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Searching for a lost frog (*Rhinoderma rufum*): identification of the most promising areas for future surveys and possible reasons of its enigmatic decline

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Abstract. Currently amphibians are more threatened than birds and mammals with more than one-third of all amphibians facing extinction. In Chile, Darwin's frog (*Rhinoderma rufum*) is classified as Critically Endangered by IUCN, and no individuals have been found since the 80's. Herein, a species distribution model (SDM) was generated to identify the most likely areas were remaining *R. rufum* populations might be detected as a first step to help to rediscover this lost frog. The SDM was based on bioclimatic and land cover information with a comprehensive collection of 19 species records compiled from literature, museum records and personal communications. The SDM suggests a potential distribution of *R. rufum*, which differs slightly from the one previously proposed. However, it shows an overlap with cultivation and artificial areas in Chile. Therefore, it seems likely that the major factor leading to *R. rufum* declines and possible extinction was anthropogenic habitat destruction.

Key words: potential habitat, species distribution modelling, habitat loss.

Introduction

In the last years, amphibians have declined dramatically in many areas of the world and as many as one-third (32%) of amphibian species are known to be threatened or extinct, and almost half (42%) are declining (IUCN 2011). The declines have various likely causes: habitat destruction, environmental pollution, UV-B irradiation, diseases, global change and introduced species (e.g. Gardner 2001, Collins & Storfer 2003, Beebee & Griffiths 2005). Once recognized current biodiversity loss, amphibian conservation action plans were initiated (Gascon et al. 2007), suggesting mitigation measurements such as lost frogs surveys (Conservation International 2011) and amphibian captive breeding programs (Gascon et al. 2007). However, for any conservation action, a critical and difficult initial step is to determine the target species' distribution (Rachlow & Svancara 2006, Gaston & Fuller 2009). Especially rare species, or those which are difficult to detect, pose unique challenges to conservationists when assessing their spatial distribution (Rachlow & Svancara 2006). This is frequently the case since many amphibians species are not well studied (Karl et al. 1999, Doherty & Harcourt 2004, Edwards et al. 2005). As a result, surveying for rare animals can be a resource-intensive activity that diverts energy and funds away from other conservation efforts (Rachlow & Svancara 2006). In spite of this, thanks to many lost frogs searching initiatives, dozens of species have been rediscovered worldwide (i.e. at the Ivory coast, Democratic Republic of Congo, Ecuador, Haiti and Mexico) even after more than two decades (Conservation International 2011).

In Chile, Darwin's frog populations have recently declined (Young et al. 2001) and are thought to be highly endangered (Glade 1998, Díaz-Páez & Ortiz 2003, IUCN 2011). *Rhinoderma rufum* is listed as number 45 on the evolutionarily distinct and globally endangered amphibian list (EDGE 2011), and it is also classified as critically endangered by IUCN (2011). Formerly, this species was distributed in small, isolated populations, and was fairly regularly seen until approximately 1978 (Baillie et al. 2004, Veloso et al. 2010). In 1980 Penna & Veloso (1990) recorded for the last time *R. rufum* in the Concepción province. Subsequently, there have been no confirmed reports despite several attempts to relocate the species, giving rise to fears that it might have become extinct in the meanwhile (Busse 2002, Baillie et al. 2004, Veloso et al. 2008).

The genus *Rhinoderma* is endemic to the Valdivian forest, a temperate rain forest, situated in the central south of Chile bordering Argentina. These frogs are unique, due to their reproductive parental care, where males brood their tadpoles inside their vocal pouch (Called Neomelia). *Rhinoderma* was described for the first time by Duméril and Bibron (1841) based on specimens collected by

Charles Darwin during his voyage around the world on the "Beagle". In his honour this species was named *Rhinoderma darwinii*. On the other hand, its sister species *Rhinoderma rufum* was described in 1902 by Philippi, as *Heminectus rufus*. However, this new species was neither recognized by Cei (1958, 1962) nor by Donoso-Barros (1970), due to the loss of the type specimen, its confusing original description, and physical similarities shared by both species. It was only revalidated by Formas (1975) after Jorquera et al. (1972, 1974) conducted an embryological and developmental study and detected differences in egg and larval development. While larvae of *R. darwinii* develop in the vocal pouch until the metamorphosis is completed, *R. rufum* larvae have only a short stay in the vocal pouch and pass through a long lasting free living tadpole stage before metamorphosis. Furthermore, unique morphological characteristics are present in *R. rufum*, such as different tympanum morphology, obtuse fingers with a bearing under (Philippi 1902, Formas et al. 1975), and more conspicuously webbed toes, different colour pattern on the ventral side of the feet, more pronounced heel appendices, and less developed metatarsal tubercles (Formas et al. 1975). Based on these developmental and morphological differences, Formas et al. (1975) concluded that they actually are two different species. As a consequence of this systematic confusion, *R. rufum* was not well studied and scarce scientific material is available. The few existing studies have described its taxonomy (Phillipi 1902, Formas et al. 1975), distribution (Barros 1918, Torres & Castillo 1973, Formas et al. 1975), development (Jorquera et al. 1974, 1981, Jorquera 1986), digestive track development (Jorquera et al. 1982, Jorquera 1986) and karyology (Formas 1976).

Once, *R. rufum* could be found between Zapallar (Moreno in Formas et al. 1975) and Concepción (Jorquera et al. 1974) in coastal areas, ravines and high hills next to small water seep protected by shady leafy trees (Barros 1918). It has been documented in leaf-litter in temperate mixed forests, and also in bogs surrounded by forests (Veloso et al. 2008). Barros (1918) stated that *R. rufum* swims with agility, but normally stays quite by the water shore, or hiding in some hole by the shore under the dead leaves that float in some eddy. Under this last shelter it is almost always floating vertical, touching the leaves with the snout. When the running water disappears, they hide themselves under stones where humidity is conserved and they group and shelter together. They are never present in sunny and open areas of the ravine, but rather in shaded areas protected by foliage, where direct sunbeam filters through only occasionally. Specimens at "Barranca alta" were collected during summer in shadowed ravines skirted by *Aristotelia chilensis* (Torres & Castillo in Formas (1975)). Specimens from Vichuquén were collected during summer in humid ravines that were covered by vegetation (Barros 1918). The animals from Chiguayante were collected during winter and spring under stones and between humid vegetation next to the water in a *Nothofagus* forest, which was relatively disturbed by people (Barros 1918).

Based on all available information on habitat preferences and the spatial distribution of *R. rufum* compiled through extensive literature surveys, museum specimens and personal communications, we herein develop a quantitative spatially explicit species distribution model (SDM) to assess its potential distribution by comparing environmental features at species record sites with those within its general area of occurrence (O'Connor 2002). Such models have been described to serve as useful tools for evaluating potential distributions and habitat suitability over large areas and also for guiding survey efforts to those areas with higher probabilities of species occurrence (Edwards et al. 2005, Peterson et al. 2005, Le Lay et al. 2010). Our results highlight areas where further field surveys may have the highest chance to detect further (last?) populations of *R. rufum*.

Materials and Methods

Species records and climate data

A total of 19 *Rhinoderma rufum* records (Table 1) were compiled from literature and material collections data. Geo-referencing was conducted when necessary with Google Earth (http://www.google.com/earth/index.html). All data was checked in the DIVA-GIS 7.4 software for possible errors (Hijmans et al. 2005a).

Current climate conditions for Chile was obtained from the WorldClim database, version 1.4 (www.worldclim.org, Hijmans et al. 2005b). This weather conditions were recorded between 1950 and 2000 interpolated to a grid cell resolution of 30 arcsec (equivalent to ca. 1 x 1 km). Bioclimate variables were calculated with DIVA-GIS, which are suitable for the SDM application (Beaumont et al. 2005, Busby 1991). As temperature is a key factor for ectothermic species, we selected the "annual mean temperature" and "temperature annual range (BIO5-BIO6)" as important variables related to energetic balances and digestive turnover rates (Rödder 2010). Humidity is the key factor limiting the distributions for

Table 1. Location of *Rhinoderma rufum* populations included in this study. Museum collections: Instituto de Zoología de Universidad Austral, Chile (IZUA); Museo de Zoología de la Universidad de Concepción, Chile (MZUC=IZUC=ICBUC); Museo de Historia Natural de Santiago, Chile (MNHN); Zoologisches Museum der Christian-Albrechts-Universität, Kiel, Germany (ZMK); Zoologisches Forschungsmuseum Alexander Koenig, Germany (ZFMK); National Museum of Natural History, Smitsonian Institute, USA (USNM=NMNH).[a] collection material lost (According to Cei (1958) in Formas et al. 1975),[b] collection material lost by fire 2008, [c] collection material not found.

Locality	Description	Coordinates	Source	Museum material (Collector, Museum (year))
Zapallar	Zapallar creek (Prov. Petorca)	32°34′S/71°25′W	Moreno in Formas et al. (1975)	Moreno, IZUA (1966)[b]; ZFMK (1926)
Pichilemu		34°20′S/71°52′W	Parra (2003) pers. comm.	
Paredones	(O'higgins region)	34°39´S/71°53′W	Penna (1978) pers. comm.	
La rufina		34°44′S/70°45′W	Perez (2000) pers. comm.	
Vichuquén		34°49′S/72°01′W	Philippi (1902), Donoso Barros 1972 in Torres & Castillo (1973)	Philippi, MNHS[a]; Timmermann, ZMK (1926); Timmermann, ZFMK (1926); Morán & Tapia, IZUA-A (1969)[b] Neotype
Curicó	Coast		Barros (1918), Donoso-Barros (1970), Formas et al. 1975	
	Nilahue valley	34°34′S/71°47′W	Barros (1918)	
	Ranguilí canyon	34°39′S/71°57′W	Barros (1918)	
	Cutemu/Catemo (spelling correction by Torres & Castillo 1973)	34°48′S/71°53′W	Barros (1918)	
	Hualañé	34°55′S/71°46′W	Barros (1918), Donoso Barros (1970), Formas et al. 1975	
San Fernando	Five league to the east of san Fernando in "Los Quillayes" (Prov. Colchagua)	34°35′S/71°45′W	Barros (1918)	
Barranca Alta	Four km North from Bucalemu beach and two km from South Boyeruca beach (Prov. Colchagua)	34°40′S/72°01′W	Wilhelm (1927), Torres & Castillo (1973)	Torres, IZUA-A (1972)[b]
Constitución	Coastal lagoons	35°19´S/72°24′W	Barros in Krieg (1924)	
Concepción			Jorquera et al. (1974, 1981), Penna & Veloso (1980)	Formas, IZUA (1974)[b]
	English avenue	36°50′S/73°02′W	Ortiz (2010) pers. comm.	Donoso Barros, MZUC (1962)
	Chiguayante	36°55′S/73°01′W	Formas et al. (1976)	Pugin, IZUA[b]; Cekalovic, MH (1974); USNM (1973)
	Caracol hill	36°37′S/72°56′W	Formas et al. (1975)	ICBUC 1964[c]
	Nonguén	36°51′S/72°59′W	Gobierno de Chile, comision nacional del medio ambiente, región Bio-bío (2008)	
Mocha island		38°28′S/74°18′W	Ibarra-Vidal (1989)	
Ramadilla		37°18′S/73°15′W	Formas (1995)	
Nahuelbuta		37°47′S/73°09′W	Ortiz & Ibarra-Vidal (2005)	

amphibians (Wells 2007), hence we included "annual precipitation", "precipitation of warmest quarter" and "precipitation of coldest quarter" as important predictor variables for the potential distribution of *R. rufum*.

Species Distribution Models

The Maxent software for species habitat modelling, version 3.3.3e (www.cs.princeton.edu/~shapire/maxent) (Phillips et al. 2004, Phillips & Dudík 2008) was applied to assess the potential distribution of Darwin's frog. In the past, Maxent has been proposed to be one of the best algorithms when the number of species records is rather low (Hernandez et al. 2006, Wisz et al. 2008). Its capacity to predict novel presence localities for poorly known species has been previously confirmed (Pearson et al. 2007). SDMs developed herein were computed using the default Maxent settings using the logistic output format (Phillips & Dudík 2008), whereby Maxent automatically selected 10.000 randomly chosen background points within an area defined by a 150 km radius enclosing all known records. The predictive performance of the SDM was assessed with the Area under the Curve (AUC), referring to the ROC (Receiver Operation Characteristic) curve assessing the SDM's ability to distinguish background points from training points (Hanley & McNeil 1982, Phillips et al. 2006). Therefore, we used 70% of the species records as training points to train the model and the remaining 30% for testing it. Re-sampling was replicated 100 times and the average predictions per grid cell were used for further processing. Values of AUC ranged from 0.5 (prediction is not better than random) to 1.0 for models giving perfect discrimination between presence and pseudo-absence records. Maxent internally allows for an assessment of the relative contribution of variables to the final model. Possible uncertainties due to extrapolation onto climatic conditions exceeding the training range of the SDM were assessed using multivariate environmental similarity surfaces (MESS; Elith et al. 2010).

Integration of land cover and human footprint data

The potential distribution of *R. rufum* as proposed by Maxent based on bioclimatic conditions include areas with apparently unsuitable micro-habitats, which can be incorporated by clipping the potential distribution derived from climate information, i.e. with land cover types available though the Global Land Cover Information Facility (GLC2000, available through http://bioval.jrc.ec.europa.eu/products/glc2000/glc2000.php).

According to literature data, the altitude where *R. rufum* can be found ranges between 0-500 m a.s.l. (Veloso et al. 2008) or between 50-500 m a.s.l. (Díaz-Páez & Ortiz 2003). Based on all available information on micro-habitat preferences (see introduction), we considered tree cover (broadleaf and needle leaf, mixed leaf type, evergreen and deciduous, open and closed, regularly flooded by fresh water) corresponding to the GLC2000 classes 1 to 7; mosaic tree cover and other natural vegetation (class 9); shrub cover (closed and open, evergreen and deciduous) corresponding to GLC200 classes 11 and 12) as potentially suitable. The following land cover classes were categorized as unsuitable for *R. rufum*: tree cover regularly flooded by saline water (class 8), tree cover burnt (10), herbaceous closed open (13), spare herbaceous or shrub cover (14), regularly flooded shrub land or herbaceous coverage (15), bare areas (19), water bodies (20), snow and ice (21), and all anthropogenically modified areas comprising cultivated and managed areas (16), mosaic cropland/tree cover/other natural vegetation (17), mosaic cropland/shrub and /or grass cover (18), and artificial surface and associated areas (22).

Information on the degree of anthropogenic disturbances was integrated using the human footprint index developed by Sanderson et al. (2002) ("Atlas of the human footprint" available at www.wcs.org/humanfootprint). This index was developed based on data gathered from 1960 to 1995 on the land surface comprising geographic data describing human population density, land transformation, access, and electrical power infrastructure. It was normalized to reflect the continuum of human influence across each terrestrial biome defined within biogeographic realms (Sanderson et al. 2002).

Results

We obtained a good SDM with an AUC value of 0.8017 although the number of available species records was rather low. The potential distribution suggested by our SDM covers well the IUCN (Veloso et al. 2008) estimate, wherein the latter is situated within higher Maxent classes (<0.422), also confirming the predicting power of the model. The lowest observed Maxent value at the presence point used for the model training was 0.3578. Analyses of variable contributions in the SDM revealed that "temperature annual range" with 39.5% had the highest explanative power, followed by "annual mean temperature" (31.2%), "precipitation of the coldest quarter" (19.2%), "precipitation of warmest quarter" (9.9%) and "annual precipitation" (0.2%).

Comparing the land cover classes wherein the species records are situated also increases the available information about *R. rufum* habitat preferences. The species records are situated in the forest areas comprising in the northern parts shrub and sclerophyll forests and deciduous forests in the south. The shrub and sclerophyll forest is characterized by Mediterranean climate with cold and rainy winter and warm and dry summer. The deciduous forest has a temperate weather with droughts during the summer, which decreases from North to South. In this area the main characteristic is the presence of deciduous species such as *Nothofagus*, which in the north are mixed with sclerophyll vegetation and in the south with laurels (Gajardo 1994).

The SMD appears to be consistent with the natural history of *R. rufum* (Fig. 1). Our map suggests that *R. rufum* can find climatically suitable habitats under current conditions in the coastal area between political region IV and northern part of region IX (Fig. 2). However, no *R. rufum* specimens have been found in the IX region, probably due to interspecific competition with *R. darwinii* occupying this area. Climatically suitable areas (Fig. 2a) are much larger than those providing suitable microhabitats (Fig. 2b) due to a pronounced impact of anthropogenic habitat modification (Fig. 2c and 2d).

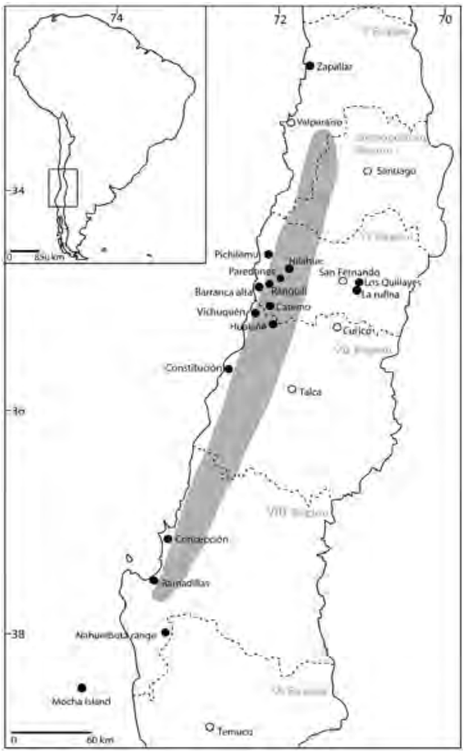

Figure 1. Distribution of *Rhinoderma rufum*. In grey solid area, the distribution of *R. rufum* as suggested by Veloso et al. (2008); dashed lines, regional borders; black circles, analyzed localities based on literature data, museums records and personal communications (see Table 1).

Discussion

This paper provides the first map of the potential distribution of *Rhinoderma rufum* derived from bioclimatic data combined with land cover information and summarized species records compiled from specimens kept in zoological collections, publications and personal communications. Environmentally suitable areas suggested by the model were situated close to the coast whereas its distribution, suggested by the Veloso et al. (2010), covers larger parts of the central valley. However, this is the area in Chile where most of the larger cities are conglomerated. The original landscape has been mostly anthropogenically modified making it difficult to find patches of unmodified vegetation (Gajardo 1994). At the same time, the distribution of *R. rufum* suggested by Veloso et al. (2010) is very similar to the Chilean cultivation areas during the 90's (Gajardo 1994). The land cover information gathered between 1999 and 2001 indicates also a high degree of anthropogenic disturbance in these areas, well mirrored by high human footprint scores. All of these former land impacts are probably currently extended in most parts of the former distribution range of *R. rufum*. Therefore, the most likely cause of population decline and possible extinction of *R. rufum* is anthropogenic habitat destruction.

Previously, Veloso et al. (2010) suggested that the possible reasons that were promoting the decline of *R. rufum* were "*the destruction of the native vegetation through the planting of pine plantations and building of second homes. However, this is unlikely to fully explain its apparent disappearance. Declines that may have taken place within environmentally suitable habitats might be the result of threats such as climate change or emerging infectious disease (possibly chytridiomycosis, although this has not previously been reported from Chile)*". In spite of this, the chytrid fungus may find suitable environmental conditions within the ranges of both *Rhinoderma* species (Rödder et al. 2009). In the meanwhile, chytridiomycosis has been recorded in Chile in an introduced species (*Xenopus laevis* by Solis et al. 2010) and in three native species including the only sister species of *R. rufum*, *R. darwinii* (Bourke et al. 2010, 2011). Previously, Bourke et al. (2010) examined histologically 115 *R. rufum* specimens (from Chiguayante collected in 1975 and Zapallar in 1926) to detect chytrid zoospores. These authors did not find chytrid-infected specimens of *R. rufum*, but further examinations were suggested to comprise more individuals, localities and a wider year range, including the most recent possible samples to establish when this disease was introduced in Chile.

Up to now, habitat destruction due to increased land use for cultivation and artificial areas are the most plausible reason for the declines of *R. rufum* and its possible local extinction. In spite of this, the potential distribution of *R. rufum* and the data presented in this paper may guide further *R. rufum* surveys helping to focus on areas where the species is likely to be rediscovered. More specifically, our model provides a valuable baseline for further analyses of *R. rufum* habitat preferences in Chile.

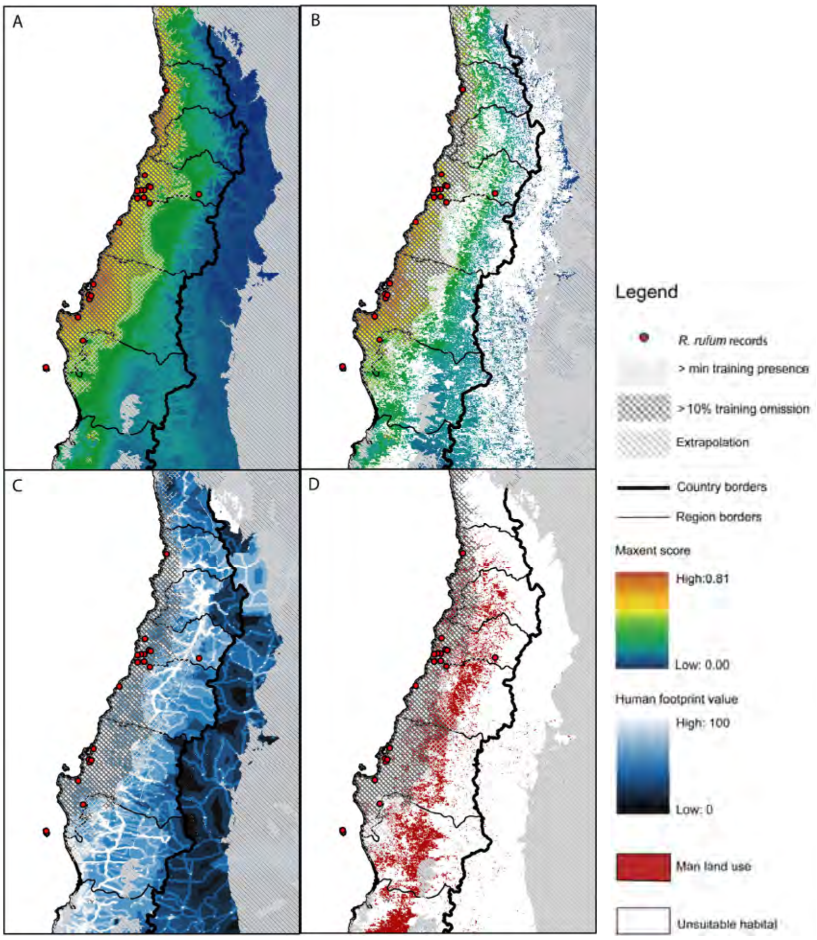

Figure 2. The potential distribution of *Rhinoderma rufum* based on bioclimatic information (A); *R. rufum* potential distribution based on bioclimatic and land use information (B; see material and methods); *R. rufum* potential distribution and human footprint (C; Sanderson et al. 2002, "Atlas of the human footprint" available at www.wcs.org/humanfootprint); *R. rufum* potential distribution and anthropogenic habitat modifications in terms of cultivated and artificial areas (D; source: Global Land Cover Information Facility 2000, GLC2000).

Hoping that in the future this frog will be found as has been the case of many other lost or thought to be extinct frogs (see introduction), it might be possible to establish an ex situ breeding program. Such facilities do exist already for its sister species, i.e. the Concepción's *Rhinoderma darwinii* breeding facility (Bourke 2010). The knowledge gathered from this species could be used to help to preserve *R. rufum* and try to save it from extinction.

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