

Eco–geographical characterization of aquatic microhabitats used by amphibians in the Mediterranean Basin

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Abstract

Eco–geographical characterization of aquatic microhabitats used by amphibians in the Mediterranean Basin.— Small freshwater ecosystems, whether of natural or artificial origin, are aquatic microhabitats for many species and are particularly important in the Mediterranean region. This study characterizes the aquatic microhabitats suitable for amphibian reproduction in the Andalusian Mediterranean Basin and identifies the environmental and geographical features that determine the presence of different amphibian species in these water bodies. Geographical and environmental favourability models were performed to determine the relationship between characteristics of the microhabitats and species presence. The characteristics analysed were geographical location, external environment (climate and topography), surrounding conditions (connectivity and conservation), type of water body, water conditions, and water dimensions. Microhabitats located in the western and central part of the study area were geographically favourable for most species. In descending order, the most common environmental factors characterizing the microhabitats were typology, surrounding conditions, water condition, external environment and size of the water body. The most common variables in the models were the connectivity between water bodies and old wells, a frequent type of microhabitat in areas of traditional cultures. Management plans should take these results into account in efforts to preserve these habitats for wildlife and especially amphibians.

Key words: Water bodies, Environmental favourability, Freshwater ecosystem, Iberian peninsula, Conservation

Resumen

Caracterización ecogeográfica de los microhábitats acuáticos utilizados por los anfibios en la cuenca mediterránea.— Los ecosistemas de agua dulce de pequeño tamaño, independientemente de su origen natural o artificial, constituyen microhábitats acuáticos de gran valor para muchas especies, especialmente en la región mediterránea. En este estudio se caracterizan los microhábitats acuáticos disponibles para la reproducción de los anfibios en la cuenca mediterránea andaluza y se identifican las características ambientales y geográficas que determinan la presencia de las distintas especies de anfibios en ellos. Se utilizaron modelos de favorabilidad geográfica y ambiental para determinar la relación entre las características de los microhábitats y la presencia de especies. Las características analizadas fueron la ubicación geográfica, el ambiente externo (clima y topografía), las condiciones del entorno (conectividad y conservación), el tipo de masa de agua, las condiciones del agua y las dimensiones de la masa de agua. Los microhábitats ubicados en la parte occidental y central de la zona de estudio fueron geográficamente favorables para la mayoría de las especies. En orden decreciente, los factores ambientales más comunes que caracterizaron los microhábitats fueron la tipología, las condiciones del entorno, las condiciones del agua, el ambiente externo y el tamaño de la masa de agua. Las variables más comunes en los modelos fueron la conectividad entre las masas de agua y un tipo de microhábitat frecuente en zonas de cultivos tradicionales: los pozos antiguos. Los planes de gestión deberían tener en cuenta estos resultados en las iniciativas encaminadas a conservar estos hábitats para la fauna y especialmente para los anfibios.

Palabras clave: Masas de agua, Favorabilidad ambiental, Ecosistemas de agua dulce, Península ibérica, Conservación

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Introduction

Water is an essential element for wildlife but human exploitation of this resource can lead to changes in freshwater ecosystems and even to their disappearance (Blondel et al., 2010). This conflict between humans and other species is especially critical in areas such as the Mediterranean region due to summer xericity, high biodiversity values (Grillas et al., 2004; Blondel et al., 2010) and high levels of species endemism (Zacharias & Zamparas, 2010). Maintenance of such aquatic habitats in Mediterranean climatic regions is thus essential for life and biodiversity (Grillas et al., 2004; Zacharias & Zamparas, 2010). Many of these freshwater ecosystems are small and they are often characterized as microhabitats.

Humans have traditionally built a vast number of artificial water bodies such as pools, ponds, basins, fountains, and water troughs. In Andalusia, the first of these water bodies were constructed in the Bronze Age (about 3,700 BP) with the rise of the metallurgical practices of the Argar culture (García-Alix et al., 2013). In the Andalusian Mediterranean Basin, in particular, artificial water bodies have been used extensively by wildlife, especially amphibians, to supply their physiological requirements and have constituted important breeding habitats for many amphibian species (Beja & Alcázar, 2003; Casas et al., 2012). Thus, both natural and artificial water bodies must be preserved to maintain the breeding habitat of amphibian species (Beja & Alcázar, 2003; García-Muñoz et al., 2010). This is particularly important given that amphibians are the most threatened vertebrates on the planet (Beebee & Griffiths, 2005).

In recent decades, the conflict between humans and amphibians over water has intensified due to changes in human water use linked to economic activity (Brühl et al., 2013), especially due to intensive agricultural practices. These practices have led to the replacement of outdoor, open water infrastructures with pipelines or underground water bodies, with the aim of increasing efficiency. These new infrastructures do not provide the conditions necessary for amphibian breeding. Traditional water infrastructures have been consequently abandoned, and amphibian conservation status has become a concern, as much as the conservation of natural microhabitats.

Amphibians are good indicators of the state of conservation of aquatic habitats, because part of their life cycle is linked to water bodies (Duelman & Trueb, 1986). Different species take advantage of different characteristics of the water bodies for their breeding success (Egea-Serrano et al., 2006; Richter-Boix et al., 2006). For this reason, characterization of water bodies is important to determine the role that their features have on the maintenance of specific amphibian populations (Calhoun & Hunter, 2003).

The relationship between amphibian presence and aquatic habitat characteristics is widely recognized, but remains vaguely defined. A key issue in amphibian conservation planning is to identify the conditions that determine the presence of each amphibian species at each water body. In this context, habitat models can be

used to identify the environmental features that explain species distributions (Fielding & Haworth, 1995). These models use mathematical algorithms to reveal the characteristics of the habitats that are most relevant for the physiological and biological requirements of the species (Barbosa et al., 2001; Muñoz et al., 2005; Romero et al., 2013). However, the geographical context used to build the models affects the relationship between environmental variables and species distribution that are retained in the models (Acevedo et al., 2012). Previous studies have analysed amphibian distribution and habitat characteristics separately. Some authors have studied reproductive habitats of species without taking into account the spatial structure of the populations (Egea-Serrano et al., 2006), whereas others have analysed species distribution without taking into account the specific characteristics of the microhabitats (Guerrero et al., 1999). Few authors have used local variables measured *in situ* as predictors in local distribution models (Gómez-Rodríguez et al., 2012; Ferreira & Beja, 2013).

The aim of this study was to characterize the types of aquatic microhabitats available for amphibian reproduction in the Andalusian Mediterranean Basin and to identify the environmental and geographical features that determine the presence of amphibian species in these water bodies. We also discuss the role of the characteristics of the microhabitats in the conservation of amphibian species.

Material and methods

The study area

This study was carried out in the Andalusian Mediterranean Basin, located in the south of the Iberian peninsula, and comprising the hydrographical basins within the Autonomous Community of Andalusia that flow into the Mediterranean Sea (Fig. 1). This territory has a surface area of 18,193 km² roughly contained in a 50 km by 350 km strip between the Strait of Gibraltar (Cadiz) and the Almanzora river basin (Almeria), and includes 652 km of coastline (MMARM, 2008). This geographical area includes large altitudinal differences and rugged mountains. The climate is Mediterranean with geographically-driven subclimates: subtropical, subdesert, continental, and mountain. The rainfall gradient ranges from 2000 mm in the west, due to the Atlantic influence, to 200 mm in the east in the Tabernas Desert (CAPMA, 2012). The extremes of the ombroclimatic belt are hyper-humid in the west and arid in the east (López et al., 2008). The temperature gradient ranges from an annual average of 18°C on the coast to 2.5°C in the mountains of the Sierra Nevada (Ninyerola et al., 2005).

Sampling of microhabitats

The classification of aquatic ecosystems is difficult due to their great variety in size, typology, hydroperiod, and vegetation. The aquatic microhabitats analysed include Williams's mesohabitats (Williams, 2006),

which were defined as temporary streams and ponds, snow–melt pools, monsoon rain pools, floodplain pools, dewponds, and wetland pools. Additionally, the aquatic microhabitats investigated in this work differ according to whether they are artificial, natural, or mixed (Beja & Alcázar, 2003; Grillas et al., 2004) and range from temporary aquatic ecosystems to water storage infrastructures of different sizes that are scattered in agricultural landscapes (Zacharias & Zamparas, 2010). We defined aquatic microhabitats as systems linked to epicontinental, non–lotic, temporary or permanent waters, linked to upwelling, drainages, or natural or artificial ponds, with an approximate maximum volume of 200 m³ and surfaces of up to 500 m² and with an associated biological community (Benítez et al., 2011).

The following literature was consulted to identify potential sites for amphibian breeding within the study area: (i) official cartographic sources (Junta de Andalucía, 2004; Instituto Geográfico Nacional, 2009); (ii) databases and scientific collections of amphibians (Asociación Herpetológica Española; colecciones del Museo Nacional de Ciencias Naturales; Estación Biológica de Doñana; Estación Experimental de Zonas Áridas; colección del departamento de Zoología de la Universidad de Granada); and (iii) theses and unpublished reports on amphibians (Reques et al., 2006).

We identified a total of 13,650 water bodies. All these points were small and 43% of them had the confirmed presence of amphibians, or suitability for them. The location of these points was processed using ESRI ArcMap 9.2 software. The itineraries for sampling were designed to include the maximum number of representative and accessible water bodies in all the river basins. Between 2009 and 2011, we sampled 568 water bodies over 64 days, travelling a total of 14,500 km (see fig. 1). Two biologists observed and sampled each water point for an average of 15 minutes. Each sampler recorded information on the presence of species, spatial situation, and environmental variables at each water body as well as the external environment (table 1). Geographic coordinates and altitude were obtained by GPS (Garmin X12). The following sampling method was used: (1) searching for and counting the number of individual amphibians present in the vicinity of the water body; (2) searching for and counting the eggs in the water body; and (3) examining the entire water body to detect larvae (Heyer et al., 1994).

Given that amphibians are difficult to detect because their reproductive cycles are closely linked to weather (Mazerolle et al., 2007), some authors propose different methods in order to increase the probability of amphibian detection (Mazerolle et al., 2007; Gómez–Rodríguez et al., 2012). However, in our study these methods were not feasible due to the large extension of the territory and the great difficulty of access to each water body. We considered instead the phenology of each species in each geographical location to visit the microhabitats when detectability was highest. Sampling efforts were concentrated in winter and spring in most locations, but in summer for sites above 1,500 meters of elevation. To locate

amphibians, we looked for adults, larvae or breeding calls in both the water body and the surroundings in a radius of 10 m. We detected larvae by dip–netting (Heyer et al., 1994). When low detectability of a particular species was an issue, the water bodies were visited several times; approximately 14.4% of the water bodies were revisited. The number of visits was included in the analysis to determine whether the different sampling effort had an effect on each species distribution.

Variables and explanatory factors

The presence of each amphibian species around each water body and its surroundings was recorded when eggs, larvae, juveniles, adults, calls, or identifiable remains (skin, bones, dry larvae, etc) were detected at the water bodies or in their vicinity. We obtained *in situ* data on 38 variables related to seven explanatory factors: five environmental factors, one geographical factor and one related to sampling effort (table 1). These environmental and geographical factors were used to investigate the relationship between the characteristics of the microhabitats and the presence of the species at each water body. We opted for an analytical approach that evaluated the role of each factor individually, because the investigation included the whole range of microhabitat characteristics that are relevant for amphibians. This is particularly important in the context of the current decrease of water bodies in the Mediterranean region, as all the critical water bodies relevant to amphibian species must be preserved and this cannot be achieved without knowing their critical characteristics separately. In a synthetic model that combined the effect of all factors (Romero et al., 2015) it would be more difficult to determine the role of each individual factor, because the most relevant factors might overshadow the effect of those of lesser, yet relevant, importance.

The location of each microhabitat, identified by longitude (X) and latitude (Y), was considered in order to evaluate the effect of the geographical factor. In this way, latitude and longitude were used to build nine spatial variables that were useful only to perform a trend surface analysis (Legendre, 1993). Thus, we included a series of polynomial expansions —X, Y, X², Y², X³, Y³, X × Y, X² × Y, Y² × X— in a logistic regression to detect the spatial structure of the water bodies used by each amphibian species. The climatic variables (air temperature and wind) and the topographic variable (altitude) were included in the external environmental factor. The variables related to connectivity between water bodies, according to their proximity to each other, and the degree of conservation of the microhabitat, according to the number of identified threats, were included in the surrounding factor. Connectivity was estimated by considering a 2 km buffer for each point, given that the species with the greatest displacement is the Natterjack toad (*Bufo calamita*), whose maximum detected displacement is 2.6 km during the reproductive period (Sinsch, 1992).

The other environmental factors describe the characteristics of the water bodies, such as the type of

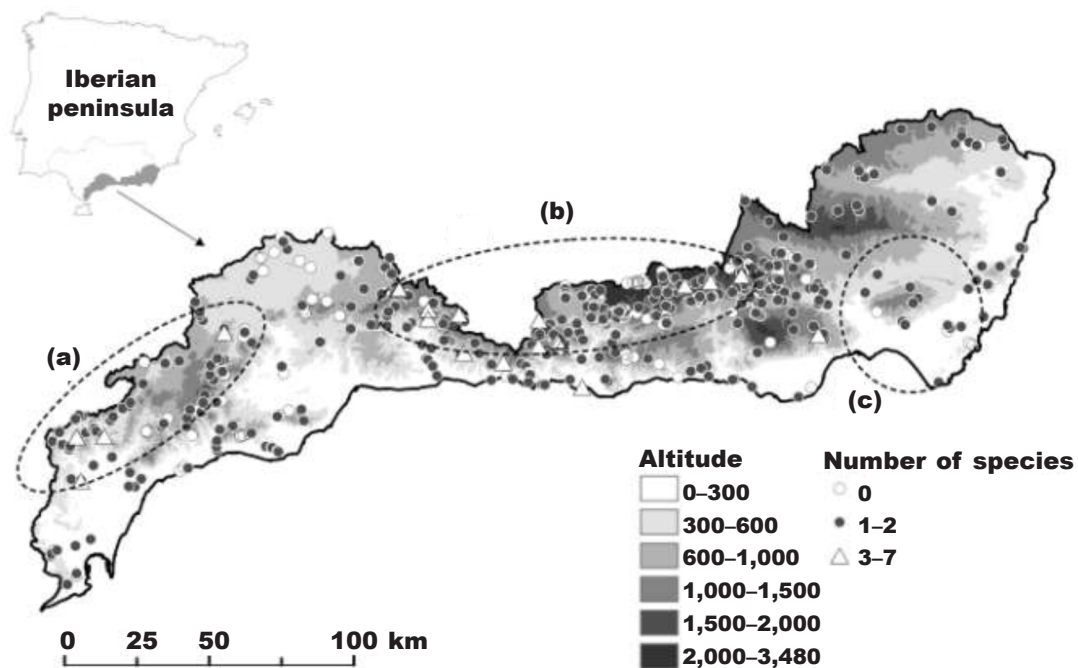


Fig. 1. Study area and distribution data showing number of species found in each water point. Altitudinal levels are indicated. The dotted ovals (a) and (b) show the most favourable zones for most of species. The dotted circle (c) shows the most favourable zone for two species (*P. perezi* and *B. calamita*).

Fig. 1. Zona de estudio y distribución de los datos, indicando el número de especies encontradas en cada punto de agua. Se indican los niveles altitudinales. Las líneas de puntos ovals (a) y (b) indican las zonas favorables para la mayoría de las especies. La línea de puntos circular (c), indica la zona más favorable para dos de ellas (*P. perezi* y *B. calamita*).

water body (Morrell, 2008), the biological and physical conditions of the water, and the size of the water body. Nine natural and nine artificial types of water bodies were identified in the study area (table 1), including, for the first time in this kind of study, old shallow wells with stone or brick walls, whose width allowed sufficient light to enter for aquatic flora and fauna to proliferate (Lanz & Greenpeace España, 1997). Water temperature, pH, and conductivity were measured using a thermometer (Eutech, ECScan, accuracy: $\pm 0.5^\circ\text{C}$), a pH meter (Eutech, pHScan 2, accuracy: ± 0.1 pH), and a conductimeter (Eutech, ECScan, SE: ± 0.01 mS), respectively. Each instrument was immersed to a depth of 2 cm. The vegetation at water bodies was assessed according to the macrophyte index of Suárez et al. (2005) and greater value was placed on the dominant taxa to assess water quality. Similarly, the macroinvertebrates were assessed according to the index of Alba-Tercedor et al. (2002). Water colour was measured on a gradient from transparent to opaque (table 1). The movement of the water mass was characterized as a function of water velocity in cm/s in an ascending gradient. The small microhabitats were measured on site and those associated to large ponds, lagoons, or reservoirs were measured using orthophotos (aerial photos corrected to represent

an orthogonal projection without perspective effects, published by Junta de Andalucía, 2004).

Wind, connectivity, conservation, vegetation, macroinvertebrates, colour, and movement are presented as semi-quantitative categorical variables (table 1). We derived wind and connectivity semi-quantitatively from speed in km/h and distance in meters from the closest water point, respectively, by considering only substantial dissimilarities in them that make a real difference for amphibians, and which go unnoticed in a continuous gradient. The typology of water bodies is presented as 18 binary variables and provides information on the presence (1) or absence (0) of each type at each body.

Geographic and environmental favourability models

A trend surface analysis was performed to obtain information on the relationship between the presence/absence of each amphibian species at each water body and geographical location (Legendre, 1993). We used backward stepwise logistic regression of the analysed water bodies on the nine spatial variables to identify the geographical probability trend and to remove the components of longitude and latitude that were redundant. Finally, the geographical pro-

Table 1. Description of variables used to build the environmental models: P/A. Presence/absence.

Tabla 1. Descripción de las variables utilizadas para elaborar los modelos: P/A. Presencia/ausencia.

Factors		
Variables	Units	Explanations
Geographical location		
Longitude (X)	m	The Universal Transverse Mercator (UTM) coordinate system
Latitude (Y)	m	projected onto the zone 30 S
External environment		
Air temperature	°C	
Altitude	m	
Wind	Range of values 1–6	Values according to speed in km/h at the following intervals: 1. Windless, 0–2 km/h, smoke rises vertically; 2. Light air, 2–6 km/h, wind direction is defined by the smoke; 3. Breeze, 7–11 km/h, wind is noticeable on face, tree leaves move; 4. Light breeze, 12–19 km/h, tree leaves move continuously; 5. Moderate breeze, 20–29 km/h, small branches move, dust rises; 6. Strong wind, > 30 km/h, small trees move, waves form in pools
Surroundings		
Connectivity	Range of values 0–3	Degrees of distance in metres of each buffer zone around the microhabitat, with the following values: 0. Isolated point, the closest point, the closest points are 2,000 m away; 1. The closest points are 1,000 m away; 2. The closest points are between 500 and 1,000 m; 3. The closest points are between 0 and 500 m
Conservation	Range of values 1–3	Values according to degree of threat: 1. Points with more than 3 types of threats; 2: Points with 1, 2 or 3 types of threats; 3. Highest level of conservation, void of threats. Types of threats: chemical pollution organic pollution, construction excavation, residual waste water, alien species, wild boar impact, neglect, floods, drought, water harvesting, cleaning or emptying, excessive livestock
Typology		
River	P/A	Permanent drainage system. In this study, river also refers to small ponds formed on the banks of the riverbed and the pools in the headwaters
Stream	P/A	Short water flow, almost continuous
Spring–fed river	P/A	Bank–side spring that gives rise to or adds water in significant quantities to a river or stream
Small wetland	P/A	An area of land that is permanently wet due to shallow superficial or subterranean sources of water including roadside water ditches
Spring	P/A	Natural upwelling of groundwater
Mine water	P/A	Artificial underground gallery that collects groundwater by gravity. If this gallery is not fully enclosed the it is called a ditch
Seepage	P/A	Small quantities of groundwater which flow from a non–permanent sources that may produce small wetlands
Temporary pond	P/A	Accumulation of non–permanent water, resulting from periods of heavy rain or other overflow water

Table 1. (Cont.)

Factors

Variables	Units	Explanations
Natural pond	P/A	Accumulation of permanent water, including high mountain lakes and other naturally occurring small bodies of water
Fountain	P/A	Simple man-made construction to raise water from a spring for daily use
Drinking trough	P/A	A receptacle built at a water source to provide livestock with drinking water or to wash clothes
Plastic-lined pond	P/A	An artificial hole lined with plastic for water storage
Earth-lined pond	P/A	An artificial hole lined with soil that can be filled naturally or artificially for water storage
Concrete pool	P/A	An artificial water tank or pool with masonry walls to store water for utilitarian purposes, such as irrigation and fish breeding or for ornamental purposes
Cistern	P/A	Underground tank
Irrigation ditch	P/A	Stone, concrete, or earth-lined ditch or channel for irrigation and other purposes
Well	P/A	Hole excavated to locate a usable vein of water. In this study, well refers to old shallow holes that are suitable microhabitats for wildlife (as opposed to modern, covered wells)
Dyke or Levee	P/A	Low transverse barriers built across ravines or streams to stop sedimentation and erosion during periods of heavy rain, in contrast to dykes built for controlling water flow with dams
Water conditions		
Vegetation	Range of values 0–3	Values according to presence of plant species that are indicators of water quality: 0. No vegetation, clear or turbid due to sediment and/or water phytoplankton; 1. Presence of helophytes and/or benthic filamentous algae; 2. Characeae algae; 3. Presence of other macrophytes
Macroinvertebrates	Range of values 0–3	Values based on the presence of species that are indicators of water quality: 0. No macroinvertebrates; 1. Presence of Diptera and/or Hemiptera and/or Annelids; 2. Molluscs and/or Odonata and/or Ephemeroptera and/or Coleoptera and/or Platyhelminthes; 3. Plecoptera and/or Trichoptera
Colour	Range of values 1–7	Values based on opacity: 1. Clear; 2. Semi-transparent green; 3. Semi-transparent brown; 4. Semi-transparent black or gray; 5. Opaque green (unicellular algae); 6. Opaque brown (due to ground solutes); 7. Opaque gray (pollution)
Movement	Range of values 1–5	Values according to velocity in cm/s: 1. Stagnant without renewal; 2. Stagnant with renewal (laminar flow without moving the entire water mass); 3. Slow movement < 20 cm/5 s; 4. Fast moving >20 cm/5 s; 5. Fast moving > 20 cm/1 s

Table 1. (Cont.)

Factors		
Variables	Units	Explanations
pH		
Conductivity	mS	
Water temperature	°C	
Dimensions		
Length	m	
Width	m	
Depth	m	
Water level	m	
Surface	m ²	
Pond volume	m ³	
Water volume	m ³	

bability values were transformed into geographical favourability values using the equation provided by Real et al. (2006):

$$F = [p/(1-p)] / [(n_1/n_0) + (p/[1-p])]$$

where n_1 is the number of water bodies where the species was found, n_0 is the number of water bodies where the species was not found, and p is the geographic probability. The favourability function reflects the degree (between 0 and 1) to which the probability values obtained in each model differ from that expected according to the species' prevalence, where 0.5 indicates no difference between both probability values. Probability depends both on the response of the species to the predictors and on the overall prevalence of the species, whereas favourability values reflect only the response of the species to the predictors. Geographic favourability values range from 0 indicating a completely unfavourable location for the water. In a similar manner, an environmental favourability model was obtained for each environmental factor and species. In this case, conditional forward stepwise logistic regression ($p < 0.05$ to include a variable, $p > 0.1$ to exclude a previously included variable) was used to comply with the parsimony principle by not including unnecessary explanatory variables in the models. Wald's test (1943) was used to assess the relative importance of each variable in the models. Wald's test relates the coefficient β of each variable with the coefficient of variation of that coefficient β in order to know the importance of each variable in the model. Akaike's information criterion (Akaike, 1973) was used to test if the model selected in the last step was more parsimonious than models in previous steps.

When performing our models, both type I and type II errors were possible. The risks of these two errors are inversely related and a researcher should determine which error has more severe consequences for the analysed situation. In our case, a type II error entails more severe consequences, as we are trying to identify the characteristics of the water bodies that are relevant for amphibian conservation. The acceptable probability of making a type I error is α , which is the level of significance established for a hypothesis test (0.05 in our case). The issue here is that when making multiple tests the type I error increases. To lower this risk, a lower value for α must be used, as the Bonferroni correction, for example, does. However, Bonferroni correction increases enormously the type II error (Pearce & Ferrier, 2000; Nakagawa, 2004). Our approach to deal with both types of error was to increase the power of the test by visiting as many water bodies as feasible, in order to reduce type II error, and to identify a low number of critical factors to test, to reduce type I error. We used 568 sampled water bodies to obtain each model, so that the power of the tests would be high, and grouped the variables into seven factors to reduce the number of models tested per species. Within each model, the stepwise procedure ensures that there is no increase in type I errors at individual steps with the number of variables tested, because at each step only the most significant variable is allowed to enter the model, whereas the significance of the rest of variables is re-analysed in the following step.

We avoided excessive multicollinearity by checking the variable inflation factor (VIF) and pair-wise variable correlations. Therefore, in variables included in any model the VIF was considered acceptable up

to 10 (Montgomery & Peck, 1992) and Spearman correlation coefficient up to 0.7. The amount of spatial autocorrelation in the variables was assessed with Moran's I coefficient (Moran, 1950).

We mapped the locations of favourable microhabitats for each species according to the characteristics related to each factor, including sampling effort. These favourable microhabitats were those with favourability > 0.5 in each favourability model.

Results

We found three urodela species (*Pleurodeles waltl*, *Salamandra salamandra longirostris* and *Triturus pygmaeus*) and eight anuran species (*Aytes dickhilleni*, *Discoglossus galganoi jeanneae*, *Pelobates cultripes*, *Pelodytes ibericus*, *Bufo spinosus*, *Bufo calamita*, *Hyla meridionalis* and *Pelophylax perezi*). All correspond to the amphibian species previously recorded in the study area. About 45% of these species are endemic to mainland Spain and the remainder are distributed throughout southwestern Europe and/or northern Africa (Pleguezuelos et al., 2002).

Most of the sampled water bodies (78%) contained at least one species and 4% contained three or more (3–7). Microhabitats with three or more species were located in mountain areas, except for one that was located in a coastal natural reserve (fig. 1). Spatial autocorrelation of all variables was very low, with Moran's I values below 0.1 except for altitude, which had a Moran's I value of 0.5.

Environmental characteristics of the microhabitats significantly favoured the presence of all species except the spadefoot toad (*P. cultripes*) (table 2; figs. 1s–10s in supplementary material).

We thus obtained significant models about favourable geographic locations of the microhabitats for every species. Favourable geographic locations for amphibians were concentrated in two areas, one westward and one central (supplementary material). The western area was favourable to *D. galganoi jeanneae*, *P. ibericus*, *H. meridionalis*, *P. perezi*, *P. waltl*, *S. salamandra longirostris*, and *T. pygmaeus* whereas the central zone was favourable to all species except *S. salamandra longirostris*. The western zone includes the medium–altitude mountains of the Serranía de Ronda and those of the Grazalema and Alcornocales Natural Parks; and the central zone includes the mountain ranges of the Sierra Tejeda, Alhama, Almjara, Lújar, and the southern slopes of the Sierra Nevada range.

About 85% of water bodies were visited once, but a significant correlation was found between the number of recorded species and the number of visits using Spearman's correlation ($r_s = 0.241$; $P < 0.05$). Sampling effort significantly favoured recording the presence of 70% of species (table 2). The location of favourable microhabitats (favourability > 0.5) for each species according to sampling effort are represented in figures 1s–10s in supplementary material.

We did not obtain a significant model for each environmental factor and species. Only for *H. meridionalis*,

P. perezi and *P. waltl* we obtained significant characterizations according to every environmental factor (table 2). As regards the variables used to measure each factor, the models for *H. meridionalis* included more variables than those obtained for other species (36.8% of all variables). The fewest characterizations (three models) were obtained for *S. salamandra longirostris* and these models included the fewest variables (7.9% of all variables).

In descending order, the most common environmental factors in the models were typology, surrounding conditions, water condition, external environment, and size of the water point (table 2). Typology entered the models for all the species, and at least one type of natural and one type of artificial microhabitat were relevant for each of them. Surrounding conditions (in terms of connectivity with other microhabitats and conservation status) were relevant for 90% of species (the exception was *B. calamita*), whereas dimensions of the water point were relevant for only 50% of species (table 2). Regarding the external environment, higher air temperature was unfavourable for 50% of amphibian species (table 2).

Regarding the number of variables, the models for *H. meridionalis* included more variables than those obtained for other species (36.8% of all variables). The fewest characterizations (three models) were obtained for *S. salamandra longirostris* and these models included the fewest variables (7.9% of all variables) (table 2).

Discussion

The richness of amphibian species in the study area follows a longitudinal gradient, with the highest number of species in the wetter west (CAPMA, 2012). However, we found that favourable geographic locations for amphibians were concentrated in two mountainous areas, one western and one central (fig. 1). This may be because the rest of the study area has been subject to more change and degradation from human activities, agricultural activities and urban development, while the eastern zone contains few natural water points due to its more arid climate. In the arid eastward zone, water bodies were only favourable to *B. calamita* and *P. perezi* (table 2). It could be because *B. calamita* reproduces in any ponds with a short hydroperiod (Reques & Tejedo, 2002), while *P. perezi* is able to use even abundant, agricultural plastic-lined man-made ponds (Llorente et al., 2002). The central area of the Guadalhorce River and the coastline of Malaga and Cadiz used to host numerous streams and wetlands with optimal characteristics for amphibians (Real et al., 1993), but many of them have disappeared due to urban, agricultural, and industrial development. *Hyla meridionalis* and *P. cultripes* are the species most affected by this process, although the geographical favourability for *H. meridionalis* still suggests a coastal distribution. However, *Pelobates cultripes* is in decline in the study area (Benítez et al., 2012), because the sandy and loose soils needed by the species to burrow in have become increasingly scarce.

Table 2. Variables included in each environmental favourability model with the signs of the coefficients (β), + or -: Ad. *Alytes dickhilleni*; Dgj. *Discoglossus galganoi jeanneae*; Pi. *Pelodytes ibericus*; Bs. *Bufo spinosus*; Bc. *Bufo calamita*; Hm. *Hyla meridionalis*; Pp. *Pelophylax perezii*; Pw. *Pleurodeles waltli*; Ssl. *Salamandra salamandra longirostris*; Tp. *Triturus pygmaeus*.

Tabla 2. Variables incluidas en cada modelo de favorabilidad ambiental con el signo del coeficiente (β), + o -. (Para consultar las abreviaturas de las especies, véase arriba.)

	Air temperature	Altitude	Wind	Connectivity	Conservation	River	Stream	Small wetland	Spring-fed river	Spring	Mine water	Seepage	Temporary pond	Natural pond	Fountain	Drinking trough	Plastic-lined pond	Earth-lined pond	Concrete pool	Cistern	Irrigation ditch	Well	Dike or Levee	Vegetation	Macroinvertebrates	Colour	Movement	pH	Conductivity	Water temperature	Length	Width	Depth	Water level	Surface	Pond volume	Water volume			
Ad	-	+		+									-																											
Dgj			+	+					+			+	+								+											+			-					
Pi				+				+														+		+																
Bs	+			+					+															+	-		-													
Bc	-												+									+																+		
Hm	-	-	-	+		+						-	+	+								+		+	+													+		
Pp	-			+		+	+																					+	+					+						
Pw	-			+										+									+			+													+	
Ssl					+										+										+															
Tp	-			+										+										+	+			-												

The study confirmed the importance of the type of aquatic habitat for amphibian species (Calhoun & Hunter, 2003; Zacharias & Zamparas, 2010). Wells entered into 60% of the models and were the most frequent typology. These structures were essential to anuran and urodele reproduction and provided shelter during dry periods (Lanz & Greenpeace España, 1997). The second most frequent aquatic microhabitat in the models was the earth-lined pond (table 2), commonly used by fauna and specifically by amphibians in the south-eastern Iberian peninsula (García-Muñoz et al., 2010).

Regarding the variables that characterized water bodies, temperature was a frequent variable in the models (appearing in 50% of them), with low values being more favourable for most of the species (1–10°C). This could be due to their nocturnal behaviour and to their location in mountain zones (Fig. 1) where there is less evaporation and therefore water bodies last longer. Our results also support the importance of connectivity between microhabitats (Tabla 2). The connection between water bodies should be maintained to allow gene flow between the different populations (Stevens et al., 2006), increasing the diversity of species (Semlitsch & Bobie, 1998).

Connectivity was more important for species that were more closely associated with water, such as *D. galganoi jeanneae*, *P. ibericus*, *H. meridionalis*, *P. perezii*, *P. waltli* and *T. pygmaeus*. Water bodies were rather isolated in some eastern watersheds (Almanzora River, Tabernas Desert, and Campo de Níjar). We suggest that management measures in these areas should be undertaken to ensure the long-term conservation of amphibians.

Some authors consider the water body size of microhabitats to be an important factor for the colonization of new water bodies by amphibians (Semlitsch & Bobie, 1998). However, in this study, water body size was only significant for 50% of the species, i.e., *D. galganoi jeanneae*, *H. meridionalis*, *P. perezii*, and *P. waltli*, probably because they are the most aquatic species and select large water bodies that are more favourable to their biological activity. Regarding water conditions, vegetation and colour were the variables most frequently included in the models. This could be because vegetation is essential for *P. ibericus*, *T. pygmaeus*, and *B. spinosus* to lay their eggs (García-Paris, 2004; Montori & Herrero, 2004), and because *H. meridionalis* needs helophytic vegetation

for its biological activity (Díaz–Paniagua, 1986). The intensity of colour was important for *P. walt* and *T. pygmaeus*, possibly because turbid water is a refuge from predators for these species.

Detectability of individuals differs among species, since some of them are conspicuous due to habits such as jumping into the water or singing, whereas others are more cryptic (De Solla et al., 2005). Some authors propose different methodologies in order to increase the detection probability of amphibians (Mazerolle et al., 2007; Gomez–Rodriguez et al., 2012). However, these methods were not feasible in our study due to the heterogeneity of the territory and water bodies. For this reason, we scheduled our visits according to the phenology of each species in each area to maximize detectability and 85.6% of water bodies were visited only once. However, when the microhabitat was visited in the appropriate phenological period for one species but not for others, the water body was revisited, thereby increasing the likelihood of finding more species. These multiple visits revealed additional species in some microhabitats, but not in others (supplementary material).

Regarding the species individually, microhabitats favourable for *A. dickhilleni* were characterized by their good conservation status, low temperatures in air and water, and higher altitudes, indicating its adaptation to mountain areas where habitat protection is also greater. *Discoglossus galganoi jeanneae* has been reported to reproduce in any shallow water with helophytic vegetation where females can lay eggs (García–París, 2004). Natural microhabitats such as small streams or temporary ponds or springs were more favourable to this species than artificial ones, though the species was also found in some artificial microhabitats, such as old wells. It was the only species favoured by wind, possibly because selecting windy places may avoid competition with species such as *P. perez*i and *H. meridionales*, which make greater use of calls and are therefore more affected by frequent strong winds (table 2).

The most important characteristics of favourable microhabitats for *B. spinosus* were the presence of vegetation, transparent water, and low conductivity. In contrast, sites with a large surface area but little volume were selected by the other congeneric species, *B. calamita*, which preferred temporary ponds. This typology is associated with the more frequent climate fluctuations to which this species is better adapted (Romero & Real, 1996).

Hyla meridionalis and *P. perez*i are closely associated with large masses of water and selected water bodies with large dimensions and with high connectivity (table 2). The water bodies that were most favourable to *H. meridionalis* were temporary ponds and natural ponds with surrounding vegetation (Sillero, 2009), whereas artificial ponds were more favourable to *P. perez*i (García–Muñoz et al., 2010). Although *P. perez*i is considered a generalist species (Llorente et al., 2002) that can adapt to conditions unfavourable to the other amphibian species and that can be present at almost any water body, our results show that this species is favoured by certain types

of microhabitats, particularly rivers, streams, plastic-lined pools and concrete pools. In addition, over 50% of characteristics of the *P. perez*i microhabitats, such as high water temperature, high pH, and width of the site, were different from those selected by other species. Thus, the models for this species indicated their preference for sites at a medium or low altitude with warm temperatures. This could be due to their preference for basking on aquatic vegetation or banks during the day, or to the fact that the reproduction of this species correlates positively to ambient temperature (Richter–Boix et al., 2006). The selection of sites of a certain width could be due to their behaviour of jumping away from threats (García–París, 2004) or to the territorial disputes between males for places to call (Díaz–Paniagua et al., 2005).

Microhabitats favourable to *P. walt*i and *T. pygmaeus* shared certain characteristics such as low temperature and connectivity and the same type of habitat: natural ponds and wells. These species are nocturnal, reproduce in natural ponds, and hide in damp places during the terrestrial phase (Montori & Herrero, 2004).

Regarding *S. salamandra longirostris*, fewer variables entered the models and only shared the conservation status of the microhabitat with other species. This was the only species that selected the presence of macroinvertebrates and fountains as a habitat; both these variables are related to the high-quality water needed by salamanders. Their larvae prefer permanent bodies of water (Baumgartner et al., 1999), but this species occupies fountains or ponds in the south of the Iberian peninsula (table 2), where water availability is lower (Egea–Serrano et al., 2006).

Conclusion

This study identified some of the geographical and ecological characteristics to take into account to maintain the conservation value of small water bodies for one of the most endangered vertebrate groups: amphibians (Beebee & Griffiths, 2005). The combination of high sampling field effort, the on-site characterization of water bodies, and modelling tools is a useful and applicable methodology. The results suggest that typology and surrounding conditions of the water bodies are critical for constituting a breeding habitat for the amphibian species that inhabit the study area. The main results of this study emphasize the importance of the typology of aquatic microhabitats and the need for connectivity between them. These results should be used to develop management tools to regulate land use, making it more compatible with the conservation of amphibians (Scocciati, 2001), especially in the most isolated habitats in medium and high mountain areas. In these areas, it is necessary to maintain existing water bodies and even to create new artificial bodies that would maintain and improve the situation of amphibians in the southern basin. Finally, coastal wetlands should be protected by encouraging moderate urban development that is more compatible with the environment and that respects the breeding sites of amphibians and their shelters.

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Supplementary material

Distribution of each species (A) and location of their favourable microhabitats (favourability > 0.5) according to the predictive factors (B to H).

Distribución de cada especie (A) y ubicación de sus microhábitats favorables (favorabilidad > 0,5) en función de los factores de predicción (de B a H).

Alytes dickhilleni

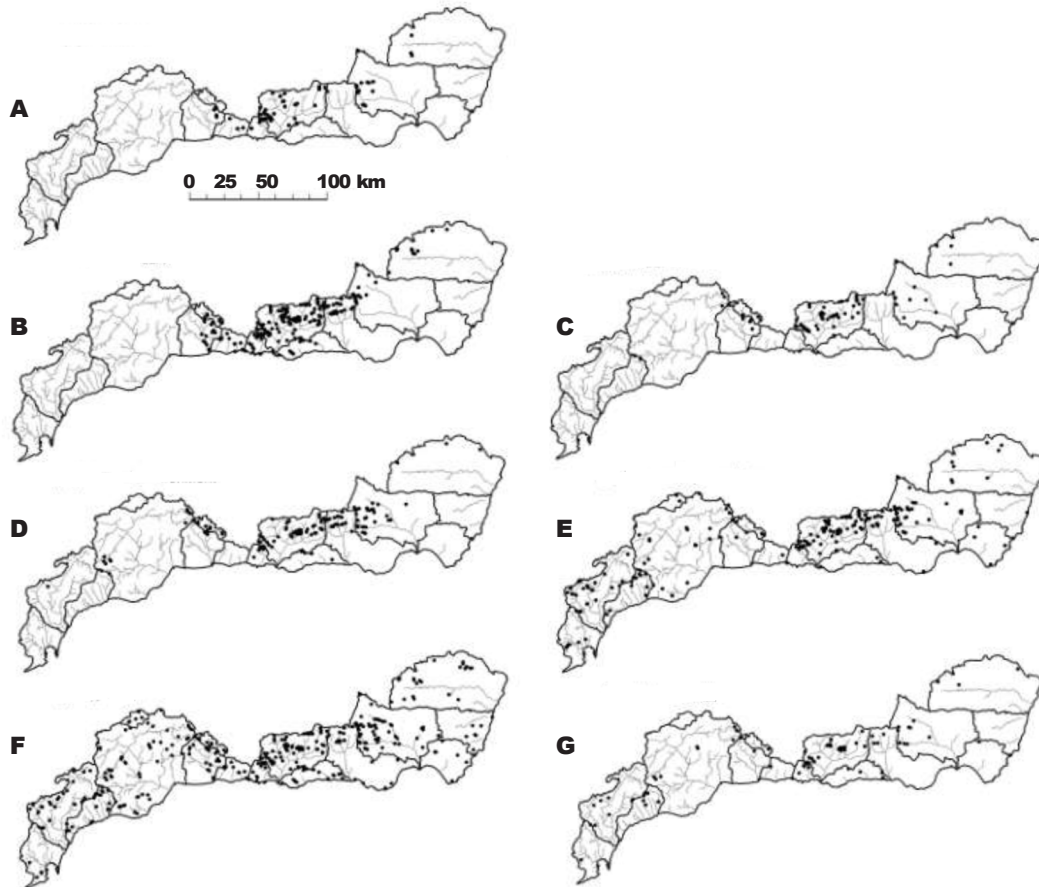


Fig. 1s. Presence of *Alytes dickhilleni* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; D. External environment; E. Surrounding conditions; F. Typology; G. Water conditions.

Fig. 1s. Presencia de Alytes dickhilleni en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; D. Entorno externo; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua.

Supplementary material. (Cont.)

Discoglossus galganoi jeanneae

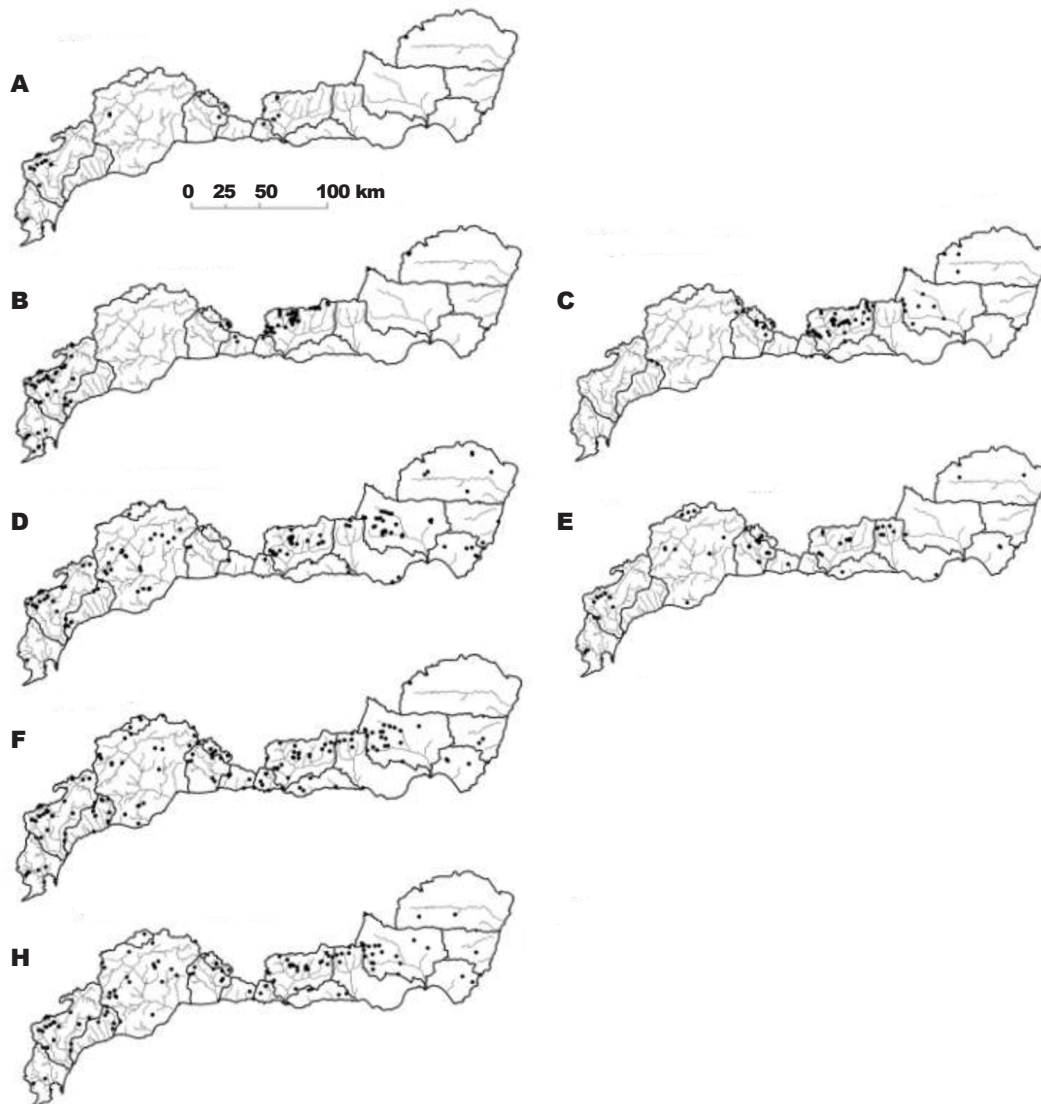


Fig. 2s. Presence of *Discoglossus galganoi jeanneae* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; D. External environment; E. Surrounding conditions; F. Typology; H. Dimensions of microhabitat.

Fig. 2s. Presencia de Discoglossus galganoi jeanneae en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; D. Entorno externo; E. Condiciones circundantes; F. Tipología; H. Dimensiones de microhabitat..

Supplementary material. (Cont.)

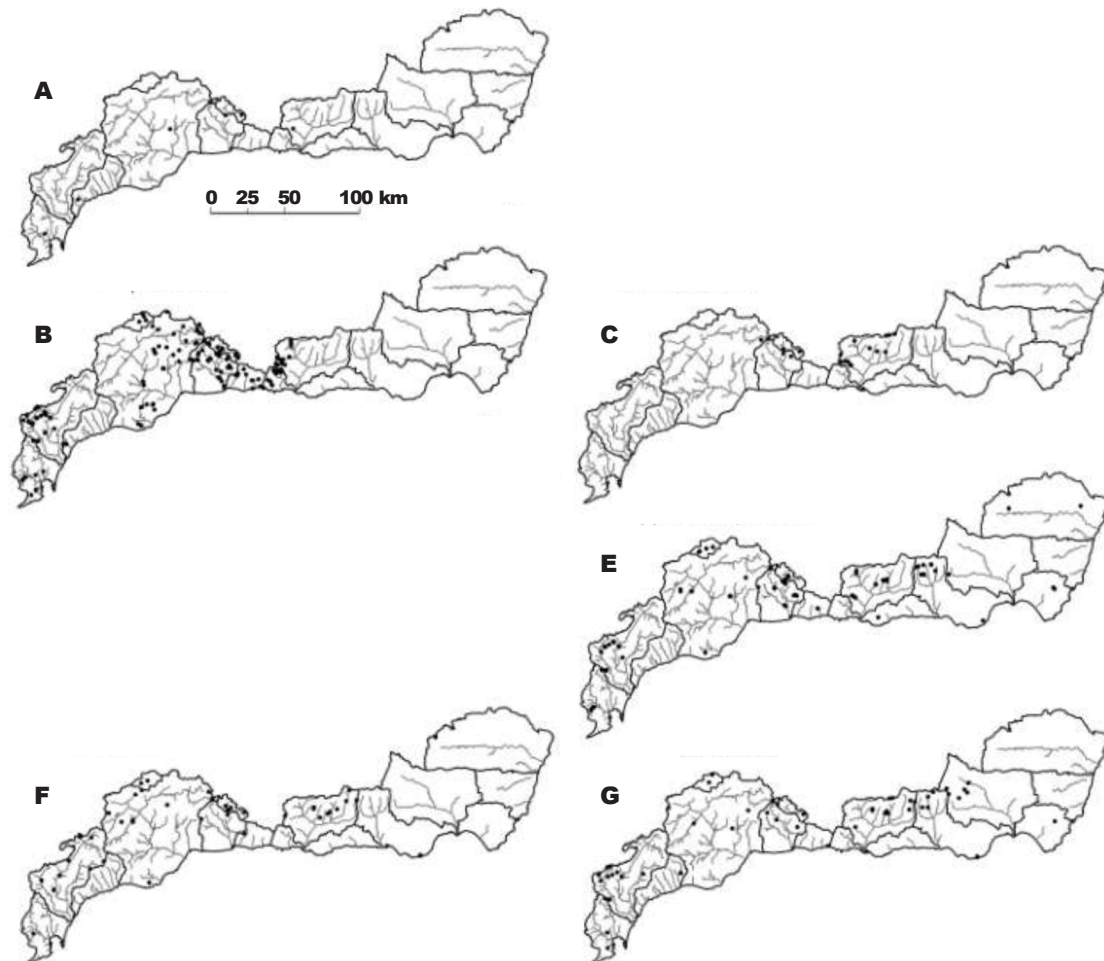
Pelodytes ibericus

Fig. 3s. Presence of *Pelodytes ibericus* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; E. Surrounding conditions; F. Typology; G. Water conditions.

Fig. 3s. Presencia de Pelodytes ibericus en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua.

Supplementary material. (Cont.)

Bufo spinosus

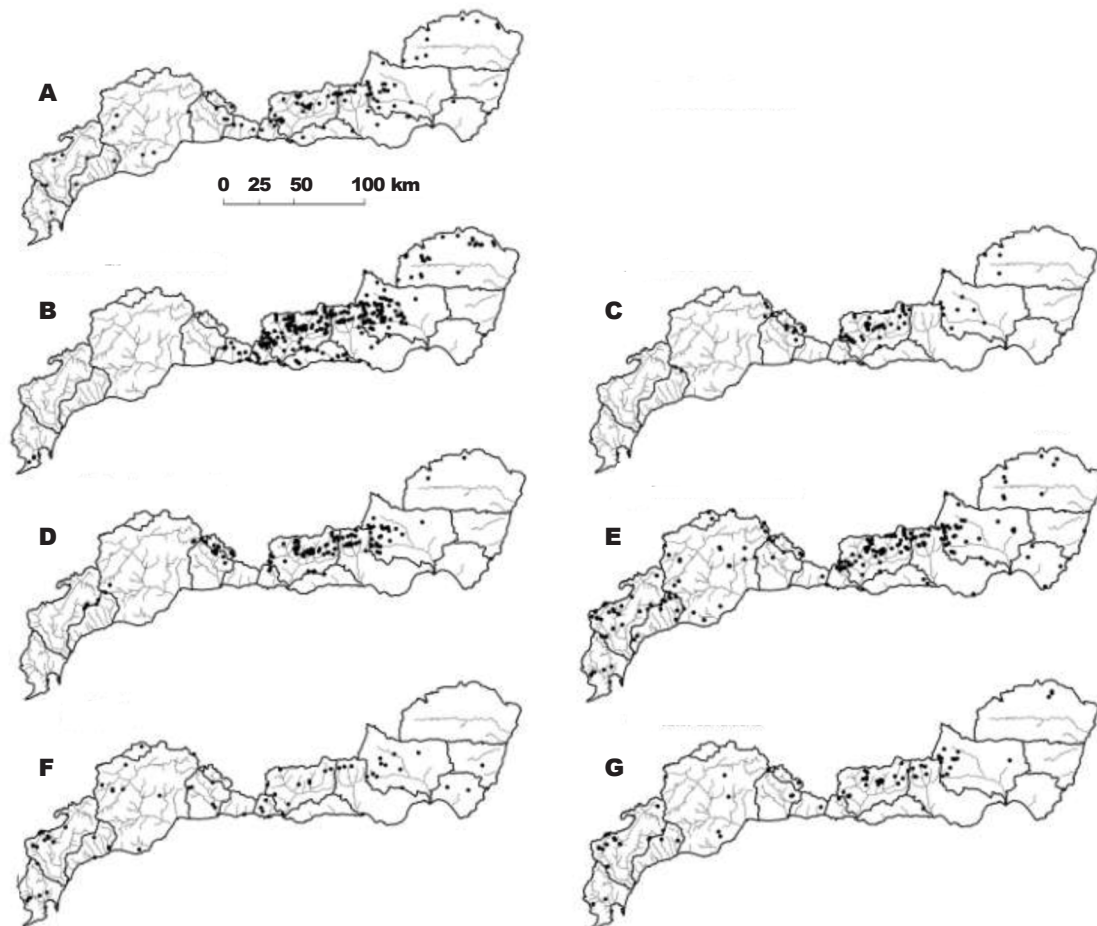


Fig. 4s. Presence of *Bufo spinosus* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; D. External environment; E. Surrounding conditions; F. Typology; G. Water conditions.

Fig. 4s. Presencia de *Bufo spinosus* en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; D. Entorno externo; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua.

Supplementary material. (Cont.)

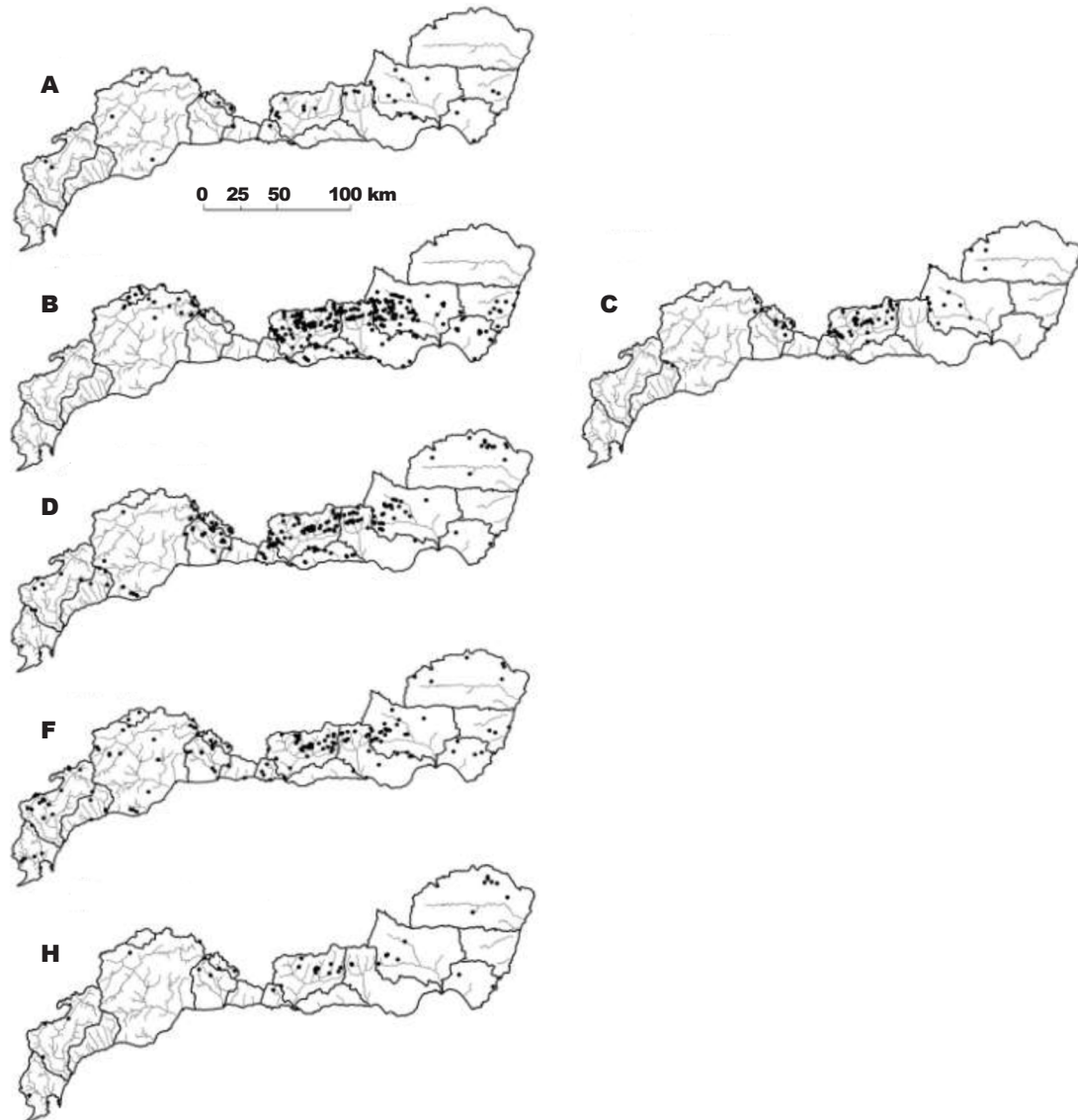
Bufo calamita

Fig. 5s. Presence of *Bufo calamita* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; D. External environment; F. Typology; H. Dimensions of microhabitat.

Fig. 5s. Presencia de *Bufo calamita* en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; D. Entorno externo; F. Tipología; H. Dimensiones de microhabitat.

Supplementary material. (Cont.)

Hyla meridionalis

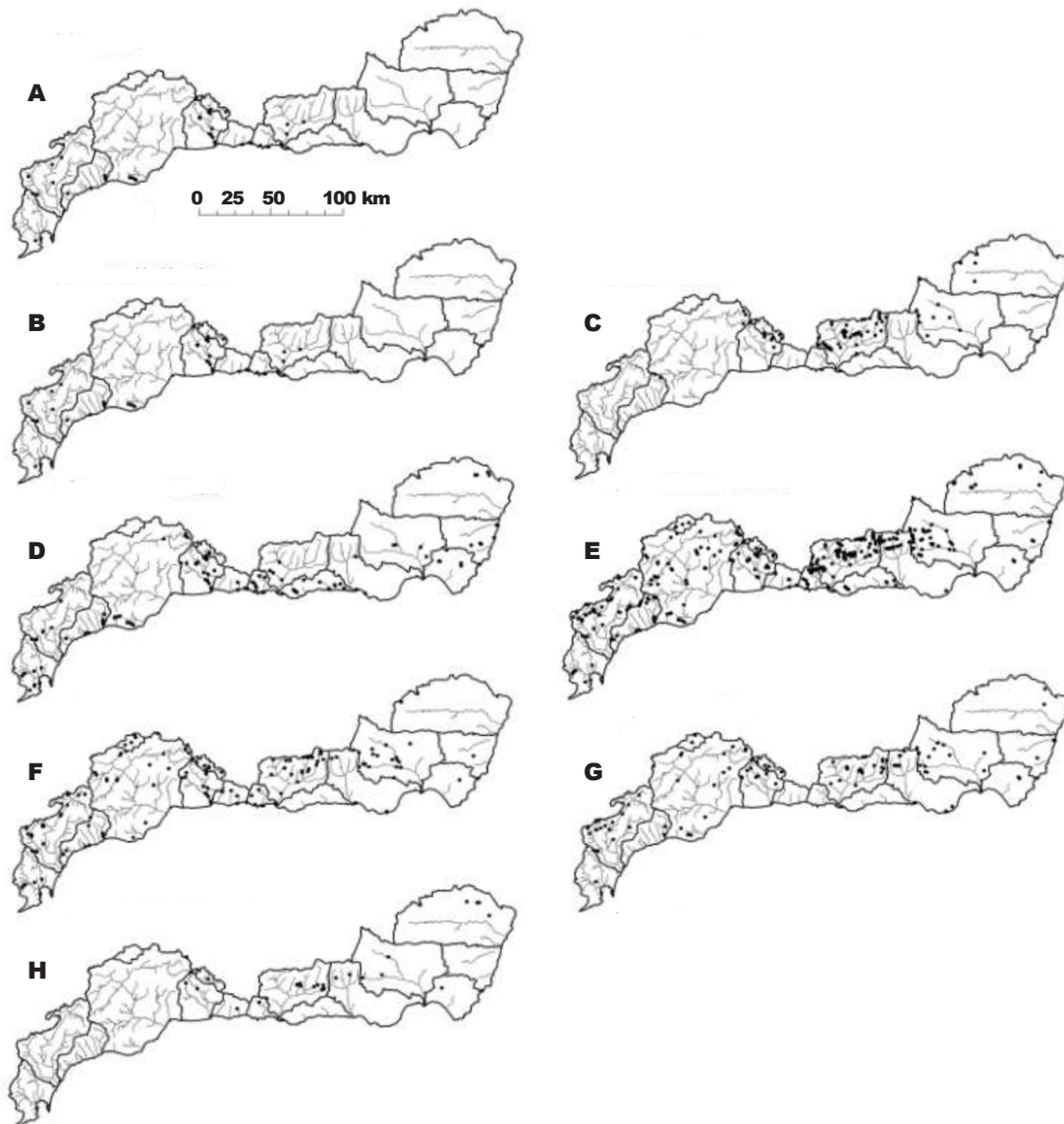


Fig. 6s. Presence of *Hyla meridionalis* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; D. External environment; E. Surrounding conditions; F. Typology; G. Water conditions; H. Dimensions of microhabitat.

Fig. 6s. Presencia de *Hyla meridionalis* en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; D. Entorno externo; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua; H. Dimensiones de microhabitat.

Supplementary material. (Cont.)

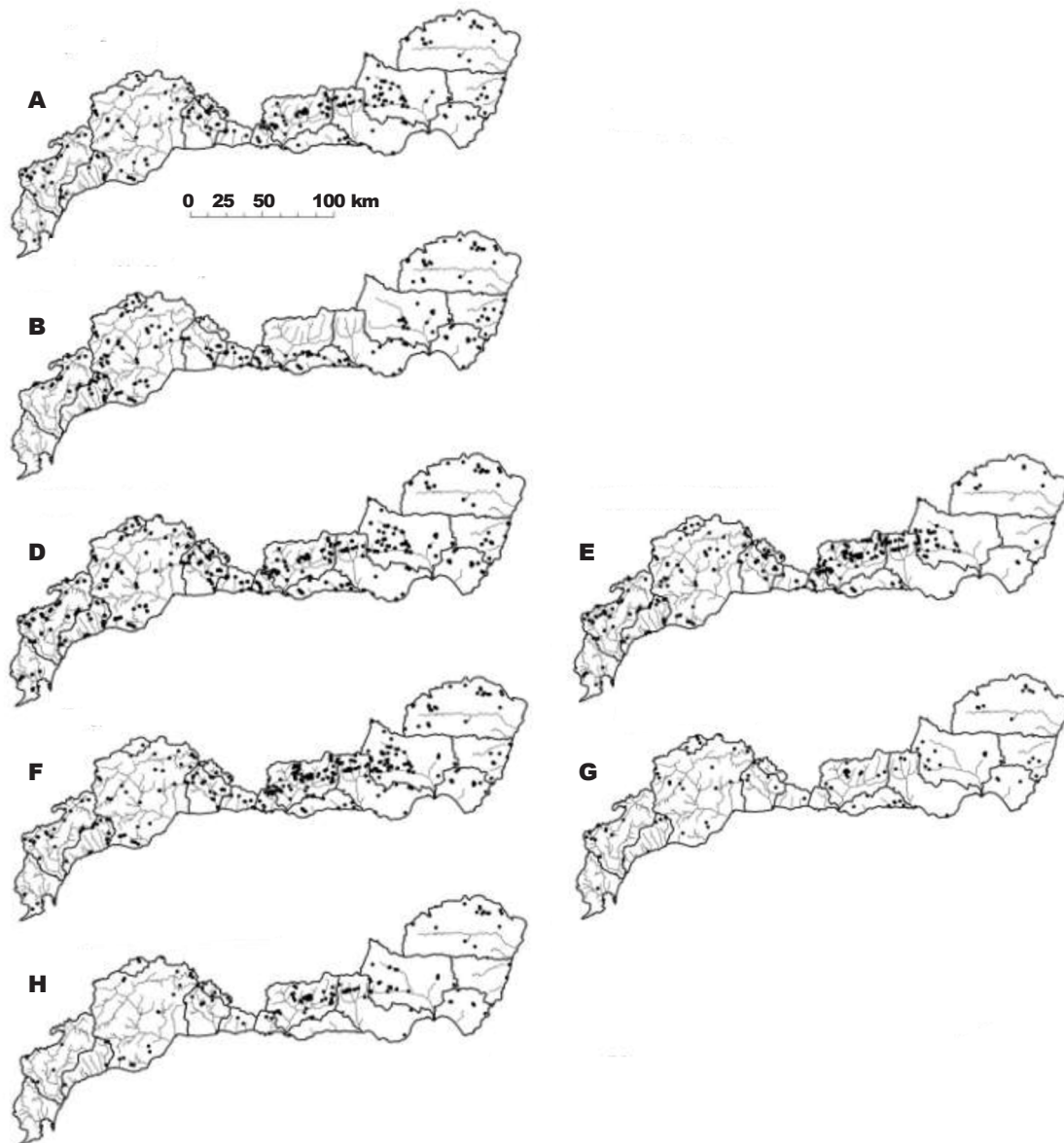
Pelophylax perezii

Fig. 7s. Presence of *Pelophylax perezii* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; D. External environment; E. Surrounding conditions; F. Typology; G. Water conditions; H. Dimensions of microhabitat.

Fig. 7s. Presencia de *Pelophylax perezii* en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; D. Entorno externo; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua; H. Dimensiones de microhabitat.

Supplementary material. (Cont.)

Pleurodeles waltl

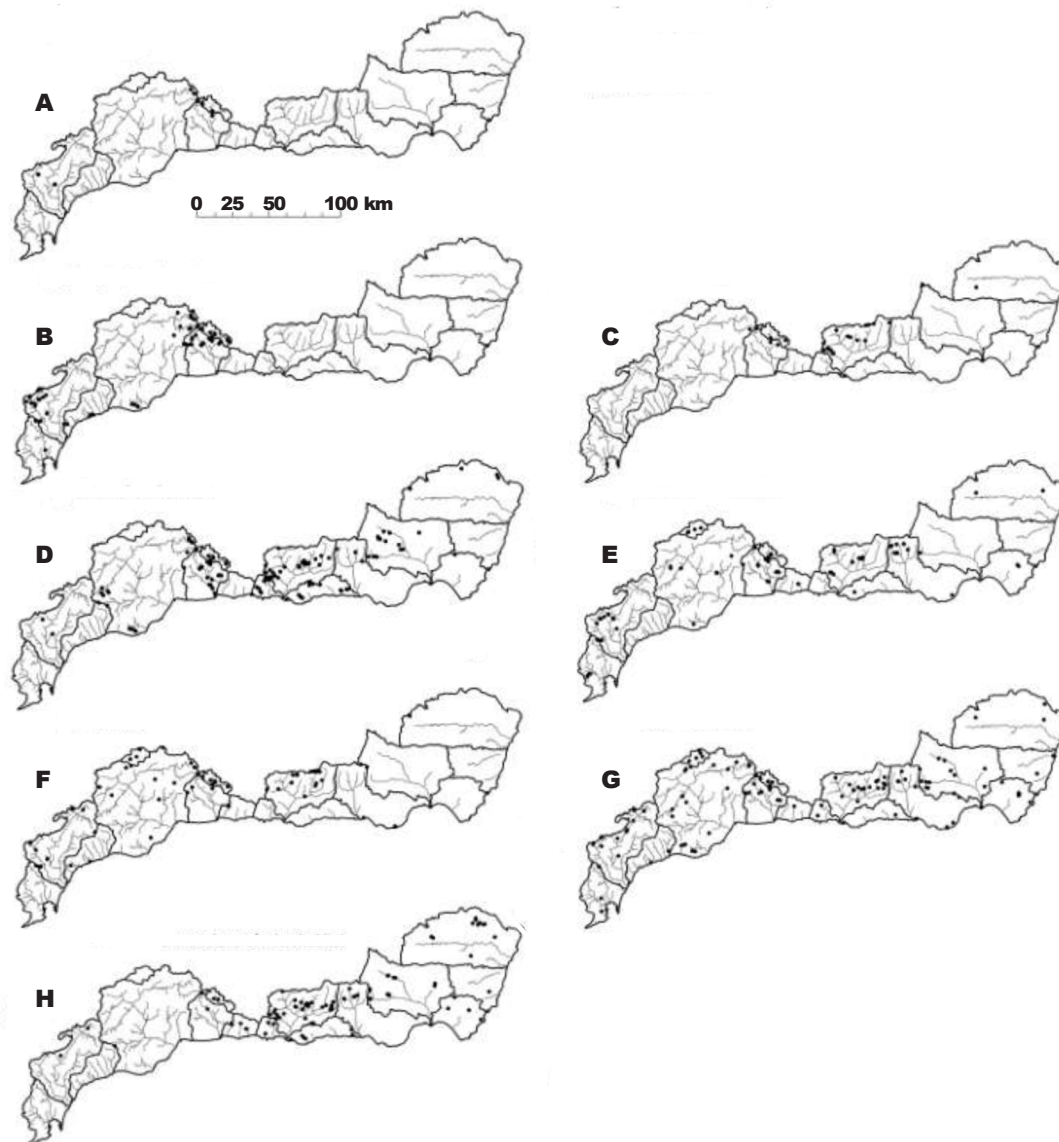


Fig. 8s. Presence of *Pleurodeles waltl* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; C. Number of visits; D. External environment; E. Surrounding conditions; F. Typology; G. Water conditions; H. Dimensions of microhabitat.

Fig. 8s. Presencia de *Pleurodeles waltl* en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; C. Número de visitas; D. Entorno externo; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua; H. Dimensiones de microhabitat.

Supplementary material. (Cont.)

Salamandra salamandra longirostris

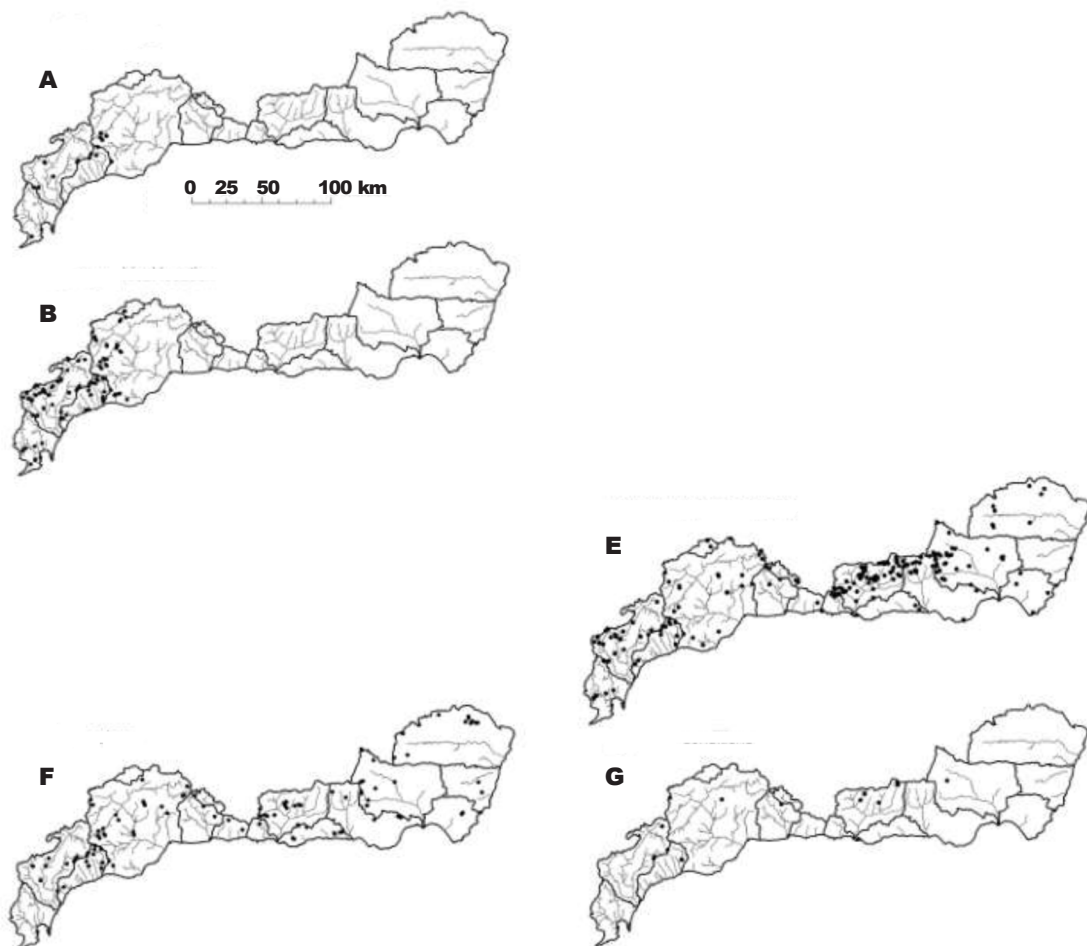


Fig. 9s. Presence of *Salamandra salamandra longirostris* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; E. Surrounding conditions; F. Typology; G. Water conditions.

Fig. 9s. Presencia de Salamandra salamandra longirostris en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua.

Supplementary material. (Cont.)

Triturus pygmaeus

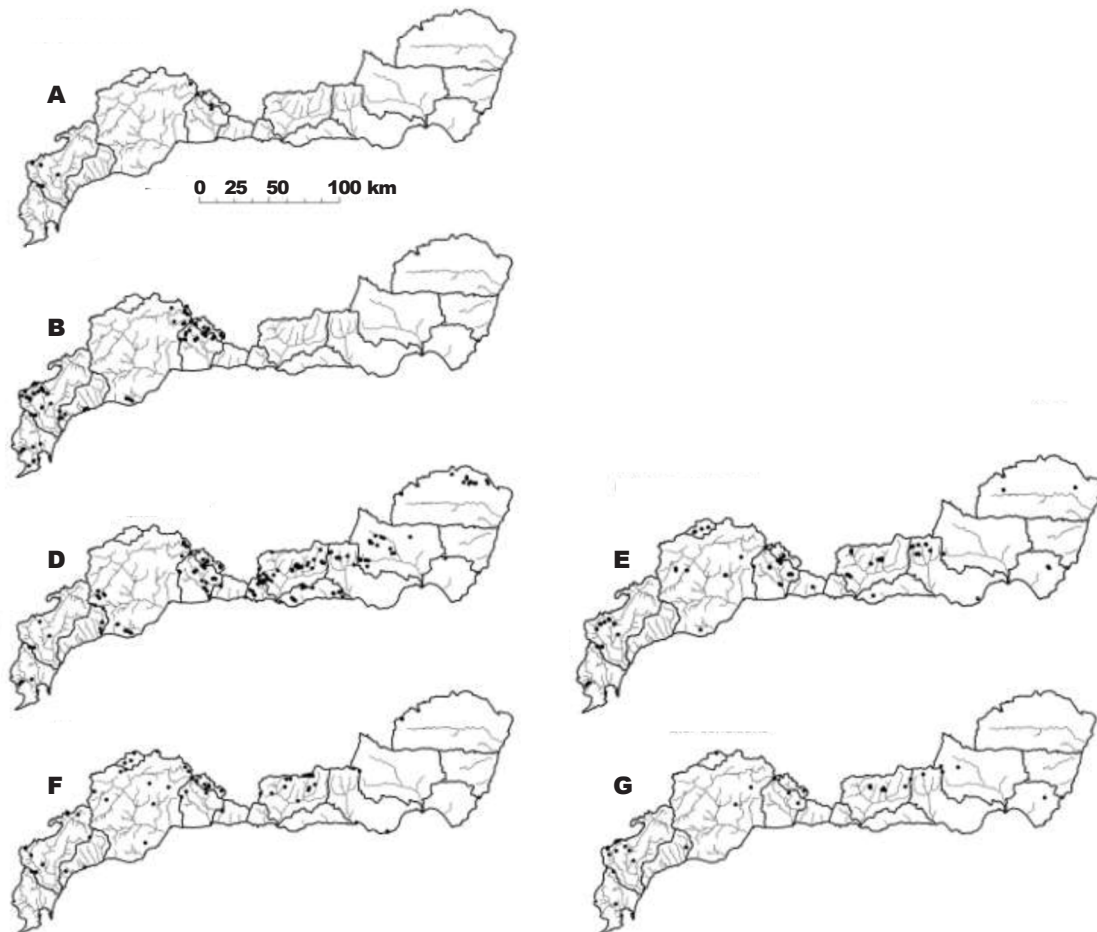


Fig. 10s. Presence of *Triturus pygmaeus* in the sampled microhabitats (A), and favourable microhabitats ($F > 0.5$) according to the factors that characterize them significantly: B. Geographical location; D. External environment; E. Surrounding conditions; F. Typology; G. Water conditions.

Fig. 10s. Presencia de *Triturus pygmaeus* en los microhábitats muestreados (A) y microhábitats favorables ($F > 0,5$) en función de los factores que los caracterizan de forma significativa: B. Localización geográfica; D. Entorno externo; E. Condiciones del entorno; F. Tipología; G. Condiciones de agua.