ANURANS FROM IGUAZÚ NATIONAL PARK AND BUFFER AREA (ARGENTINA): REVIEW OF SPECIES LIST AND ECOLOGICAL NOTES ON THE LEAF-LITTER ASSEMBLAGES

ANUROS DEL PARQUE NACIONAL IGUAZÚ Y SU ÁREA DE AMORTIGUACIÓN: REVISIÓN DE LA LISTA DE ESPECIES Y NOTAS SOBRE SUS ENSAMBLES TERRESTRES

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Resumen.— En este aporte actualizamos la lista de especies de anfibios del Parque Nacional Iguazú y su área de amortiguamiento, en el noreste de Argentina, y evaluamos de manera complementaria la distribución temporal de los anuros terrestres que habitan en el parque. Muestreamos mensualmente ocho cuerpos de agua, y ocho trampas con cercas de deriva entre abril de 2013 y febrero de 2016. Complementamos nuestros datos con datos publicados e inéditos sobre los anuros. Registramos 27 especies en nuestras muestras, en las que tres especies son nuevos registros para el Parque Nacional Iguazú y la zona de amortiguamiento: Ololygon aromothyella, Melanophryniscus devincenzii y Lithobates catesbeianus. Registramos diez especies en el conjunto de anuros de hojarasca, siendo Physalaemus cuvieri y Leptodactylus elenae las especies más frecuentes, que representan el 63% de los individuos atrapados. A pesar de los cambios mensuales, la riqueza no varió estacionalmente, sin embargo, en los meses con las temperaturas más altas, encontramos una mayor riqueza de especies posiblemente relacionada a eventos estocásticos asociados a variables estacionales. Nuestros resultados confirman la importancia de la conservación del Parque Nacional Iguazú y de la provincia de mayor biodiversidad, Misiones, que alberga más del 50% de las especies de anuros de Argentina.

Palabras clave.— Anfibios, clima, temperatura, variación temporal.

Abstract.— In this contribution we update the amphibian species list from Iguazú National Park and the buffer area, Misiones Province in northeastern Argentina, and we evaluate the temporal distribution of leaf-litter anurans that inhabit the park. We sampled monthly eight water bodies and eight pitfall traps with drift-fences between April 2013 and February 2016. We supplement our data with published and unpublished data. We recorded 27 species in our samples, of which, three species are new records for Iguazú National Park and the buffer area: Ololygon aromothyella, Melanophryniscus devincenzii and Lithobates catesbeianus. We recorded ten species in the leaf-litter anuran assemblage, Physalaemus cuvieri and Leptodactylus elenae being the most frequent species, representing 63% of the individuals trapped. Despite monthly climate changes, richness did not vary seasonally; however, we found a higher species richness in months with the most elevated temperatures possibly related to stochastic events interacting with seasonal variables. Our results confirm the conservation importance of Iguazú National Park and the most biodiverse province, Misiones, which houses more than 50% of the anuran species of Argentina.

Keywords.— Amphibians, climate, temperature, temporal variation.
INTRODUCTION

Ecological communities are dynamic in space and time (Leibold & Chase, 2018). In anuran assemblages, this dynamism is associated with the high species diversity and variety of life histories (Wells, 2007). Biotic and abiotic factors influence spatial and temporal variations in communities. In general, anuran assemblages change over time due to climatic conditions (e.g. Ryan et al., 2014; Ryan et al., 2015). Temporal variation seems to be most influenced by temperature, and precipitation (Hartel et al., 2007; Garey & Silva, 2010; Bolzan et al., 2019), but also by pressure from interspecific interactions (e.g. Toft, 1985) and environmental changes (Bonecker et al., 2013). However, this information is based mainly on samples in water bodies used by anurans for reproduction, and data on leaf-litter anuran assemblages are more scarce (e.g., Rocha et al., 2013), with a precedent for northeastern Argentina (region that we sampled in the present study) in the work of Gangenova et al. (2018), where they compared anuran assemblages from pine plantations with those of the native forest, but they did not assess the community’s temporal variation.

We sampled the anurans of the INP and in northwest and east regions of the buffer area. Cabure-í (25.697986° S; 54.141343° W) is a town in the eastern part of the INP buffer area. There the local community develops agricultural activities. In the northwest buffer area - known as "600 Hectareas" (25.615479° S; 54.55806° W) - the activity is mainly tourism.

We report the patterns of temporal variation in species richness, and composition of leaf-litter anuran assemblages in the INP. MATERIALS AND METHODS

Study Site

The Iguazú National Park (25.690758° S; 54.477886° W) is known worldwide for Iguazú Falls, which is one of the main tourist centers of the country. Associated with the Iguazú River, on the border with Brazil, the INP protects 67,620 hectares of Atlantic Rain Forest, which is currently reduced to approximately 13% of its original extension and is the largest remaining forest of that landscape (Ribeiro et al., 2009). The INP is under the influence of a warm and humid subtropical climate, with an average annual rainfall of 2000 mm, without a dry season. However, there is usually more rain in spring and late autumn. The average annual relative air humidity is 78.6%. INP is located in the southwestern foothills of the Brazilian basaltic shield, and its relief is undulating with heights between 200-350m asl. The streams’ hydric regimes are quite variable, depending on rainfall. The average annual temperature is 20.7 °C, with an average maximum of 31.8 °C and an average minimum of 10.7 °C. The basalt exposed to temperature and humidity results in the characteristic red soil -altisols- of this region (SIB, 2017; Oyarzabal et al., 2018). The INP has the most diverse flora of Argentina with high structural complexity (Sru, et al., 2009).

Due to the extreme importance of the Iguazú National Park region for the conservation of the biodiversity, including amphibians, and the existing unconsolidated data on the anuran fauna, we now provide an update of the amphibian species list from INP and the surrounding buffer area. In addition, we report the patterns of temporal variation in species richness, and composition of leaf-litter anuran assemblages in the INP.
Inventory and monitoring

We applied different methods to obtain a more complete inventory of anurans, including primary and secondary data collection. For primary data, we use the visual encounter survey during night and day (Crump & Scott, 1994), the acoustic survey of breeding sites (Scott & Woodward, 1994), and passive sampling, specifically for the study of leaf-litter assemblies, using eight pitfalls traps with drift-fences (Corn, 1994). These traps were numbered from one to eight. We sampled eight environments associated with water bodies (pitfall traps numbered 1, 3, 5 and 8 - as part of the study of the leaf-litter community-, La Cantera, Apepú, Old Airport and INP roadides). In these, eight sites sampling effort was between 1 to 9 hours/researcher per site. Servicing pitfall traps 1, 3, 5 and 8 required 5 hours/researcher, and sampling the La Cantera, Old Airport and INP roadsides 8 to 27 hours/researcher of total effort. In Apepú sampling effort was 59 hours/researcher at the site.

To sample leaf-litter anuran assemblages, we used eight pitfall traps with drift-fences (PT) (Table 1), each one with four buried plastic 80-liter buckets joined together by plastic net drift fences 5½ meters long and ½ meter tall (drift-fences) in a Y-shaped design (Corn, 1994), or a linear design where vegetation did not allow the Y-design. Despite the difference in shape of the pitfall traps due to local conditions, we assume that each trap, in Y-shaped or linear design, has the same capture probability. During the sampling period, the pitfall traps were checked daily in the morning or afternoon, totaling 504 days/trap or 12,096 buckets/night between November 2013 and April 2015. PT were distributed in different landscape units of INP according to the surface drainage (fast drainage or flood-prone units), presence of a water body (lentic, lotic, or none) and vegetation type (native or exotic).

We used only visual encounter surveys in buffer areas. The samplings were carried out during the day and night. In the “600 Hectares” site, 13 daily and 12 nocturnal searches were made, which represented 52 hours/sampling. We sampled two water bodies, (i) a lagoon (25.603518° S; 54.548575° W), (ii) a flooded area in a low jungle environment (25.600175° S; 54.550586° W). In the western region, Cabure-i (25.680780° S; 54.556854° W), we spent eight days and six nights of fieldwork totaling 48 hours/ researcher searching for anurans in six family-owned production facilities (25.70668° S; 54.1415° W – 25.69912° S; 54.14017° W – 25.67773° S; 54.155513° W - 25.67729° S; 54.15141° W – 25.69129° S; 54.15282° W and 25.85747° S; 54.9754° W). Outside of the protected area, there is an abrupt change in the landscape, with the soil mainly dedicated to agriculture and livestock, eliminating the native vegetation, and replacing it with pasture. In this area, we found small strips of native vegetation protecting the banks of countless streams, and there are artificial lagoons of various dimensions that serve as water reservoirs for human consumption and livestock, which we sampled in search of anurans.

For each anuran captured, we took three photographs - dorsal, lateral, and ventral views - for the comparison of designs, coloring, and gland distribution (Hagström, 1973; Doody, 1995; Pereira & Maneyro, 2016). After data collection, the individuals were released no less than 10 meters from the pitfall trap. Since the collection of individuals was not allowed, the determination of the species trapped was made in the field or in the laboratory by analyzing the photographs of the individuals and the bibliography (keys, original descriptions, field guides). Weather data was provided by the National Weather Service (https://www.smn.gob.ar/). We obtained three climatic variables: monthly mean temperature, precipitation and air moisture.

| Variable       | PT1 | PT2 | PT3 | PT4 | PT5 | PT6 | PT7 | PT8 |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Drainage       | low | high| low | high| low | high| high| low |
| Vegetation     | native | native | native | native | native | exotic | native | exotic |
| Water body     | lentic | none | lentic | none | lentic | lotic | none | lentic |
| Latitude       | 25° 40.6' | 25° 40.4' | 25° 40.4' | 25° 39.2' | 25° 39.4' | 25° 41.4' | 25° 40.9' | 25° 41.1' |
| Longitude      | 54° 26.9' | 54° 26.9' | 54° 26.9' | 54° 27.6' | 54° 27.8' | 54° 27.5' | 54° 27.7' | 54° 28.2' |

Table 1. Main variables and position of the pitfall-traps used for the study of leaf-litter assemblages in the Iguazú National Park.
We obtained secondary data by searching for published articles using Google Scholar platform applying the terms "Iguazú National Park", "Amphibians" and "anurans" in both Spanish and English. Also, we search for technical reports of National Parks Administration staff to consolidate an amphibian species list from INP.

Statistical processing
To assess sampling efficiency of the leaf-litter anurans survey, we used sample-based rarefaction curves by randomizing the samples 1,000 times (Gotelli & Colwell, 2001) using Past 3.0 software (Hammer et al., 2001). We applied Circular Statistical analysis to assess whether there was seasonality in leaf-litter anuran capture using software Oriana 2.02 (Kovach, 2004). To evaluate which climate variables (i.e. mean temperature, precipitation and air moisture) explain the temporal variation of anuran richness, we used Generalized Linear Models (GLM) analysis using quasi-Poisson distribution due to data overdispersion. We standardize climate variables using the Z-score transformation. These analyzes were implemented in the R software (R Development Core Team, 2019).

RESULTS

Anuran species list.— We recorded 32 species of anurans based on primary and secondary data from the INP and surrounding buffer areas. The anurans belonged to 14 genera, in seven families: Hylidae (six genera, 15 species), Leptodactylidae (two genera, eight species), Bufonidae (two genera, four species), Odontophrynidae (two genera, two species), and Alsodidae, Centrolenidae and Ranidae with one species each (Table 2, Figs. 1 and 2). We recorded 27 species from our sampling efforts, including three species that had not been found before in this area. The secondary data provided five species that we did not record.

Leaf-litter anuran assemblage. In the eight PT we recorded 373 individuals from ten species, only two recaptures. According to the rarefaction curve, we found that the sampling was representative of the leaf-litter anuran assemblage (Fig. 3). The most frequent species were Physalaemus cuvieri Fitzinger, 1826 (n= 128, 34% of the individuals) and Leptodactylus elenae Heyer, 1978 (n= 111, 29% of the individuals) represented 63% of the sampling the other abundant species recorded were Rhinella ornata (Spix, 1824) (n= 54), Leptodactylus mystacinus (Burmeister, 1861) (n= 28), Proceratophrys avelinoi Mercadal de Barrio and Barrio, 1993 (n= 22) and Elachistocleis bicolor (Guérin-Méneville, 1838) (n= 19). P. cuvieri, L. elenae, P. avelinoi and L. mystacinus were recorded in most of the PT, with records in 90% of the PT for the first two species and 60% of the PT for the last two. The PT captured between 20-80% of the total leaf litter species recorded in the study, species trapped ranges from PT1 (eight species, n= 81) to PT7 (two species, n= 5) (Table 2).

Although the highest species richness was recorded in the hottest and wettest period, the temporal variation did not show a well-marked seasonal pattern (Rayleigh Test Z= 0.585; P=0.56); September was the month with the highest number of registered species (six species), while May was the month with the lower number of recorded species (1 species) (Fig. 4). Moreover, the absence of seasonality, temporal variation in species richness was marginally positively associated with temperature, in months with a higher temperature, higher species richness was recorded (Table 3, Fig. 5).

DISCUSSION

The current number of 32 anuran species of Iguazú National Park and surrounding buffer area is remarkable and represents approximately 52% of the species of anurans from Misiones Province, and 18% of the anurans from Argentina (Vaira et al., 2012; Cardoso & Pereyra, 2018, Baldo et al., 2019). Research in other protected areas of Misiones reported 15 species of anurans 8 km apart from INP (López & Kubisch, 2008), 21 species 110 km apart from INP (López & Nazer, 2009), 20 species 45 km apart from INP (Lescano et al., 2013) and recently Gangenova et al., (2018) reported 18 species in the native forest of the INP and the Uruguaí Provincial Park –adjacent to INP. Our results confirm the importance of the INP in the conservation of the anurans.

Our species list resolved some differences among previous anurans species list of INP (Montanelli & Acosta (1991) and unpublished technical reports). Our results added two native species to the INP (Ololygon aromothyella and Melanophryniscus devincenzii): besides, we verified the presence of 16 species in the surrounding buffer area, one of which is an exotic invasive: Lithobates catesbeianus (Lowe et al., 2000; Dassak et al., 2003). Ololygon aromothyella is distributed, according to the original description, only in the center of the province of Misiones, in two localities in Uruguay, two localities in Brazil, and it is probably present in Paraguay (Frost, 2020). Our record is the northernmost for the O. aromothyella in Argentina. One of the authors (CAL) obtained records of the species in the margins of National Route 19 (25, 856274° S; 54, 167898° W) within the Uruguaí Provincial Park (unpublished data). O. aromothyella has been categorized as Not Threatened for Argentina (Vaira et al., 2012), while at the global level, it is classified as Data Deficient (Stuart, 2006). Melanophryniscus devincenzii is distributed in
Table 2. Amphibian species registered in the Iguazú National Park in pitfall-traps (PT) and the literature review. Sources: a = Montanelli & Acosta, 1991, b= Bosso & Céspedes, 1994, c= Gangenova & Guzmán, 2011, d= Apepú, 2013, e= López & Grassi, 2019, D= disturbed edges of access roads, O= old airport, A= Apepú, Ct= Cantera, *= buffer area, X= outside the pitfall-traps.

| Species                                    | PT1 | PT2 | PT3 | PT4 | PT5 | PT6 | PT7 | PT8 | Site          | Reference     |
|--------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|---------------|---------------|
| *Bufonidae*                                |     |     |     |     |     |     |     |     |               |               |
| *Melanophryniscus devincenzii* (Klappenbach, 1868) | 2   |     |     |     |     |     |     |     | D, O          | Present study |
| *Rhinella ornata* (Spix, 1824)              | 15  | 10  | 10  | 5   | 9   | 5   |     |     | a, b          | Present study |
| *Rhinella diptycha* (Cope, 1862)            |     |     |     |     |     |     |     |     | A, D, O, *    | a, b, d        |
| *Rhinella icterica* (Spix, 1824)            |     |     |     |     |     |     |     |     | *             | Present study |
| *Hylidae*                                  |     |     |     |     |     |     |     |     |               |               |
| *Boana albopunctata* (Spix, 1824)           |     |     |     |     |     |     |     |     | A, Ct         | a, b, c        |
| *Boana cainguia* (Carrizo, 1991)            |     |     |     |     |     |     |     |     | Ct            | b, c          |
| *Boana faber* (Wied-Neuwied, 1821)          |     |     |     |     |     |     |     |     | x             | Ct, *          |
| *Boana raniceps* (Cope, 1862)               |     |     |     |     |     |     |     |     | D, O, *       | c, Present study|
| *Dendropsophus minutus* (Peters, 1872)       |     |     |     |     |     |     |     |     | A, *          | a, b, c, d     |
| *Dendropsophus nanus* (Boulenger, 1889)      |     |     |     |     |     |     |     |     | A, *          | a, b, c, d     |
| *Dendropsophus sanborni* (Schmidt, 1944)     |     |     |     |     |     |     |     |     | D, O, *       | c, Present study|
| *Itapotihyla langsdorffi* (Duméril and Bibron, 1841) |     |     |     |     |     |     |     |     | x             | A, Ct, D, O    |
| *Scinax fuscovarius* (Lutz, 1925)           |     |     |     |     |     |     |     |     | A, D, O, *    | a, b, c, d     |
| *Scinax nasicus* (Cope, 1862)               |     |     |     |     |     |     |     |     | *             | c, Present study|
| *Scinax perereca* Pombal, Haddad, and Kasahara, 1995 |     |     |     |     |     |     |     |     |               | c             |
| *Scinax squalirostris* (Lutz, 1925)          |     |     |     |     |     |     |     |     |               | a, b, c        |
| *Ololygon aromothyella* (Faivovich, 2005)    |     |     |     |     |     |     |     |     |               | Present study  |
| *Ololygon berthae* (Barrio, 1962)           |     |     |     |     |     |     |     |     | A             | d             |
| *Trachycephalus typhonius* (Linnaeus, 1758)  |     |     |     |     |     |     |     |     | A             | a, b, c        |
| *Microhylidae*                              |     |     |     |     |     |     |     |     |               |               |
| *Elaeistoeleis bicolor* (Guérin-Méneville, 1838) | 11  | 1   | 2   | 1   | 3   |     |     |     | *            | b, c, Present study|
| *Leptodactylidae*                           |     |     |     |     |     |     |     |     |               |               |
| *Leptodactylus eilenae* Heyer, 1978          |     |     |     |     |     |     |     |     | 19            | 17, 36         |
| *Leptodactylus fuscus* (Schneider, 1799)     |     |     |     |     |     |     |     |     | A, D, O, *    | a, b, c, d     |
| *Leptodactylus luctator* (Steffen, 1815)     |     |     |     |     |     |     |     |     | 1             | 3, 1           |
| *Leptodactylus podicipinus* (Cope, 1862)     |     |     |     |     |     |     |     |     | A             | Present study  |
| *Leptodactylus mystacinus* (Burmeister, 1861) | 2   | 4   | 9   | 7   | 3   | 1   |     |     | A, b, c        |
| *Physalaemus gracilis* (Boulenger, 1883)     |     |     |     |     |     |     |     |     | 1             | *             |
| *Physalaemus cvieri* Fitzinger, 1826         |     |     |     |     |     |     |     |     | 28            | 27, 38         |
the southern (AmphibiaWeb, 2020) and the central areas of Misiones (López & Nazer, 2009), and in Corrientes Province of Argentina, with a few records in southern Brazil, Paraguay, and Uruguay (Frost, 2020). Although at the country level it is categorized as Not Threatened, at the global level it is listed as Endangered (Stuart, 2006). Two species previously listed for INP are not included in our species list. The species *Scinax x-signatus* reported by Montanelli & Acosta (1991) is not included in the list of amphibians in the INP, as it does not inhabit the country (AmphibiaWeb, 2019; Vaira et al., 2012). *Dermatonotus muelleri* is not included either, as the species should not be present in the province of Misiones (Vaira et al., 2012), and there are no vouchers in the Center for Subtropical Ecological Research (CIES) collection. Individuals assigned to *Odontophrynus americanus* require future studies, given the recent description of *O. reigi* distributed in the study area (Rosset et al., 2021).

We observed the absence of a well-marked seasonality in anurans richness, although not seasonal, temporal changes in species richness was positively associated with temperature variation. The positive relationship between richness and temperature has already been observed in other anuran assemblages in different biomes (e.g. Conte & Rossa-Feres, 2006; Garey & Silva, 2010; Bolzan et al., 2019). Anurans are ectothermic (Wells, 2007), so it is expected that higher the temperature in the northeast of Misiones might be exacerbated by an invasive wave that comes from southern Brazil, which seems to have already occurred in different areas at the east of the province of Misiones (Both et al., 2011).

We recorded the presence of an exotic species, *Lithobates catesbeianus*, in the surrounding area of INP. *L. catesbeianus* has already been recorded in other areas in Argentina (see López & Grassi, 2019); nonetheless, this is the first record of the species near the INP. *L. catesbeianus* can cause a reduction in anuran biodiversity in the sites it colonizes (Silva et al., 2011; Leivas et al., 2013; Laufer & Gobel, 2017). Moreover, this species was associated with *Batrachochytrium dendrobatidis* transmission (Daszak et al., 2003), a disease often lethal to anurans. In addition to this complex situation, the invasion by *L. catesbeianus* in the northeast of Misiones might be exacerbated by an invasive wave that comes from southern Brazil, which seems to have already occurred in different areas at the east of the province of Misiones (Both et al., 2011).

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Figura 1. Algunos anuros del Parque Nacional de Iguazú, Argentina.
A) Limnomedusa macroglossa; B) Melanophryniscus devincenzii; C) Rhinella dypticha; D) Rhinella icteric; E) Rhinella ornata; F) Scinax fuscovarius; G) Boana albopunctata; H) Boana caingua; I) Boana faber; J) Boana raniceps; K) Dendropsophus minutus; L) Dendropsophus nanus; M) Dendropsophus sanborni; N) Itapotihyla langsdorfi; Ñ) Ololygon aromothyella. Fotos: CAL.

Figure 1. Some anurans from Iguazú National Park, Argentina. A) Limnomedusa macroglossa; B) Melanophryniscus devincenzii; C) Rhinella dypticha; D) Rhinella icteric; E) Rhinella ornata; F) Scinax fuscovarius; G) Boana albopunctata; H) Boana caingua; I) Boana faber; J) Boana raniceps; K) Dendropsophus minutus; L) Dendropsophus nanus; M) Dendropsophus sanborni; N) Itapotihyla langsdorfi; Ñ) Ololygon aromothyella. Photos: CAL.
Figure 2. Some anurans from Iguazú National Park, Argentina. A) *Scinax nasica*; B) *Trachycephalus typhonius*; C) *Leptodactylus elenae*; D) *Leptodactylus fuscus*; E) *Leptodactylus luctator*; F) *Leptodactylus mystacinus*; G) *Leptodactylus podicipinus*; H) *Physalaemus cuvieri*; I) *Physalaemus gracilis*; J) *Elachistocleis bicolor*; K) *Odontophrynus americanus*; L) *Procetophrys avelinoi*. Photos: CAL.
there is an increase in the anuran activity, resulting in a higher probability of capture, especially in areas with seasonal climate (e.g. northeastern Argentina). We verified that some months of the hot-wet season held the highest species richness. However, this variation indicates a random pattern due to the absence of seasonal variation in species richness between the two seasons, hot-wet and cold-dry. The absence of a seasonal pattern is due to unexpected anuran activity in the winter. It may be associated with the weather during sampling events, where days of winter temperatures close to summer highs were associated with increased anuran activity, although in the last 20 years the average annual temperature of the province of Misiones was higher than the normal climatological reference value (https://www.smn.gob.ar/clima/anomalia), constituting more of a climatological trend than a climatological event. We concluded that the activity of the INP anuran assemblages throughout the study period would be temperature driven. However, the response of the anuran assemblage could be related to a stochastic combination of climatic variables and by the strongly seasonal presence of certain resources and conditions (e.g. photoperiod).

CONCLUSIONS

Our anuran species list is crucial because it unifies information that was fragmented in several sources and is essential for the elaboration of management and conservation plans in protected areas and their vicinity. Furthermore, we encourage studies with anurans in the areas near to the park where additional species are expected and for monitoring *L. catesbeianus* population. Despite Misiones being home to the highest anuran species richness in Argentina (Lopez & Prado, 2012; Vaira et al., 2012), there are still many areas that have not been inventoried, and we believe that many species have not yet been recorded. Finally, the application of basic biosecurity protocols during field work is suggested.

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