Diversity of the Adult Frogs (Amphibia: Anura) in Prince of Songkla University, Pattani Campus, Thailand

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Abstract

An investigation of adult frog diversity in Prince of Songkla University, Pattani Campus, Pattani Province of Thailand was carried out during March 2018 – February 2019 to provide the species checklist, habitats, relative abundance, Shannon–Wiener's diversity, evenness indices, conservation status and to construct an area identification key to adult frog species and diagnosis morphological description. The surveys were conducted approximately 3–4 days each month. Five families, 8 genera and 11 species: Bufonidae (1 species), Dicroglossidae (3 species), Microhylidae (5 species), Ranidae (1 species), and Rhacophoridae (1 species) were found in this research. They were found in temporary ponds except *Hylarana erythraea* found in freshwater ponds only. The analysis of relative abundance revealed that *Duttaphrynus melanostictus* and *Fejervarya limnocharis* showed the highest value (78.38%) whereas *Micryletta inornata* showed the lowest value (2.70%). Additionally, the results of the Shannon–Wiener's diversity and evenness indices were 2.01 and 0.68, respectively. In this study, the conservation status of most species was the least concern, except for *Polypedates discantus* was not evaluated. Moreover, the area identification key to adult frog species and diagnosis morphological description were provided.

Keywords: Adult frogs, Anura, Diversity, Pattani Campus, Prince of Songkla University

Introduction

There are many geographic areas in Southeast Asia that would be appropriate widespread species of frogs (Rujirawan, Stuart, & Aowphol, 2013). Thailand is located in the tropical area of Southeast Asia, which is considered a diverse source of frogs. They are strongly associated with wetlands and forest ecosystems (Mulatu & Getahun, 2018). The frogs play an important role in ecological and economic fields (Muslim, Sari, & Yasir, 2017). Ecologically, most larval frog stages live in the water, and the adult stages live on the ground. Meanwhile, they are predators on invertebrates as well as small vertebrates. On the other hand, they are food for other animals, such as reptiles, birds, and mammals (Schriever & Williams, 2013; Hocking & Babbitt, 2014). Moreover, the frogs can be used as a bioindicator for changing the environmental condition, due to their ability to live in various habitats (Muslim et al., 2017). The diversity of frog in a habitat is highly dependent on various factors, such as the food, the stream for reproduction and the predators (Ndriantsoa, Riemann, Raminosoa, Rödel, & Glos, 2017). Economically, the frogs are widely harvested for food, traditional medicines, and the pet trade in many localities (Rodríguez, Rollins-Smith, Ibanez, Durant-Archibold, & Gutiérrez, 2017). Recently, the frogs in Thailand are more than 100 species and chance to be discovered more (Chan-ard, 2003).

Prince of Songkla University, Pattani Campus is located in Pattani Province of Thailand, where identity area is present. The 1.46 km² approximated area set aside 2 kilometers muddy beach, where is the mangrove forest. Every year, sediment including organic waste and other waste materials from land and ocean (Yuliana,

Hewindati, Winata, Djatmiko, & Rahadiati, 2019) will cover up the land by the influence of Pattani River, expanding the area of the university. More piles of the sediment on the ground became substrates, which are known for their fertility, functioning as a habitat for various types of life (Yuliana et al., 2019; Thomas et al., 2017). Equally, there are 2 seasons of this province: the dry season (February – April) and the rainy season (May – January), hence, that may influence frog species to be different from other locals. However, checklists and surveys about the frog species in Prince of Songkla University, Pattani Campus still remains unknown causing people not to attach great importance to the frog. Academicians and researchers are interesting on large reptiles, migratory birds and visible mammals more than on the small animals, mainly frogs. Moreover, they are often threaten by human creating construction in the area. The loss of natural area and poor pollution are serious problems (Disi, Amr, & Hamidan, 2014), chance of frogs is exterminated from this locality. Thus, there is essential need of the frog diversity in this university as a baseline information for provide conservation in the future.

Therefore, the purposes of this study were to provide the species checklist, habitats, relative abundance, Shannon–Wiener's diversity, evenness indices, conservation status and to construct an area identification key to adult frog species and diagnosis morphological description in Prince of Songkla University, Pattani Campus, Pattani Province. Knowledge of diversity and organization is essential for the development of conservation policies and a sustainable environmental management system. (Riyanto, 2011; Searcy, Gabbai–Saldate, & Shaffer, 2013).

Methods and Materials

Study area

The location of the study was in Prince of Songkla University located in Rusamilae Subdistrict, Mueang Pattani District, Pattani Province, and it cover 1.46 km². The map of the research location is presented in Figure 1. The research was conducted for 1 year from March 2018 – February 2019.

The climate data base on Pattani Province statistic for 30 years, modified from Thai Meteorological Department (2018). The rainfall pattern is highest between May and January (rainy season) and its lowest between February and April (dry season), the average annual temperature of this province is 27.1 °C.

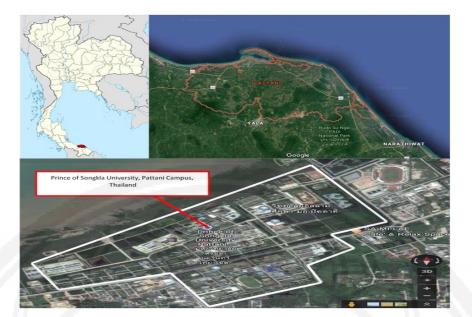


Figure 1 Map of Thailand showing research location is Rusamilae Subdistrict, Mueang Pattani District, Pattani Province, Thailand

Procedures

The survey of the frogs was carried out from March 2018 – February 2019, approximately 3–4 days each month in the night (7.00 – 10.00 pm, GMT +7) by opportunistic visual and acoustic encounter surveys in all potential microhabitats of adult frogs (Mulargia, Corti, & Lunghi, 2018). The survey assistance equipments were applied such as grab stick, camera, amphibian identification books, and frog sampling collected tools. The techniques for nocturnal searches of the adult frogs is using flashlight. The adult frogs would be temporarily blinded when the flashlights shine in their eyes, making easy to catch and identify species. The adult frogs were identified to species by following Chan–ard (2003), Rujirawan et al. (2013), Alhadi et al. (2019, p. 111), Garg et al. (2019, p.1), and Yodthong, Stuart, & Aowphol, (2019). The field specimens collected for identification were kept at the Zoology Laboratory, Prince of Songkla University, Pattani Campus. Moreover, species checklist, population counts and habitats of adult frogs were recorded. The construction of an area identification key to adult frog species in this study site was considered about the special structure of field specimens collected from each species, as well as a conservation status of each adult frog species, was cited from IUCN Red List (IUCN, 2019).

Data analyses

The survey data were offered in 1 year analysis, also separated information for analyzing in rainy season and dry season. This data was analyzed by using Microsoft Excel, described to provide information on relative abundance (RA), Shannon–Wiener's diversity index (H'), and evenness index (E).

Relative abundance (RA)

The relative abundance was analyzed by following Pettingill (1950) as follow: "% Relative abundance = (animals meet times / survey times) x 100"

Relative abundance use Chumrieng & Kongthong (2006) standard that can separate 6 degrees as follows:

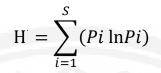
1-15% the least relative abundance value

16-30% a few relative abundance values

31-45% quite a few relative abundance values

- 46-60% moderate relative abundance value
- 61–90% many relative abundance values
- 90-100% the most relative abundance value
- Shannon-Wiener's diversity index (H')

Shannon-Wiener's diversity index (H') (Krebs, 1989) is used to analyze the diversity of frogs as follow:



H' = Shannon-Wiener's diversity index

S = Number of species

Pi = Proportion number of each species to the number of all species

Evenness index (E)

Evenness index reveals a pattern of distribution in somewhere. We used a formula that calculated by Buzas and Gibson (Krebs, 1989), this formula as follow:

$$E = e^{H'}/S$$

E = Evenness index

e = 2.71828

H' = Shannon-Wiener's diversity index

S = Total of species

Results

Diversity of the adult frog habitats

They can be observed in different habitats, there were artificial canals (AC), agriculture zones (AZ), building zones (BZ), freshwater ponds (FP), temporary ponds (TP), and yard zones (YZ).

Artificial canals and constructor zones were found around the university and near the roadside. At night, they always turn on the light cause attracted many insects and moreover in rainy season.

Agriculture zones, temporary ponds, and yard zones, which have different vegetation that grows following the habitats potentially serve for the adult frogs. The majority of the agriculture zone is dominated by herbs and vegetables while temporary ponds, and yard zone are dominated by grass.

Freshwater ponds have many aquatic plants and always fulfill with water all year but other habitats are completely dried up in dry season.

Checklist of adult frog species

In this study, 5 families, 8 genera and 11 species of the adult frogs were found. Checklist of adult frogs species are as detailed below (Table 1).

100 M T N 200

Family	Species list	Habitat						Conservation	
		AC	AZ	BZ	FP	TP	YZ	status	
Bufonidae	Duttaphrynus melanostictus	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	least concern	
Dicroglossidae	Fejervarya cancrivora	\checkmark	\checkmark		\checkmark	\checkmark	\checkmark	least concern	
	Fejervarya limnocharis	\checkmark	\checkmark	-	\checkmark	\checkmark	\checkmark	least concern	
	Occidozyga martensii	\checkmark	\checkmark	-	\checkmark	\checkmark	-	least concern	
Microhylidae	Kaloula pulchra	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	least concern	
	Microhyla butleri	-		-	-	\checkmark	-	least concern	
	Microhyla heymonsi	-			-	\checkmark	-	least concern	
	Microhyla mukhlesuri	76		V		\checkmark	·	least concern	
	Micryletta inornata	-	-	14	\	\checkmark		least concern	
Ranidae	Hylarana erythraea	-	-		\checkmark			least concern	
Rhacophoridae	Polypedates discantus	-	\checkmark	-	\checkmark	\checkmark	23 <u>-</u> 12	not evaluated	

Table 1 Species list of the adult frogs in Prince of Songkla University, Pattani Campus, Thailand

Area identification key to adult frog species in Prince of Songkla University, Pattani Campus, Pattani Province

An area identification key to adult frog species was considered about the special structure of field specimens collected from each species. Then, the information was modified from Chan-ard (2003), Rujirawan et al. (2013), Alhadi et al. (2019, p. 111), Garg et al. (2019, p. 1) and Yodthong et al. (2019). Hence, the area identification key to adult frog species is detailed below: 1B. Paratoid gland absent......2 3A. Maxillary teeth present......4 8A. Whole dorsum brown appear with two dark-edged light brown or yellowish stripes from eye to 9A. Dark stripe from snout tip along eyes to near groins, lower edge becomes gray.....

10B. Presence of numerous large tubercles similar to dorsolateral fold along dorsolateral region.....



Figure 2 Adult frog species in Prince of Songkla University, Pattani Campus, Pattani Province. A. Duttaphrynus melanostictus;
B. Polypedates discantus; C. Hylarana erythraea; D. Occidozyga martensii; E. Fejervarya cancrivora; F. Fejervarya limnocharis; G. Micryletta inornata; H. Kaloula pulchra; I. Microhyla heymonsi; J. Microhyla butleri; K. Microhyla mukhlesuri.

Species accounts and diagnosis morphological description

Family: Bufonidae (1 species)

Duttaphrynus melanostictus

The common name is "Asian Common Toad". They are stout and brown or grey-brown. Dry skin covers with spines, the tip of spines is black. Pair of supraorbital ridges continue as strong parietal ridges from snout to edge of the eyelid. Paratoid glands present, these are great and long above tympanum. The belly is mottled brown. The measurements of snout-vent length (SVL) of this species are about 56 mm. The adults of this species are found in every habitat. In rainy season, male often sits around the wetland and calls for mating.

Family: Dicroglossidae (3 species)

Fejervarya cancrivora

The common name is "Crab-eating Frog or Mangrove Frog". They have truncate snout or obtusely pointed, maxillary teeth present, vomerine teeth present, tympanum distinct. Color is brown or gray-brown, a white spot on head, sometimes with orange or green mind dorsal band from tip of snout to the vent, black spot spread around body, has interrupted dorsolateral folds on the back and outer metatarsal tubercle absent. The belly is white, ventrolateral line present. The measurements of SVL of this species are about 47.8 mm. This frog species was mostly found around moist land, with an activity inside the water at the pond bank near muddy beach.

Fejervarya limnocharis

The common name is "Grass Frog". They have truncate snout or obtusely pointed, maxillary teeth present, vomerine teeth present, tympanum distinct. There are small tubercles presenting in skin of body, interrupted dorsolateral folds on the back, and outer metatarsal tubercle present. Color is brown or olive, sometimes with orange or green mind dorsal band from the tip of snout to the vent, black spot spread around the body, the belly is white, ventrolateral line present, and vocal sacs are mottled with black or brown. The measurements of SVL of this species are about 35.6 mm. This species was commonly found around university.

Occidozyga martensii

The common name is "Marten's Puddle Frog". This species is short and thick frog with a broad head, maxillary teeth present, and vomerine teeth absent, tympanum is covered with skin, and great tubercle beside the dorsolateral line. The color is brownish gray. The head is brownish gray and flecked with the darker spots. There are lighter spots on the tip of the snout. The mid-dorsal part of body is an orange band from the tip of snout to vent. The belly is white. The measurements of SVL of this species are about 22 mm. In this study, they were found around the wetland.

Family: Microhylidae (5 species)

Kaloula pulchra

The common name is "Painted Bullfrog". The body is stocky, with a small head and rounded snout, maxillary teeth and vomerine teeth absent. The dorsum is dark brown, sometimes with irregular yellowish-brown spots. Whole dorsum brown appears with two dark-edged light brown or yellowish stripes from eye to groin, which may itself be punctuated with several spots. The belly is mottled dirty yellowish-brown, males have a black throat. The measurements of SVL of this species are about 45 mm. Its habitat is the temporary pond, the male always calls for mating when raining.

Microhyla butleri

The common name is "Noisy Frog". They have a small head, rounded snout, maxillary teeth and vomerine teeth absent. On metatarsals, there are two metatarsal tubercles (inner and outer). Their skin is brownish, fade color on body side, and presence of numerous large tubercles on dorsal. The belly is white. The measurements of SVL of this species are about 14.3 mm. In this study, they were found sitting under grass leaves near temporary ponds.

Microhyla heymonsi

The common name is "Dark-side Chorus Frog". They have a small size, rounded snout, maxillary teeth and vomerine teeth absent. On metatarsals, there are two metatarsal tubercles (inner and outer). Their skin is brownish, dark stripe from snout tip along with eyes to near groins, the lower edge becomes gray, and there is a black line across from snout to vent on dorsal. The belly is white. The measurements of SVL of this species are about 14.3 mm. They were found lying concealed in the grass or under leaf litter near temporary ponds. In the surveyed, male always calls for matting in rainy season that sounds like repeated clicking sounds.

Microhyla mukhlesuri

The common name is "Mukhlesur's Narrow-mouthed Frog". They have a small size, rounded snout, maxillary teeth and vomerine teeth absent, tympanum hidden and presence of two metatarsal tubercles (inner and outer). Skin presents of numerous large tubercles similar to dorsolateral fold along the dorsolateral region. Their dorsum is dark gray to brownish, dark X-shaped mark on the dorsum, U-shaped black mark above the

anus, and black band start from the tip of snout to the groin. The belly is white. The measurements of SVL of this species are about 17.4 mm. They were found in the grass near the bank of the temporary pond.

Micryletta inornata

The common name is "Inornate Chorus Frog". They have small size and rather flattened, rounded snout, maxillary teeth and vomerine teeth absent. Skin is smooth, brownish-gray on dorsum, three black stripes from tip of snout alongside and head to near groins. The belly is white and sometime mottled with black spots. They have inner metatarsal tubercle, no outer tubercle. The measurements of SVL of this species are about 12 mm. They were found co-exited with *Microhyla heymonsi, Microhyla mukhlesuri*, and *Occidozyga martensii* in the grass near the bank of the temporary pond.

Family: Ranidae (1 species)

Hylarana erythraea

The common name is "Green-backed Frog". They are medium-sized frog. They have rather rounded snout, maxillary teeth present, vomerine teeth present. Tips of fingers and toes are rounded disks. Skin is smooth and green, dorsolateral fold present. The belly is white and ventrolateral line is absent. The measurements of SVL of this species are about 23.1 mm. In this study, *Hylarana erythraea* always live in freshwater pond that is dense vegetation and also co-excited with *Duttaphrynus melanostictus*, *Fejervarya cancrivora*, *Fejervarya limnocharis*, and *Occidozyga martensii*.

Family: Rhacophoridae (1 species)

Polypedates discantus

The common name is "Malayan Slender Tree Frog". They are medium-sized tree frog with pointed snout and presenting of maxillary teeth and vomerine teeth. Tips of fingers and toes are rounded disks, intercalary cartilage presenting in fingers. Brown body skin and limbs are shagreened, smooth skin in the orbital region. Sometimes, dark X-shaped marking visible on interorbital region, neck, and shoulders. Black spots are scattered on dorsal. The belly is white and absence of ventrolateral line. The measurements of SVL of this species are about 49.2 mm. They were found above 1 - 2 meters on trees near the temporary pond and also found around shrub grass.

Data analysis

Analysis of relative abundance was performed all year, the highest value was *Fejervarya limnocharis* and *Duttaphrynus melanostictus* (78.38%), the lowest value was *Micryletta inornata* (2.70%). Additionally, the results showed that Shannon–Wiener's diversity and evenness indices were 2.01 and 0.68, respectively (Table 2).

In the dry season, when relative abundance was analyzed, the highest value was *Fejervarya limnocharis* (100.00%), the second was *Duttaphrynus melanostictus* (80.00%) and the third was *Fejervarya cancrivora* (70.00%). The lowest value was *Microhyla butleri* (10.00%). Additionally, the results showed that Shannon–Wiener's diversity and evenness indices were 1.83 and 0.62, respectively (Table 2).

In the rainy season, when relative abundance was analyzed, the highest value was *Duttaphrynus melanostictus* (77.78%), the second was *Fejervarya limnocharis* (70.37%) and the third was *Kaloula pulchra* (66.67%). The lowest value was *Micryletta inornata* (2.70%). Additionally, the results showed that Shannon–Wiener's diversity and evenness indices were 2.04 and 0.70, respectively (Table 2).

Familar	Species 1:-4]	Relative abundance (%)				
Family	Species list	Dry season	Rainy season	1 year			
Bufonidae	Duttaphrynus melanostictus	80.00	77.78	78.3			
	Fejervarya cancrivora	70.00	59.26	62.1			
Dicroglossidae	Fejervarya limnocharis	100.00	70.37	78.3			
	Occidozyga martensii	40.00	33.33	35.1			
Microhylidae	Kaloula pulchra	60.00	66.67	72.9			
	Microhyla butleri	10.00	11.11	10.8			
	Microhyla heymonsi	20.00	25.93	24.3			
	Microhyla mukhlesuri	20.00	33.33	29.3			
	Micryletta inornata	0.00	2.70	2.70			
Ranidae	Hylarana erythraea	20.00	22.22	21.6			
Rhacophoridae	Polypedates discantus	50.00	25.93	32.4			
Shannon	-Wiener's diversity index (H')	1.83	1.83 2.04				
	Evenness index (E)	0.62	0.70	0.68			

Table 2 One year data analysis of the adult frog diversity in Prince of Songkla University, Pattani Campus, Thailand

Discussion

This study was recorded during both rainy and dry season in March 2018 - February 2019, 5 families, 8 genera and 11 species, followed by Bufonidae (9.09%), Dicroglossidae (27.27%), Microhylidae (45.46%), Ranidae (9.09%), and Rhacophoridae (9.09%) were found. Data analysis was considered in 1 year, the four species showed many relative abundance values (RA = 61-90%), Fejervarya limnocharis (RA = 78.38%), Duttaphrynus melanostictus (RA = 78.38%), Kaloula pulchra (RA = 72.97%), and Fejervarya cancrivora (RA = 62.16%). The first three species were abundant in the lowlands of Thailand. They were found hiding, foraging, sitting, and matting within grass near temporary ponds, and sometimes they jumped to water when were disturbed. They are also distributed in university campuses area such as artificial canal, agriculture zone, building zone, and other human settlements (Blackburn et al., 2013; Ibrahim, Nur-Hafizah, Nurul-Dalila, Choimmber, & Muin, 2012; Yodthong et al., 2019). On the other hand, Fejervarya cancrivora can distribute in specific area that set aside muddy beach. Chan-ard (2003) and Yodthong et al. (2019) reported that this species can survive in the intertidal zone such as on the bank or water of brackish shrimp ponds. In the survey, this species of frog was found to live in freshwater zone or wetland such as, freshwater ponds, artificial canals according to Muslim et al. (2017) reported that Fejervarya cancrivora lives in a natural aquatic habitats and presents many activity inside the water. Therefore, this study showed that these four species show adaptability serving to explain the widespread, co-exiting with another species of the adult frogs in this university. The quite a few relative abundance values (RA = 31-45%) are Occidozyga martensii (RA = 35.14%) and Polypedates discantus (RA = 32.43%), they were found in aquatic habitats where covered by grasses or shrubs. Meanwhile, *Polypedates discantus* were often found on vegetation 1-2 meters above the temporary ponds (Rujirawan et al., 2013). Other species (RA < 30%) has been sighted in under grasses or dense plant areas around freshwater and temporary ponds that affect these species to present relative abundant value less than others (Hasan, Islam, Kuramoto, Kurabayashi, & Sumiaa, 2014).

When species between seasons (dry and rainy) were considered, every species were found in both seasons, also known to be closely associated with and dependent on water habitats, except *Micryletta inornata* were recorded in rainy season only. In this study, all specimens of this species has been found hiding and sitting on mud under leaves grass beside temporary pond that is usually behaviors of this species (Alhadi et al., 2019, p. 111). In dry season, water in temporary pond quantity decreased until completely dried out and soil has been hard. On the other hand, the behavior of *Micryletta* will forage and mate relatively surrounding water source (Poyarkov, Nguyen, Duong, Gorin, & Yang, 2018). Cause this species was found in rainy season easier. Some species of the adult frogs present relative abundance values in rainy season lower than dry season. In rainy season, vegetation more grew up that made dense in many habitats. Because of obstacle to survey, access has been constrained to focus on many habitats (Sanguila et al., 2016).

The analysis of Shannon–Wiener's diversity and evenness indices were highest in the rainy season (H' = 2.04, E = 0.70). In rainy season, the adult frogs were reproductive period, they will mate and forage food easier. Every microhabitats have fulfilled with water, preys, and low temperature which are suitable for the adult frogs. Therefore, the amount of the adult frogs in the rainy season were abundant more than those in the dry season (H' = 1.83, E = 0.62). From there, Shannon–Wiener's diversity and evenness indices were increased. The differences in the analysis of Shannon–Wiener's diversity and evenness indices between dry and rainy seasons were probably caused by distribution changes of the amphibians as the response of climate difference in two seasons (Riyanto, 2011). From some of the results of this study, it can conclude that the presence of water and the plant area play an important role of forming an ecosystem for amphibians (Solsky, Smolova, Dolezalova, Sebkova, & Vojar, 2014) to foraging, reproduction, and cover from predators (Auguste & Hailey, 2018, p. 86). Each habitat is influence amphibian diversity (Jongsma, Hedley, & Dura^{*}, 2014) and different environmental factors affect to habitat are important for analyzed relative abundance values, Shannon–Wiener's diversity, and evenness indices in the surveyed (Auguste & Hailey, 2018).

In general, Prince of Songkla University comprises natural and artificial wetlands including artificial canals, agriculture zones, building zones, freshwater ponds, temporary ponds, and yard zones. The majority of the wetlands are temporary ponds. In the study, most adult frogs were found in these areas because there was various vegetation such as glasses and 1-2 meters high shrubs around. Temporary ponds with shrubs and grasses are suitable habitat for many insect species such as grasshoppers, butterflies, moths, and dragonflies (Gunawan, Sugiarti, Rianti, & Sihombing, 2016) accord with the adult frog behavior which often foraging, sitting, and matting near temporary ponds. Therefore, in the surveyed can found species of the adult frog more than other habitats.

Conclusion and Suggestions

In this study, it could be supported the conservation status of amphibians, which is less information. From investigation, almost species was the least concern, except for *Polypedates discantus*, which was not evaluated (IUCN, 2019). At present, there are many areas in Prince of Songkla University (Pattani Campus), where will be prepared for construction. Thus, it is hoped that this study could be helped to conversation amphibians for survival and existence. These species play very important role in food web which is carnivore in ecological



processes of ecosystem. Future study should more focus on habitats and behavior of each species that will contribute conservation efforts anuran species in the university.

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