

## Resting Site Selection and Microhabitat Use of an Asiatic Toad (*Bufo gargarizans*; Anura: Amphibia) in South Korea

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**Abstract:** By selecting appropriate microhabitats, animals gain various benefits. For amphibians, which are ectothermic, appropriate microhabitat selection is directly related to their survival. This study aims to characterize the microhabitat type selected by *Bufo gargarizans* in South Korea, which has not yet been examined, using radiotelemetry. We analyzed the percent cover of different microhabitat features at sites where *B. gargarizans* was found. The microhabitat was categorized into eight types: stacked dry leaves litter or hay, grass, naked field, stones/pebbles, fallen branches, alive or dead trees/shrubs, wetland, and other. We collected a total of 60 location data, all of which had closed cover. From these, nine hibernation sites and six post-breeding dormancy sites were identified. Dry leaf litter was found to be the most frequently selected microhabitat type. We also found significant differences in microhabitat type between the hibernation period and the post-breeding dormancy period. We considered that these differences can be explained by the ecological characteristics of *B. gargarizans*.

**Key words:** Amphibian conservation; Bufonidae; Dry leaves; Habitat characteristics

### INTRODUCTION

Habitat selection not only reflects the location preferences and choices of an animal but also is crucial for its survival (Bélisle and Desrochers, 2002; Semlitsch and Bodie, 2003;

Marques et al., 2017; Calatayud et al., 2020). Habitat selection may vary depending on food availability, the presence of predators, vegetation cover, the availability of shelters for hiding, climatic conditions, etc. (Bélisle and Desrochers, 2002; Semlitsch and Bodie, 2003; Szekely et al., 2018). It may also influence breeding success, population dynamics, and behavior of the species (Yan and Chen, 1998; Yang et al., 2017). Thus, the habitat of an

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animal may change seasonally and is considered a key aspect in understanding its ecology (Baldwin et al., 2006; Groff et al., 2016; Ljubisavljević et al., 2017).

In the era of human mediated habitat alteration and destruction (Rahman et al., 2020, 2022; Nneji et al., 2021), studying habitat selection has become even more important. Habitat selection is particularly significant for ectothermic animals, such as amphibians, which need different types of habitats through different seasons (Roznik and Reichling, 2021; Valdez et al., 2021; Tong et al., 2023). Although most amphibians remain inactive only in the winter hibernation period, many species also undergo estivation to avoid extremely high temperatures (Jared et al., 2019), and some become dormant after the breeding season (Yu and Guo, 2010; Lee et al., 2013; Tong et al., 2023). Because amphibians cannot control their body temperature, which fluctuates with the environment (Rahman, 2016), they need to be choosy when selecting the habitats in which they spend their dormant periods (Santana et al., 2015; Calatayud et al., 2020; Hammond et al., 2021). Detailed information about habitat, such as microhabitat, increases our understanding of the species (Searcy et al., 2013). Microhabitat selection by amphibians has been shown to vary depending on species, sex, age class (Valdez et al., 2016), and landscape characteristics such as natural and human-modified habitats (Valdez et al., 2017). Consequently, amphibians utilize microhabitats in a wide variety of ways (Griffin and Case, 2001; Crawford and Semlitsch, 2008), including both terrestrial and aquatic environments. Because the local survival and abundance of amphibians are intrinsically related to the availability of shelters, conservation plans need to take microhabitat requirements into account (Basile et al., 2017). Understanding microhabitat selection by amphibians during periods of low activity and dormancy is crucial as it may differ from their habitat use during the breeding season and could be overlooked as important for population success (Garnham et al., 2015). Hence, it

is very important to recognize the characteristics of the microhabitats that amphibians use during their dormant period for the better understanding of their ecology and formulating conservation policies (Okamiya and Kusano, 2018).

An Asiatic toad (*Bufo gargarizans*) is a widely distributed species, found in Russia, China, and Korea, which mainly lives under fallen leaves or dead trees in forest valleys (Jang and Seo, 2010; Lee et al., 2011). Although the International Union for Conservation of Nature (IUCN) has mentioned a wide geographic distribution of this species and categorized its conservation status as least concern (LC), a recent study indicated that the Korean population may differ from the Russian and Chinese populations (Othman et al., 2022). Additionally, despite a determination that this species faces less risk from an invasive species, *Lithobates catesbeianus*, in South Korea than other anurans (Park et al., 2022), the rapid development of residential areas and industrialization is threatening its habitats. However, the two previous studies focusing on the habitat selection of *B. gargarizans* are only from China. One of them highlighted habitat selection in hibernation and post-breeding dormancy (Yu and Guo, 2010), and the other focused only on the hibernation period (Su et al., 2020). Thus, to fill the knowledge gaps in habitat selection of this species in South Korea, we studied the characteristics of its microhabitat use before the hibernation periods and post-breeding dormancy periods. This is one of only a few studies that explore the post-reproductive dormancy of amphibians. It could clarify the ecology of *B. gargarizans* and help in preparing conservation and management policies needed to respond to the high pressures of rapid land development and urbanization in South Korea.

## MATERIALS AND METHODS

### *Study site*

The study was conducted in Bichon village (35.048327° N 127.715536° E, 80–130 m asl)

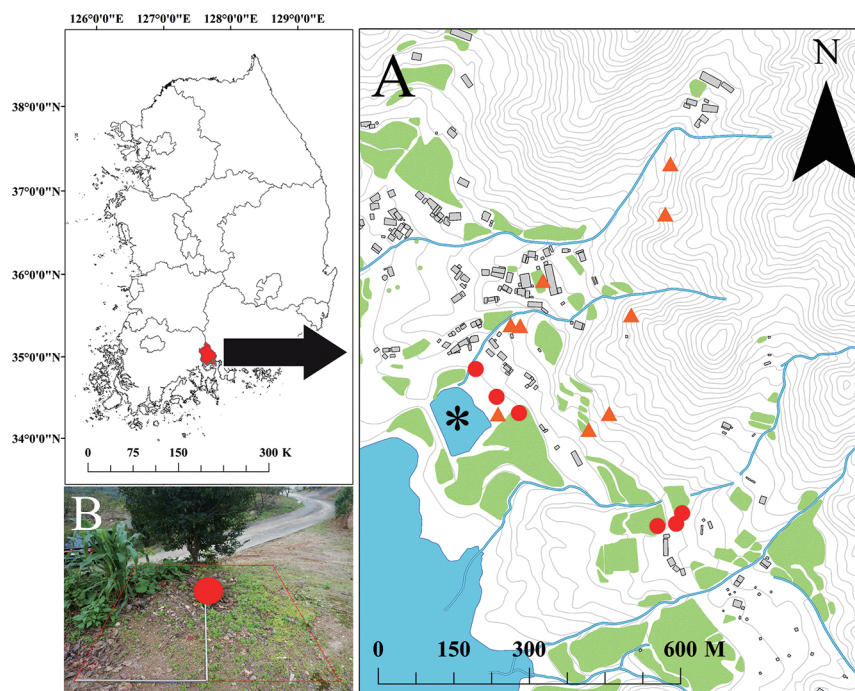


FIG. 1. Study site and example of a microhabitat. A: A contour map of the study site. Asterisk indicates the breeding site, orange triangles are hibernation sites, and red circles are post-breeding dormancy sites. B: Photograph of a microhabitat quadrat. Red circle in the middle indicates the spot where *B. gargarizans* was located. We laid a 2×2 m quadrat (red square) centered on the red circle and measured the percentages of each microhabitat type within the quadrat.

in Gwangyang, Jeollanam-do, South Korea (Fig. 1A). The village is bounded by orchards on the North, a 500 m mountain on the west, and a lake (Sueo) on the Southeast. There is a small reservoir (Bipyong Pond) between Sueo Lake and the village, which is used by the toad for breeding every year (Park et al., 2021). The reservoir covers 5,300 m<sup>2</sup> with a depth of ca 5 m.

#### Field surveys

The survey was conducted between August 2016 and May 2017. Radio telemetry was used to confirm the selected microhabitats of *B. gargarizans*. Following previous studies, we used BD-2 transmitters (1.6 g, Holohil, Canada), maintaining a transmitter weight to body mass ratio of a less than 5% for the experimental individuals (Brown et al., 2006; Lee et al.,

2013; Park et al., 2021; Park et al., 2022). Therefore, we selected only individuals with body mass of 30 g or more. For this, after capture, we first ensured the size and body mass of each individual by measuring the snout-to-vent length (SVL) and body mass (BM) using vernier calipers (0.05–150 mm, XP tool, Indonesia) and an electronic scale (0.1–1,000 g, Wessglobalm, Korea), respectively. Then, we attached the transmitter to the individuals at the waist with a fishing line and a stainless-steel ring in accordance with the methods of previous studies (Lee et al., 2013; Park et al., 2021; Park et al., 2022). We allowed the individuals to acclimatize with the transmitter, observing them for over 30 minutes in the box, and after ensuring their good physical condition and no abnormal behavior, we released each individual at the same site

where it was captured. After release, we tracked the individuals four times a week using a receiver (138–174 MHz, Biotrack, UK) and an antenna (Yagi Antenna, Biotrack, UK). Considering that *B. gargarizans* are nocturnal (Lee et al., 2011), night tracking would not be suitable to confirm their selected habitats due to their activity. Therefore, individual tracking confirmation was conducted during the day-time.

#### Data analysis

After the tracking *Bufo gargarizans* left for another site, we placed a quadrat at the point where the individual was stayed (Fig. 1B) and took a photograph. Based on previous studies (Rodrigues, 1994; Green et al., 2004) and the size of *B. gargarizans*, the quadrat was set to 2×2 m. To enhance objectivity, the analysis of microhabitat use was conducted by three researchers with research experience. The analysis was conducted using quadrat photographs. Three researchers independently determined the ratio of each microhabitat, and the average values of all the researchers were calculated. The categories included dry leaf (stacked humus, hay, and/or fallen leaves), grassland (only low growing plants), naked field (only bare soil, not included in other categories such as grassland or fallen leaves), stone tomb (stacked large and small stones and pebbles), fallen branches (piled-up branches), timbers and the tree remains (alive or dead woody vegetation, including both trees and shrubs), wetland (covered by a water system or wet place, i.e., a pond, marsh, etc.), and others (not included in the above categories, e.g., artificial objects, a road, etc.). We also collected a control group, used by probability sampling method. A small cloth was thrown within the area where *B. gargarizans* has been stayed, and the site where the cloth fell was selected as the control site. To enhance the reliability of the experiment, for each area, two control sites were selected and photographed in the same manner in a 2×2 m quadrat.

The microhabitat data were divided for a comparative analysis. Considering the survey

period, we divided the period defining collected from August through November 2016 as “before the hibernation period” and from March through May 2017 as the “post-breeding dormancy period”. In addition, we considered a microhabitat to be a temporary dormancy site, if the individual stayed between 3 and 10 days, and considered it a permanent dormancy site, if the individual spent over 10 days there.

Because all datasets did not conform to the normal distribution, we conducted Mann–Whitney *U* test to determine statistically significant differences between groups. The statistical analyses were conducted using IBM SPSS statistics (ver. 20, IBM, USA).

#### RESULTS

A total of 33 individuals were tracked using radiotelemetry. We captured 27 individuals (16 males and 11 females) before the hibernation period (August and September 2016) and six individuals (five males and one female) in the post-breeding dormancy period (March 2017).

Among the 27 individuals tracked before the hibernation period, we collected data from 19 individuals (10 males and nine females), excluding those only stayed at microhabitat for less than 3 days or the toads of which we lost the signal. From these individuals, we confirmed nine hibernation sites (three males and six females). Based on previous research, we determined the place as the hibernation site if the individual was stayed over 10 days and was found in the burrow with its eyes closed and the burrow’s entrance was blocked. Hibernation started sequentially in October, and all nine individuals went into hibernation by early November. All toads burrowed to over 10 cm below the surface and blocked the burrow entrance. Additionally, we calculated the minimum linear distance between the breeding site and hibernation site using a Geographic Information System program (ArcMap, ver. 10.7, ESRI, USA), resulting in  $282.3 \pm 186.3$  m (mean±SD). The average elevation was  $107.0 \pm 31.0$  m asl.



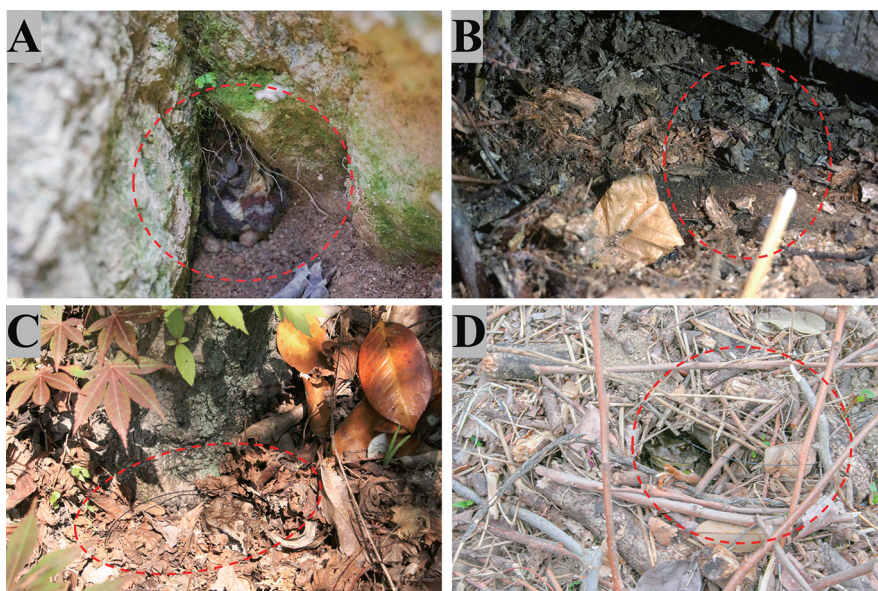


FIG. 2. Microhabitats where the presence of *B. gargarizans* was confirmed (red dashed line). Photographs A and B were taken before or during the hibernation period, and C and D were taken during the post-breeding dormancy period. A: Resting site before the hibernation period. An individual was located in the crevice between rocks. B: A hibernation site. The entrance was blocked, and only the transmitter antenna was exposed. C: Resting site in the post-breeding dormancy period. An individual resting nestled in fallen leaf litter. D: A post-breeding dormancy site, with an individual in an underground burrow. The entrance was open, contrasting with hibernation points, and the toad was resting with its eyes open.

The six individuals found during the post-breeding dormancy period were collected while moving out from the pond, and we collected microhabitat data of them. Post-breeding dormancy started in April. Based on previous research, we determined the place as a post-breeding dormancy site if a toad was found in the post-breeding period and stayed over 10 days and if the burrow's entrance was open. We confirmed a total of six post-breeding dormancy sites, one per individual. In all sites *B. gargarizans* kept their eyes open while sheltering in small cracks or under fallen leaves or sheltering by digging shallow, open burrows to cover themselves. For post-breeding dormancy sites, the average distance from the breeding site was  $227.7 \pm 195.6$  m and the average elevation was  $82.3 \pm 14.6$  m asl (the calculation method is the same as the hibernation site).

We selected a total of 60 microhabitat

quadrats of *B. gargarizans*, and 120 sites were collected as a control group. The all microhabitat quadrats had canopies, and all had open entrances except for the hibernation sites. All individuals were confirmed to rest while hiding in specific shelters, such as underground burrows, under fallen leaves or humus, or in stone crevices (Fig. 2). The percentages of each microhabitat type in the 60 quadrats were 35% dry leaf, 30.4% grassland, 14.3% naked field, 9.4% stone tomb, 5.8% fallen branches, 3.8% timbers and the tree remains, 1.1% other, and 0.2% wetland (Table 1). As a result of comparative analysis with the control group, three of the eight categories, that is, stone tomb, fallen branches, and timbers and the tree remains, were found to be significantly higher than the control quadrats (Fig. 3A;  $P < 0.05$ ). Of the 60 quadrats, 43 were identified before the hibernation period and 17 during the post-breeding

TABLE 1. Percentages of each microhabitat type in the microhabitat quadrats (n=60), hibernation sites (n=9), and post-breeding dormancy sites (n=6) of *B. gargarizans*. Microhabitat types include dry leaf (stacked humus, hay, and/or fallen leaves), grassland (only low growing plants), naked field (only bare soil, not included in other categories such as grassland or fallen leaves), stone tomb (stacked large and small stones and pebbles), fallen branches (piled-up branches), timbers and tree remains (alive or dead woody vegetation, including both trees and shrubs), wetland (covered by a water system, i.e., a pond, marsh, etc.), and others (not included in the above categories, e.g., artificial objects, a road, etc.).

	Dry leaf	Grassland	Naked field	Stone tomb	Fallen branches	Timbers and tree remains	Wetland	Others
Microhabitat quadrats (n=60)	35.0	30.4	14.3	9.4	5.8	3.8	0.2	1.1
Hibernation sites (n=9)	42.1	31.2	11.1	3.0	7.5	4.9	0.0	0.2
Post-breeding dormancy sites (n=6)	43.5	9.7	17.2	23.1	0.0	0.7	2.2	3.6

dormancy period. Comparing the microhabitat type percentages by period, grassland was greater in the hibernation period than in the post-breeding dormancy period (Fig. 3B;  $P<0.05$ ).

When the results were divided by the stayed days, 45 and 15 temporary dormancy and permanent dormancy sites were recorded, respectively. All hibernation sites and all post-breeding dormancy sites were included as the permanent dormancy sites. The statistical analyses showed that there was no significant difference in microhabitat type between the temporary dormancy and permanent dormancy sites, but the comparison between the hibernation and post-breeding dormancy sites showed significant differences (within permanent dormancy sites). The percentage of fallen branches was higher in hibernation sites (Fig. 3C; Table 1;  $P<0.05$ ). The microhabitat type percentages of hibernation sites (n=9) were 42.1% dry leaf, 31.2% grassland, 0% naked field, 3.0% stone tomb, 7.5% fallen branches, 4.9% timbers and the tree remains, 0.2% others, and 0.0% wetland. For the post-breeding dormancy sites (n=6), those were 43.5% dry leaf, 9.7% grassland, 17.2% naked field, 23.1% stone tomb, 0.0% fallen branches, 0.7% timbers and the tree remains, 3.6% others, and 2.2% wetland (Table 1).

DISCUSSION

In this study, we confirmed microhabitat type selected by *B. gargarizans*. We collected data on a total of 60 microhabitats during the entire experimental period, and the most frequently used (35%) microhabitat type was dry leaf (stacked humus, hay, and/or fallen leaves). Species of Bufonidae are adapted to terrestrial life (Bartelt et al., 2004), possessing adaptations such as thick skin (Lee, 2003) and the ability to store water (Boutilier et al., 1992) and withstand relatively large amounts of dehydration (Jones et al., 1992). After the reproductive period, they move out from the breeding sites (e.g., ponds) and migrate toward mountainous areas (Sung et al., 2007; Lee et al., 2013). In amphibians with limited dispersal ability, a lot of energy is consumed for movement (Yu et al., 2009; Luo et al., 2014, 2015). For this reason, terrestrial amphibians, such as species of Bufonidae, need to periodically absorb water in their habitat (Lillywhite and Licht, 1974; Jorgensen, 1991). In this study, the major microhabitat elements of *B. gargarizans* were accumulated fallen leaves, humus, and hay as dry leaf. This environment offers many easily accessible hiding spots and readily maintains a relatively high humidity and temperature. Therefore, similar to the

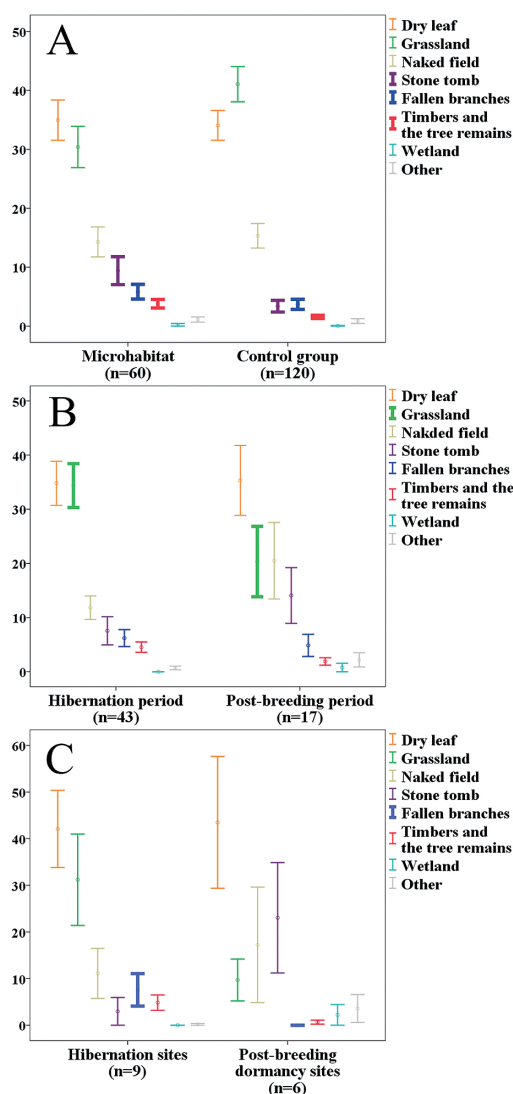


FIG. 3. Comparisons of microhabitat type percentages. A: Comparison between all microhabitat quadrats and the control sites. Significant difference was found in stone tomb, fallen branches, and timbers and the tree remains. B: Comparison of microhabitat quadrats by the period. A significant difference was found in grassland. C: Comparison between hibernation and post-breeding dormancy sites. A significant difference was found in the fallen branches.

results of previous studies, species of Bufonidae would prefer the microhabitat with the high ratio of fallen leaves, humus, and hay

(Bartelt et al., 2004; Yu and Guo, 2010; Su et al., 2020).

We confirmed that nine individuals started hibernation from mid-October. Su et al. (2020) reported the standard hibernation temperature of amphibians as 5°C, with pre-hibernation ambient temperatures close to 5°C, ambient temperatures during hibernation dropping to less than 5°C, and ambient temperatures of 5°C or higher after hibernation. Additionally, previous studies have shown that *B. gargarizans* hibernates in rock crevices, hollow tree trunks, dense vegetation, and even underwater (Denton and Beebe, 1993; Schwarzkopf and Alford, 1996; Seebacher and Alford, 1999; Yu and Guo, 2010). However, a recent study (Su et al., 2020) has observed a preference for low grass habitat (i.e., a mean herbaceous vegetation height < 60 cm), which is similar to the results of our study. In our study, the percentage of dry leaf was the highest around hibernation sites. At the ground level, the accumulated humus, hay, and fallen leaves act as a canopy, providing a good shelter and high humidity (Moore et al., 2018). We presume that this is the reason why *B. gargarizans* selected the microhabitat with humus, hay, and fallen leaves for hibernation sites.

According to previous studies, *B. gargarizans* prefers sandy soil as post-breeding dormancy sites, and the toad burrows to a shallower depth for post-breeding dormancy than for hibernation sites (Yu and Guo, 2010). Additionally, some individuals do not return to the previous hibernation habitats after breeding and selected a poorer site, with some individuals seen without any cover (Yu and Guo, 2010). We observed similar results in our study although preference for sandy soil was not observed. This difference is simply due to the differences in available microhabitat types: sandy soils did not exist in our study area. Yu and Guo (2010) also explained that *B. gargarizans* consumes a lot of energy to breed, and thus the toad stays close to the breeding sites and avoids digging directly. We observed individuals in open-entrance burrows and consuming food. At the post-breeding dormancy

sites, the highest microhabitat type was again dry leaf. We suspect that due to the lack of energy following breeding, this habitat was chosen for the post breeding dormancy partly because it provides easily found food sources (Yu and Guo, 2010).

Our analysis found differences of chosen microhabitats in the proportion of the low growing plants (grassland) between the periods. This difference would be related to the fact that the post-breeding period in this area is March–April, when the grass has not yet grown much there. In another study of the hibernation habitats of *B. gargarizans*, it was found that farmland or vegetable gardens were preferred (Su et al., 2020). These habitats are likely to be comparable to the grassland in our study. In these habitats, individuals would be able to avoid predators. In addition, generally, the soil contents of grasslands are loose due to the influence of tillers (Gerson, 1982). Su et al. (2020), thus, suggested that it is easier for toads to breathe while hibernating in areas with high water content, and our results would be explained similarly.

The differences in the permanent dormancy sites between the periods showed that a relatively high percentage of fallen branches during hibernation. Our study area is mostly made up of orchards, and the period of before hibernation is the harvest time for the fruit. Thus, during the harvest, branches remain and pile up in the orchards, and it seems that *B. gargarizans* used this as an adequate hiding place. Notably, all the chosen habitats of *Anaxyrus borea*, a species of the same family, Bufonidae, had tree fragments and tunnels (Browne and Paszkowski, 2018). In the post-breeding period in South Korea, plants do not grow much. Therefore, the percentage of dry leaf and naked field are relatively higher. Thus, we consider that *B. gargarizans* digs burrows or chooses places near piles of stones where they are easy to hide.

In this study, we characterized the microhabitat type selected by *B. gargarizans* based on radiotelemetry. Our result suggests that accumulated humus, hay, and fallen leaves are the

most preferred microhabitat of *B. gargarizans*. This finding provides basic information on this species and is expected to be used for toad habitat conservation.

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