

## RESEARCH ARTICLE

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# Effects of pine invasion on land planarian communities in an area covered by *Araucaria* moist forest

Simone Machado de Oliveira<sup>1</sup>, Piter Kehoma Boll<sup>1</sup>, Vanessa dos Anjos Baptista<sup>1,2</sup> and Ana Maria Leal-Zanchet<sup>1\*</sup>**Abstract**

**Background:** Cultivation of exotic pine species has increased in areas of *Araucaria* moist forest, which is an ecosystem with high biological diversity. Land planarians are generally susceptible to replacement of natural habitat by exotic tree species plantations. However, how planarian communities respond to the invasion of exotic species in natural habitats is unknown: (1) Are there differences in richness and abundance of land planarians between sites of *Araucaria* moist forest with (AMF-P) and without (AMF) pine trees and between these areas and pine plantations (P)? (2) Does the species composition of land planarians vary among AMF, AMF-P, and P?

**Results:** This study shows that mean richness and abundance of land planarians per sample unit are higher in AMF sites than AMF-P sites. In addition, AMF sites have a higher estimated richness per sample unit than pine plantations and AMF-P sites. In AMF-P sites, mean richness and abundance are higher in sample units not influenced by pine trees than in those with pine needles. An ordination analysis indicated that the communities of each site are distinct, especially the communities of the AMF and P sites.

**Conclusions:** The present work demonstrated negative impacts from a recent invasion of an exotic species of pine on the richness and abundance of land planarians in a site of *Araucaria* moist forest and significant variation in the planarian community composition between sites of *Araucaria* moist forest and pine plantation.

**Keywords:** Community structure; Species composition; Flatworms; Exotic species; Conservation

**Background**

Until the beginning of the 20th century, *Araucaria* moist forest was listed as critically endangered in terms of environmental conservation (MMA 2002; Guerra et al. 2002; Castella and Brites 2004). It covered about 25% of the state of Rio Grande do Sul, Brazil (Guerra et al. 2002; Koch and Corrêa 2002). It currently covers an estimated 3.25% of Brazil, for a total of 919,565 ha (SEMA/UFSM-RS 2001), only 14.9% of the original vegetation remaining (Mähler Junior and Larocca 2009). One of the causes of this reduced geographic range of *Araucaria* moist forest is its progressive replacement by extensive areas of exotic monocultures, such as pine species (Pinheiro and Ganade 2009).

Although they are economically important, monocultures negatively affect native ecosystems due to their low diversity of plant species (Vallejo et al. 1987). Not only do monocultures potentially negatively affect biodiversity but they also have increased vulnerability to pests and are prone to exotic species invasions (Russel 1989; Engelmark et al. 2001; Pinheiro and Ganade, 2009).

Land planarian communities are highly diverse in areas of *Araucaria* moist forest (Leal-Zanchet and Carbayo 2000; Baptista et al. 2006; Fick et al. 2006; Leal-Zanchet and Froehlich 2006; Antunes et al. 2008; Leal-Zanchet and Baptista 2009; Leal-Zanchet et al. 2011, 2012). They are important elements of forest communities and are considered to be top predators in their microhabitats, where they feed on other invertebrates, such as annelids, gastropods, insect larvae, other arthropods, and even other land planarians (Du Bois-Reymond Marcus 1951; Froehlich 1955; Ogren 1995; Jones and Cumming 1998; Winsor et al. 1998; Sluys 1999; Carbayo

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and Leal-Zanchet 2003; Prasniski and Leal-Zanchet 2009). These flatworms do not have any water retention mechanism and have low tolerance to extreme changes in temperature and humidity. Thus, they are considered to be good bioindicators of the degree of impact on the areas where they are found, and they are used in studies on diversity and conservation (Sluys 1998, 1999; Winsor et al. 1998).

Previous studies have indicated that land planarians are highly sensitive to the replacement of *Araucaria* moist forest by monocultures of exotic species (Carbayo et al. 2002; Fonseca et al. 2009). However, the response of planarian communities to the invasion of exotic species in their original habitat remains unknown. Considering the high diversity of land planarians in areas of *Araucaria* moist forest and the increasing impact of pine plantations on these areas, our study attempts to answer the following questions: (1) Is there a difference in richness and abundance of land planarians between sites of *Araucaria* moist forest with and without pine trees and between these areas and pine plantations? (2) Does species composition of land planarians vary between sites of *Araucaria* moist forest and pine plantations?

## Methods

### Study area

Sampling took place in São Francisco de Paula National Forest, located in the northeastern region of the state of Rio Grande do Sul, Brazil (29°23' to 29°27' S, 50°23' to 50°25' W). It consists of a conservation unit of sustainable use included in an area covered by the Atlantic Forest Biosphere Reserve, a core area that has high to very high conservation priority (MMA 2000). This conservation unit is composed of a heterogeneous mosaic of environments, including remnants of *Araucaria* moist forest, plantations of exotic species of pine (*Pinus* spp.) and eucalyptus (*Eucalyptus* sp.), and plantations of the native species *Araucaria angustifolia*. It has a total area of 1,606 ha and altitude higher than 900 m, with an altitudinal variation of 300 m. The climate is Cfb (i.e., mesothermic, super humid, with mild summer and cold winter), mean annual temperature is 14.5°C, and mean annual rainfall is 2,252 mm (Backes 1999; ICM-BIO -unpublished data).

### Sampling design

Three sites were sampled in São Francisco de Paula National Forest. Two sites were composed of *Araucaria* moist forest, located on opposite sides (west and east) of a 0.5- to 1-m wide stream, and the third was composed of an exotic pine species plantation. Each site had an area of 0.5 ha and was subdivided into 200 5 m × 5 m plots, from which 50 were randomly selected in each site for sampling land planarians.

Both sites of the *Araucaria* moist forest slope slightly towards the stream and have different structural features. The western site (AMF) is dominated by plants in the canopy layer and almost completely lacks an understory. The eastern site (AMF-P) is dominated by young plants that form a dense understory and suffers from the invasion of an exotic pine species. Part of the site is directly affected by this impact. The third site (P), a pine plantation, is located about 2.5 km east of the *Araucaria* moist forest sites, has a well-developed understory, and is surrounded by areas with native forest and *Araucaria* plantations. Site P differs from AMF-P by lower abundance and richness of tree species associated with pine trees and by a thicker leaf litter layer, due to the amount of pine needles on the soil.

Each site was sampled five times between January and July 2010. In each plot, divided into four quadrants, land planarians were sampled by four previously trained persons by directly searching in the mainland planarian refuges: under stones, logs, fallen branches, and leaf litter.

**Table 1 Land planarian richness and abundance at three sites in a southern Brazilian conservation unit**

	AMF	AMF-P	P	Total
<i>Obama ladislavii</i> (Graff, 1899)	26	16	11	53
<i>Obama</i> sp. 1	19	11	9	39
<i>Luteostriata ernesti</i> (Leal-Zanchet & Froehlich, 2006)	0	0	36	36
<i>Paraba franciscana</i> (Carbayo & Leal-Zanchet, 2001)	28	6	1	35
<i>Obama josefi</i> (Carbayo & Leal-Zanchet, 2001)	22	2	1	25
<i>Choeradoplana iheringi</i> (Graff, 1899)	4	6	2	12
<i>Matuxia</i> sp.	4	3	5	12
<i>Choeradoplana</i> sp.	2	5	4	11
<i>Pasipha backesi</i> (Leal-Zanchet et al., 2012)	0	0	9	9
<i>Luteostriata graffi</i> (Leal-Zanchet & Froehlich, 2006)	3	3	2	8
<i>Cratera</i> sp.	0	7	0	7
<i>Pseudogeoplana</i> sp. 1	0	2	5	7
<i>Obama</i> sp. 2	2	2	2	6
<i>Pasipha</i> sp.	5	1	0	6
<i>Pseudogeoplana</i> sp. 4	5	1	0	6
<i>Luteostriata arturi</i> (Lemos & Leal-Zanchet, 2008)	1	2	1	4
<i>Obama</i> sp. 3	2	2	0	4
<i>Pasipha brevilineata</i> (Leal-Zanchet et al., 2012)	0	0	3	3
<i>Supramontana irritata</i> (Carbayo & Leal-Zanchet, 2003)	0	3	0	3
<i>Pseudogeoplana</i> sp. 2	0	0	2	2
<i>Rhynchodemus</i> sp.	0	1	1	2
<i>Xerapoa</i> sp.	0	2	0	2
<i>Cephaloflexa araucariana</i> (Carbayo & Leal-Zanchet, 2003)	0	0	1	1
<i>Pseudogeoplana</i> sp. 3	1	0	0	1
Unidentified specimens	8	1	13	22
Total	132	76	108	316

AMF, *Araucaria* moist forest; AMF-P, *Araucaria* moist forest with pine needles; P, pine plantation.

A sampling effort of 10 min was used for each plot in each round of sampling.

The animals were identified in the field, whenever possible, according to characteristics of external morphology, such as color pattern, size, and body form. In the lab, the animals were measured and the analysis of the external morphology was complemented. Specimens were fixed with 10% formalin. For identification at species level, histological preparations of the copulatory apparatus and the pharynx were made. Adult specimens without any of the morphological characteristics required for identification at the genus level, which could be differentiated from specimens of other species occurring in the study area, were placed in the collective group *Pseudogeoplana* Ogren and Kawakatsu, 1990. Unidentified flatworms were either immature specimens or those lost before fixation. Voucher specimens of each species were deposited in the scientific collection of the laboratory (Instituto de Pesquisas de Planárias, UNISINOS, São Leopoldo, Brazil) in order to do morphologic and/or taxonomic studies.

#### Data analysis

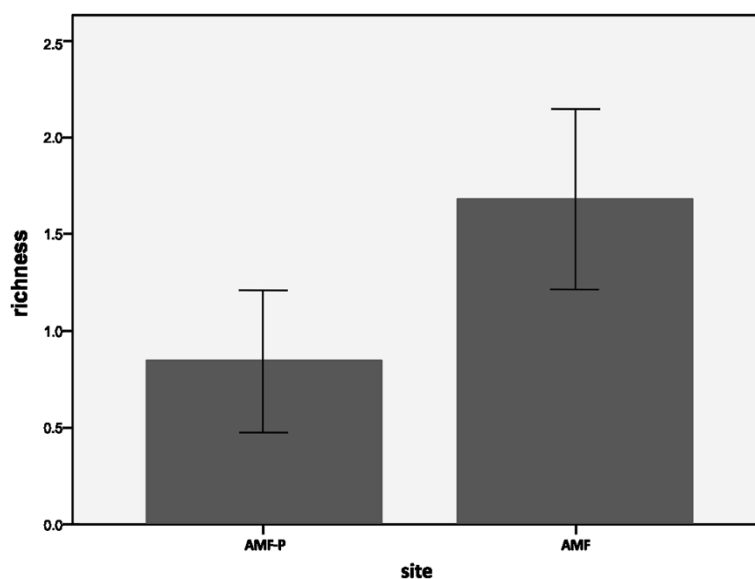
The analyses were performed at site level (alpha diversity) using the sample units (plots) as repetitions. To verify the existence of significant differences in mean richness and abundance of land planarians by plot in the three study sites, an analysis of variance (ANOVA) was applied, followed by a Tukey test (with SPSS-19; IMB Corporation, Somers, NY, USA). Rarefaction curves based on individuals were built with EcoSim, version 7.72 (Gotelli and Entsminger 2012), in order to correct

richness to the same abundance of specimens, aiming to compare the communities of study sites. The specimens were used as sample units to build the curves, thus more clearly showing richness patterns (Gotelli and Colwell 2001). The Chao2 method with EstimateS (Colwell 2005) was used to estimate richness by site. Unidentified specimens were considered only in the calculation of abundance.

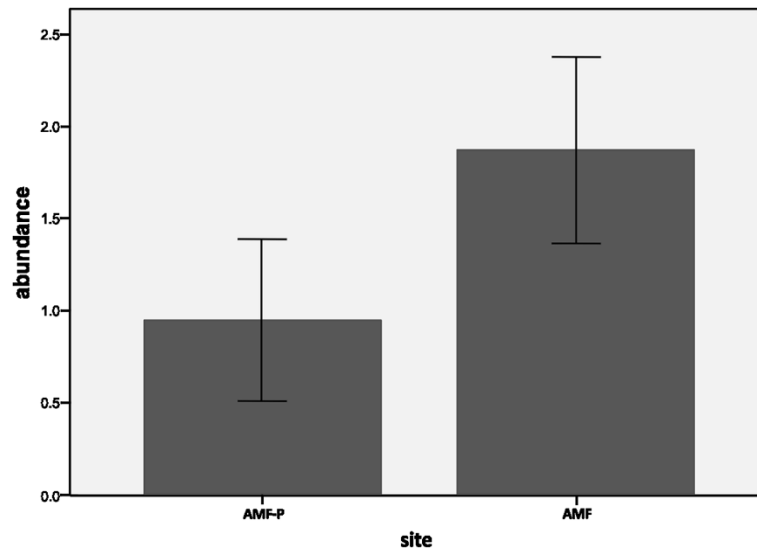
An ordination analysis (nonmetric multidimensional scaling (NMDS)), using species abundance data, was applied to explore differences in flatworm assemblage composition. NMDS was carried out with R-software, version 2.15, on a similarity matrix based on the Bray-Curtis dissimilarity measure. Data were logarithmized and relativized. Species with abundance lower than 0.9% of the total of individuals were excluded from the analysis in order to reduce ordination stress. Only stress values lower than 0.20 provide an adequate description of the distance matrix (Legendre and Anderson 1999). The Berger-Parker dominance index was used to verify the relative importance of the most abundant species of land planarians in the studied sites.

#### Results

In the three study sites, 316 specimens, distributed over 24 flatworm species, were sampled (Table 1). The mean richness ( $F_{(1,2)}$ , 3.885;  $p = 0.017$ ) and mean abundance ( $F_{(1,2)}$ , 6.698;  $p = 0.001$ ) of land planarians per sample unit were higher at the site not influenced by pines (AMF) than at the site influenced by pines (AMF-P), and there were no significant differences between the pine plantation site (P) and AMF or AMF-P sites. In AMF-P, mean richness ( $t$  test,  $p = 0.006$ ) and mean abundance



**Figure 1** Mean richness of land flatworms per sample unit in plots with and without *Pinus* needles. AMF, *Araucaria* moist forest; AMF-P, *Araucaria* moist forest with pine needles.  $P = 0.006$ .



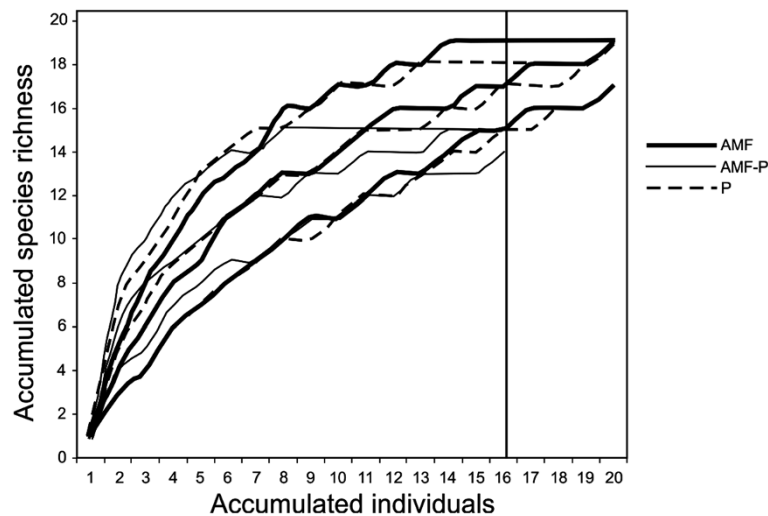
**Figure 2** Mean abundance of land flatworms per sample unit in plots with and without *Pinus* needles. In a southern Brazilian conservation unit. AMF, *Araucaria* moist forest; AMF-P, *Araucaria* moist forest with pine needles.  $P = 0.007$ .

( $t$  test,  $p = 0.007$ ) were higher in plots not influenced by pines than in those with pine needles (Figures 1 and 2).

Considering total species richness per site, the observed richness at sites AMF-P and P were similar and higher than what was observed in AMF. The rarefaction curve, with corrected richness under the same abundance, supported these results (Figure 3). However, taking into account the results per sample unit in each studied site, AMF showed a higher estimated richness. Thus, a larger species increment was estimated for AME, followed by P and AMF-P (Table 2).

#### Composition of the communities

The recorded species are distributed among 13 genera and a collective group. *Obama* and *Luteostriata* were the richest genera (Table 1). The three sites had ten species in common, but the dominant species were different for each site. In AME, *Paraba franciscana* ( $n = 28$ ) and *Obama ladislavii* ( $n = 26$ ) showed dominance indices of 21% and 19%, respectively, while in AMF-P, *O. ladislavii* ( $n = 16$ ) and *Obama* sp. 1 ( $n = 11$ ) showed dominance indices of 21% and 14%, respectively. In P, the dominant species was *Luteostriata ernesti* ( $n = 26$ ), with a dominance index of



**Figure 3** Species accumulation curves with confidence intervals for three sites in a southern Brazilian conservation unit. AMF, *Araucaria* moist forest; AMF-P, *Araucaria* moist forest with pine needles; P, pine plantation.

**Table 2 Species richness and abundance of land planarians in a southern Brazilian conservation unit**

	AMF	AMF-P	P
Abundance	133	76	108
Average abundance per plot	2.66	1.52	2.16
Observed richness	14	18	17
Average richness per plot	2.02	1.36	1.64
Estimated richness (Chao 2)	113.2	24.5	29
Species increment (%) (Chao 2)	708.60%	36.10%	70.60%

AMF, *Araucaria* moist forest; AMF-P, *Araucaria* moist forest with pine needles; P, pine plantation.

33%, while *O. ladislavii* was the second most abundant flatworm ( $n = 11$ ), with 10% dominance.

Some species had very different dominance indices between the sites. *P. franciscana* had a high dominance in AMF but a dominance index of only 8% in AMF-P; *Obama josefi*, the third most dominant species in AMF (17%), had only 3% dominance in AMF-P. In pine plantations, both species had dominance lower than 1%, with only one specimen recorded, while *L. ernesti* was recorded only in P.

The ordination analysis supports these results by revealing a significant separation of land planarian communities between sites of *Araucaria* moist forest and pine plantation (Figure 4), indicating that the communities from the three sites are distinct. The ordination stress was 0.09, which ensures reliable interpretation of the results.

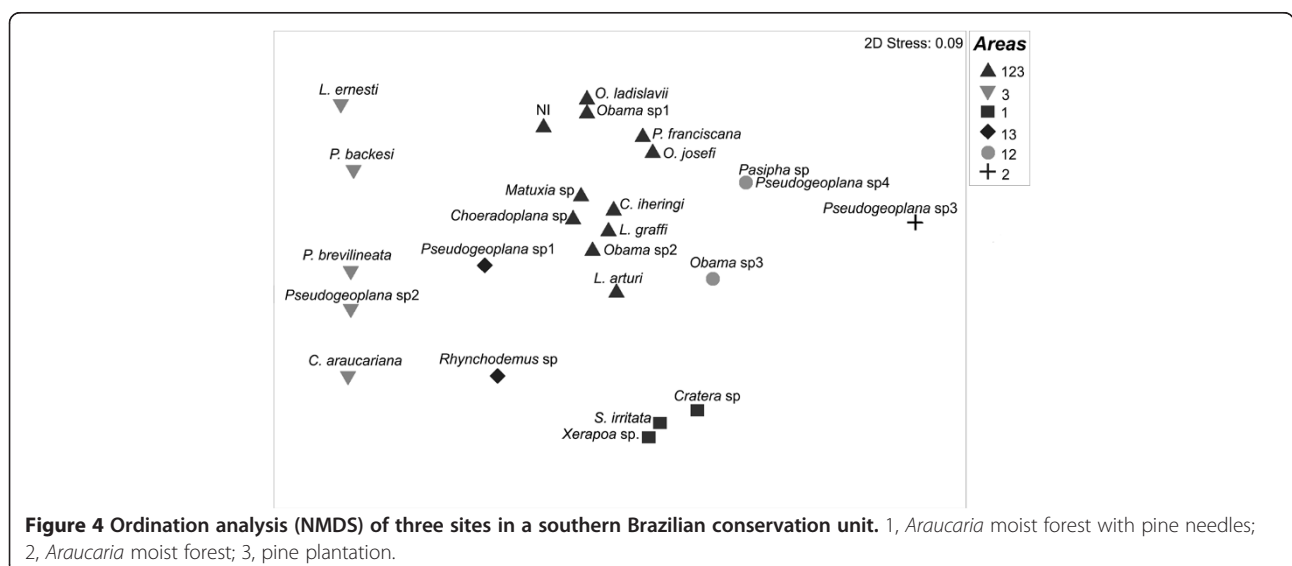
## Discussion

The results indicate the existence of significant differences in species richness and mean abundance of land planarians per sample unit between the sites of *Araucaria* moist

forest. The alterations in microhabitat caused by the accumulation of pine needles on the soil in AMF-P, which changed the microclimate, probably caused the differences found for these two sites. Similar situations have been found in other studies, where the abundance of several invertebrate groups was reduced in plantations where values of pH, humidity, and C:N ratios were altered by the different leaf litter composition (Bonham et al. 2002; Robson et al. 2009; Castro-Díez et al. 2012).

The impact on the land planarian community was clear in AMF-P plots without pine, which had significantly higher values of richness and abundance than plots with pine. However, total richness per site was probably not influenced since the invasion was recent and the surrounding areas are conserved. In other studies, these characteristics were important for diversity in areas dominated by exotic species (Bonham et al. 2002). Furthermore, AMF-P has a more developed understory than AMF, which may favor a higher richness in AMF-P.

Mean richness and abundance per sample unit in the pine plantation did not differ significantly from the other sites, probably due to the occurrence of intact native forest in the surrounding area. This intactness is a result of the plantation being about 40 years old and having a well-developed understory. Thus, it seems to be in a secondary stage of ecological succession. In fact, understory diversity is an important factor for diversity conservation in plantations (Humphrey et al. 1999). Older plantations also support a larger number of native terrestrial invertebrate species (Bonham et al. 2002). Additionally, the small extension of pine plantations in the study area and the dispersal ability of land planarians (Carbayo et al. 2002) together with the landscape features of this conservation unit (Fonseca et al. 2009) facilitate occupation of these sites by planarians. Nevertheless, the land





planarian communities are highly sensitive to the replacement of *Araucaria* moist forest by monocultures of exotic species (Carbayo et al. 2002; Fonseca et al. 2009).

With respect to species composition, the community in the pine plantation clearly differed from that of the *Araucaria* forest. Five species were exclusive to the pine plantation, and *L. ernesti* was dominant and only recorded in this site. However, previous studies have recorded a low abundance of *L. ernesti* in *Araucaria* moist forest (Carbayo et al. 2002; Antunes et al. 2012).

Among the dominant species in the *Araucaria* forest sites, the generalist species *O. ladislavii* (Carbayo et al. 2002; Fick et al. 2006) has a high dominance index at both sites and at the pine plantation. These results suggest that the structural differences between sites did not affect populations of *O. ladislavii*. Similar results were found in a previous study in the same study area (Carbayo et al. 2002). In studies at a larger spatial scale, *O. ladislavii* abundance was low at other sites of *Araucaria* moist forest (Leal-Zanchet and Baptista 2009; Leal-Zanchet et al. 2011).

*P. franciscana* and *O. josefi*, the first and third most dominant species in AMF, respectively, are among the five most abundant species in areas of *Araucaria* moist forest (Leal-Zanchet et al. 2011). Their low dominance or abundance in AMF-P and P suggest that both may prefer preserved areas, which has already been indicated for *P. franciscana* (Carbayo et al. 2002).

## Conclusion

This work demonstrated negative impacts of the recent invasion of an exotic pine species on the richness and abundance of land planarians at a site of *Araucaria* moist forest and also revealed significant variation in community composition between *Araucaria* moist forest and pine plantations. However, the factors that influence land planarian species composition at sites with different degrees of impact and in areas with exotic tree species are still unknown, in addition to the microhabitat features that may influence occupation by land planarian species.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

SMO participated in the field samplings, made the histological preparations, performed part of the statistical analyses, made the figures, and drafted the manuscript. PKB participated in the field samplings, contributed in the statistical analyses, edited the figures, and reviewed the manuscript. VAB performed part of the statistical analyses and reviewed the manuscript. AMLZ conceived the study, made intellectual contributions, determined the sampling design, coordinated the study, and reviewed the manuscript. All authors read and approved the final manuscript.

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