The Diversity of Anuran Species in Urban Forest in Selangor

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Abstract. This study aimed at determining anuran diversity in Compartment 12 and 13 of Ayer Hitam Forest Reserve (AHFR), Puchong, Selangor. The study determining species diversity, richness and evenness, analyzing correlation between the anuran species with the microclimate and updating anuran species status. Fieldwork was carried out from October 2016 to February 2017 investing six person of sampling effort. Fifteen of 300 m nocturnal transect lines have been constructed in this inventory at three different study areas; walking trails, streams, and swampy. A total of 119 individuals belonging to 18 species were recorded. Most abundant species recorded were Fejervarya cancrivora, Leptobrachium hendricksoni, and Pulchrana laterimaculata. Compartment 12 found to have the highest species diversity and evenness compared to Compartment 13. Stream has the highest species composition with Ingerophrynus parvus and Leptobrachium hendricksoni each with eight individuals respectively. Most anuran species recorded were highly associated with temperature such as Limnonectes malesianus, Amnirana nicobariensis, Limnonectes plicatellus, and Fejervarya limnocharis. This study also recorded Amnirana nicobariensis, Kalophrynus pleurostigma, and Pulchrana laterimaculata for the first time for AHFR. Therefore, this further research is needed by increasing forest coverage for the anuran diversity study as a reference for future study.

1. INTRODUCTION

Anura is the most widely represented of any group of amphibians comprising the tailless frogs and toads. They possessed dual life cycle and known to be vulnerable to environmental pollutants. The anurans are largely distributed in the tropical and Peninsular Malaysia is one of known as regions of high amphibian fauna diversity and endemism due to its complex and rugged topography [2]; [3]; [8]; [5]. Currently, 111 anuran recorded in Peninsular Malaysia comprising six families: Bufonidae (19), Dicroglossidae (20), Megophryidae (12), Microhylidae (21), Ranidae (18), and Rhacophoridae (21) [20]. While in Borneo, there are 138 species of amphibians which includes eight families of anuran which are Bombinatoridae, Bufonidae, Ceratobatrachidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae and Rhacophoridae [15].

Anuran inventory studies in Ayer Hitam Forest Reserve (AHFR) begun during 1975 to 1985 for museum collections and continued from April 1998 to January 1999 along the Rasau River. The inventories revealed 10 and 13 anurans respectively. Recent inventory study were initiated back on 2015 in compartment 15 which recorded 24 anurans. The most dominant species inhabit the compartment were Polypedates leucomystax (Four-lined Tree Frog) and Chalcorana ranicep (White-lipped Frog) each with 9% respectively ([17]; [18]). Being in the center of urbanization is threatening to Ayer Hitam Forest Reserve. Further rapid development will causes the forest become smaller and offers greater habitat loss to the wild animals. Besides, habitat loss has shown to be one of the causes of amphibian declines worldwide [10]. Owing to its semi-permeable skin, the species is
environmentally sensitive which important as bioindicator to human and ecosystem health [1], [9]. Among compartments in the forest, compartment 12 and 13 of the forest is categorized as less disturbed [25] and information on the anuran is unknown. Therefore, this study aimed to update the species inventory and reports on its diversity in AHFR for future anuran studies.

2. Materials and methods

2.1 Study site
Compartment 12 and 13 of Ayer Hitam Forest Reserve is situated at 03° 00' 792'' N, 100° 38' 821'' E and 03° 00' 941'' N, 100° 38' 874''E with elevation of 233 m above sea level (Fig. 1). The study area is a lowland dipterocarp forest (Paiman & Amat, 2007) and the level of disturbance is low [25]. The topography of the area is rugged and hilly with the presence of clear water flowing stream. This in turn provide conducive area for the anuran to survive. Dominant plant species found here were Dipterocarpus spp. (Keruing), Agrostitachys spp. (Jenjulong), Pandanus spp. (Pandan), Calameae spp. (Rotan), and Eugeissona spp. (Bertam).

![Figure 1. Map of Ayer Hitam Forest Reserve, Puchong, Selangor](source: [7].)

Three habitats were selected in this study: walking trail, stream, and swampy. Each habitat was surveyed twice a week, for five months. Active sampling were conducted at night for three hours (20:00h-23:00h) with the involvement of six man-effort. The frog sampling was carried out along trail and stream for a distance of 300 m transect line while random method was used for swampy area. The handheld torchlight and head lamps were used during the activity. Sample captured by bare hands and placed in the plastic container with holes on the lid. Each location of the species sampled was marked by using Garmin GPSMAP60CSx.
The containers were labelled and informations such as microhabitat and microclimate were also recorded. The parameters measured in microclimate were temperature, relative humidity, soil pH, and soil moisture by using Extech Meter for Humidity, Temperature, Airflow, and Light Level 45179 and pH soil and Moisture Tester Takemura DM-15. Samples were brought back to the laboratory for further identification. Identifications by referring to Berry (1975) and [20]. Measurements such as snout-vent length (SVL) and tibia length (TL) were obtained by using vernier caliper while the weight was measured by using spring balance. The photograph of each samples were taken by using a compact camera which included the dorsal and ventral sides. Samples that were identified were marked using paint mark (Neitfeld, Barrett, & Silvy, 1994; [18]) and released back in the original habitat.

2.2 Statistical analysis
Non-parametric comparison between habitats were determined by using Kruskal-Wallis in IBM SPSS Statistic Version 22. The Canonical Correspondance Analysis (CCA) and Species Accumulation Curve (SAC) were applied and both generated by using PAST software version 3.14 [6]. Diversity analysis was generated by using MVSP version 3.1.

3. Results
Eighteen species from five families were recorded with a total of 119 individuals in compartment 12 and 13 of Ayer Hitam Forest Reserve (Table 1). These included the family of Bufonidae (8.40%; n=10), (42%; n=50) from the family of Dicroglossidae, (14.29%; n=17) from the family of Megophryidae, the family of Microhylidae with (11.76%; n=14), and the family of Ranidae with (23.53%; n=28). The family Dicroglossidae was found most abundant with *Fejervarya cancrivora* (16%; n=19) followed by the family Ranidae with *Pulchrana laterimaculata* (9.2%; n=11) and *Leptobrachium hendricksoni* (9.2%; n=11).

While in three habitat types, stream (53.57%; n=60) was found to be most species recorded compared to trail (32.14%; n=36) and swampy (14.29%; n=16). Species recorded higher in the stream were *Ingerophrynus parvus* and *Leptobrachium hendricksoni* each with eight individuals respectively. Based on Kruskal-Wallis test, showed that there was statistically significant difference in species between these three habitats, $x^2 (2)=22.707$, $p= 0.00$.

**Table 1.** Species and relative abundance of anuran recorded at Ayer Hitam Forest Reserve Puchong, Selangor (numbers in brackets are in percentage).

| No. | Taxa                        | Common name               | Total | Habitat        |
|-----|-----------------------------|---------------------------|-------|----------------|
| Bufonidae                      |                            |                           |       |                |
| 1   | *Ingerophrynus parvus*      | Dwarf Stream Toad         | 10 (8.4)  | T, ST          |
| Dicroglossida                  |                            |                           |       |                |
| 2   | *Fejervarya cancrivora*     | Crab-eating Frog          | 19 (16.0) | T, ST          |
| 3   | *Fejervarya limnocharis*    | Asian Grass Frog          | 9 (7.6)   | T, ST, SW      |
| 4   | *Limnonectes ibanorum*      | Rough-backed Frog         | 7 (5.9)   | T, ST, SW      |
| 5   | *Limnonectes malesianus*    | Lesser Swamp Frog         | 4 (3.4)   | ST             |
| 6   | *Limnonectes plicatellus*   | Rhinoceros Frog           | 3 (2.5)   | ST             |
| 7   | *Occidozyga laevis*         | Puddle Frog               | 8 (6.7)   | ST             |
| Megophryidae                   |                            |                           |       |                |
| 8   | *Leptobrachium hendricksoni*| Spotted Litter Frog       | 11 (9.2)  | T, ST          |
| 9   | *Leptobrachium nigrops*     | Black-eyed Litter Frog    | 6 (5.0)   | T, ST, SW      |
| Microhylidae                   |                            |                           |       |                |
| 10  | *Kalophrynus palmatissimus* | Lowland Grainy Frog       | 9 (7.6)   | T, ST          |
| 11  | *Kalophrynus pleurostigma*  | Black-spotted Sticky Frog | 3 (2.5)   | T              |
| 12  | *Microhyla berdmorei*       | Berdmore’s Narrow-mouthed Frog | 1 (0.8) | ST             |
Table 2. Diversity indices of anuran between two sites

| No. | Species                     | Description                         | Count (Diversity) | Location |
|-----|-----------------------------|-------------------------------------|-------------------|----------|
| 13  | Microhyla mantheyi          | Manthey’s Narrow-mouthed Frog       | 1 (0.8)           | SW       |
| 14  | Amnirana nicobariensis      | Nicobar Frog                        | 3 (2.5)           | ST       |
| 15  | Chalcorana labialis         | White-lipped Frog                   | 10 (8.4)          | T, ST, SW|
| 16  | Pulchrana glandulosa        | Rough-sided Frog                    | 2 (1.7)           | T, ST    |
| 17  | Pulchrana laterimaculata    | Side-spotted Swamp Frog             | 11 (9.2)          | SW       |
| 18  | Pulchrana baramica          | Brown Marsh Frog                    | 2 (1.7)           | SW       |

**Total species**: 18  
**Total number**: 119 (100)

Note: T= trail, ST= stream, SW= swampy

Species accumulation curve shown in Figure 2 explained that the pattern between species diversity were almost the same in both compartment. The curve was increasing and begins to decreasing slowly as more rare taxa were recorded [6]. However, no asymptote was obtained in the species accumulation curve which means the sampling did not define the true species diversity in the habitat [21]. The Shannon-Wiener Diversity Index (H) shows that compartment 12 has highest species diversity compared to compartment 13 with 2.658 and 2.108 respectively. This may due to the absence of swampy in Compartment 13 which reduce the number of individuals recorded. The Evenness (E) also shows the community in compartment 12 was spread evenly among species compared to compartment 13 with 0.938 and 0.879 respectively. Based on Table 2, low Simpson’s Dominance Index (D), was identified in Compartment 13 with 0.08. In contrast, species dominance, D, was detected at 0.15 with the presence of Fejervarya cancrivora which recorded 29% of total anuran species in Compartment 13. An independent sample t-test conducted to compare the species recorded between Compartment 12 and 13 shows that there was a significant difference, t(54)=10.09, p=0.00.

![Species accumulation curve (SAC) of anuran in (a) Compartment 12 and (b) Compartment 13 in Ayer Hitam Forest Reserve, Puchong, Selangor.](image)

**Figure 2.** Species accumulation curve (SAC) of anuran in (a) Compartment 12 and (b) Compartment 13 in Ayer Hitam Forest Reserve, Puchong, Selangor.
Table 2. Diversity indices of anuran between two sites.

| Site                                      | Compartment 12 | Compartment 13 |
|-------------------------------------------|----------------|----------------|
| Shannon-Wiener Index, $H'$                | 2.658          | 2.108          |
| Evenness Index, $E$                       | 0.8391         | 0.7482         |
| Simpson Dominance Index, $D$              | 0.08           | 0.15           |
| $1/D$                                     | 0.92           | 0.85           |

Table 3. Mean±SD of environmental variables within habitats in Ayer Hitam Forest Reserve.

| Microclimate          | Trail          | Stream         | Swampy         | Trail          | Stream         |
|-----------------------|----------------|----------------|----------------|----------------|----------------|
| Temperature (°C)      | 25.14±0.58     | 26.86±1.39     | 25.86±0.89     | 26.30±1.35     | 25.81±0.68     |
| Relative Humidity (%) | 70.05±7.94     | 78.98±3.36     | 78.88±2.82     | 84.18±3.50     | 84.18±5.43     |
| Soil pH               | 2.97±2.30      | 4.64±2.36      | 6.37±0.60      | 6.44±1.17      | 3.20±2.52      |
| Soil moisture         | 5.65±1.07      | 4.92±1.09      | 4.02±0.06      | 4.13±0.53      | 5.65±1.00      |

There was considerable variation in microclimate attributes within habitats (Table 3, Figure 3, 4, 5, 6, and 7). For example, the average of temperature (°C) in Compartment 12 and 13 were between 25.14°C to 26.30°C, relative humidity (%) ranged from 70.05% to 84.18%, soil pH ranged from 2.97 to 6.44, and soil moisture ranged from 4.02 to 5.65.
Figure 3. Histograms showing variations of average in microclimate variables measured at three sampling trails in Compartment 12.
Figure 4. Histograms showing variations of average in microclimate variables measured at three sampling streams in Compartment 12.
Figure 5. Histograms showing variations of average in microclimate variables measured at three sampling swampy in Compartment 12.
Figure 6. Histograms showing variations of average in microclimate variables measured at three sampling trails in Compartment 13.
Figure 7. Histograms showing variations of average in microclimate variables measured at three sampling streams in Compartment 13.
Canonical Correspondance Analysis (CCA) was conducted to investigate the relationship between anuran species with the environmental variables and habitats (Figure 8 and Figure 9). In Compartment 12, *Leptobrachium nigrops* was positively correlated towards relative humidity and soil pH. *Kalophrynus palmatissimus* and *Pulchrana glandulosa* prefers higher soil moisture. *Chalcorana labialis* shows correlation with temperature and relative humidity. *Limnonectes malesianus, Annirana nicobariensis, Limnonectes plicatellus*, and *Fejervarya limnocharis* were associated with temperature. While in Compartment 13, *Fejervarya cancrivora* was positively correlated towards soil pH and temperature. *Leptobrachium nigrops* was associated to soil moisture and relative humidity. Lastly, *Kalophrynus palmatissimus* stands away from the associations.

![Figure 8](image.png)

**Figure 8.** Canonical Correspondence Analysis (CCA) of 17 anuran species with four environmental variables and habitats recorded in Compartment 12.
Fig 9. Canonical Correspondence Analysis (CCA) of 11 anuran species with four environmental variables and habitats recorded in Compartment 13.

Of the total anurans were sampled in the compartments, three species were identified as new records in AHFR, namely *Amnirana nicobariensis* (Ranidae), *Kalophrynus pleurostigma* (Microhylidae), and *Pulchrana laterimaculata* (Ranidae). The updated checklist of anuran in AHFR is represented in Table 4. Based on the checklist shown in Table 3, AHFR now hosts to 35 anuran species which covers 31.5% of anuran species recorded in Peninsular Malaysia. In addition, few species recorded from previous research by [17] were sampled during this study namely *Occidozyga laevis* (Dicroglossidae), *Limnonectes plicatellus* (Dicroglossidae), and *Ingerophrynus parvus* (Bufonidae).
Table 4. Updated checklist of anuran in Ayer Hitam Forest Reserve, Puchong, Selangor.

| No | Taxa                     | IUCN Status    | Along Rasau River [17] | Comp 15 [18] | This study, Comp 12 and 13 |
|----|--------------------------|----------------|-------------------------|-------------|---------------------------|
|    |                          |                | 1975        | 1979          | 1981          | 1984        | 1998          | 1999          | 2015          | 2016          |
| 1  | *Duttaphrynus melanostictus* | Least Concern  |             |              |              |              |             |              |              |              |
| 2  | *Ingerophrynus divergens*  | Least Concern  |              |              |              |              |             |              |              |              |
| 3  | *Ingerophrynus parvus*     | Least Concern  |              |              |              |              |             |              |              |              |
| 4  | *Ingerophrynus quadriporcatus* | Least Concern | 1           |              |              |              |             |              |              |              |
| 5  | *Phrynoidis aspera*        | Least Concern  |              |              |              |              |             |              |              |              |

**Bufonidae**

| 6  | *Fejervarya cancrivora*   | Least Concern  | 3           |              |              |              |             |              |              |              |
| 7  | *Fejervarya limnocharis*  | Least Concern  | 1           |              |              |              |             |              |              |              |
| 8  | *Fejervarya sp.*          |                | 1           |              |              |              |             |              |              |              |
| 9  | *Limnonectes blythii*     | Near Threatened| 1           | 1            |              |              |             |              |              |              |
| 10 | *Limnonectes leporinus*   | Least Concern  |              |              |              |              |             |              |              |              |
| 11 | *Limnonectes malesianus*  | Near Threatened| 1           | 1            |              |              |             |              |              |              |
| 12 | *Limnonectes paramacrodon*| Near Threatened|              |              |              |              |             |              |              |              |
| 13 | *Limnonectes ibanorum*    | Near Threatened|              |              |              |              |             |              |              |              |
| 14 | *Limnonectes doriae*      | Least Concern  |              |              |              |              |             |              |              |              |
| 15 | *Limnonectes plicatellus* | Least Concern  |              |              |              |              |             |              |              |              |
| 16 | *Occidozyga laevis*       | Least Concern  |              |              |              |              |             |              |              |              |
| 17 | *Occidozya lima*          | Least Concern  |              |              |              |              |             |              |              |              |

**Dicroglossidae**

| 18 | *Leptobrachium hasseltii* | Least Concern  |              | 1            |              |              |             |              |              |              |
| 19 | *Leptobrachium hendricksoni* | Least Concern |              | 1            |              |              |             |              |              |              |
| 20 | *Leptobrachium nigrops*   | Least Concern  |              | 3            |              |              |             |              |              |              |
| 21 | *Leptotalax sp.*          |                |              |              |              |              |             |              |              |              |

(continued)
| Microhylidae                  |                     |   |   |   |   |   |
|------------------------------|---------------------|---|---|---|---|---|
| 22 Kalophrynus palmatissimus | Endangered          | 1 | 2 | 9 |   |   |
| 23 Kalophrynus pleurostigma  | Least Concern       |   |   | 3 |   |   |
| 24 Microhyla berdmorei       | Least Concern       | 3 | 1 |   |   |   |
| 25 Microhyla mantheyi        | Least Concern       | 2 | 1 |   |   |   |
| Ranidae                      |                     |   |   |   |   |   |
| 26 Amnirana nicobariensis   | Least Concern       |   |   |   |   | 3 |
| 27 Hylarana chalconota      | Least Concern       | 1 | 1 |   |   |   |
| 28 Hylarana erythraea       | Least Concern       |   | 1 |   |   |   |
| 29 Hylarana labialis        | Least Concern       |   | 2 | 10|   |   |
| 30 Hylarana raniceps        | Least Concern       |   | 4 |   |   |   |
| 31 Hylarana signata         | Least Concern       | 1 | 1 |   |   |   |
| 32 Odorrana hosii           | Least Concern       |   | 1 |   |   |   |
| 33 Pulchrana baramica       | Least Concern       | 2 | 2 |   |   |   |
| 34 Pulchrana glandulosa     | Least Concern       | 1 | 2 |   |   |   |
| 35 Pulchrana laterimaculata | Least Concern       |   | 11|   |   |   |
| Total species               |                     | 1 | 1 | 4 | 3 | 7 |
| Total individuals           |                     | 1 | 1 | 4 | 3 | 7 |

Total individuals: 119
4. DISCUSSION

The most abundant species of all recorded was *Fejervarya cancrivora*. This species can be found in each sub locations of trails and streams of the compartments. The species is the only living amphibian that can adapted to any environment and widely different salinity [24]. This Crab-eating frog was first discovered in Compartment 15 by [18] but not documented during [17] along the Rasau River. In these area, the presence of clean water species such as *Chalcorana labialis* and *Pulchrana glandulosa* (Ibrahim et al., 2012a; 2012b) showed that the compartments were considered as low in anthropogenic disturbances and providing this species to a conducive habitat. *Pulchrana laterimaculata*, a member of family Ranidae is known as a species which predominantly an inhabitant of peat or inland swampy forest (IUCN, 2014) was found dominating in the swampy area of the compartment.

Stream has the highest recorded species among the three habitats. Water is undoubtedly essential to amphibian abundance [22]. In this study, quite a number of *Leptobrachium hendricksoni* and *Ingerophrynus parvus* were using this habitat to breed. *Leptobrachium hendricksoni* were seen mating near a slow-moving water beside a fallen log on a litter while *Ingerophrynus parvus* were seen mating using a small puddle near the slowly moving water. This was reported by [16] while female of *Ingerophrynus parvus* will lay clutches of eggs in stagnant or slowly moving water of rocky pools alongside the small streams less than 10 m wide [14].

Higher species diversity in Compartment 12 compared to Compartment 13 may due to the absence of swampy area in Compartment 13. This in turn, reduces the number of species recorded during the survey by seven species. The species namely *Amnirana nicobariensis*, *Kalophrynus pleurostigma*, *Limnonectes malesianus*, *Limnonectes plicatellus*, *Microhyla mantheyi*, *Pulchrana glandulosa*, and *Pulchrana laterimaculata*. *Amnirana nicobariensis* were found usually perching on the leaf and tree branch. *Kalophrynus pleurostigma* often can be found camouflaged on the forest litter. Species such as *Limnonectes malesianus*, *Limnonectes plicatellus*, and *Pulchrana glandulosa* were recorded near the sandbank and forest litter while *Microhyla mantheyi* and *Pulchrana laterimaculata* were seen in the swampy habitat.

The CCA has shown that species such as *Limnonectes malesianus*, *Amnirana nicobariensis*, *Limnonectes plicatellus*, and *Fejervarya limnocharis* were highly associated to temperature concentrated within 25°C to 27°C in Compartment 12 suggesting that these species prefers to inhabit a habitat within this range of temperature. Except in Compartment 13, *Kalophrynus palmatissimus* was recorded the only species weakly correlated with the parameters. However, this forest-litter species was found quite a number in the forest litter and was hardly seen because of its ability to camouflage [18].

Several existing species were assessed under IUCN Red List of Threatened Species discovered Near Threatened (NT) species such as *Limnonectes ibanorum* and *Limnonectes malesianus* while *Kalophrynus palmatissimus* was classified as Endangered (EN) (IUCN, 2014).

5. CONCLUSION

Ayer Hitam Forest Reserve hosted to 18 number of anuran species and microclimate plays a role to the presence of anuran in a habitat. Besides, throughout the survey, several of new recorded species to the forest were also documented suggesting that more species recorded previously or new may still existed. The presence of clean water species showed the compartments are low in anthropogenic activity.

Increasing human activity in the forest may cause damage and awareness towards conserving our urban forest should be inserted. This fragmented forest offers many benefits ecologically to the surrounding. Thus, a policy should be designated to reassure that AHFR will be consistently be a forest reserve, education, and research centre for the next coming generations.

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