



# Progressively approaching the distribution of *Passiflora ischnoclada* (Passifloraceae) from a single occurrence record

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**Abstract:** Until recently, *Passiflora ischnoclada* was only known from a single occurrence record. In this paper we describe how different ecological niche modelling techniques were successively used to generate better potential distribution models for the species and guide field work. At each step, new records were found until the species' real distribution was approximated based on a model ensemble created with five different algorithms. The estimated distribution is concentrated on a single area of 84 km<sup>2</sup> where the species is considered endangered.

**Key words:** ecological niche modelling, species distribution modelling, ensemble forecasting, endangered species

*Passiflora ischnoclada* Harms (Harms 1929: 812) (Passifloraceae) is a woody vine species of passion flower from the Neotropics with a peculiar taxonomic history. The species was described using a specimen without any fully developed flowers and without indication of holotype or herbarium (Harms 1929; Bernacci 2001). Almost ten years later, Killip (1938) mentioned that a voucher from the original collection was deposited at B herbarium. Unfortunately that specimen was destroyed during wartime in 1943, and for many years *P. ischnoclada* was incorrectly synonymised with *P. jilekii* Wawra (von Fernsee and Maly 1863: 110–111) (Cervi 1997), which is currently considered a synonym of *P. mediterranea* Vell. (Vellozo 1831: 72) (Cervi and Rodrigues 2010), until another voucher from the original collection was found at SP herbarium (Bernacci 2001).

Despite the historical confusion, *P. ischnoclada* can actually be easily identified in the field for being relatively abundant where it occurs and for its exuberant flowers (November–April; Figure 1) and pinkish fruits (January–April). It can be distinguished from other species of *Passiflora* L. for having filiform stipule, petiole with 1–2

pairs of nectaries, simple entire oval leaves, cylindrical stem and verticillate bracts (Bernacci 2003). However, until recently the only two specimens containing precise coordinates (*H. Lorenzi* 3282, IAC; *I. Cordeiro* & *R. Mello-Silva* 2787, IAC) were collected so close to each other that the species could be considered known from a single locality in the upper montane Atlantic Forest in São Paulo state (SP), Brazil. For this reason, as soon as it was rediscovered, *P. ischnoclada* was immediately considered endangered in SP (SMA 1998; Bernacci 2001, 2003) and in the latest national assessment it was considered critically endangered (Bernacci et al. 2013; MMA 2014).

In cases when so little data is available for a species, such assessments may contain a fair amount of precaution, as species with small population size or restricted distribution are known to face a higher risk of extinction (IUCN 2012, 2014). Moreover, recent



Figure 1. *Passiflora ischnoclada* flower. (Photo: R. Giovanni, December 2010).

studies indicate that rare species also tend to perform irreplaceable ecosystem functions (Mouillot et al. 2013), making them even more important conservation targets. For these reasons, our main goal was to obtain more data for *P. ischnoclada* and get a better approximation of its real distribution.

Since ecological niche modelling can be successfully used to predict and to understand species distributions—including situations when only a few occurrence points are available (Pearson et al. 2007; Siqueira et al. 2009), we used different ecological niche modelling techniques to guide new field work and gradually obtain more occurrence points for the species. The final potential distribution model was post-processed to approximate the real distribution of *P. ischnoclada* for the first time.

Taking the coordinates of one of the original records (23°37'49" S, 045°41'41" W), an environmental dissimilarity model was initially generated with openModeller (Muñoz et al. 2011) using the Euclidean metric. This initial model and all subsequent ones used the same set of environmental layers. Layer selection was carried out through a more general correlation analysis across the Brazilian territory, so that other ecological niche models could be later generated with the same layers for other plant species in Brazil. The analysis was performed with nineteen bioclimatic variables and altitude from WorldClim (Hijmans et al. 2005), as well as potential evapotranspiration and aridity index from the Global High-Resolution Soil-Water Balance dataset (Zomer et al. 2008) — all layers in high resolution (30 arc-seconds). For each high correlation detected between two layers ( $r > 0.75$ , including visual inspection), one of them was discarded, always favouring simpler (non-composite) variables that could better explain plant distribution and facilitate understanding environmental preferences. The final selection consisted of eight variables from WorldClim: mean diurnal temperature range, maximum temperature of warmest month, minimum temperature of coldest month, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, precipitation of coldest quarter and altitude.

Starting with the first model, the main idea was to guide new field work by looking at areas with similar environmental conditions where more individuals or maybe new populations could potentially be found. After each expedition, whenever there were enough new points in distinct pixels to generate another model with more robust algorithms, the new model replaced the old one in subsequent field work. For algorithms that produced continuous output (with the exception of the first environmental dissimilarity model), the projected model was transformed into a binary map showing suitable/unsuitable areas based on the Lowest Presence

Threshold (LPT) above zero, ensuring that all presence points would be inside suitable areas. When more than one algorithm was used, the corresponding resulting models were converted into binary models based on the LPT and then aggregated to build a model ensemble, also known as consensus model (Thuiller 2004; Araújo and New 2007). Only regions with agreement between most algorithms were considered suitable for the species.

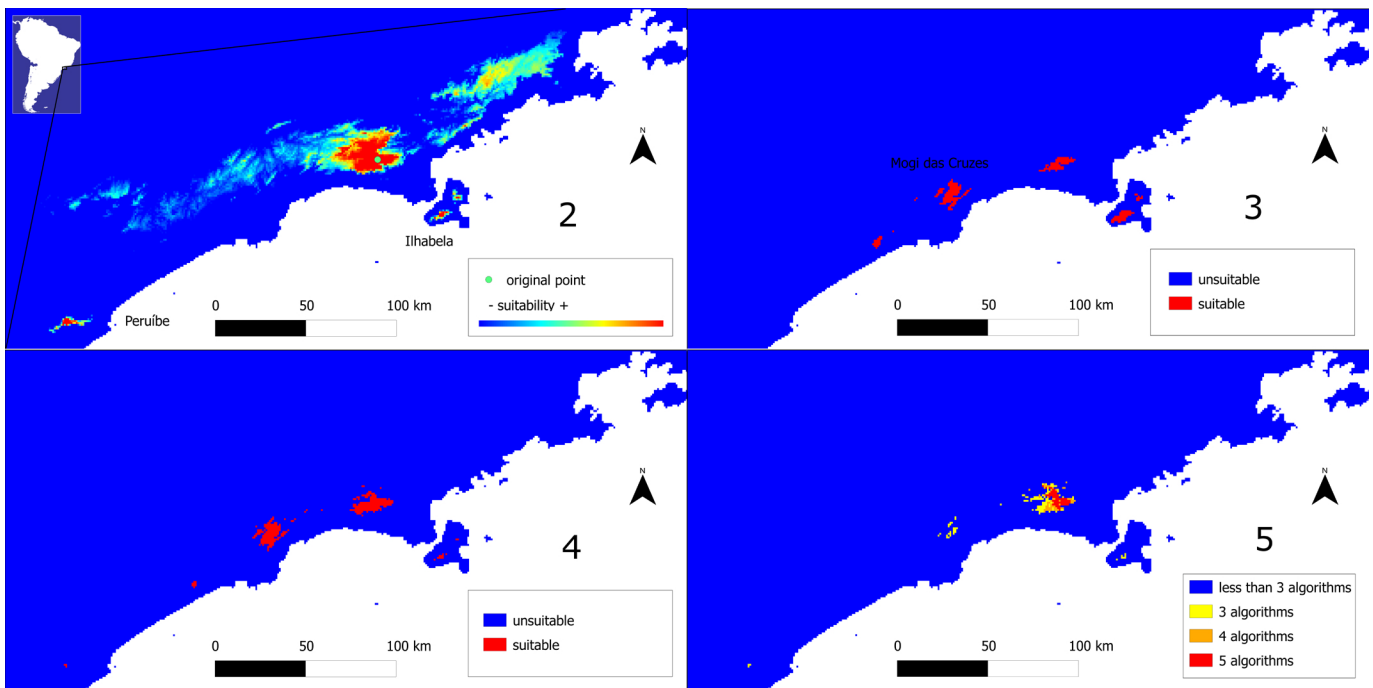
In the end, the estimated real distribution of *P. ischnoclada* was approximated by: 1) taking from the final consensus model only the core area where the species was known to occur (discarding distant predicted areas), 2) removing all disjunct pixels predicted by the model (keeping only a single continuous area), 3) discarding model predictions (suitability values) without any associated species occurrence, 4) merging the remaining model predictions into a single category, 5) transforming the raster into a vector file by taking the outer polygon generated by *gdal\_contour* (GDAL 2015), 6) discarding areas outside vegetation remnants mapped by Fundação SOS Mata Atlântica (SOSMA 2013) and 7) making final adjustments to the resulting polygon by manually treating minor shape singularities, such as removing small internal rings.

All individual models generated in each different context—with the exception of the first model that was based on a single point—were tested for their significance using the leave-one-out procedure (Pearson et al. 2007). Since this test depends on proportional areas of projected models (predicted area divided by the total study area), we assumed a relatively small region around the original point (circular area with a radius of 50 km) to be the study area. Tests were therefore based on model projections limited by that circular mask, which also excluded marine and insular areas.

Additionally, an environmental profile was generated for the species based on the same environmental layers used to generate the models, showing minimum and maximum environmental values considering all occurrence points.

Besides the original location, the initial model (Figure 2) highlighted two other places with very similar environmental conditions: 1) higher elevations in Ilhabela Island, not so far from the original point (45 km), but isolated from it; and 2) another area at the top of Serra do Mar mountain range 190 km away from the original point southwest, at Pedro de Toledo, near Peruíbe. Both regions were visited in a first expedition in February 2010, always searching towards higher elevations such as Baepi peak in Ilhabela, but no individuals were found.

A second expedition in December 2010 used a more conservative approach. Based on the same environmental dissimilarity model, the goal was to find more individuals close to the original location but on



**Figures 2–5.** Potential distribution maps for *Passiflora ischnoclada*. **2:** Environmental dissimilarity model generated with the original point. **3:** Maxent model generated with 8 points and transformed into a binary model based on the LPT. **4:** Consensus model generated with Maxent and GARP BS using 14 points (both models were converted into binary models based on the LPT before aggregation). **5:** Consensus model generated with Maxent, GARP BS, ENFA, Mahalanobis Distance and one-class SVM using 19 points (when necessary, individual models were converted into binary models based on the LPT before aggregation).

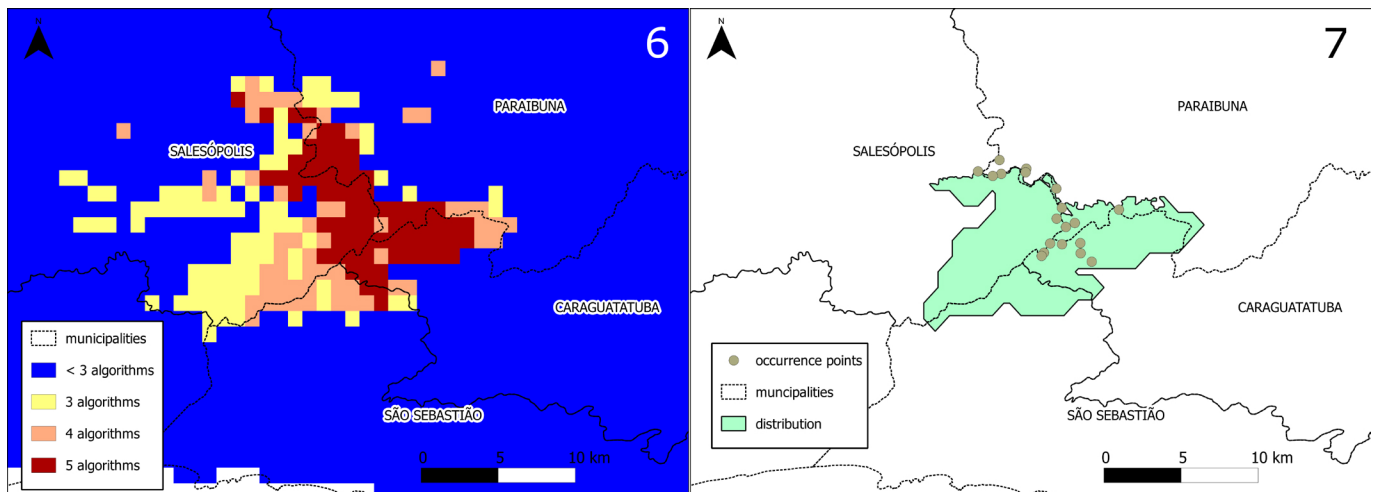
different pixels with distinct environment conditions. This time many individuals were found, seven of them meeting the desired distinct pixel condition (R. Giovanni & L.C. Bernacci 4, 5, 6, IAC; and four recorded observations at 23.62584° S, 045.70009° W; 23.63950° S, 045.69697° W; 23.63886° S, 045.68629° W; and 23.62012° S, 045.69701° W). In one of our searches, we crossed the known area of occurrence at the top of Serra do Mar (1,250 m above sea level [a.s.l.]) and started descending the mountain range looking for individuals along the road. They were continuously found until the altitude of 970 m a.s.l., from where no other individual was observed.

The new set of eight distinct pixels was used to generate another ecological niche model now using Maxent (Phillips et al. 2006). This time a larger area located 60 km southwest of the original point, close to Mogi das Cruzes, was being clearly indicated as suitable for the species (Figure 3). Before exploring it, a third expedition was carried out in March 2012 to find more distinct pixels close to the original location, but now guided by the new model and exploring other paths in different directions. Six individuals were found in the desired conditions (L.C. Bernacci & R. Giovanni 4758, 4760, 4765, 4766, 4768, IAC; and one recorded observation at 23.644004° S, 045.707477° W). That suitable region close to Mogi das Cruzes was later visited on a fourth expedition in April 2013 to Parque das Neblinas (with special permit from Instituto Ecofuturo and Instituto Florestal), with no individuals found, leading to the conclusion that the

species only occurred in a single conjunct area around the original point.

Having now a total of fourteen distinct pixels where the species was known to occur, a new ecological niche model (Figure 4) was created by combining a new Maxent model with a GARP Best Subsets model (Anderson et al. 2003). Two final expeditions (April 2013 and June 2013) were carried out to find more occurrences for the species and generate a better model using more algorithms. The expeditions explored more areas near the borders of the model (outside and inside it), resulting in five new pixels with recorded occurrence (23.594600° S, 045.733364° W; 23.601972° S, 045.732337° W; 23.603089° S, 045.737305° W; 23.600663° S, 045.745882° W; and 23.601188° S, 045.718174° W). Having a total of 19 distinct pixels, a final model was generated with openModeller combining new Maxent and GARP Best Subsets models with ENFA (Hirzel et al. 2002), Mahalanobis Distance (Farber and Kadmon 2003) and one-class Support Vector Machines (Schölkopf et al. 2001) models. This last model (Figures 5 and 6) shows only regions where there is agreement between 5, 4 or 3 algorithms (red, orange and yellow, respectively).

The final estimate for the real distribution of *P. ischnoclada* (Figure 7) includes a continuous area of 84 km<sup>2</sup>, most of it protected by the Parque Estadual da Serra do Mar (87%). Only one specimen was collected on a highly degraded area (fence of eucalyptus plantation) and the corresponding location was left out the



**Figures 6–7.** Approximating the real distribution of *Passiflora ischnoclada* from the potential distribution model. **6:** Native projection of the final consensus model, now showing only the core area where the species is known to occur. **7:** Estimate of the real distribution showing all occurrence points used in the modelling procedure.

estimated distribution for being outside the vegetation remnants map. The environmental profile (Table 1) also reflects the small distribution, showing relatively restricted value ranges.

During the process of estimating the real distribution, when disjunct areas are excluded from the final model projection, the remaining continuous core area can be considered an approximation of the original distribution of the species (before any major human impact). Therefore, when deforested areas were excluded from that core area in the final step, we also got an estimate of habitat loss for the species over the years: 26%.

**Table 1.** Environmental profile for *P. ischnoclada*. Most values are based on the same environmental layers used during the modelling procedure considering all occurrence points, with the only exception of minimum altitude that was replaced with a more precise GPS measurement.

Variable	Minimum	Maximum	Unit
Mean diurnal temperature range	7.6	7.9	°C
Maximum temperature of warmest month	21.6	22.9	°C
Minimum temperature of coldest month	7.4	8.6	°C
Precipitation of wettest quarter	760	791	mm
Precipitation of driest quarter	229	256	mm
Precipitation of warmest quarter	740	775	mm
Precipitation of coldest quarter	229	256	mm
Altitude	970	1243	m

In the end, all algorithms produced significant models ( $p < 0.01$ , Poisson-binomial test; see Table 2). The estimated real distribution, all previous potential distribution models and the occurrence points were made available in a public data repository (Giovanni 2015).

The estimated distribution still contains areas that could not be sampled due to lack of resources and difficult access, although the whole altitudinal gradient was fully inspected in one direction. This is a typical situation where ecological niche modelling can be a valuable technique. Since each algorithm usually requires a minimum number of points to reach an acceptable level of stability and produce more accurate results (Mateo et al. 2010) and considering that existing comparative evaluations between different algorithms are still far from definitive (Peterson et al. 2011), the approach described here tries to gradually obtain more points based on the latest model, until an ensemble model with more algorithms and potentially better results can be generated (see Marmion et al. 2009). However, even when more efficiency can be gained by guiding field work with potential distribution models, obtaining enough points in distinct pixels can be a difficult task depending on the accessibility of the area, species rarity, distribution extent, spatial resolution and resources available. In total, six expeditions were carried

**Table 2.** Model significance calculated with the leave-one-out procedure for each algorithm.

Context	Total points	Algorithm	Correct predictions (leave-one-out)	Average proportional area	Significance
model2	8	Maxent	6	0.028	$p < 0.01$
model3	14	Maxent	13	0.032	$p < 0.01$
		GARP BS	14	0.039	$p < 0.01$
model4	19	Maxent	18	0.026	$p < 0.01$
		GARP BS	19	0.030	$p < 0.01$
		Mahalanobis	14	0.008	$p < 0.01$
		one-class SVM	17	0.012	$p < 0.01$
		ENFA	18	0.039	$p < 0.01$

out to explore some of the regions indicated by models and detect the species in nineteen distinct pixels.

Even considering that most part of the estimated distribution for the species is inside a protected area, given the relatively small predicted range, the history of devastation in the Atlantic Forest (Victor et al. 2005) and the persisting threats on that particular region, such as land pressure, eucalyptus plantation and illegal exploitation of natural resources, *P. ischnoclada* can be considered endangered by IUCN (2012, 2014) criteria (EN-B2ab[ii,iii,iv]). Global warming can be an additional threat—especially in mountainous ecosystems, where species ranges are continuously forced toward higher elevations, eventually leading to mountain-top extinctions (Colwell et al. 2008; Dullinger et al. 2012). A conservation strategy for this species could include specific actions in the management plan of Parque Estadual da Serra do Mar, which encloses most of its distribution and ex situ conservation efforts by local botanical gardens.

This risk assessment differs from the latest Red List of the Brazilian Flora assessment, which estimated an area of occupancy (AOO) of only 8 km<sup>2</sup> and classified it as critically endangered (Bernacci et al. 2013). The Red List of the Brazilian Flora calculates AOOs by counting pixels containing occurrence points and multiplying by a pixel area of 4 km<sup>2</sup> (Martinelli et al. 2013). In large countries where most species are poorly collected, such as Brazil, this approach can easily underestimate the real AOO. In contrast, potential distribution models based on environmental conditions tend to overestimate the real distribution by not taking into account movement and biotic interaction factors (Peterson et al. 2011). When using potential distribution models, more realistic AOOs may be obtained by considering only areas inside vegetation remnants and then removing disjunct areas without any registered occurrence. The same approach can be used when field work is not possible, in which case it is safer (in terms of not overestimating the species range for risk assessment purposes) to assume that the species does not occur in unsurveyed areas far away from known occurrence sites.

While contributing to a better understanding of the distribution of *P. ischnoclada*, this study also provided essential insights to build a new online system dedicated to the biogeography of plants and fungi in Brazil (Biogeo 2015), where other researchers can now use almost the same modelling strategy (algorithms, parameters, environmental layers and model ensembles) to generate ecological niche models for other plant species based on herbarium records, guiding new field work, research and conservation efforts.

### Material examined

*Passiflora ischnoclada*—BRAZIL. SÃO PAULO: Caraguatubá: beira de estrada, 13 December 2010, R.

Giovanni & L.C. Bernacci 5 (IAC 53132); idem, R. Giovanni & L.C. Bernacci 6 (IAC 53133); Paraibuna: em beira de capoeira, 16 April 2002, H. Lorenzi 3282 (IAC 42199); limite com Salesópolis, estrada para a torre de retransmissão, km 49 da Estrada Intermediária, cerca de 21 km do entroncamento com a Rodovia SP-088, 10 October 2003, I. Cordeiro & R. Mello-Silva 2787 (IAC 44081); beira de estrada, 13 December 2010, R. Giovanni & L.C. Bernacci 4 (IAC 53131); Salesópolis: limites do Parque Estadual da Serra do Mar, 17 March 2012, L.C. Bernacci & R. Giovanni 4758 (IAC 54314); idem, L.C. Bernacci & R. Giovanni 4760 (IAC 54316); idem, L.C. Bernacci & R. Giovanni 4765 (IAC 54320); idem, L.C. Bernacci & R. Giovanni 4766 (IAC 54339); fazenda próxima ao Parque Estadual da Serra do Mar, 18 March 2012, L.C. Bernacci & R. Giovanni 4768 (IAC 54341).

### ACKNOWLEDGEMENTS

We are grateful to Marinez Ferreira de Siqueira and Flávia Souza Rocha for all ideas and suggestions and to Daniel A.V. Montero for field assistance during the last expedition.

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**Authors' contribution statement:** RG and LCB collected the data and made the analysis. RG generated the models and wrote the text, which was revised by LCB.

**Received:** 15 April 2015

**Accepted:** 14 July 2015

**Academic editor:** Gustavo Hassemer