

**FORAGING BEHAVIOR AND IDENTIFICATION OF FUNGI
ASSOCIATED WITH *Rhinella marina* OF CENTRAL LUZON
STATE UNIVERSITY ECOSYSTEM**

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ABSTRACT

AGUSTIN, ANGELI T., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, **JUNE 2019, FORAGING BEHAVIOR AND IDENTIFICATION OF FUNGI ASSOCIATED WITH *Rhinella marina* OF CENTRAL LUZON STATE UNIVERSITY ECOSYSTEM**

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The study was focused on the determination of foraging behavior and identification of fungi associated with frogs in the ecosystem of CLSU.

The collected frogs in the undisturbed and disturbed study areas in CLSU were classified as *Rhinella marina*. The frogs were swabbed in the dorsal surface and appendages using a sterile cotton swab and this were transferred in a dilution bottle containing 10ml of sterile water. Isolation of fungi was done by pour-plate technique. Isolated pure cultures of fungi were identified through cultural and morphological characterization. Cultural characterization was done by three-point inoculation technique. Morphological characterization was done by slide culture technique. Identified fungi associated with this frog were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Aspergillus oryzae*, *Aspergillus brasiliensis*, *Fusarium semitectum*, and *Rhizopus microsporus*. Chytrid fungus was not found in *R. marina* collected in the ecosystem of CLSU.

On the other hand, foraging behavior was determined by dissecting the frog and analyzing its stomach content. The gut content analysis revealed that *R. marina* feeds on plant materials, pebbles, and insects belonging to order Coleoptera and Hemiptera.

LITERATURE CITED

- Abarca, J. G., Zuniga, I., Ortiz-Morales, G. & Lugo, A. (2018). Characterization of the skin microbiota of the cane toad *Rhinella cf. marina* in Puerto Rico and Costa Rica. *Frontiers in Microbiology*, 8(2624).
- Abdel-Azeem, A. M., Salem, F. M., Abdel-Azeem, M. A., Nafady, M. T., Mohesien, M. T. & Soliman, E. A. (2016). Biodiversity of the genus *Aspergillus* in different habitats. *New and Future Developments in Microbial Biotechnology and Bioengineering*, 3-28.
- Alexopoulos, C. J., Mims C. W. & Blackwell, M. (1996). *Introductory mycology*, Wiley and Sons, New York, NY, 4th edition.
- Apayor-Ynot, C. L. A., Tan, S. N., Lim, N. K., Baron, E. M. D. & Mohagan, A. B. (2017). Diet of cane toads (*Rhinella marina*) collected from areas adjacent to human dwellings in Davao City, Philippines. *Imperial Journal of Inetrdisciplinary Research*, 3(11).
- Arenz, B., Blanchette, R. A. & Farrell, R. L. (2014). Fungal diversity in Antarctic soils. *Antarctic Terrestrial Microbiology: Physical and Biological Properties of Antarctic Soils*, 35-53.
- Balbool, B. A., Abdel-Azeem, A. M., Khalil, W. F. & El-Kazzaz, W. F. (2013). Bioprospecting as a conservation tool: the genus *Aspergillus* (*Eurotium*) in Egypt. *Third International Congress on Fungal Conservation*, 36.
- Barr, D. J. S. (1990). Phylum Chytridiomycota. In: *Handbook of Protoctista*, ed. Margulis L, Corliss JO, Melkonian M, Chapman DJ. Jones and Bartlett, Boston, MA. 454-466.
- Beebee, T. C. J. & Griffiths, R. A. (2005). The amphibian decline crisis: A watershed for conservation biology?. *Biological Conservation*, 125, 271-285.
- Berger, L., Speare, R. & Hyatt, A. (1999). Chytrid fungi and amphibian declines: Overview, implications and future directions. *Environment Australia*, 23-32.
- Bishop, D. & Haas, C. (2009). Frog Biodiversity and Conservation. *Communications and Marketing, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University*, 420-527.
- Blaustein, A. R. & Kiesecker, J. M. (2002). Complexity in conservation: lessons from the global decline of amphibian populations. *Ecology Letters*, 5, 597-608.
- Blaustein, A. R., Romansic, J. M., Kiesecker, J. M. & Hatch, A. C. (2003). Ultraviolet radiation, toxic chemicals, and amphibian population declines. *Diversity and Distribution*, 9, 123-140.

- Bosch, J., Martinez-Solano, I. & Garcíaparis, M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation*, 97, 331-337.
- Bonugli-Santos, R. C., Vasconcelos, M. R., Passarini, M. R. Z., Vieira, G. A. L., Lopes, V. C. P. & Mainardi, P. H. (2015). Marine-derived fungi: diversity of enzymes and biotechnological applications. *Frontiers in Microbiology*, 6(269).
- Brown, R. M. & Diesmos, A. C. (2009). Philippines, biology. in R. Gillespie and D. Clague, eds., *Encyclopedia of Islands*. University of California Press, Berkeley, California, USA. 723-732.
- Brown, R. M., Siler, C. D., Oliveros, C. H., Esselstyn, J. A., Diesmos, A. C., Hosner, P. A., Linkem, C. W., Barley, A. J., Oaks, J. R., Sanguila, M. B., Welton, L. J., Blackburn, D. S., Moyle, R. G., Peterson, A. T. & Alcalá, A. C. (2013). Evolutionary processes of diversification in a model island archipelago. *Annual Review of Ecology, Evolution, and Systematics*, 44, 411-435.
- Butinar, L., Frisvad, J. C. & Gunde-Cimerman, N. (2011). Hypersaline waters – a potential source of foodborne toxigenic *Aspergilli* and *Penicillia*. *FEMS Microbiology Ecology*, 77, 186-199.
- Chinchar, V. G., Hyatt, A., Miyazaki, T. & Williams, T. (2009). Family Iridoviridae: poor viral relations no longer. *Current Topics in Microbiology and Immunology*, 328, 123-170.
- Croll, N. A. & Cross, J. H. (2013). *Human Ecology and Infectious Diseases*. Academic Press, 1st edition.
- Cunningham, A. A., Daszak, P. & Rodríguez, J. P. (2003). Pathogen pollution: defining a parasitological threat to biodiversity conservation. *Journal of Parasitology*, 89, 78-83.
- Cunningham, W. P. & Saigo, B. W. (1999). *Environmental Science: A Global Concern*, McGraw-Hill Companies, USA, 5th edition, 650.
- Daszak, P., Berger, L., Cunningham, A., Hyatt, A. D., Green, D. E. & Speare, R. (1999). Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases*, 5(6), 735-748.
- Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science*, 287, 443-9.
- Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica*, 78, 103-16.

- Diesmos, A. C., Alcala, A. C., Siler, C. D. & Brown, R. M. (2014). Status and conservation of Philippine amphibians. in H. Heatwole and I. das, eds., *Conservation Biology of Amphibians of Asia. Status and Decline of Amphibians: eastern Hemisphere. Natural History Publications (Borneo), Kota Kinabalu, Malaysia*, 310-336.
- Diesmos, A. C., Diesmos, M. L. & Brown, R. M. (2006). Status and distribution of alien invasive frogs in the Philippines. *Journal of Environmental Science and Management, Philippines*, 9(2), 41-53.
- Diesmos, A. C., Diesmos, M. L. & Brown, R. M. (2010). *Philippine Daily Inquirer*.
- Diesmos, A. C., Watters, J. L., Huron, N. A., Davis, D. R., Alcala, A. C., Crombie, R. I., Afuang, L. E., Gee-Das, G., Sison, R. V., Sanguila, M. B., Penrod, M. L., Labonte, M. J., Davey, C. S., Leone, E. A., Diesmos, M. L., Sy, E. Y., Welton, L. J., Brown, R. F. & Siler, C. D. (2015). Amphibians of the Philippines, Part I: Checklist of the Species. *Proceedings of the California Academy of Sciences*, 62(20), 457-539.
- Diesmos, A.C., & Brown, R. M. (2011). Proceedings of the Conference "Biology of the Amphibians in the Sunda Region, Southe-east Asia," (Univ. of Malaysia Sarawak, Kota Samarahan) 26-49.
- Diesmos, M. L. L., Diesmos, A. C., Siler, C. D., Vredenburg, V. T. & Brown, R. M. (2012). Detecting the distribution of the chytrid fungus in the Philippines. *Froglog*, 20(5), 48-49.
- Dobson, A. & Foufopoulos, J. (2001). Emerging infectious pathogens of wildlife. *Philosophical Transactions of the Royal Society B Biological Sciences*, 356, 1001-12.
- Dörfelt, H. & Schmidt, A. R. (2005). A fossil *Aspergillus* from Baltic amber. *Mycological Research*, 109, 956-960.
- Early, R. (2009). Pathogen control in primary production: crop foods. *Foodborne Pathogens*, 2nd edition.
- Elvers, K. T., Leeming, K., Moore, C. P. & Lappin-Scott, H. M. (1998). Bacterial-fungal biofilms in flowing water photo-processing tanks. *Journal of Applied Microbiology*, 84, 607-618.
- Findley, K., Oh, J., Yang, J., Conian, S. and Deming, C. (2013). Topographic diversity of fungal and bacterial communities in human skin. *Nature*, 22.
- Gautam, A. K., Sharma, S., Avasthi, S. & Bhadauria, R. (2011). Diversity, Pathogenicity and Toxicology of *A. niger*: An Important Spoilage Fungi. *Research Journal of Microbiology*, 6(3), 270-280.

- Global Invasive Species Database (GISD) 2015. Species profile *Rhinella marina*.
- Gugnani, H. (2003). Ecology and taxonomy of pathogenic *Asperilli*. *Frontiers in Bioscience*, 8(1-3), 346-357.
- Hirai, T. & Matsui, M. (2002). Feeding ecology of *Bufo japonicas formosus* from the montane region of Kyoto, Japan. *Journal of Herpetology*, 36, 719-723.
- Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H. & Kuzmin, S. L. (2000). Quantitative evidence for global amphibian population declines. *Nature*, 404, 752-755.
- Hocking, D. J. & Babbitt, K. J. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, 9(1), 1-17.
- Icochea, J., Quispitupac, E., Portilla, A. & Ponce, E. (2002). Framework for assessment and monitoring of amphibians and reptiles in the lower Urubamba Region, Peru. *Environmental Monitoring and Assessment*, 76, 55-67.
- Inger, R. F. (1954). Systematics and zoogeography of Philippine Amphibia. *Fieldiana: Zoology*, 33, 183-531.
- Jaime-Garcia, R. & Cotty, P. J. (2010). Crop rotation and soil temperature influence the community structure of *Aspergillus flavus* in soil. *Soil Biology and Biochemistry*, 42(10), 1842-1847.
- Kiesecker, J. M., Blaustein, A. R. & Miller, C. R. (2001). Transfer of a pathogen from fish to amphibians. *Conservation Biology*, 15(4), 1064-1070.
- Lever, C. (2001). The cane toad: the history and ecology of a successful colonist. *Westbury Academic and Scientific Publishing, Otley, West Yorkshire, UK*, 230.
- Lips, K. R., Brem, F., Brenes, R., Reeve, J. D., Alford, R. A. & Voyles, J. (2006). Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proceedings of the National Academy of Sciences USA*, 102, 3165-3170.
- Longcore, J. E., Pessier, A. P. & Nichols, D. K. (1999). *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia*, 91(2), 219-227.
- Maciel, M. N., Collevatti, R. G., Colli, G. R. & Schwartz, E. F. (2010). Late Miocene diversification and phylogenetic relationships of the huge toads in the *Rhinella marina* (Linnaeus, 1758) species group (Anura: Bufonidae). *Molecular Phylogenetics and Evolution*, 2, 787-797.
- Mazzoni, R., Cunningham, A. A., Daszak, P., Apolo, A., Perdomo, E. & Speranza, G. (2003). Emerging pathogen in wild amphibians and frogs (*Rana catesbeiana*)

- farmed for international trade. *National Center for Biotechnology Information*, 9(8), 995-998.
- Meijer, M., Houbraeken, J. A. M. P., Dalhuijsen, S., Samson, R. A. & De Vries, R. P. (2011). Growth and hydrolase profiles can be used as characteristics to distinguish *Aspergillus niger* and other black aspergilli. *Studies in Mycology*, 69, 19-30.
- Money, N. P. (2016). Fungi and Biotechnology. *The Fungi*, 3rd edition.
- Moslem, M. A., Mashraqi, A., Abd-Elsalam, K. A., Bahkali, A. H. & Elnagaer, M. A. (2010). Molecular detection of ochratoxigenic *Aspergillus* species isolated from coffee beans in Saudi Arabia. *Genetics and Molecular Research*, 9(4), 2292-2299.
- Mousavi, H. A. E., Soltani, M., Khosravi, A., Mood S. M. & Hosseinifard, M. (2009). Isolation and Characterization of Saprolegniaceae from Rainbow Trout (*Oncorhynchus mykiss*) Eggs in Iran. *Journal of Fisheries and Aquatic Science*, 4(6), 330-333.
- Muthomi, J. W., Mureithi, B. K., Chemining, G. N., Gathumbi, J. K. & Mutit, E. W. (2012). *Aspergillus* species and aflatoxin B1 in soil, maize grain and flour samples from semi-arid and humid regions of Kenya. *International Journal of Agriculture Science*, 2(1), 22-34.
- Nelson, P. E., Dignani, M. C. & Anaissie, E. J. (1994). Taxonomy, biology and clinical aspects of *Fusarium* species. *Clinical Microbiology Reviews*, 7, 479-504.
- Nichols, D. K., Lamirande, E. W., Pessier, A. P. & Longcore, J. E. (2001). Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. *Journal of Wildlife Diseases*, 37, 1-11.
- Pessier, A. P. (2002). An overview of amphibian skin disease. *Seminars in Avian and Exotic Pet Medicine*, 11, 162-174.
- Pessier, A. P., Nichols, D. K., Longcore, J. E., & Fuller, M. S. (1999). Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and white's tree frogs (*Litoria caerulea*). *Journal of Veterinary Diagnostic Investigation*, 11, 194-199.
- Pitt, J. I. & Hocking, A. D. (1997). *Fungi and Food Spoilage*. Springer, New York, ISBN-10: 0834213060, 2nd edition, 593.
- Pitt, J. I. & Hocking, A. D. (2009). *Fungi and Food Spoilage*. Springer Dordrecht Heidelberg London New York, 3rd edition.
- Pounds, J. A. (2001). Climate and amphibian declines. *Nature*, 410, 639-640.
- Pounds, J. A., Bustamante, M. R., Coloma, L. C., Consuegra, J. A., Michael, P. L. & Fogden, M. P. L. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, 439, 161-167.

- Powell, M. J. (1993). Looking at mycology with a Janus face: A glimpse of chytridiomycetes active in the environment. *Mycologia*, 85, 1-20.
- Roberts, R. J. (1989). *Fish Pathology*. Balliere Tindall, London. 4th edition.
- Rosenblum, E. B., Voyles, J., Poorten, T. J. & Stajich, J. E. (2010). The Deadly Chytrid Fungus: A Story of an Emerging Pathogen. *Public Library of Science*, 6(1).
- Rowley, J., Brown, R., Bain, R., Kusrini, M., Inger, R., Stuart, B., Wogan, G., Thy, N., Chanard, T., Trung, C. T., Diesmos, A., Iskandar, D. T., Lau, M., Ming, L. T., Makchai, S., Truong, N. Q. & Phimmachak, S. (2010). Impending conservation crisis for Southeast Asian amphibians. *Biology Letters*, 6, 336-338.
- Salcedo, L. D. P., Correa, M. F. & Galvis, A. R. A. (2011). First record of *Saprolegnia* sp. In an amphibian population in Columbia. *Universitas Scientarium*, 16(3).
- Santos, E. M., Almeida, A. V. & Vasconcelos, S. D. (2004). Feeding habits of six anuran (Amphibia: Anura) species in a rainforest fragment in Northeastern Brazil. *Inheringia Serie Zoologia*, 94 (4).
- Schuster, E., Dunn-Coleman, N., Frisvad, J. & Van Dijck, P. (2002). On the safety of *Aspergillus niger*-A review. *Applied Microbiology and Biotechnology*, 59, 426-435.
- Skerratt, L. F., Berger, L., Speare, R., Scott, C., McDonald, K. R., Phillott, A. D., Hines, H. B. & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth Journal Consortium*, 4, 125-134.
- Sodhi, N. S., Koh, L. P., Brook, B. W. & Lng, P. K. (2004). Southeast Asian biodiversity: an impending disaster. *Trends in Ecology and Evolution*, 19, 654-660.
- Solis, F., Ibañez, R., Hammerson, G., Hedges, B., Diesmos, A., Matsui, M., Hero, J. M., Richards, S., Coloma, L., Ron, S., La Marca, E., Hardy, J., Powell, R., Bolanos, F., Chaves, G. & Ponce, P. (2009). *Rhinella marina*. *The IUCN Red List of Threatened Species*.
- Sparling, D. W., Linder, G. & Bishop, C. A. (2000). Ecotoxicology of amphibians and reptiles. *Pensacola, FL: Society of Environmental Toxicology and Chemistry (SETAC)*, pp. 904.
- Sreelatha, K. S., Natarajan, P. and Ritakumari, S. D. (1990). Studies on the food and feeding behaviour of *Bufo melanostictus*. *Journal of Ecobiology*, 2(3), 213-221.
- Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L. & Fischman, D. L. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, 306, 1783-1786.

- Stueland, S., Hatai, K. & Skaar, I. (2005). Morphological and physiological characteristics of *Saprolegnia* spp. strains pathogenic to Atlantic Salmon, *Salmo salar* L. *Journal of Fish Diseases*, 28, 445-453.
- Sutton, D. A., Fothergill, A. W. & Rinaldi, M. G. (1998). Guide to clinically significant fungi, Williams & Wilkins, Baltimore, 1st edition.
- Tinsley, R. C., Coxhead, P. G., Stott, L. C., Tinsley, M. C., Piccinni, M. Z. & Guille, M. J. (2015). Chytrid fungus infections in laboratory and introduced *Xenopus laevis* populations: assessing the risks for U.K. native amphibians. *Biological Conservation*, 184, 380-388.
- Toft, C. A. (1980). Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. *Oecologia*, 45(1), 131-141.
- Tripathi, M. & Joshi, Y. (2015). Endolichenic fungi in Kumaun Himalaya: A Case Study. Recent Advances in Lichenology Volume 2: Modern Methods and Approaches in Lichen Systematics and Culture Techniques, 111-120.
- Valencia-Aguilar, A., Cortes-Gomez, A. M. & Ruiz-Agudelo. (2013). Ecosystem services provided by amphibians and reptiles in Neotropical ecosystems. *International Journal of Biodiversity Science, Ecosystem Services and Management*, 257-272.
- Varga, J., Kocsube, S., Toth, B., Frisvad, J. C., Perrone, G., Susca, A., Meijer, M. & Samson, R. A. (2007). *Aspergillus brasiliensis* sp. nov., a biseriate black *Aspergillus* species with world-wide distribution. *International Journal of Systematic and Evolutionary Microbiology*, 57(8).
- Voyles, J., Berger, L., Young, S., Speare, R. & Webb, R. (2007). Electrolyte depletion and osmotic imbalance in amphibians with chytridiomycosis. *Diseases of Aquatic Organisms*, 77, 113-118.
- Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W. F., Dinudom, A., Cook, D., Webb, R., Alford, R. A., Skerratt, L. F. & Speare, R. (2009). Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science*, 326, 582-585.
- West, B. C., Oberle, A. D. & Kwon-Chung, K. J. (1995). Mucormycosis caused by *Rhizopus microsporus* var. *microsporus*: cellulitis in the leg of a diabetic patient cured by amputation. *Journal of Clinical Microbiology*, 33, 3341-3344.
- Williams, T., Barbosa-Solomieu, V. & Chinchar, V. G. (2005). A decade of advances in iridovirus research. *Advances in Virus Research*, 65, 173-248.