**SUPPORTING INFORMATION**

**Perspectives in Ecology and Conservation**

**How habitat loss and fragmentation are reducing conservation opportunities for vertebrates in the most threatened savanna of the World**

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**Appendix S2:** Extended methods

*Study area*

The Cerrado is the second largest South American phytogeographical domain, surpassed in extension only by Amazonia and occupying a central position in the Neotropical region, being dominated by upland savannas and grasslands (Ab’Sáber, 1998). It is bordered in the northwest by the Amazon and in the east by the Atlantic Forest, and forms the South American diagonal of open vegetation together with the Caatinga in the northeast and the Gran Chaco in the southwest. This savanna ecoregion is dominated by heterogeneous xeromorphic vegetation ranging from areas dominated by grasslands, with small shrubs (*campo limpo*), to areas formed by almost closed canopy woodland (*cerradão*; Eiten, 1972; Ratter *et al.* 1997). However, the Cerrado has been intensively modified by the conversion of its natural vegetation into croplands and planted pastures, which implies deforestation rates higher than the Amazon, coupled with less legal protection of its outstanding endemic biodiversity (Strassburg *et al.* 2017).

We adopted the limits of the Cerrado ecoregion as proposed by Dinerstein *et al.* (2017), which is an ecoregion approach initially based on the Cerrado limits of the Instituto Brasileiro de Geografia e Estatística (IBGE, 1993; see Olson *et al.* 2001). As we included a variable containing land use information, available mainly for the Brazilian territory (MapBiomas, 2022; see below), we retained the Brazilian portion of the ecoregion corresponding to 99.23% of the Cerrado, after removing its small portions in Paraguay and Bolivia.

*Species and occurrence records*

Our database is composed of 13,790 unique records of 337 Cerrado endemic terrestrial vertebrates, including 124 amphibian anurans, 66 lizards, 63 snakes, 45 birds and 39 mammals, with a mean of 2,758 distribution records per group (sd=2,097), and a mean of 41 records per species (sd=93). This is the most comprehensive database of geographic information on Cerrado endemic terrestrial vertebrates to date. These records are based on planned field surveys to cover previous sampling gaps and revision of vouchered specimens deposited in scientific collections (see details in Nogueira, Colli & Martins,2009; Valdujo *et al.* 2012; Nogueira *et al.* 2019; Carmignotto, Pardini & Vivo, 2022), complemented by revised literature data. We considered endemic species, those with ranges fully or largely coincident with the approximate limits of the Cerrado provided in Dinerstein *et al.* (2017). Species with marginal records in transitional areas between the Cerrado and other domains, but with local ranges associated with typical environments of the Cerrado were also considered endemic, due to their possible historical association to once continuous areas of Cerrado. The nomenclature follows specific literature for each vertebrate group (Frost, 2020 for anurans, Uetz *et al.* 2020 for lizards/amphisbaenians, Nogueira *et al.* 2019 for snakes, Pacheco *et al.* 2021b for birds, and Abreu *et al.* 2021 for mammals).

We departed from verified point-locality records and created normalized heatmaps to represent species distributions. Heatmaps represent a simple extrapolation of a species point occurrence, highlighting regions with a high density of records and giving less weight to pixels towards the edge of the buffered heat core. The highest values are attributed to the exact location where the species was recorded and lower values are continuously attributed to pixels further away from the verified occurrence (QGIS Development Team, 2022a). This approach allows us to give relatively less importance to pixels distant from the original species record without disregarding the potential of surrounding areas to contain suitable environmental conditions for a given species. Also, compared with other commonly used methods based on correlative extrapolations, and considering the completeness of our database, heatmaps are only based on the distributional records, minimizing potential commission errors as a consequence of spurious projections. Finally, using heatmaps avoids overlooking potentially important areas around a species record (e.g. decreasing the effect of omission errors) while also dealing with putative commission errors by decreasing the importance of a pixel according to its distance from the verified occurrence.

We created the heatmaps using the Kernel Density Estimation tool, available in QGIS 3.24 (QGIS Development Team, 2022b). In order to create a spatially representative extrapolation we defined the radius of 0.5º providing a 1º circular area around the species record. In the Cerrado, 1ºx1º grid systems have been used in studies on vertebrates diversity and historical biogeographical patterns (e.g. Diniz-Filho et al. 2008a, Azevedo et al. 2016), and prioritization analyses for conservation purposes (Diniz-Filho et al. 2008b). We used the resolution of ~20km2 (0.041667º) and grid origin based on the WorldClim 2.5 arc minutes bioclimatic database (Fick and Hijmans, 2017), to allow further comparative analyses that might consider those climatic variables (see for example Lemes et al. 2020) and to guarantee a spatial resolution that would optimize computational requirements without excessively downgrading land use variables. We normalized the estimated heat values by dividing the resulting raster file by the maximum value of the raster, therefore obtaining a continuous output from zero to one for all species.

*Estimation of Priority Areas*

We used Zonation 4.0 (Moilanen *et al.* 2014) to identify priority areas for the conservation of Cerrado endemic terrestrial vertebrates. The software implements hierarchical prioritization of areas based on the distribution of biodiversity features (e.g. species, ecosystem services) considering predefined user input weights for each feature. In this case, each pixel contains information on the occurrence of a given biodiversity feature, and the algorithm continuously removes pixels with smaller values of the features of interest, progressively recalculating the importance of the remaining pixels and repeating this procedure until the last pixel in the study area is removed. Then, the pixels are hierarchically classified and the output of highly important areas can be displayed according to user-defined conservation thresholds.

Zonation allows for different “cell-removal rules”. Prioritizations were run under the *Core Area Zonation* (CAZ) rule. In short, the CAZ rule identifies high-priority areas as those that present a high occurrence level of a single rare or highly weighted feature (for a more detailed explanation on different prioritization rules see Di Minin *et al.* 2014). This removal rule was selected given that selected input species are endemic to the Cerrado, and many are restricted to small portions of the study region. In this sense, Zonation is more likely to create an output that represents all species highlighting portions of the Cerrado that must be preserved to protect highly irreplaceable biodiversity features. To select areas optimal for expanding the PA network, we included existing PAs as a hierarchical mask (Di Minin *et al.* 2014). This approach leads to minimum costs to achieve conservation targets as it selects the best part of the landscape surrounding existing PAs, which are preferably retained as the first option in the analysis. The shapefile of PAs was downloaded from the World Database on Protected Areas (IUCN & UNEP, 2020) and cropped to the limits of the Cerrado. We included all PA categories, with strict and non-strict conservation goals, such as National and State Parks, Ecological Stations, and Private areas such as “APAs” and “RPPNs”, in our analysis. Only PAs with detailed geographic information were considered, excluding those represented only as a point locality.

Zonation accounts for feature-specific weights prioritizing the protection of highly weighted biodiversity features (in this case species), which allows us to adapt the prioritization to our specific aims. To emphasize the importance of microendemic, threatened (VU, EN, CR) or poorly known (DD) taxa as a precautionary measure (see Nori et al. 2018), we generated a simple index including both categories: distributional pattern and extinction risk. Our weighted index is the result of a multiplication of values from 1 to 3 (“widespread” = 1, “partial” = 2, and “restricted” = 3, see Nogueira *et al.* 2019) for range size, and values from 1 to 5 according to the IUCN categories (LC = 1, NT = 2, VU and DD = 3, EN = 4 and CR = 5; IUCN, 2022). Additionally, DD species described since 2010 and species currently not assessed by IUCN received the value of “2” in the extinction risk part of the index. This value represents a lower weight than weights assigned to species that remained classified as DD even after a decade of their description, while also represents a higher weight than that of taxa indisputably regarded as “Least Concern” for conservation purposes. The endemic rodent *Juscelinomys candango* was not included in the analysis because according to IUCN it is classified as extinct.

In order to penalize pixels covered by anthropic land-uses, we included reclassified binary land-use maps (obtained from MapBiomas, 2022) as a negative variable with a strong weight (equal to the sum of all positive variables weights). These rasters preclude or minimize the possibility to assign a high conservation value to pixels covered by crops or urban areas (see details of the raster reclassification in the supporting information, Appendix S1). To assess how priority areas (and conservation opportunities) have changed as a result of land use and land cover (LULC) changes throughout the last decades, we repeated the analyses using land-use map scenarios from 1985 to 2020 (MapBiomas, 2022) in intervals of five years and also considered a pristine Cerrado scenario (e.g. without any LULC changes). To simulate a “pristine Cerrado scenario” we classified the whole Cerrado area as “Natural”, so no pixel was down-weighted due to the presence of anthropic uses.

According to the Aichi Biodiversity Targets (CBD, 2010), protected area networks should represent at least 17% of the world's landmass (see Target 11; CBD, 2010). We also mapped a recently proposed threshold of 30%, for the post-2020 global biodiversity framework (Woodley *et al.* 2019). Finally, to analyze the effect of LULC changes and resulting fragmentation on priority areas across time, we grouped patches of priority areas (i.e. connected pixels of the top 17% of priority areas in each scenario) depending on their area. We used the following categories: "Large" for priority nucleus with area coverage equal to or larger than 1,000 km2; "Medium" for priority nucleus with area coverage equal to or larger than 250 km2 and smaller than 1,000 km2; and "Small" for priority nucleus with area coverage smaller than 250 km2. We consider that continuous areas of more than 1,000 km2 represent enough available habitat for maintaining a viable population of most of the included species.

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