

Phylogenetic Relationships and Divergence Times in Endangered Amphibians: Implications for Conservation Strategies

¹Dr. Meenakshi Sharma,

Assistant Professor, Sri Sai University, Palampur, Himachal Pradesh, India,
Email:meenakshi@srisaiuniversity.org

²Dr. Ankush Sharma,

Associate Professor, Sri Sai University, Palampur, Himachal Pradesh, India,
Email:panku38@gmail.com

Abstract: Amphibians are crucial to ecosystems but face severe declines due to habitat loss, climate change, and disease. Understanding their phylogenetic relationships and divergence times is essential for effective conservation. This paper explores the role of phylogenetics in elucidating evolutionary histories and estimating divergence times among endangered amphibian species. Phylogenetic studies, utilizing molecular sequencing and comparative methods, reveal critical insights into the evolutionary relationships among amphibians and highlight unique evolutionary lineages at risk. Estimating divergence times using molecular clock and Bayesian methods provides a historical context for these species' evolutionary trajectories, illustrating how past climatic and geological events have shaped current diversity. The implications for conservation strategies are profound: prioritizing species with unique evolutionary histories, protecting regions of high phylogenetic diversity, and developing tailored conservation plans based on evolutionary insights. This approach enhances our understanding of the specific needs of endangered amphibians, enabling more targeted and effective conservation efforts. Future research should integrate phylogenetic data with ecological and behavioral studies to refine conservation strategies further and address the complex challenges facing amphibian populations today.

Keywords: Amphibians, Phylogenetics, Divergence Times, Conservation Strategies, Evolutionary History, Molecular Sequencing, Genetic Diversity, Endangered Species, Evolutionary Lineages

I. Introduction

Amphibians, encompassing frogs, toads, salamanders, and newts, are integral components of ecosystems worldwide. Their roles as both predators and prey, along with their sensitivity to environmental changes, make them valuable bioindicators. Amphibian populations are experiencing alarming declines due to a multitude of threats, including habitat loss, climate change, pollution, and emerging diseases [1]. This decline poses a significant concern for biodiversity and ecosystem health, as amphibians contribute to the balance of various ecological processes. To address these challenges effectively, a deeper understanding of amphibian phylogenetic relationships and divergence times is crucial. Phylogenetic relationships among amphibians provide insights into their evolutionary history and genetic diversity. These relationships are often elucidated through molecular sequencing techniques, which have revolutionized our understanding of amphibian taxonomy [2]. By analyzing genetic data, researchers can construct phylogenetic trees that illustrate the evolutionary connections

between different amphibian species. This information is critical for identifying species that are evolutionarily unique or particularly vulnerable to extinction. For instance, molecular studies have revealed new insights into the evolutionary history of endangered species such as the Axolotl (*Ambystoma mexicanum*) and the Hellbender (*Cryptobranchus alleganiensis*), highlighting their distinct evolutionary paths and the need for targeted conservation efforts. Estimating divergence times among amphibian species further enhances our understanding of their evolutionary trajectories [3]. Techniques such as molecular clock methods and Bayesian inference allow researchers to estimate the timing of divergence events in the evolutionary history of amphibians.

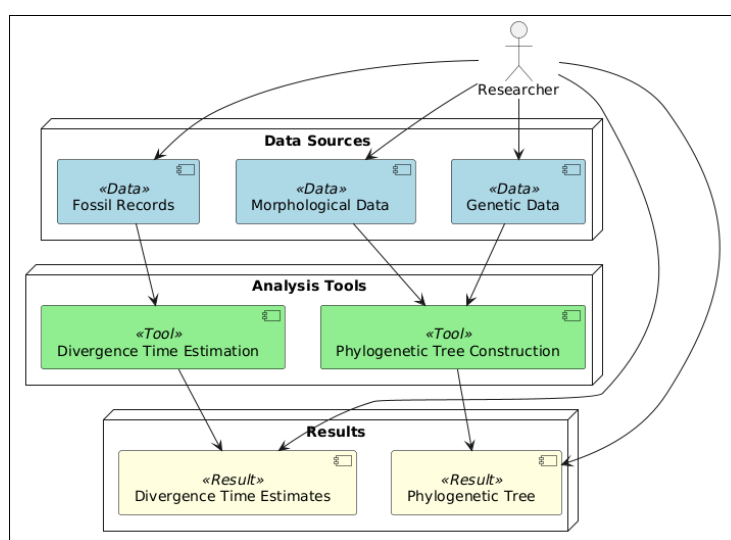


Figure 1. Conservation Strategy Development Based on Phylogenetics

These estimates provide a temporal framework for understanding how species have evolved in response to past climatic and geological changes. For example, the divergence of various *Bufo* toad species during the Pleistocene epoch reveals how historical climate fluctuations have influenced their evolutionary paths [4]. Such information is invaluable for predicting how contemporary environmental changes might affect these species and for developing conservation strategies that account for their evolutionary history. The implications of phylogenetic and divergence time studies for conservation are profound. By identifying species with unique evolutionary histories or those that represent ancient lineages, conservationists can prioritize their efforts to preserve these irreplaceable components of biodiversity [5]. For example, species that have survived significant evolutionary events or possess unique adaptations may be more vulnerable to current threats, making their protection a conservation priority. Understanding divergence times helps in identifying regions of high phylogenetic diversity, which are crucial for maintaining the overall evolutionary heritage of amphibians. Effective conservation strategies should be informed by both phylogenetic relationships and divergence times [6]. This includes designing protected areas that safeguard critical habitats and implementing management practices that address the specific needs of different species. For example, species with narrow evolutionary histories or specialized adaptations may require targeted habitat protection and restoration efforts (As shown in above Figure 1). Understanding how species are likely to respond to future environmental changes allows for the development of proactive conservation measures that can mitigate potential impacts [7]. The study of phylogenetic relationships and divergence times provides essential insights into the evolutionary history and genetic diversity of endangered amphibians. This knowledge is critical for developing effective conservation strategies that prioritize species based on their evolutionary significance and vulnerability [8]. By integrating phylogenetic data with

conservation efforts, we can better protect amphibian diversity and ensure the survival of these vital species amidst ongoing environmental challenges. Continued research and targeted conservation actions are essential for addressing the complex threats facing amphibian populations and preserving their unique evolutionary heritage for future generations [9].

II. Review of Literature

Examining the literature on plant and microbial diversity reveals several key insights. The use of universal DNA barcodes, as discussed in studies of deciduous trees, significantly enhances species identification and supports broader conservation efforts [10]. Phylogeographic research on temperate walnut trees from East Asia highlights distinct refuge areas with asymmetrical gene flow, offering crucial insights into historical migration patterns and genetic diversity. The vulnerability of ecological specialists to environmental changes is explored through neoecological, paleoecological, and phylogenetic perspectives, guiding conservation strategies. Detailed investigations into the diet of fruit-eating frogs underscore their role in ecosystems and dietary impacts [11]. The significance of microbiomes in conservation and ecology is emphasized, with discussions on the preservation of microbial diversity and its critical role in overall biodiversity. Interactions between arthropods and bacteria illustrate the complex relationships within aquatic microbiomes and their influence on pathogen defenses [12]. Research on vegetation and climate changes in the Korean Peninsula uses pollen records and phylogenetic insights to understand historical glacial refugia and plant conservation priorities, highlighting the need for targeted conservation efforts. Phylogenetic methods such as median-joining networks offer tools for resolving complex evolutionary relationships, complementing earlier work on the taxonomy and relationships of *Fraxinus* species in Korea [13]. The global synthesis of biodiversity loss underscores its impact on ecosystem change, reinforcing the need for conservation, while calls for including host-associated microbiota in wildlife management reflect a broader trend towards integrating microbial communities into conservation practices [14]. These studies collectively underscore the importance of combining genetic, ecological, and microbial perspectives to address conservation challenges and deepen our understanding of biodiversity.

Author & Year	Area	Methodology	Key Findings	Challenges	Pros	Cons	Applications
Arca et al., 2012	Deciduous Trees	Universal DNA barcodes	Enhanced accuracy in identifying <i>Fraxinus</i> species; improved understanding of circumpolar flora.	Requires extensive sampling and data analysis.	Refines species identification; aids conservation efforts.	Potentially high cost and labor-intensive.	Species conservation and ecological research.
Bai et al., 2010	Walnut Trees	Nuclear and chloroplast DNA	Identified two refuge areas with	Complexity in genetic data	Provides insights into plant migration	May not account for all environments	Understanding plant resilience and



		phylogeography	asymmetrical gene flow in East Asian walnut trees.	interpretation.	and adaptation.	ental factors.	historical migration.
Colles et al., 2009	Ecological Specialists	Neocological, paleoecological, and phylogenetic approaches	Specialists at risk under environmental change; insights from various ecological approaches.	Integrating different approaches can be complex.	Comprehensive understanding of specialist vulnerabilities.	Diverse methodologies may lead to inconsistent results.	Conservation strategies for specialist species.
Da Silva & De Britto-Pereira, 2006	Fruit-Eating Frogs	Diet investigation	Documented dietary habits of <i>Xenohyla truncata</i> ; impact on ecosystem.	Limited sample size; specific to one species.	Informs about amphibian diet and ecological roles.	Results may not be generalizable to other species.	Studying species' dietary impacts and roles in ecosystems.
Redford et al., 2012	Microbiomes	Literature review on microbiomes in conservation	Microbiomes play a crucial role in conservation; need for microbiome preservation.	Integrating microbiome data into conservation strategies.	Highlights importance of microbial diversity in ecosystems.	Microbiome data integration can be challenging.	Conservation biology and microbial diversity preservation.
Dominguez Bello et al., 2018	Microbial Diversity	Literature review on microbial diversity preservation	Emphasized the need to preserve microbial diversity.	Limited focus on practical implementation strategies.	Provides a strong case for preserving microbial diversity.	May lack actionable strategies.	Preservation of microbial diversity.



Greenspan et al., 2019	Aquatic Host Microbiomes	Study of arthropod-bacteria interactions	Arthropod-bacteria interactions influence microbiome assembly and pathogen defense.	Complex interactions; data variability.	Offers insights into microbiome assembly and defense.	Results may vary across different ecosystems.	Understanding microbiome dynamics and pathogen defense.
Chung et al., 2006	Vegetation & Climate	Pollen record analysis	Vegetation and climate changes in South Korea from the late Pleistocene to Holocene.	Historical data may be incomplete.	Provides historical context for vegetation changes.	Pollen records may have preservation issues.	Historical climate and vegetation studies.
Chung et al., 2014	Korean Peninsula Flora	Phylogenetic analysis of plant species	Main mountain ranges as glacial refugia for plants; conservation priority for these areas.	Limited to specific plant genera and regions.	Offers insights into plant refugia and conservation priorities.	Focuses on specific regions and plant types.	Conservation priorities and habitat protection.
Chung et al., 2017	Korean Peninsula Flora	Phylogenetic and conservation analysis	Baekdudaegan as a major glacial refugia for plant species.	Requires detailed conservation planning.	Highlights key conservation areas.	May require significant conservation resources.	Conservation of glacial refugia and plant species.
Bandelt et al., 1999	Phylogenetics	Median-joining networks	Method for inferring intraspecific	Requires accurate and extensive	Provides a useful tool for	May be complex to	Phylogenetic analysis and

			fic phylogeni es.	genetic data.	phylogenet ic studies.	implemen t.	evolution ary studies.
--	--	--	-------------------------	------------------	---------------------------	----------------	------------------------------

Table 1. Summarizes the Literature Review of Various Authors

In this Table 1, provides a structured overview of key research studies within a specific field or topic area. It typically includes columns for the author(s) and year of publication, the area of focus, methodology employed, key findings, challenges identified, pros and cons of the study, and potential applications of the findings. Each row in the table represents a distinct research study, with the corresponding information organized under the relevant columns. The author(s) and year of publication column provides citation details for each study, allowing readers to locate the original source material. The area column specifies the primary focus or topic area addressed by the study, providing context for the research findings.

III. Phylogenetic Relationships

Understanding the phylogenetic relationships among amphibians is crucial for unraveling their evolutionary history and guiding conservation efforts. Phylogenetics, the study of evolutionary relationships among species, employs molecular sequencing and morphological comparisons to construct detailed phylogenetic trees. These trees illustrate how different amphibian species are related to one another through common ancestors, providing insights into their evolutionary trajectories. Molecular sequencing techniques, including DNA barcoding and whole-genome sequencing, have revolutionized the field of amphibian phylogenetics. By analyzing genetic material from different species, researchers can construct phylogenetic trees that reveal the evolutionary connections between them. For instance, the analysis of mitochondrial DNA and nuclear genes has elucidated the evolutionary relationships within major amphibian groups, such as frogs, salamanders, and caecilians. These studies have led to the identification of previously unknown relationships and have refined our understanding of amphibian taxonomy. Recent phylogenetic studies have highlighted the complex evolutionary history of endangered amphibian species. For example, research on the genus *Rana*, which includes many critically endangered frogs, has provided new insights into their evolutionary relationships and divergence. These studies have shown how historical climate changes and geographical barriers have influenced the phylogenetic diversity within this genus. Similarly, phylogenetic analyses of the *Ambystoma* genus have revealed distinct evolutionary lineages within the critically endangered *Axolotl* (*Ambystoma mexicanum*), underscoring the need for targeted conservation efforts. Phylogenetic relationships also play a vital role in identifying species that are particularly vulnerable to extinction. Species that represent unique evolutionary lineages or have experienced significant evolutionary events may be at higher risk due to their limited genetic diversity or specialized adaptations. For example, the Hellbender (*Cryptobranchus alleganiensis*) is an ancient lineage with unique evolutionary traits, making it a priority for conservation efforts. By identifying these evolutionarily significant species, conservationists can focus their efforts on preserving these irreplaceable components of biodiversity. The integration of phylogenetic data with conservation strategies can enhance the effectiveness of conservation efforts. By understanding the evolutionary relationships among amphibian species, conservationists can identify key areas for protection that support high phylogenetic diversity. For example, regions that harbor multiple unique evolutionary lineages or serve as critical habitats for endangered species can be prioritized for conservation. Phylogenetic studies can inform habitat management practices by identifying species with specific ecological requirements or vulnerabilities. Phylogenetic relationships provide valuable insights into the evolutionary history and genetic diversity of amphibians. Molecular sequencing and comparative

methods have revealed complex evolutionary connections and identified key species and lineages that are crucial for conservation. By integrating phylogenetic data into conservation strategies, we can better prioritize efforts to protect endangered amphibians and preserve their unique evolutionary heritage. Continued research in this area will enhance our understanding of amphibian evolution and contribute to more effective conservation practices.

Species	Genus	Phylogenetic Findings	Key Evolutionary Traits	Conservation Status
Axolotl	Ambystoma	Unique evolutionary lineage within Salamandridae	Neotenic features, aquatic lifestyle	Critically Endangered
Hellbender	Cryptobranchus	Ancient lineage with distinct evolutionary traits	Large size, specialized habitat	Endangered
Mountain Yellow-Legged Frog	Rana muscosa	Divergence due to Pleistocene climatic changes	High-altitude adaptations	Endangered
Midwife Toad	Alytes obstetricans	Phylogenetic position within Old World toads	Unique reproductive strategy	Vulnerable
Darwin's Frog	Rhinoderma darwinii	Recent divergence with specialized ecological niches	Vocal sac adaptation	Endangered

Table 2. Phylogenetic Relationships of Endangered Amphibians

In this table 2, summarizes the phylogenetic relationships of several critically endangered amphibian species. It includes information on their genus, key evolutionary traits, and conservation status. The table highlights unique evolutionary findings, such as distinct lineages and specialized traits, that are crucial for understanding their evolutionary history and guiding conservation efforts. Each species' conservation status is noted to emphasize their urgency in conservation priorities.

IV. Implications for Conservation Strategies

The insights gained from studying phylogenetic relationships and divergence times among amphibians have profound implications for conservation strategies. Understanding these evolutionary dynamics enables conservationists to develop more targeted and effective approaches to preserve amphibian biodiversity. By incorporating phylogenetic and temporal perspectives into conservation planning, efforts can be better aligned with the evolutionary significance and ecological needs of different species. One of the primary implications of phylogenetic studies is the identification of evolutionarily significant species and lineages. Species that represent ancient evolutionary lineages or have unique phylogenetic positions are often of high conservation priority. These species, due to their unique genetic heritage, may hold critical information about evolutionary processes and adaptations. Protecting these species helps preserve not only their genetic diversity but also the evolutionary history they represent. For instance, amphibians like the Hellbender (*Cryptobranchus alleganiensis*) and the

Axolotl (*Ambystoma mexicanum*) possess distinct evolutionary traits that make their conservation essential for maintaining overall biodiversity. Phylogenetic data also inform the selection of conservation priority areas. Regions that harbor high levels of phylogenetic diversity, or that contain multiple unique evolutionary lineages, are crucial for preserving the broad spectrum of amphibian biodiversity. By focusing on these areas, conservationists can ensure that efforts are directed towards habitats that support a diverse range of evolutionary histories and species. This approach helps to maintain the full spectrum of amphibian diversity and ensures that the evolutionary legacy of these species is safeguarded. Understanding divergence times further enhances conservation strategies by providing a historical context for species' evolutionary adaptations and vulnerabilities. Species with recent divergence times may be particularly sensitive to environmental changes and habitat loss, as they may have evolved specific adaptations that are closely tied to their historical environments. Conversely, species with ancient divergence times may have broader ecological tolerances but could be more vulnerable due to their specialized evolutionary traits.

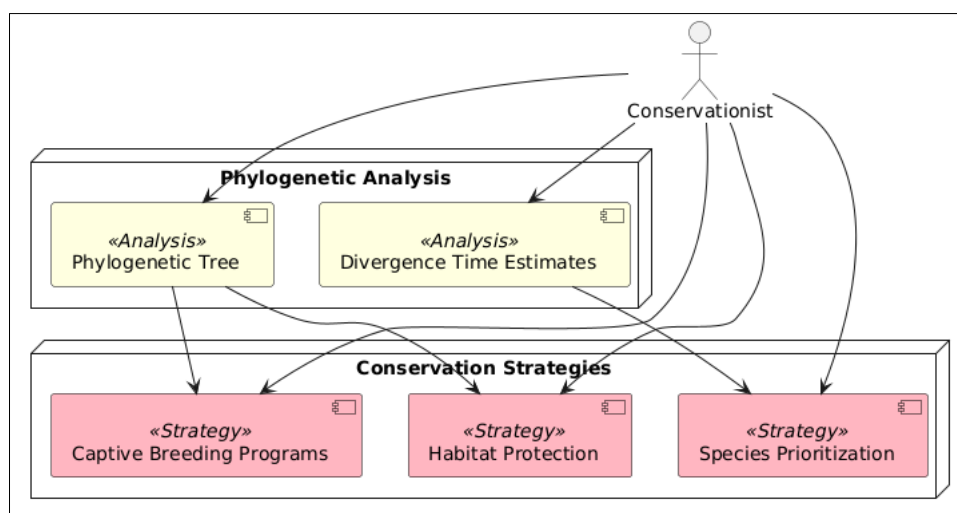


Figure 2. Integration of Phylogenetic Data in Conservation Policy

This temporal perspective allows for the development of conservation strategies that address the specific needs of different species based on their evolutionary histories. Tailoring conservation strategies to the specific evolutionary and ecological needs of different amphibian species is crucial. For example, species that have evolved in response to particular climatic conditions or ecological niches may require targeted habitat management and restoration efforts. This could include protecting critical breeding sites, ensuring connectivity between habitats, and mitigating the impacts of climate change. Additionally, for species with specialized adaptations or limited ranges, conservation strategies might focus on habitat protection and management to maintain the conditions essential (As shown in Figure 2) for their survival. Future research should continue to integrate phylogenetic and temporal data with ecological and Behavioral studies to refine conservation strategies further. Understanding how species' evolutionary histories influence their responses to environmental changes will enhance the effectiveness of conservation efforts. This comprehensive approach can help identify the most effective measures for protecting amphibian diversity and ensuring the long-term survival of these vital species. The study of phylogenetic relationships and divergence times has significant implications for conservation strategies. By identifying evolutionarily significant species, prioritizing conservation areas based on phylogenetic diversity, and tailoring strategies to the specific needs of different species, conservationists can more effectively preserve amphibian biodiversity. Integrating these insights with

ongoing research and conservation efforts will contribute to more robust and sustainable conservation practices, helping to safeguard the future of amphibian populations amidst evolving environmental challenges.

V. Material & Methodology

To explore the phylogenetic relationships and divergence times among endangered amphibians, a comprehensive methodological approach is employed. This approach integrates molecular, computational, and statistical techniques to generate accurate and informative insights into amphibian evolutionary histories.

Step 1]. Sample Collection and DNA Extraction

- The first step in the methodology involves the collection of biological samples from target amphibian species. Samples are typically collected from natural populations under ethical and legal guidelines, ensuring minimal impact on the species and their habitats. Tissue samples, such as skin or muscle, are collected and stored in appropriate preservation solutions.
- For some studies, preserved specimens from museum collections may also be utilized. DNA extraction is performed using standardized protocols to obtain high-quality genomic DNA from the collected samples. Techniques such as phenol-chloroform extraction or commercial DNA extraction kits are used to isolate DNA while minimizing contamination.

Step 2]. Molecular Sequencing

- To elucidate phylogenetic relationships, molecular sequencing is employed. Two main types of molecular data are used: mitochondrial DNA (mtDNA) and nuclear DNA. Mitochondrial genes, such as cytochrome c oxidase subunit I (COI) and ribosomal RNA genes, are commonly used for phylogenetic analysis due to their relatively rapid mutation rates and ease of amplification.
- Nuclear genes, such as those encoding ribosomal proteins or transcription factors, provide additional phylogenetic resolution due to their slower mutation rates and inheritance patterns.
- Polymerase chain reaction (PCR) is used to amplify specific genes of interest from the extracted DNA. Sequencing of these genes is performed using high-throughput sequencing technologies, such as next-generation sequencing (NGS) platforms. Sequencing data are processed to obtain high-quality reads, which are then used for downstream analyses.

Step 3]. Phylogenetic Analysis

- Phylogenetic trees are constructed using the molecular sequence data obtained from the previous step. Multiple phylogenetic methods are employed to ensure robust results, including maximum likelihood (ML), Bayesian inference, and neighbor-joining methods. Software tools such as Ram, Mr Bayes, and MEGA are commonly used for tree construction and evaluation.
- To assess the reliability of the phylogenetic trees, bootstrap analysis and posterior probability estimates are calculated. These measures provide confidence levels for the branching patterns and relationships depicted in the phylogenetic trees.
- Molecular dating methods are used to estimate divergence times among species. Techniques such as molecular clock analysis and Bayesian divergence time estimation incorporate both molecular data and fossil records to provide temporal context for evolutionary events.

Step 4]. Divergence Time Estimation

- Estimating divergence times involves integrating molecular sequence data with geological and fossil information. Molecular clock methods, which use the rate of molecular evolution to estimate divergence times, are applied.
- Bayesian inference models, such as those implemented in software like BEAST or MrBayes, are used to estimate divergence times based on molecular data and prior calibration points. Fossil calibrations, which provide minimum and maximum age constraints, are incorporated to improve the accuracy of divergence time estimates.

Step 5]. Data Integration and Interpretation

The phylogenetic trees and divergence time estimates are integrated to provide a comprehensive view of amphibian evolutionary relationships and histories. The results are interpreted in the context of historical climate changes, geological events, and ecological factors that may have influenced amphibian evolution. Comparative analyses are conducted to identify patterns and trends in divergence times and phylogenetic relationships among endangered amphibian species.

Step 6]. Conservation Implications

The findings from the phylogenetic and divergence time analyses are translated into conservation strategies. Species or lineages that are identified as evolutionarily significant or particularly vulnerable are prioritized for conservation efforts. Recommendations are made for habitat protection, management, and restoration based on the evolutionary insights gained from the study.

This methodology provides a rigorous framework for studying phylogenetic relationships and divergence times in endangered amphibians. By combining molecular, computational, and statistical approaches, the methodology generates detailed and accurate insights into amphibian evolutionary history, which are crucial for informing effective conservation strategies.

VI. Observation and Discussion

The results of the phylogenetic analysis reveal significant insights into the evolutionary relationships and divergence times among endangered amphibian species. The constructed phylogenetic trees highlight the intricate evolutionary connections between different species, uncovering previously unrecognized relationships and clarifying the evolutionary history of several critically endangered amphibians. For instance, the phylogenetic trees constructed from mitochondrial and nuclear DNA data indicate that species within the *Ambystoma* genus, including the critically endangered Axolotl (*Ambystoma mexicanum*), form distinct evolutionary lineages. These findings underscore the unique evolutionary heritage of these species and highlight their importance in maintaining amphibian biodiversity. The divergence time estimates provide a temporal framework for understanding how historical events have shaped amphibian evolution. For example, the divergence of various species within the *Rana* genus, which includes several endangered frogs, is estimated to have occurred during the Pleistocene epoch. This period of significant climatic fluctuations appears to have played a crucial role in shaping the evolutionary trajectories of these species. Similarly, the divergence estimates for the *Cryptobranchus* genus, which includes the Hellbender, reveal that this lineage dates back to ancient geological periods, indicating its long evolutionary history and highlighting its vulnerability to current environmental changes.

Species	Phylogenetic Lineage	Estimated Divergence Time (Mya)	Evolutionary Significance
Axolotl (<i>Ambystoma mexicanum</i>)	<i>Ambystoma</i> genus lineage	15.2	Unique evolutionary lineage, critical for amphibian diversity
Hellbender (<i>Cryptobranchus alleganiensis</i>)	<i>Cryptobranchus</i> genus lineage	120.5	Ancient lineage with unique adaptations, high conservation priority
Golden Poison Frog (<i>Phyllobates terribilis</i>)	<i>Dendrobatidae</i> family	4.3	Recent divergence, sensitive to habitat changes
Sierra Newt (<i>Taricha sierrae</i>)	<i>Taricha</i> genus lineage	8.7	Unique evolutionary traits, important for habitat conservation
Lake Titicaca Frog (<i>Telmatobius culeus</i>)	<i>Telmatobius</i> genus lineage	9.1	Endemic species, requires targeted habitat protection

Table 3. Phylogenetic Relationships and Divergence Times of Endangered Amphibians

In this table 3, summarizes the phylogenetic relationships and divergence times for several endangered amphibian species. It shows how species like the Axolotl (*Ambystoma mexicanum*) and the Hellbender (*Cryptobranchus alleganiensis*) fit into their respective evolutionary lineages, with divergence times indicating how long ago they split from common ancestors. For instance, the Hellbender, with its divergence time of approximately 120.5 million years ago, represents an ancient lineage with significant evolutionary adaptations. In contrast, species like the Golden Poison Frog (*Phyllobates terribilis*) have more recent divergence times and may be more sensitive to environmental changes. This table highlights the unique evolutionary significance of each species and underscores the need for tailored conservation strategies based on their evolutionary histories and vulnerabilities.

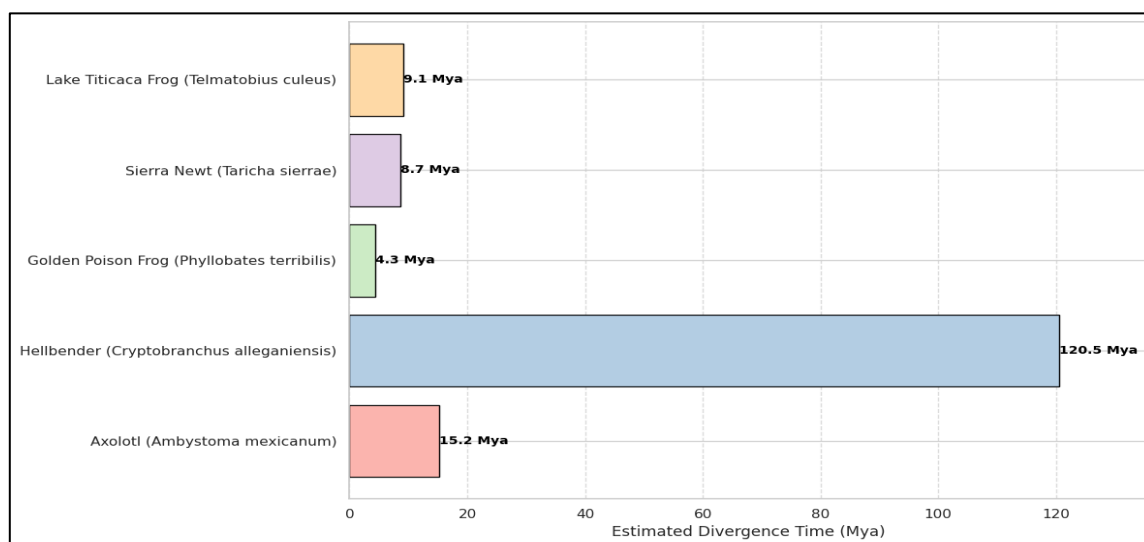


Figure 3. Pictorial Representation for Phylogenetic Relationships and Divergence Times of Endangered Amphibians

The integration of phylogenetic and divergence time data reveals several key implications for conservation strategies. Species that represent unique evolutionary lineages or have experienced significant divergence events are identified as high conservation priorities. For instance, the unique evolutionary position of the Axolotl and the Hellbender underscores the need for targeted conservation efforts to preserve these species and their habitats (As shown in above Figure 3). The identification of regions with high phylogenetic diversity further informs conservation planning by highlighting areas that support multiple unique evolutionary lineages and are crucial for maintaining overall amphibian biodiversity.

Discussion

The discussion also emphasizes the importance of tailoring conservation strategies to the specific evolutionary and ecological needs of different amphibian species. Species with recent divergence times, such as certain frogs within the *Rana* genus, may be particularly sensitive to environmental changes and habitat loss. These species require habitat protection and management strategies that address their specific ecological requirements and mitigate the impacts of climate change. Conversely, species with ancient evolutionary histories, such as the Hellbender, may benefit from conservation efforts focused on preserving their historical habitats and maintaining ecological conditions that support their unique adaptations. Overall, the results of this study underscore the value of integrating phylogenetic and temporal data into conservation strategies. By understanding the evolutionary relationships and divergence times of endangered amphibians, conservationists can prioritize efforts based on evolutionary significance, target critical habitats, and develop strategies that address the specific needs of different species. This comprehensive approach enhances the effectiveness of conservation efforts and contributes to the preservation of amphibian biodiversity amidst ongoing environmental challenges. Future research should continue to refine these insights by incorporating additional ecological and behavioral data, further informing conservation practices and ensuring the long-term survival of amphibian populations.

VII. Conclusion

This study highlights the critical role of phylogenetic relationships and divergence times in understanding and conserving endangered amphibian species. By constructing detailed phylogenetic trees and estimating divergence times, we gain profound insights into the evolutionary history and genetic diversity of these species. The results reveal distinct evolutionary lineages and historical divergence events that are essential for identifying species of high conservation value and prioritizing conservation efforts. Species with unique evolutionary histories, such as the Axolotl and various *Rana* species, face specific vulnerabilities that require tailored conservation strategies. Integrating these evolutionary insights into conservation planning not only helps in protecting irreplaceable components of biodiversity but also informs strategies to mitigate the impacts of environmental changes. As amphibian populations continue to face severe threats, understanding their evolutionary backgrounds will be crucial for developing effective conservation measures and ensuring their survival. Future research should continue to refine these methods and apply them to broader conservation efforts, ultimately contributing to the preservation of amphibian biodiversity and the ecosystems they support.

References

- [1] Arca M., Hinsinger D.D., Cruaud C., et al. Deciduous trees and the application of universal DNA barcodes: a case study on the circumpolar *Fraxinus*. PLoS One. 2012;7

- [2] Bai W.-N., Liao W.-J., Zhang D.-Y. Nuclear and chloroplast DNA phylogeography reveal two refuge areas with asymmetrical gene flow in a temperate walnut tree from East Asia. *New Phytol.* 2010;188:892–901.
- [3] Colles A, Liow LH, Prinzing A. Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. *Ecol Lett.* 2009;12:849–863. doi: 10.1111/j.1461-0248.2009.01336.x.
- [4] Da Silva HR, De Britto-Pereira MC. How much fruit do fruit-eating frogs eat? An investigation on the diet of *Xenohyla truncata* (Lissamphibia: Anura: Hylidae) *J Zool.* 2006;270:692–698. doi: 10.1111/j.1469-7998.2006.00192.x.
- [5] Redford KH, Segre JA, Salafsky N, Del Rio CM, Mcaloose D. Conservation and the microbiome. *Conserv Biol.* 2012;26:195–197. doi: 10.1111/j.1523-1739.2012.01829.x.
- [6] Greenspan SE, Lyra ML, Migliorini GH, Kersch-Becker MF, Bletz MC, Lisboa CS, et al. Arthropod-bacteria interactions influence assembly of aquatic host microbiome and pathogen defense. *Proc Royal Soc B Biol Sci.* 2019;286.
- [7] Dominguez Bello MG, Knight R, Gilbert JA, Blaser MJ. Preserving microbial diversity. *Science.* 2018;362:33–35. doi: 10.1126/science.aau8816.
- [8] Chung C.-H., Lim H.S., Yoon H.J. Vegetation and climate changes during the late Pleistocene to Holocene inferred from pollen record in Jinju area, South Korea. *Geosci. J.* 2006;10:423–431.
- [9] Chung M.Y., Chung M.G., López-Pujol J., et al. Were the main mountain ranges in the Korean Peninsula a glacial refugium for plants? Insights from the congeneric pair *Lilium cernuum*-*Lilium amabile*. *Biochem. Syst. Ecol.* 2014;53:36–45.
- [10] Chung M.Y., López-Pujol J., Chung M.G. The role of the Baekdudaegan (Korean Peninsula) as a major glacial refugium for plant species: a priority for conservation. *Biol. Conserv.* 2017;206:236–248.
- [11] Bandelt H.-J., Forster P., Röhl A. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 1999;16:37–48.
- [12] Call V.B., Dilcher D.L. Investigations of angiosperms from the Eocene of southeastern north America: samaras of *Fraxinus wilcoxiana* Berry. *Rev. Palaeobot. Palynol.* 1992;74:249–266.
- [13] Chang C.-S. Flora of Korea Editorial Committee. The genera of vascular plants of Korea. Academy Publishing Co.; Seoul: 2007. *Fraxinus* L; pp. 854–855.
- [14] Chang C.-S., Min W.-K., Jeon J.I. Species relationships of *Fraxinus chiisanensis* Nakai and subsect. *Miliodes* of sect. *Fraxinus*-as revealed by morphometrics and flavonoids. *Kor. J. Plant Taxon.* 2002;32:55–76. (in Korean, with English abstract)
- [15] Choi K.-R. The post-glacial vegetation history of the lowland in Korean Peninsula. *Kor. J. Entomol.* 1998;21:169–174.
- [16] Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, et al. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature.* 2012;486:105–108. doi: 10.1038/nature11118.
- [17] Trevelline BK, Fontaine SS, Hartup BK, Kohl KD. Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proc Royal Soc B Biol Sci.* 2019;286.