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TRINH THI HONG

RESEARCH ON DISTRIBUTION AND NUTRITIONAL CHARACTERISTICS OF AMPHIBIANS IN BEN EN NATIONAL PARK AND PU LUONG NATURE RESERVE, THANH HOA PROVINCE

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Supervisors:

1. Supervisor 1: Dr. Pham Thi Nhi
2. Supervisor 2: Dr. Dau Quang Vinh

Referee 1: Assoc. Prof. Hoang Van Ngoc

Referee 2: Assoc. Prof. Le Trung Dung

Referee 3: Assoc. Prof. Tran Duc Hau

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INTRODUCTION

1. The necessity of the research

Amphibians are a group of vertebrates with diverse habitats and are recorded to be distributed across most habitat types worldwide. They play a crucial role in food chains and webs within various ecosystems [1,2].

Aggregate up-to-date data on species composition, habitat-based distribution, and the nutritional characteristics of amphibians is an urgent task. Implementing such research will not only deepen our understanding of their ecological roles and help predict the impacts of amphibian population declines but also provide essential information for conservation programs. This, in turn, will support the prioritization of strategies and enhance amphibian conservation efforts.

In Thanh Hoa, Ben En National Park represents a lowland mountain ecosystem (the highest mountain peak is 497m above sea level) belonging to the Northeast lowland sub-region [12] [13] [14] and Pu Luong Nature Reserve represents a highland mountain ecosystem belonging to the Northwest highland sub-region [13] [14] [15], both of which have diverse and rich fauna and flora. There have been a number of studies on amphibians here. However, studies have focused on species diversity [16] [17], studies on distribution characteristics are incomplete [16] [18] and no studies have analyzed the food composition of amphibians here.

Based on that practical situation, the topic: "*Research on distribution and nutritional characteristics of amphibians in Ben En National Park and Pu Luong Nature Reserve, Thanh Hoa province*" aims to update the list of species composition, analyze the distribution and nutritional characteristics of amphibians in the two special-use forests above.

2. Objectives

General Objective: This study aims to evaluate the species diversity, distribution pattern and diet of amphibians in Ben En National Park and Pu Luong Nature Reserve in Thanh Hoa province.

Specific objectives:

- Assess the diversity of amphibian species in Ben En National Park and Pu Luong Nature Reserve.

- Analyze the distribution characteristics of amphibian species according to their habitat types, altitudes and habitats in Ben En National Park and Pu Luong Nature Reserve.

- Analyze the nutritional characteristics of amphibian species according to their habitat types, altitudes and habitats in Ben En National Park and Pu Luong Nature Reserve.

3. Contents

Contents 1: Assessment of amphibian species diversity in Ben En National Park and Pu Luong Nature Reserve

- List of amphibian species in Ben En National Park and Pu Luong Nature Reserve

- Assessment of rarity and conservation of amphibian species in Ben En National Park and Pu Luong Nature Reserve

- Comparison of species diversity and similarity with neighboring protected areas

Contents 2: Evaluation of distribution characteristics of amphibians in Ben En National Park and Pu Luong Nature Reserve according to: macrohabitat type, elevation levels and microhabitat.

Contents 3: Assessment of nutritional characteristics of some amphibian species in Ben En National Park and Pu Luong Nature Reserve. Analysis of nutritional characteristics of some species representing macrohabitat type, elevation levels and their microhabitat in Ben En National Park and Pu Luong Nature Reserve.

4. Scientific and conservation significances

The research results of the project will provide updated data on the species composition, distribution, and food spectrum of amphibians in Ben En National Park and Pu Luong Nature Reserve, Thanh Hoa province.

These research results will serve as an important scientific basis for planning the conservation and sustainable development of biodiversity in Thanh Hoa province.

5. New findings

Updated and supplemented the list of amphibian species in Ben En National Park (30 species) and Pu Luong Nature Reserve (28 species). Recorded additional 02 species for Ben En National Park and 19 species for Pu Luong Nature Reserve.

Analyzed the distribution characteristics of amphibian species in Ben En National Park and Pu Luong Nature Reserve based on macrohabitat type, elevation levels and microhabitat.

Analyzed the nutritional characteristics of 19 amphibian species in Ben En National Park and Pu Luong Nature Reserve through their food composition.

Analyzed the nutritional characteristics of selected amphibian species representing different macrohabitat type, elevation levels and microhabitat.

Chapter 1. LITERATURE REVIEW

1.1. Overview of research on amphibians in Indochina and in Vietnam

1.1.1. Research on amphibian species diversity in Indochina and in Vietnam

The number of newly described species and new records for Vietnam has increased significantly in recent years. In 2009, the number of recorded species was 176, and it has now increased to 300 species. Research areas have been expanded to cover almost all biogeographical regions of amphibians in Vietnam.

1.1.2. Taxonomic revision studies

In Thanh Hoa province, the species *Microhyla fissipes* was reidentified as *Microhyla mukhlesuri*; *Amolops cremnobatus* was renamed as *Amolops tanfuilianae*; *Xenophrys major* was renamed as *Xenophrys lancangica*; and *Ichthyophis bannanicus* was reidentified as *Ichthyophis kohtaoensis*.

1.1.3. Research on the distribution patterns of amphibians in Indochina and Vietnam

1.1.3.1. Biogeographical regionalization of amphibians

Vietnam has 11 biogeographical sub-regions of amphibians out of the 23 sub-regions in the Indochina region. Pu Luong Nature Reserve belongs to the Northwest Highland sub-region, while Ben En National Park is located in the Northeast Lowland sub-region. The elevation is divided into three elevation levels (<300 m; 300–800 m; >800 m). The habitat is categorized into two types: forest and open forest. There are three types of amphibian microhabitats: aquatic (underwater), terrestrial (on the ground), and arboreal (on trees).

1.1.3.2. Study on the distribution of amphibian species according to macrohabitat type, elevation levels and places of residence

The food spectrum of amphibians mainly consists of insects, with coleopterans, hymenopterans, orthopterans, and termites being the dominant prey. In particular, in some large amphibians, remains of small amphibians and reptiles have also been found in their stomachs.

1.1.5. Overview of Amphibian Research in Thanh Hoa Province

1.1.5.1. Studies on the species composition and distribution of amphibians

In Ben En National Park and Pu Luong Nature Reserve, research has primarily focused on species diversity. However, no comprehensive study has been conducted on the distribution characteristics of amphibians, nor has there been any analysis of their food composition.

1.2. Overview of natural and socio-economic conditions of the research area

1.2.1. Overview of natural and socio-economic conditions at Ben En National Park

1.2.1.1. Natural conditions

1.2.1.2. Population, socio-economics

1.2.2. Overview of natural and socio-economic conditions in Pu Luong

Nature Reserve

1.2.2.1. Natural conditions

1.2.2.2. Population, socio-economics [81]

Chapter 2. RESEARCH METHODOLOGY

2.1. Field surveys and study sites

2.2. Methods

2.2.1. Sampling

A routine method in amphibian research.

* Method of collecting amphibian food samples

Method 1: Gastric resection

Method 2: Direct dissection to remove the stomach

2.2.2. Laboratory research methods

- Morphological analysis

- Morphological criteria used in amphibian identification[83]

- Species identification:

- Methods for identification of prey samples

2.2.3. Analysis of distribution characteristics of amphibians

Distribution by macrohabitat type; Distribution by elevation levels; Distribution by microhabitat

2.2.4. Evaluation of rare species with conservation value

2.2.5 Indexes used in the study of food composition of amphibians

- Frequency (F) represents the abundance of some prey species obtained from stomach samples:

- Number (N) is the number of food samples of a prey species:

- Volume (V, mm3) of each food sample is estimated using the formula according to the documents of Ngo et al. (2014) [92] and Magnusson et al. (2003) [93]:

- Importance index (Ix) of each food species is calculated using the formula described in the documents of Caldart et al. (2012) [94] and Ngo et al. (2014) [92]:

- Shannon – Weiner biodiversity index (H'); Dominance index (D) [96]; Simpson evenness index (1-D); Pianka index; Inheriting previous research documents.

- Data processing In this study, we used PAST software to analyze the data on feed composition. In which, we used the Dice index (Nei, 1979) to calculate the similarity between feed components.

+ Shapiro-Wilk test was used to test the distribution of variables.

+ Mann-Whitney test was used to post-test the difference between two independent groups but not normally distributed.

Chapter 3. RESULTS AND DISCUSSION

3.1. Amphibian species composition in the study area

3.1.1. List of amphibian species

Based on the analysis of specimens collected through field trips and referring to published documents, it was found that in Ben En National Park and Pu Luong Nature Reserve, there are a total of 42 amphibian species, belonging to 8 families, of which the Rhacophoridae family has the most diverse number of species with 9 species (accounting for 23.26%), followed by the Microhylidae family and the Ranidae family with 8 species (accounting for 18.6%), the Dicroglossidae family with 7 species (accounting for 16.28%), the Megophryidae family with 6 species (13.95%), the Bufonidae family with 2 species (4.65%), the lowest are the Hylidae family and the Ichthyophiidae family with 1 species (2.33%).

Some changes in taxonomic revision: *Microhyla fissipes* was re-identified as *Microhyla mukhlesuri* [50]; *Amolops cremnobatus* was renamed *Amolops tanfuilianae* [56]; *Xenophrys major* was renamed *Xenophrys lancangica* [55]; *Ichthyophis bannanicus* was re-identified as *Ichthyophis kohtaoensis* [51].

The research results recorded 30 amphibian species belonging to 7 families of the Anura order at Ben En National Park. Among them, the Microhylidae family had the highest species diversity, with 8 species (26.67%), followed by the Ranidae and Dicroglossidae families, each with 6

species (20.00%). The Rhacophoridae family recorded 4 species (13.33%), Megophryidae had 3 species (10.0%), and Bufonidae had 2 species (6.67%). The family with the lowest diversity was Hylidae, with only 1 species (3.33%).

Compared with the studies of Nguyen Van Sang et al. (2000) [17], this study recorded two additional species for Ben En National Park: Kalophrynus interlineatus and Ophryophryne microstoma.

3.1.2. Identification characteristics of newly recorded species for the study area

In this study, rapid identification characteristics based on the specimens collected in the research area were provided for each species. Information about the specimens (date of collection, coordinates of collection, and location of collection). The study described identification characteristics of 17 amphibian species in the research area.

The following are the identification characteristics of a representative species:

1) *Limnonectes bannaensis* – Banna frog.

Research sample: (n=1): 1♀ HDU 03659 collected in Pu Luong Nature Reserve.

Identification characteristics: Body size is average [♀ SVL = 64.4 mm (n = 1)]. Head length is shorter than head width. Teeth are short, thick, and arranged obliquely in a V-shape; tongue is bifurcated; eardrum is not visible.

– **Forelimbs:** Forelimb I is equal in length to forelimb II and lacks webbing. Hindlimbs are fully webbed.

– **Body coloration and characteristics:** Skin is smooth and flabby; dorsal surface is grayish-brown to black; the interorbital region exhibits dark streaks; 2–3 dark streaks are present on the lips; the belly is creamy white; brown streaks are found on the chin and chest. The lower legs have small granules, each with a central white dot.

Recording location: Forest near Eo Ken village, Pa Ban, Dong Dieng,

Thanh Son commune, Khuyn village, Co Lung commune, Ba Thuoc district.

2) *Leptobrachella petrops* - Eyebrow toad petrops
3) *Leptobrachium chapaense* - Chapa spadefoot toad
4) *Boulenophrys parva* – Lesser horned toad
5) *Ophryophryne microstoma* – Small-mouthed Toad
6) *Boulenophrys palpebralespinosa* - Spiny eyelid horned toad
7) *Xenophrys lancangica* – Lancang horned toad
8) *Kalophrynus interlineatus* – Striped sticky Frog
9) *Microhyla mukhlesuri* - Mukhlesur's narrow-mouthed Frog
10) *Microhyla* cf. *heymonsi*- Heymon's narrow-mouthed Frog
11) *Microhyla pulchra* - Beautiful narrow-mouthed frog
12) *Odorrana chloronota* - Bright green Frog
13) *Amolops tanfuilianae* - Fui Lian's Lao torrent frog
14) *Hylarana guentheri* - Guenther's frog
15) *Rana johnsi* - Johns' groove-toed frog
16) *Polypedates megacephalus* - Big Headed Tree Frog
17) *Theloderma lateriticum* - Brick-red Bug-eyed Frog

3.1.3. Species of conservation concern

Six species of conservation value (13.95%) were recorded in the study area, including two species in Ben En National Park and four species in Pu Luong Nature Reserve, as detailed below:

Five species are listed in the Vietnam Red Book (2007) [91], including one classified as Critically Endangered (CR) (*Boulenophrys palpebralespinosa*), two as Endangered (EN) (*Quasipaa delacouri, Rhacophorus kio*), and two as Vulnerable (VU) (*Ingerophrynus galeatus, Ichthyophis kohtaoensis*).

One species is listed in the IUCN Red List (2024) [9] as Near Threatened (NT) (*Leptobrachella petrops*).

3.1.4. Comparison of amphibian species composition similarity between Ben En National Park and Pu Luong Nature Reserve and adjacent protected areas

The results indicated that the highest similarity in amphibian species composition was observed between Pu Luong Nature Reserve and Nam Dong Nature Reserve (djk = 0.74), followed by Ben En National Park and Cuc Phuong National Park (djk = 0.70). The lowest similarity was recorded between Ben En National Park and Nam Dong precious gemstone conservation area (djk = 0.52).

3.2. Distribution Patterns of Amphibian Species in the Study Area

3.2.1. Distribution patterns of amphibian species Ben En National Park

3.2.1.1. Distribution by macrohabitat type

The research findings indicate that the secondary regenerating forest habitat supports the highest amphibian species richness, with a total of 26 species, accounting for 86.67% of the total recorded species. This is followed by the residential and agricultural land habitat, where 18 species were recorded (60%), while the least diverse habitat was the minimally disturbed evergreen forest, with 12 species, representing 40% of the total.

A total of five species were recorded across all three macrohabitat types, namely *Microhyla mukhlesuri*, *Microhyla cf. heymonsi*, *Polypedates megacephalus*, *Polypedates mutus*, and *Rhacophorus orlovi*. Additionally, six species were found to occur exclusively in two macrohabitat types—secondary regenerating forest and minimally disturbed evergreen forest—including *Limnonectes bannanensis*, *Quasipaa delacouri*, *Leptobrachium chapaense*, *Ophryophryne microstoma*, *Xenophrys lancangica*, and *Odorrana nasica*.

These results are consistent with those reported by Le et al. (2020) [64], as both study sites are located within the same lowland Northeastern biogeographic subregion and represent conservation areas with similar ecological characteristics.

3.2.1.2. Distribution by elevation levels

Ben En National Park is characterized by lowland hill ecosystems, situated within the Northeastern lowland subregion [13,14]. The highest hill peak in the park reaches an elevation of approximately 497 meters above sea level. However, the stream systems within the forest are predominantly located at elevations below 300 meters. Consequently, the majority of field surveys and research activities were conducted within this altitudinal range.

3.2.1.3. Distribution by microhabitat

The study on microhabitat distribution of 30 amphibian species in Bến En National Park revealed a clear stratification of ecological niches and habitat use. This vertical and horizontal partitioning among species contributes to maintaining balance within the biological community in terms of both shelter and food resources. Amphibians were predominantly distributed in areas with high humidity, such as beneath forest canopies, along stream banks, in ponds, and in swampy areas.

The investigation into microhabitat preferences aimed to assess the degree of spatial separation among species within the ecosystem, as well as their adaptability to different habitat types. However, species distribution was found to fluctuate frequently, largely depending on environmental factors such as humidity, air temperature, and food availability.

The results recorded that terrestrial habitats supported the highest species richness, with 24 species (80%). The remaining two habitat types each recorded 5 species (16.67%). Notably, three species: *Hoplobatrachus chinensis, Limnonectes bannaensis,* and *Odorrana nasica*—were observed to inhabit both aquatic and terrestrial environments.

3.2.2. Distribution patterns of amphibian species Pu Luong Nature Reserve

3.2.2.1. Distribution by macrohabitat type

The research findings recorded in Pù Luông Nature Reserve indicate that amphibian species richness was highest in the minimally disturbed evergreen forest habitat, with 19 species, accounting for 67.86% of the total. This was

followed by the secondary regenerating forest, which supported 17 species (60.71%)**,** while the residential and agricultural land habitat recorded the lowest diversity, with only 10 species (35.71%).

3.2.2.2. Distribution by elevation levels

In Pu Luong Nature Reserve, amphibian species richness was highest at elevations ranging from 300 to 800 meters, with 23 species, accounting for 85.19% of the total. This was followed by elevations above 800 meters, where 16 species (59.26%) were recorded. The lowest species richness was observed at elevations below 300 meters, with 14 species, representing 51.85% of the total.

3.2.2.3. Distribution by microhabitat

In Pu Luong Nature Reserve, terrestrial amphibians accounted for the highest species richness, with 17 species (62.96%). This was followed by arboreal species, with 9 species (33.33%), and aquatic species, with 7 species (25.93%). The lowest number of species was recorded in fossorial habitats, with only one species, the caecilian *Ichthyophis kohtaoensis*, representing 3.7% of the total.

3.3. Dietary composition of amphibian species

3.3.1. Dietary composition of selected amphibian species

1) Dietary composition of the asian common toad (Duttaphrynus melanostictus)

Regarding prey occurrence frequency, Formicidae was the most frequently encountered prey item, recorded 14 times (F = 87.50%), followed by Scarabaeidae with 10 occurrences (F = 62.5%), and Rhinotermitidae with 8 occurrences (F = 50%). Other prey families were recorded less frequently, ranging from 1 to 5 times, F: 6.25–31.25%.

In terms of prey abundance, Rhinotermitidae accounted for the highest number of individual prey items with 405 specimens (45.97%), followed by Kalotermitidae with 263 specimens (29.85%), and Formicidae with 101 specimens (11.46%). The remaining prey types were represented by only a

single individual.

With respect to prey volume, Rhinotermitidae also contributed the largest total volume at 21,323.20 mm^3 (58.54%), followed by Scarabaeidae at 2,878.18 mm^3 (7.9%). Other prey taxa contributed relatively small volumes, ranging from 0.001% to 5.4% of the total.

Rhinotermitidae was identified as the most important prey taxon in the diet of *Duttaphrynus melanostictus*, with the highest Index of Relative Importance (Ix = 51.5%), followed by Formicidae (Ix = 34.32%) and Scarabaeidae (Ix = 24.34%).

2) Dietary composition of the rice field frog (Fejervarya limnocharis)

Regarding prey occurrence frequency, Formicidae was the most frequently encountered prey group, recorded 21 times (F = 46.66%), followed by Rhinotermitidae with 13 occurrences (F = 28.88%). Other prey families appeared less frequently in the stomach contents, ranging from 1 to 11 occurrences, F: 2.22%–24.44%.

In terms of prey abundance, Rhinotermitidae accounted for the highest number of individual prey items with 49 specimens (18.28%), followed by Formicidae with 40 specimens (14.92%). The lowest counts were associated with prey types recorded only once.

With respect to prey volume, Gryllotalpidae contributed the largest proportion, comprising 31.25% of the total prey volume, followed by Kalotermitidae with 9.47%. Other prey items contributed between 0.004% and 8.76%.

Based on the Index of Relative Importance (Ix), Formicidae was identified as the most significant prey group in the diet of *Fejervarya limnocharis* (Ix = 21.43%), followed by Rhinotermitidae (Ix = 17.9%), Scarabaeidae (Ix = 12.88%), and Gryllotalpidae (Ix = 12.15%).

3) Dietary composition of the banna frog (Limnonectes bannaensis)

The dietary composition of two individuals of *Limnonectes bannaensis* from the Pu Luong Nature Reserve was examined. The results indicated that

the Banna wrinkled frog consumed five different prey types**.** Formicidae was identified as the prey item with the highest frequency of occurrence**,** highest number of individuals**,** and highest relative importance. Specifically, it was recorded with a frequency (F) = 100%**,** number of individuals (N) = 3, and an Index of Relative Importance (Ix) = 51.91%**.**

4) Dietary composition of the lesser tree frog (Hyla simplex)

Stomach content analysis of 21 individuals of *Hyla simplex* collected from Ben En National Park revealed a total of 20 arthropod families representing various invertebrate prey taxa. The species exhibited a broad diet, preying on a wide range of invertebrates. However, ants belonging to the family Formicidae were identified as the most dominant prey group, characterized by the highest frequency of occurrence (F = 90,48%), the highest number of individuals consumed (N = 80), and the highest Index of Relative Importance (Ix = 57.04%). These results indicate a strong trophic dependence on ants, reflecting both their ecological availability and potential ease of capture in the species' arboreal habitat.

5) Dietary composition of the Sapa spadefoot toad (Leptobrachium chapaense)

Stomach content analysis was conducted on 29 individuals of *Leptobrachium chapaense*, including 7 specimens from Ben En National Park and 22 specimens from the Pu Luong Nature Reserve. A total of 19 distinct invertebrate prey types were identified across all samples. Among these, 11 prey types were found in individuals from both sites, whereas 3 prey types were exclusive to the Ben En population and 5 prey types were found only in the Pu Luong population.

This variation suggests potential spatial differences in prey availability or foraging behavior between the two sites. Notably, ants of the family Formicidae were identified as the most important prey group in the diet of *L. chapaense*, contributing the highest values across key feeding indices. This dominance reflects both the ecological abundance of ants and their

accessibility within the species' *Leptobrachium chapaense* microhabitat.

6) Dietary Composition of the Small-eyed Toad (Boulenophrys parva)

Stomach content analysis of three individuals of *Boulenophrys parva* collected from the Pu Luong Nature Reserve revealed the presence of three distinct prey types, including representatives of the molluscan group Pulmonata, the invertebrate family Formicidae (ants), and the annelid family Lumbricidae (earthworms).

7) Dietary composition of the Small-mouthed Toad (Ophryophryne microstoma)

Stomach content analysis of five individuals of *Ophryophryne microstoma* revealed prey items belonging to five invertebrate families. Among these, Lepidoptera larvae were identified as the prey type with the highest encounter frequency and number (F = 40%; N = 4).

8) Dietary composition of the Spiny eyelid horned Toad (Boulenophrys palpebralespinosa)

Stomach content analysis of Boulenophrys palpebralespinosa revealed three distinct prey types, including amphibian tadpoles, members of the gastropod family Euconulidae, and ants from the family Formicidae.

9) Dietary Composition of the Lancang's horned toad (Xenophrys lancangica)

Stomach content analysis of six individuals of *Xenophrys lancangica* collected from the Pu Luong Nature Reserve revealed a total of three prey categories, including terrestrial snails (Pulmonata), grasshoppers (Acrididae), and roundworms (Nematoda). Among these, Pulmonata emerged as the most significant prey group, exhibiting the highest frequency of occurrence (F = 83.33%), the greatest numerical abundance (N = 5).

10) Diet composition of the Banded Bullfrog Kaloula pulchra

During the study conducted at Ben En National Park, stomach contents from six individuals of *Kaloula pulchra* were collected and analyzed. The results revealed that the species' diet consisted of prey belonging to 15

invertebrate families. Among these, ants (Formicidae) were present in all stomach samples (100%), indicating their ubiquitous presence in the diet. However, termites (Rhinotermitidae) represented the most dominant prey group in terms of number (%N = 50.19%), volume (%V = 74.40%), and overall importance index (Ix = 63.75%), suggesting that they are the primary prey item for *K. pulchra* in the studied area.

11) Diet Composition of the Striped sticky Frog Kalophrynus interlineatus

A dietary analysis was conducted on six individuals of *Kalophrynus interlineatus* collected from Ben En National Park. The study identified prey items belonging to 12 invertebrate families. The species primarily consumed prey from the ant family (Formicidae) and three termite families: Kalotermitidae, Rhinotermitidae, and Termitidae. Among these, Rhinotermitidae was determined to be the most important prey group, contributing 34.98% to the overall diet. This was followed by Formicidae (28.15%) and Kalotermitidae (19.28%), indicating a preference for social insects in the foraging strategy of *K. interlineatus* within the study area.

12) Diet Composition of the Mukhlesur's narrow-mouthed Frog Microhyla mukhlesuri

The dietary composition of 11 individuals of *Microhyla mukhlesuri* was examined at Ben En National Park. The analysis revealed that prey items belonged to six insect families. *M. mukhlesuri* exhibited a strong feeding preference for ants, which were found to be the dominant prey type in terms of frequency of occurrence (F = 81.82%), number of prey items (%N = 94.09%), volume (%V = 54.78%), and overall importance index (Ix = 76.89%). These values were markedly higher compared to those of other prey types, indicating that ants constitute the principal food source for this species in the study area.

13*) *Diet composition of the Narrow-Mouthed Frog Microhyla* cf. *heymonsi

During the study, the diet of 13 individuals of *Microhyla cf. heymonsi* was analyzed, including 7 specimens collected from Ben En National Park and 6 from Pu Luong Nature Reserve. A total of 178 prey items were recorded, belonging to five invertebrate families. Among these, ants of the family Formicidae were identified as the preferred food source. Notably, across both study sites, Formicidae exhibited the highest values in terms of frequency of occurrence, number of prey items, volume, and Index of Relative Importance (IRI), significantly exceeding those of other prey categories. These findings highlight the prominent role of Formicidae in the dietary composition of *Microhyla cf. heymonsi*.

14*) *Diet composition of the Beautiful narrow-mouthed frog Microhyla pulchra

The diet of six individuals of *Microhyla pulchra* was analyzed, including four specimens collected from Ben En National Park and two from Pu Luong Nature Reserve. The results revealed five types of invertebrate prey. Among these, ants belonging to the family Formicidae were present in the majority of stomachs examined and were recorded in significantly higher numbers compared to other prey types, indicating their prominent role in the diet of M. pulchra across both study sites.

15) *Diet Composition of the Fui Lian's Lao torrent frog Amolops tanfuilinae*

The dietary composition of 23 individuals of *Amolops tanfuilinae* was examined at Pu Luong Nature Reserve. The analysis identified prey items belonging to 21 invertebrate families. Among these, beetles (Order: Coleoptera) and spiders (Order: Araneae) showed the greatest family-level diversity, with seven families recorded in each. Ants (Formicidae) were found to be the most frequently occurring prey (F = 52.17%), with the highest

number of prey items (%N = 35.21%) and the highest importance index (Ix = 32.77%), indicating their dominance in the diet.

16) Diet composition of Günther's Frog Hylarana guentheri

A total of 28 individuals of *Hylarana guentheri* were analyzed, including 15 from Ben En National Park and 13 from Pu Luong Nature Reserve. The stomach content analysis yielded 150 prey items belonging to 28 invertebrate families and one vertebrate family. Notably, one prey item from the family Dicroglossidae was recorded, though it was only relevant in the diet of larger individuals. The species demonstrated a broad dietary range. In addition to consuming a variety of insect prey typical of other amphibians, *H. guentheri* was found to ingest larger prey, such as gastropods (Class: Gastropoda), freshwater crabs (Parathelphusidae), and amphibians (Dicroglossidae).

17) Diet composition of the Annamite Stream Frog Hylarana annamitica

The diet of 10 individuals of *Hylarana annamitica* collected from Ben En National Park was analyzed. A total of 29 invertebrate prey types were identified. Ants (Formicidae) were found to be the most important prey item (Ix = 35.55%), followed by stink bugs (Pentatomidae) with an importance index of 17.03%, and crab spiders (Thomisidae) at 15.88%.

18) Diet composition of the Big-headed Tree Frog Polypedates megacephalus

A total of 44 individuals of *Polypedates megacephalus* were examined, including 32 from Ben En National Park and 12 from Pu Luong Nature Reserve. A combined total of 283 prey items from 28 invertebrate families were identified. At Ben En, 213 prey items from 22 families were recorded, while 68 prey items from 11 families were found at Pu Luong. Six families were shared between the two sites; 16 families were unique to Ben En, and five families were exclusive to Pu Luong. At both sites, termites (Rhinotermitidae) were the most important prey, with an importance index of 30.90% at Ben En and 57.18% at Pu Luong.

19) Diet composition of the Burmese Tree Frog Polypedates mutus

The dietary composition of four individuals of *Polypedates mutus* was examined, revealing 23 prey items belonging to four invertebrate families. Termites of the family Kalotermitidae were dominant in the diet, accounting for 17 out of the 23 prey items (73.91%), indicating a strong feeding preference for this prey type.

3.3.2. Diet composition of amphibian species across different habitat types

This study examined the diet composition of three amphibian species in Pu Luong Nature Reserve, each representing a distinct habitat type: *Fejervarya limnocharis* as a representative of residential and agricultural land, *Hylarana guentheri* for regenerating secondary forest, and *Leptobrachium chapaense* for undisturbed evergreen forest.

Stomach contents from 24 individuals of *Fejervarya limnocharis*, inhabiting residential and agricultural areas, revealed 36 prey types belonging to 12 invertebrate orders. The species exhibited a highly diverse diet. Among the prey, spiders (Order: Araneae), beetles (Coleoptera), and true bugs (Hemiptera) were the most represented, each contributing six families, accounting for 16.66% of all recorded families at this elevation. Ants (Formicidae) were identified as the most frequently encountered prey (F = 41,67%) and the most numerous (N = 20).

In the regenerating secondary forest habitat, stomach contents from 13 individuals of *Hylarana guentheri* yielded 18 prey types, primarily invertebrates. Notably, one prey item was a juvenile frog of the family Dicroglossidae, indicating occasional predation on vertebrates. The species consumed a broad and relatively even array of prey items. Formicidae again emerged as the most frequently encountered and most numerous prey (F = 23,08%, N = 6).

For *Leptobrachium chapaense*, a species associated with undisturbed evergreen forests, stomach contents from 22 individuals revealed 14 prey types. Ants (Formicidae) dominated the diet, showing the highest frequency of occurrence (F = 68,18%) and the greatest number of prey items (N = 50), resulting in the highest importance index (Ix = 46.68%) among all prey

categories.

3.3.3. Diet Composition of Amphibian Species Across Elevation Gradients

This study analyzed the dietary composition of three amphibian species in Pu Luong Nature Reserve, each representing a different elevation zone. *Fejervarya limnocharis* was selected as the representative for low elevation zones (below 300 m), *Amolops tanfuilianae* for mid-elevation zones (300–800 m), and *Boulenophrys palpebralespinosa* for high elevation zones (above 800 m).

Stomach content analysis of 20 individuals of *Fejervarya limnocharis* from below 300 m revealed 32 prey types belonging to 10 invertebrate orders. The species exhibited a diverse diet. Spiders (Order: Araneae), beetles (Coleoptera), and true bugs (Hemiptera) were the most represented, with six families each, accounting for 16.66% of all prey families recorded at this elevation. Ants (Formicidae) were the most dominant prey item in terms of frequency (F = 50%), number of individuals (N = 20), and importance index (Ix = 26.51).

In the mid-elevation zone (300–800 m), 23 individuals of *Amolops tanfuilianae* were analyzed. The results identified 21 invertebrate prey families. Beetles (Coleoptera) and spiders (Araneae) again showed the highest family-level diversity, with seven families each (33.33% of all prey families recorded). Formicidae was the most important prey group in terms of frequency (F = 52,17%), abundance (N = 25), and importance index (Ix = 32.77).

For the high elevation zone (above 800 m), stomach contents from 11 individuals of *Boulenophrys palpebralespinosa* were examined. Only three prey families were recorded. Notably, a tadpole (Amphibia) was identified among the prey items, indicating occasional predation on vertebrates. The remaining prey included snails (Euconulidae) and ants (Formicidae). Ants were again the dominant prey type, with the highest frequency (F = 90,91%) and abundance (N = 19).

3.3.4. Diet composition of amphibian groups according to habitat types

3.3.4.1. Diet composition of amphibians in aquatic habitats

The study analyzed the diet of 33 individuals representing amphibian species in aquatic habitats, including 10 *Hylarana annamitica* and 23 *Amolops tanfuilianae*. A total of 100 food samples were identified, representing 27 families across 10 orders. The order Coleoptera (beetles) was the most diverse, with 10 families recorded (accounting for 10% of the total prey families), followed by Araneae (spiders) with 7 families (7%).

Formicidae (ants) were the most frequently encountered prey, with the highest number of occurrences and the highest importance index (F = 48,48%; N = 39; Ix = 34.56%). The prey with the highest volume, however, was Tettigoniidae (crickets), accounting for 19.31% of the total prey volume.

3.3.4.2. Diet composition of amphibians in terrestrial habitats

The most frequently encountered prey type in terrestrial habitats was ants (Formicidae), with 21 occurrences (75%), followed by Scarabaeidae and Rhinotermitidae, each with 9 occurrences (32.14%). Armadillidae was recorded 7 times (25%). The remaining prey types had fewer occurrences, ranging from 1 to 6 times (accounting for 3.57% to 21.43%).

3.3.4.3. Diet composition of amphibians in arboreal habitats

In arboreal habitats, ants (Formicidae) were the most frequently encountered prey, with F = 47.17%, followed by Rhinotermitidae with F = 28.30% and Kalotermitidae with F = 22.64%. The remaining prey types had fewer occurrences, ranging from F: 1.89% to 11.32%.

In terms of prey quantity, Formicidae was the most abundant, with 110 samples collected, followed by Rhinotermitidae (87 samples) and Kalotermitidae (68 samples). The remaining prey types had fewer samples, ranging from 1 to 13 (0.29% to 3.78%).

Regarding prey volume, Rhinotermitidae accounted for the highest percentage (30.19%), followed by Kalotermitidae (20.19%) and Gryllotalpidae (14.96%). Other prey types ranged from 0.004% to 13.74%.

Rhinotermitidae was identified as the most important prey group (Ix = 27.93%), followed closely by Formicidae (Ix = 27.54%) and Kalotermitidae

(Ix = 20.87%). The importance index for other prey groups ranged from 0.73% to 5.9%.

Discussion

In this study, we observed significant dietary segregation among three amphibian groups: the aquatic frogs (*Hylarana annamitica* and *Amolops tanfuilianae*), the terrestrial frogs (*D. melanostictus* and *H. guentheri*), and the arboreal frogs (*H. simplex* and *P. megacephalus*). We identified 68 different types of prey, with 9 common prey types across all three groups; 15 prey types shared between the terrestrial and aquatic groups; 15 prey types shared between the arboreal and aquatic groups; and 22 prey types shared between the terrestrial and arboreal groups. Additionally, 9 prey types were exclusively found in the arboreal group, 19 in the terrestrial group, and 6 in the aquatic group.

The comparison of dietary composition among the aquatic, terrestrial, and arboreal amphibians revealed that these species generally consumed a diverse range of invertebrates, with even distribution (dominance index < 0.4; Simpson index > 0.6) and high diversity (Shannon index > 1.7). However, ants (Formicidae) and termites (Rhinotermitidae and Kalotermitidae) were the preferred prey types and considered the most important. This preference aligns with feeding habits observed in other amphibian species. Conversely, a study by Pham et al. (2023) [108] on the foraging ecology of *P. megacephalus* in Son La and Lai Chau provinces reported that Orthoptera was the most important prey. The high population density of ants and termites in Bến En National Park may partly explain the preference for these prey types by the amphibians in this area.

The study also noted differences in prey size preferences among amphibian species from different habitats. The aquatic group showed that *H. annamitica* (W_{TB} = 2.648; L_{TB} = 7.089; V_{TB} = 37.809) preferred larger prey compared to *A. tanfuilianae* (W_{TB} = 1.663; L_{TB} = 3.570; V_{TB} = 11.242), with statistically significant differences ($p < 0.05$). The terrestrial group showed that *D. melanostictus* (L_{TB} = 7.714 ± 5.103) preferred larger prey compared to *H. guentheri* (L_{TB} = 7.339 ± 4.82), also with significant statistical differences

($p < 0.05$). However, *H. guentheri* ($W_{TB} = 3.215$) preferred prey with a larger width compared to *D. melanostictus* ($W_{TB} = 2.662$). Similar trends were observed in the arboreal group, where *P. megacephalus* ($W_{TB} = 3.8$; $L_{TB} = 8.8$; $V_{TB} = 61.003$) preferred larger prey compared to *H. simplex* ($W_{TB} = 1.6$; $L_{TB} = 3.9$; $V_{TB} = 11.04$), with statistically significant differences ($p < 0.05$). Although *P. megacephalus* and *H. guentheri* shared similar dietary components (Dice index = 0.55) and environmental habitats, *H. guentheri* ($W_{TB} = 3.215$; $L_{TB} = 7.339$; $V_{TB} = 170.912$) preferred larger prey compared to *P. megacephalus* ($W_{TB} = 3.8$; $L_{TB} = 8.8$; $V_{TB} = 61.003$), with significant statistical differences ($p < 0.05$).

Despite coexisting within the same habitat, these species seem to exhibit niche separation in their feeding behavior. Two arboreal frog species were often observed foraging in different locations. *H. simplex* was typically found foraging on tree branches about 1.5 meters above the ground, while *P. megacephalus* foraged both below 1.5 meters and occasionally on the ground. *H. guentheri* foraged on the ground but was also observed hunting on tree branches below 1.5 meters, while *D. melanostictus* foraged exclusively on the ground. These observed differences in foraging locations likely explain the low dietary overlap between these species. Therefore, the differences in dietary composition among the species may represent an ecological niche separation that helps maintain stable coexistence between arboreal and terrestrial amphibians in Bến En National Park.

The analysis of dietary overlap using Pianka's index (Ojk-observation) yielded a high value of 0.84, but contrastive analysis indicated a very low ecological overlap (Ojk = 0.064; CI 95%: 0.007 – 0.380). This low overlap in ecological niches helps reduce competition, enabling these species to coexist in the same habitat.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study recorded a total of 42 amphibian species belonging to 8 families across the surveyed areas. Notably, two species were newly recorded for the species list of Ben En National Park, while 18 species were new records for the Pu Luong Nature Reserve. Among these, five species are listed in the Vietnam Red Data Book (2007), and one species is listed in the IUCN Red List (2024). In terms of habitat distribution, amphibians were found most commonly in recovering secondary forests (32 species, 76.19%), followed by less-disturbed evergreen forests (23 species, 54.76%), and the fewest in residential and agricultural landscapes (18 species, 42.86%). Regarding microhabitat preferences, the majority of species were ground-dwelling (31 species, 73.81%), followed by arboreal species (10 species, 23.81%), aquatic species (7 species, 16.67%), and only one species was found living underground (2.38%).

In terms of elevational distribution, all 30 species in Ben En National Park were recorded below 300 m, while in Pu Luong Nature Reserve, 14 species were recorded below 300 m (51.85%), 23 species between 300–800 m (85.19%), and 16 species above 800 m (59.26%). The diet composition of 19 amphibian species was analyzed, including 14 species from Ben En and 12 from Pu Luong. Diet analysis by habitat type showed that amphibians from residential and agricultural landscapes had the most diverse prey types (36 types, 52.94%), followed by those from secondary forests (18 types, 26.47%) and evergreen forests (14 types, 20.59%). Ants (Formicidae) were the most frequently consumed and numerically dominant prey in all three habitat types.

By elevation, amphibians at elevations below 300 m consumed the most diverse prey (36 types, 76.6%), followed by those at 300–800 m (21 types, 44.68%) and above 800 m (3 types, 6.38%). Again, Formicidae were the most commonly encountered and important prey group at all elevations. When analyzed by microhabitat, ground-dwelling amphibians showed the highest

dietary diversity with 47 prey types, followed by arboreal species (37 types) and aquatic species (27 types). Formicidae were the most important prey for aquatic amphibians, while Rhinotermitidae (termites) were the primary prey for both terrestrial and arboreal amphibians.

Recommendations

To gain a more comprehensive understanding of the dietary characteristics of amphibian species in the study area, future research should consider the following directions:

- Conducting further surveys to supplement the species composition of amphibians in the limestone mountain ecosystems of the Pu Luong Nature Reserve;

- Investigating the dietary traits of amphibians inhabiting elevations above 800 meters and aquatic species in Pu Luong, in order to provide more complete data on their feeding ecology;

- Exploring the influence of environmental factors on the dietary patterns of other amphibian species in the region.

PUBLICATIONS RELATED TO THE DISSERTATION

1. Trinh Thi Hong, Nguyen Huu Tan, Thieu Thi Huyen (2020). *Diet composition of several amphibian species in the family Microhylidae at Ben En National Park, Thanh Hoa Province*. Journal of Science, Hong Duc University, Issue 51 (2020), pp. 106–113.
2. Dau Quang Vinh, Trinh Thi Hong, Le Dinh Phuong, Ngo Van Binh (2020). *Species diversity of amphibians (Amphibia) in Pu Luong Nature Reserve, Thanh Hoa Province*. Journal of Science, Vinh University, Volume 49 – Special Issue 2A/2020, pp. 70–78.
3. **Hong Thi Trinh**, Hai Ngoc Ngo, Anh Van Pham, Nhi Thi Pham and Vinh Quang Dau, 2023, Segregation in diet composition of two syntopic tree frog species, Hyla simplex and Polypedates megacephalus, in Ben En National Park, Vietnam, *Animal Biology*, 73(4): 423-436
4. **Trinh Thi Hong**, Ngo Ngoc Hai, Dau Quang Vinh, Pham Thi Nhi, 2024, Diet of two species of the genus Hylarala Tschudi, 1838 in Ben En national park, Thanh Hoa province, *Thai Nguyen University Journal of Science and Technology*, 229 (09). tr 313-319. DOI: https://doi.org/10.34238/tnu-jst.10270 , http://jst.tnu.edu.vn.