SPECIES DIVERSITY AND ABUNDANCE OF LAND SNAILS IN KHAO BIN LIMESTONE HILLS OF RATCHABURI PROVINCE

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Abstract

Species diversity and abundance of land snails were carried out in Khao Bin limestone hills, Ratchaburi Province. Six plots of 20x20 m were set up to collect specimens from two areas of limestone hills in the rainy season from June to September 2020. All specimens of the land snails were classified into 8 species and 6 families, which belonged to 2 subclasses comprising Prosobranchia and Pulmonata. The Prosobranchia consisted of *Cyclophorus* sp. and *Cyclotus* sp. The Pulmonata consisted of *Cryptozona siamensis, Landouria* sp., *Oophana* sp., *Prosopeas* sp., *Pupina* sp., *Sarika* sp. The highest abundance species is *Cyclophorus* sp. (291 specimens or 27%), and the lowest abundance species is *Prosopeas* sp. (1 specimen or 0.1%). Ecological indices were analyzed, comprising density = 0.45 individuals/m^2, species richness (Margalef index) = 1.00, diversity index (Shannon-Weiner's index; H′) = 1.01, dominance index (Simpson's index) = 0.15, evenness index = 0.48 and similarity index (Bray – Curtis) among each plot range from 10.90 – 94.44.

Keywords: Land snails, Limestone hill, Species diversity, Abundance

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Introduction

The diversity of land snails is mostly studied in limestone hills. These habitats are rich in land snail species because of suitable environmental factors such as high calcium content and alkaline soils (Graveland et al., 1994). Limestone hills usually consist of various habitats with their specific environments, providing endemism in these areas (Clements et al., 2006). It is usually found endemic species that adapt in unique habitats for a long time. In Malaysia, Foon et al. (2017) reported that more than 100 species of land snails were discovered in only 12 limestone hills. 34 were endemic species, and 30 species are new to science. Also, Marzuki et al. (2021) studied 8 limestone outcrops in the District of Bau, the state of Sarawak in Malaysian Borneo. They discovered a total of 122 species of land snails and 13 are new to science. So, it indicated that limestone hills are hotpots for land snails' diversity.

In Thailand, the study of land snail diversity was popular in many habitats in northeastern Thailand (Tumpeesuwan & Tumpeesuwan, 2010; Tanmuangpak et al., 2012; Jumlong et al., 2013; Tumpeesuwan et al., 2014; Tumpeesuwan & Tumpeesuwan, 2015; Tanmuangpak et al., 2017; Tumpeesuwan et al., 2020). Few studies were reported in western Thailand. Ratchaburi Province is dominated by limestone hills, approximately 40% of the total area. They are densely distributed in the central region of the province (Department of Mineral Resources, 2008). Many sites are well known as tourist attractions, such as the Khao Ngu stone park, Chom Pol cave, and Khao Bin cave. Previously, there were reports about land snails in limestone hills in Photharam District such as Khao Chongpran (Panha and Burch, 2002), Wat Tham Sarika, Khao Changum, and Wat Tham Nam, as well as Wat Buri Ratchawanaram in Pak Tho District (Pholyotha et al., 2020). However, the earlier studies mainly focused on taxonomy purposes. The diversity data in these areas of limestone hills have not been reported before.

This research is focused on the diversity of land snails in Khao Bin, one of the famous limestone hills in Ratchaburi Province. Khao Bin has not been reported on land snails' diversity before. This area is enclosed by natural forests. However, some are adjacent to agricultural areas. Such areas are possibly susceptible to the loss of land snail species due to the development of areas as tourist attractions. It is urgent for studying and comparing land snails' diversity within the area. So, the objective is to study the diversity and abundance of land snails in Khao Bin limestone hills, Ratchaburi Province. The study of land snails in the Khao Bin area will fill the gap of knowledge about the ecological context between land snails and limestone hills in Ratchaburi Province and Thailand.

Materials and Methods

1. Study sites and sample collections

Aerial imagery of Ratchaburi Province, as well as field surveying, were used to choose study sites. Samples were collected during the wet season, from June to September 2020. To cover both an area with natural forests and one with agricultural areas, six plots were randomly set up at 2 different sites at Khao Bin (Figure 1). The first site is encircled by natural forests, comprising 4 plots (KB-01 to KB-04; coordinate: 13°35'35.25" N to 99°39'58.94" E). The sampling plot areas were sloping areas of limestone hills. They were adjacent limestone cliffs covered by mixed deciduous forest. The second site was bordered by agricultural areas, comprising 2 plots (KB-05 to KB-06; coordinate: 13°33'10.32" N to 99°41'1.20" E). On this site, we can only set up 2 plots because of the narrow area of forests with more sloping topography. The sampling plot area was covered by bamboo communities, which did not exist at the first site. Also, this second site tends to be more disturbed by humans than the first one.

For each site, plots of 20x20 m were set up in line transect to cover various habitats in the sampling area such as litter, under the rocks, and the tree. Sample collection was carried out in the daytime. The naked eye searched both living and empty shells in many habitats, such as on trees, rocks, and ground. In a plot, searching was spent 30 minutes and carried out by 4 people (Tumpeesuwan & Tumpeesuwan, 2010). 3 people walked through the plot in each straight line in the same direction. Then another walked in a zigzag pattern around the plot to search samples that may be overlooked by the three-person (Tumpeesuwan & Tumpeesuwan, 2015). To record the numbers of each taxon in fields, land snail specimens were identified by using guides for land snails classification (Sucharit & Panha, 2008; Sucharit et al., 2018)

Then, specimens were conveyed to the laboratory. Living specimens were drowned and preserved in 70% ethyl alcohol to study reproductive anatomy, radula morphology, and molecular genetics in the future. Empty shells were cleaned and preserved. All specimens were registred and preserved as reference collection in the faculty of Science and Technology, Muban Chombueng Rajabhat University. All samples were delicately identified to taxa by comparing to previous research.

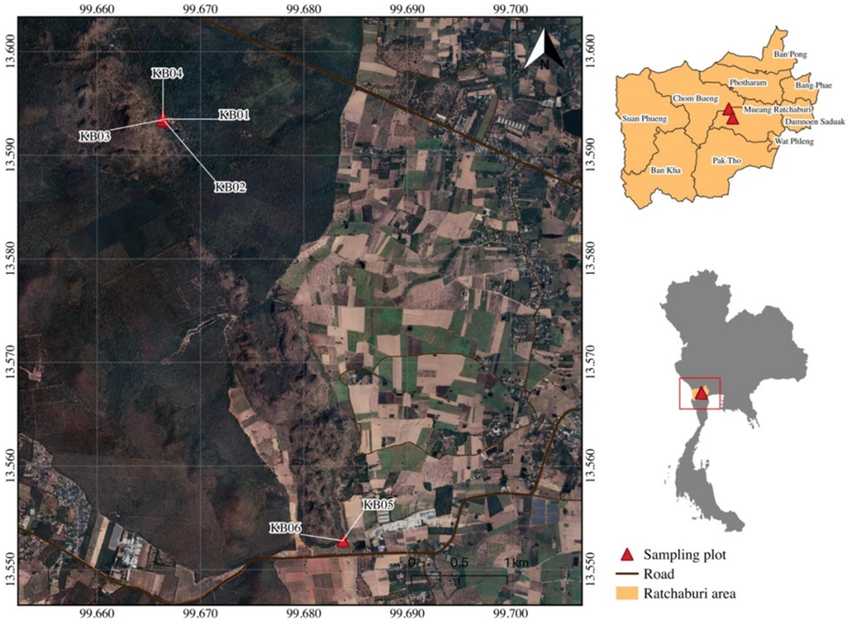

Figure 1 Map of the study site and locations of sampling plots, located in Khoa Bin limestone hills. It comprises 2 sites – the first sites (KB-01 to KB-04) with encircled forest, and the second site (KB-05 to KB-06) with the agricultural buffer zone.

2. Analysis of ecological indices

2.1 Density

Density for each plot was calculated from the number of individuals divided by plot area (Krebs, 2009).

$$D = \frac{N}{Area} \tag{1}$$

D = values of density (individuals/m^2)

N = numbers of individuals (individuals)

Area = area of plots (m^2)

2.2 Species richness

The species richness of land snails was calculated by using Margalef's index (Margalef, 1958).

$$R = \frac{S-1}{\ln N} \tag{2}$$

R = the values of Magalef's index

S = the numbers of species

N = the numbers of individuals

2.3 Diversity index (Shannon-Wiener's index)

The diversity of land snails was calculated by using Shanon-Wiener's diversity index (Krebs, 2009).

$$H' = -\sum_{i}^{S}(p_i lnp_i) \quad (3)$$

H' = the value of Shannon-Wiener diversity index

S = the number of species

p_i = the proportion of total sample belonging to the i the species

lnp_i = the natural logarithm of p_i

2.4 Dominance index (Simpson's index)

The dominance index of the land snail community was calculated by using Simpson's diversity index (Stiling, 2015).

$$D_S = \sum_{i}^{S}(p_i^2) \quad (4)$$

S = the numbers of species

p_i = the proportion of total sample belonging to the i the species

2.5 Evenness index

The evenness of the land snail community was calculated by using the Evenness index (Krebs, 2009).

$$E = \frac{H'}{H_{max}} \quad (5)$$

E = the value of evenness index (range 0–1). The closer to 1, the higher in evenness.

H' = the Shannon-Wiener's index

H_{max} = the maximum species diversity (=$\log_2$ S)

2.6 Similarity index

The similarity among plots was calculated by using the Bray-Curtis index (Bray & Curtis, 1957).

$$SI = 100 \times \left[1 - \frac{\sum|n_i - n_S|}{\sum|n_i + n_S|}\right] \quad (6)$$

SI = the coefficient value of similarity index

n_i = the number of the i species

n_S = the number of the S species

Results

A total of 1,083 individuals were collected, 1,032 of which were empty shells (95.29%) and 51 were living shells (4.71%). The specimens were identified into 8 species and 6 families, which belonged to 2 subclasses including Prosobranchia and Pulmonata. The Prosobranchia comprised 2 species including *Cyclophorus* sp. and *Cyclotus* sp. (family Cyclophoridae). The Pulmonata comprised 6 species including *Sarika* sp. and *Cryptozona siamensis* (family Ariophantidae), *Landouria* sp. (family Camaenidae), *Pupina* sp. (family Pupinidae), *Oophana* sp. (family Streptaxidae), and *Prosopeas* sp. (family Subulinidae). *Cyclophorus* sp. was mostly found from six plots (286 individuals) and the rarest species was *Prosopeas* sp. (only 1 specimen). All taxa were shown in Figures 2 and 3.

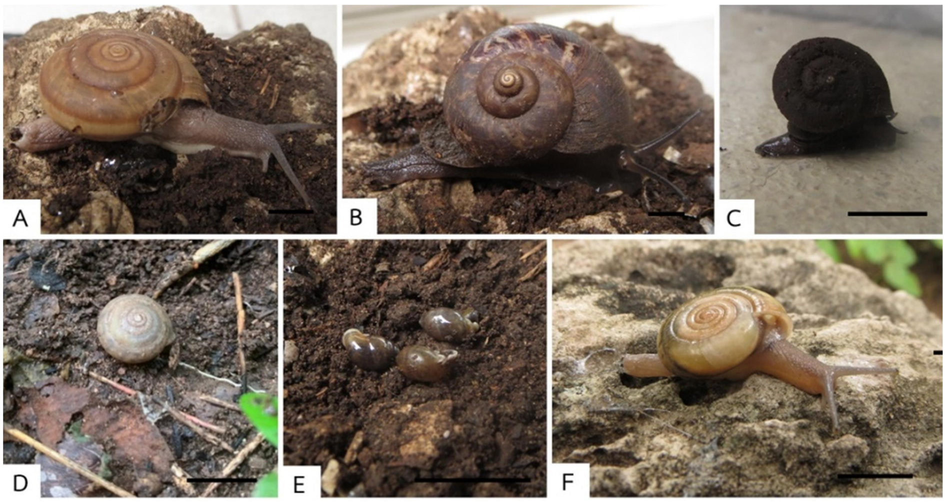

Figure 2 The living specimen of land snails in Khao Bin limestone hills

A = *Cryptozona siamensis* B = *Cyclophorus* sp. C = *Cyclotus* sp.

D = *Landouria* sp. E = *Pupina* sp. F = *Sarika* sp.

The scale bar is 10 millimeters long in all pictures.

The density was 0.45 individuals/m^2, ranging from 0.06 to 1.00 individuals/m^2 for 6 plots. The KB-01 was the highest density plot, followed by KB-04, KB-03, KB-06, and KB-05 respectively. Species richness index was 1.00 for overall areas, ranging from 0.88 to 1.59 among six plots. The KB-05 had the highest richness, followed by KB-06, KB-01, KB-02, KB-03, and KB-04 respectively (Table 2). The diversity index (Shannon-Wiener's index; H') was 1.01 (H'_{max} = 2.08) for overall areas, ranging from 1.44 to 1.73 among six plots. The KB-01 had the highest diversity, followed by KB-02, KB-05, KB-03, KB-06, and KB-04 respectively (Table 2).

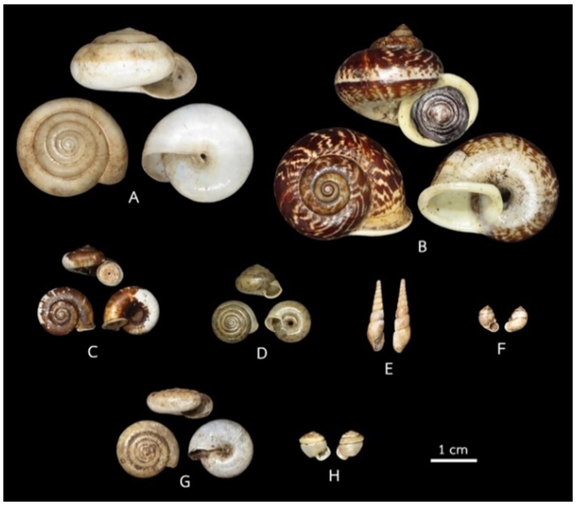

Figure 3 Empty shells of land snail in Khao Bin limestone hills
A = *Cryptozona siamensis* B = *Cyclophorus* sp. C= *Cyclotus* sp.
D = *Landouria* sp. E = *Prosopeas* sp., F = *Pupina* sp.
G = *Sarika* sp., H = *Oophana* sp.

The dominance index (Simpson's index) was 0.23 for overall areas, ranging from 0.19 to 0.28 among six plots. The KB-04 had the highest value, followed by KB-06, KB-05, KB-03, KB-02, and KB-01 respectively (Table 2). The evenness index was 0.48 for overall areas, ranging from 0.80 to 0.92 among six plots. The KB-05 had the highest value, followed by KB-03, KB-01, KB-06, KB-02, and KB-04 respectively (Table 2). The similarity index (Bray-Curtis) ranged from 10.90 to 94.44. The highest coefficient value was found between KB-01 and KB-03. The lowest coefficient value was found between KB-02 and KB-05 (Table 3).

Table 1 Land snail recorded in 6 plots in the Khao Bin limestone hills.

Species	Number of individuals in sampling plots						Number		Total
	KB-01 S(L)	KB-02 S(L)	KB-03 S(L)	KB-04 S(L)	KB-05 S(L)	KB-06 S(L)	S	L	
Cryptozona siamensis	31 (3)	90 (2)	40 (2)	70 (0)	8 (0)	4 (0)	243	7	250
Cyclophorus sp.	23 (3)	89 (2)	30 (0)	127 (0)	3 (0)	14 (0)	286	5	291
Cyclotus sp.	27 (3)	56 (9)	27 (4)	35 (1)	- (-)	- (-)	145	17	162
Landouria sp.	23 (1)	93 (4)	49 (0)	48 (0)	4 (0)	12 (0)	229	5	234
Prosopeas sp.	- (-)	- (-)	- (-)	- (-)	1 (-)	- (-)	1	-	1
Pupina sp.	3 (0)	2 (0)	- (-)	- (-)	- (-)	2 (2)	7	2	9
Sarika sp.	29 (4)	34 (10)	12 (1)	11 (0)	3 (0)	5 (0)	94	15	109
Oophana sp.	3 (0)	8 (0)	6 (0)	4 (0)	4 (0)	2 (0)	27	-	27
Total specimens	**139 (14)**	**372 (27)**	**164 (7)**	**295 (1)**	**23 (0)**	**39 (2)**	**1,032**	**51**	**1,083**
Number of taxa	**7**	**7**	**6**	**6**	**6**	**6**	**-**	**-**	**8**
Density	**0.38**	**1.00**	**0.43**	**0.74**	**0.06**	**0.10**	**0.43**	**0.02**	**0.45**

Remarks Indices were calculated from all specimens in sampling plots.

S represents the number of empty shells in sampling plots.

L represents the number of living specimens in sampling plots.

- (-) represents an absence of both in sampling plots

Table 2 Ecological indices of land snails in Khao Bin limestone hills.

Ecological indices	Number of individuals in sampling plots						Total
	KB-01	KB-02	KB-03	KB-04	KB-05	KB-06	
Species richness	1.19	1.00	0.97	0.88	1.59	1.35	1.00
Shannon-Wiener diversity index (H')	1.73	1.66	1.63	1.44	1.64	1.58	1.01
H'_{max}	1.95	1.95	1.79	1.79	1.79	1.79	2.08
Simpson diversity index	0.19	0.20	0.21	0.28	0.22	0.24	0.24
Evenness index	0.89	0.85	0.91	0.80	0.92	0.88	0.48

Table 3 The coefficient values of similarity index among 6 plots of Khao Bin.

Plot	KB-01	KB-02	KB-03	KB-04	KB-05
KB-02	55.43				
KB-03	94.44	60.00			
KB-04	68.15	85.18	73.23		
KB-05	26.14	10.90	23.71	14.42	
KB-06	42.27	18.64	38.68	24.33	71.88

Discussions

This study revealed a surprisingly lower number of living shells in Khao Bin limestone hills (Table 1). It was possibly due to many factors. Some snails were still in a period of estivation, which they have buried themselves under soils, liters, and rocks (Tumpeesuwan et al., 2014). Some species were nocturnal and could not be found in the daytime, a time of our sampling. In addition, living specimens might be eaten by mammals such as rodents and macaque (dominated species) because gnaw marks were observed on many shells. However, it is unclear about the interaction among species. The species interaction between snails and these mammals is interesting for study beyond.

For the 8 species collected in six plots, only one species was a carnivore (*Oophana* sp.). Moreover, most of the species were herbivores and detritivores. The most abundant species was *Cyclophorus* sp., accounting for 27% of total specimens. The rarest species was *Prosopeas* sp., accounting for 0.1% of total specimens, and there were no living specimens for this species.

The low density of land snails was observed in each plot (Table 1). It is indicated that land snails are sparsely distributed in Khao Bin. This study cannot relate any ecological parameter (leaf litter thickness, canopy, understory density, predator) to snail density. However, the observation during field sampling found that high-density plots were nearly located on the cliff and at slightly higher elevations than other plots. Also, there was seeping water from the cliff rocks in these plots. Sucharit & Panha (2008) suggested that most land snails always inhabit areas with high humidity or limestone hills, which are an essential source of calcium for building their shells. A high density of land snails could be associated with moisture and habitat conditions.

The difference of richness among plots is probably due to both the species number in each plot and the individuals' number of each species. High values of these numbers are generally associated with a high value of species richness (Death, 2008). According to Tanmuangpak et al. (2012), the area with a diversity score greater than one indicated highly diverse land snails. Similarly, Khao Bin (Shannon-Wiener's index = 1.44-1.73; Table 2) also tended to have a high diversity of land snails. The six plots had varying scores of diversity index (Table 2). Schilthuizen & Rutjes (2001) suggested that the difference in diversity index was due to small sample sizes, which could result in sampling error rather than true beta diversity. This case could also explain the disparity in diversity among the six plots. In Khao Bin, there were no dominant species according to the dominance index (Simpson's index=0.19-0.28; Table 2). Tumpeesuwan et al. (2014) suggested that a dominance index of less than 0.5 indicates that no dominant species is present in sampling locations with more than half of all specimens.

This research included 2 sample locations (Figure 1). The community of land snails among four plots inside the first location (KB-01 to KB-04; Table3) had high similarity index values, indicating that they were substantially similar. Two plots inside the second location (KB-05 to KB-06; Table3) also had a high similarity index. However, the similarity value across plots from 2 locations was less than 43% (Table 3), indicating that the species composition of the plots differed. Interestingly, the first location is surrounded by woodland, whereas the second is a buffer zone for agricultural land use (Figure 1). The variation in species composition between two locations might be explained by the influence of agricultural activities. The impact of agricultural land usage on land snail diversity should be investigated more in the future.

Conclusions

There was a total of 1,083 individuals gathered, with 4.71% of living shells and 95.29% empty shells. All individuals were identified into 2 subclasses, 6 families, and 8 species. The most abundant species was *Cyclophorus* sp. with 27% of total individuals. The rarest species was *Prosopeas* sp. with 0.1% of total individuals. The density of land snails was 0.45 individuals/m^2. Overall, species richness was 1.0, the diversity index (Shannon-Wiener) was 1.01, the maximum diversity index (H'_{max}) was 2.08, the dominance index was 0.23, the evenness index was 0.48, and the similarity index ranged from 10.90 to 94.44. In this study, the identification of specimens was based on external shell morphology. More study including anatomy, radula, and molecular genetics was needed to identify species precisely. More sampling plots were needed to collect at night to expand the area research and get a more accurate biodiversity assessment.

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