Species of Frogs Used in Academic Research in the Philippines and their Conservation Status

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ABSTRACT

Frogs are amphibians with notable webbed dorsal feet that allow them to swim and jump. Frogs are commonly used in academic settings, such as biology lectures. Recognizing the species of frogs, their conservation status, and the ethical standards used in research in the Philippines can help educate people about the frogs that live in the country, their existence, and appropriate frog handling. The study's goal is to learn about the specific species of frog used in research in the Philippines, its conservation status, and whether ethical standards were followed during the investigation. Descriptive, bibliographic approach was utilized in the study's research design. This study's data came from published studies, articles, and books, among other source materials. In the 15 academic studies mentioned, a total of 59 species have been identified. The most employed species of frog in academic research is the common tree frog (Polypedates leucomystax). Platymantis corrugatus (horned forest frog) was identified and used nearly six times in the fifteen scholarly studies discovered. The conservation status of 42 of the frog species used and identified is mostly Least Concern. Meanwhile, three Platymantis species have been designated as Endangered. With 23 species, most frog species were last assessed in 2018. Several species were also assessed in 2008, with one species and seven species in 2004. The conservation status and year assessed for one frog species are not obtainable because it is a recently found species. In terms of ethical standards, fourteen of the fifteen studies abided by ethical standards in the safe handling of frogs, with one study not stating due to lack of information.

Keywords: *Frogs, academic research, conservation status*

INTRODUCTION

Frogs are tailless amphibians which belongs to the order Anura. It has prominent eyes, soft, supple skin, and powerful, webbed hind feet that are ideal for jumping and swimming. Frogs and toads have long been used in experiments, although they are now primarily used as dissection animals at higher education institutions rather than for pharmacology and physiology research (Tyler, 2009).

Regarding the usage of frogs in educational institutions, it is frequently utilized in biology lectures as exemplar vertebrates with amphibian characteristics and habits. Frogs provide ideal model creatures for scientific research into anatomy, development, behavior, and physiology. The advantage of using preserved frogs in dissection is that it is a good way to learn about vertebrate anatomy and complex body systems. Studying the frog's anatomy enables conversations about evolution and how its anatomical features are connected to their functions. Dissection lessons of frogs are suitable for a wide variety of students such as college students who learn about comparative anatomy (“Frog Dissection | Carolina.com”, 2022)

The animal is beneficial and employed all around the world. Many important cell biology and developmental ideas were discovered as a result of the study with frog embryos, including the molecular cell cycle regulation and the first finding of various positive vertebrate developmental genes. The importance and adaptability of the frog Xenopus as a model organism for vertebrate developmental pathways has long been recognized and never questioned (Blum &

Ott, 2019). Further utilization of species of frogs in diverse experiments can help in the development of the society.

Distinguishing the frog species involved in animal research in the Philippines may help the citizens and future researchers in becoming more knowledgeable of the country's most common species, and the researchers believe that there is a need for awareness on the unconventional facts pertaining to the frog community in the country. Several species are only recognized and scrutinizing the species of frogs used in animal research can contribute to the scientific field in the country. Moreover, understanding the conservation status of the species of the frogs enables the researchers and future researchers to be cognizant of the frog species that are vulnerable or endangered so that misuse and exploitation of the frogs will be avoided.

Ethical standards are significant before conducting an experiment. The welfare of all animals must be given proper attention and abuse must be circumvented. Various institutions such as universities and schools are the primary users of animals such as frogs for studying and gaining knowledge. The present study aims to establish if ethical standards are implemented by the academic institutions. The study overall, focuses on evaluating the distinct species of frogs, its conservation status, and if ethical standards were applied in several academic institutions that could ameliorate and accelerate the information concerning frogs in the Philippines.

Review of Literature

Population of Frogs

The diversity of amphibians and its endemism in the Philippines are high. The country currently has 110 native taxa of frog species, with 85 percent of the local fauna, highest estimate of endemism in the Indo-Malayan region. However, because of widespread amphibian population reductions, the ecology of amphibians has attracted attention in recent decades. According to the International Union for Conservation of Nature's Red List of Threatened Species (IUCN), 41 percent of worldwide amphibians are currently threatened with extinction, owing to overexploitation, habitat loss, pollution, climate change, and illness. (Jabon et al., 2019)

Due to the occurrence of climate change in the surroundings, several organisms struggle to survive which also affects their holistic development, vulnerability and survival rate. According to a study, there are 107 amphibians species including 3 caecilians, 104 frogs and toads in the Philippines which demonstrates the results of 26 species with a percentage of 24.30% under Highly Vulnerable, covers up the 44.86% of 48 species are the Moderately Vulnerable while 27 species are under the Vulnerable side that has a percentage of 25.23% and lastly, indicated of amphibians species that are Least Vulnerable are the 6 species which covers up 5.61%, which all in all under the rate and percent of vulnerability due to climate change. In line with this, in order to attain the vulnerability rate of amphibian species in the Philippines in relation to climate change, given criteria such as the status which describes the alien, non-endemic and endemic species are observed. Secondly, elevation which shows the ranges from wide, restricted up until lower limitations. While habitat including non-forest, caves, ponds and ground prior to the reproduction from egg stage to adulting stage. Lastly, rarity which discusses the common, intermediate common to uncommon and being rare (Alcala, Bucol, Diesmos, & Brown, 2012).

As mentioned above, one of the factors affecting endemism of frogs in the Philippines are also due to loss of habitat. Hence, habitat loss can immediately affect one’s survival inclined on food, selecting a mate and ability to reproduce. In this study, ecological sites from Mindanao specifically on Mt. Apo, Mt. Kitanglad, Mt. Hamiguitan and Mt. Malindang were selected as the locale of the study to determine the richness and endemism of the said species. Moreover, the researchers randomly selected 20x20 meters plot in a 1-hectare plot based on the given sites. Based on the results, 18 species were observed and found in the four ecological sites accordingly

together with 8 species endemic. Mt. Malindang got the highest species richness and endemism while Mt. Apo had the least species richness. Subsequently, Mt. Kitanglad anf Mt. Apo showed least number of endemic species (Mohagan et al., 2018).

On the other hand, patterns among the frog species on the Philippines specifically in Sundaland (Borneo) are said to be the closest relative or a possible distinct species. Puddle frogs from northern part of Borneo, Palawan, Tawi-Tawi, the Sulu Archipelago and Zamboanga in western Mindanao were the evident sites of species patterning. Occidozyga is a genus of frogs that are commonly known as the puddle frog are kind of frog species that has higher variability and considered as widespread among the other existing frog species. Among the different sites located on Mindanao archipelago, 6 classifications of species were determined and found. O. laevis, which is commonly related to the species of O. sumatrana, O. baluensis and O. diminutive while the outgroup is the species among the classification of O. martensii and O.lima. Therefore, evidence of species patterning among the puddle frogs are more distantly related based on the selected sites of Mindanao. If possible so, questions such as evolution of said species in ecological forms or reduced competition will be focused and asked (Chan, Schoppe, Rico, & Brown, 2021).

Furthermore, the introduction of the six given amphibian species such as Lithobates catesbeianus, Kaloula pulchra, Rhinella marina, Hoplobatrachus rugulosus, hylarana erythraea and Eleutherodactylus planirostris are the possible evidence in revealing history of obsolete invasion status, geographic risk and essentiality of alien invasions in the future. Through update and assessment of current to future geographic risk, the results showed high risk in geographic in relation to invasion of the present up until future alien anuran species. Therefore, Philippines must have preventive measures and appropriate action to solve and minimize the possibility of species invasions in the near future most specifically in the Philippines (Kurniawan, 2017).

In relation to invasion, certain observations among the Xenopus species including the X. borealis, X. gilli, X. muelleri, X. fraseri and X. tropicallis were obtained in order to answer the following questions pertaining to the ranges, distance, movement and behavior occurred in the said species during migration process. Results are revealed that both native and invasive species of Xenopus does transfer from land to land which has a total distance approximately 40 meters up to 2 kilometers in maximum. Yet, evidences of migration process are not proven based on temperature nor weather conditions thus, it occurs throughout the day. Besides, migration of the said species to the various bodies of water purposely for breeding and reproducing process also occurred. In addition, there are some reports that Xenopus species does invade water-bodies such as ponds or lakes which is an evident occurrence that they transfer from land to land (Measey, 2016).

Conservation of Frogs species

Extensive species checklists are required for the successful implementation of preservation and recovery strategies, and should evaluate vulnerability degree, conservation status, and anthropogenic threats (Spier et al., 2018).

The IUCN Categories and Criteria were created in 1994 to promote impartiality and openness in assessing species conservation status, and therefore to improve uniformity and comprehension among users (IUCN Standards and Petitions Committee, 2019). IUCN's mission is Influence, support, and help civilizations all over the globe in preserving nature's integrity and variety, as well as ensuring that any use of natural resources is fair and environmentally sustainable ("Vision and mission | IUCN," n.d.).

Ethical Standard Application

The genus of Xenopus or clawed frogs are commonly used in laboratory experiments because they are easy to breed and maintained in the laboratory, they are also small that makes them take a little space in the laboratory and plus they produce eggs all year round that makes them abundant in the environment and genetically they are similar to humans and therefore make a good animal model for studying anatomy and human diseases ("Why use the frog in research? | Facts | yourgenome.org," 2021).

The Guide for the Care and Use of Laboratory Animals (the Guide) aims to guide institutions in catering and utilizing animals in ways that are scientifically, technically, and humanly acceptable. The Guide is also designed to help investigators fulfill their responsibility to organize and execute animal studies in accordance with the highest scientific, humane, and ethical standards (National Research Council et al., 2011).

In an academic research at Mindanao State University-Main Campus, Marawi City 9700, Philippines, the karyotype of one of the Oreophryne species was described. Oreophryne anulata wherein ten mature frogs were collected via acoustic and opportunistic sampling at a site bordered by Terminalia woods and raised in an enclosure at a room temperature of 23–25 °C and was artificially lighted for 12 hours each day with a lamp, and the frogs were fed with caught fruit flies every other day for one to three months before being karyotyped. By squashing intestinal epithelial cells, vapor fixation, air-drying, and labeling with aceto-orcein, analyzable metaphase spreads were routinely produced from seven colchicine-treated frogs. The chromosomal analysis revealed a typical diploid karyotype of 2n=22, with four metacentric and seven submetacentric chromosome pairs and no discernible sex chromosomes. Based on the guidelines of Guide for the Care and Use of Laboratory Animals: Eighth Edition this study didn't violate any ethical standards in handling the frogs (Balinton & De La Seña, 2015).

Another study at Luzon region, presented a thorough species reports for 59 amphibian and reptile species (frogs, skinks, agamids, gekkonid lizards, varanids, and snakes) from the Pantabangan-Carranglan Watershed, which is located inside the Caraballo Mountain Range and whose biota is little documented. This was also the first comprehensive survey of the watershed's herpetofauna. The frogs used in this study were 18 different species. In the process, Wildlife Gratuitous Permit No. III-2015-06 was used to gather voucher specimens for species. Ethyl acetate was used to euthanize the specimens. The chemical ethyl acetate used in the euthanasia process was not found in various sources available online and therefore lacked in sufficient data to verify whether it is acceptable or not for euthanasia of amphibians (Cruz, Afuang, Gonzalez, & Gruezo, 2018).

Conceptual Framework

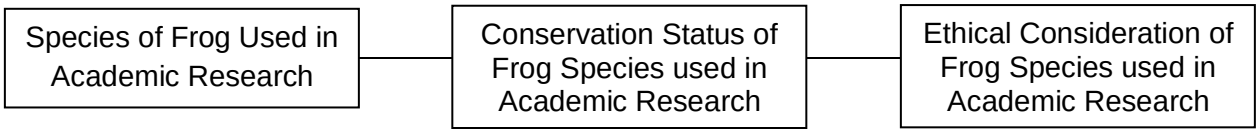

Figure 1. Conceptual framework of the Study

Figure 1 shows conceptual framework of the study. The study aims to determine the distinct species of frogs used in academic research in the Philippines as well as its conservation status and its ethical consideration which aligns proper practice done in experiments or research.

Objectives of the Study

Generally, this study aims to identify the species of frogs used in academic research in the Philippines for academic research set-up and their conservation status. Specifically, the study aims to determine the distinct species of frogs used in animal research in the Philippines. As well as to determine the conservation status of the frogs used in the research and lastly to determine if ethical standards were applied during the research procedures in academic institutions.

METHODOLOGY

The present study was conducted using a descriptive research design in bibliographic research approach which is aimed to describe a scenario or phenomena in detail and in a methodical manner. Secondary research approach was used, which is a method of gathering information from existing studies, literature, books, images, audio, and other sources. This method generated valuable data in the study in response to the objectives set by the researchers and was presented in tabular form.

In particular, fifteen academic studies from 2015 to 2021 involving frogs from the Philippines were gathered and evaluated in order to extract all of the frog species involved in the studies. In addition, the International Union for Conservation of Nature (IUCN) website was examined to determine and describe the conservation status of various frog species used in academic research in the Philippines. Meanwhile, a variety of reputable handbooks and guidelines were used to assess whether the process of preparing and euthanizing frogs in the academic studies was ethical or not, including Recommendations for euthanasia of experimental animals: Part 2 (Close, Croft, Lane, & Knoll, n.d.), Euthanasia - SPNHC Wiki (The Society for the Preservation of Natural History Collections, 2021), Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals., 2011), Measuring and monitoring biological diversity: Standard methods for amphibians (Heyer, 1994) and, AVMA Guidelines for the Euthanasia of Animals: 2020 Edition (Leary & Johnson, 2020). Manual on biodiversity assessment and monitoring system for terrestrial ecosystems: How-to guidelines (Biodiversity Management Bureau & Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, 2017) and, Recognition and Alleviation of Pain and Distress in Laboratory Animals (Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, & National Research Council, 1992).

RESULTS AND DISCUSSIONS

Table 1 presents the list of species used and their conservation status in all fifteen (15) academic research. The first species under the Family Bufonidae is the species of R. marina. While species including P. bayani, P. cornutus, P. corrugatus, P. dorsalis, P. guentheri, P. hazelae, P. isarog, P. lawtoni, P. luzonensis, P. mimulus, P. montanus, P. navjote, P. panayensis, P. polillensis, P. rabori, P. sierramadrensis and P. subterrestris are under the Family Ceratobatrachidae. The species under the Family Dicroglossidae are the F. moodiei, F. vittigera, H. rugulosus, L. leyntensis, L. macrocephalus, L. magnus, L. visayanus, L. woodworthi and O. laevis. On the Family Megophryidae, the species M. stejnegeri are used. While under the Family Microhylidae are the species of Kalophrynus pleurostigma, Kalophrynus sinensis, Kaloula conjuncta, Kaloula kalingensis, Kaloula negrosensis, Kaloula picta, Kaloula pulchra, Kaloula rigida and O. anulata. Whereas the species including H. erythraea, H.similis, P. grandocula, P. similis, S. aurantipunctata, S. igorota, S. luzonensis, S. tinpanan, S. guttatus, S. latopalmatus, S. natator, S. nubilus, S. parvus and S. tuberilinguis are under the Family Ranidae. Lastly, the

species such as K. appendiculatus, P. leucomystax, R. bimaculatus, R. pardalis, Philautus acutirostris, Philautus leitensis, Philautus surdus and Philautus worcesteri are under the Family Rhacophoridae.

Table 1. Frog species used and their conservation status with year assessed

Species Used	Conservation Status	Year Last Assessed
Family Bufonidae		
Rhinella marina	Least Concern	2008
Family Ceratobatrachidae		
Platymantis bayani	Data Deficient	2017
Platymantis cornutus	Least Concern	2017
Platymantis corrugatus	Least Concern	2018
Platymantis dorsalis	Least Concern	2018
Platymantis guentheri	Least Concern	2017
Platymantis hazelae	Vulnerable under criteria B1ab(iii)	2017
Platymantis isarog	Least Concern	2017
Platymantis lawtoni	Endangered under criteria B1ab(iii)	2017
Platymantis luzonensis	Near Threatened under criteria B1ab(iii)	2018
Platymantis mimulus	Least Concern	2017
Platymantis montanus	Vulnerable under criteria B1ab(iii)	2017
Platymantis navjoti	Not Available	Not Available
Platymantis panayensis	Endangered under criteria B1ab(iii)	2018
Platymantis polillensis	Least Concern	2017
Platymantis rabori	Least Concern	2017
Platymantis sierramadrensis	Vulnerable under criteria B1ab(iii)	2018
Platymantis subterrestris	Endangered under criteria B1ab(iii)	2018
Family Dicroglossidae		
Fejervarya moodiei	Data Deficient	2004
Fejervarya vittigera	Least Concern	2018
Hoplobatrachus rugulosus	Least Concern	2004
Limnonectes leytensis	Least Concern	2018
Limnonectes macrocephalus	Near Threatened under criteria A2acd	2018
Limnonectes magnus	Near Threatened under criteria A2acd	2018
Limnonectes visayanus	Near Threatened under criteria A2cd	2018
Limnonectes woodworthi	Least Concern	2018
Occidozyga laevis	Least Concern	2014
Family Megophryidae		
Megophrys stejnegeri	Least Concern	2017
Family Microhylidae		
Kalophrynus pleurostigma	Least Concern	2004
Kalophrynus sinensis	Least Concern	Not Stated
Kaloula conjuncta	Least Concern	2017
Kaloula kalingensis	Least Concern	2017
Kaloula negrosensis	Least Concern	Not Stated
Kaloula picta	Least Concern	2018
Kaloula pulchra	Least Concern	2004
Kaloula rigida	Least Concern	2017
Oreophryne anulata	Least Concern	2017
Family Ranidae		
Hylarana erythraea	Least Concern	2014
Hylarana similis	Least Concern	Not Stated
Pulchrana grandocula	Least Concern	2017
Pulchrana similis	Least Concern	2018
Sanguirana aurantipunctata	Vulnerable under criteria B1ab(iii)	2017
Sanguirana igorota	Vulnerable under criteria B1ab(iii)	2018
Sanguirana luzonensis	Least Concern	2018
Sanguirana tipanan	Vulnerable under criteria B1ab(iii)	2017
Staurois guttatus	Least Concern	2018
Staurois latopalmatus	Least Concern	2018
Staurois natator	Least Concern	2017
Staurois nubilus	Near Threatened under criteria B1ab(iii)	2017
Staurois parvus	Vulnerable under criteria B1ab(iii)	2018
Staurois tuberilinguis	Least Concern	2018
Family Rhacophoridae		
Kurixalus appendiculatus	Least Concern	2004
Philautus acutirostris	Least Concern	2018
Philautus leitensis	Least Concern	2018
Philautus surdus	Least Concern	2018
Philautus worcesteri	Least Concern	2017
Polypedates leucomystax	Least Concern	2004
Rhacophorus bimaculatus	Least Concern	2017
Rhacophorus pardalis	Least Concern	2004

In all the fifteen academic research found, the common tree frog (Polypedates leucomystax) are the most used species of frog in academic research. Prior to this, the Platymantis corrugatus (horned forest frog) under the Family Ceratobatrachidae was used and mentioned almost six times in all the fifteen academic research found. While species of H. rugulosus and R. marina are used for almost five times in the academic research provided. Next commonly used is the species of H. erythraea, K. pulchra and O. laevis. While the K. pleurostigma, K. kalingensis, L. leytensis, L. magnus, P. dorsalis and P. mimulus was used thrice in all the fifteen available academic research. The following species were used twice in the academic research including the F. moodiei, F. vittigera, K. conjucta, K. picta, L. macrocephalus, L. visayanus, L. woodworthi, P. leitensis, P. surdus, P. guentheri, P. montanus and S. luzonensis. While the rest of the species not mentioned are used only once in all the fifteen academic research found.

A significant number or forty-two of the frog species listed in the table has a conservation status of Least Concern. (Committee, 2021) stated that it is essential to convey that least concern, in simple words, these species are of less concern with regards to extinction risk than species in those other threat classifications. This is not to imply that these species are irrelevant in terms of conservation. All of the species in the genus Philautus, Kaloula, Pulchrana, Hylarana, and Rhacophorus are labeled as Least Concern. Some species belonging to the genus Oreophryne, Kalophrynus, Hoplobatrachus, and Rhinella are considered Least Concern.

Besides that, Platymantis bayani and Fejervarya moodiei are categorized as Data Deficient. As reported by (Committee, 2021), whenever there is insufficient evidence to form an evaluation of its danger of extinction, it is considered Data Deficient. One frog species, Platymantis navjoti, doesn't yet have a conservation status. News from (Anderson, 2020), reported that Platymantis navjoti is a newly discovered Platymantis species that inhabit the forests of Leyte and Samar islands in the Philippines.

There are five species that have been designated as Near Threatened. Limnonectes macrocephalus, Limnonectes magnus, Limnonectes visayanus, Platymantis luzonensis, and Staurois nubilus are the five species. A taxon is considered Near Threatened when it's been assessed against the criteria but doesn't currently eligible for Vulnerable, Endangered, or Critically Endangered, but is near to or is likely to be selected for a threatened classification in the coming years (Committee, 2021). For instance, Limnonectes macrocephalus is categorized as Near Threatened, as it is prevalent in suitable habitats, but has reduced in some areas because of overharvesting. The most significant threats to this species are loss of habitat as a result of logging, widening agriculture, and human habitation, as well as pollution of rivers and streams (Stuart et al., 2008). An additional example is Limnonectes magnus, as per (Stuart et al., 2008), which is Near Threatened due to over-exploitation. Threats in the Philippines involve loss of habitat caused by logging, agriculture, agricultural pesticides on rivers and streams, and collecting for human utilization and global export.

Vulnerable, is when the best current information demonstrates that a taxon fulfills any criteria A to E for Vulnerable, it is deemed to be encountering a high danger of extinction in the wild (Committee, 2021). Seven vulnerable species include Platymantis montanus, Platymantis sierramadrensis, Platymantis hazelae, Sanguirana aurantipunctata, Sanguirana igorota, Sanguirana tipanan, and Staurois parvus. One case is Platymantis sierramadrensis, it is considered to be Vulnerable because the scope and condition of its forest habitation on Luzon are deteriorating (Resources, 2018).

Ultimately, when the chief existing evidence signifies that a taxon satisfies any of the Endangered criteria A to E, it is perceived to be undergoing a very high risk of extinction in the wild or is considered to be Endangered (Committee, 2021). Three species from the genus Platymantis are identified as Endangered namely Platymantis subterrestris, Platymantis panayensis, and Platymantis lawtoni. According to (Wrinkled & Frog, 2018), Platymantis subterrestris is listed as Endangered for the reason that its scope of occurrence is 4,235 km2 and

all individuals are in less than five locations which are threat-defined. In addition, the magnitude and quality of its environment are both decreasing.

The year the conservation status of the frog species was last assessed is also illustrated. The table shows that the majority of species were last assessed in 2018, with 23 species. 22 species were evaluated in 2017, two in 2014, one in 2008, and seven in 2004. The year last assessed for two of the three species was not specified, and the year last assessed for one species was not available. There are examples of species belonging to the same genus with a ten-year gap or more from the most recent assessment year. Fejervarya moodiei and Fejervarya vittigera are two examples. Fejervarya moodiei was last assessed in 2004, but Fejervarya vittigera was last assessed in 2018. In addition, Limnonectesis is a great example of a genus whose species are all assessed in the same year, 2018.

The ethical frog handling guidelines were evaluated in fifteen academic studies from the Philippines. The three main Philippine islands were studied equally: Luzon, Visayas, and Mindanao. A checklist was used to assess adherence to ethical guidelines (see Table 2 note). The findings found out that fourteen of the fifteen research successfully followed ethical guidelines in handling frogs, with the other one classified as undetermined due to a lack of supporting data.

To elaborate on the findings, starting with Luzon, in the study of Merculio & Arvin, 2017 (1L), the frogs were obtained using hand/dip nets to avoid injury. According to the Manual on biodiversity assessment and monitoring system for terrestrial ecosystems: How-to guidelines, hand/dip nets will assist the safe handling of herpetofauna species such as frogs. As a result, (1L)'s procedure was deemed ethical. Meanwhile, the study of Cruz, Afuang, Gonzalez, and Gruezo, 2018 (2L) was categorized as undetermined since the chemical ethyl acetate utilized in the euthanasia process lacked sufficient data and studies to verify whether it is acceptable for use as euthanasia for amphibians. Moving forward, studies by Rivera & AM Paz-Alberto - e Business Manager et al, 2020 (3L), C. T. Gonzalez et al., 2020 (4L) and Vincent et al., 2021 (5L) utilized improvised traps and handpicking/reach and grab method to capture the surveyed frogs, then after gathering the essential data, the animals gathered in (3L) were released back to their capture point, whereas (5L) only photographed the surveyed frogs on-site. Both (3L) and (5L) did not gather voucher specimens. (3L) was considered ethical as frogs were released back to their capture site since unnecessary collection and slaughter of any species could disrupt the food web in the ecosystem (E. Gonzalez, 2017). Purposive sampling was used on (4L), which is a method that was elaborated in the Manual on biodiversity assessment and monitoring systems for terrestrial ecosystems: How-to instructions. Given all of the aforementioned steps, (3L), (4L), and (5L) were recognized as studies that successfully conformed with ethical guidelines in frog handling.

Moving to the studies from Visayas, In Supsup et al., 2016 (6V), captured frogs were recognized and measured, and the common species were released after the data was collected. Unidentified frog species were humanely collected and euthanized with aqueous chloretone, then fixed in 10% buffered formalin and transferred to 70% ethanol. The voucher specimens were eventually deposited at the Philippine National Museum's Herpetology Collections in Manila. Furthermore, Diesmos, Scheffers, Mallari, Siler, and Brown, 2020 (8V) also employed aqueous chloretone for the euthanasia of voucher frog specimens in their study. The processes utilized by (6V) and (8V) were classified as humane and ethical based on the Heyer et al., 1994 handbook, which is the most commonly used reference of the fifteen studies gathered in the Philippines. The use of aqueous chloretone was determined as the most efficient technique of killing adult amphibians in Heyer et al.'s handbook. Also, in the book of The Amphibians and Reptiles of Costa Rica: A Herpetofauna between Two Continents, between Two Seas, the use of chloretone was said to cause painless death in amphibians. As a result, (6V) and (8V) were labeled to be ethical. Then, in the study by Aureo and Bande, 2017 (7V), the frogs were captured directly with bare hands and released back into their habitat after measurements and photographs were acquired.

Additionally, Villanueva et al., 2021 (10V) clearly mentions that the procedures for capturing the frogs were based on the Manual on biodiversity assessment and monitoring system for terrestrial ecosystems: How-to guidelines. The study used purposive sampling to capture and observe the amphibians. Since the process of (7V) and (10V) was similar to that of (3L) and (4L), the research can likewise be considered ethical. Finally, in the study of Decena, Avorque, Decena, Asis, and Pacle, 2020 (9V), captured frog species were marked using the method of toe clipping, and after measuring and photographing the animals, they were returned back to the capture point unharmed. The toe clipping procedure, in which toes were removed in distinct combinations, was the least expensive method for marking anurans (Heyer, Donnelly, McDiarmid, Hayek, & Foster, 1994). According to the Guide for the Care and Use of Laboratory Animals, toe clipping is only acceptable if no other method of individual identification is available. Based on the supporting data, (9V) can be categorized as a study that complied to ethical frog handling guidelines.

On Mindanao, the sample frogs in Balinton & De La Sea, 2015 (11M) study were adapted and placed in an open enclosure at an air temperature of 23–25 °C for one to three months before being sacrificed for chromosomal analysis. The enclosure was artificially illuminated for 12 hours each day with a lamp, and the frogs were fed captured fruit flies (Drosophila melanogaster) every other day. Rainwater was sprayed on them at least twice a day to keep them moist. Water was poured into the cage and then drained via a hole at the bottom to drain waste. The study also stated that all institutional and national criteria for laboratory animal care and usage were followed, which was in fact true. According to the Guide for the Care and Use of Laboratory Animals, the microenvironmental temperature for amphibians should sustain their physiological process, air ventilation should compensate for thermal and moisture loads, and amphibians should have access to adequate food. (11M) shown effort and attention to detail in handling the sample frogs, for that reason, the study was labeled as ethical. On the other hand, the frog samples were handpicked in the study by Delima-Baron, Mohagan, Leano, and Amoroso, 2019 (12M). The Department of Environment and Natural Resources Region XI has granted the study a Gratuitous Permit for the gathering of voucher specimens. Given the study's procedure of obtaining an authorization permit before collecting voucher specimens, it is evident that (12M) followed ethical procedures on collecting frogs species Meanwhile, captured frogs were processed for identification and measurement in the studies by (Jabon et al., 2019 (13M) and Roselle et al., 2020 (14M). Frog samples that were not necessary for the next step in the procedure were returned to their natural habitat. The species subjected to stomach analysis were euthanized using the double-pithing procedure. Although, the efficiency of the pithing technique for painless death was questioned. According to the guidebook of Recognition and Alleviation of Pain and Distress in Laboratory Animals, double pithing is an effective method of killing some coldblooded animals since it destroys both the brain and the spinal cord. However, in order to be considered ethical, this technique should only be carried out by trained individuals. Assuming that the double-pithing technique was performed by a trained individual/s. h (13M) and (14M) were considered ethical. Lastly, Maglangit et al., 2020 (15M) collected voucher specimens of the surveyed frogs by euthanizing them using aqueous chloretone, which has previously been considered as a humane method in frog euthanasia. As a result, this study was also recognized as ethical.

Table 2. List of fifteen academic research in the Philippines that used frogs with an ethical standard checklist

REF#	Titles	Checklist
1L	Bioaccumulation of Cadmium in Selected Tissues of Hoplobatrachus rugulosus Wiegmann (2017)	✓
2L	Amphibians and Reptiles of Luzon Island, Philippines: the Herpetofauna of Pantabangan-Carranglan Watershed, Nueva Ecija Province, Caraballo Mountain Range (2018)	—
3L	Assessment of the Diversity of Animals in Mt. Tapulao, Palauig, Zambales (2020)	✓
4L	Understorey to canopy vertebrate fauna of a lowland evergreen forest in Mt. Makiling Forest Reserve, Philippines (2020)	
5L	Diversity of a highly variable landscape: The Herpetofauna of Central Luzon State University, Nueva Ecija, Philippines (2021)	✓
6V	Amphibians and Reptiles of Cebu, Philippines: The Poorly Understood Herpetofauna of an Island with Very Little Remaining Natural Habitat (2016)	✓
7V	Anurans Species Diversity and Composition along the Successional Gradient of the Evergreen Rainforest in Silago, Southern Leyte, Philippines (2017)	✓
8V	A new forest frog of the genus Platymantis (Amphibia: Anura: Ceratobatrachidae: subgenus Tirahanulap) from Leyte and Samar islands, eastern Philippines (2020)	✓
9V	Impact of habitat alteration on amphibian diversity and species composition in a lowland tropical rainforest in Northeastern Leyte, Philippines (2020)	✓
10V	Biodiversity in Forests over Limestone in Paranas,Samar Island Natural Park (SINP), A UNESCO World Natural Heritage Site Nominee (2021)	✓
11M	Karyotype of a rare minute frog, Oreophryne cf. anulata (Anura: Microhylidae), in Agusan Marsh, Mindanao, Philippines (2015)	✓
12M	Philautus (Bush Frogs) Species from Montane Forest of Marilog District, Davao City, Southern Mindanao, Philippines (2019)	✓
13M	Density and diet of invasive alien anuran species in a disturbed landscape: A case in the University of the Philippines Mindanao, Davao City,Philippines (2019)	✓
14M	Native and invasive alien anuran species in urbanized areas in Davao City, Philippines, with preliminary study of feeding biology (2020)	✓
15M	Leech parasitism on the Mindanao foot-flagging frog Staurois natator (Günther, 1858) on Mindanao Island, Philippines (2020)	✓

Legend. Main geographical area of the study: L=Study from Luzon, V=Study from Visayas, M=Study from Mindanao; Compliance to ethical guidelines in handling frogs: ✓=Successfully followed the ethical guidelines, X=Failed to follow the ethical guidelines, — = Undetermined

CONCLUSIONS

There are total of fifty-nine (59) species in the fifteen (15) academic research mentioned. A total of one (1) species in the Family Bufonidae, seventeen (17) Platymantis sp. classified on the Family Ceratobatrachidae, nine (9) species on the Family Dicroglossidae, one (1) species on the Family Megophryidae, nine (9) species under the Family Microhylidae, Family Ranidae consists of fourteen (14) species and eight (8) species on the Family Rhacophoridae.

In connection with the conservation status of each species used in academic research, two species are data deficient due to uncertainties in occurrence, populations status, and ecological conditions while forty-one were least concern, two species were classified as near threatened under critetia A2acd, one species on near threatened under criteria A2cd and two species stated as near threatened under criteria B1ab(iii). There were also approximately seven species identified as vulnerable under criteria B1ab(iii) and three species were mentioned as endangered under criteria B1ab(iii). Lastly, in one of the academic research mentioned, one newly discovered species, Platymantis navjoti, was classified as not available since this species was newly discovered. Furthermore, the conservation status of forty-eight (48) IUCN species was considerably updated from 2017 to 2018, except for seven (7) species whose status is outdated spanning from 2014 to 2004, and four (4) species whose status is not stated or not available.

In the concern of ethical standards, all academic research found applied proper ethical considerations except for one study which was considered undetermined since the chemical used, ethyl acetate, lacked sufficient data confirming that it is acceptable for euthanasia in amphibians.

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