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Population dynamics of the southern short-tailed opossum (*Monodelphis dimidiata*) in the Pampas of Argentina

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Abstract. The genus *Monodelphis* is one of the most species rich among Neotropical marsupials. Despite this, little is known about most of the species. One of the most enigmatic species is *M. dimidiata*, a small terrestrial opossum that inhabits the Pampean region of Argentina, which is suspected to be a semelparous breeder. From 2005 to 2008, we conducted seasonal live trapping in near-pristine marshy grasslands and agroecosystems of the Pampean region in order to evaluate the population trends of this species and the occurrence of semelparity. *M. dimidiata* was characterised by a low abundance in the study area. The average density was higher in grasslands than in agroecosystems, and it appeared to be influenced by vegetative cover. The onset of the breeding season occurred during spring when the opossums showed a sudden increase in body size. Given that mature individuals were not found beyond autumn, the findings suggest an annual cycle for this species. In addition, this abrupt maturation resulted in a pronounced sexual dimorphism that, together with the strong reproductive seasonality and a likely polygynous mating system, supported previous claims that the species is semelparous. Our results also emphasise the importance of native grassland habitat for the maintenance of stable populations of *M. dimidiata*.

Additional keywords: marsupial, neotropical, Pampean grasslands, semelparity, South America.

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Introduction

South American and Australian marsupials share many aspects of their life history and ecology (Springer *et al.* 1998). Fossil and molecular evidences indicate that marsupials were originally distributed in Gondwana, and that Antartica may have served as the bridge for the earliest marsupials' transit between South America and Australia (Redford and Eisenberg 1992; Nilsson *et al.* 2004; Beck *et al.* 2008). The subsequent geographic isolation resulted in convergent evolution, with different marsupial groups occupying similar adaptative zones in the Neotropical and Australasian regions (Tyndale-Biscoe 2005). Although extant marsupials are well represented in both regions, Australasian marsupials have been more intensively studied, with much less known about the basic biology of Neotropical species.

It has been observed that both Australasian and Neotropical marsupials may show an array of breeding strategies, with iteroparous species (i.e. reproducing many times and participating in several breeding seasons) on one extreme, and semelparous species (i.e. reproducing once in a lifetime) on the opposite end of the spectrum (Morton *et al.* 1989; Taggart *et al.*

1997). Although the majority of species are iteroparous in both regions, a subset of species show semelparous life cycles. Australian marsupials show a special type of semelparity known as 'male die-off', in which only males die after one breeding event and most females may survive more than one breeding season and reproduce more than once (Tyndale-Biscoe 2005). In comparison, all Neotropical opossums that have been reported as semelparous breeders show massive post-mating mortality in both sexes (McAllan 2008).

Currently, three orders of marsupials are found in the Neotropics: Didelphimorphia (opossums and mouse opossums), Paucituberculata (shrew opossums) and Microbiotheria (a monotypic order represented by *Dromiciops gliroides*) (Palma 2003). The didelphimorphs are the most diverse and widely distributed of the Neotropical marsupials, occupying nearly all habitat types (Redford and Eisenberg 1992). Among them, the short-tailed opossums of the genus *Monodelphis* are the most diverse both in species number and in the variety of habitats occupied (Gardner 1993; Patton and Costa 2003; Solari 2010). The 22 species of short-tailed opossums are found in forests and grasslands from Panama to Argentina, where

they appear to occupy the small terrestrial, insectivorous and omnivorous mammal niche (Redford and Eisenberg 1992; Flores *et al.* 2007; Pine and Handley 2008). Despite being diverse and widespread, *Monodelphis* is one of the least known didelphid genera in respect to both the ecology and geographic distribution (Patton and Costa 2003).

Seven species of Monodelphis have been recorded for the southern Cone of South America. The best known species in the genus is *M. domestica*, which has been the object of several studies mainly in Brazil (e.g. Bergallo and Cerqueira 1994). The southern short-tailed opossum (M. dimidiata) is the southernmost species in the genus. Its distribution includes southern Brazil and Uruguay, and it is the only Monodelphis that extends to the Pampas region of Argentina (Redford and Eisenberg 1992; Nowak 1999; Massoia et al. 2000). This region is characterised by extensive areas of native grasslands and has a conspicuous and unique biodiversity, including nearly a hundred species of mammals (Bilenca and Miñarro 2004). M. dimidiata is considered a typical grassland opossum, although it has also been found sporadically in other environments, such as crops and pastures, creek edges, and rocky areas (Reig 1964; Ojeda and Mares 1989; Bárquez et al. 1991; Redford and Eisenberg 1992; Comparatore et al. 1996; Morando and Polop 1997; Altrichter et al. 2001; Bó et al. 2002; Pardiñas et al. 2004; Gardner 2008). In spite of being the second best known species in the genus, little is known about the population dynamics and ecology of M. dimidiata, perhaps because of its low densities and the difficulty of observing individuals in the field (Reig 1964).

Monodelphis dimidiata has a very short life span and it is believed to be a semelparous breeder (Pine et al. 1985). Semelparity was first proposed for this opossum by Reig (1964) and, more importantly, it was the first report of this sort of life history trait for any mammalian species (Pine et al. 1985). On the basis of field observations, these authors indicate that males die during the summer shortly after a sharp seasonal reproductive period, and adult females, after rearing their litters during early autumn, do not survive to reproduce in the following year. In addition, they found that individuals undergo a sudden change in body size during the mating period when they reached maturity (Reig 1964, 1965; Pine et al. 1985). They concluded that this species was an 'apparently' semelparous annual marsupial. Although the data from these studies were provocative, they were not specifically designed to evaluating the existence of semelparity, and Redford and Eisenberg (1992) suggested that more detailed laboratory and field studies were needed. Afterwards, a study performed in captivity found that adult individuals showed senescence, a trait compatible with a semelparous life cycle (González and Claramut 2000). However, no studies have been conducted explicitly to evaluate the occurrence of semelparity and the life-history traits linked to this unusual reproductive mode in wild populations of M. dimidiata. Interestingly, semelparity seems to be a widespread strategy among Australasian dasyurids. Massive post-mating male mortality has been reported in several dayurid genera (e.g. Phascogale, Dasykaluta, Parantechinus, Dasyurus), including Antechinus, a genus that shows similar habits and ecology to Monodelphis (Tyndale-Biscoe 2005). Although breeding strategies of Neotropical species are much less known, similar life-history traits have been found in several related didelphid

genera. For instance, two species of *Marmosops* have been demostrated to be obligate semelparous breeders (Lorini *et al.* 1994; Leiner *et al.* 2008), and one species of *Gracilinanus* has been claimed to be a partial semelparous breeder (i.e. only some populations and years) (Martins *et al.* 2006*a*, 2006*b*).

We undertook a study aimed at assessing the population dynamics of *M. dimidiata* in the Pampas of Argentina in order to evaluate its population trends and the main life-history traits associated to this reproductive mode. In particular, if a semelparous life cycle occurs in this species, we expect to find that all individuals belonging to one breeding year disappear from the population in the following year.

Materials and methods

Study area

The study was carried out in Mar Chiquita Biosphere Reserve (south-eastern Buenos Aires Province, Argentina; 37°32'- $37^{\circ}45'$ S, $57^{\circ}19'-57^{\circ}26'$ W). It is located in the biome known as Rio de la Plata Grasslands, a prairie of temperate grasslands that originally covered the plains of South America (Soriano et al. 1991) and represents one of the most extensive areas of grasslands of the world (Chaneton et al. 2002). The study area includes the subregion of the Flooding Pampas (sensu Soriano et al. 1991), which is characterised by lowlands plains and the occurrence of periodic floodings, the intensity and duration of which change according to topography. The Mar Chiquita Biosphere Reserve consists of both governmental and private domain areas. The core zone is a publicly-owned restricted area that consists of a coastal lagoon surrounded by marshes and grasslands (8600 ha: Isacch 2001). Surrounding this section, there is a multiple-use zone with privately-owned ranches dedicated mainly to livestock production, which comprises ~60% of the reserve area (16 200 ha: Isacch 2008).

In the restricted area the vegetation is almost pristine and shows zonation determined by the chemical properties of the soil and by the structure of the landscape (Vervoorst 1967; Perelman et al. 2001; Chaneton et al. 2002). There are three main halophitic plant communities that occur through the low, middle, and upper marsh zones. In the flood-prone areas around the lagoon shoreline, there is low intertidal marsh vegetation, characterised by the presence of cord grass (Spartina densiflora). The middle marsh, which is inundated only temporarily, supports brackish vegetation with spiny rush (Juncus acutus) as the dominant species. In the upper marsh, where inundations are infrequent, the halophitic vegetation is dominated by extensive areas of Pampas grass (Cortaderia selloana) (Vervoorst 1967; Isacch et al. 2006). The privately-owned areas are located on the highland plains, which have been extensively modified by agricultural activities. Since cattle grazing is the main form of agriculture, most of this land is devoted to pastureland and, to a lesser extent, to cropland that is cultivated for corn, soybean and wheat production (Isacch 2008).

Capture of individuals

From 2005 to 2008, we performed seasonal capture–mark– recapture censuses in the study area (Gurnell and Flowerdew 1994). Trapping sessions were designed to cover three breeding cycles of *M. dimidiata* (i.e. winter of one year to autumn of the

following year), thus completing four sessions for each breeding year and 12 for the entire period. During each trapping session, we set 180 Sherman traps distributed in five square grids. Each grid consisted of 6×6 trap-stations 10 m apart from each other, and at each trap-station one trap was located on the ground hidden by vegetation. We baited each trap with rolled oats coated in beef fat, and we provided synthetic bedding material as needed for protection from heat and cold (Coppeto et al. 2006). Seasonal trapping sessions consisted of four consecutive nights, and traps were checked for captures every day early in the morning (Gurnell and Flowerdew 1994). We adhered to guidelines for use of animals in research and to the legal requirements of our country. In addition, this work has been approved by the Institutional Ethics Committee and Review Board of the National Council of Scientific and Technical Investigations (CONICET) and by the National University of Mar del Plata. Our trapping effort was designed to cover the natural areas as well as the agriculturally modified areas in the study area. We established three grids in near-pristine grassland areas following the natural zonation of the marsh (Isacch et al. 2006). One grid was set in the lowland marsh (cord grass patch), another grid was set at middle brackish marsh (spiny rush patch) and the third grid was set in the upper marsh (Pampas grass patch). Two other grids were located in agriculturally modified areas: one plot used for cattle grazing (pastureland patch) and other grid in a cornfield.

Each captured individual was removed from the trap and lightly sedated with ether to reduce stress during the handling procedure (Schubert et al. 2009). We recorded grid number, sex, and reproductive condition of each individual. The reproductive condition (mature or inmature) was determined on the basis of hair coloration (adult individuals have a darker coat and lack the reddish cast of immature individuals: Pine et al. 1985) and external characters, taking into account the state of the vagina, nipples and pregnancy as indicators of mature females, and the relative size of testes for mature males (Gurnell and Flowerdew 1994). We also recorded standard morphometric measures of each individual including total length (TL) and tail length, which were measured to the nearest millimetre using a plastic rule, and the weight (W) was measured to the nearest gram using a spring scale. In addition, each individual was marked using numbered metallic ear tags. In all cases, sedating, measuring and attaching the ear tag took less than 2 min. After that, we released the animal in the same trap-station where it was captured (Gurnell and Flowerdew 1994).

Data analysis

We estimated abundance of the *M. dimidiata* population for each habitat and each season by means of the Jackknife method (Manning *et al.* 1995; Williams *et al.* 2002), using the program CAPTURE2 (Rexstad and Burnham 1991). From this information we estimated density (number of individuals per hectare) for each habitat and season, accounting for edge effects (Wilson and Anderson 1985). To evaluate the spatial and temporal variability of the *M. dimidiata* population, we compared density among habitats and breeding years (i.e. from winter to autumn in the following year) using two-way ANOVA followed by an all pairwise multiple-comparison procedure (Student–Newman–Keuls method: Zar 2010). In addition, the recapture

rate was calculated as the ratio between the number of recaptured individuals and the total number of captured individuals, expressed as a percentage (D'Andrea *et al.* 1999).

We analysed the differences in body measures (TL and W) of opossums for the different combinations of sex and reproductive condition, using two-way ANOVA followed by an all pairwise multiple-comparison procedure (Student–Newman– Keuls method: Zar 2010). Data were transformed as needed in order to fulfill test assumptions. Furthermore, we plotted the relationship between TL and W for the entire population and for each sex. The regression slopes of log-transformed variables were compared between sexes using ANCOVA (Zar 2010).

We calculated the proportion of mature/immature individuals for each season (Krebs 1989). Due to our limited sample size, seasonal captures over all habitats were grouped to allow seasonal comparisons. In addition, we compared the changes in sex ratio in order to determine biases from the expected ratio 1 : 1 (Krebs 1989). In this case, seasonal captures from the same breeding year were grouped to allow comparisons. These analyses were performed using Chi-square tests (Zar 2010). Finally, the variation in TL and W was analysed by season and compared using Kruskal–Wallis tests and Dunn's multiple comparisons (Zar 2010). For all analyses we used R software, ver. 2.7.0 (R Development Core Team 2008).

Results

Seventy-five individuals of *M. dimidiata* were captured and marked, 26 females, 45 males and 4 unsexed juveniles. The mean seasonal density of *M. dimidiata* for the entire sampling area was quite low, averaging 5.1 ha⁻¹ (\pm 4.9, s.d.). Marked individuals were rarely recaptured ($6.3\% \pm 9\%$, s.d.). However, it is important to note that all recapture events always occurred within the same breeding year (i.e. no one individual belonging to one cohort was recaptured in the following year).

Abundance of *M. dimidiata* was variable both at the temporal and spatial level (Fig. 1). We found that this species occurred at greatest density in Pampas grass, intermediate in density in spiny rush, and lowest in density in cord grass and pastureland $(F_{3,43}=11.24, P<0.001)$. No opossums were captured in the cornfield. We also found differences among years, with the second breeding year showing higher density than the third breeding year $(F_{2,43}=4.48, P<0.019)$. There was no statistically significant interaction between habitat and breeding year (P=0.913).

Mature individuals were larger in size than immature ones ($F_{2,68} = 53.2$, P < 0.0001), and males were larger than females ($F_{2,68} = 1083.9$, P < 0.0001). Interaction between maturity and sex also showed significant differences for both TL and W ($F_{2,68} = 38.9$, P < 0.0001). Post hoc comparisons indicated that mature males were significantly larger than mature females and immature individuals of both sexes (P < 0.001), whereas mature females showed intermediate size and were significantly larger than immature individuals (P < 0.001) (Table 1). We did not find significant differences in TL and W values between immature males and immature females (TL: P = 0.06; W: P = 0.83).

We found a significant relationship between TL and W variables ($R^2 = 0.87$, $F_{71} = 483.4$, P < 0.0001) (Fig. 2). Body size showed an abrupt change between 125 and 160 mm TL for



Fig. 1. Seasonal variation of the abundance $(\pm s.e.)$ of southern short-tailed opossum (*Monodelphis dimidiata*) during three breeding years (winter 2005 to autum 2008) in the Flooding Pampas of Argentina.

 Table 1.
 Morphometric measurements by age and sex of the southern short-tailed opossums (Monodelphis dimidiata) caught during the sampling period in the Flooding Pampas of Argentina

 Values are given as mean ± s.d.

Sex	п	Total length (mm)			Weight (g)		
		Inmature	Mature	Overall mean	Inmature	Mature	Overall mean
Female	26	108.5 ± 7.3	172.6 ± 9.1	129.0 ± 31.5	17 ± 3.5	50.4 ± 5.8	28 ± 16.6
Male	46	112.9 ± 6.6	200.8 ± 24.4	140.3 ± 43.6	16 ± 3.1	91.3 ± 22.4	40 ± 37.2
Overall mean	72	111.4 ± 7.1	190.5 ± 24.3	136.2 ± 39.8	17 ± 3.3	76.4 ± 27.0	35 ± 31.9



Fig. 2. Relationship between total length (TL) and weight (W) of the southern short-tailed opossums (*Monodelphis dimidiata*) caught during the sampling period in the Flooding Pampas of Argentina.

females and between 127 and 182 mm TL for males. This change was even more evident for W, with a threshold at 23–37 g for females and 26–71 g for males (Fig. 2). Slope values for TL–W curves were not significantly different between males and females (ANCOVA: $F_{1,69}$ =3.25, P=0.076). In addition, males showed an increase in testes size relative to TL when they reached maturity. Mature males had large testes, which represented 27.5% (±3%) of TL (diameter 13.2 ± 3.6 mm, n = 3), whereas immature males had testes that represented 17.6% ± 1.3% of TL (diameter 3.2 ± 0.46 mm, n=7).

The ratio of immature/mature individuals showed a cyclic fluctuation each year (n=30, n=35, and n=10 for the first, second and third breeding year respectively) (Fig. 3). Immature individuals of both sexes were most commonly caught during summer and autumn ($\chi^2 = 17.4$, d.f. = 1, P < 0.05, and $\chi^2 = 7.6$, d.f. = 1, P < 0.05, respectively), whereas mature individuals were most commonly caught during spring ($\chi^2 = 7.6$, d.f. = 1, P = 0.005). No differences were observed in the maturity ratio during winter ($\chi^2 = 0.38$, d.f. = 1, P = 0.54). In addition, we registered differences between sexes in the dates of appearance



Fig. 3. Seasonal variation in the ratio of immature/mature individuals of southern short-tailed opossums (*Monodelphis dimidiata*) caught during the sampling period in the Flooding Pampas of Argentina.

of mature individuals. Mature males were captured only during spring (26 November to 15 December), whereas mature females were captured from mid-spring to late summer (26 November to 16 March). It is important to note that mature individuals captured during summer were exclusively females (15–16 March 2007, n=2; 22 February 2008, n=1). In addition, we registered cases of pregnancy in females captured in mid-spring (29 November 2006, n=3), and mid-summer (22 February 2008, n=1).

The seasonal change in the ratio of mature to immature individuals was also reflected in the values of TL and W. The size of individuals was significantly lower during summer and autumn, in relation to spring when both TL and W suddenly increased (TL: $H_{3,75}$ =42.5, P=0.0001; W: $H_{3,75}$ =40.97, P=0.0001) (Fig. 4). The size of the individuals showed intermediate values during winter, and did not differ from other seasons.

Although males were more prevalent than females in most seasons, sex ratio did not differ from the expected 1 : 1 in any habitat. However, the year-based analysis (grouping captures of the same breeding year) resulted in a male-biased sex ratio (1 : 4.4) only for the first year ($\chi^2 = 4.6$, d.f. = 1, P = 0.03).

Discussion

We found M. dimidiata to be a low-density species in the southeastern Flooding Pampas of Argentina. This is typical for this opossum since similar density values have been reported throughout its range (Pine and Handley 2008), and generally for all Neotropical marsupials (Redford and Eisenberg 1992). In addition, the abundance of M. dimidiata was higher in native Pampean grasslands than in agroecosystems. In this sense, it has been reported that the dense vegetation cover that characterises Pampean grasslands may serve as natural refugia for many small mammals (Comparatore et al. 1996), and they may provide both a shelter from avian predation and a suitable site for nest construction required by this opossum (Redford and Eisenberg 1992). Agroecosystems, in contrast, would be less suitable habitats for Pampean small mammals, given that they are unstable environments and have low vegetation cover during most of the year (Bilenca et al. 2007).

Our study is the first specifically designed to evaluate the population dynamics of *M. dimidiata* and, despite the low population numbers and recapture rates, our findings support a semelparous life cycle for this species. In each year sampled all mature individuals of one cohort disappeared in the following year. This result confirms previous reports (Reig 1964, 1965; Pine *et al.* 1985; González and Claramut 2000) and is an important advance in the knowledge of the ecology of this species, with implications for all neotropical marsupials in general.

Furthermore, our study also found several other life-history traits (discussed below) of M. dimidiata that support a semelparous life cycle. These traits are similar to those found for the Australian dasyurid marsupial genus Antechinus (Braithwaite and Lee 1979; Holleley et al. 2006; Naylor et al. 2008). First, M. dimidiata showed an annual life cycle characterised by a repetitive pattern of change in the maturity ratio within each breeding year, with dominance of immature individuals in most seasons with the exception of spring, when mature individuals were dominant. This seems to support a short mating period for this opossum, which would be constrained to the warm months between late November and mid-March. All these findings suggest the occurrence of an annual replacement of generations probably attributable to post-mating mortality of adults, as has been reported for several marsupial species (Bradley 2003; Holleley et al. 2006; Martins et al. 2006a, 2006b). Reig (1964) suggested that the disappearance of mature individuals of M. dimidiata during autumn may be attributable to a massive mortality caused by the beginning of the first frosts. In comparison, massive post-mating mortality of males in Australian antechinus has been attributed to severe physiological stress (Bradley 2003). Whether endogenous (e.g. stress, hormonal control) or exogenous (e.g. low temperatures, food limitation) factors are more important in determining the life cycle of the semelparous South American didelphids is still an unanswered question and merits further investigation (González and Claramut 2000; Leiner et al. 2008).

Second, our results indicate a sudden change in body size by *M. dimidiata* when they reached maturity. We found males underwent a more pronounced change in body size than females, as evidenced by a noticeable increase in body and gonadal size.



Fig. 4. Seasonal average values of (a) weight and (b) total length of southern short-tailed opossums (*Monodelphis dimidiata*) caught during the sampling period in the Flooding Pampas of Argentina.

In the case of males these traits would be indicative of male–male competition, given that larger males have competitive advantages over smaller individuals, such as increased mating opportunities (González and Claramut 2000), and it is also a typical feature among mammalian species with polygynous and promiscuous mating systems (Clutton-Brock 1988). In the case of females, the body mass gained at maturity could have been related to the high energetic costs of maternity (Pine *et al.* 1985). The energetic effort of females would be focussed in providing a succesful protection, lactation and rearing of the offspring during their once-in-a-lifetime reproductive opportunity (Clutton-Brock 1989; González and Claramut 2000; Fisher and Blomberg 2011). It is important to note that these are all factors proposed as driving forces on the evolution of semelparity (Holleley *et al.* 2006).

All other examples of semelparity among Neotropical marsupials have been found in two related insectivorous genera in Brazil. These include two cases of obligate semelparity in Marmosops (e.g. M. incanus: Lorini et al. 1994; M. paulensis: Leiner et al. 2008), and a case of partial semelparity in Gracilinanus microtarsus (Martins et al. 2006a). Thus, although iteroparity seems to be the general rule among Neotropical opossums (e.g. in the genera Caluromys, Marmosa, Metachirus, Micoureus, and Thylamys: Gardner 2008), these examples indicate that semelparity might represent a frequent reproductive mode among Neotropical opossums. Moreover, recent didelphid phylogeny indicates that all these genera of mouse opossums belong to different lineages, one including Monodelphis, Micoureus and Marmosa, and other including Marmosops, Gracilinanus, Lestodelphys and Thylamys (Palma 2003). This suggests that semelparity in didelphids may have evolved either once (in the ancestor of these genera) or twice (once in each lineage) and has probably been lost multiple times. In this sense, studies on the reproductive strategies of poorly known species of these genera would be important to elucidate the evolution of semelparity among Neotropical marsupials.

In Autralasian antechinuses, the weaned young grow slowly through the winter, their growth accelerates during spring and they then produce the next generation in summer (Holleley *et al.* 2006). This is similar to the strategy we found in *M. dimidiata*, except that only male antechinuses die after one mating season and most females can survive more than one breeding year and breed more than once (Naylor *et al.* 2008; Fisher and Blomberg 2011). Thus, although Neotropical marsupials did not show a radiation comparable with that of Australian dasyurids, both have developed similar reproductive strategies, which represents an interesting example of convergence between South American and Australasian marsupial faunas.

Although the conservation status of *M. dimidiata* has been changed from Near Threatened to Least Concern (Brito et al. 2011), it is possible that its conservation status has not been adequately evaluated due the lack of appropriate information to make an assessment of its risk of extinction (Díaz and Ojeda 2000). Brito et al. (2011) also indicate that the major threat for M. dimidiata populations is habitat conversion to agriculture. In this context, several of our findings such as low densities, strong reproductive seasonality, and possible dependence on native grasslands, are indicative that this species may show a higher vulnerability to land-use than previously supposed. Since in recent years agriculture has expanded in the Pampean region (Viglizzo et al. 2006; Aizen et al. 2009), the remanent nearpristine grassland fragments would probably be the only refuge for stable population of M. dimidiata at this area (Comparatore et al. 1996), and increased connectivity among these fragments should be a priority for the conservation of this species.

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