

## **Species Richness and Endemism of Amphibians along the Riparian System of Clarin River, Misamis Occidental, Philippines**

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### **Abstract**

Taking into account that amphibians are very good indicators of ecosystem health, a study of frog and toad species diversity was conducted along the riparian environment of Clarin River, Misamis Occidental Province, Philippines. We used visual encounter surveys to determine species richness of amphibians present in the area. With an initial goal of establishing a baseline about amphibian species diversity in this river drainage, we conducted surveys that resulted in records documenting the occurrence of twelve species in ten genera and five families. Seven species (58.33%) are endemic to the archipelago; these additionally include a Mindanao Faunal Region endemic, *Pulchrana grandocula* and two Mindanao Island endemics, *Ansonia muelleri* and *Ansonia mcgregori*. Among the recorded endemic species, three are classified as “Vulnerable” to extinction. The highest species richness (N=8) and calculated species diversity ( $H'=1.829$ ) were recorded at sampling Site Two (midstream). Threats to amphibian population persistence in the Clarin River drainage include anthropogenic disturbances such as land conversion and overhunting for human subsistence. The presence of endemic and formally classified endangered species indicates the need to protect this riparian system as an important habitat resource for endemic western Mindanao amphibian populations.

**Keywords:** diversity, endangered, indicators, threats, vulnerable

## Introduction

With more than 7,100 islands, the Philippines is the second-largest archipelago in the world (Ambal et al., 2012) and a megadiverse country with exceptionally high amphibian species richness and endemism (Diesmos et al., 2002a; Brown & Diesmos, 2009). The amphibian fauna in the Philippines is composed of three caecilians (Gymnophiona; in a single genus and one family), plus frogs and toads (anurans), with approximately 112 species in 23 native genera in nine families (Brown et al., 2012; Diesmos et al., 2015). Among the fauna, exceptionally high levels of endemism (85%) have been estimated, with possibly as much as 23% of the native amphibians being Mindanao endemics (Relox et al., 2010; Diesmos et al., 2015).

Many secretive species are poorly known due to their restricted ranges and reproductive biology, which is so tightly coupled to local microclimates (Diesmos et al., 2002a). However, studies conducted in the different ecosystems of Mindanao show that the region supports exceptionally high levels of diversity and endemism (Taylor, 1920, 1922 a, b; Brown & Alcala, 1970; Plaza & Sanguila, 2015).

One recent study in Mt. Kalatungan Mountain, Bukidnon recorded 15 forest amphibian species, 60% of which are Philippine endemics (Warguez et al., 2013). In Mt. Malindang, 26 species of amphibians (42% endemic) were reported by Nuñez et al. (2010). In caves of Northern Mindanao, Nuñez et al. (2015) recorded four amphibian species, including one Philippine endemic. In Bega Watershed of Agusan del Sur, 13 species of anurans were documented (Calo & Nuñez, 2015); and in the wetland ecosystem in Agusan Marsh, 11 species (six of which were Philippine endemics) from six families have been reported (Sularte et al., 2015). Very recently, Brown (2015) described a new species of stream frog of the genus *Hylarana* (= *Pulchrana*) from Mt. Busa, Sarangani and South Cotabato provinces, southern Mindanao Island. Clearly, herpetological diversity of the southern Philippines remains incompletely documented, with new data adding to our understanding of the diversity on a regular basis (Brown et al., 2013).

However, habitat destruction in the form of forest conversion, environmental pollution (Sularte et al., 2015), and destruction of riparian environments (Almeida-Gomes et al., 2015) seriously threatened amphibian biodiversity in the country (Brown et al., 2012). Presently, there are 48 threatened species of amphibians in the Philippines (IUCN 2015). Amphibians are known to be sensitive to environmental changes and are easily affected by forest fragmentation, logging, and changes in microclimatic variables and habitat structure (Almeria & Nuñez, 2013). Amphibians are also sensitive to changes in their natural environment brought on by pollution and climate change that cause the rise in temperature and changes in humidity (Alcala et al., 2012; Bickford et al., 2010). Hence, amphibians are particularly sensitive bioindicators of the health of their environment.

Many species of amphibians rely on riparian (stream and river) habitats for reproduction (Haddad & Prado, 2005) and some of them live their entire life cycles in close association with bodies of water near riparian systems (Ficetola et al., 2009).

Riparian habitats are important for amphibian populations, and facilitate everything from individual foraging to dispersal and colonization of new areas (Cohen, 2014; Surasinghe & Baldwin, 2015). Riparian habitats also provide connectivity between forest patches (Rodríguez-Mendoza & Pineda, 2010). Structural changes in water courses and their margins due to the removal of riparian vegetation have been shown to cause shift in amphibian species composition (Rodríguez-Mendoza & Pineda, 2010). In spite of the relatively small area, riparian habitats are extremely important for amphibian conservation because they maintain stable community composition and high overall biodiversity (Almeida-Gomes et al., 2015).

The Clarin River drainage of northwestern Mindanao is a major water system of Misamis Occidental Province, which provides ecosystem services in the form of fresh water for amphibian and human populations alike. The river drainage includes different sub-types of waterways (small streams, springs, seeps), and is associated with vegetation, which supports an abundance of species, from insects to amphibians. However, this river system has been modified for agricultural and other human uses. Untreated human sewage,

urban development, logging, and pollution clearly have adverse impacts on the river (*personal observations*), and the amphibian fauna of the area remain poorly understood.

In this study we assessed the amphibian communities along the riparian Clarin River drainage system, focusing on species composition, abundance, diversity, endemism, and threats to their community. We conducted the following study to generate data, which characterize the amphibian diversity of riparian systems of Misamis Occidental, northwest Mindanao. Further, through this endeavor, this will help structure the conservation policy of flora and fauna species present in the area.

## Materials and Methods

### Study area

The Clarin River drainage is located on the southeastern side of Misamis Occidental Province in Northern Mindanao ( $123^{\circ} 37' 30''$  to  $123^{\circ} 13' 10''$  East longitude and  $8^{\circ} 7' 30''$  to  $8^{\circ} 13' 10''$  North latitude). It is the natural boundary of the Municipality of Clarin and Ozamiz City (Figure 1).

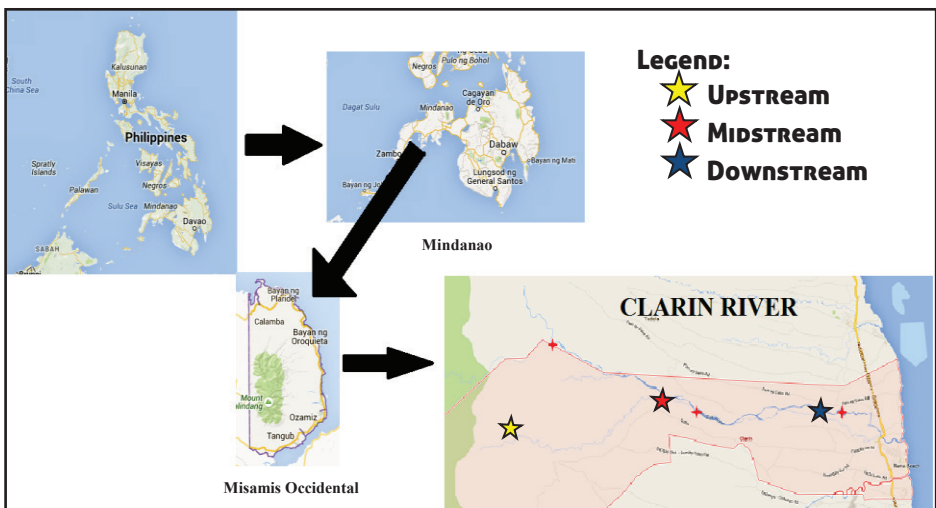


Figure 1. Map of Clarin River, Misamis Occidental ([www.google.com.ph/maps2015](http://www.google.com.ph/maps2015)).

### ***Sampling sites***

Three sampling sites were established in the Clarin River drainage. Sampling Site 1 (upstream) is located in Barangay Stimson Abordo, Ozamiz City. This site is located 521.51 meters above sea level (masl) at  $8^{\circ} 12' 19.13''$  N  $123^{\circ} 42' 46.82''$  E. This site consists of a forested area and can be reached via existing human-made trails or by river access. The area provides one of the primary sources of water in Ozamiz City. Farming of upland vegetables and coconuts is very common in the area. Sampling Site 2 (midstream) is situated in Barangay Guba, Clarin. This site is located at 140.89 masl at  $8^{\circ} 13' 05.47''$  N  $123^{\circ} 46' 24.31''$  E. Large tracts of agroforestry and quarrying are common in the area. Sampling Site 3 (downstream) is located in Barangay Pan-ay, Clarin. The site is located at 37.19 masl at  $8^{\circ} 12' 55.39''$  N  $123^{\circ} 49' 55.94''$  E. Cultivation (rice fields) are common in the area. An irrigation canal is located adjacent to the river system. Domestic solid and liquid wastes from households and quarrying in commercial scale were the largest sources of pollution.

### ***Entry protocol***

A gratuitous permit from the Department of Environment and Natural Resources (Region 10) was obtained for the conduct of the study. Barangay officials in Ozamiz City and the Municipality of Clarin who were informed about the study provided their consent.

### ***Sampling procedure***

A visual encounter survey (“cruising”) method was used to sample amphibians from approximately 1900 hours to 2200 hours. Our sampling effort was maximized, with several searches done in between and beyond this period, and resulting in a non-uniform sampling time for each site.

Collected samples were identified based on the available field guides of Nuñez (2005, 2012) and Alcala and Brown (1998) and species identity was verified by consultation with R. M. Brown (University of Kansas). Morphometric variables were measured using a vernier caliper, and body weight was taken using a spring balance. Samples that were identified in the field were marked and released. Voucher specimens

were deposited in the Department of Natural Sciences, College of Arts and Sciences, Misamis University. The conservation status and geographical distribution of each anuran species were checked using the 2015 IUCN (International Union for Conservation of Nature) Red list of Threatened Species. Determination of threats to Clarin River and amphibians was based on direct observations.

### ***Statistical analysis***

Biodiversity indices and other statistical analysis were determined using Paleontological Statistics (PAST) software. BioDiversity PRO software version 2.0 was used for the Bray-Curtis cluster analysis for identifying the similarity of the different sampling sites.

## **Results and Discussion**

Twelve species of amphibians belonging to the anuran families Bufonidae, Dicroglossidae, Microhylidae, Ranidae, Rhacophoridae were recorded in the three sampling sites (Table 1). The highest species abundance was recorded in the upstream river site. The area is covered with forest patches and the least disturbed among the three sampling sites. Forest vegetation was composed of a large number and variety of plants and trees that create a wide range of canopy cover. The canopy cover may provide maintenance of temperature and moisture that is critical for the survival of the anurans and other amphibian species (Knapp et al., 2003). Amphibian assemblages in such natural landscapes may have a lower probability of local or metapopulation extinction than those positioned in disturbed landscapes, despite the elevated abundance of some species (Gray et al., 2004). Although the upper reaches of the river had the highest species abundance, it has lesser species richness (N=6) compared with our midstream area. The lesser species richness may be related to higher altitude and its steep undulating slope, which may limit the dispersion of anurans in the area. Our results from sampling Site 1 (upstream) can be associated with the results of faunal inventories of several mountains, which reveal a decreasing trend in amphibian richness with increasing elevation (Siler et al., 2011; Nuñez et al., 2010; Diesmos et al., 2002b).

**Table 1. Species composition, number of individuals, and relative abundance of amphibians in different sampling sites.**

Anurans	Number of Individuals (Relative abundance in %)				Total
	Sampling Sites			Downstream	
	1 Upstream	2 Midstream	3		
<b>Bufonidae</b>					
<i>Ansonia muelleri</i>	5(13.15)	0(0.0)	0(0.0)	0(0.0)	5(6.49)
<i>Ansonia mcgregori</i>	20(52.63)	1(7.14)	0(0.0)	0(0.0)	21(27.27)
<i>Rhinella marina</i>	0(0.0)	3(21.43)	21(80.77)		24(31.17)
<b>Dicroglossidae</b>					
<i>Hoplobatrachus rugulosus</i>	0(0.0)	1(7.14)	0(0.0)	0(0.0)	1(1.30)
<i>Fejervarya moodiei</i>	0(0.0)	5(35.71)	0(0.0)	0(0.0)	4(5.19)
<i>Limnonectes magnus</i>	7(18.42)	0(0.0)	1(3.85)	1(3.85)	8(10.39)
<i>Limnonectes leytensis</i>	0(0.0)	1(7.14)	0(0.0)	0(0.0)	1(1.30)
<b>Microhylidae</b>					
<i>Kaloula pulchra</i>	0(0.0)	1(7.14)	3(11.54)		4(5.19)
<b>Ranidae</b>					
<i>Pulchrana granducola</i>	3(7.89)	1(7.14)	0(0.0)	0(0.0)	4(5.19)
<i>Staurois natator</i>	1(2.63)	0(0.0)	0(0.0)	0(0.0)	1(1.30)
<b>Rhacophoridae</b>					
<i>Polypedates leucomystax</i>	0(0.0)	1(7.14)	1(3.85)	1(3.85)	2(2.60)
<i>Rhacophorus bimaculatus</i>	2(5.26)	0(0.0)	0(0.0)	0(0.0)	2(2.60)
<b>Total number of individuals</b>	<b>38(49.35)</b>	<b>14(18.18)</b>	<b>26(33.76)</b>		<b>77</b>
<b>Total number of species</b>	<b>6</b>	<b>8</b>	<b>4</b>		<b>12</b>

The highest species richness (8) was documented in the midstream area, but this site had the lowest species abundance. The low species abundance in the midstream could be attributed to its habitat condition. The site was dominated by grasses; light penetration is maximal, providing a presumably less suitable microhabitat for exothermic anurans. The tree stands at this site are fewer, and widely spaced, creating a relatively lower canopy cover. Intense sunlight is not suitable for amphibians given their physiological constraints and reliance on consistently moist microhabitats (Almeria & Nuñez, 2013). Agricultural disturbance may positively influence the abundance of some (invasive) amphibian species (Diesmos et al., 2006). Less mobile amphibians that breed explosively could be competitively dominant in the absence of predation and may benefit disproportionately from landscape cultivation. Elevated abundances in croplands may not necessarily imply better conditions for amphibian populations (Yeagers et al., 2013). Both positive and negative patterns of amphibian population influences have been documented to occur in natural and urban streams (Riley et al., 2005). Studies have reported negative effects of agriculture on total amphibian abundance (Mensing et al., 1998), and both positive and negative effects on the occurrence and abundance of individual species and guilds.

The lowest species richness was observed in our downstream site, close to heavy disturbance, agriculture, and human habitation. At the periphery of the area, human disturbances, rice farming, and irrigation canal were common. Agricultural and urban land conversion has been shown negatively to affect amphibian species richness (Atauri & de Lucio, 2001). In addition to habitat loss, fertilizer and pesticide use in agricultural areas have been shown to affect the biodiversity and functioning of ecosystems (Benton et al., 2003). Species richness is lower in areas situated in agricultural and urban landscapes as compared to forested landscapes (Houlahan & Findlay, 2003).

*Rhinella marina*, an introduced and invasive amphibian from South America had the highest relative abundance (31.17%). This species was first introduced in the Philippines in the 1930s as a biological pest control agent. It now occurs in most of the major islands



throughout the Philippines except in the Palawan island group. Studies on introduced populations of *R. marina* from other parts of the world show that the species has had adverse ecological impacts on native species of frogs (Diesmos et al., 2006, 2015).

*Ansonia mcgregori* was most abundant in our upstream site and commonly found above rocks covered with mosses. This species was reported from the Western Mindanao Pleistocene Aggregate Island Complexes or PAIC (Taylor, 1920; Brown & Alcalá, 1970; Sanguila et al., 2011), which includes the Mt. Malindang Range. The upstream area is located in the buffer zone of this mountain range. *Ansonia mcgregori* is categorized “Vulnerable” (IUCN 2015). The distribution of this species has been characterized as “severely fragmented”, and continuing decline in the extent and quality of its forest habitat in Mindanao has been extrapolated from forest cover reductions (IUCN 2015).

A relatively high level of endemism (58%) was recorded in Clarin River (Table 2). There are seven Philippine endemic of amphibians, one of which is “Near-threatened” (IUCN 2015) and three taxa are classified as “Vulnerable” (IUCN 2015; Figure 2). This level of endemism is slightly higher compared to the 54% endemism during the recent survey conducted by Calo and Nuñez (2015) in Bega Watershed, Prosperidad, Agusan del Sur.

One species, *Limnectes magnus*, was categorized as “Near-threatened” (IUCN 2015) because of an inferred decline based on habitat loss (forest cover reduction) throughout much of its range, plus a possible over-harvesting for food (Baillie et al., 2004). The species was observed by us to occur in low numbers and in limited distribution.

Introduced and invasive species of amphibians (Diesmos et al., 2006) were encountered in all of our sampling sites. Anthropogenic disturbance in landscapes surrounding wetlands can affect resident organisms (Findlay & Houlihan, 1997). With some human disturbances, insect populations have been shown to increase, and available food sources for amphibians also increased—favoring survival of introduced amphibians (Reh & Seitz, 1990).

**Table 2. Habitat, geographic distribution and conservation status of amphibian species in Clarin River.**

Anuran Species	Common Name	Habitat	Geographic Distribution (IUCN 2015)	Conservation Status (IUCN 2015)
<i>Ansonia muelleri</i>	Mueller's Toad	Terrestrial, Freshwater	Mindanao Island Endemic	Vulnerable
<i>Ansonia mcgregori</i>	McGregor's Toad	Terrestrial, Freshwater	Mindanao Island Endemic	Vulnerable
<i>Rhinella marina</i>	Cane Toad, Giant Marine Toad	Terrestrial, Freshwater	Introduced	Least Concern
<i>Hoplobatrachus rugulosus</i>	Chinese Edible Frog	Terrestrial, Freshwater	Introduced	Least Concern
<i>Limnonectes magnus</i>	Giant Philippine Frog	Terrestrial, Freshwater	Philippine Endemic	Near Threatened
<i>Limnonectes leytensis</i>	Small Disked Frog, Swamp Frog	Terrestrial, Freshwater	Philippine Endemic	Least Concern
<i>Kaloula pulchra</i>	Asian Painted Frog	Terrestrial, Freshwater	Introduced	Least Concern
<i>Fejervarya moodiei</i>	Crab Eating Frog	Terrestrial, Freshwater	Philippine Endemic	Data Deficient
<i>Pulchrana grandocula</i>	Big-eyed Frog	Terrestrial, Freshwater	Mindanao Faunal Endemic	Least Concern
<i>Staurois natator</i>	Mindanao Splash Frog	Terrestrial, Freshwater	Non-Endemic; Native	Least Concern
<i>Polypedates leucomystax</i>	Common Tree Frog	Arboreal, Terrestrial, Freshwater	South East Asia	Least Concern
<i>Rhacophorus bimaculatus</i>	Asiatic Tree Frog	Arboreal, Terrestrial, Freshwater	Philippine Endemic	Vulnerable
<b>Total no. of endemic species (endemism)</b>			<b>7 (58.33%)</b>	



**Figure 2.** Some endemic species of amphibians in riparian system of Clarin River, Misamis Occidental (A. *Rhacophorus bimaculatus*–Vulnerable, B. *Pulchrana granducola*, C. *Ansonia mcgregori*–Vulnerable, D. *Limnonectes magnus*–Near Threatened, E. *Fejervarya moodiei*, F. *Limnonectes leytensis*)

Urban impacts on stream amphibian communities may occur in arid environments, where the extra inputs of water represent a great departure from the natural hydrological regime. Decreased flow variability due to changes in stream hydrology and climate can facilitate the establishment of exotic species with accompanying damage to native communities (Eby et al., 2003).

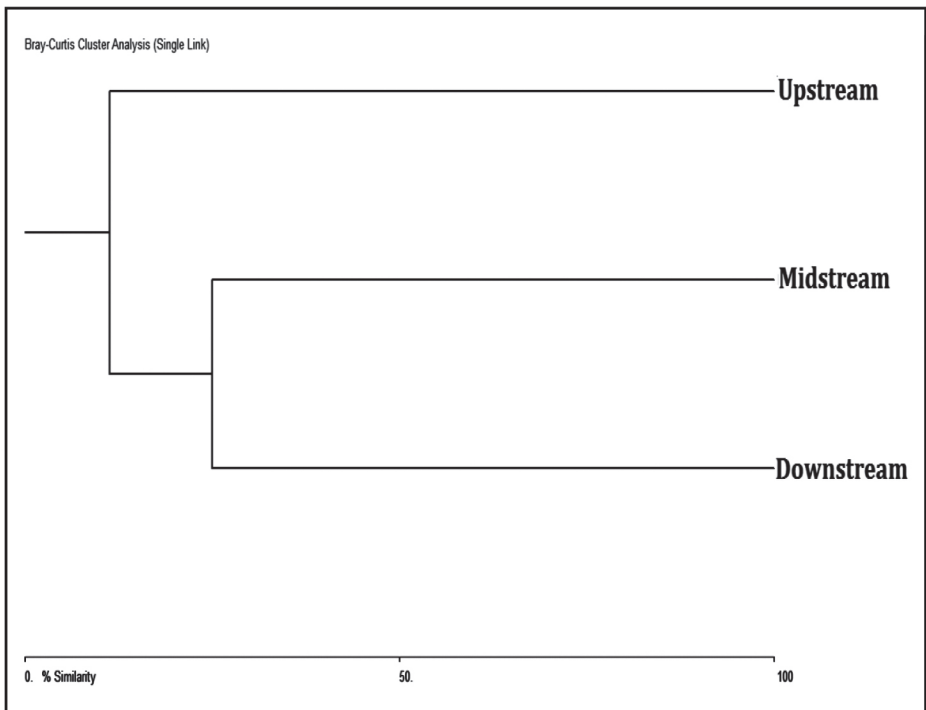
We recorded low to moderate diversity in our three sampling sites (Table 3). Sampling at Site 2 (midstream) recorded the highest species diversity ( $H' = 1.829$ ), with downstream the lowest ( $H' = 0.6723$ ). Modifications of aquatic and terrestrial environments may have positively influenced the amphibian populations in agroforests. Smith and Haukos (2002) noted that plant diversity and structure could be greater in farmland, increasing food resources and escape cover for larvae. Nitrogen influx from fertilizers may also increase food resources for larval amphibians in agricultural areas (Leibold & Wilbur, 1992). Postmetamorphic body size, temporal demographics, and diet diversity may be negatively affected by agricultural cultivation (Gray, 2002; Gray & Smith, 2005; Smith et al., 2004).

A more or less even distribution was recorded in all sampling sites except Site 3, which had a relatively low evenness, probably due to the abundance of *Rhinella marina* which dominates the site. The differences in evenness of distribution may result from variation in community geographical and physical factors, given that the distribution of organisms involves selection of habitats, which provide the resources required for the survival of a particular species (Bryant, 2004).

Our midstream and downstream sites had the highest similarity (25%), which could indicate a relatively high number of species in common. The effects of habitat destruction and modification, such as those caused by land conversion to an agricultural area have long been recognized as major threats to biodiversity (Pimm & Raven, 2000; Brooks et al., 2002), including that of amphibians. Site 1 anuran communities were more unique because the area is a forested with lower levels of disturbance.

**Table 3. Biodiversity indices of amphibians in the three sampling sites of Clarin River, Misamis Occidental.**

Biodiversity Indices	Upstream (Site 1)	Midstream (Site 2)	Downstream (Site 3)
Species Richness	6	8	4
Individuals	38	14	26
Dominance	0.338	0.2041	0.6686
Shannon H'	1.367	1.829	0.6723
Evenness	0.6542	0.7783	0.4897



**Figure 3. Dendrogram showing the similarity of the three sampling sites based on the presence and absence of anuran species.**

### ***Threats to the amphibians in Clarin River***

Major threats to amphibian populations in Clarin River may include habitat destruction and overexploitation. Quarrying and land conversion are common, and alteration of habitat favors invasive species establishment across the landscape (Diesmos et al., 2006; D'Amore et al., 2010). The presence of introduced and invasive species may pose a threat to native amphibians. The ability of alien frogs to invade natural forests has tremendous implications for amphibian conservation in the Philippines given that over 80% of its amphibian species (mostly endemic) are forest obligates (Brown et al., 2001, 2012).

### **Conclusion and Recommendations**

The Clarin River of Misamis Occidental hosts a significant number of endemic Philippine amphibian species. The river has moderate species diversity and a relatively high level of anuran endemism. The presence of potentially threatened anurans implies a need for conservation measures for species protection. Other river systems in Misamis Occidental (and western Mindanao in general) need to be surveyed to complete conservation assessments of amphibians riparian systems of the province.

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