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Original Article

Modeling Potential Distribution of the Nguyen's Fanged Frog *Limnonectes Nguyenorum*

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Abstract: The Nguyen's Fanged Frog (*Limnonectes nguyenorum*) was originally discovered from Vietnam in 2015, and it was recently recorded in China in 2021. In Vietnam, this species has been recorded in Ha Giang, Tuyen Quang, Lao Cai, Yen Bai, Son La, and Vinh Phuc provinces. Although information on ecology and distribution of the Nguyen's Fanged Frog is still limited, hunting for food and habitat destruction are major threats to the populations of *L. nguyenorum*. In this study, we used species occurrence data from previous studies and our field surveys to predict the potential distribution of the species using MaxEnt approach. The model results show that the potential distribution of *L. nguyenorum* may include other national parks and nature reserves in northern Vietnam: Hoang Lien – Van Ban and Bat Xat (Lao Cai); Muong La and Ta Xua (Son La); Che Tao and Na Hau (Yen Bai); Xuan Son (Phu Tho); Tam Dao (Vinh Phuc-Thai Nguyen-Tuyen Quang); Na Hang (Tuyen Quang); Ba Be (Bac Kan); as well as Bac Me, Du Gia, Phong Quang, and Bat Dai Son (Ha Giang). Based on the optimal model, the predicted populations of the Nguyen Fanged Frog in Xuan Son and Tam Dao national parks in Vietnam, and Daweishan in Yunnan Province of China, if exists, may represent unique evolutionary clades. Therefore, future research and conservation initiatives may focus their efforts in such areas to find new populations of the Nguyen's Fanged Frog, as well as assess the distribution, habitat, and ecological patterns of this newly discovered amphibian species.

Keywords: Limnonectes nguyenorum, MaxEnt, Species Distribution Modeling.

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1. Introduction

The Nguyen's Fanged Frog, *Limnonectes nguyenorum* McLeod, Kurlbaum & Hoang, 2015, was originally described from Cao Bo Commune in Tay Con Linh Nature Reserve, Vi Xuyen District, Ha Giang Province, northern Vietnam [1]. The species could be identified based on the following morphological characteristics: snout-vent length: 43.0-46.5 mm in males, 36.5-43.6 mm in females; head longer than wide; canthus rostralis indistinct and rounded, loreal region flat; supratympanic fold indistinct; prominent white bar extending from nares to insertion of arm, upper lip with distinct white spots and dark brown bars; skin on top of head and venter smooth, skin on dorsum and flanks feebly crenulate; pericloacal area, and dorsal surfaces of shank and foot covered with heterogeneous tubercles; males with nuptial pads on first and second fingers; males with slightly enlarged heads [1-3] (Figure 1). This species was found in small treams or pools in evergreenforests. The Nguyen's Fanged Frog is classified as Near Threatened in the IUCN Red List because of habitat destruction and hunting pressures from most known sites [4].

Figure 1. A female Nguyen's Fanged Frog, recorded during our field surveys.

Species distribution modeling (SDM) is an approach that explores and examines potential interactions between species occurrences and influencing environmental variables, and it has been demonstrated to be useful in studying ecology and distribution patterns of rare and understudied species [5]. SDM can help to identify undiscovered distributions for elusive and poorly known species [6], design protected areas that account for future inpacts on endangered taxa [7], uncover new species lineages whose other traits such as morphological and genetic diagnostic characters, may be inconclusive [8], and understand effects of important abiotic factors on species distribution [9]. Therefore, many SDM approaches have been developed, and among the most commonly used is Maximum Entropy (MaxEnt) [5, 10]. MaxEnt has been shown to be capable of producing good predictive performance even with a low number of available records, and it can determine hidden interactions between environmental variables [11]. Hence, MaxEnt has been recommended as a standard tool for studying species distribution [10, 12].

In this paper, we colleted occurrence records of the Nguyen's Fanged Frog from previous studies and our field surveys, and constructed MaxEnt models to generate a distribution map and help advance understanding and conservation measures for this recently discovered frog species.

2. Methods

2.1. Field Surveys

Field surveys were conducted at five sites in Muong Do and Muong Bang communes, Phu Yen District; Ca Nang Commune, Quynh Nhai District; and Nam Pam Commune, Muong La District; Son La Province, northern Vietnam between 2015 and 2020. Individuals were observed in water between 19:00 and 22:00h in small streams or pools in evergreen forest with large, medium, and small hardwoods mixed with shrubs and vines. For taxonomic identification, four individuals were collected for morphological analysis and other frogs were released at the collecting sites. After being photographed in life, collected animals were anesthetized and euthanized in a closed vessel with a piece of cotton wool containing ethyl acetate

[13], fixed in 85% ethanol, and subsequently stored in 70% ethanol. Voucher specimens were deposited in the collection of the Faculty of Environmental Sciences, University of Science, Vietnam National University, Hanoi, Vietnam.

2.2. Data Pre-processing

Records of the Nguyen's Fanged Frog from published papers, field reports, and proceedings were also collated. All records were then integrated and cleaned as suggested by Chapman (2005) [14]. To reduce spatial autocorrelation issues, we used the spThin package [15] in R [16] to thin out localities within five kilometers distance [7]. This created the final set of 11 localities as the input data for MaxEnt model. We used 19 bioclimatic variables at 30-arcsec resolution available at the WorldClim 2.1 database [17], and restricted the extent by using a three degree buffer around minimum convex polygon of the occurrence localities [18].

2.3. Model Construction and Evaluation

We ran all analyses in MaxEnt version 3.4.4 [5]. We used all feature classes' combinations, and tested a range of regularization multipliers values from 0.5 to 10.0 with increments of 0.5. All other parameters followed recommendations from MaxEnt developers [19]. We then used the jackknife method, recommended for models with a low number of occurrence records, to construct MaxEnt models [20]. To assess model performance and select the optimal one, we used the 10% omission rate threshold to select models that showed the least overfitting. From this set, we then chose the models with the highest Area Under the Curve (AUC) values. The last set of models were then selected based on the Akaike information criterion, which balances complexity with model fitness [21]. For the final model, we used the 10% training presence threshold to classify between suitable and unsuitable areas for the Nguyen's Fanged Frog [20].

3. Results and Discussions

3.1. Potential Distribution for the Nguyen's Fanged Frog

For the SDM, MaxEnt models showed reasonable prediction power for the distribution of the Nguyen's Fanged Frog, with the average AUC values > 0.85. The best model had the regularization multiplier value of 2.5 and a combination of linear, quadratic, and hinge feature classes, and AUC value of 0.869. All final models were quite similar in terms of predicting the overall distribution of *L. nguyenorum* with only trivial differences in the edge locations of the species.

However, the regularization multiplier value of 2.5 for the optimal model means that the final model was slightly overfitted. The final prediction should therefore be carefully interpreted as "high potential zones", and it may exclude regions that are likely to be suitable for the Nguyen's Fanged Frog, especially in the edge areas. The total climatically suitable distribution area of the frogs is estimated at about 57,000 km^2; but similar to other bioclimatic-based models, our results do not account for possible human impacts and other biotic interactions on the actual range of *L. nguyenorum*. Hence, it is likely to overpredict where human activities are prevalent, and interpretion of the results in such areas should be done with caution.

The current potential distribution range of the Nguyen's Fanged Frog can be broadly divided into two main regions, and they are roughly divided by the Red River system. In particular, one part of the potential distribution range of the species covers parts of Lao Cai, Lai Chau, Dien Bien, Son La, Yen Bai, and Phu Tho provinces. The other part covers Ha Giang, Tuyen Quang, Vinh Phuc, Thai Nguyen, Bac Kan, and Cao Bang provinces. Based on this result, we suggest the protected areas, which lie within the largely continuous and climatically suitable habitats for the fanged frogs, to be

prioritized for future surveys. They are presented in Figure 2 and include:

Hoang Lien – Van Ban Nature Reserve, Bat Xat Nature Reserve (Lao Cai, Vietnam), and Jinping Fenshuiling Nature Reserve (Yunnan, China): Together with Hoang Lien National Park (Lao Cai and Lai Chau, Vietnam), they form a largely continuous block of protected forests. With the discovery of the *L. nguyenorum* in Sa Pa, it is possible that significant population of the Nguyen's Fanged Frog inhabits this area [3].

Muong La and Ta Xua nature reserves (Son La, Vietnam), Che Tao and Na Hau nature reserves (Yen Bai, Vietnam): Previous study has found a population of the Nguyen's Fanged Frog in Che Tao, and our survey discovered a new record of the species in nearby Muong La. While the habitat degradation in this region is noticeable, they still play an important role in maintaining existing populations of the fanged frogs [22].

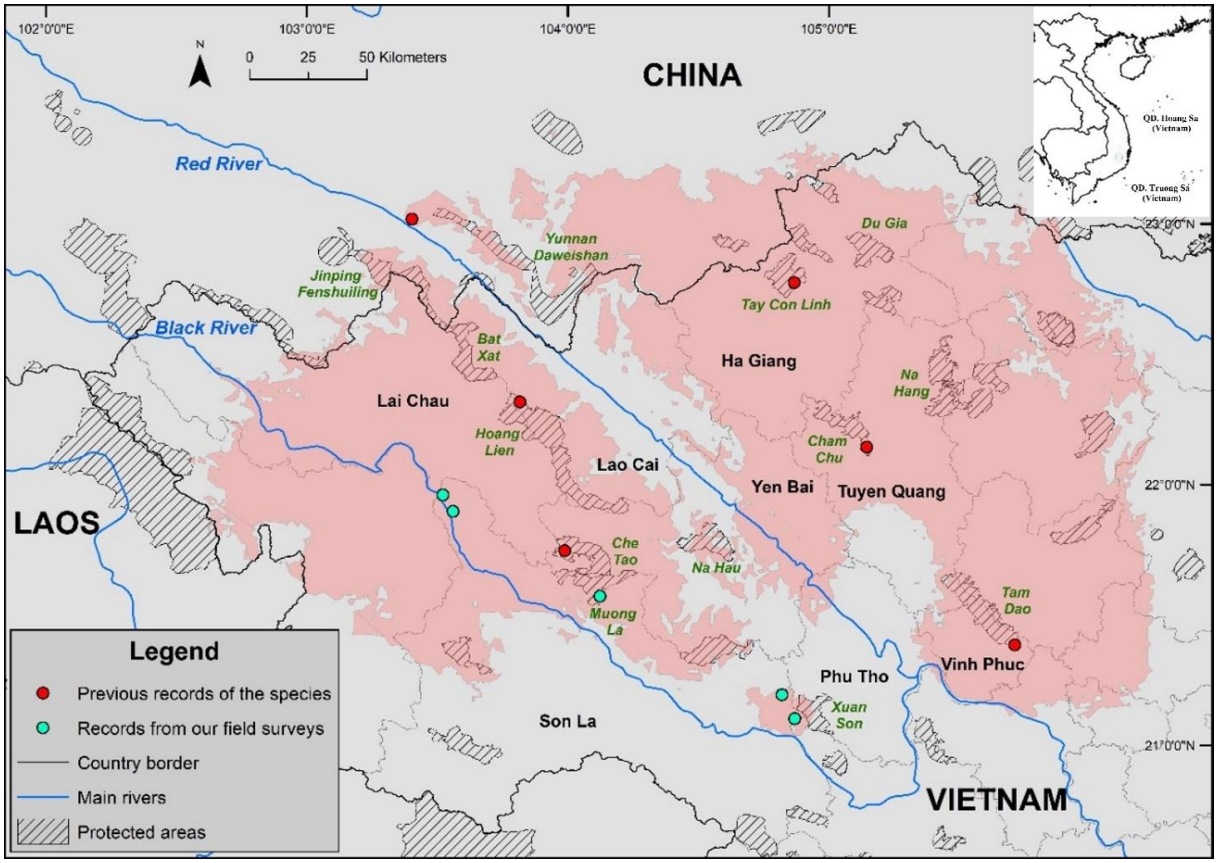

Figure 2. Potential distribution of the Nguyen's Fanged Frog.

Du Gia National Park, and Phong Quang, Bat Dai Son, and Bac Me nature reserves (Ha Giang, Vietnam), Na Hang Nature Reserve (Tuyen Quang, Vietnam), Ba Be National Park (Bac Kan, Vietnam), and Malipolaojunshan and Xiaoqiaogou nature reserves (Yunnan, China): Together they form another relatively continuous forest block that is close to Tay Con Linh Nature Reserve, the type locality of the species. Except for Du Gia National Park, other sites have not been well studied in terms of biodiversity research. Hence, they should be priotized for finding new populations of the Nguyen's Fanged Frog [1, 2].

Tam Dao National Park (Vinh Phuc, Thai Nguyen, and Tuyen Quang, Vietnam): Tam Dao is very close to another known site of *L. nguyenorum* in Me Linh Biodiversity Research Station . Given the distance between this locality and other record of the fanged frog, and the relative isolation of Tam Dao to other important sites [23], it is possible that Tam Dao supports an evolutionarily unique clade of *L. nguyenorum*. Future surveys may focus on confirming the existence of the species in Tam Dao, as well as comparing its phylogenetic relationships with other populations.

3.2. Discussions

In our modeling process, we did not include any topographic or hydrologic variables when constructing the models. Despite that fact, our results still showed that the Red River system plays a significant role as a zoogeographical barrier for the Nguyen's Fanged Frog (Figure 2). Therefore, the Red River, besides its geographic impacts, does have other influences on the distributions of herpetofauna. Other studies have proposed that while the Red River system might promote speciation in some species, the ecological divergence in downstream areas could help maintain the species diversification [23-25].

We note that in our modeling results, beside two main distribution regions, there are several small "break-away" parts. Two prime examples are Xuan Son National Park (Phu Tho, Vietnam), and Daweishan (Yunnan, China) with significant distances and natural barriers between them and the main parts. Hence, two populations of the Nguyen's Fanged Frog in those two sites, similar to Tam Dao, may represent two distinct populations. Current data support our hypothesis, with the *L. nguyenorum*'s population near Daweishan (Yunnan) forming a separate clade [26]. However, we also notice that DNA sequences of the Sapa and Vinh Phuc samples are a bit short, and hence do not allow for a more rigorous comparison. Therefore, future phylogenetic studies should further investigate the populations from the areas using additional molecular and morphological data to resolve the taxonomic issue of this fanged frog.

4. Conclusion

In this study, we combined occurrence records of the Nguyen's Fanged Frog from literature and our field survey data to construct MaxEnt models for the species distribution. The optimal MaxEnt model showed reasonable prediction power with the average AUC values > 0.85, and the best model had an AUC value of 0.869. The model results suggest that *L. nguyenorum* is mostly distributed in Ha Giang, Tuyen Quang, Lao Cai, Lai Chau, Yen Bai, Son La, Vinh Phuc, Phu Tho, and Yen Bai provinces, Vietnam, and a small part in southern Yunnan Province, China. Therefore, protected areas in this regions, especially those that form a large continiuous forest such as Hoang Lien – Van Ban, Hoang Lien, and Bat Xat (Lao Cai, Lai Chau); or Bac Me, Tay Con Linh, Du Gia, Phong Quang, and Bat Dai Son (Ha Giang), may support important populations of the fanged frogs. Future research and conservation initiatives should focus their efforts on such areas to find new populations of the Nguyen's Fanged Frog, as well as address the outstanding taxonomic issues of this poorly known amphibian.

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