



Integrating phylogenetic and functional biodiversity facets to guide conservation: a case study using anurans in a global biodiversity hotspot

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Abstract

The Cerrado is one of the most threatened biomes in Brazil, with little spatial representation within the Protected Area network. Recently, proposed conservation plans worldwide have advocated for the use of multiple biodiversity facets to protect unique evolutionary and functional processes. Our aim was to identify areas with high biodiversity representativeness applying this multifaceted perspective, and propose conservation plans based on the joint analysis of taxonomic, functional and phylogenetic diversity. We used a database of the Brazilian National Program for Research in Biodiversity, which employs a standard protocol for sampling tadpoles. The Cerrado database includes samples from 165 water bodies spread over 15 localities, covering most of the Central Brazilian Cerrado. We selected four morphological traits to calculate functional diversity and used a dated phylogeny available in the literature to compute phylogenetic diversity. Our approach selected five priority areas for conservation, one of which is already protected. Our results highlighted the importance of four new areas which show high values of diversity, including original lineages and traits, and urgently need conservation prioritization. Furthermore, unlike the current protected network, our approach performs significantly better than random at protecting sites with high phylogenetic and functional diversity. We therefore discuss how the multifaceted indices considered can help protect key ecosystem functions and evolutionary legacy in anuran communities of the Brazilian Cerrado.

Keywords Cerrado hotspot · Multifaceted analysis · Tadpoles · Functional and phylogenetic diversity

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Introduction

Since the early 80' s, different strategies have been applied to guide conservation decisions in a rational and systematic process, to conserve as much biological diversity as possible (Pressey et al. 2007). However, these approaches have been largely based on taxonomic diversity, namely the protection of individual species or the protection of hotspots of species richness (Margules and Pressey 2000; Myers et al. 2000; Rodrigues et al. 2006). While these approaches have certainly advanced greatly the field of conservation biology, they usually implicitly assume that the extinction or decline of one species or the other are equivalent (i.e., all species are phylogenetically and functionally equivalent) (Iknayan et al. 2014), and they usually focus on endemic and rare species as units of conservation (e.g., IUCN conservation categories; IUCN 2017). These simplifications may be problematic for two reasons. First, abundant and widespread species sometimes perform key or unique ecosystem functions or represent highly distinct evolutionary lineages (Lavorel et al. 1997; Devictor et al. 2010; Mouquet et al. 2012). Their decline or extinction would therefore have disproportionately important consequences for ecosystem function or for evolutionary distinctiveness. In this context, focusing solely on rarity, range size or population decline as criteria for prioritization may lead us to ignore potential tipping points for ecosystem functions. Second, regardless of the species range or abundance, not all species are equivalent. For example, keystone species may cause large shifts in ecosystem functions (Hooper et al. 2005). A growing body of literature suggests that species with different life history traits and ecological properties complement each other providing different ecosystem functions (i.e., the niche complementarity hypothesis: Cadotte 2017; Hautier et al. 2018). Conversely, the insurance hypothesis within the biodiversity ecosystem-function (BEF) framework predicts that some level of redundancy guarantees that the system resists to individual extinctions without compromising ecosystem processes (Reiss et al. 2009; Isbell et al. 2018). Furthermore, recent studies have shown that species that seem redundant when looking at one particular function, are not redundant when analyzing multiple functions at the same time (Reiss et al. 2009; Cadotte 2017; Hautier et al. 2018) or when compared through time (Loreau et al. 2003). All these lines of evidence point to the need to consider the maximization of species differences within an ecosystem as a suitable conservation goal if we are interested in the maintenance of ecosystem functions (e.g., Mace et al. 2012; Trindade-Filho et al. 2012).

Along the same lines, phylogenetic considerations may be relevant in conservation biology for several reasons (Mouquet et al. 2012). First, traits are sometimes highly conserved along evolutionary lineages. Because of this, phylogenies have been advocated as a proxy for functional composition (Cadotte et al. 2010; but see Venail et al. 2015). Indeed, given that we lack complete knowledge regarding the functional traits that are relevant for particular ecosystem functions, and given recent advances in molecular biology, phylogenies are sometimes more complete and of easier access than functional traits (Mouquet et al. 2012). Second, phylogenetic diversity and distinctiveness may be considered as conservation goals per se (Pavoine et al. 2005; Mouquet et al. 2012). For example, the extinction of a species from an old, species-poor clade will result in a greater loss of evolutionary information than the extinction of a less distinct species from a young and species-rich clade (Redding and Mooers 2006; Winter et al. 2013). On top of that, some species' extinctions can also cause evolutionary cascades, implying changes in the evolutionary paths of the remaining species (Dirzo et al. 2014). Finally, and more speculatively, it has also been proposed that phylogenetic diversity represents evolutionary potential during episodes of rapid

environmental change, such as the one current climate change is imposing on ecosystems (Forest et al. 2007).

These recent developments also highlight the fact that there are at least four different dimensions of phylogenetic and functional composition that need to be considered to fully characterize the conservation value of a site within each facet. We will call them here functional or phylogenetic richness, divergence, regularity and originality (Table 1). Richness refers to the total number of species, or the total accumulated functional traits and evolutionary history represented in a community (Mason et al. 2005; Tucker et al. 2016); divergence refers to the average or overall phylogenetic or functional separation between species (Mason et al. 2003; Tucker et al. 2016); regularity is another type of divergence that focuses on how evenly spaced (phylogenetically or functionally) species within a community are (Mason et al. 2005; Tucker et al. 2016); and finally originality refers to how different one particular species is from all others (Pavoine et al. 2005). Notice that while richness, divergence and regularity are diversity measures and apply to the full community represented in a site, originality is species-specific and allows identifying species that are unique in either their functional or phylogenetic identities. Recent studies have shown that including functional and phylogenetic approaches into ecological studies ensure predictability on communities' sensitivity and resilience (e.g., Balvanera et al. 2006; Srivastava and Bell 2009; Mace et al. 2012). However, they have also shown that phylogenetic and functional diversity measures are often very highly correlated to taxonomic diversity (Meynard et al. 2011; Mouquet et al. 2012; Pardo et al. 2017). Therefore, the analysis of functional and phylogenetic diversity often requires comparing the observed diversity value to what would be expected solely from species richness (Laliberté and Legendre 2010; Vellend et al. 2011; Pardo et al. 2017). In other words, the conservation value of multiple sites need to be evaluated against what would be expected based on a random draw of the same number of species, potentially revealing areas with more trait functions or phylogenetic history than expected solely by the number of species present in that site.

The Cerrado biome is the richest savanna region in the world (Silva and Bates 2002; Silva et al. 2006) and comprises high species richness and endemism, therefore being considered as a global biodiversity hotspot (Myers et al. 2000). It is also one of the most endangered biomes of the world, threatened mainly by the expansion of the agricultural frontier (Strassburg et al. 2017). Amphibians, to which anurans belong, show a higher proportion of threatened species compared to other vertebrates, with 32.4% of all known species being in either the “threatened” or “extinct” categories (2030 species out of 6260 amphibians) (IUCN 2016). Large declines of amphibian populations have been documented, demanding urgent public and governmental efforts for effective amphibian conservation in Brazil (Becker and Loyola 2008). Anurans are highly dependent on the existence of high humidity and the persistence of water sources throughout their life cycles, resources that are limited in the Cerrado during the dry season. On top of their ecological vulnerabilities, adult anurans and tadpoles may fulfill important ecosystem functions in natural communities. For example, adult anurans and tadpoles feed on eggs (Bowatte et al. 2013), larval stages (Mokany 2007) and adult mosquitos (Raghavendra et al. 2008), some of which are important vectors of tropical diseases (e.g., *Aedes spp.*, *Anopheles spp.* and *Lutzomyia spp.*). They can therefore play a role controlling obnoxious populations and can help disease control (e.g., yellow fever, dengue fever, zika virus, malaria, leishmaniases; Raghavendra et al. 2008), especially in tropical areas. Most tadpoles also feed on microalgae, therefore playing an important role in the maintenance of water quality of ponds and streams by preventing water eutrophication (Ranvestel et al. 2004). Given this evidence, and although anuran functional roles have not been thoroughly studied and quantified, there is no question

Table 1 Dimensions of diversity (biodiversity facets), respective definition and diversity metrics used to quantify them

Facet	Diversity			Originality
	Richness	Divergence	Regularity	
Taxonomic	Number of species: SR	The degree of species similarities in a community: TD_{RAO} (Rao 1982)	The regularly distribution of species abundances in a community: TD_{RAO} (Rao 1982)	–
Phylogenetic	Total evolutionary history accumulated in a community: PD_F (Faith 1992)	Mean phylogenetic distance of all species in a community: PD_{RAO} (Rao 1982) MPD (Webb et al. 2002)	The regularly distribution of species in the phylogenetic tree in a community: Rao's functional entropy (Rao 1982) Hed and Haed (Cadotte et al. 2010)	How unique a species is according to its evolutionary history: Phylogenetic Originality (Pavoine et al. 2005)
Functional	Total trait space accumulated in a community: FRic (Villéger et al. 2008)	Mean divergence of functional characters in a community: FD_{RAO} (Rao 1982) FD_{IV} (Mason et al. 2003)	The regularly distribution of species traits abundances in a community: FD_{RAO} (Rao 1982)	How unique a species is according to its functional traits: Functional Originality (Pavoine et al. 2005)

SR Species richness, TD_{RAO} Rao's taxonomic diversity, FRic Functional richness, FD_{IV} functional divergence, FD_{RAO} Rao's functional diversity, PD_F Faith's phylogenetic diversity, MPD mean pairwise phylogenetic distance, PD_{RAO} Rao's phylogenetic diversity, Hed and Haed evolutionary distinctiveness

that their conservation has also functional implications at the ecosystem level. Loyola et al. (2008) identified priority areas for the conservation of endangered anurans in the Neotropics and found that species with an aquatic reproductive mode are often underrepresented in the conservation planning process. In fact, many protected forest remnants are isolated, meaning that forest habitats where adults spend most of their life, and water bodies where they reproduce, are often disconnected, a process called habitat splitting and which constitutes one of the greatest threats to amphibians (Becker et al. 2007). This makes anurans in the Cerrado a very highly vulnerable group of species.

In this study, we aimed at applying a taxonomic, phylogenetic and functional approach including as many of these different facets as possible to a highly vulnerable group of species, anurans, in a global biodiversity hotspot, the Cerrado biome in Brazil, to prioritize sites for conservation of water-dependent anurans. To our knowledge, this is the first integrative study using anurans' taxonomic, phylogenetic and functional components to consider conservation recommendations in the Cerrado. We derive recommendations on highly valuable sites for conservation as well as focal species, highlighting the advantages and shortcoming of the multifaceted approach.

Methodology

Study area

The Cerrado is a Savannah biome, with a mosaic of savanna, grasslands and forests (Eiten 1972). The climate is tropical, with precipitation varying between 800 and 2000 mm/year, with a pronounced dry season from April to September (Peel et al. 2007), and average temperatures between 18 °C to 28 °C (Dias 1992). The Brazilian Cerrado is the second largest biome in South America, covering 2036.448 km² (Ministério do Meio Ambiente 2017). Despite its biological importance, the Cerrado is the hotspot with the lowest percentage of fully protected areas in Brazil (Ministério do Meio Ambiente 2017). Although 8.21% of its territory is currently under some kind of legal protection, only 2.85% is under full protection, the other 5.36% being designated under sustainable use conservation units, including private reserves (Ministério do Meio Ambiente 2017).

Database

We used the database of the Brazilian National Program for Research in Biodiversity (SISBIOTA-Brazil) project that employed a standard protocol for sampling tadpoles in five Brazilian biomes. The Cerrado biome database gathers information on tadpoles from 165 waterbodies (ponds and streams), located in 15 distinct areas (Fig. 1). The sampling effort of the SISBIOTA focused on filling the gaps in tadpole inventories that were already existent, and is therefore appropriate to focus on the most vulnerable species, the ones that depend on waterbodies for reproduction (Lips et al. 2003; Bustamante et al. 2005). Additionally, to standardize and allow comparisons at large spatial scales from the SISBIOTA database, we considered only isolated lentic and lotic waterbodies, i.e., those without communication among them or with other types of waterbodies. The standard protocol consisted of sampling tadpoles with a hand dipnet (32 cm diameter and 1.5 mm² mesh) through all surface of each pond (Skelly and Richardson 2010) and along a 100 m transect in streams between November 2011 and May 2014. We sampled each water body during

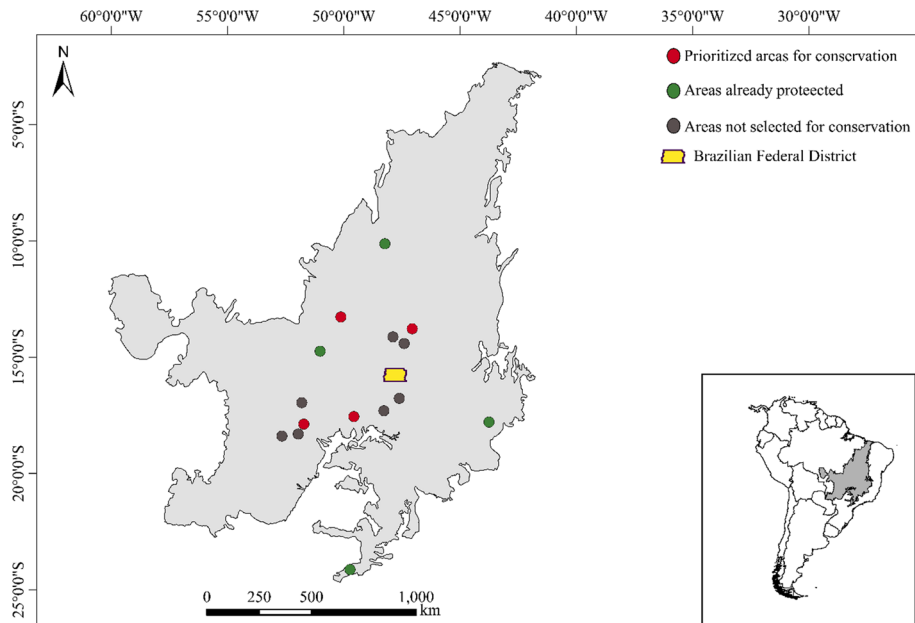


Fig. 1 Distribution of 15 sampled areas in Brazilian Cerrado. Green dots represent already protected areas. Red dots are the areas selected by us as the priorities for conservation of multiple dimension of diversity. Gray dots are areas not selected as a priority in our conservation approach

1 h, trying to maximize the total area covered as well as representing all types of microhabitats in each survey (Skelly and Richardson 2010). The total number of tadpoles per species for each water body was then considered as a reliable abundance estimate, which was subsequently used for the calculation of diversity indices requiring relative abundances. All the species included in our study present direct dependence of water bodies during some part of their life cycle. In our dataset there are two species that are generally considered as terrestrial or semi-terrestrial that are in fact dependent of water: *Ameerega flavopicta*, which exhibits parental care of the eggs, but where the adult males need to take the hatched tadpoles to nearby ponds associated to streams to complete their metamorphosis (Haddad and Martins 1994; Toledo et al. 2004); and *Thoropa* tadpoles, which present a semi-terrestrial development, but can only occur in rocks within or at the immediate proximity of streams (Caramaschi and Sazima 1984; Eterovick and Barros 2003).

Functional traits

We chose four morphological traits (total length, body compression, number of teeth rows and reproductive mode) available in the SISBIOTA database, which represent good predictors of the functional role of tadpoles on water ecosystems (Table S1). This database holds categorical and continuous morphological traits, determined in five to ten tadpoles per species, between 33 to 38 developmental stages (Gosner 1960). For species sampled out of this interval, we consulted the respective description papers or we made the measurements in tadpoles deposited in Brazilian scientific collections (“Coleção do Departamento de Zoologia e Botânica da UNESP de São José do Rio Preto”, “Museu de Zoologia da

Universidade Estadual de Campinas”, “Coleção Célio F. B. Haddad”, “Coleção Zoológica da Universidade Federal de Goiás”). We excluded three species of our functional analysis because of the lack of information on all the four traits. The remaining species have complete information for all trait values.

Phylogeny

We constructed the phylogenetic topology for anuran species of the Cerrado based on Pyron (2014)’s dated phylogeny. Species unrepresented on that phylogeny were replaced with a congeneric species for which the same age was assumed. If no congeneric species was represented, we bound the species on the likely phylogenetic placement based on cladistics literature (Faivovich 2002; Faivovich et al. 2005, see topology in Fig. S1). Of the 67 species included in the analysis, 88% were represented in Pyron’s phylogeny, 9% were added at the congeneric level, and 3% were bound based on the cladistics literature. We then used *bladj* (Branch Length Adjuster) on the software Phylocom 4.2 (Webb et al. 2008) to estimate the age of taxa that were bound on the Pyron’s dated phylogeny. *bladj* sets the age of the nodes of unknown age by estimating the age of clade diversification from the branches of known age (see also Chamberlain et al. 2012). To calculate phylogenetic diversity, we considered the distance between species in millions of years.

Functional, phylogenetic and species diversity

We chose a variety of diversity indices to represent taxonomic, functional and phylogenetic richness, divergence and regularity, for the 15 sites studied, plus an index of species originality to identify functionally or phylogenetically unique species (Table 1). Among the indices considered, the Rao diversity index is the only one that can be calculated for taxonomic (TD), functional (FD) and phylogenetic diversity (PD), allowing comparisons between these three facets. All other indices are specific to either the taxonomic, functional or phylogenetic components (see Table 1, Fig. 2, for details).

For functional and phylogenetic originality of species, we used the originality index proposed by Pavoine et al. (2005). This index is also based on the Rao quadratic entropy (QE), but specifically weights in the uniqueness of each species in different communities. We selected the top 5% of species with the highest originality values for further discussions.

Defining conservation priorities for protected areas

To identify priority areas for conservation of anuran species, we used a ranking method that integrates the different facets of diversity mentioned above. We did not consider spatial prioritization methods, such as systematic conservation planning, because we do not have continuous distribution information for the species or their habitats at the scale and resolution required, and optimization software commonly used to implement it are not currently well adapted to incorporate phylogenetic and functional diversity and their different variants. However, our dataset is the most complete in the region to include representation of early life-stages of anurans and evaluate important independent waterbodies. A score-based ranking method therefore seemed like a sensitive choice, given the good response regarding conservation prioritization in particular contexts (Volkman et al. 2014; Chen et al. 2017). The ranking followed these steps:

- (1) We computed Pearson's correlation among diversity indices, using the Bonferroni correction for significance levels (Table S2). When the indices were correlated to > 0.8 , we eliminated from further analysis one of them, prioritizing the index that represented more diversity dimensions and that was most used in the literature. A Principal Components Analysis (Legendre and Legendre 2012) complemented this analysis to visually assess the relationship among indices and the evidence of which one is representing the same diversity component (Fig. 3). This led us to remove four correlated diversity indices. Therefore, the rest of the ranking was carried out considering the remaining six diversity indices: Rao's Functional, Phylogenetic and Taxonomic diversities, species richness, functional divergence and evolutionary distance (Hed).
- (2) We standardized each selected index with the following formula proposed by Maire et al. (2013):

$$Div_{stand} = \frac{\alpha_x - \alpha_{\min x}}{\alpha_{\max x} - \alpha_{\min x}}$$

where α is the value of the index in each area x , and $\alpha_{\min x}$ and $\alpha_{\max x}$ are the lowest and highest values of the index α in the area x . Therefore, the standardized indices $Div_{stand(\alpha)}$ vary between 0 and 1 with in each of the 15 sampled areas.

- (3) We calculated a synthetic index (SI) of conservation prioritization by summing up each of the standardized indices in each site (Maire et al. 2013). SI varies from a minimum value (different from zero, that represents the area with the lowest values for all indices) to six (the maximum value obtained if one area has the highest value for all indices).
- (4) Finally, we ranked the areas based on their SI values. Sites with $SI > 3.0$ were considered as high priority, since those represent the highest diversity values for at least four of the six indices.

To ensure that the areas for conservation prioritization are representing singular sites that add new diversity to the previously selected ones, we estimated the cumulative percentage of Rao's functional and phylogenetic diversity when adding new sites. We implemented this by ranking the sites from the most to the least diverse (in terms of Rao FD and PD), and vice versa, and calculated the pulled Rao FD and PD by aggregating the sites in order along the diversity gradient (Devictor et al. 2010). We compared these curves to a Whisker plot that represents the random expectation given the same species richness by randomly selecting areas for protection and calculating the accumulation of diversity sorting three sites each time and repeating the process 20 times.

Finally, we performed a sample-based rarefaction analysis to verify if sample effort influenced our synthetic metric for prioritization of sites (Fig. S2). The classic rarefaction of species richness (Gotelli and Colwell 2001) has only been implemented to Rao's FD and PD (Ricotta et al. 2012), so we based this analysis only using Rao in all three facets.

Spatial autocorrelation

We tested spatial autocorrelation using Moran's I correlograms for all biodiversity facets (Legendre and Legendre 2012). We choose a priori six distance classes, each one composed by similar number of samples and the significance test for autocorrelation was based in 1000 randomizations. Moran's I correlogram was calculated in the software *Spatial Analysis in Macroecology* (SAM; Rangel et al. 2006, 2010).

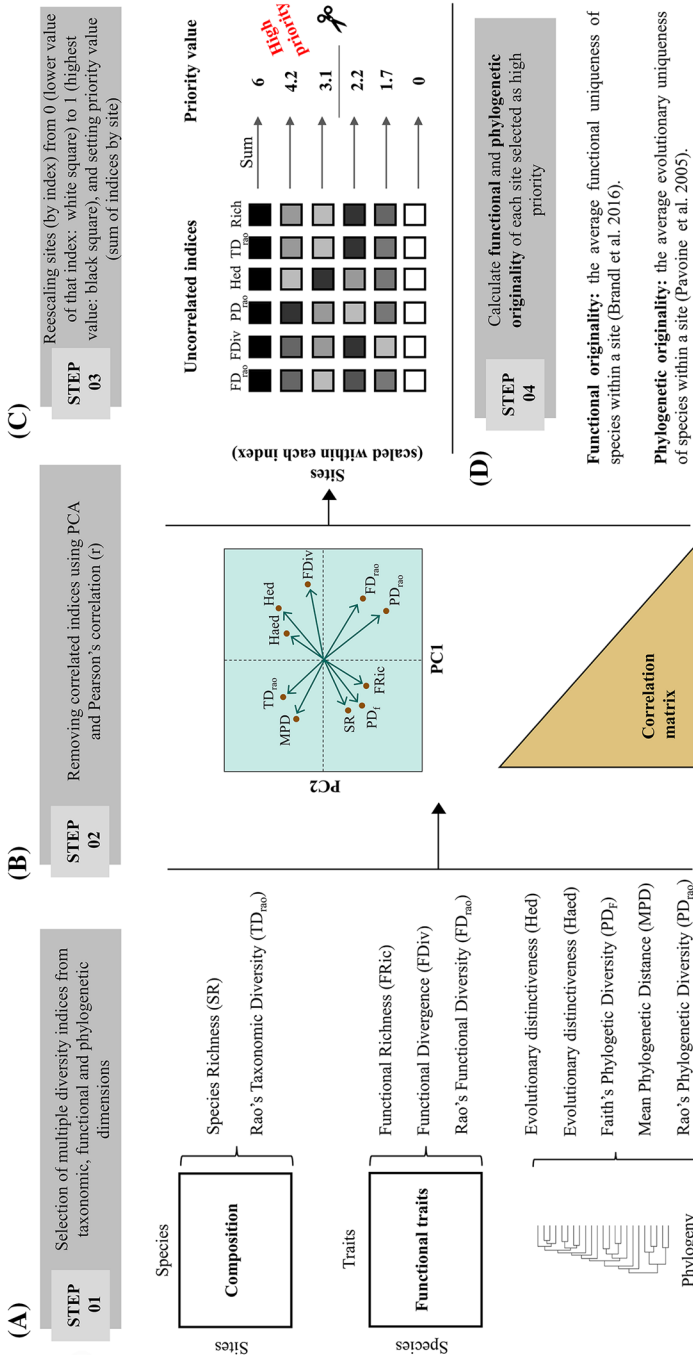


Fig. 2 Main steps used in the prioritization for conservation in the Cerrado. First, we performed the selection of multiple diversity indices using PCA (Fig. 3) and Pearson's correlation corrected by Bonferroni criteria (Table S2). Second, we tested and removed the correlated indices. Third, we standardized and summed up the six indices selected for each area to obtain a synthetic index (SI) of conservation prioritization (Maire et al. 2013). SI varies from a minimum value (different from zero, that represents the area with the lowest values for all indices) to six (the maximum value obtained if one area has the highest value for all indices). We rank the areas based on their SI values and considered as areas with high priority for conservation those with $SI > 3.0$. Last, we calculated functional and phylogenetic originality

most original species was *Leptodactylus labyrinthicus*, followed by *Physalaemus marmoratus*, *L. gr. labyrinthicus* and *Physalaemus centralis* (Table S4). The functional originality analysis gave higher weights to Leptodactylidae frogs (the four most functionally original species).

Five areas presented high priority values according to the synthetic index. One of them, Lago Cedro Extractive Reserve (LAG, Table 2, Fig. 2), is already protected. The remaining four areas are not currently protected: Nova Roma (NOV), São Miguel do Araguaia (MIG), Pontalina (PON) and Jataí (JAT, Table 2, Fig. 2). The sample-based rarefaction analysis showed that the recommendation of JAT as high priority area was partly driven by sampling bias, whereas the other sites were robust to such biases. Indeed, when we rarefied the species richness, Rao's FD and PD, JAT did not have the highest value of functional and phylogenetic diversity (Fig. S2). Conversely, even after rarefying these diversity dimensions, the other three areas still have the highest diversity values.

Related to the accumulation of functional and phylogenetic diversity, we found that our prioritization approach always does significantly better at representing the diversity facets as compared to choosing the sites randomly (Fig. 4). We also found that areas already protected fall within the range of the random selection, which means that those sites do not protect functionally and phylogenetically diverse sites more efficiently than a random draw of sites (Fig. 4).

Discussion

As hotly debated in the last decade (Mason et al. 2005; Tucker et al. 2016), we demonstrated that the use of species richness is not necessarily a good proxy for other biodiversity dimensions. Here, by combining different biodiversity dimensions into a synthetic metric,

Table 2 Conservation priority values for each area in the Brazilian Cerrado

Areas	Synthetic index
Nova Roma	4.11
São Miguel do Araguaia	4.07
<i>RESEX Lago Cedro</i>	4.07
Pontalina	3.41
Jataí	3.19
<i>PE Lajeado</i>	3.00
Alto Paraíso de Goiás	2.52
Pires do Rio	2.35
São João da Aliança	2.15
Caiapônia	2.08
Chapadão do Céu	1.76
Cristalina	1.52
<i>PE Cerrado</i>	1.43
<i>PE Sempre Vivas</i>	1.42
Serranópolis	1.14

Those in italic are already included in the Brazilian protected areas network and in bold are the areas identified as priorities for conservation

RESEX extractive reserve, *PE* state park

we were able to select five areas deserving high conservation priority for anurans in the Cerrado biodiversity hotspot. More importantly, four of these areas (Nova Roma—NOV, São Miguel do Araguaia—MIG, Pontalina—PON and Jataí—JAT: Figs. 1 and 2) are not included in the current network of Brazilian protected areas (Ministério do Meio Ambiente 2004), highlighting the under-representation of phylogenetic and functional composition in the current protected area planning. Our results fall in line with other regions and taxonomic groups, such as birds (Devictor et al. 2010), fishes (Strecker et al. 2011), spiders (Gonçalves-Souza et al. 2015) and plants (Pardo et al. 2017), reinforcing the idea that there is a strong mismatch among different biodiversity facets, and that our current conservation planning system focusing on taxonomic units disregards the importance of community properties. This is well illustrated in our Fig. 4, which shows that by considering 4–9 sites for conservation (depending on the facet, Fig. 4a vs. b), we would be doing a significantly better job than a random draw by systematically using a compound index such as the one used here. Conversely, that same analysis also shows that the areas that are currently protected do not do a better job as compared to a random selection, when considering functional and phylogenetic diversity.

The mismatch between taxonomic diversity, which is usually the only facet considered in conservation planning, and the other facets of diversity, can have important consequences in terms of the selection of sites of high priority (Devictor et al. 2010; Gonçalves-Souza et al. 2015). For instance, some areas could have high species richness but low phylogenetic diversity when there is geographic isolation and recent colonization with fast in situ diversification. Similarly, when species are functionally redundant, there is an incongruence between species richness and functional diversity (Gillespie 2004; Weinstein et al. 2014). Furthermore, phylogenetic and functional originality could also affect whether species richness will work properly as a proxy for other diversity dimensions (Swenson et al. 2007). When there is strong trait conservatism, sister groups will show similar traits; conversely, under low conservatism, traits can be more labile within a group of species. It is therefore the combination of evolutionary history as well as ecological mechanisms currently affecting community assembly, which will determine the interplay and relevance of taxonomic, phylogenetic and functional components in a conservation context. Therefore, and as others have done before us (Devictor et al. 2010; Gonçalves-Souza et al. 2015), we also recommend the use of complementary indices in conservation decisions. Our arguments are further reinforced by the recent study by Pollock et al. (2017), in which they demonstrated, at a global scale, that linking multifaceted biodiversity provides large conservation gains for birds and mammals by ensuring a rich array of further effects for the ecosystem functioning and human well-being.

Implications for the Brazilian protected network: the case of the Cerrado hotspot

Limited funding available for nature conservation usually jeopardizes our ability to protect biodiversity over large areas (Bruner et al. 2004). By using different approaches, and different diversity measures, we may avoid overlooking important features in deciding conservation prioritization. However, increasing human, social and academic effort to cover different conservation approaches is a double-edged sword, because it improves information assessment, but it increases conservation costs. The most common approach to identify conservation priority areas is to try to represent the maximum biodiversity while minimizing the total cost or area allocated to protected areas (Margules and Pressey 2000). Our

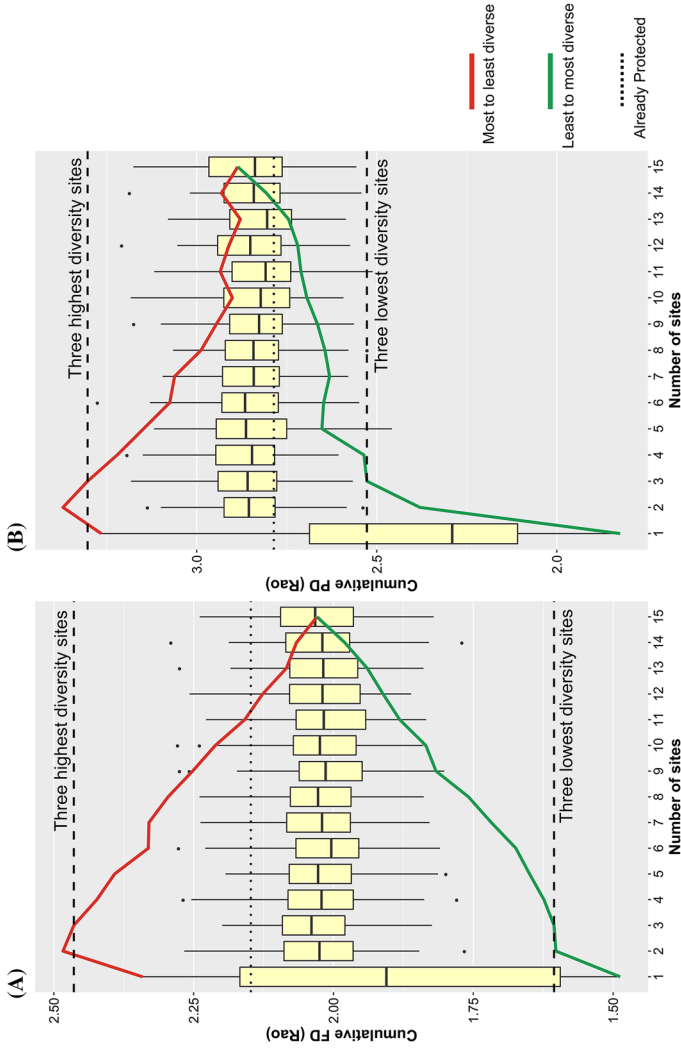


Fig. 4 Diversity accumulation curves for **a** Functional diversity (FD) and **b** Phylogenetic diversity (PD) as calculated with Rao's index. Whisker boxes represent FD or PD for the same number of randomly selected sites. The red line represents the strategy where sites are added in order, from the most diverse to the least diverse. The green line represents the diversity if we added sites in the opposite order (from the least to the most diverse). The dotted line represents diversity in the sites already protected, and the two dashed lines represent cumulative diversity in the three sites containing either the highest or the lowest diversity. Therefore, the green line would represent adding sites in the least efficient possible way, whereas the red line is the most efficient way. Notice that the red line is above the whisker boxes (random expectation) and above the dotted line (already protected) until we have added almost all the sites to the protected area network, meaning that our strategy would, for any given number of protected sites, protect more functional and phylogenetic diversity than either the current protected areas or the least-to-most diversity strategy

approach uses different diversity facets, such as evolutionary history and functional traits, to increase the robustness of the selection of priority areas without significantly increasing conservation costs.

There are good reasons to create new protected areas in the Cerrado hotspot, including the small percent of land currently protected (only 2.85% of the Cerrado hotspot in integral protection conservation units) (Ministério do Meio Ambiente 2017) and the high deforestation rates, which make it one of the most threatened biomes on the South American continent (Silva and Bates 2002; Klink and Machado 2005). Interestingly, the areas JAT and NOV, were already defined as high priority areas for conservation in 2016 by the Brazilian Ministry of the Environment. These areas should be urgently protected because they have high anuran diversity in multiple facets (this study), as well as presence of traditional human communities, high esthetic value given by the caverns in the region, and presence of dry forests (Ministério do Meio Ambiente 2017). The other two areas, MIG and PON, were not included in this government document, and we argue that they should be added. On top of their high multifaceted biodiversity value, these two sites are located in areas under high agriculture and pasture pressure (Ministério do Meio Ambiente 2017), which cause water silting by soil erosion and contamination of water bodies, increasing the threats to anuran species.

Implications for anuran conservation

According to the IUCN red list (IUCN 2017), most of the anuran species present in our study area are not under any type of threat (excepted *Hypsiboas cipoensis*, which is considered “near threatened”). Thus, even if red lists are a useful and effective tool for conservation of vertebrates in general at large scales (Rodrigues et al. 2006), here they would not have added any useful insights regarding the conservation of anuran biodiversity. As Hidasi-Neto et al. (2013) have demonstrated, IUCN red lists are not able to capture on their own the ecological and evolutionary importance of species. This emphasizes the need of adding additional criteria to the IUCN red lists. Here, we argue that those lists should systematically include, both at the site level and at the species level, the value of phylogenetic and functional differences between species in reinforcing ecological processes.

In that context, an important criterion is species originality, which prioritizes species based on unique functional traits or evolutionary history (Pavoine et al. 2005). The pattern we have found suggests that some species contributing disproportionately to the functional or phylogenetic originality pattern of some areas deserve conservation attention, even if some of them are widespread. In the Cerrado hotspot, Microhylidae frogs, *Chiasmocleis albopunctata*, *Dermatonotus muelleri*, and *Elachistocleis cesarii* showed the most distinctive evolutionary history, which goes along the lines of Silvano et al. (2016) who had already characterized this group as evolutionary distinct in the Cerrado. This clade diversified about 70 million years ago during the late Cretaceous and early Tertiary, and has been an important source of diversification ever since (Roelants et al. 2007). Microhylidae frogs are fossorial species, and many of them present explosive breeding. Thus, these frogs only get out of the ground for reproduction, which occurs just once in a year (Wells 1977). These biological and behavioral characteristics allow them to deal with the severe dry season and frequent fires occurring in the Cerrado.

Opposite arguments regarding phylogenetically distinctiveness of groups have been made in the literature. On one hand it has been argued that recently diversified clades represent taxonomic groups that have higher evolutionary potential to adapt quickly to environmental changes, because they already show quick adapted evolutionary history (Erwin 1991; Mace et al. 2003). This would mean little phylogenetic originality at the species level would be compensated by high functional originality within a group and/or high phylogenetic and functional diversity at the community level. On the other hand, it has also been argued that older and more distinct clades carry high evolutionary potential to deal with stressful and stochastic events because of the accumulation of genetic diversification through time (Sgrò et al. 2011; Mouquet et al. 2012). This would mean that we should value phylogenetic originality over other diversity considerations. Despite these contradictions, two facts remain clear throughout both arguments. First, losing a species that represents an old and unique lineage is of conservation concern on itself, since its disappearance will eliminate a branch of evolutionary history that will remain unrepresented. In that context, highlighting the phylogenetic originality of these Microhylidae frogs can help us consider their conservation under a new perspective. And second, whether phylogenetic diversity represents on itself a desirable quality in conservation sites will depend on its relationship to functional properties of the ecosystems.

The four new areas identified here have at least one of these phylogenetic original species in their composition: *D. muelleri* (Microhylidae) and *E. cesarii* (Microhylidae) in NOV, *E. cesarii* in MIG, *D. muelleri* and *E. cesarii* in PON, and *E. cesarii* in JAT. Despite their functional originality, *L. labyrinthicus* and *P. centralis* were the only two functionally original species present in the selected areas, both were found in MIG, and *P. centralis* in JAT. These species exhibit traits that are making them unique in the sampled areas: the type of reproductive mode, the ability of their tadpoles to live in the bottom of ponds, and, in the case of *L. labyrinthicus*, reduced labial tooth rows (de Sousa et al. 2014). Their eggs are embedded in a foam nest, avoiding their desiccation (Zina 2006). The benthonic foraging of their free-living tadpoles and the foam nests of both species allow them to survive even when water volume decreases, a common event in a savanna biome. Although the functional roles of these species have not been thoroughly quantified in these ecosystems, tadpoles in general are crucial for nutrient cycling in water bodies because they affect the abundance and diversity of basal resources and primary consumers (Ranvestel et al. 2004), and abundant species are particularly important in influencing trophic links. Benthic tadpoles facilitate periphyton production by grazing, being key organisms for the food web dynamics and energy flow (Ranvestel et al. 2004). These kinds of traits seem to be associated with idiosyncratic functions that increase ecosystem functioning. Furthermore, as either functionally or phylogenetically original species have a significant contribution to the diversity of these high priority areas, losing these species in still unprotected areas could erode regional biodiversity. We therefore believe that future studies should focus on the role of these functionally unique species in maintaining water ecosystem health in the region.

Conclusions

In November 2016, the Brazilian Ministry of the Environment published a guide to enlarge, implement and consolidate the Brazilian national system of conservation units (SNUC) aiming to include new areas in the conservation system. Here we recommend that in a

near future four areas in the Cerrado hotspot should be legally protected by Brazilian laws. More broadly, we strongly argue for the inclusion of phylogenetic and functional considerations in conservation planning. Selecting protected areas based on traditional biodiversity metrics that ignore the uniqueness of each species, such as species richness and endemism, disregards evolutionary and functional considerations that may be key for ecosystem resilience. Taxonomic criteria can be misleading because (i) they usually ignore evolutionary patterns, (ii) they do not ensure community persistence in the long term (Forest et al. 2007; Faith 2008) and, (iii) they do not allow us to make predictions about functional consequence of environmental changes (Díaz et al. 2007; Reiss et al. 2009). Ultimately, we can only guarantee the long-term maintenance of the community if we can protect key ecosystem functions, rather than species or sites selected at random or through rarity criteria, and we have to admit that species that are sometimes abundant or widespread may play key roles in the maintenance of ecosystems functions. One of the key advantages of our approach is that it allows incorporating such criteria, even without a full understanding of species evolutionary and functional roles within the community. One could argue that allocating limited conservation funds to species and sites that are unique on either one of these dimensions is a sensible investment for future resilience of ecosystems.

In addition, it is important to highlight that our study tried to fill the gap existing in anuran conservation regarding water-dependent larval stages. By conserving early developmental stages, we are increasing the probability that anurans reach their adult phase. Adult anurans exhibit an important role in mosquito control, the most common vectors of diseases in tropical systems.

Our study certainly has some potential limitations, such as the fact that we cannot tease apart the contribution of terrestrial, semi-terrestrial, and phytotelma species to ecosystem functioning, or how do species (and lineages) respond to different environmental habitats (e.g. ponds, streams). Thus, we encourage future studies to expand this approach to terrestrial and aquatic habitats. Also, there might be other more efficient ways to combine phylogenetic and functional information into a compound index, or even specific situations under which we might want to consider one or the other facet as more important for prioritization. However, this case study clearly demonstrates that the establishment of conservation strategies for anurans in the Cerrado can be improved by considering multiple biodiversity facets to maximize anuran protection. A major field that remains to be explored for a better assessment of the functional approach is the quantification of ecosystem services that are performed by anurans, including the key links to other trophic levels (e.g., how many mosquitos do they eat and what would happen with certain diseases if anurans disappear).

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