

Speeding up the discovery of unknown plants: a case study of *Solanum* (Solanaceae) endemics from the Brazilian Atlantic Forest

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RESUMO: (Acelerando a descoberta de plantas desconhecidas: um estudo de caso com espécies de *Solanum* (Solanaceae) endêmicas da Floresta Atlântica brasileira.) O Brasil possui uma das mais ricas floras do mundo, o que faz com que seja desafiador documentar sua totalidade. Estimativas recentes apontam que as áreas com maior diversidade de plantas devem também conter o maior número de espécies não descritas, e o Brasil está entre estas áreas. Neste estudo nós apresentamos uma aplicação de Modelagem de Nicho Ecológico (MNE) que ainda não foi amplamente discutida em literatura: acelerar a descoberta de espécies de plantas em regiões megadiversas e com baixa densidade de coletas por área. Foi utilizado o algoritmo MaxEnt no intuito de auxiliar na descoberta de novas populações de espécies de *Solanum* de distribuição restrita da Floresta Atlântica brasileira. Nós utilizamos o método *Model-Based Search* para espécies e para um clado como um todo, o que nos permitiu a descoberta de novos pontos de ocorrência de espécies raras e nos guiou a uma área onde foi encontrada uma espécie ainda não descrita. O emprego de MNE associado ao conhecimento da biologia e história evolutiva de espécies ou clados pode ser útil para acelerar descobertas em áreas de alta diversidade e conhecimento escasso.

Palavras-chave: Modelos de Distribuição Preditiva de Espécies, conservação, biodiversidade

ABSTRACT: Brazil holds one of the richest floras in the world, what makes it challenging to document its entirety. Recent estimates point that the richer the area, the more undescribed plant species it might hold, and therefore Brazil is

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among the areas that might have a representative number of plant species yet to be discovered. In this study we present an application of Ecological Niche Modelling (ENM) not exhaustively discussed in literature: to accelerate plant species discovery in megadiverse but not well known regions. We used MaxEnt algorithm in order to help finding new populations of narrowly distributed *Solanum* species from the Brazilian Atlantic Forest. We applied the Model-Based Search approach to species and a clade as a whole and it enabled us to find new presence points to rare species, and guided us to an unexpected area that actually held a new species. The use of ENM allied with biological and evolutionary knowledge of species or clades can be helpful to accelerate discoveries in rich but poorly known areas.

Key words: Ecological Niche Modelling, conservation, biodiversity

Introduction

Brazil is known to hold one of the most diverse floras in the world (about 32,500 vascular plant species) and efforts to document it more precisely in a continuously updated working list of known plants (JBRJ, 2014), highlighted several poorly known taxa and conservation challenges for the future (Forzza *et al.*, 2012).

Encompassing an area of more than 8.5 million square kilometers, with the highest species/area ratio for vascular plants known thus far (Forzza *et al.*, 2012), the country is yet listed among predicted areas that might hold a considerable number of species to be discovered (Joppa *et al.*, 2011). With two of the world hotspots of biodiversity (Mittermier *et al.*, 2005) and numbers of new plant discoveries per year that not seem to diminish (Sobral & Stehmann, 2009), the scientific community urges for better planning of its policymakers to attain the Global Strategies for Plant Conservation (GSPC) targets established for 2020 (CDB, 2010).

Aware of its commitments, the Brazilian government did its first moves, as publishing the Livro vermelho da flora do Brasil (Martinelli & Moraes, 2013), after a questionable red list of threatened species (MMA, 2008; Scarano & Martinelli, 2010; Sousa-Baena *et al.* 2014), and discretely started efforts towards an online flora. Nevertheless, in such a vast country with an astonishing plant diversity and the landscape being rapidly transformed, is undeniable the alarming possibility that species might disappear before even being scientifically described.

Recent estimates for species of flowering plants yet to be discovered shows that unknown species within Brazil could contribute to an increase of 4 to

63% in the number of species, varying among regions (Joppa *et al.*, 2011). These high numbers are not surprising, considering the low collection/area ratio for some regions (Sobral & Stehmann, 2009). But if the models are to be realistic, an enormous effort is necessary to increase the number of systematists (or taxonomists) studying the Brazilian flora, in order to accelerate the discoveries. Another complicating but encouraging factor is that most undescribed species are likely to be of limited distribution, mostly rare and potentially endangered and therefore of particular usefulness in determining conservation priorities (Thomas, 1999; Pimm *et al.*, 2010). In this case it is preponderant to increase the number of collections available in some areas, to better understand its real diversity and document species distributions more precisely.

Given the exposed, and considering that training human resources could be time consuming and increasing collections in vast remote areas can represent a significant cost, some strategies should be implemented to optimize or speed up the process of cataloguing species diversity. In 2010 the SISBIOTA Program was created by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) to support biodiversity studies. The *Rede Integrada em Taxonomia de Plantas e Fungos* conducted part of it since then, and here we report a case study allied with the program, of a method that can be used to accelerate species discovery. We use the Model-Based Sampling (MBS) approach described in Guisan *et al.* (2006) that consists on using Ecological Niche Modelling (ENM) or Species Distribution Models (SDMs) to generate environmental suitability maps for poorly known species. The predicted areas by the models as having similar suitable conditions are then targeted for additional field work. The efficiency of MBS to map unknown populations of rare plant species (e.g. Kamino *et al.*, 2012; Le Lay *et al.*, 2010; Särkinen *et al.*, 2013; Williams *et al.* 2009) and even to guide species discovery (Raxworthy *et al.*, 2003) was previously reported.

The group used as a model here is called the *Solanum inornatum* species group (Giacomin & Stehmann, 2014), a subclade of herbs to shrubs within the larger Brevantherum clade (*sensu* Weese & Bohs, 2007), one of the main lineages of the giant genus *Solanum* L. (Solanaceae). The Brevantherum clade is a Neotropical group of about 95 species, with a center of diversity in southeastern Brazil. The *S. inornatum* species group was only recently recognized as part of the Brevantherum clade (Giacomin, 2010) and is so far composed by four species of subshrubs, endemic to the mountain ranges of Mantiqueira and Serra do Mar (Giacomin & Stehmann, 2014). While revising the group, very restricted species distributions were revealed, and incited us to look for additional populations in other areas. We used MBS to predict areas that could contain unknown populations and while trying to validate the models

in the field unexpected results were achieved. The assumption that closely related species tend to have a conserved niche (Peterson *et al.*, 1999) guided our findings and conclusions.

Materials and Methods

Biotic data and study area. Presence points for the species of the *Solanum inornatum* species group were gathered from 2008 to 2010, during revisionary work in the following collections (acronyms from Index Herbariorum, <http://sweetgum.nybg.org/ih/>): BHCB, CESJ, ESA, RB and SPSF. Considering the rarity of the group, even with a vast number of herbaria being revised along continued taxonomic studies in the Brevantherum clade (more than 42 Brazilian and foreign collections), we could only gather trustable points from 23 sheets, in the cited collections, of which 15 were for *Solanum bradei* Giacomini & Stehmann, three for *S. inornatum* Witasek, three for *S. kriegei* Giacomini & Stehmann, and two for *S. friburgense* Giacomini & Stehmann.

Considering the group distribution (see Giacomini, 2010), we adopted a study area encompassing the Southeastern region of Brazil that contains the states of Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo.

Models and the Model-Based Sampling approach. Following the MBS approach (Guisan *et al.*, 2006) and as our primary goal was to search for additional populations of species of the *S. inornatum* group, but only a few points were available, we have chosen to generate models for *S. bradei* and *S. kriegei* separately using the herbaria-gathered points, to test the efficiency of the models to the group and the environment, considering distinct situations, with more (15) and less (three) points, respectively.

As phylogenetic data for the group was available (Giacomini, 2010; Giacomini *et al.*, in prep.), it was previously known that the group had a strongly supported monophyly within the Brevantherum clade and was morphologically and molecularly distinct from all the other species of it, so we also generated a model using the points of all species together (23 in total), including the points for *S. friburgense* and *S. inornatum*, to compare with the others models. The decision to model the whole clade was also supported by the fact that the species had very similar habitat conditions, that were observed in the field, and therefore could have at least some dimensions of its niches conserved. All the species of the *S. inornatum* group inhabits mostly mid to high elevation moist forests in Serra do Mar and Mantiqueira (see Giacomini, 2010 for more details) and show a somewhat similar spreading capacity. This approach is treated as an alternative to cases where very few points are available for species, as in

here, what could influence the model accuracy or even make it not feasible to generate a model, depending on the employed method or algorithm.

We have chosen the MaxEnt algorithm (version 3.3.3a; Philips *et al.*, 2006), that uses the principle of maximum entropy, to generate the habitat suitability maps. MaxEnt is known to perform better than other methods in complex landscapes, even with limited biological information (e.g. Gogol-Prokurat, 2011; Pearson *et al.*, 2007). Even with the few points available for *S. kriegeri*, our decision to use the same algorithm for all models was based on that would allow a comparison among them, and also that previous authors had successfully applied MaxEnt to as few points as we use here (e.g. Särkinen *et al.*, 2013) and in a similar study area (Kamino *et al.*, 2012). How MaxEnt works was described elsewhere, by Elith *et al.* (2011).

To select the predictor variables used to generate the models, we departed from a set of 28 environmental layers, all of them with a 30 arc second resolution ($\sim 1 \text{ km}^2$): 19 bioclimatic predictors obtained from the Worldclim database (Hijmans *et al.*, 2005; <http://www.worldclim.org/>), models of the slope and aspect that was created using the WorldClim elevation data, an digital elevation model (United States Geological Survey Hydro-1K Elevation Derivative Database; <http://eros.usgs.gov/>), means of actual and potential annual evapotranspiration (Consortium for Spatial Information-Database; <http://www.cgiar-csi.org/>) and four layers of Vegetation Index Isolines (EVI Images) composed of 16-day satellite images NASA-MODIS (<https://earthexplorer.usgs.gov/>). Explanations on how each predictor is generated and what it represents, can be found on each source.

For each of the species or the whole species group, the data were evaluated to avoid colinearity. A principal components analysis (PCA) was performed and the axis to which each pair was most correlated was determined. Variables that were found to be correlated with Pearson coefficients of 0.7 or higher were excluded. Also, some of the 19 Bioclimatic predictors of the Worldclim database were pre-selected by running MaxEnt with its default parameters and excluding the variables that made no contributions to the model. We retained five predictors for the *S. bradei* model, seven for *S. kriegeri* and nine for the model generated to all clade points. The variables chosen for each one is listed in Table 1.

To generate the models, we used the default settings of MaxEnt, and just started the run allowing a random seed and with a number of replications of $n-1$, where n is the number of occurrences available. To evaluate the model performance, we used the cross-validation, randomly splitting the occurrences into two groups which one of them is used for creating the model and the other for validating the model. We also used the receiver operating characteristic

Table 1. Variables chosen for each generated model. The predictors chosen for each species or the whole clade were used in both models (see text). What each predictor represents is cited in each respective source.

Species or group modeled	Predictors
<i>Solanum bradei</i> Giacomini & Stehmann	Annual Mean Temperature, Minimum Temperature of Coldest Month, Elevation, EVI_193 and Actual Annual Evapotranspiration
<i>S. kriegeri</i> Giacomini & Stehmann	Annual Mean Temperature, Precipitation of the Driest Month, Elevation, EVI_97, EVI_273, Aspect and Potential Annual Evapotranspiration
<i>S. inornatum</i> species group (4 species)	Annual Mean Temperature, Mean Diurnal Range, Precipitation of the Driest Month, Elevation, EVI_01, EVI_97, Aspect, Slope and Actual Annual Evapotranspiration

(ROC) curve analysis for validation. The ROC analysis characterizes the predictive performance of the model at all possible thresholds by a single number, the area under the curve (AUC; Phillips *et al.*, 2006).

With the first models generated, we have chosen the lowest presence training threshold (LPT) to draw the maps that would guide us to areas that could be used to validate them. But instead of generating a binary map, the areas with pixel values lower than LPT were classified as zero (as white in the maps) and the areas with pixel values above LPT had their suitability values kept. As the LPT values for *S. bradei* and the whole clade were too low (a possible artifact of low confidence points from herbaria) we decided to adopt as suitable, areas with values greater than 0,3.

Areas of interest, that held similarities with the ones where the species occur, and that were predicted by the models, were then visited to seek for new populations, during the years of 2011-2013. A potential habitat distribution map was generated for *S. bradei*, *S. kriegeri* and the clade as a whole (four species), using the same predictors and parameters used in the first generated models, but now with 30 points, seven newly acquired.

Results

All the models generated with herbaria-gathered points had a good performance, considering the yielded AUC values of 0,96 for *S. bradei*, 0,99 for *S. kriegeri* and 0,99 for the clade. The most important variable in all models was the Annual Mean Temperature, with a contribution of 69% in the *S. bradei* model and of 49% and 77% in the *S. kriegeri* and the clade models, respectively.

The resulting potential distribution maps of the first three models are

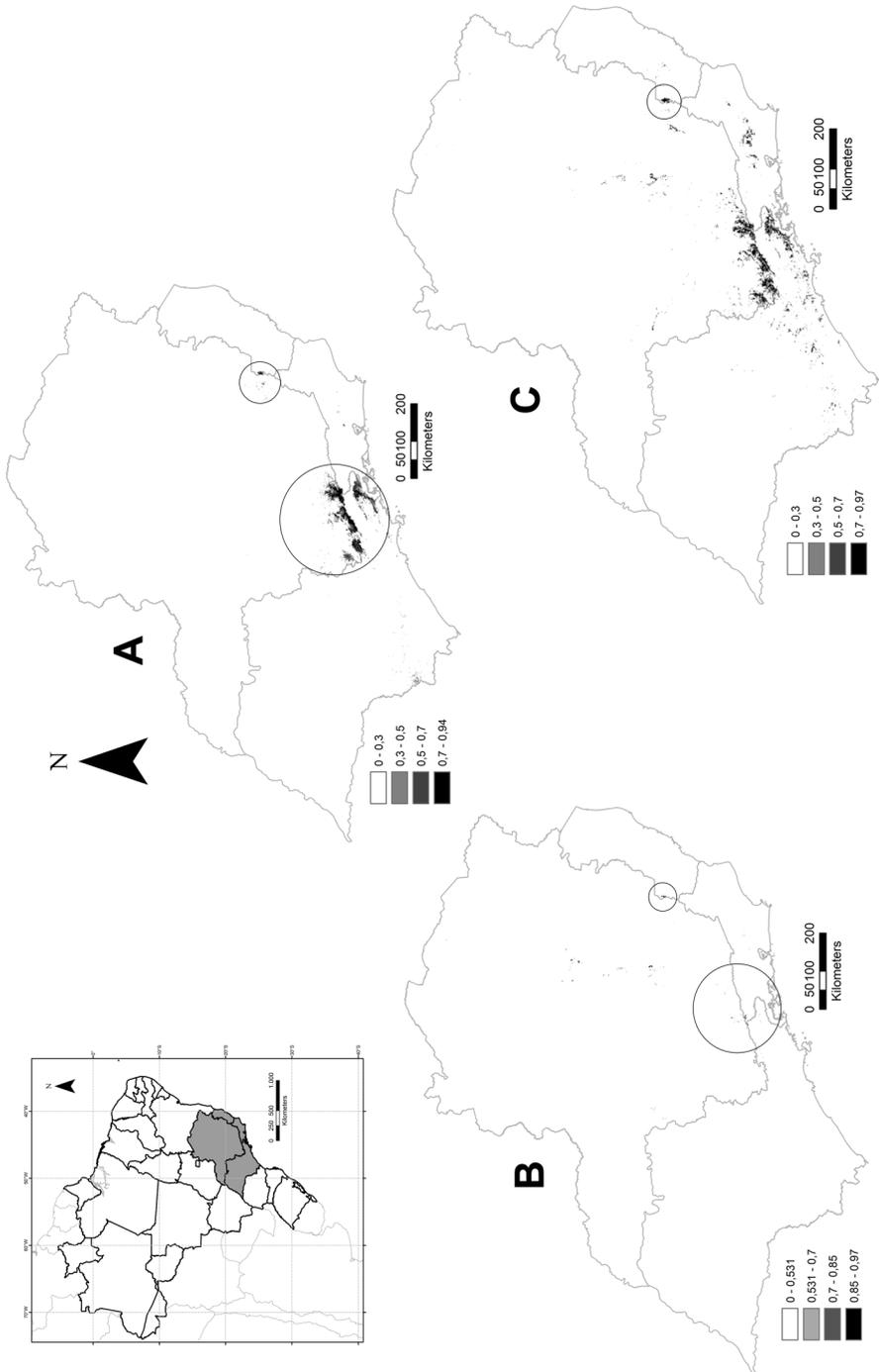
shown in Figure 1. The model generated for *S. bradei* (Fig. 1-A) presented suitable areas mainly in the southern Mantiqueira range and north-central Serra do Mar, close to the borders of Minas Gerais, Rio de Janeiro and São Paulo states (bigger circle in Fig. 1-A). The predicted areas coincide with the known distribution considering the points from herbaria and are mainly 1000 m above sea level. The map also indicated suitable areas in the Serra do Mar range in Rio de Janeiro state and in the border of Minas Gerais and Espírito Santo state (small circle in Fig. 1-A), in Serra do Caparaó, where no points were known thus far for the clade.

The model generated for *S. kriegei* (Fig 1-B) was very restrictive, considering the high LPT value (0,531). It predicted few areas in southern Mantiqueira, including Serra do Ibitipoca, where all herbarium points are from (bigger circle in Fig. 1-B). The model predicted as suitable high elevation areas in the Espinhaço range, where no collections are known, even with some sites with a considerable number of collections/area rate, such as Serra do Cipó. As for *S. bradei*, an environmentally suitable area was predicted at Serra do Caparaó (small circle in Fig. 1-B).

The model generated for the whole clade (Fig. 1- C) was less restrictive than the species ones and predicted areas that coincide with the known occurrences of *S. friburgense* and *S. inornatum* in Nova Friburgo region in Rio de Janeiro state and in Serra do Mar range in São Paulo, respectively. As the other models, Serra do Caparaó was predicted as having suitable areas for the species (circle in Fig. 1-C).

Based on the results of the first models, we targeted a few areas to visit in order to try to locate new populations of the species. As not all areas could be visited, due to time spent and costs involved, some were focused, based on the characteristics and habitat specificity of each species. From south to north, new populations were found of *S. inornatum* in Serra da Bocaina (mun. São José do Barreiro, SP), of *S. bradei* in the Mantiqueira core (mun. Delfim Moreira and Itamonte, MG), of *S. kriegei* in Serra Negra (mun. Rio Preto, MG) and of a morphologically distinct species at Serra do Caparaó (mun. Ibitirama, ES).

The new models generated associating the herbarium data and the new occurrences mapped in the years of 2011-2013 are presented in Figure 2 and also presented a high performance, with AUC values of 0,98 for *S. bradei*, 0,99 for *S. kriegei* and 0,98 for the clade. The model generated for *S. bradei* was more restrictive (Fig. 2- A) even with a low LPT (0,01), and the predictor that contributed most this time was the Minimum Temperature of the Coldest Month (80%). The mainly predicted area was in the Mantiqueira core (circle in Fig. 2- A).



The model generated for *S. kriegeri* (Fig. 2- B), on the other hand, presented an expanded area when compared to the first model and presented a lower LPT (0,354), and the predictor that contributed most remained the same. The model predicted more suitable areas in Mantiqueira range and in western Minas Gerais, as well expanded the predicted areas in Espinhaço range. Nevertheless, the couple new populations found lies close to the only one known before, at Serra Negra (circle in Fig. 2-B).

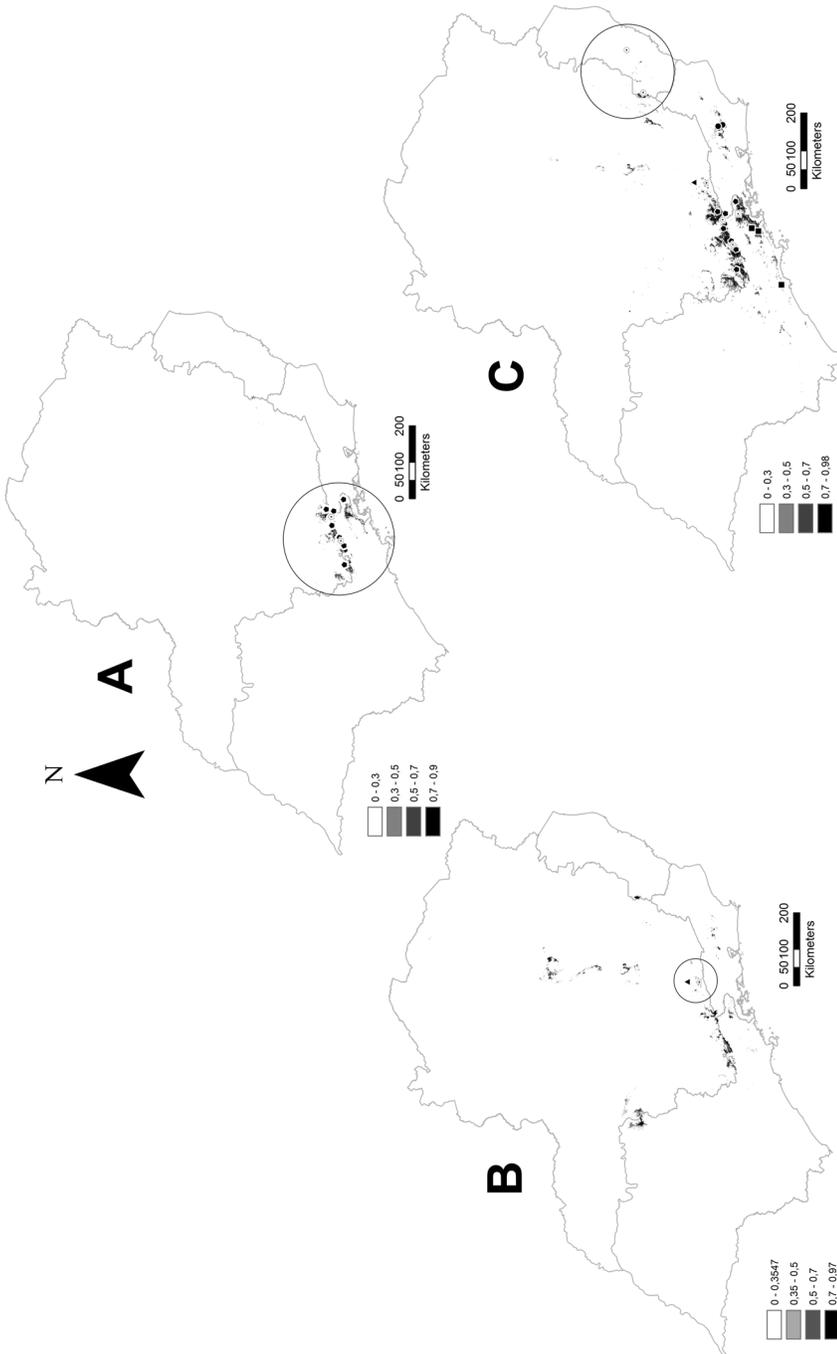
The model generated for the clade (Fig. 2- C) contracted slightly when compared to the previous one, but again presented a low LPT (0,01). The predictor that contributed most kept the same. The suitable areas predicted in Serra do Caparaó expanded discretely (circle in Fig. 2- C).

Discussion

It has been previously demonstrated in literature how ENMs can help to increase the detection rates of rare species in the field (such as in Raxworthy *et al.*, 2003; Kamino *et al.*, 2012; Särkinen *et al.*, 2013) and the MBS approach has proven its efficiency in this scenario (Guisan *et al.*, 2006). Again, the case study reported here corroborates the use of ENM or of SDM methods to locate new populations of rare species from few records.

Nevertheless, the results and models presented were carefully interpreted by taxonomists, with expertise in the focused group, and the targeted areas where new populations were found were carefully chosen, considering each known habitat specificity and the modeled distribution. Although the models guided us to supposedly more assertive decisions, even in restrictive modeled distributions, like Fig. 1- B for *S. kriegeri*, the prediction of areas in the Espinhaço range for example could lead to unsuccessful collection efforts. Based on continued visits

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Figure 1. Study area (up left) with the four states of Southeastern region of Brazil in grey, and maps of potential habitat distribution for: A. *Solanum bradei* Giacomini & Stehmann [the circles indicate the known distribution area (bigger circle) in Mantiqueira range and an unexpected predicted area (smaller circle) in Serra do Caparaó]; B. *S. kriegeri* Giacomini & Stehmann [the circles indicate where the known occurrences lies (bigger circle) at Serra do Ibitipoca and an unexpected predicted area (smaller circle) in Serra do Caparaó] and C. *S. inornatum* species group (the circle indicates the same unexpected predicted area in Serra do Caparaó). The legend represents the degree of suitability predicted: the darker the pixel, the greater the value of environmental suitability. For each model, a different threshold was used (see text for details).



to some areas at Espinhaço and considering that *S. kriegeri* grows in undisturbed dwarf cloud forests, with sandy soils (Giacomin & Stehmann, 2014), and has a very ineffective spreading capacity, it is unlikely that it occur as far north as predicted. This could either represent a case of model overfitting, due a large training area used (Barve *et al.*, 2011), or a simplistic view of factors that act in the distribution range size of species, when considering only bioclimatic predictors (Särkinen *et al.*, 2013).

Even if we consider that caution should be taken when dealing with a small number of records or with biased sampling, our results were encouraging, and the comparison of the modeled distributions presented in Figure 1 and Figure 2 might show a possibly more realistic distribution than the observed, with the herbarium gathered points. The question presented here, if there is still more populations of the species out there and if other predictors should be used to a more reliable prediction, will be left to be answered.

The new populations found for three of the four rare species of the *S. inornatum* species group shows that even areas in the Atlantic Forest that held one of the highest collection/area ratio of the country and where the main herbaria and research institutes are located, might be undercollected. The high number of new species described in the last two decades also indicates that the region is not enough taxonomically known (Sobral & Stehmann, 2009). If we take into account that Atlantic Forest retains only about 10% of its original forest cover (Ribeiro *et al.*, 2009) and holds more than half of all threatened species of the Brazilian flora (Martinelli *et al.*, 2013), efforts to locate new populations of these species are imperative. It is also important to highlight that the majority of the species considered threatened in the Brazilian red list are

← **Figure 2.** Maps of potential habitat distribution, after newly acquired data, for: A. *Solanum bradei* Giacomin & Stehmann (the circle indicates that new records were found only in the same known distribution area in Mantiqueira range); B. *S. kriegeri* Giacomin & Stehmann (again, the circles indicates where the known occurrences lies at Serra do Ibitipoca and Serra Negra) and C. *S. inornatum* species group (the circle indicates a predicted area where a morphologically distinct species was collected, at Serra do Caparaó). The legend represents the degree of suitability predicted: the darker the pixel, the greater the value of environmental suitability. For each model, a different threshold was used (see text for details). The symbols represent *S. bradei* (dark pentagons for points gathered in herbaria; open pentagons for newly acquired points), *S. inornatum* Witasek (dark squares for points gathered in herbaria; open squares for newly acquired points), *S. kriegeri* (dark triangles for points gathered in herbaria; open triangles for newly acquired points), *S. friburgense* Giacomin & Stehmann (dark circles) and a morphologically distinct undescribed species (open hexagons).

known by only few population and the extinction risks were assessed mainly or exclusively by the criterion B of IUCN (IUCN, 2013), based on restrict ranges and decreasing habitat quality. This means that increasing geographic distribution data available can keep or change the conservation status for several species in future reassessments.

Non-geographical biases can also be affecting the lower presence of the specimens in herbaria, once all four species could be considered unattractive plants for botanists (Schmidt-Lebuhn *et al.*, 2013). Most species of *Solanum* are shrubs, found on the border and gaps of forests, and showy whitish corolla contrasting with the yellowish anthers, while the *S. inornatum* group is represented by subshrubs, inhabiting the understory of moist forests, with relatively non-showy flowers (Giacomin & Stehmann, 2014).

In this study we presented an approach not broadly used in literature: to model more than one species together. Our intention while implementing this was to look for an alternative to predict suitable areas for species with so few occurrences that algorithms such as MaxEnt could or should not be used, although other methods can be employed (e.g. Siqueira *et al.*, 2009). For this approach we considered that the species had similar habitats when observed in the field and through information from herbarium labels, and that they were closely related, and therefore correspond to a natural group. The model results led us to find new populations of *S. inornatum*, that could not be modeled alone using MaxEnt, and gave us a clear view of an area in Serra do Caparaó that was supposedly suitable for the group to occur, but no points were known, leading us to an undescribed species discovery.

We accepted that some dimensions of the niche could be conserved among species, if the speciation processes were relatively recent (Peterson *et al.*, 2009). Considering that a huge amount of phylogenetic information has become available in the past decades, it seems that modeling clades could be of some use, to both rare and undescribed species.

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