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Rapid Survey of Anuran Species in Baguio-Benguet Area and Isolation of their Fungal Symbionts

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Baguio City is one of the fastest-growing centers of urbanization in the Philippines. As part of the Cordillera Mountain Range, it is a biodiversity hotspot that is largely unexplored. This study is a preliminary investigation using visual encounter surveys of anuran species in various localities of the Baguio-Benguet area (BBA). These include Loakan Airport (LA), Camp John Hay (CJH), and Soroptimist Compound (SC) at the Military Cut Off, all in Baguio; Nangalisan, Tuba (NT), Benguet; and Master’s Garden (MG) at Puguis, La Trinidad, Benguet. We documented three Luzon-endemic species of frogs – namely, *Kaloula rigida* (Family Microhylidae), *Sanguirana luzonensis* (Family Ranidae), and *Limnonectes macrocephalus* (Family Dicroglossidae); one Philippine-endemic species, *Kaloula picta* (Family Microhylidae); and an invasive species, *Rhinella marina* (Family Bufonidae). The pathogenic fungi *Batrachochytrium dendrobatidis* was not detected in this study, but several cosmopolitan fungal species were isolated from the anuran integument – including *Penicillium* spp., *Cladosporium* sp., *Talaromyces* sp., *Mucor* sp., and *Rhodotorula* sp. The results of the study highlight the importance of regional surveys of urbanized areas in the country in aiding the conservation and preservation efforts for the remaining wildlife. Anthropogenic factors like logging and conversion of forested areas to commercial spaces, which lead to habitat degradation and catastrophic events, are still the leading threats to this understudied but presumed species-rich area. To date, this is the first update on anuran biodiversity in BBA after Inger’s study in 1954, and the first survey of fungi association with the amphibian integument in the Cordillera Administrative Region. Hence, further taxonomic and conservation research is recommended.

Keywords: biodiversity, conservation, Cordillera, frog, endemic, herpetofauna

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Little is known about the herpetofauna in Baguio and its vicinities. Recent efforts were mainly focused on central Cordillera (Diesmos et al. 2005) and in the north (Brown et al. 2012). The last published account of anuran sighting within Baguio was 60 years ago (Inger 1954). This study aids in updating species biodiversity assessments in the Cordillera that would contribute to the baseline information for conservation efforts in the BBA. Also, it promotes awareness and initiative to conduct herpetofaunal studies in one of the more poorly understood areas in Northern Luzon concerning amphibian and reptilian fauna (Brown et al. 2012).

In addition to a poor understanding of herpetofauna in the area, their role as host to microbes is also largely unknown. For instance, the pathogenic chytrid fungus B. dendrobatidis greatly affects frog populations on a global scale (Wake and Vredenburg 2008). It was confirmed that B. dendrobatidis is already present on Luzon (Smith et al. 2019). The possible occurrence of this pathogen, in addition to anthropogenic practices leading to habitat destruction especially in urban areas (e.g. logging and conversion of forest patches into commercial spaces), make the local anuran species vulnerable to the threat of extinction (Wake and Vredenburg 2008). Permission to collect was acquired from the authorities in each site of interest below (Figure 1). Visual encounter surveys were conducted in the afternoons and evenings during selected days and nights from November 2015 to February 2016. To avoid cross-contamination, sterile procedures were observed as described by Phillott et al. (2010), while swabbing of the anurans to determine the fungal community followed that of Brem et al. (2007). Consequently, all anurans were safely released immediately after swabbing and photo-documentation.

Culture-dependent methods of fungal isolation were performed following the protocols described by Longcore (2000), Piotrowski et al. (2004), and Brem et al. (2007). In brief, each anuran integument was swabbed using sterile cotton tips then inoculated onto potato dextrose agar (PDA) plates supplemented with chloramphenicol. After incubation, fungal colonies were subcultured to PDA slants and potato dextrose broth for morphological characterization and DNA extraction (Liu et al. 2000). DNA extracts were subjected to amplification targeting the ITS region using ITS1 and ITS4 primers. Amplicons were sent to First BASE Laboratories (http://www.firstbaselab.com/) for sequencing. MEGA 6 was used in processing the sequences and initial fungal identification

Figure 1. Sampling sites in the BBA.
Table 1. Summary of anuran species encountered from all five collection sites.

<table>
<thead>
<tr>
<th>Anurans</th>
<th>CJH</th>
<th>SC</th>
<th>MG</th>
<th>LA</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinella marina a</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Limnonectes macrocephalus b</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaloula rigida b</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaloula picta (first locality record) c</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Sanguirana luzonensis (first locality record) c</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

a Invasive species; b Luzon-endemic; c Philippine-endemic

FAMILY BUFONIDAE

Rhinella marina (Linnaeus, 1758)
Common name: cane toad, marine toad

Rhinella marina (Figure 2A) was found at only two sites. In NT, numerous individuals were seen in flooded rice paddies. Several individuals were also seen in LA foraging on the runway.

Figure 2. Anuran species: (A) Rhinella marina, (B) Limnonectes macrocephalus, (C) Kaloula rigida, (D) Kaloula picta, and (E) Sanguirana luzonensis.
FAMILY DICROGLOSSIDAE

*Limnonectes macrocephalus* (Inger, 1954)
Common name: giant Philippine frog

A brown morph of *Limnonectes macrocephalus* (Figure 2B) was observed in CJH while a black morph was observed in MG.

**Figure 3.** Fungi from the anuran integument: (A–C) *Penicillium* spp., (D) *Cladosporium* sp., (E) *Talaromyces* sp., and (F) *Mucor* sp.

FAMILY MICROHYLIDAE

*Kaloula rigida* (Taylor, 1922)
Common name: Luzon narrow-mouthed frog

*Kaloula rigida* (Figure 2C) was originally documented in Baguio City as early as 1922 (Brown et al. 2013). In this study, this frog was recorded in SC, which is a partially disturbed habitat due to the presence of an establishment building with a man-made garden.

*Kaloula picta* (Duméril and Bibron, 1841)
Common name: painted narrowmouth frog

*Kaloula picta* (Figure 2D) was seen in LA foraging on the runway like *R. marina.*
FAMILY RANIDAE

*Sanguirana luzonensis* (Boulenger, 1896)
Common name: Luzon frog

*Sanguirana luzonensis* (Figure 2E) was observed inside a water tank near a stream behind the administration building of LA.

From the swabbed frogs, several genera of fungal symbions were isolated, mostly ascomycetes. The isolates were identified at the genus level – namely, *Penicillium* spp. (96-100%), *Cladosporium* sp. (90%), *Talaromycetes* sp. (100%), *Mucor* sp. (90%), and *Rhodotorula* sp. (94%) (Figure 3). Though the association of these fungi and anuran integument are still not established, most were cosmopolitan species and are generally present in the environment such as soil, air, and water, while some are endophytic in nature (Lanzafame 2001; Frisvad and Samson 2004; Stewart and Munday 2005; Zalar et al. 2007; Yilmaz et al. 2012).

Regional surveys are as important as major biogeographic surveys, particularly in highly threatened urbanized areas where species richness is still understudied. In this study, we have established that several Luzon- and Philippine-endemic species thrive in vulnerable areas and the threat of *B. dendrobatidis* was not observed locally. However, anthropogenic factors that lead to habitat degradation continue as threats to the survival of these endemic frog species, and biodiversity conservation remain as urgent imperatives.

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