 1983:61-82.

Everitt BL. 1980. Ecology of saltcedar: a plea for research. Environmental Geology 3(2): 77–84.

Jiang Z, Chen Y, Bao Y (2012) Population genetic structure of Tamarix chinensis in the Yellow River Delta, China. Plant Syst Evol (2012) 298:147–153 DOI 10.1007/s00606-011-0532-1.

Ranjan Kumar Sahoo, Mohammad Wahid Ansari, Madhusmita Pradhan, Tushar K Dangar, Santanu Mohanty, Narendra Tuteja (2014) A novel *Azotobacter vinellandii* (SRIAz3) Functions in Salinity Stress Tolerance in Rice. Plant Signaling & Behavior 2014; 9:e29377.

Storey R, Thomson WW (1994). An X-ray Microanalysis Study of the Salt Glands and Intracellular

Calcium Crystals of *Tamarix*. Annals of Botany **73**: 307-313.

Wilkinson, R. "Seasonal Development of Anatomical Structures of Saltcedar Foliage," Botanical Gazette, Vol. 127, No. 4, 1966, pp. 231-234.

Yang Q-E, Gaskin J (2007) Tamaricaceae. In: Wu Z-Y, Raven PH (eds) Flora of China, vol 13. Science, Beijing, p 63.

Cultural Experiences

HOLLIS DAHN

Trip and Cultural Report
REUG China 2015

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This year's traveling in China as part of the REU-China program was a deeply enriching experience. I ventured to Shanghai, Nanjing, Beijing, Chengdu, Wuyishan, and finally Shennongjia, with Wuyishan and Shennongjia being my main study sites. In the larger cities, and in transit between them, I found many new sights and opportunities that I had not in previous travels. In the cities I learned about how modern Chinese herpetological research is conducted, made new colleagues and friends, and sampled cultural staples of these different regions. During the weeks spent conducting my research in the more remote locations, I was able to glimpse an entirely new side of Chinese life. There we interacted with farmers, villagers, and their local customs on a daily basis. I spoke to local people about our research and the animals that live around them, and I got to witness first-hand the impact of local management decisions on Chinese wildlife. Being able to interact with the people of China in both urban and rural settings was invaluable for informing my understanding of Chinese cultural and perspective for my future research.

As of this summer, I have studied the Mandarin Chinese language off and on for a total of seven years. It began with an introductory course during my freshman year of high school and continued into my undergraduate career. My level of fluency increases dramatically with every visit to China. This year, I was able to put that knowledge and experience to use translating for colleagues as I learned more with each interaction. For example, as my mentor, Kevin Messenger, and I were walking a remote trail looking for snakes we came across a farmer and his herd of goats. I was able to communicate with the farmer that we were there to study the snakes on his land. We had a short conversation with him and then went on our way. As we parted, I told the farmer "我们走." (pronounced "wǒ mén zǒu" translating to "we'll go") and he responded "慢走." (pronounced "màn zǒu" meaning "go slowly"). His response struck me as very kind and reflected the generally open and courteous sentiment we had been experiencing with people all over China.

Beijing

Kevin and I visited the capital city of China before our major field research sites in order to allow me the chance to train in safely working with snakes in the field. While there, we stayed at the home of a friend of Kevin's, Scott Lupien. Scott has been conducting business in China for nearly a decade. From him I gleaned much about the struggles of living and conducting business in China as a foreigner. Scott introduced me to his client Wulong. Wulong had met Kevin in

previous years and had been particularly impressed with Kevin and his research. Wulong treated us to several extravagant Beijing-style meals including Peking duck and fish hot-pot. At meals with Wulong, we toasted each other in typical Chinese fashion and Wulong expressed his eagerness to see Kevin and my research progress. He even went as far as saying that, after learning about Kevin's research and his appreciation of reptiles and amphibians, Wulong will no longer eat snakes. This is despite snakes being, as he said, Wulong's "favorite animal to eat." I found this change of heart to be very encouraging.

My training in Beijing consisted of searching for and capturing Gloydius brevicaudus on a remote, ruined section of the Great Wall of China. We took all day to make the strenuous hike up and down steep, crumbling sections of the wall. We safely caught five G. brevicaudus that day. The snakes were photographed and then released where we had found them on the wall. Finding that many snakes in a relatively small area in one day speaks volumes about their high abundance in the area. That and the ruins of the wall led me to consider the deep roots of Chinese culture and its interactions with the wildlife of China. I wondered how the laborers building the wall dealt with the venomous snakes.

Chengdu

After leaving Beijing, Kevin and I visited Chengdu for one week so that I could take morphological measurements from the specimens at Chengdu's Museum of Herpetology. Chengdu is widely known as the birthplace of modern Chinese herpetology. We were hosted there by another friend of Kevin's, Wang Xiaohe. Xiaohe is a graduate biology student at the Chengdu Institute of Herpetology. We had a wonderful time in Chengdu thanks to her hospitality. Xiaohe showed us where she works in the institute and helped us gain access to the samples we needed to measure. She also took us out to try the Sichuan-style cuisine of Chengdu.

I spoke with Xiaohe at length about what it's like to conduct graduate research in China. She told me about various aspects of collecting data, writing, and publishing papers in China that I had never heard of before. Wang Xiaohe leant me a new perspective on academia in China and how it is changing.

Nanjing

While in Nanjing I experienced another new facet of China: student life. I lived in a student dorm on the Nanjing Forestry University campus for many weeks while going to classes, eating at the cafeterias, and exploring the city. One evening I was even able to visit Purple

Mountain near campus to collect toads for my research. I met many students at NFU from all over the world.

Wuyishan NNR

In Wuyishan, as in most places, we were often treated with enthusiastic hospitality, often being invited into peoples' homes for tea and conversation. The villagers were very curious about us and our purpose for being there. I was able to communicate well enough to satisfy some of their curiosity and learn a bit myself. A common first question was "What country are you from?" to which I usually answered "Take a guess." in a joking manner. Top guesses for our homeland were Australia, France, and Canada. I had no doubt that we were the first Americans that many people we'd met had encountered.

We visited the home of a tea seller in a village known as Seven Li. I ended up buying three canisters of it to bring home. The tea was grown in Wuyishan (probably in the same fields I was surveying for research) and was dried and oxidized over an open fire that gave the tea a strong smoky smell and flavor. I'm a fan of black tea and this is by far the favorite of my collection.

In that same home of the tea seller, I was surprised to see an image of Jesus on the rear wall. Later I noticed more and more Christian imagery in the area. I'm told that Wuyishan was the origin of tea for the western world. That is, the first tea brought to Europe was gathered in Wuyishan. I wonder now if it was done by missionaries.

Shennongjia NNR

It was very interesting to hear what the villagers had to say about their local wildlife and its management with regard to their own rapidly-expanding construction projects in Shennongjia. There Kevin and I witnessed a slightly unsettling instance of local management decisions affecting that wildlife. As part of my research, we spent many evenings surveying one particular icy-cold stream in search of a certain species of frog. During these surveys we also commonly encountered fish, toads, arthropods, small mammals, and (a personal favorite) numerous salamanders in and around the rushing water. On our last evening at that location, Kevin and I arrived at the stream to find these same fish, toads, arthropods, and salamanders either dead or exhibiting convulsions. We gathered up and photographed each species we found affected (apparently only those of small body size that had been in the water). As for the cause, there was a road near the stream that was being expanded at the time. Three hours before we had arrived at

the stream, Kevin and I had heard an explosion from the construction site where they were clearing away a small section of the mountain. The choice to build that road so near to the stream had resulted in the devastation of that stream's inhabitants that day. It was unclear to us how many times that had happened or how capable the stream's ecosystem was in recovering from these events, but the simple destruction was plainly evident. While this was unfortunate, I'm very grateful to have been present to witness and help record the event for science.

While that event illustrates part of the negative aspect of studying wildlife near remote villages, in Shennongjia, and elsewhere in my travels, we did encounter what I would consider positive changes. This was especially evident in the mindsets of those who had met Kevin in previous years. Kevin was instantly recognizable to many of the communities who had heard about him visiting every year and heard that he is interested in snakes, but specifically wanted to see them alive and in their natural setting. I believe that Kevin's demonstration of valuing wildlife in situ has gone a long way in instilling that sentiment among the villagers.

To witness both the good and the bad in small Chinese villages interacting with local wildlife was a treasured privilege for me. I value those conversations I was able to have with them very highly because, from a conservation perspective, it is these villagers that it's most important to understand. The people that are going to have the most dramatic impact on the wildlife we study are those that live there. I believe this experience will dramatically affect my future career as a biologist.

Future directions

My time in China this year has exposed me to so many new facets of the country and its people that I can't help but want to return to see more. I hope to continue my research on the wildlife of China into the future, interacting with local friends and villagers along the way. I am both excited and nervous for what China's future might look like. As the needs of the country's economy grow and standards of living rise, more and more pressure will undoubtedly be placed on its natural resources.

However, being able to make friends and interact with people in China has boosted my optimism. I look forward to seeing what solutions the researchers of China develop for these issues and to seeing how the minds of smaller communities might be swayed towards a more sustainable path.

JEREMY ISABELLE

Trip and Cultural Report
REUG China 2015

Alabama A&M University
Normal, Alabama 35762

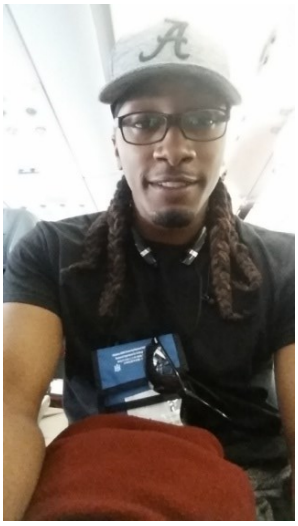
Hello, my cultural experience came a little earlier then everyone else's. My experience was meeting my roommate John. John is a graduate student from the state of Florida who's still wondering how he was led to Alabama A&M University. He has a lot of family from Haiti so he can speak Haitian and he has also lived in France so he knows French. Back home school wasn't the only thing that he was facing on day to day bases.

There were parties, friends, family and a lot of fun. I mean he was living in Miami so what do you expect! He found himself talking to a stranger about going back to school at this random University in Normal, Alabama and they both made their minds up to go. Well John traveled and enrolled but the other guy was nowhere to be found…That's strange right?

John and I met for the first time when we started our REUG program because we were roommates. I found it very challenging at first when I was trying to understand what he was saying due to his Florida accent but I caught on! In fact, I'm probably the only student who knows what he is saying most to the time. This was a cultural experience that would last forever

because we became very close and good friends throughout our trip. My second experience was actually flying from the United States to another country.

Not many people can say they took a flight and actually witnessed 3 or 4 different sunrises while advancing forward in time. That was amazing and crazy at the same time. What made the flight so different and special to me is the fact that each seat had a flat screen on the back of it. The flat screen had movies, shows, cartoons, games and music on them. I wish I could personally say thanks to whoever thought of that because it took the place of sleeping and boredom.



After a 13 hour flight we then landed at the Shanghai airport. I've never felt so watched in my life! People were looking, pointing and there were cameras everywhere. After arriving to the hotel and going out to eat, my perception of Chinese food changed. It seemed as if the Chinese food we eat in America was like… the reject food. My classmates and I found ourselves looking eye to eye at animal heads, feet and everything else. The **third wave** was the food. After that first dinner we knew what we were facing for the rest of this internship.

We weren't prepared for this at all!! So the next morning we all went down to have breakfast in the hotel and it was Chinese food! Ok, I was hoping this wasn't going to be an everyday thing, if so I was going to starve. I soon find out the breakfast was too bad, they had a few things that was pretty good, but I was still upset. After leaving the hotel and going downtown to visit the Pearl Tower, I came to my senses that I'm surrounded by people who I

can't communicate with, at all! I caught myself a few times asking questions and just being stared at…..

I couldn't enjoy the history that was presented inside of the pearl tower because one of our "Instructors" was rushing us… which I will never understand, crazy right? One thing I liked and enjoyed about Shanghai was the people. Everywhere we went people were nice, smiling and taking pictures with us. They seem to be very nice and friendly, I guess because they are used to foreign people, but that would be backwards right?

While we were inside of the tower we ran into a group that was visiting from Nigeria, they were pretty friendly and excited to meet people from the U.S.A. It really felt like we were a part of a scene out of "Coming to America" or something. I never expected to actually have to tell someone that I was from the United States of America. The question of where we were from was so unexpected, for the first time in my life I almost forgot where I was from.

Not really, but I felt like I had to think about it. That was a true game changer! "I'm from the United States of America" proudly…. Haha! For some reason, they made us feel like we were special. That was a very humbling experience.

We later had dinner at the hotel and it really wasn't that bad. I guess I just had to get over the fact that I wasn't going to eat anything I was use to. Of course rice was served but they also had a few different dishes like chicken, duck and shrimp. I didn't really care too much about the duck but it was my first time trying it so that was awesome! It wasn't all that good to me but I'm thankful for the experience.

As we all got together and went downtown to shop, I didn't feel as if I was in a new country. Downtown Shanghai reminded me of downtown New York. The lights, people, cars, shopping malls were all familiar. I even enjoyed talking to the street hustlers. One thing I had never experienced was the malls being eight floors, which blew me away! So dope! We took so many pictures with the people walking on the streets. People taking pictures were one of the main things talked about but you don't think about it until it really happens. My hair even got pulled a few times but it was all good.

The first time I looked back and almost got mad but I had to realize where I was. The next exciting moment was arriving to the Nanjing Forestry University campus where we would be staying. I didn't know what to expect nor did I have any ideas of how I thought the campus would look. The campus life was real different and boring at times but I had my best moments when my classmates and I were all together talking, playing cards, joking and leaving campus. I really had a great time with all of them (John "John Gotti", Jessica "Red", Eboney "Eb", La'teasha "Teasha" and Michelle "Chelle". These guys were great!

Do Something!

I remember one night we all planned to go to Walmart but John and I got left… We woke up and were like where is everybody? So we decided to get up and so something. We walked all over campus and found things we wouldn't have found if we would have stayed in the rooms. As we were walking to notice a huge basketball court, it had to be about 30 basketball goals inside. After finally finding a way inside, we asked one of the Chinese students could we shoot with him and we did…. Well we didn't actually ask…but the point is we played ball with him! That was a pretty cool moment, playing basketball with a guy who doesn't speak any English and he was good! HAHA! We left the court and also found a pool hall.

We all went out and had fun multiple times during the trip. We met another group who were visiting from Georgia and started kicking it with them. They came to the "Boom Boom Room" a few times to play cards and talk with us. They were leaving in a few days so we all went out for dinner with them and ended eating and going out after. We found a karaoke bar on the way back to the bus stop and stayed all night!! H

HAVE FUN!!

During the time we spent on campus, going to class, meeting with our classmates and mentors we all needed a break! We decided one day that we would work on our research projects during the day and have a party at night, since we were off the next day. The party was banging!!! Everybody had a really good time and enjoyed relaxing from work.

1912

I had a very fun group of students who like to have fun and do their work. If you like to go out and have fun then 1912 is the place you want to go. This is a strip of clubs and bars and its FREE to enter! The drinks may be expensive but you can always have something to drink before you go.

The research project

I really enjoyed working with Emma, who's a graduate student. Surprisingly she spoke English good enough that I would understand her. I felt really lucky because some of the other students had a hard time working with their graduate student. This was my first time ever doing a research project and I didn't understand it at first but it grew on me. I wish I had more time to do more research but our time was limited. Emma was really nice, even though I was pressing for time and we didn't have a lot of data ready, she never stressed about rushing the research. That allowed me to relax and try to get as much info from her as I could to end this internship on a positive note.

The program

There were positive and negative aspects throughout this internship. I really think the communication between Alabama A&M University and Nanjing Forestry University could have been much better. We as students were put in different situations when we got to the campus of the Nanjing Forestry University that could have been avoided. Some of us didn't know what our research would be on, we didn't know who we were working with and one student had her whole project switched from what she was told it would be on. Those situations could drive a student to think and react negatively, but our students handled the pressure very well and completed their research the best way they could. This program is very challenging, personally, mentally, physically and spiritually but at the end it's all worth it!

CHRYSTAL LI

Trip and Cultural Report
REUG China 2015

Alabama A&M University
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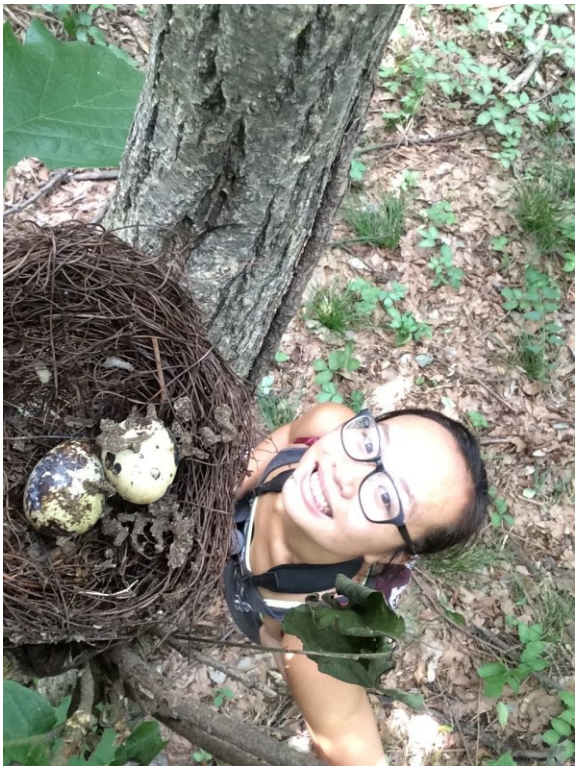

Having lived in and attended high school in China, my cultural experience was different from everybody else's since I was pretty familiar with Chinese culture coming in. I would say the most interesting part of my "cultural experience" was helping people translate things and adjust – seeing what they were uncomfortable with or found different made me more aware of the differences between Chinese and American culture.

A big adjustment I noticed everyone had to make was eating local food. A lot of the other students weren't used to eating rice with everything nor were they used to the variety of dishes served with rice. The first few days, when we were eating at restaurants or in more formal

settings, we were served a lot of tea. Tea is a huge part of Chinese culture, and some people were definitely not used to it. To be fair, Chinese people do drink a lot of tea, especially if you don't like it. I think the stepping stones to enjoying Chinese food were foods that were similar to things that we ate at home in America, such as the fried chicken in the cafeteria or the 'shao kao' barbeque that we went to during one of the first weeks.

Wanting the other students to enjoy their time in China, I tried to introduce them to foods that I liked and thought they might enjoy. For breakfast I bought 'pineapple bread,' which is sweet bread with some kind of flavored crust on top that looks a little like the scales on a pineapple. One of my suitemates tried some and said it tasted like sweet bread from home, and bought her own and ate it for the rest of the trip. 'Hong shao' beef noodles were a hit after I recommended them to someone in the cafeteria, and they became a staple lunch food. Another food item I found a lot of people raved about was Chinese yogurt, which is more liquid and drinkable than they sell it in the States and comes in different flavors. Some things I love myself that other people didn't like are porridge, stinky tofu – or tofu in general – and mochi, although mochi ice cream was okay.

Some cool foods we ate while out were this spiral potato thing, which is a fried potato cut in a spiral on a stick. At Confucius Temple we saw people eating them and spent half the night trying to find one because they were so cool. We also went out with someone's lab mates to eat hot pot, which everybody loved because hot pot is the bomb. Back to cafeteria food, I noticed that people really liked the sliced potato dish. One thing I found about food almost everywhere that we ate was that the portion sizes were hilariously large. We always had way too much rice or too many dishes or just generally too much – it was almost customary to leave leftovers. I think part of this has to do with a custom where not being able to finish your food shows that your host has been extremely generous and filled you with so much food you couldn't eat any more – but that's just an idea.

Of course, living in Nanjing was also very cool because there were a lot of historical places to see. We were lucky to be here for the dragon boat festival, and while I didn't go see the dragon boat races, our group went out to Confucius Temple later that night. It's a beautiful little shopping district with ancient architecture and there were dragons on display at the center area. We walked around and bought some souvenirs and food. We also went to see the Nanjing museum, which was enormous and had all sorts of artifacts from ancient China all through the

dynasties. There were more things than we were able to look at at the museum, but we spent a few hours there and saw ancient Chinese armor, tools, pottery, and so on. There was also a lot of art at the museum. Another place to go to was Purple Mountain, which I went up frequently for my project, but only once went up to the top and to see the mausoleum.

A place that our group frequently went to at night was Xinjiekou, the urban hub of the city. It was a large shopping district with multi-story malls and enormous stores lining the streets, and at the center of it was an underground flea market-type shopping area called Fashion Lady. Fashion Lady is your typical Chinese market, packed floor by floor with a grid of booths selling similar wares – mostly women's clothing for as cheap as you could bargain for. Certain stores or alleys specialized in hats, shoes, jewelry, toys, children's clothes, and so on – there was no end to the variety of things that could be sold. You could also buy food, get a manicure, and play games. My favorite booths in Fashion Lady were an arcade filled with claw machines with weird toys, an old lady selling pets on a corner – hamsters, fat rabbits, turtles, even a squirrel – and a trinket store on the bottom floor.

Xinjiekou was where people went when they wanted to eat Western food. The first few days, people went almost every day to buy things at Walmart and eat Pizza Hut or McDonalds for a taste of home. Pizza Hut in China is not like Pizza Hut at home, however, and is a (relatively) fancy sit-down restaurant with waiters, metal cutlery and a full menu of food and drink. We also found a Burger King and Subway in subway basement. I frequented Happy Lemon, an Asian drink stall, Oasis, an American restaurant/bar with excellent salad and pizza, and a gelato store whose name I can't remember in the basement of a fancy mall called Deji Plaza.

Back on the topic of food, there were a few restaurants that we found online and tried out. The first was Ciao Italia, an authentic Italian restaurant on a little back street in Nanjing. I shared a salad, ravioli and pizza and it was some of the best Italian food I've had in China. Another that wasn't new for me but was new for other people was Ajisen Ramen, which is one of my favorite chains in China because I love ramen. Abreeotta didn't want to try the ramen but got some really flavorful fried rice, which she loved because, as she said, it "tasted like jambalaya." So that was good. We also went to another restaurant/bar called Jimmy's, which we were recommended by an American girl we ran into at Xinjiekou, and they had all manner of greasy American food and

a decent selection of beer (or, more than 2). I ordered the "Tropic Lava Thunder Burger" which came with a grilled pineapple.

Living semi-independently as a student in NFU was the biggest new experience for me. The last time I had to hand wash laundry on a regular basis was at summer camp, also in China, nearly ten years ago, and then I barely did it. I think neither I nor the other students were used to the bathroom, where the shower and sink were in one big room and, while I knew it was clean enough, just always looked grimy. The toilets had one Western and one squatting toilet, which is the standard in most parts of China. Another thing that I know but always forget about – in China, you need to bring your own toilet paper. Since it was always raining outside and extremely humid otherwise, I dried my clothes in my room on a line hung over my bed. The bed had extended bars with loops at the end that I am pretty sure were put there for that exact purpose.

Getting around Nanjing was so easy because the subway system was efficient and cheap, and best of all, air conditioned. The only thing that you had to be careful of was that it could get crowded, or very crowded, as it was the night before Dragon Boat Festival when everybody was trying to get somewhere to be with their families. There was no such thing as waiting or being polite on the subway, because you would be pushed out of the way and be unable to make it on or off the train.

One cultural difference that I noticed was that Chinese girls want to be pale, as opposed to how people in the U.S. want to be tan. Having just left a class on a tropical island where I spent a lot of time in the water, I was roasted head to toe and had many people comment on how dark I was. In comparison, whenever it was sunny out people would walk around with parasols to prevent getting tan and many face and skin products had skin-whitening formula in them.

Other things that were fashionable in China were platform shoes and jelly sandals. A lot of Chinese women wear high heels casually, and not just reasonable-comfort short wedges or block heels but full on stilettos and four inch pumps on the subway and in the street. People also liked dyeing their hair lighter shades of brown or having highlights or an ombre tone on the bottoms. I also often saw girl friends walking around holding hands or arm in arm, which girls in the U.S. don't really do. Couples were also more public with their affection as it seemed everywhere I went there were couples holding hands, hugging, whispering in each other's ears, and generally being loving. It wasn't uncommon to see couples or pairs of friends wearing

matching clothes as well. Some wore entire identical outfits, or had the same pants, shirt, hat, or shoes. This was interesting since in the States it's considered tacky to match, and girls will even make an effort to check that they aren't wearing the same outfit as friends.

In the same way that Chinese characters look cool to us, English words look cool to Chinese people and they're all over t-shirts and packaging and all sorts of things. A lot more Chinese people can speak English than Americans can speak Chinese, however, and I think that really helped in getting around and for the other students to get to know their lab mates. I found that even though they weren't fluent, nearly everybody in the university spoke enough English to hold a proper conversation. However, they were very shy and thought their English was worse than it was, and whenever they found out I was around and was able to translate they would stop talking and speak through me, which was a little disappointing. Most people had English names, some of them different from names that you would hear in the U.S. – for example, Caro, Cici, and Peggy who helped me out with my project.

Since a lot of things are censored in China, there are alternatives for almost every sort of social media. Instead of Google, there is Baidu. Instead of Youtube, people use Baidu video or Youku or Sohu. Instead of Twitter, people use Weibo. Instead of Facebook, people use QQ or Renren. But the most popular form of social media that I know of doesn't really have a Western equivalent, and it's Wechat, or Weixin. Wechat is a messaging service like Whatsapp, but also has a newsfeed-style profile and friends feed where you can post updates on your life and pictures like Twitter, and anyone you're connected to can see it. You can message people individually or in group messages and it's a popular tool between friends, families and even businesses. Much in the same way that almost everybody has a Facebook in the States, everybody in China has a Wechat and it's the first thing you exchange when you meet someone new and want to get their contact.

Since China has such a huge population, social media presence is proportionally large and things go viral on an even larger scale than in the U.S. While we were in Nanjing, the "belly button challenge" was popular – a challenge where you had to wrap an arm around your back to touch your belly button from the other side of your body. If you could do it, it was a sign that you were fit and skinny and Chinese social media was flooded with pictures of tiny girls touching their belly buttons. The most popular picture, however, was of a large Chinese man with a beer gut doing the belly button challenge – long arms and flexibility, perhaps? Another

thing that blew up on Chinese social media was an extremely good-looking man in Taiwan who made a living selling bean curd on the street. His Internet fame drew hundreds of people to his stall, and pictures showed a line of young women wrapping around the block at the stall waiting for bean curd man.

Overall it was fun being back in China again and I enjoyed being able to show people new things. I also had quite an adventure acquiring 150 quail eggs for my project. Here is a picture of me with a nest.

MICHELLE PHILLIPS

Trip and Cultural Report
REUG China 2015

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LA'TEASHA WATKINS

Trip and Cultural Report
REUG China 2015

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Hi Folks!

My name is La'Teasha Watkins and I am from a small country town known as York, Alabama. I was 19 years old, and a rising sophomore, when I embarked on my journey to China. It was my first internship as well as my first international experience. It was **AMAZING!** If you are a student and you are considering applying for this internship, I would like to encourage you to take advantage of this wonderful opportunity. You will not regret it. If you are a student and you have been accepted for this internship, congratulations! Within this paper, you will find a personal reflection of how living in China has impacted my life. You will get a glance of a typical day in China. Again, apply/congratulations! And enjoy!

The Flights. Get your double mint ready because this will be a long flight lady and gents. Considering that I had only flown once prior to this internship, I think I handled the 1 hour, 5 hour, and even the 13 hour flight, from America to Asia, pretty decently. Although my ears ached for an extremely long period of time, I found chewing gum a (little) relieving. I would advise you to bring a few snacks for the air plane rides and a pair of headphones. Although 13 hours seems long, by the time you watch 4 movies, time flies by. Also, mentally prepare yourself the night before because you don' realize how much energy is released while sitting down until you've sat down for 19 hours of the day (haha!).

Arrival in China. Living in China was truly an eye-opening experience. I learned so many new things, met so many beautiful souls, and formed lifelong relationships. Upon my arrival to Shanghai, China I remember asking myself "Is this real...am I really here?" Yes it was real and yes I was there… I got off the plane, retrieved my luggage, and followed our tour guide to our destination. I remember the bus ride… There were lots of sky scrapers and huge billboards- everything written in Chinese. It was very beautiful. I remember taking selfies to capture the moments and the joy painted all over my face… And I remember my first time walking the streets in China. It was adventurous. Locals were outside selling fruit and there were a lot of motorcyclists roaming the streets. Fun fact! For every single car there is in China, there are 10 motorcycles, 20 bikes, and **0** traffic laws. Those people will run you over and never look back if you are not careful. And honking the horn every 1-3 minutes is tradition- do not get offended. I repeat do not get offended. I also remember our dinner that night. We ate at a restaurant near our

hotel. We had tea, pork, and soup. Simple right? **NO, WRONG, VERY-VERY WRONG**. The waitress could not speak English, I did not know how to ask for water or ice, and on top of that the food was horrible. Of course, it later grew on me. As I prepared for bed that night, I was reminded of how blessed I was for receiving the opportunity to come to China. I said my prayers and I slumbered. On the second day we went site seeing. The Pearl tower was my favorite because it's where I earned my celebrity status. When I got off the bus to go to the Pearl tower, I literally felt like a super star. All of the people wanted to take pictures with me and it was so unanticipated. I mean, I had heard the rumors, but I did not believe it. I saw for myself that day. We spent 3 days in Shanghai and then we departed.

People. The citizens of China are really interesting and cool. There are 3 things I would like to point out about the natives of China. 1) They can dress really well! Every day I witnessed trendsetting styles. Lots of platform, prints, and polyester. It was like a live fashion show. 2) People in China like to stare but 3) they are really friendly. Now, these two points are joined together because they go hand in hand. Everywhere I went people stared at me. I was not use to that type of attention, but I liked it. I felt like the Chinese people were really fascinated with our nationality and complexions. It was a humbling experience-really. I believe the thing I liked the most about the natives of China would have to be how kind they are. Everyone was so kind to me. If I needed help, there was always somebody available to assist me. Everyone always greets you with a smile. And they are so genuine and caring.

Food (and Tea). Everything comes with rice. No ifs, ands, or buts about it. But relax! It is okay; I promise. Rice will become your best friend in China, unless, however, you are one of those adventurous eaters. In that case, you may think rice is boring. If you are anything like me, and don't eat meat unless you can verify what kind it is, eating in China may be a frustrating experience. That's okay too. Because there are tons of food and drinks to try in China. You are bound to find something or many things that you will like. Speaking of drinks, I hope you like "cha". "Cha" translates to "tea" in English. And no I am not referring to that diabetes in a jug sweet juice we have back home, called tea. I am referring to that very hot, very herbal, very green-tea. It is often served in the place of water or soft drinks. I did not like the tea very much, but on the bright side I did receive a chance to participate in a tea party. We had black tea, green

tea, and red tea. It all tasted like boiled water to me, but at least I made an honest effort to try it. So heads up now- your options will be either tea or alcohol. Take your pick! The peer pressure to drink alcohol in China is not as bad as people say it is. However, if you do like to drink, then good. You will be provided several opportunities to drink collectively and alone because beer is always offered at dinners and it's also sold in the school's store. Overall, I give the food in China a 4 on a scale of 1-5. I am very proud of my friends and myself because we all overcame our fear of trying new foods. In turn discovered I like Pineapple bread!

Places. I really enjoyed our outings! As a group, we often found ourselves traveling to destinations with our mentors, friends, lab mates, and professors from both Alabama Agricultural and Mechanical University and Nanjing Forestry University. We went to the mall, restaurants, karaoke bar, deer reservation, the Brocade Museum, the Poplar Plantation, Confucius Temple and many other places. One thing is for sure, wherever we went, it was always a learning experience. We took the subway back and forth from one destination to the next. We would often stopped and asked for directions. We were 50.01 successful at always receiving directions. We even had a few people lead us all completely to our destination. We also took the bus once, but the subway is just better.

Classes. During our stay in China, our mentors arranged for us all to take a Mandarin and Chinese culture classes. Our first class was the Mandarin class. The Chinese language is very interesting and different from our language. For instance, the Manderin language is spoken in 4 tones and each tone can have a major impact on the meaning of each word, whereas the English language is only spoken in one tone, and the inflection of our voices depict our moods/ expressions and not the meaning of a word. I learned how to say the magic word, "shui"-which means water. The second class held at Nanjing Forestry University was a Chinese cultural class. Chinese culture was influenced by Wuxing- metal, fire, earth, wood, and wind. The Chinese has a famous symbol called the Taji which refers to Yin and Yang. There are also 5 colors that are significant to the Chinese culture-yellow, white, red, ching, and black. Yellow often symbolizes emperor or palace. It is good, hence the Yellow River. Next is white which means pure and clean. The Chinese culture has an expression called the "White Face" which means devious…contradicting, I know! But it's all so cool! Third is the color red. Red is good. It is

often related to big celebrations such as weddings, festivals, and New Year's. Fourth is ching which is a mixture between green and blue. Ching symbolizes life, spring, and it is viewed as a positive color. Lastly there is black. Black means serious, respectful, ceremony, death, sad, unlucky. In Chinese culture, they have a phrase called "Black money" which means dirty money.

Barriers. There were many barriers that I faced in China. I think the biggest thus far would have to be the language barrier. You will find it very frustrating, at first, attempting to relate information to people who don't speak English; that is, if you are impatient. One very important thing to remember is that, English is your first language-not theirs. Remember to remain patient and learn how to use your surroundings to describe what you are trying to say. I suggest brushing up on your charade skills, because body language can be a game changer in China.

Cool Things That Happened. When we went to the mall in Shanghai, we noticed a Forever 21. Immediately, we're all like "oh, cool. A Forever 21. Lets go!". Dude, that Forever 21 had **4** floors. I have never see so many clothes, from one particular store, in one building, in my entire life. I was like, "I have never seen a Forever 21 this big in the States!" Everybody all laughed in agreeance. So if you are wondering what we did next, here it is… We all shopped of course- one American tradition we could not ignore. This goes back to my statement earlier. People in China are trendsetters. I, by the way, am still in awe of that Forever 21.

I also remember the time we went to the mall, Fashion Lady, in Shanghai. So we all decided to have an outing. It was Michelle, Ebony, Abreiota, John, Jeremy, and myself. So of course, the natural thing to do here is to head to the subway. But the guys told us to follow them. They literally led us through someone's house, corner after corner, until we came out on the other side of the school. It was so hilarious and fun because some of us had no clue where they were leading us. Has to be the neatest short cut ever.

Lastly, I remember when we all ventured to this Karaoke bar. This is my most favorite memory because we had an incredible time that night. It was a Tuesday night and we were leaving this pizza parlor, when we came across this spot with lights and loud music. I said "heyyyyy, it's a karokee bar y'all!" So we walked in and we decided we would only stay 20 minutes. Well 20 minutes turned into 3 in a half hours. It's so funny, because when we walked in everyone was

sitting down. By the time we left, people were singing and dancing! They took photos, recorded videos with us, and cheered us on. It was good, clean, wholesome fun. I will never forget it because it was my first karaoke bar experience. We song all American songs and the crowd recognized a few of them. One thing I remember about that night is the Chinese girl who walked onto stage and sung Celine Dion "My Heart Will Go On". That did it for me! It was like time stopped. We had oodles of fun that night!

Goodbye Folks! I hope I have provided you a solid idea of what you can expect while living in China-strange food, good times, and nice people! This cultural experience will always be a cherished one. I got a chance to live in a different culture and experience new foods, lifestyles, and so much more! I am so thankful to God for bestowing this opportunity upon me- as well as the USDA, NIFA, NFU, and Alabama A&M. Best of wishes to you on your journey. ☺

La'Teasha A. Watkins

Some photos of our good times ☺

JESSICA WILLIAMS

Trip and Cultural Report
REUG China 2015

Alabama A&M University
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6 June 2015

A New Perspective: Cultural Experience While In China

To whom it may concern:

Traveling to a new country for the first time can be exciting, fun, frustrating, and overwhelming all at once. As a first time traveler I really had no idea what to expect. Although I had general assumptions about what was to come, I began this journey as open minded as I could. China is where I was traveling to and I was excited to go. Now that I am here and my journey is almost over, I have learned and gained respect for many things from both my home country, the United States, and China. This cultural paper will elaborate on my first hand experiences as a first time traveler abroad.

Before I left for China and had many images and ideas of what it would look and be like. I thought that China would be well developed and have many advances that the United States had never seen. New technologies, cars, and buildings that touch the sky every place we went is all that would be in my view. The latest version of every game system, cell-phone, laptop, and game would be out. I also had the impression that there would be street arts and performers. Dancers, artist, singers, magicians, and musicians would all have their hats laid out for a tip as I walked by. I envisioned friendly people wanting to get to know me and interested in where I was from. China didn't live up to all of my expectations.

Once I was in China I arrived in Shanghai, a city area, there were many of the sky scrapers and lights that I had imagined. The first thing that I observed was that there was no diversity at all in China. I got a lot of stares from on lookers who had no idea what to make of my skin color along with other people on the trip, which have an even darker completion than myself. They took lots of pictures of use almost every place we visited. If there was it was work or tourist related. I was able to stay for the short period of two days before moving to my real destination, Nanjing Forestry University. When I arrived here I noticed the difference in the dull surroundings and forested areas. Having dinner with the President and other officials of the school made me realize that social classes are really important here. With that being said, I also observed that there wasn't very much individuality.

As I continued my journey in this strange land I was able to try new things. One of the first new things I encountered was food of the Chinese cuisine. This could take up its own paper but here is a summary. The rice is always present at every meal here, like bread is to an

American meal. My favorite way the rice dish was fried rice with egg and some type of chopped grilled chicken over in it. I have no formal name but I have eaten it every day and it never fails to satisfy me. Dumplings were a little interesting though they could be good, I just can't get over the way the meat looks in the inside. A steam stuffed bun with sweeten egg yolks served as a tasty treat every once and a while. I also tried very exotic delicacies that are quite common for china. Tofu was among one of those things but did not sit well at all on my stomach. I tried blood duck soup, and toad ovaries unknowingly and felt that my taste buds had been violated after finding out what it was, I didn't like either dish anyway. Talk about adding insult to injury. I did willingly try a snail and I wouldn't eat it again, but if I ever had to survive in the wilderness snails would be the go to. I also tried riding the subway for the first time and quickly learned that it is a quick and easy way to get around. One of the places I traveled to the most is Xhigenko. I also ventured out to other places that I couldn't began to tell you because I can't spell them. I had to google the one place I just mentioned. The final new thing I tried was to learn a new language. I learned I guess what we would call the alphabet but only for the Mandarin language. I tried short phrases but this thing called tones in China made me incapable of saying anything I intended to.

The most wonderful part about being somewhere different is seeing places that mean a lot to that region. Like how Washington D.C. has memorials and Texas has the Alamo. In Shanghai, China there was this great building with a lot of funky architecture called the Pearl Tower. It gave us a full circle view of the city. There a spot in the highest part of the building with glass flooring for you to get over your fear of heights if you had any. There were temples we visited that were like tourist traps because they had shopping in them. I also visited the Nanjing museum about the history of China and got to walk around in many elaborate parts of the museum. It was so big we didn't get a chance to visit each building they had holding different artifacts, arts, and historically valued items. We got to go on a field trip with people outside of our group to the Dafeng Milu National Nature Preserve. It was a very cool place where we got to see Davis deer and poplar tree plantations that have a great economic impact on China.

For some people it's hard to make friends, but in china its super hard to make friends even if your friendly. Especially not being able to speak the native language. It definitely wasn't impossible though. A lot of the people on this trip with REUG had never even seen each other before. Some didn't even attend the same school as the few that did. We were still able to come

together and create bonds that people in a stressful environment can create. Ebony, Michelle, Chrystal, John, La'Teasha, Jeremy, Janine, Hollis, Kevin, and Janine are cool group of people. I also made friends also with people from my lab Diwu Fan, Xiuzhi Li, and Sha Xu. They were all very kind to me and were very willing to help with anything I need inside outside of the lab. One of my most fund memory of a time we had together was when they took me to diner at Pizza Hut. The waitress brought out an appetizer that had black olives in it that, I'm guessing, is not very popular I China. Diwu Fan was intrigued to try one but Xiuzhi Li quickly said no don't do it- it will be gross. Of course, I pushed him to try, which really Li grossed out by just the sight of them. Diwu Fan didn't like them anyway though. I did make a few friends outside of the program. I am not able to get everyone's name so please bear with the nicknames. The first friend I encountered would have to be Laura which I met in Dafeng. She is a painter and had noticed some of my sketches. She says that she has been painting since the age of three, and she showed me some of her work. I felt much honored that she thought that my armature mediocre drawings could be something. The next two people I encountered have been deemed "Come On" and "Pretty Hair". I mean I'm not good with American names in English, so did you really have that high of expectations of me getting their names? Anyhow, they took me to play baton mitten at the campus gym where I was showed up by the athletic Jeremy. Then Pretty Hair came to my dorm and watched some of her favorites shows with me and ordered takeout of McDonald's. I was able to show her some pictures of my family and so did she. We even took silly selfies.

Although there were many fun adventures, like anywhere, I did come across problems. The first problem the most easily to recognize would be the language barrier. Surprisingly, a lot of people here do speak a little English and they are a lot better they want to admit. I was able to learn very, very short phrases that would not help in carrying a conversation at all just hello, goodbye and thank you. I also attended a language and culture class. It was like pre-school level, learning the alphabet and vowels as a basic start. I was able to catch on to the numbers really quickly. Another problem was when I was out shopping people would try to get over on me sometimes. I recall an incident in Fashion Lady, a mall, I was looking at some shoes. There was a sign up clearly displaying 60 RMB or the pair I was looking at, the lady told me 80 RMB. I looked at the sign and pointed and she quickly reverted her statement to 60RMB. Ting about this incident has given me buyer's remorse. Overall, I believe that the hardest problem I had to overcome was getting used to new customs and social norms. There is no such thing as personal

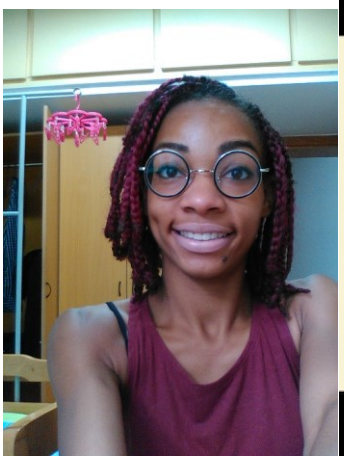
space at all in China and the word no doesn't exist either, take what you want from that. Hand washing and hang drying clothes are not very bizarre to me but not having access to a washer or dryer finitely is very hard to get used to when it becomes a way of life. I talked about the food earlier but adjusting to the foods they have to offer was very frustrating in some cases and I'm sure I won't eat rice for a while when I get home. To overcome this I kept an open mind about the foods that would be introduced as new to me and tried as much as was acceptable to look at. These few problems listed did allow me to grow and have greater appreciation for what is available to me in my home land.

In order to grow you must first go throw a period of discomfort. In China, I was definitely out of my comfort zone in a lot of instances. However, I did learn to make the best of everything I have. Beds in China are not that comfortable overall, but they literally had given us a type of padded mat. Did I like it? No, but it is way better than what the other students get so I was very grateful for my little mat. I also learned to be cordial with people when their behavior is not the best or when I wanted to on my worst behavior. I learned to always look or the silver lining in the sky, because it is truly there if you look for it. Most importantly, I learned that it's more than one way to get something done if you look for them and not get discouraged the most ridiculous hiccups bound to be in any journey you go on.

In conclusion, I did enjoy myself. Even though I was homesick for majority of the time. I was able to see what China was really like. I tried many new things and I'm sure that was the first and last time for some. I got to see things that are important to China as a country. I made new friends and overcame my problems by looking for the silver lining in the sky. Traveling to China was never on my bucket list but I could add it and cross it off. I am honored to have had this opportunity because I don't think there is a large number of people in comparison with the earth's population that can say that they have traveled to China, let alone out of their home country. I hope this paper was entertaining and enlightening as I recalled many experiences and encounters that I endured while in China.

Sincerely, Jessica Williams

p.s. Here are a few pictures. Enjoy.








JOHN WATSCHEN FLEURIMOND

Trip and Cultural Report
REUG China 2015

Alabama A&M University
Normal, Alabama 35762

China, the world fastest growing economy encourages many countries to support their student exploring their amazing culture. It was happiness, when I received the acceptance letter from Dr. Moss to participate in the cultural exchange scholarship program. A scientific research opportunity while we will be visiting different touristic sites. At that point, there were no turning back; I dropped all the plans for the summer and was ready to go to China. My family and friends were very supportive, while many others were absolutely negative toward me going to China. I visited so many sites in China I lost count. This country uniqueness makes it a great place to explore.

My landing in China. When we get to the airport, I still couldn't believe I was in China. After checking point, I felt awkward how the people was staring, and pointing at us. In the bus going to the Hotel I was exploring already the view of China, and couldn't wait for dinner. As excited I was for dinner, I ended up eating mango from a lady shop on the side of the road. Most of the meals are cooked with pork, which I don't eat. They serve wild duck, special made shrimp, meals that I've never seen before. They look nice but were not my specialties. While I was waiting for some rice and fried chicken, the server brought out the watermelon meaning "end of dinner". Some of my friend enjoyed the food, but I just enjoyed my first learning lesson "how to use our chopstick". After our meal there were still excitement exploring the beauty of one of the most popular city of China "Shanghai".

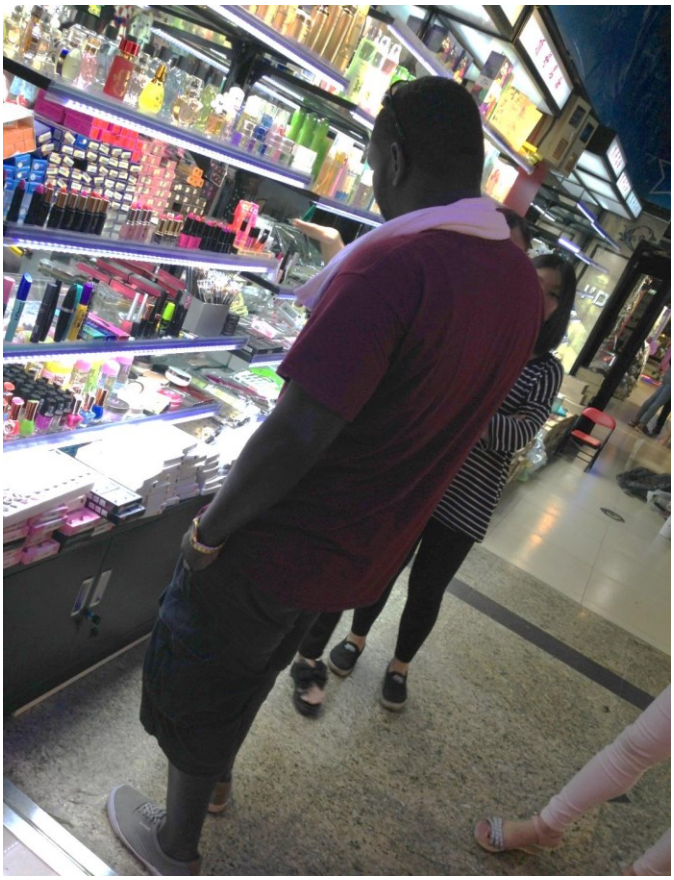
Wonderful moments in Shanghai. Shanghai is a well known city, it have many things for tourists to explore. The Pearl tower was my favorite spot. Two day in China I was already in love with the Shanghai. The people are very nice, the children were amazing. They were fighting to take pictures with us, they fell in love with my friends dreadlocks. The beauty of the pearl tower is phenomenal; the height makes you feel above the city. Everything was very sophisticated; almost everybody can hold a conversation in English which make it easy to get around. We met some African friends at the Pearl tower who were extremely happy to take some pictures with us. They invite us to Nigeria; it was amazing holding a conversation with people from different continent where we had the opportunities to learn about our differences including the culture of China. The underground museum at the Pearl tower is beautiful; they had some iconic figures at the museum.

Beautiful view of the pearl tower, I had a great time hanging out at the pearl tower.

A nice view from the top floor at Pearl tower.

Time to bargain for a better price.

In china, most merchants give you a price for their products where you have to bargain for it. This is very common in different outdoor markets in the United States, but both prices are reasonable. Shanghai the people asks for a ridiculous price, and you're staring at them thinking "China supposes to have stuffs for cheap". They do have stuffs for cheap but you have to know the worth to bargain for them. Sometimes you can look at the stuffs and give them a ridiculous offer. There is no such of thing as bargain too low in China specially Shanghai. Most of the time, they have you paying a lucrative amount for something they sold to somebody else 75 percent less.



I'm trying to bargain that lady to buy that Versace perfume for my fiancée. NO LUCK!!!

Welcome to Nanjing.

Nanjing is very nice and unique; it is not as popular as Shanghai. But I still had a great time. The Cafeteria was my only problem which I adapted to it fast. They had my favorite beef noodle that I enjoyed every day.. The people in Nanjing were really shy; they rather speak between them when they see us instead of asking question. The second week I went to Walmart to explore the city by taking the subway, the ticket was very cheap. I bought groceries, cereals and milk. We went to Pizza Hut for dinner, it was delicious.

I'm trying to ask information about the new Jurassic Park movie right beside Walmart.

Trip to the Poplar Museum and Dafeng National Nature Preserve.

Being sick riding on a bus for seven hours can be painful, but once you see the beauty of the deer at the national reserve nothing matters but them. The history of the deer was devastated, but this iconic man made history when he helped the deer returned to their homeland. We took a lot of pictures of the deer, some of us really want to get closer to touch the deer but it was

prohibited. It is amazing how China evolving every part of their history to benefit their tourism. Everything you see in China makes you think of 20 years from that initial moment. This is a country in a broad recovery, everything is booming. They adopted some of the western culture to their own advantage. While many countries are setting back on so many things, China is growing everything excessively. They are not scared to spend money in their country, they are building fast trains, new subway stations, and welcoming everybody to explore their greatness.

Pere David's deer back to their homeland.

Purple Mountain.

One morning four of us decide to explore the mountain everybody been talking about. It was amazing when we got there, everything was priceless. There were so many steps to get to

the top of the mountain. While we were there a lot of Chinese tourists came to visit with their family.

Food and cooking.

Being in China was a test for my cooking skills. The food was different in each province. In Nanjing the food was better, but I was tired with the seasoning smells. People in China eat a lot of pork which I don't eat so that was a major problem. The meals were looking nice but some of them aren't too tasty. One day we decided to go to Walmart to buy chicken so we can cook some fried chicken. We settle ourselves by getting pots from each other room to season the chicken then fry it. I was the chef at the moment hoping my chicken came out good because everybody stand in the kitchen waiting for the food to be done. It was great!!!!

I'm showing my chopstick skills at our first banquet. .

EBONEY STALLWORTH

Trip and Cultural Report
REUG China 2015

Alabama A&M University
Normal, Alabama 35762

Prior to Travel

My nerves were in a bundle after learning that I would be traveling to China this summer. I was nervous and didn't really know what to expect, considering that I have never traveled out of the United States before. I did not know what I needed to buy or what products I could still have access to outside of America. I frantically shopped to make sure that I would have some of the creature comforts abroad that I am accustomed to at home. I bought everything from

suitcases to lotion and toothpaste. Just like magic, the day for our little group from Alabama to board the plane to China came swiftly. I was excited and apprehensive at the same time; hoping that I would like living abroad for the summer. Since I was a small child I could only imagine of far away places, but now through academia that dream was about to become a reality.

Shanghai, China

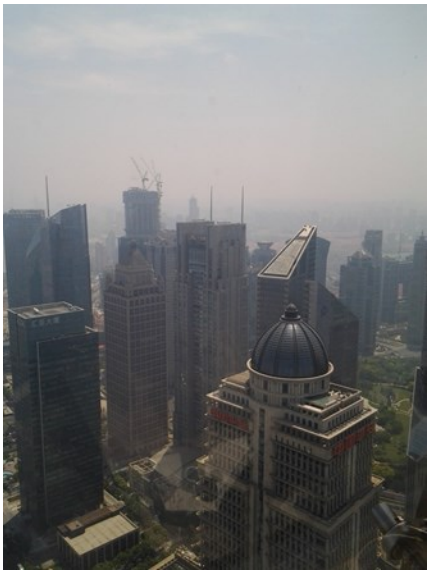
The plane ride was smooth and surprisingly comfortable considering it was eleven hours across the Pacific Ocean. The plane food was not very yummy, but it was food none the less. Once the flight attendant came over the intercom to announce our arrival into China, I immediately felt the nervous butterflies bouncing and flying around in my stomach. They were an ever-present reminder of how little I knew about China and Chinese culture as a whole. Once we stepped into the airport from the plane, I was immediately confronted with hot, sticky air. I wondered if the air conditioning was off inside of the whole airport? Once outside of the airport, the air was even more humid, and I noticed that the air smelled totally different than what I was used to in the states. It smelled heavy and thick with things I cannot name.

Once we got on the bus to take us to hotel, I thought that it would be a relatively short ride, but it was surprisingly long. The city was full of life, lights and sounds. I watched the buildings and people as we drove and couldn't wait to explore the city once we were settled in. I was shocked at how most of the drivers make up their own driving rules and lanes even though lines are clearly visible. Horns constantly blared and honked from differing driving styles among the motorists. We arrived at the hotel at 10:30pm that night. I was very interested in learning how the inside of the hotel looked so that I could note similarities and differences from America. Inside of the room, the keycard controlled not only the locks but also the electricity in the rooms. In order to turn on lights and the air conditioner, the keycard had to be inserted into a special slot next to the room entrance. If, at any time, the card was to be removed, the electricity would go off in the room. That is very efficient, in my opinion. Many of my applications on my devices did not work well on the Wifi and some did not work at all.

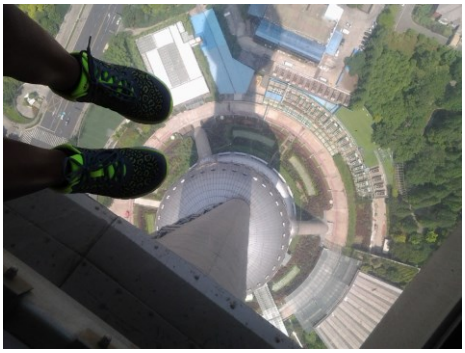
After we were settled in, we went to dinner at a restaurant next to the hotel. The waitresses brought out so many dishes that I lost count. There were pork dishes, beef dishes, two soups and more that I cannot even name. I tried jellyfish for the first time and I do not think that

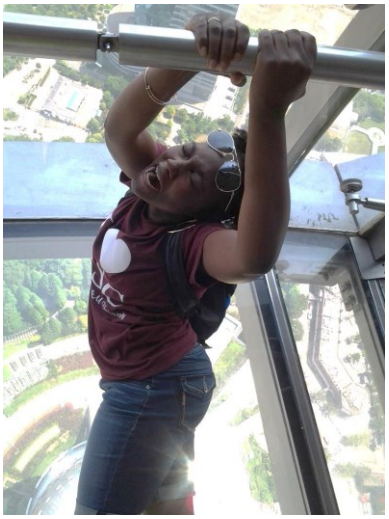
it is for my pallet. It was a little transparent and the texture was surprisingly crunchy, and the seasoning tasted like vinegar.

Day two of Shanghai was much more interesting. We went to a popular tourist attraction called the Pearl Tower. It was an amazingly tall building with glass floors at the top. Inside of the tower, there is a museum on the bottom level. The museum was one of the best parts about the tower, aside from the glass floors.

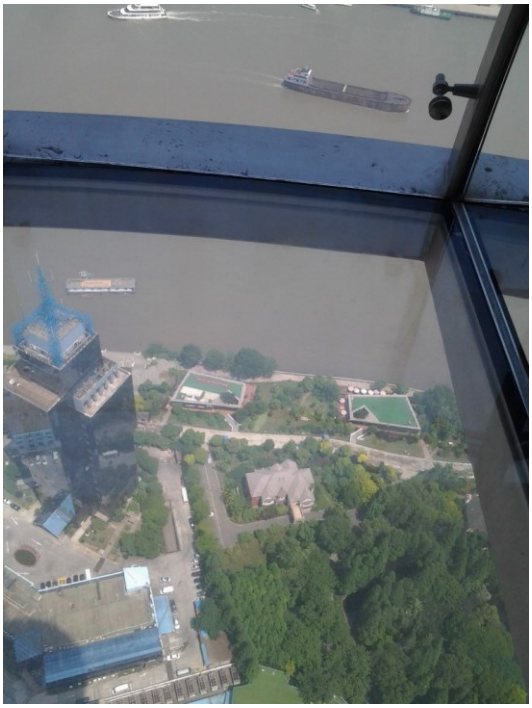


View from the top of Pearl Tower.





Ahhh, glass floors!!! Where is a superhero when you need one?


More views from the top.

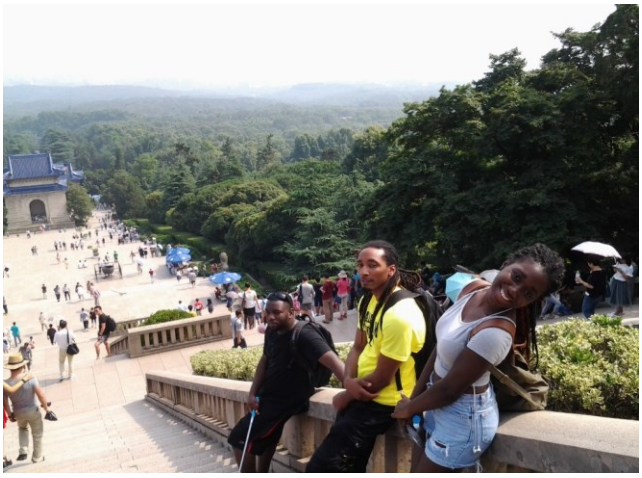
As I stated before, the museum was one of my favorite memories of the Pearl Tower in Shanghai. Below, there are pictures from the museum that really sparked my interest.

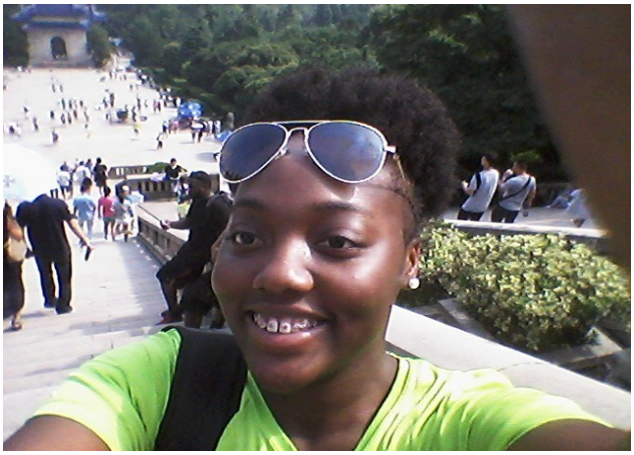

Figurines in the museum.

 Cool depiction of ancient China.

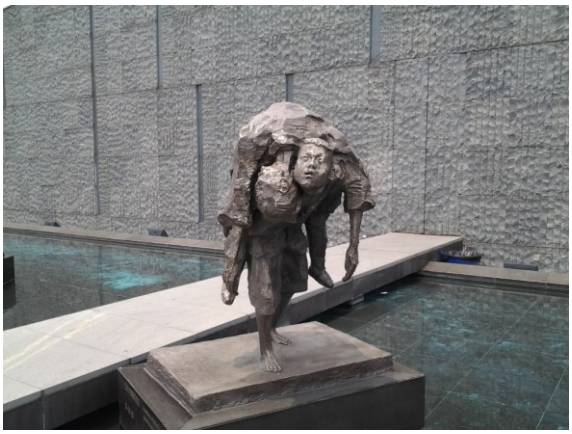
Nanjing, China

My favorite memories of Nanjing are Xinjeiko and Purple Mountain! I loved the hike up to the top of the lookout point at the Mausoleum. I enjoyed the architecture and view from the top. There were so many stairs to climb, but it was worth braving the heat and sun to get there.

 Purple Mountain.

 Me at Purple Mountain.

Another memory that stands out to me is the one of the Nanjing Massacre Museum (see photos below). Over 300,000 people died on the day that Japan invaded the capital city, which was Nanjing at the time. Women, children, babies and men lost their lives in the invasion. The mood of the museum was very melancholy and somber. The whole experience was very humbling. It was a definite eye opener.



Dafeng, China

Dafeng National Nature Reserve was very inspiring. The Milu deer was almost extinct, but their population has grown from mere double digits into the thousands. Another name for them is the David Deer; because of the man that rescued them.

Happy and thriving Milu Deer.

Conclusion

All in all, I made enough friendships and memories for a lifetime. The culture and history of China resonates with my heart. China now has a special place in my heart and mind. This country is full of cuisine and culture. I am happy to say that I have met people that I plan on keeping in contact with. This place has definitely had such challenges as language barriers and the food has not always been to my liking, but it feels like home away from home. I cannot wait to get to Beijing and discover everything the city has to offer.

The End

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