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# Multiscale endemism analysis for amphibians of Paraguay

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Although there are many studies that analyse and describe the distribution patterns of diverse organisms in South America at different scales, Paraguay has been poorly assessed from a biogeographic point of view. Some of the available contributions on the biogeography of Paraguay are based on different taxonomic groups, such as mammals, birds, reptiles, and plants, describing relationships between species and their habitats by using indices of similarity and cluster analysis. The main objective of this contribution is to identify areas of endemism based on the distribution of the 87 amphibian species known from Paraguay, and to compare the results with the three schemes of ecoregion proposed for the country. Eight areas of endemism were identified at different size of grids/scales, congruent with Dry Chaco, Atlantic Forest, Cerrado, Grasslands of Mesopotamia, Ñeembucú, and the Great American Chaco ecoregions.

*Keywords:* Anura, Areas of Endemism, Biogeography, Distribution data, NDM/VNDM, South America

## INTRODUCTION

There are several studies that analyse and describe the distribution of different organisms at different scales in South America (e.g. Cabrera & Yepes, 1960; Cabrera & Willink, 1973; Morrone, 2001; Diniz-Filho et al., 2006; Guedes et al., 2014; Xavier et al., 2014; Azevedo et al., 2016; Hoffmeister & Ferrari, 2016), however, two information gaps are still evident. Despite the known worldwide population declines in amphibians (Blaustein et al., 1994; Corns, 1994; Stuart et al., 2008), detailed distribution analyses of this group are scarce; and the distributional biodiversity patterns of Paraguay are still poorly known. In general, available biogeographical information for Paraguay is based on either studies that include the country as a part of a wider area (i.e. continental or regional analyses, for example; Lundberg et al., 1998; Leynaud & Bucher, 1999; Oakley et al., 2005; Cáceres, 2007; Werneck, 2011; Nascimiento et al., 2013; Giarla & Jansa, 2014; Silva et al., 2014; Arzamendia & Giraudo, 2015; Nori et al., 2015; Hoffmeister & Ferrari, 2016), or local and fragmentary studies based on different taxonomic groups (mammals: López-González, 2004; 2005; Stevens et al., 2007; Rumbo, 2010; birds: Hayes, 1995; reptiles: Bauer, 2014; Cacciali & Ubilla, 2016; and plants: Keel et al., 1993; Spichiger et al., 1995; Chernoff et al., 2004). These contributions are mostly focused on describing the relationships between species and their habitats using indices of similarity, cluster analysis and predefined areas. Despite the contribution on the distribution of amphibians' species in Paraguay provide by Weiler et al. (2013), no progress has been made in the direction of formal analyses of distributions. The need for detailed studies on the distribution of amphibians in Paraguay is urgent in order to develop efficient conservation policies, especially in the biomes affected by the advance of agricultural frontiers, as in the Chaco region.

The concept of "areas of endemism" is used in biogeography to refer to those geographic areas delimited by the congruence in the distributions of at least two taxa (Platnick, 1991). These areas describe particular characteristics of biodiversity (Grehan, 1993; Carvalho, 2011) and their identification constitutes an important tool for conservation and a fundamental step in the understanding of the evolutionary history of taxa (Casagranda & Grosso, 2013; Warren et al., 2014). Several methodologies have been proposed in the last years for the identification of areas of endemism (Morrone, 1994; 2014; Hausdorf, 2002; Dos Santos et al., 2008; Veech, 2014; Da Silva et al., 2015; Guerin et al., 2015; Oliveira et al., 2015; Vilhena & Antonelli, 2015), however Parsimony Analysis of Endemicity (PAE; Morrone, 1994) and Endemicity Analysis (EA; Szumik et al., 2002 and Szumik & Goloboff, 2004) are the most used (Da Silva & Oren, 1996; García-Barros et al., 2002; Nori et al., 2011; Aagesen et al.

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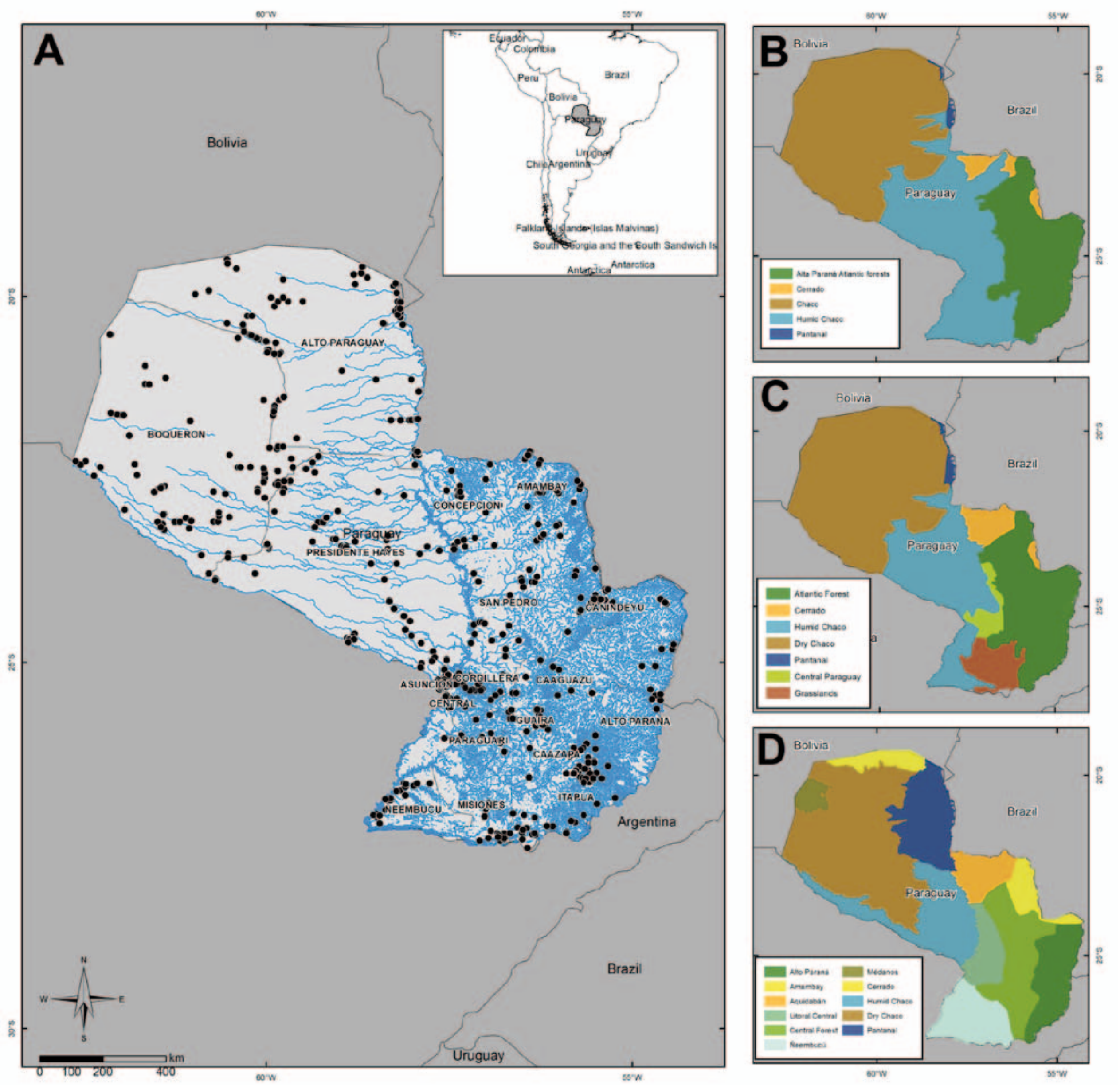


**Figure 1.** Reference Maps. **A)** Amphibian records used mapped on natural watercourses with political boundaries of Paraguay; **A-B-C)** Ecoregion schemes proposed for Paraguay by Dinerstein et al.(1995) **(B)**; del Castillo & Clay (2005) **(C)**; Secretaría del Ambiente (2013) **(D)**.

2012; Escalante, 2015; Cacciali & Ubilla, 2016; Andrade-Díaz et al., 2017). Different to other methods, EA has been exclusively developed for the identification of areas of endemism and shows advantages over other methods due to inclusion of spatial information in the searches (Casagranda et al., 2012).

Multiple definitions of "endemic" and "endemism" can be found in the literature, generating confusion and misunderstanding around the term (see Anderson, 1994). In the present study, we adopt the definition of Platnick (1991), considering an area of endemism as a geographic area defined by the congruent distribution of two or more taxa. Following this definition, a species will be considered as endemic when, together with other(s) species, it participates in the delimitation of an area of endemism. Since any species can contribute to the delimitation of areas of endemism at some geographic scale, no species were discarded from the analyses in the present study, even if its distributional range exceeded the area of study.

**Table 1.** Parameters used in NDM/VNDM during the search of areas of endemism and consensus areas

| | Grid Sizes | | |
|---|---|---|---|
| | 0.5°x0.5° | 0.7°x0.7° | 1°x1° |
| Fill | 70 | 30 | 10 |
| Assumed | 100 | 50 | 30 |
| Minimum species score | 0.5 | | |
| Sets with | 2 or more endemic species | | |
| Sets with score above | 2 | | |
| Random seed | 1 | | |
| Repeat search | 20 | | |
| Loose consensus rule | 40 % | | |

The main goals of this papers are: 1) to identify areas of endemism based on the distribution of the 87 amphibian species known from Paraguay; and 2) to compare them with three ecoregion schemes proposed for Paraguay: (a) Dinerstein et al. (1995); (b) del Castillo & Clay (2005) and (c) Secretaría del Ambiente (2013) (Fig. 1B–D) and; 3) to provide updated and complete information on the distributional range of the amphibian species in the country, covering spatial gaps initially observed in the data.

## METHODS

**Study site**
Paraguay is located in the centre of South America (Fig. 1A), occupying an area of 406,752 km$^2$. The Paraguay River divides the country in two main regions: the Oriental region and the Occidental region or Chaco, which covers more than the 60 % of the national territory. The Oriental region presents an average temperature of 23°C and 1200–800 mm of annual precipitation (ENPAB, 2016), while the Chaco shows a similar average temperature (25°C) but an annual precipitation of approximately 400–200 mm. Paraguay does not contain large orographic chains or high elevations, with the greatest altitude at the Cerro Peró (840 m.a.s.l).

**Data**
Our database included 4744 records of 87 anuran species (32 genera, 10 families) distributed across Paraguay (Supplementary Table). These data were obtained from the main museum collections in the country: the Museo Nacional de Historia Natural del Paraguay (MNHNP) and the Instituto de Investigación Biológica del Paraguay (IIBP), as well as data from recent publications (i.e. Brusquetti & Lavilla, 2006; Weiler et al., 2013; Caballero et al., 2014; Brouard et al., 2015; Lavilla et al., 2016). The distribution records were revised and corrected, with taxonomy updated following Pyron & Wiens (2011), Duellman et al. (2016) and Dubois (2017). Records of doubtful taxonomic identity and imprecise localities were discarded. Records including the description of collection localities, but lacking geographic coordinates, were georeferenced with the help of Google Earth and ArcGis 10.1. Finally, records of the same species for the same collection locality were deleted in order to obtain a matrix of unique records. Our final database included 2560 unique localities for 87 anuran species distributed in Paraguay (Fig. 1A).

**Areas of Endemism**
In order to identify areas of endemism, the distributional dataset was analysed with the software NDM/VNDM ver. 3 (Goloboff, 2004), which applies the optimality criteria described by Szumik et al. (2002) and Szumik & Goloboff (2004). Since geographic scale (grid size) influences pattern recognition (Casagranda et al., 2009; Szumik et al., 2012; Ocampo et al., 2019), in the present paper we analysed the data under three different grid sizes: 0.5° x 0.5°, 0.7° x 0.7° and 1° x 1°.

The information gaps in the distributions of species are mostly due to incomplete inventories (the Wallacean shortfall). To deal with data gaps in our matrix, we used the fill option available in VNDM. This function infers potential presences of a species in cells that are surrounded — within a certain radius— by cells where that species is observed. Values used for the fill function are detailed in Table 1. In the case of species where automatic fill function was not enough to cover important data gaps, a hand-made fill was made, guided by the distribution maps of amphibians published by the International Union for the Conservation of Nature (IUCN, 2016).

During the search of areas of endemism, VNDM calculates an Endemicity Index for each species (EIs) distributed within the set of the cells evaluated (areas). The IEs measures the congruence among the distribution of a species and the given area, and varies from 0 to 1; where the maximum value of 1 is assigned to a species distributed uniformly and exclusively in the evaluated area, that is, a perfect fit. The IEs value decreases as the distribution of the taxon increases outside of the area and/or its distribution inside the area is scattered. The Endemicity Index of an area of endemism (EIa) is equal to the sum of the IEs of the endemic species it contains. The search parameters used in the analyses are detailed in Table 1 (parameters keeping their default values are not included in the table).

Areas of endemism similar in spatial structure and/or species composition (fide Casagranda et al., 2012) were grouped in consensus areas (CAs, see Aagesen et al., 2013) to summarise the results obtained. Two rules have been proposed for the construction of CAs: the tight and loose consensus rules (more details in Aagesen et al., 2013). Both rules group the areas according to a percentage of shared species defined by the user; in this work we used a loose consensus rule of 40 % of similarity. The general patterns described here are based on CAs and are compared with three ecoregion schemes proposed for Paraguay: (a): Dinerstein et al. (1995); (b) del Castillo & Clay (2005); (c): Secretaria del Ambiente (2013) (Fig. 1B–D).

## RESULTS

**Identified Areas of Endemism**
The searches resulted in 17, 27 and 57 individual areas of endemism (IA) for the 0.5°, 0.7° and 1° grid sizes, respectively; that were grouped in 6, 10, and 17 CAs. The CAs obtained under different grid sizes are mostly congruent among them (Fig. 2). The size of grid in which each CA was identified is indicated by a subscript number. The EI values for each area, the endemic species and consensus values are detailed in Table 2.

**Grid 0.5° x 0.5°**
17 IAs were identified under this grid size and grouped into 6 CAs (Fig. 3A-B). The $CA0_{0.5}$ covers all the country (Fig. 3B), while $CA1_{0.5}$ is located in the south-eastern Oriental region, (Fig. 3A). $CA2_{0.5}$ and $CA4_{0.5}$ are found in the southern part of the country, defined by species characteristic of forested and open areas, respectively (Fig. 3A–B). $CA3_{0.5}$ covers the western part of the Occidental region. Finally, $CA5_{0.5}$ is located in the northern Oriental region (Fig. 3A).

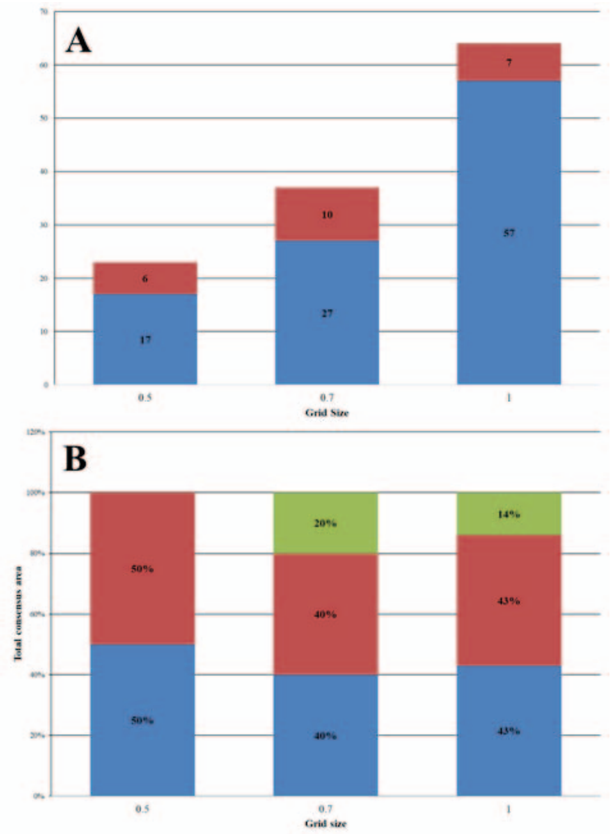


**Figure 2. A)** Total number of individual areas (IA, blue) and consensus areas (CA, red) recognised at different scales (0.5°x0.5°, 0.7°x0.7°, 1°x1°). **B)** Total number of CAs recognised at different scale of analyses (0.5°x0.5°, 0.7°x0.7°, 1°x1°). Different colours in the bars represent the percentage of CAs exclusively recognised under each scale (green); percentage of CAs recognised under two scales (red); and percentage of CA recognised under all scales of analyses (blue).

**Grid 0.7° x 0.7°**

With the grid size of 0.7° x 0.7°, 27 IAs were identified and grouped in 10 CAs (Fig. 3C-F). CA0$_{0.7}$ is defined by species associated with forest and is located in the south-eastern part of the Oriental region (Fig. 3E). CA1$_{0.7}$ and CA4$_{0.7}$contain species widely distributed in the country (Fig. 3F). The limits of CA2$_{0.7}$ coincide with the Oriental region and part of the Occidental region, with species related to open areas (Fig. 3C). CA5$_{0.7}$, located in the Occidental region (Fig. 3E); CA6$_{0.7}$ covers the entire Occidental and part of the Oriental region (Fig. 3D). CA7$_{0.7}$ is located in the northern Oriental region, with species characteristic of both forested and open areas (Fig. 3E). Finally, CA8$_{0.7}$ and CA9$_{0.7}$ are located in the south of the Oriental region, with species typical of forested and open areas (Fig. 3D–E).

**Grid 1° x 1°**

In this grid size, 57 IAs were identified and grouped in 7 CAs (Fig. 3G-I). CA0$_{1}$ covers all the country, with species of wide distribution that are associated with many different types of habitats (Fig. 3G); while the CA1$_{1}$ and CA2$_{1}$ are located in the centre of the country, with species associated mostly with open areas; however, some species related to forested areas are also present (Fig. 3H). CA3$_{1}$ is located in the north of the Oriental region, defined by both open and forested area species, while CA4$_{1}$ covers the entire Occidental region. CA5$_{1}$ is located in the south of the Oriental region, with species characteristic of forested and open areas (Fig. 3I). CA6$_{1}$ covers the entire Oriental region and the northern Occidental region (Fig. 3G).

**CAs compared to ecoregions**

Several CAs found are congruent with different ecoregions proposed for Paraguay. The Dry Chaco (sensu Dinerstein et al., 1995) was recovered by CA3$_{0.5}$, CA5$_{0.7}$ and CA4$_{1}$ (Fig. 4A); the Atlantic Forest (sensu Dinerstein et al., 1995) was recovered with CA1$_{0.5}$ and CA0$_{0.7}$ (Fig. 4B); and the Cerrado (sensu Dinerstein et al., 1995) was recovered by CA5$_{0.5}$, CA7$_{0.7}$ and CA3$_{1}$ (Fig. 4C). The Mesopotamian Grasslands (del Castillo & Clay, 2005) was recovered by CA2$_{0.5}$ and CA9$_{0.7}$ (Fig. 4G), while the Ñeembucú ecoregion (Secretaria del Ambiente, 2013) was recovered with CA4$_{0.5}$ and CA8$_{0.7}$ (Fig. 4F). Also, the Oriental region was recovered as an area of endemism by CA2$_{0.7}$ (Fig. 4E) and the Dry Chaco + Humid Chaco (sensu Dinerstein et al., 1995) was recovered as a single area in the CA6$_{0.7}$ (Fig. 4D). The species scores for each CA and the corresponding values are found in Table 2.

**Dry Chaco**

The Dry Chaco ecoregion (sensu Dinerstein et al., 1995) (Fig. 4A) is defined by taxa traditionally recognised as endemic to this ecoregion (Table 2), such as *Leptodactylus laticeps* (De Sá et al., 2014), *Chacophrys pierottii*, *Lepidobatrachus laevis* and *Lepidobatrachus llanensis*, and *Ceratophrys cranwelli* (Brusquetti & Lavilla, 2006; Faivovich et al., 2014). Also inhabiting part of this ecoregion are the species *Dermatonotus muelleri*, *Elachistocleis haroi*, *Leptodactylus bufonius*, *Rhinella major*, and *Odontophrynus lavillai*.

**Atlantic Forest**

Our results recover this ecoregion by the congruent distributions of *Melanophryniscus atroluteus*, *Melanophryniscuskrauczuki*,*Chthonerpetonindistinctum*, *Boana pulchella*, *Boana curupi*, *Crossodactylus schmidti*, *Itapotihyla langsdorffii*, *Argenteohyla siemersi*, *Phyllomedusa tetraploidea*, *Proceratophrys avelinoi*, and *Luetkenotyphlus brasiliensis* (Table 2). Almost all these species are associated with the Atlantic Forest (Fig. 4B) in Argentina, Brazil and Paraguay (Brusquetti & Lavilla, 2006; Brusquetti & Lavilla, 2008; Caldart et al., 2010; Motte et al., 2011), except for *Melanophryniscus devincenzii* that has a disjunct distribution, with populations in Uruguay separated from those in north-eastern Argentina and southern Paraguay (Maneyro & Kwet, 2008; Airaldi et al., 2008; Boeris et al., 2010), and *A. siemersi* with a unique record known from Paraguay (Villarrica, Department of Guairá; see Brusquetti & Lavilla, 2006), in the transition area among Atlantic Forest and Humid Chaco (sensu Dinerstein et al., 1995).

**Cerrado**

The Cerrado ecoregion (sensu Dinerstein et al., 1995) (Fig. 4C) is characterised in our analyses by *Physalaemus*

**Table 2.** Consensus areas in congruence with the ecoregions proposed for Paraguay. By column: Grid size of analysis (Grid), Consensus Areas ID (CA), Endemic Species, ID of the Individual Areas of Endemism included in the CA; Consensus Endemicity Value (CEV).

| Grid | CA | Species | AEI | CEV |
|---|---|---|---|---|
| **Dry Chaco ecoregion** (Dinerstein et al., 1995) | | | | |
| 0.5 | 3 | *Ceratophrys cranwelli, Chacophrys pierottii, Lepidobatrachus laevis, Leptodactylus laticeps* | 10 | 3.04-3.29 |
| 0.7 | 5 | *Ceratophrys cranwelli, Chacophrys pierottii, Lepidobatrachus laevis, Lepidobatrachus llanensis, Leptodactylus laticeps, Odontophrynus lavillai* | 8, 10, 15 | 2.82-3.32 |
| 1 | 4 | *Ceratophrys cranwelli, Chacophrys pierottii, Lepidobatrachus laevis, Lepidobatrachus llanensis, Leptodactylus laticeps, Leptodactylus bufonius, Odontophrynus lavillai, Elachistocleis haroi, Dermatonotus muelleri, Rhinella major, Phyllomedusa sauvagii* | 6, 13, 37, 39, 56 | 3.42-4.92 |
| **Atlantic Forest ecoregion** (Dinerstein et al., 1995) | | | | |
| 0.5 | | *Melanophryniscus atroluteus, Melanophryniscus krauczuki, Chthonerpeton indistinctum, Boana pulchella, Crossodactylus schmidti, Phyllomedusa tetraploidea* | 3, 8, 15, 17 | 2.95-3.45 |
| 0.7 | | *Melanophryniscus atroluteus, Melanophryniscus krauczuki, Melanophryniscus devincenzii, Rhinella ornata, Proceratophrys avelinoi, Luetkenotyphlus brasiliensis, Boana curupi, Itapotihyla langsdorffii, Crossodactylus schmidti, Argenteohyla siemersi, Phyllomedusa tetraploidea* | 0, 3, 11, 12, 21, 25 | 2.02-3.88 |
| **Cerrado ecoregion** (Dinerstein et al., 1995) | | | | |
| 0.5 | 5 | *Physalaemus centralis, Physalaemus marmoratus, Leptodactylus furnarius, Rhinella icterica, Dendropsophus jimi* | 14 | 3.71-3.96 |
| 0.7 | 7 | *Physalaemus centralis, Physalaemus marmoratus, Leptodactylus furnarius, Rhinella icterica, Rhinella scitula, Dendropsophus jimi* | 18 | 4.64-4.89 |
| 1 | 3 | *Physalaemus centralis, Physalaemus marmoratus, Leptodactylus furnarius, Rhinella icterica, Rhinella scitula, Dendropsophus jimi, Dendropsophus elianeae, Elachistocleis matogrosso, Siphonops paulensis* | 3, 25, 51, 52 | 2.93-5.24 |
| **Mesopotamian grasslands ecoregion** (Del Castillo & Clay, 2005) | | | | |
| 0.5 | 2 | *Melanophryniscus atroluteus, Melanophryniscus krauczuki, Chthonerpeton indistinctum, Boana pulchella, Crossodactylus schmidti, Phyllomedusa tetraploidea* | 7, 12, 13 | 2.25-3 |
| 0.7 | 9 | *Melanophryniscus atroluteus, Melanophryniscus krauczuki, Chthonerpeton indistinctum, Boana pulchella, Crossodactylus schmidti* | 22 | 4.29-4.54 |
| **Ñeembucu ecoregion** (Mereles et al., 2013; Secretaria del Ambiente, 2013) | | | | |
| 0.5 | 4 | *Pseudopaludicola mystacalis, Physalaemus santafecinus, Scinax similis* | 11 | 2.39-2.64 |
| 0.7 | 8 | *Pseudopaludicola mystacalis, Physalaemus santafecinus, Scinax similis* | 19 | 2.43-2.68 |
| **Oriental region** | | | | |
| 0.7 | 2 | *Boana albopunctata, Boana caingua, Boana faber, Dendropsophus minutus, Ololygon berthae, Proceratophrys avelinoi, Leptodactylus labyrinthicus, Rhinella ornata* | 2, 4, 9, 13, 17 | 2.21-3.71 |
| **Great American Chaco** (TNC et al., 2005) | | | | |
| 0.7 | 6 | *Physalaemus biligonigerus, Leptodactylus bufonius, Dermatonotus muelleri, Elachistocleis haroi, Rhinella major* | 16 | 4.05-4.30 |

*centralis*, *Physalaemus marmoratus*, *Lepodactylus furnarius*, *Rhinella scitula*, *Dendropsophus elianeae*, and *Dendropsophus jimi* all formerly described as endemic to the Cerrado (Table 2) (Napoli & Caramashi, 1999; Caramaschi & Niemeyer, 2003; Nascimento et al., 2006; Baldo et al., 2008; Vasconcelos et al., 2014; Loebmann et al., 2017). Other species occurring in this area are *Elachistocleis matogrosso*, associated with the Pantanal and the Cerrado (Caramashi, 2010; Brouard et al., 2015), and *Rhinella icterica*, which occurs both in Cerrado and Atlantic Forest (Brusquetti & Lavilla, 2006; Valdujo et al., 2012).

### Mesopotamian grasslands

The Mesopotamian grasslands ecoregion is defined in our results by the congruent distributions of *C. schmidti*, *P. tetraploidea* and *M. krauczuki*, species with distributions related to the Atlantic Forest (Table 2; Fig. 4G; Baldo & Basso, 2004; Brusquetti et al., 2007; Caldart et al., 2013). The fact that these species, predominantly distributed in the Atlantic Forest, score for an open formation ecoregion could be a consequence of poor sampling. In Paraguay, these three species are only known from only a single locality each, and all these localities are on the geographic boundaries of the Mesopotamian Grasslands (see Fig. 1C). Species of wider distribution such as *C. indistinctum* —an aquatic species associated with the Parana River system — (Brusquetti & Lavilla, 2006; Cajade, 2012), *B. pulchella* and *M. atroluteus* — which are associated with open areas in Brazil, Argentina and Paraguay— (Cei, 1980; Brusquetti & Lavilla, 2006) also contribute to define this ecoregion.

### Ñeembucú

*Scinax similis*, *Physalaemus santafecinus* and *Pseudopaludicola mystacalis* (Table 2) defined the Ñeembucú ecoregion (Secretaría del Ambiente, 2013; Fig. 4F), all three are species associated to open areas (Brusquetti & Lavilla, 2006; Brusquetti et al., 2009; Ingaramo et al., 2011).

### Oriental region

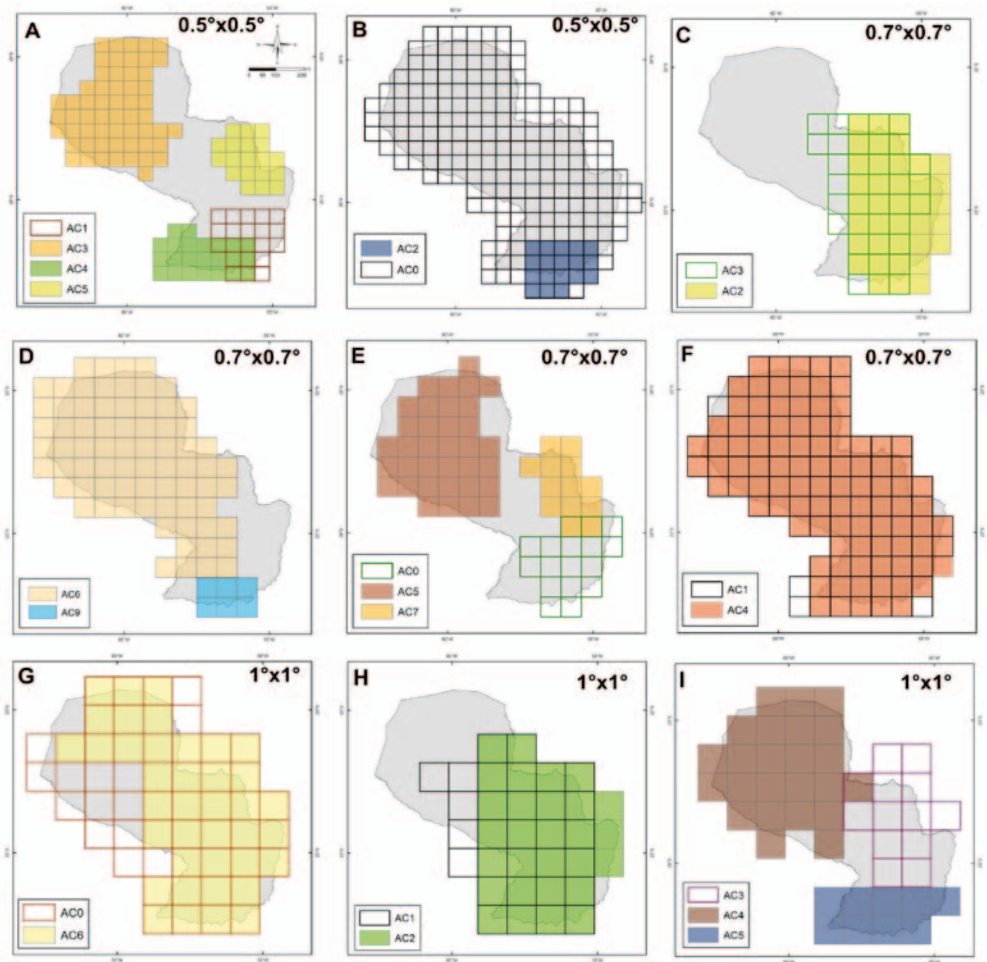


**Figure 3.** Consensus areas identified under different scales: **(A-B)** CAs identified when using grids of 0.5°x0.5°; **(C-F)** CAs identified when using grids of 0.7°x0.7°; **(G-I)** CAs identified when using grids of 1° x 1°

The Oriental region is extended from the eastern margin of the Paraguay River to the Rio Parana (Fig. 4E) and was defined by widely distributed species such as *Dendropsophus minutus*, *Boana albopunctata* and *Leptodactylus labyrinthicus* (Cei, 1980; Brusquetti & Lavilla, 2006; de Sá et al., 2014; Gehara et al., 2014) and species associated with the Atlantic Forest, such as *Boana caingua*, *B. faber*, *P. avelinoi* and *Rhinella ornata* (Table 2, Cei, 1980; Kwet & Faivovich, 2001; Baldissera et al., 2004; Brusquetti & Lavilla, 2006; Lavilla & Brusquetti, 2010). *Ololygon berthae* is also included as endemic to this area, a species characteristic of open areas and the Atlantic Forest in Argentina, Brazil, Uruguay and Paraguay (Lopez et al., 1999; Brusquetti & Lavilla, 2006; Duellman et al., 2016). The distributions of all these species reach the Paraguay River in the Oriental region, but none of them has been found in the Occidental region.

**Great American Chaco (Dry Chaco + Humid Chaco)**
The Dry Chaco + Humid Chaco (Great American Chaco sensu Dinerstein et al., 1995; Fig. 4D) is identified by typical Chacoan species like *E. haroi* and *L. bufonius* (Table 2) (Narvaes & Rodrigues, 2009; Caballero et al., 2014; de Sá et al., 2014; Pereyra et al., 2016). *Dermatonotus muelleri*, which has a wide distribution in Argentina, Bolivia, Brazil and Paraguay (Brusquetti & Lavilla, 2006) and is associated with the dry diagonal of open formations (Prado & Gibbs, 1993), *R. major* and *Physalaemus biligonigerus*, widely distributed in Argentina, Bolivia, Brazil and Paraguay (Brusquetti & Lavilla, 2006; De La Riva et al., 2000; Narvaes & Rodrigues, 2009), also contribute to define this CA.

## DISCUSSION

**Areas of endemism and ecoregions**
Our results indicate the existence of two large areas of endemism in Paraguay: the Dry Chaco (AC3$_{0.5}$, AC5$_{0.7}$, and AC4$_{1}$) and the Oriental region (AC2$_{0.7}$) (Figs 4A, E). The Dry Chaco was identified under all the grid sizes used (0.5°, 0.7°, 1°) and matches with the definitions of several authors (Dinerstein et al., 1995; Mereles et al., 2013). This area is characterised by species strongly linked with Chacoan environments such as *C. cranwelli*, *Ch. pierottii*, *L. laevis*, *L. llanensis*, *Le. laticeps* and *O. lavillai* (De la Riva et al., 2000; Brusquetti & Lavilla, 2006, Faivovich et al. 2014).

In the Oriental region five CAs, related to previously defined ecoregions, were identified for amphibians (Atlantic Forest, Cerrado, Ñeembucú, Mesopotamian grasslands and Oriental region), in contrast with previous studies that identified only two areas of endemism for birds: Campos Cerrados to the north and Paraná to the east (Cracraft, 1985; Hayes, 1995). The Campo Cerrado and Parana (Cracraft, 1985; Hayes, 1995) were also recovered in our analysis and are congruent with the Cerrado and

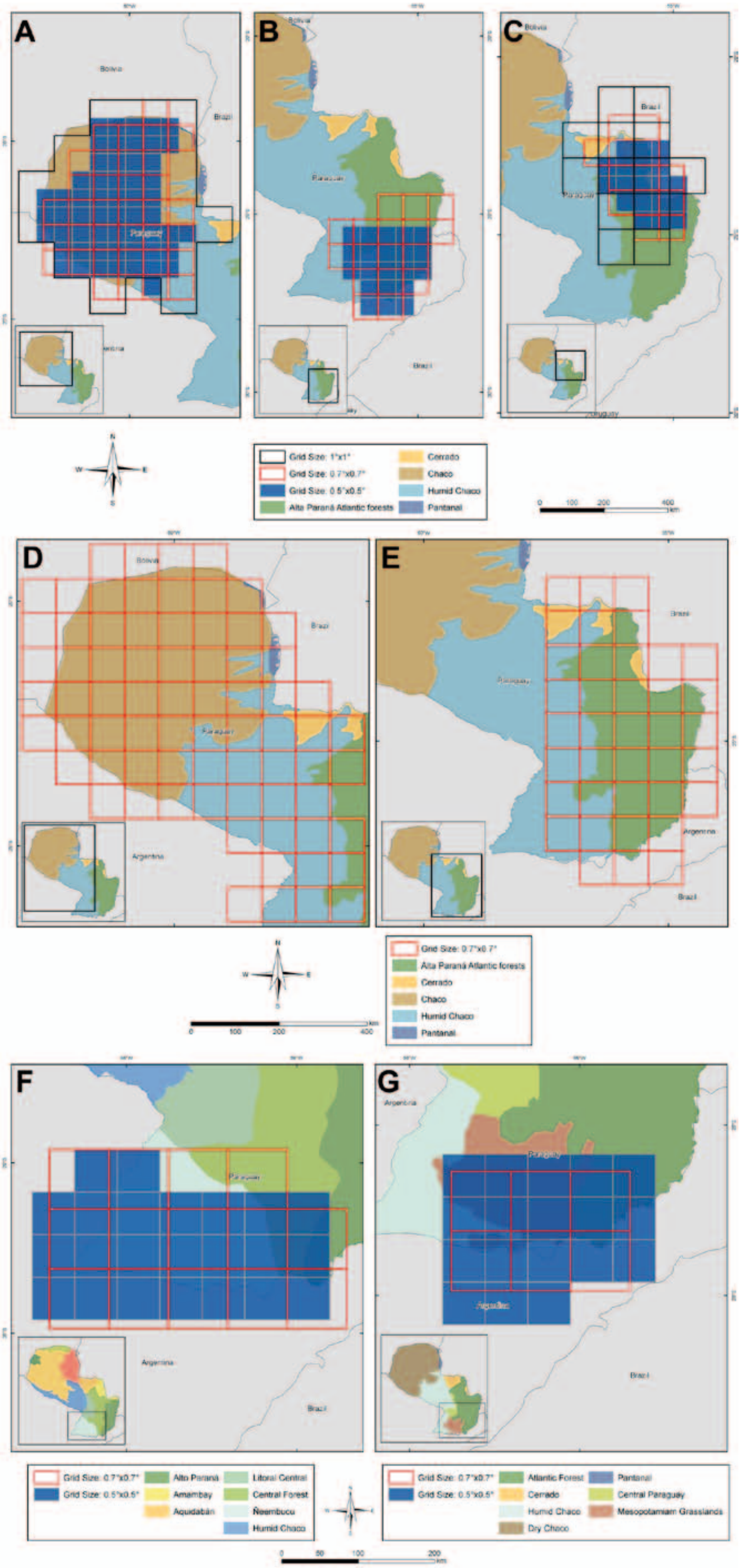


**Figure 4.** Consensus area identified in congruence with the ecoregions proposed by different authors. **(A-E)** Dinerstein et al. (1995). **A)** Dry Chaco; **B)** Atlantic Forest; and **C)** Cerrado; **D)** Great American Chaco (Dry Chaco + Humid Chaco); **E)** Oriental region no formal proposal as an ecoregion; **F)** Ñeembucú according to Secretaría del Ambiente (2013); and **G)** Mesopotamian grasslands according to del Castillo & Clay (2005).

Atlantic Forest ecoregions (Figs. 4C and B). The Cerrado is an ecoregion related to xerophyte environments of South America (Prado & Gibbs, 1993; Cacciali & Ubilla, 2016). In this work, we identify the Cerrado as an area independent from the Atlantic Forest, each one identified by unique and characteristic species, thus sustaining the identity of each area. This differs from what was found by Cacciali & Ubilla (2016) for reptiles, where the Atlantic Forest was recognised as an area of endemism with the Cerrado nested inside. However, these authors suggested that some sampling problems could have influenced on their results, like sampling concentration in specific localities and total absence of records in large areas.

The Paraguay River dates from the early Miocene (Potter, 1997) and it has been proposed as a physical barrier to the distribution of the species of *Thylamys* (Mammalia) (Giarla & Jansa, 2014). This was also observed by Piatti (2017) for different species of the genus *Xenodon*, which can be found on opposite sides of the river in natural areas with different biotic characteristics (e.g. *Xenodon pulcher* and *X. semicinctus* on the western side, and *X. histricus* and *X. dorbignyi* in the east). As stated by Myers (1982) the differences between both sides of the Paraguay River are attributed to distinct biotic characteristic, which determine different habitats on each side: forested humid habitats in the east and xerophytic and arid habitats in the west. In line with the observed for mammals and reptiles, major differences in the general composition of amphibian species can be observed between the eastern and western sides of the river. However, large areas along the river with similar habitats in both margins, present certain species characteristic of the Great American Chaco (Dry Chaco + Humid Chaco) occurring on both sides (see Souza et al., 2010; Sugai et al., 2013; Weiler et al., 2013; Brusquetti et al., 2018). This fact indicates that, although the river acts as a barrier for several taxa, its effectiveness differs among groups and among species. More studies are needed to better understand the role of the river as a barrier in the distribution of amphibians and to determine which factors make it more or less effective in limiting dispersal of different groups.

Our results also recover the Great American Chaco (Dry Chaco + Humid Chaco) as a single area of endemism, in concordance with those described by Szumik et al. (2012). Unlike the Dry Chaco, this area is defined by widespread species, which besides the Chaco also occurs in the Yungas (e.g., *E. haroi*), Cerrado and Caatinga (e.g., *D. muelleri*), and Amazonia (e.g., *R. major*). A similar area of endemism was also identified for birds by Hayes (1995) and for reptiles by Cacciali & Ubilla (2016).

**Different scales in the identification of areas of endemism**

As discussed by several authors, the use of different scales/grid sizes influences the search and identification of areas of endemism (Aagesen et al., 2009; Casagranda et al., 2009; Szumik et al., 2012). Our results show an increase in the number of areas of endemism identified when increasing the grid size (Fig. 2), furthermore, some areas of endemism are only identified when using a specific grid size (Table 2). The effects of the grid size on the identification of areas of endemism is especially relevant when analysing datasets with sampling gaps (Casagranda et al., 2009; Szumik et al., 2012), and species with discontinuous distributions, as is the case of some amphibians in Paraguay. The filling tools offered by NDM/VNDM helped to deal effectively with this problem, diminishing the impact of data incompleteness.

In their identification of areas of endemism for reptiles of Paraguay, Cacciali & Ubilla (2016) found only three areas of endemism when applying the Parsimony Analysis of Endemicity (PAE). These authors suggested that poor sampling efforts in some areas, together with intensive collection in urban centres and along access roads, would be the cause for the poor pattern recognition and questioned the validity of the results. Although similar sampling problems were observed in our amphibian dataset, the use of different grid sizes together with the manual filling tool allowed us to ameliorate the gap information problem, identifying more areas of endemism and characterising these better. A possible cause of the limited number of AEs identified by Cacciali & Ubilla (2016) - not explored by the authors- is their methodological choice. As discussed by several authors, PAE has shown to be very sensitive to incomplete sampling (a common problem in distributional databases; Arias et al., 2010), as well as a to have a poor performance when dealing with the identification of overlapping and disjunctive patterns, relatively common in nature (Casagranda et al., 2012; Szumik et al., 2018).

**Final considerations**

Our paper presents the first delimitation of areas of endemism in Paraguay based on amphibians. Most ecoregions previously proposed for Paraguay (such as Pantanal and Humid Chaco; Dinerstein et al., 1995; Mereles et al., 2013) show a high congruence with the CAs recovered in our analyses, however, the $CA2_{0.7}$ does not show correspondence with the ecoregions but represent an original distributional pattern, related to the transition zone between the Atlantic Forest and the Cerrado. This CA indicates a biotic cline area characterised for a mixture of species: from species adapted to more humid regions (e.g. *B. caingua*, *B. faber*, *O. berthae*, *P. avelinoi*, *R. ornata*) to species widely distributed that mainly inhabit much drier environments (e.g. *Le. labyrinthicus*, *B. albopunctata*).

Our results corroborate that classic ecoregions, qualitative defined on the base of flora, also represent natural patterns for amphibians. The application of a quantitative method delivered hypothesis of endemism feasible to be tested, as well as made available a list of endemic amphibian species for each area of endemism, facilitating future discussion of results. Quantitative studies, like the present, allow the replication of analyses, facilitating the discussion of hypotheses under the light of new evidence. In this sense, much is still to be done in biogeography of Paraguay and we hope this contribution will be a first step in this direction.

The description of areas of endemism for amphibians provides fundamental information to discuss the evolution of these taxa across time and space, and opens new questions about the incidence of ecological and historical factors on their distributional ranges. Understanding the processes involved in shaping the distribution of amphibians are important in a time where their existence seems to be threatened from multiple fronts (Scheele et al., 2019). Furthermore, about 50 % of the amphibian species of Paraguay are distributed in the Chaco, one of the most diverse biomes in South America (WWF, 2015), hosting a wide diversity among which about a quarter are endemic (Redford et al., 1990; Nori et al., 2016) and subjected to strong environmental pressures. In the last 10 years, the great American Chaco has reached the highest rate of deforestation in the world, with more than 1500 hectares of habitat destroyed every day (Hansen et al., 2013; Caballero et al., 2014). This ecoregion has been set as a priority for conservation of Neotropical terrestrial vertebrates (Loyola et al., 2009), that is why studies that resume and formalise the knowledge on the geographic distribution of the species inhabiting the Chaco become urgent as a first step to preserve these.

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