

**ISOLATION, IDENTIFICATION AND GERMPLASM COLLECTION OF
WATERBORNE FUNGI IN CENTRAL LUZON STATE UNIVERSITY**

ELYZA A. EVILLA

An Undergraduate Thesis Submitted to the Faculty of the Department of Environmental
Science, College of Arts and Sciences, Central Luzon State University,
Science City of Muñoz, Nueva Ecija, Philippines
in Partial Fulfillment of the Requirements
for the Degree of

**BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE
(Environmental Biology)**

FEBRUARY 2020

ACCEPTANCE SHEET

This undergraduate thesis entitled **“ISOLATION, IDENTIFICATION AND GERMPLASM COLLECTION OF WATERBORNE FUNGI IN CENTRAL LUZON STATE UNIVERSITY,”** prepared and submitted by **ELYZA A. EVILLA** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE (ENVIRONMENTAL BIOLOGY),** is hereby accepted:


JERWIN R. UN DAN, Ph.D.
Adviser

1-22-20
Date Signed

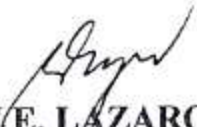

CESAR V. ORTINERO, Ph.D.
Critic

1. 22. 2020
Date Signed

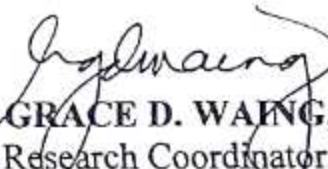

LUZVIMINDA S. QUITOS, Ph.D.
Department Research Coordinator

1-22-2020
Date Signed

Accepted as partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN ENVIRONMENTAL SCIENCE (ENVIRONMENTAL BIOLOGY):**


SHARON E. LAZARO, Ph.D.
Chair, Department of Environmental Science

22 January 2020
Date Signed


KRISTINE GRACE D. WANG, M.Sc.
College Research Coordinator

1/23/2020
Date Signed


EVARISTO A. ABELLA, Ph.D.
Dean, College of Arts and Sciences

1/23/20
Date Signed

BIOGRAPHICAL SKETCH

The author of this undergraduate thesis proposal was named Elyza A. Evilla by her parents, Evelyn A. Evilla and Elmer A. Evilla. She lives in Barangay Ricos, Umingan, Pangasinan. She was born on May 2, 1999 at Manila. She graduated her elementary at Mary Cause of our Joy Catholic School located at Soldiers Hills Village, Muntinlupa City and graduated her secondary level at Guadalupe Catholic School located at Guadalupe Nuevo, Makati City. She is in her tertiary level now at Central Luzon State University taking up Bachelor of Science in Environmental Science major in Environmental Biology.

ACKNOWLEDGMENT

The author would like to give her grateful appreciation to the following persons who paved the ways towards the accomplishment of this paper.

Her thesis adviser Dr. Jerwin R. Undan and her critic Dr. Cesar V. Ortinero for the motivation, advise, support, patience and guidance. She appreciates their knowledge and vast expertise to help her improve this paper.

To the department research coordinator Dr. Luzviminda S. Quitos, sincere thanks given for the tireless efforts, guidance, support, patience and advice given to the author.

To Dana Theresa De Leon, Mike Andre Malonzo and the research assistants and staffs of Tuklas Lunas Center and Biotechnology Laboratory for their guidance, patience and advice.

Her regards to her friends, Kathleen, Camille, Eddieson, Kim, Erika, Zhandon, Cyril and Florence for their support and tireless efforts given.

She would like to express her deepest gratitude and sincere thanks given to her parents, Mr. Elmer A. Evilla and Mrs. Evelyn A. Evilla and her siblings Elymer A. Evilla and Elisha A. Evilla for their understanding, encouragement, love, and endless support.

To our ALMIGHTY GOD who perpetually gives her strength and guidance.

TABLE OF CONTENTS

	PAGE
LIST OF TABLE	vii
LIST OF FIGURES	viii
LIST OF APPENDICES	ix
LIST OF APPENDIX TABLES	x
LIST OF APPENDIX FIGURES	xi
ABSTRACT	xii
INTRODUCTION	1
Background of the Study	1
Objectives of the Study	2
Significance of the Study	2
Scope and Limitation of the Study	3
Time and Place of the Study	3
REVIEW OF RELATED LITERATURE	4
Freshwater Fungi	4
Habitat of Freshwater Fungi	5
Biogeography of Freshwater Fungi	6
Ecological Adaptations of Freshwater Fungi	6
Role of Fungi	7
Reproduction of Fungi	8
Importance of Fungi	8
Fungi in the Philippines	9
Molecular Identification	10
METHODOLOGY	12
Study Area	12
Collection of Samples	13
Sampling Stations	13
Pure Culture Media	13

Plating	14
Fungal Isolation	14
Identification of Fungal Isolates	14
Morphological Characteristics	14
Molecular Identification	15
DNA Extraction	15
DNA Quality Check and Gel Electrophoresis	16
Polymerase Chain Reaction	16
Mycelia Preservation	17
Taking Pictures of the Fungi Colonies	17
Determination of Environmental Factors	17
RESULTS AND DISCUSSION	19
Morphological Characteristics of Fungal Isolate	19
<i>Diaporthe neotheicola</i>	19
<i>Aspergillus sydowii</i>	21
<i>Edenia gomezpompae</i>	22
<i>Microsphaeropsis arundinis</i>	24
Molecular Identification	25
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS	27
Summary	27
Conclusion	28
Recommendations	28
LITERATURE CITED	29
APPENDICES	32

LIST OF TABLE

TABLE		PAGE
1	Morphological characteristics of each fungus	25
2	Identities of the fungal species using BLAST with Genbank accession numbers	26

LIST OF FIGURES

FIGURE		PAGE
1	Map of CLSU retrieved from openstreetmap.org	12
2	Conceptual framework of the primary focus of the study	18
3	Cultured characteristics of <i>Diaporthe neotheicola</i> in PDA; Front (left); Back (middle); Conidia (right, 100x)	20
4	Cultured characteristics of <i>Aspergillus sydowii</i> in PDA; Front (left); Back (middle); Conidia (right, 100x)	21
5	Cultured characteristics of <i>Edenia gomezpompae</i> in PDA; Front (left); Back (middle); Conidia (right, 100x)	23
6	Cultured characteristics of <i>Microsphaeropsis arundinis</i> in PDA; Front (left); Back (middle); Conidia (right, 100x)	24

LIST OF APPENDICES

APPENDIX		PAGE
I	Environmental Factors	33
II	Molecular Identification	34

LIST OF APPENDIX TABLES

APPENDIX TABLE	PAGE
1 Environmental factors of sampling sites	33

LIST OF APPENDIX FIGURES

APPENDIX FIGURE		PAGE
1	Temperature and pH of water; Lagoon 1 (left); Lagoon 2 (right)	33
2	Temperature and pH of water; Pond 1 (left); Pond 2 (right)	33
3	Digital dry bath for DNA Extraction	34
4	PCR profile	34
5	DNA Quality check	35
6	Loading procedure for DNA Quality check and Gel Electrophoresis	35
7	<i>Aspergillus sydowii</i> (Hyphae length)	36
8	<i>Microsphaeropsis arundinis</i> (Hyphae length)	36
9	<i>Edenia gomezpompae</i> (Hyphae length)	37
10	<i>Diaporthe neotheicola</i> (Hyphae length)	37
11	<i>Edenia gomezpompae</i> (Hyphae length)	38

ABSTRACT

EVILLA, ELYZA A., Department of Environmental Science, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **February 2020, ISOLATION, IDENTIFICATION AND GERMPLASM COLLECTION OF WATERBORNE FUNGI IN CENTRAL LUZON STATE UNIVERSITY**

Adviser: JERWIN R. UNDAN, Ph.D.

Fungi serve as decomposers in freshwater ecosystem. Fungi also include some of the most important organisms, both in terms of their ecological and economic roles. By breaking down dead organic material, they continue the cycle of nutrients through ecosystems. This study aimed to determine and identify the different species of water fungi present in selected ponds and lagoon in Central Luzon State University (CLSU), purify the fungal isolates describe the morphological characteristics of the collected water fungi and establish the molecular identification of waterborne fungus. The study was able to identify four species of isolated fungi using morphological and cultural characterization. L2R4 was *Diaporthe neotheicola* with %Identity of 97.74% with accession number KC145873.1. L1R2 was *Aspergillus sydowii* with %Identity of 100.00% with accession number MH793856.1. P2R3 was *Edenia gomezpompae* with %Identity of 97.79% with accession number EF565744.1. P1R1 was *Microsphaeropsis arundinis* with %Identity of 100.00% with accession number MH911414.1. This is the first report on the presence of water fungi in CLSU. This study represents the data and can be a reference by the future field researchers regarding what species of waterborne fungi are present in selected freshwater of CLSU.

Keywords: water fungi; morphology; molecular identification

LITERATURE CITED

- Alias, S. A., Jones, E. B. G., & Torres, J. (1999). Intertidal fungi from the Philippines, with a description of *Acrocordiopsis sphaerica* (Ascomycota). *Fungal Diversity*, 2, 35-41.
- Alvarenga, N., Birolli, W. G., Selegim, M. H. R., & Porto, A. L. M. (2014). Biodegradation of methyl parathion by whole cells of marine-derived fungi *Aspergillus sydowii* and *Penicillium decaturense*. *Chemosphere*, 117, 47-52. doi.org/10.1016/j.chemosphere.2014.05.069
- Astrero J. B. (2018). Isolation, morphological characterization and molecular identification of associated fungi in diseased onion (*Allium cepa* L.) leaf. (Unpublished undergraduate thesis). Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.
- Botero, W. B., De Amorim, M. R., Carlos, I. Z., Polesi, M. C., & Dos Santos, L. C. (2019). Aromatic Polyketides and Macrolides from *Microsphaeropsis arundinis*. *Journal of the Brazilian Chemical Society*, 1-6. doi.org/10.21577/0103-5053.20190189
- Chauhan, R., Lone, S. A., & Beigh, A. H. (2014). Pathogenicity of three species of *Aspergillus* (*A.fumigatus*, *A.niger* & *A.sydowii*) on some fresh water fishes. *Life Sciences Leaflets*, 48, 65-72. <http://lifesciencesleaflets.ning.com/>
- Chen, R. & McDonald, B. A. (1996). Sexual Reproduction Plays a Major Role in the Genetic Structure of Populations of the Fungus *Mycosphaerella graminicola*. *Genetics* 142: 1119-1127 Texas AHM University, College Station, Texas.
- Chen, S. F., Morgan, D. P., Hasey, J. K., Anderson, K., & Michailides, T. J. (2014). Phylogeny, Morphology, Distribution, and Pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. *Plant Disease*, 98(5), 636-652.
- Dighton, J. (2016). Fungi in Ecosystem Processes. Mycology Series Rutgers University Pinelands Field Station, New Lisbon, New Jersey, USA 13: 978-1-4822-4906-4.
- Eo, J., Choi, M., & Eom, A. (2014). Diversity of Endophytic Fungi Isolated from Korean Ginseng Leaves. *Mycobiology*, 42(2), 147-151. doi:10.5941/MYCO.2014.42.2.147
- Gao, Y., Liu, F., Duan, W., Crous, P. W., & Cai, L. (2017). *Diaporthe* is paraphyletic. *IMA Fungus*, 8(1), 153-187.
- Golzar, H., Tan, Y. P., Shivas, R. G., & Wang, C. (2012). First report of shoot blight of persimmon caused by *Diaporthe neotheicola* in Australia. *Australasian Plant Dis. Notes*, 7, 115-117

- Guarnaccia, V., Groenewald, J.Z., Woodhall, J., Armengol, J., Cinelli, T., Eichmeier, A., ... Crous, P.W. (2018). *Diaporthe* diversity and pathogenicity revealed from a broad survey of grapevine diseases in Europe. *Persoonia*, 40, 135–153.
- Gulis, V., Kuehn, K. & Suberkropp, K. (2006). The role of fungi in carbon and nitrogen cycles in freshwater ecosystems. *Fungi in Biogeochemical Cycles*, 46, 404-435.
- Halliday, C. L. & Chen, S. (2015). Medical mycology: closing the gap in diagnostics and emerging fungal species? *A Centre for Infectious Diseases and Microbiology*, 38, 1-19.
- Hyde, K. D., Jones, E. B. G., Leano, E., Pointing, S. B., Poonyth, A. D., & Vrijmoed, L. L. P. (1998). Role of fungi in marine ecosystems. *Biodiversity and Conservation*, 7, 114-1161.
- Joshi, M. & Deshpande, J. D. (2010). Polymerase Chain Reaction: Methods, Principles and Application. *International Journal of Biomedical Research*, 1(5), 81-97.
- Łoniewska, A. G., Kowalska, T. K., Wardzyńska, G., & Boryn, K. (2007). Occurrence of Fungi in Water Distribution System. *Polish Journal of Environmental Studies*, 16(4), 539-547.
- Monchy, S., Sancier, G., Jobard, M., Rasconi, S., Gerphagnon, M., Chabé, M., ... Viscogliosi, E. (2011). Exploring and quantifying fungal diversity in freshwater lake ecosystems using rDNA cloning/sequencing and SSU tag pyrosequencing. *Environmental Microbiology*, 13(6), 1433–1453.
- Nakai, Y., Abe, T., Kohda, C. & Ando, T. (1999). Water Characteristics and Microbial Flora in a Lagoon System for Waste water from a Paddock of Dairy Cattle. *Animal Science Journal*, 70(1), 38-42.
- Parveen, S., Lanjewar, S., Sharma, K. & Kutti, U. (2011). Isolation of fungi from the surface water of river. *Journal of Experimental Science*, 2(10), 58-59.
- Reppas, G., Gottlieb, T., Krockenberger, M., Halliday, C., & Malik, R. (2015). *Microspheeropsis arundinis*: an emerging cause of phaeohyphomycosis in cats and people. *Microbiology Australia*, 10, 74-78.
- Rosana, Y., Matsuzawa, T., Gonoj, T., & Karuniawati, A. (2014). Modified Slide Culture Method for Faster and Easier Identification of Dermatophytes. *Microbiology Indonesia*, 8(3), 135-139.
- Rypien, K. L. (2008). The origins and spread of *Aspergillus sydowii*, an opportunistic pathogen of caribbean gorgonian corals. (Published doctoral dissertation). Cornell University, Ithaca, New York.

- Spring, O. & Thines, M. (2010). *Molecular Identification of Fungi* (pp. 35-50). Springer: London, United Kingdom. DOI 10.1007/978-3-642-05042-8
- Su, G. S., Dueñas, K., Roderno, K., Sison, M. A., Su, M. L. S., Ragragio, E., ... Heralde, F. M. III (2014). Distribution and Diversity of Marine Fungi in Manila Bay, Philippines. *Annual Research & Review in Biology*, 4(24), 4166-4173.
- Sun, B., Chen, A., Gao, W., Zhou, Y., Ghazanfar, M., & Wakil, W. (2013). A new endophytic ascomycete associated with the medicinal plant, *Achyranthes Bidentata Blume* (Amaranthaceae). *Pakistan Journal of Botany*, 45(1), 319-323.
- Thomas, M. & Thangavel, M. (2017). Isolation of fungi from the surface water of Vembanadu wetland agroecosystem. *International Journal of Applied Microbiology and Biotechnology Research*, 5, 52-58.
- Westermeier, R. (2005). Gel Electrophoresis. *Encyclopedia of Life Sciences* (pp. 1-6). Amersham Biosciences Europe GmbH, Freiburg, Germany. doi:10.1038/npg.els.0005335
- Wong, M., Goh, T., Hodgkiss, I. J., Hyde, K., Ranghoo, V. M., Tsui, C. K. M., ... Yuen, T. (1998). Role of fungi in freshwater ecosystems. *Biodiversity and Conservation* 7, 1187-1206. DOI: 10.1023/A:1008883716975
- Yamaguchi, M. U., Rampazzo, R. C. P., Yamada-Ogatta, S. F., Nakamura, C. V., Ueda-Nakamura, T. & Filho, B. P. D. (2007). Yeasts and filamentous fungi in bottled mineral water and tap water from municipal supplies. *Brazilian Archives of Biology and Technology*, 50, 1-9.