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Raccoon (*Procyon lotor*) in Portugal: forecasting pathways for an invasive carnivore

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RESUMO ALARGADO

As espécies invasoras constituem um grave problema ecológico, sendo consideradas uma das cinco principais causas de perda de biodiversidade mundialmente. São responsáveis pela introdução de novas doenças e pela alteração de diferentes interações bióticas e abióticas em ecossistemas nativos. As razões pelas quais as espécies exóticas são introduzidas em novos ecossistemas são variadas, mas muitas devem-se a fugas de cativeiro ou libertações intencionais. O seu controlo com sucesso nestas áreas depende sempre do quão precoce é a sua deteção e implementação de um plano de erradicação.

Um exemplo de uma espécie invasora com impactos negativos na biodiversidade nativa, é o guaxinim (*Procyon lotor*), um carnívoro generalista. Nativa da América do Norte e Central, esta espécie é considerada, atualmente, invasora em vários países do mundo, principalmente na Europa, onde foi detetada pela primeira vez em 1927 na Alemanha. Atuando como um vetor de zoonoses, perigosas tanto à saúde pública como à fauna nativa, e tendo uma dieta bastante flexível, este carnívoro invasor tem uma capacidade incrível de adaptação a novos ambientes, o que lhe permite prosperar num amplo espectro de habitats invadidos. Está geralmente associado a galerias ripícolas e ambientes com abundância de água, pois é nesses que encontra alimento (invertebrados, anfíbios, répteis, aves e pequenos mamíferos) e refúgio (árvores ocas ou caídas). Assim, os rios representam rotas de dispersão importantes, conectando habitats favoráveis a esta espécie em diferentes tipos de paisagens. É também uma espécie muito associada à presença humana, aproveitando-se do lixo e de outras fontes de alimento de origem antrópica. Assim, devido ao seu comportamento e dieta pode vir a competir com diferentes carnívoros nativos e aumentar a pressão predatória a algumas espécies já ameaçadas, como o cágado-de-carapaça-estriada (*Emys orbicularis*), o cágado-mediterrânico (*Mauremys leprosa*), o mexilhão-de-rio (*Margaritifera margaritifera*), ou espécies de aves aquáticas, especialmente anseriformes.

Na Península Ibérica existem já várias populações reprodutoras estabelecidas, todas localizadas em Espanha. A primeira área de ocorrência foi localizada perto de Madrid e Guadalajara, no centro de Espanha, tendo origem em fugas de cativeiro. A espécie foi inicialmente detetada em 2001, mas, entretanto, deram-se novos eventos de introdução, acidental ou propositada, em diferentes zonas do país. Até agora, foram registadas seis populações reprodutoras estabelecidas e vários registos isolados de guaxinins. A maior população encontra-se na região onde se deu o primeiro registo deste invasor, perto de Madrid e Guadalajara. As restantes foram registadas no Parque Nacional de Doñana (Andaluzia), Galiza, Cantábria e País Basco, e Alicante. Em Portugal, são conhecidos apenas dois registos confirmados, ambos referentes a avistamentos de indivíduos isolados no noroeste do país que escaparam das suas instalações de cativeiro, em 2008 e 2014.

Face à necessidade de atualizar a distribuição deste carnívoro invasor na Península Ibérica, algo que não é feito desde 2012, recolhemos registos de guaxinim em Portugal e Espanha, de populações reprodutoras estabelecidas e indivíduos isolados em liberdade, assim como espécimes mantidos em cativeiro. Com esta informação construímos modelos estatísticos preditivos, através do software Maxent, criando mapas que assinalam quais as áreas com maior probabilidade de ocorrência de populações reprodutoras desta espécie e, assim, identificar quais as regiões mais vulneráveis para onde esta espécie se poderá expandir em Portugal. Os modelos Maxent são uma técnica de modelação de distribuição de espécies que utiliza apenas dados de presença e que, através do princípio da máxima entropia, estima a probabilidade de presença da espécie em estudo, utilizando variáveis relacionadas com a sua ecologia e os dados de presença da mesma. No decorrer do processo de modelação ecológica, avaliámos quais as variáveis ambientais determinantes para a presença de guaxinim, de modo a perceber quais melhor preveem a sua distribuição, num contexto ibérico. Após analisar as áreas mais vulneráveis em Portugal, realizámos uma prospeção de campo onde procurámos indícios de presença de guaxinins, com ajuda de uma equipa de biólogos especialistas em guaxinins e um cão pisteiro especificamente treinado para a deteção desta espécie. Colocámos também câmaras de foto-armadilhagem numa das zonas, de modo a aumentar a eficácia de deteção deste carnívoro exótico. Fizemos também, um inventário das instalações que tenham um historial de guaxinins em cativeiro por toda Península Ibérica. Finalmente, para sensibilizar a população em geral quanto ao perigo desta espécie como ameaça à biodiversidade e na tentativa de obter registos de presença da espécie através de uma abordagem de ciência-cidadã, foram também publicados dois artigos numa revista online (Wilder), onde foi solicitado aos leitores que, caso detetassem esta espécie em meio natural, comunicassem com a equipa do projeto e facultassem os dados da sua ocorrência.

No total, coletámos 1090 registos de presença de guaxinim em toda a Península Ibérica entre 2005 e 2020. Destes, 1025 correspondem a registos de populações reprodutoras estabelecidas. A maior parte dos registos corresponde à grande população do centro de Espanha e provêm de animais capturados, mas foram igualmente obtidos vários registos isolados de guaxinim, alguns deles perto da fronteira com Portugal. Registámos também, na Península Ibérica, pelo menos 15 instalações que possuem, ou possuíram num passado recente, guaxinins em cativeiro. Destas, pelo menos 2 estão associadas a fugas confirmadas de guaxinins em cativeiro, mas suspeita-se que este número seja superior. Em Portugal, recolhemos informação de 8 possíveis registos desta espécie em liberdade. No entanto, não conseguimos comprovar a sua presença devido à falta de dados que corroborem os avistamentos. Em Portugal existem, pelo menos 3 instalações com presença comprovada de guaxinins em cativeiro, contudo, duas delas asseguram que têm os animais esterilizados e em nenhuma há evidências de uma possível fuga.

Identificámos a proximidade com cursos de água e valores médios de precipitação anual entre 400 e 1450 mm como os melhores preditores da presença de guaxinim. Assim, regiões com estas duas características serão aquelas para onde o guaxinim se poderá expandir, e fixar, com sucesso. É no noroeste de Portugal que a maioria dos rios apresenta habitats adequados para esta espécie e, por isso, esta região é a mais vulnerável a uma possível invasão por parte do guaxinim. No entanto, a zona centro do país, junto à fronteira com Espanha também foi identificada como de risco de invasão por parte desta espécie.

Durante a nossa prospeção de campo em Portugal, incidindo no noroeste e na zona centro, não encontrámos evidências da presença da espécie em Portugal, mesmo em zonas em que há registos possíveis e isolados de guaxinim, ou onde existem registos confirmados em Espanha, junto à fronteira portuguesa, nomeadamente ao longo do rio Minho, em Cáceres e na vertente galega da Serra do Gerês (Ourense). No entanto, outras zonas podem também estar sujeitas a uma invasão por este carnívoro, que utiliza os rios como rotas de dispersão, como é o caso das zonas portuguesas da bacia hidrográfica do rio Tejo, que alberga, na porção espanhola, a maior população ibérica. Portanto, é fundamental garantir que as autoridades competentes assegurem uma monitorização regular das áreas selecionadas pelos nossos modelos como altamente vulneráveis à invasão, principalmente as que ficam perto da fronteira com Espanha e das áreas próximas de instalações que alberguem guaxinins em cativeiro, uma vez que existem fugas confirmadas em Espanha e em toda a Europa neste tipo de instalações. Paralelamente, sugerimos o acompanhamento constante da situação em Espanha, em especial no que diz respeito a registos de presença de guaxinim junto à fronteira Portuguesa, para evitar ou detetar precocemente uma eventual invasão desta espécie em Portugal. Assim, será possível minimizar eventuais impactos deste carnívoro invasor na fauna nativa portuguesa.

Palavras-chave: Conservação, Espécies Invasoras, Maxent, Modelos de Distribuição de Espécies, Prevenção

ABSTRACT

Raccoons (*Procyon lotor*) are generalist carnivores native to North and Central America. Currently, this species is considered invasive across several countries in the world, especially in Europe, being a vector of zoonoses and an effective predator of native species, given its flexible diet that enables to prosper in a wide range of invaded habitats. In the Iberian Peninsula, there are already several established breeding populations, all of them located in Spain. The largest population and the first to be established in the wild is located near Madrid and Guadalajara, in Central Spain. In Portugal, so far there are only two confirmed records and both referring to isolated events of escaped individuals. Given the need for updating the population status of this invasive carnivore in the Iberian Peninsula, we collected records from the established breeding populations and isolated individuals and, by using the Maxent ecological modelling approach, we identified the most vulnerable areas to where this species might expand its range. Within these areas, in Portugal, we identify two high risk and priority regions, where we conducted a field survey in search of raccoon's signs of presence. In total, we collected 1090 records of raccoon presence throughout all Iberia, between 2005 and 2020. Of those, 1025 records corresponded to established breeding populations with some isolated records located in areas near the Portuguese border. Our model results enabled us to identify the most important environmental variables in predicting raccoon presence in an Iberian context. Areas in proximity to water bodies and with mean annual precipitation values between 400 and 1450 mm seem to gather suitable conditions to host raccoons, and therefore are higher invasion risk areas, where this carnivore can expand to. In northwestern Portugal, most rivers present suitable habitats for this species, although during our field assessment there was no clear evidence of any new sign of the presence of raccoons in Portugal. However, there are some records near the Portuguese border, linked to isolated individuals and breeding populations, near the Minho river and the Spanish slope of Serra do Gerês, respectively. Therefore, it is crucial to ensure that regular monitoring of the areas identified in our models as highly vulnerable to invasion risk, as well as those near facilities with captive raccoons, since there are confirmed escapes of individuals from captivity in Spain, and all across Europe. Furthermore, constant monitoring of the Spanish situation is also crucial, especially to assure an early warning regarding the establishment of breeding populations near the Portuguese border, to prevent and minimize the effects of an eventual invasion of this species into the Portuguese territory.

Keywords: Conservation, Invasive Species, Maxent, Prevention, Species Distribution Models

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1. INTRODUCTION

Invasive species have ecological consequences worldwide, inducing changes in many of the world ecosystem and being one of the five major causes of biodiversity decline globally (Reid *et al.* 2005). These species generally have a high ability to adapt to new environments and can bring several ecological effects on native communities as niche displacement, competitive exclusion, and loss of mutualistic interactions between native species, thus altering different biotic and abiotic interactions and processes in the ecosystems where they are introduced. Furthermore, invasive species are often responsible for the introduction or transmission of several wildlife diseases, contributing to the reduction of fitness and abundance in native species populations (Mooney and Cleland 2001; Keller *et al.* 2011), as well as acting as vectors of zoonotic diseases with a risk to public health (Hulme 2014). In extreme cases, especially where the invader is a predator, these impacts may even lead to the extinction of native species (Mooney and Cleland 2001). However, the presence of invasive species does not only pose a threat to native biodiversity, but they may also constitute a significant challenge and menace to different human activity sectors, such as agriculture, fishing, aquaculture, among others (Pyšek and Richardson 2010). Furthermore, invasive species may also raise negative emotional perceptions and attitudes by humans, when affecting pets and other domestic animals. The multidimensional effects of invasive species are reaching such a paramount scale that the Convention on Biological Diversity (CBD) deliberated that participant parties, such as the European Union (EU), should take action to prevent the introduction, spread and trade of invasive species, as well as control and/or eradicate those who pose a threat to native ecosystems (https://www.cbd.int/).

Biological invasions are structured in distinct population processes (Colautti and MacIsaac 2004; Keller *et al.* 2011). For a species to become invasive it needs, first, to be moved to regions located outside its native range, through direct or indirect human activity (i.e. arrival phase). Then, if it survives transportation, it is called an introduced species. However, only when the introduced species manages to escape and establish in the wild, reproducing independently, and its populations grow to levels that prevent local extinction, it becomes an established species (i.e. establishment phase). Thereafter, if it spreads widely through its new range, occupying new habitats or areas (i.e. spread phase), and causes significant negative ecological and/or economic impacts it is referred to as an invasive species (i.e. impact phase). Thus, prevention of arrival is the best approach to avoid the establishment of invasive species and to reduce the magnitude of their ecological and economic impact. Past that point, the control of invasive species should take place at an early stage of the invasion, so that the problem does not reach irreversible proportions (Pyšek and Richardson 2010; Keller *et al.* 2011). Usually, early invasion stages are the most critical, as invasive species are more easily controllable by actions targeting the founder populations, which are often small and more prone to local extinction (Mehta *et al.* 2007).

Invasions from terrestrial vertebrates can occur due to intentional releases into the wild (Perrings et al. 2010), or accidental escapes from captivity or domestication (Hulme 2007). Escapes into the wild are mostly related to the pet trade, live animals for food, fur farms, and even zoos activities (Hulme *et al.* 2008; Keller *et al.* 2011). So, international animal trade is indirectly responsible for the introduction of exotic species in several countries across the world (Westphal *et al.* 2008; Hulme *et al.* 2008). Many of these escapes or releases from their captive facilities (Hulme et al. 2008) resulted in expanding wild populations, which later became invasive.

Raccoons (*Procyon lotor*) (Figure 1.1) are a great example of an invasive species, widely used in the pet trade and fur market, which are still bred in captivity in many European countries (Biedrzycka *et al.* 2014). These activities can lead to accidental escapes or even intentional releases (e.g. for hunting purposes) of some captive animals into the wild (Kauhala 1996; Beltrán-Beck *et al.* 2012; García *et al.* 2012; Biedrzycka *et al.* 2014; Mori *et al.* 2015). The scale and impact of these releases are so high that this carnivore is listed as one of the 100 most invasive species in Europe (Winter 2009). Pet trade is often a consequence of this species' aesthetics and funny behaviours, like washing food before eating it (Lyall-Watson 1963), which enhances raccoon popularity among humans. Raccoons are generalist mesocarnivores native to North and Central America, being able to live in a broad spectrum of habitats with water available nearby, a major limiting factor for its presence and abundance (Gehrt and Fritzell 1998; Beasley *et al.* 2007). The high ecological adaptive plasticity in raccoons allows its coexistence with humans in urban areas, which is reinforced by the high food abundance in these anthropic environments (Bartoszewicz *et al.* 2008). This adaptability is linked to the fact that, although it is a carnivore, generally behaves as an opportunistic omnivore, feeding on fruits, invertebrates, fish, amphibians, reptiles, birds, other mammals and even human wastes (Lotze and Anderson 1979; Kauhala 1996). Such a highly flexible diet can be an important advantage in adapting to new environments and when facing new challenges in the range where they are introduced (Ikeda *et al.* 2004; Bartoszewicz *et al.* 2008). Their high water requirement makes wetlands and riparian zones highly suitable habitats for this invasive species. Furthermore, besides water, these riparian habitats also provide a wide variety of food resources that can be used by raccoons (e.g. berries and aquatic invertebrates; Stuewer 1943; Lotze and Anderson 1979) and refuges structures (e.g. hollow trees; Stuewer 1943; Gehrt and Fritzell 1998; Bartoszewicz *et al.* 2008). Besides being resource provider habitats, riparian areas can also play an important role in dispersion and colonization to new areas (García *et al.* 2012; Mori *et al.* 2015). However, both large rivers and mountain ranges seem to represent geographical barriers to the dispersal of this invasive species (Cullingham *et al.* 2009; Puskas *et al.* 2010; Mori *et al.* 2015; Fischer *et al.* 2015). Raccoons' ability to invade successfully new territories is also linked to their reproductive strategy. Their cubs are, generally, born between April and May in litters of one to seven individuals (Lotze and Anderson 1979; Ritke 1990), which is a high number of cubs for a carnivore with the size of raccoons, possibly due to greater food ingestion during gestation or the reduction of cubs body mass (Ritke 1990). Before the next breeding season, juvenile males migrate to new territories, while young females are philopatric (Gehrt and Fritzell, 1998).

Figure 1.1- A raccoon in the wild. *(Photo credits: Jorge Layna).*

Raccoons are a widely distributed invasive species, with breeding populations occurring in countries like Japan, Russia, Iran, Azerbaijan, Uzbekistan and much of Europe, including the Iberian Peninsula (Figure 1.2) (Ikeda *et al.* 2004; Frantz *et al.* 2005; Beltrán-Beck *et al.* 2012; García *et al.* 2012; Farashi *et al.* 2013; Timm *et al.* 2016; Bencatel *et al.* 2019; Louppe *et al.* 2019). In Europe, the first documented occurrence of a raccoon in the wild was on 1927 in Germany, where its numbers and range have increased dramatically throughout the last decades, given the absence of any larger predator, or competitor, to control its populations (Lutz 1996). Consequently, the species is already widely spread across Central and Eastern Europe, with reported presence in at least 27 countries (Figure 1.2), and with few where this carnivore is not yet established, such as the United Kingdom, Slovenia or Portugal (Salgado 2018). The consequences associated with the raccoon's widespread invasion encompasses several conservation concerns: 1) competition with native species for resources, as reported in Japan by Ikeda *et al.* (2004), where raccoons compete with native raccoon-dogs, owls and possibly foxes for food and refuge (although there is, to date, a lack of evidence of such impacts for European ecosystems); 2) increase predatory pressure upon small vertebrates due to its high diet adaptability, adding additional risks to already threatened species, including fish, amphibians, reptiles and small birds (Ikeda *et al.* 2004; Bartoszewicz *et al.* 2008; Salgado 2018). In particular, this carnivore poses a real threat to the tree and, especially, the ground-nesting birds, due to the high predation of eggs and nestlings that are easily accessible to this skilled and resourceful predator (Kauhala 1996; Ikeda *et al.* 2004; Ellis *et al.* 2007; Bartoszewicz *et al.* 2008; García *et al.* 2012). Furthermore, raccoons also constitute a threat to public health. This species is a transmission vector of *Balisascarys procyonis*, a nematode fatal to humans, and also carry other dangerous diseases that can affect both humans and animals, such as rabies, canine distemper, and others (Ikeda *et al.* 2004; Bartoszewicz *et al.* 2008; Beltrán-Beck *et al.* 2012). Lastly, this species induces economic impacts on human communities causing damages in different primary sectors, such as poultry production and agriculture, and also on public and animal health (including animal recovery centres), due to their role in the transmission of zoonoses, their diversified diet and anthropophilic behaviour (Ikeda *et al.* 2004; Beltrán-Beck *et al.* 2012).

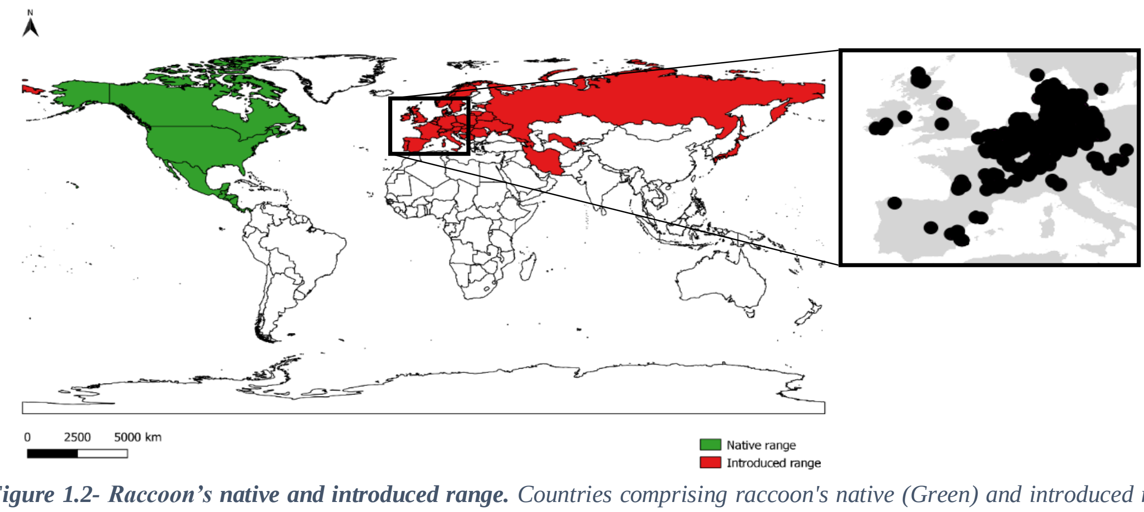

Figure 1.2- Raccoon's native and introduced range. *Countries comprising raccoon's native (Green) and introduced range (Red). Inset map with records of raccoons occurring in the wild in Europe (black dots) (source: Louppe* et al. *2019).*

In the Iberian Peninsula, the raccoon was first detected in the wild in 2001, in the province of Madrid, and since then has been spreading across continental Spain (García *et al.* 2012). The largest population is distributed between the provinces of Madrid and Guadalajara, whose founders seem to be originated from, at least, two distinct episodes of introduction based on genetic evidence: one in Jarama river and another in the Henares river, both included in Tagus river basin (García *et al.* 2012; Alda *et al.* 2013). Wild populations of this species were also detected in the Doñana National Park (Andalusia), Alicante, Galicia, Biscay, Cantabria, and other Spanish regions (Figure 1.3), indicating a series of independent introduction events throughout the country (Fernández-Aguilar *et al.* 2012; García *et al.* 2012). However, the published information on raccoon presence and distribution in Iberia is sparse and outdated (>7 years; e.g. García *et al.* 2012; Alda *et al.* 2013), a pattern of great concern especially when dealing with a species with a high spreading invasion potential (Louppe *et al.* 2019). Furthermore, the rivers in the Iberian Peninsula seem to offer an abundance of food and refuge while acting as dispersion corridors, increasing the risk of natural colonisations to neighbouring areas, including Portugal (García *et al.* 2012; Alda *et al.* 2013). However, native carnivores also use such environments and these sympatric spatial patterns may increase competition for resources. Particularly, native prey that are important food resources to Iberian predators, are often more abundant in these riverine environments (Rosalino *et al.* 2009). Furthermore, these systems have a generally high abundance of the introduced red swamp crayfish, *Procambarus clarkii*, that became an important nutritional source for many carnivores in Iberia (Gherardi 2006; García *et al.* 2012; Almeida *et al.* 2012; Barrientos *et al.* 2014; Melero *et al.* 2014). Consequently, raccoons may compete for food and foraging areas with several Mediterranean carnivores, such as the Eurasian otter (*Lutra lutra*) and the European polecat (*Mustela putorius*), a species that is suffering a population decline in recent years (Skumatov *et al.* 2016; Salgado 2018). Raccoons may also compete for resting sites with other Iberian predators, such as owls (Ikeda *et al.* 2004), common genets (*Genetta genetta*) or stone martens (*Martes foina*). Predation by raccoons in Iberian landscapes might increase the extinction risk of several threatened Iberian species somewhat linked to riparian environments, such as the European pond turtle (*Emys orbicularis*), the Mediterranean turtle (*Mauremys leprosa*), and aquatic birds, especially duck species, which are more vulnerable when resting in their ground nests or when are flightless for a certain amount of time (Blanco and González 1992; Cabral *et al.* 2005; Alvarez 2008; García *et al.* 2012). When available, raccoons also feed on bivalves (Simmons *et al.* 2014), and thus the freshwater pearl mussel (*Margaritifera margaritifera*), an endangered Iberian species (Moorkens *et al.* 2018) could face another new threat.

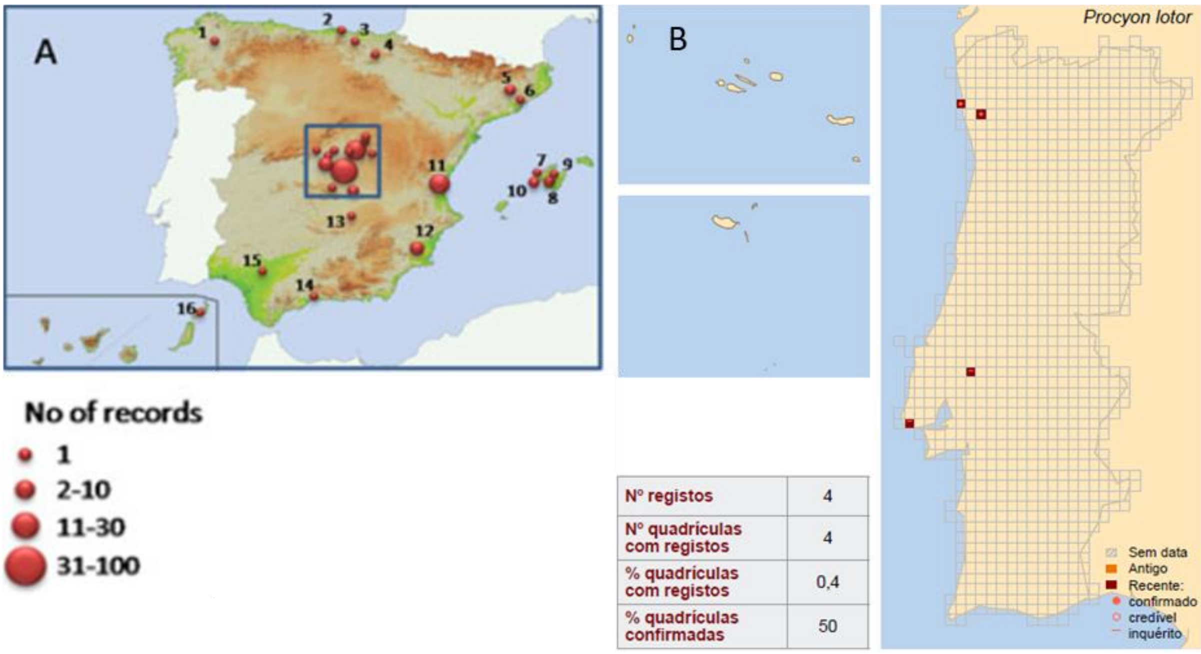

Figure 1.3- Known distribution of raccoons in the Iberian Peninsula. *A: Records of occurrences in Spain until 2012 (source: García* et al. *2012). B: Records of confirmed and possible occurrences in Portugal until 2018 (source: Bencatel* et al. *2019).*

Raccoons were detected in the wild in Portugal only recently (Figure 1.3), comprising four records of single individuals, but only two of them, located in the coastal region at north of Porto (in 2008 and 2014), were confirmed to be an effective raccoon presence by photographs and a capture (Bencatel *et al.* 2018, 2019). These detections seem to be related to different isolated events of introduction and do not probably correspond to breeding populations (Bencatel *et al.* 2019). This seems to indicate that, at least in Portugal, the invasion is still in the arrival phase and, therefore, a successful invasion event from this species can still be prevented, since only a few isolated animals were recorded. Furthermore, given the steady expansion and establishment of raccoon populations in Spain, it can be expectable that this species will soon occur in areas near the Portuguese border. However, the confirmation of this expectation is hampered by the lack of updated knowledge, since the last study that compiled all available information on raccoon presence in Spain is dated nearly a decade ago (García *et al.* 2012). These pieces of evidence render the urgent need to update the distribution of raccoons in the Iberian Peninsula, including of specimens held in captivity and occurring in the wild (considering both occasional records or breeding populations), as well as identify possible invasion routes to predict the most suitable and expectable areas for its occurrence in Portugal. This knowledge will allow more efficient management of this invasive carnivore in the scope of current Spanish and Portuguese legislation related to control measures to prevent the introduction and proliferation of exotic species (Decree-law nº 565/1999; Decree-law nº 92/2019; Blasco Hedo 2012).

Species distribution models (SDMs) are an excellent tool to predict the potential risk of invasion by an exotic species, such as the raccoon (Araújo and Guisan 2006; Sax *et al.* 2007). In recent years, these models have become central to several methodological approaches in ecology and conservation biology (Guisan and Thuiller 2005). These are empirical models that statistically relate the ecological niche of a given species to environmental variables that are collected in a known location where that species occurs, and allow projecting the potential distribution for the species in question to non-sampled areas (Guisan and Zimmermann 2000; Peterson 2001). Absence data are often biased, either due to absence uncertainties, linked to the fact that the area might include habitats favourable to the species, but it has not yet arrived, to ambiguities associated to record's origin, or even to the inexistence of this type of data for some regions. Therefore, using only presence data, especially with invasive species, prevents this type of bias and does not ignore potentially favourable habitats (Hirzel *et al.* 2002). Modelling the distribution of species using a Maxent approach (Phillips *et al.* 2006) has shown to be a very promising modelling technique for dealing with presence-only data and achieving good and robust results when predicting invasive species distributions (Phillips *et al.* 2006; Elith *et al.* 2006). Maxent is based on a machine-learning technique that estimates species niches using environmental predictors together with species occurrence data. Through the maximum entropy principle, it calculates the distribution probability, given that the expected value of each environmental variable matches its empirical average measured over the presence records (Phillips *et al.* 2006).

Considering this methodological approach to model the risk of invasion and the current knowledge gaps related to the invasive raccoon presence in the Iberian Peninsula, this study aims to: 1) update the current distribution pattern of this species in Spain and Portugal, based on all available records, including reference to wild and captive animals; 2) identify the drivers that determine the presence of raccoon's breeding populations in Spain and, based on those, pinpoint the regions with a higher risk/probability of harbouring raccoon breeding populations in the Iberian Peninsula; and 3) highlight what areas, in Portugal, should be prioritized for raccoon monitoring, and conduct the first survey to enhance the detection of an early invasion in the country. We hypothesize that: 1) the Iberian distribution of the raccoon is, today, larger than it was when the last census took place (in 2012), with records already near the Portuguese border; 2) Raccoon presence is mostly determined by the proximity to water bodies with high productivity, due to its high water requirement (Stuewer 1943; Lotze and

Anderson 1979); and 3) the northwestern region of Portugal is the most vulnerable area to a raccoon invasion since it presents a high density of permanent rivers (Stuewer 1943) and higher precipitation (Farashi and Naderi 2017).

By fulfilling our objectives and identifying the areas with a higher risk of raccoon occurrence in Portugal, this study will contribute to effective future management actions aiming to prevent and control an eventual expansion of raccoon occurrence into Portugal. In the end, our results will enhance the efficacy of an early warning raccoon invasion network system and, thus contribute to the conservation of Portuguese biodiversity.

2. MATERIALS AND METHODS

2.1 Study Area

The study area encompasses the continental part of the Iberian Peninsula, including Portugal and Spain (Figure 2.1). It is the westernmost peninsula in Europe, connected to central Europe through the Pyrenees mountain range and bordered by the Atlantic Ocean to the north, west and part of the south, and by the Mediterranean Sea to the south and east. Iberia's southwest is characterized by mostly plains while the northern half and the southeast regions have several mountain ranges. Many major rivers, such as Minho, Lima, Douro, in the north, Tagus in the centre, and Guadiana in the south, have their source in Spain and reach the Atlantic Ocean in Portugal, often forming estuarine systems. The Tagus River is the longest Iberian river, crossing through central Spain and Portugal, reaching the Atlantic in Lisbon. The Iberian Peninsula has three distinct climatic zones: 1) the Atlantic zone, in the northwest, characterized by mild temperatures and abundant rainfall in the wet season; 2) the Mediterranean area, in the South and East, with milder winters with less precipitation and a hot and dry season; 3) the Continental zone, in a more central region, with a more extreme climate, i.e. hotter and drier summers and more rigorous winters (Sillero *et al.* 2012; Lorenzo-Lacruz *et al.* 2013). Derived from its privileged position, Iberia is a pathway between Europe and Africa and one of the main Pleistocene glacial refugia in Europe (Gómez and Lunt 2007), harbouring a diversified fauna, flora, and habitats, including several endemic species. Vegetation is dominated by pine trees, cork and holm oaks predominantly in the northwest, and olive trees and cork/holm oaks in the east and south, respectively (Loidi 2017). The Iberian Peninsula is included in the Mediterranean basin biodiversity hotspot with a high number of endemic species, containing 0.9% of the world's endemic vertebrates (Myers *et al.* 2000). Furthermore, Iberia shelters around 50% of the European terrestrial vertebrate species with a rate of endemism of 31% (Williams *et al.* 2000).

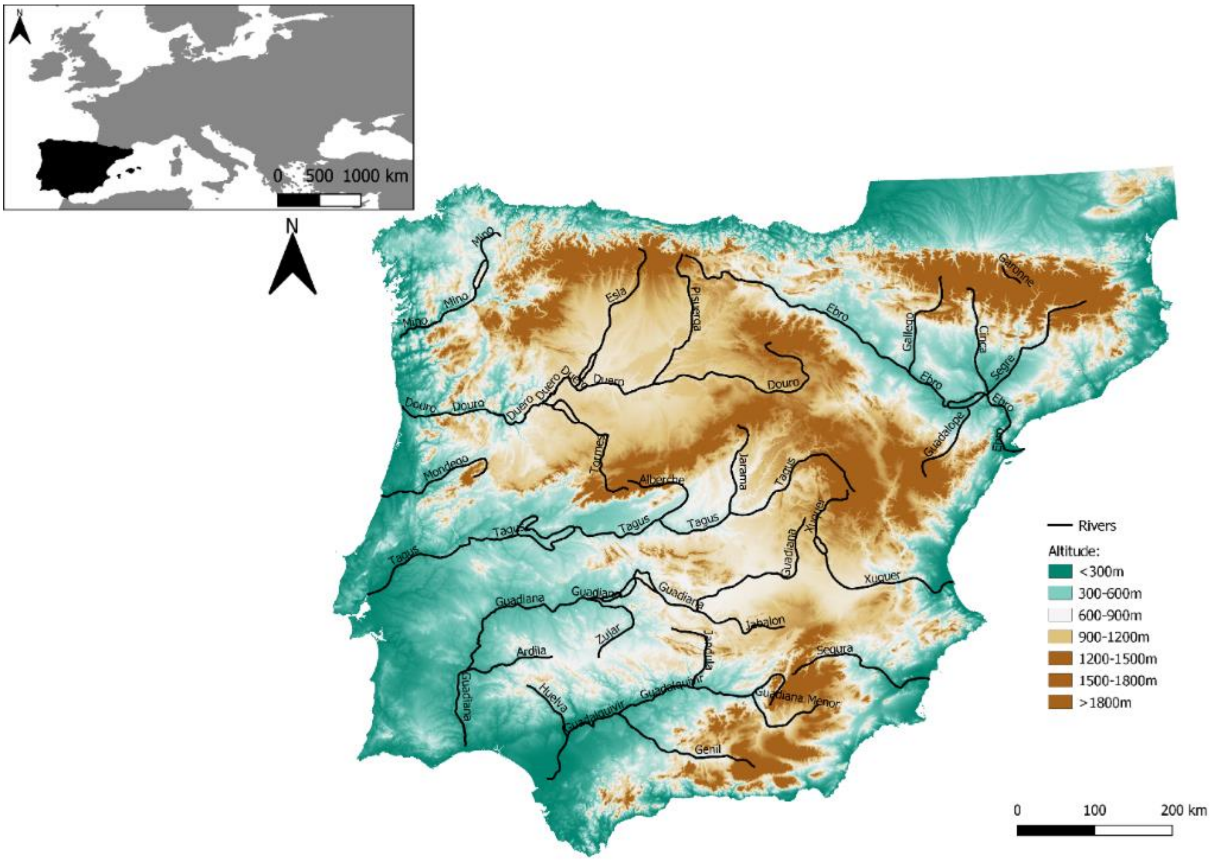

Figure 2.1- Location of the Iberian Peninsula within Europe, and representation of the Iberian topography and major rivers.

2.2 Raccoon data collection

To assess the current distribution of wild raccoons in Iberia we collected records of its presence between 2005 and 2020 from published documents through a literature review and from other different reliable sources (e.g. websites, experts, unpublished reports, zoological collections, etc.). We classified the compiled records of raccoons in the wild, considering either presences from breeding populations (i.e. where evidence of pregnant females or cubs were reported) or occasional detections of isolated individuals, where there was no evidence of an established breeding population present at the time. The collected data from wild occurrences comprised captures, camera-trap photos or videos, footprints, scats, direct sightings, animals detected with a scent-detection dog, roadkills, radio-tracked animals, and photos (J. F. Layna *pers. comm.*; Santiago Palazón/Generalitat de Cataluña *pers. comm.*; Fernández-Aguilar *et al.* 2012; Generalitat Valenciana 2012, 2013; Layna *et al.* 2013; Vazquez 2013; Morán *et al.* 2015; Layna and Prieto 2017; Suances *et al.* 2018; Bencatel *et al.* 2019; Dana *et al.* 2020). Dubious records including unreliable sources (e.g. unconfirmed sightings without photographic proof) or linked to some degree of uncertainty in raccoon's identification (e.g. difficulty in footprint identification) were only represented for Portugal (as possible records) but were excluded for the modelling procedures.

We also compiled information on captive raccoons, both in private (e.g. pet owners) and public collections (e.g. zoos), by contacting Iberian wildlife centres that kept or still keep raccoons in captivity (e.g. as pets or for public exhibition), since this invasive species is known to escape from some of these facilities. Whenever possible we obtained information about the number of captive individuals, their sex, origin, and reproductive status in the mentioned facilities. Furthermore, we identified which of these facilities provide a high abundance of animal food that may attract wild or recently escaped raccoons to their locations, which could induce a high raccoon abundance in the areas surrounding those facilities, over the years. Thus, by assessing the conditions of this species in the enquired facilities we intended to predict the risk of future escapes or to localize the source of recently escaped individuals.

Additionally, we promoted together with NATIVA Association (http://anativa.org/) a crowdfunding campaign to raise funds to support our fieldwork and to help collect data on raccoon sightings from the general public by using a citizen science approach. To help further this cause, Wilder, a Portuguese online magazine, wrote two articles related to this study, one to publicize and explain the project goals (https://www.wilder.pt/historias/biologos-estao-a-estudar-risco-de-expansao-do-guaxinim-em-portugal/) and another to help people knowing how to identify a raccoon in the wild (https://www.wilder.pt/seja-um-naturalista/como-identificar-um-guaxinim/). Both articles were used to divulge the project and create public awareness of this invasive carnivore as well as to enhance the chance of receiving new occurrence information regarding raccoons in the wild in Portugal.

2.3 Drivers of raccoon presence in Iberia

We selected 12 environmental predictors with documented relevance on this species ecology that we hypothesize to influence raccoon's occurrence in the Iberian Peninsula (Table 2.1). These predictors were clustered into three main categories: climatic, land cover, and topographic data. We selected these variables based on two-folded reasoning. First, a literature review that included previous studies targeting habitat selection and ecological requirements of introduced (including within the Iberian Peninsula) and native raccoon populations, which have identified some of the variables as influential (Stuewer 1943; Baldwin *et al.* 2006; Bartoszewicz *et al.* 2008; García *et al.* 2012; Farashi *et al*. 2013, 2016; Mori *et al.* 2015; Farashi and Naderi 2017; Duscher *et al.* 2018; Louppe *et al.* 2019). On the other hand, we also selected predictors that were highlighted by the expert-based opinion of Spanish wildlife biologists, highly experienced in raccoon population control in the introduced range.

2.4 Species distribution modelling

To assess which drivers might be determining raccoon's presence in Iberia we used a Maxent approach, based on the theory of maximum entropy applied to presence-only data (De Martino and De Martino 2018) and which has shown to be an adequate modelling technique already used in several studies predicting the distribution of invasive species (Phillips *et al.* 2006; Elith *et al.* 2006; Ficetola *et al*. 2007; Farashi and Naderi 2017). In the modelling procedure, we selected as presence data the records only corresponding to breeding populations (see Figure 3.1), because escaped isolated animals could provide wrong assumptions regarding their habitat preferences (as they might just be moving away from the confining facilities). Furthermore, evidence of reproduction proves that the habitat in which they were recorded is suitable enough for them to breed (i.e. presence of partners and sufficient resource to rear offspring). The 12 environmental predictors were incorporated into a Geographical Information System, as different layers, built using the software QGIS (v 3.6.2) (QGIS Development Team 2019) and R (v 3.6.0) (R Core Team 2019), and manipulated for modelling purposes, i.e. converted into the same geographic coordinate system (projection EPSG:3035-ETRS89/LAEA Europe) and scale (pixels of 1km^2), after being rasterized.

Before the modelling procedure itself, we assessed the adequacy of the entire dataset, based on the modelling requirements related to multicollinearity and spatial autocorrelation (Blalock 1963;

Segurado *et al.* 2006). Therefore, we first identified and excluded variables that were correlated with each other, as multicollinearity can overinflate the standard errors, thus leading to biased estimations (Farrar and Glauber 1967). To test correlations between environmental variables, we used the *usdm* package (Naimi *et al.* 2014) in R (v 3.6.0) (R Core Team 2019) to estimate each variable's Variance Inflation Factor (VIF). We chose as threshold value VIF<5 (Zuur *et al.* 2010). We started with a set that included all variables and estimated the VIF for every variable. If any variables reached a VIF value higher than 5, it was excluded and the VIF values were estimated again for the remaining variable subset. This process was repeated until no variable reached a VIF of 5 or higher. Mean precipitation of wettest quarter, annual mean temperature, and mean temperature of the warmest quarter were removed from the modelling procedure, as they did not meet the VIF<5 criteria (see VIF results in Table S.1). We then assessed the spatial distribution of our presence-only data to investigate data spatial autocorrelation. As we detected some data geographical clustering, since the majority of our records are located in Madrid and Guadalajara regions, we corrected this sampling bias by following the recommended systematic sampling approach described by Fourcade *et al.* (2014). This method reduces the spatial aggregation and usually improves species distribution models performance by removing random neighbouring occurrences and randomly sampling one record per grid cell (1km^2) (Fourcade *et al.* 2014).

Modelling analyses were performed using the Maxent software (v 3.4.1) (Phillips *et al.* 2019). To evaluate the model fit, we used different approaches, corresponding to all three types of replication settings present in the software, which lead to the three different fitting outputs: 1) cross-validation; 2) bootstrapping; 3) subsampling. In cross-validation the records are randomly divided into groups of the same size (i.e. the number of presences), leaving each group out in each run. These groups are, latter, used for evaluation of the model predictions. In the bootstrapping and subsampling approaches, presence data is split into training and test groups randomly, with and without replacement of the presence records, respectively. In the training tests, we split randomly the species presence records into two subsets: 75% of the records were used to calibrate the model while the remaining 25% was used to evaluate it. This procedure was replicated 10 times, and therefore each time we run the model, a different set of presence representing 75%-25% proportion of the records were selected, except for the cross-validation approach, which uses all the presence data to evaluate the model (Phillips 2017). By averaging the 10 runs we obtained the final model. We accessed the models' fit using the averaged Area Under the Curve (AUC) of a Receiver Operating Characteristic (ROC) plot and the related Standard Deviation (SD), provided by Maxent outputs (Phillips *et al.* 2006). Finally, we assessed which variables contributed the most in each model, by creating response curves and performing jackknife tests to assess variable's importance (Elith *et al.* 2011; Phillips 2017). The jackknife test is based on the creation of different models sets. One where it excludes each of the environmental variables in turn, creating a model with the remaining ones; another, univariate, where each one of the variables is tested individually; and, finally, a model including all variables (Phillips 2017). We also examined the variables relative importance according to their permutation importance, which focuses on the drop of AUC in models when each variable is excluded, providing a precise ranking of the environmental variables chosen (Phillips *et al.* 2006; Searcy and Bradley Shaffer 2016; Phillips 2017).

We then produced maps of predicted habitat suitability for raccoons' breeding populations in the Iberian Peninsula, based on the three types of fitting outputs.

*Table 2.1- **List of environmental predictors used in the modelling procedure.** Also, their acronym, description, type of influence (+: positive; -: negative), reasoning supporting their selection, and data source.*

Category	Environmental Variables	Code Name	Description	Influence	Reasoning	Data Source
Land Cover	Distance to agricultural fields (km)	DAF	Distance to: Rice fields, Vineyards, Fruit trees, and berry plantations, Olive groves, Annual crops associated with permanent crops, Complex cultivation patterns, Land mainly occupied by agriculture with significant areas of natural vegetation, and Agroforestry areas	-	Associated to the high availability of food resources and/or human waste (Ikeda *et al.* 2004; Bartoszewicz *et al.* 2008; J. F. Layna *pers. comm.*)	https://land.copernicus.eu/pan-european/corine-land-cover/clc2018
	Distance to urban areas (km)	DUA	Distance to: Continuous urban fabric, Discontinuous urban fabric, Industrial or commercial units, Road and rail networks and associated land, Port areas, Airports Mineral extraction sites, Dumpsites Construction sites, Green urban areas, and Sport and leisure facilities	-		
	% of tree cover in a grid cell	TC	Percentage of Broad-leaved forest, Coniferous forest, and Mixed forest in each pixel (1 km^2)	+	Associated to shelter and dispersal (Bartoszewicz *et al.* 2008; Fischer *et al.* 2017; J. F. Layna *pers. comm.*)	
	Distance to water bodies (km)	DWB	Distance to the nearest river, lake, lagoon, etc.	-	Associated to the high availability of food resources and shelter (Stuewer 1943; Bartoszewicz *et al.* 2008; J. F. Layna *pers. comm.*)	https://www.diva-gis.org/gdata; https://www.miteco.gob.es/es/cartografia-y-sig/ide/descargas/agua/red-hidrografica.aspx
	% of riparian vegetation in a grid cell	RV	Percentage of riparian vegetation in each pixel (1 km^2)	+		https://land.copernicus.eu/local/riparian-zones/riparian-zones-delineation
Topographic	Altitude (m)	ALT	Altitude a.s.l.	-	Associated with less suitable bioclimatic conditions for occurrence (Mori *et al.* 2015; J. F. Layna *pers. comm.*)	http://srtm.csi.cgiar.org/srtmdata/
Climatic	Mean temperature of the warmest quarter (ºC)	TWQ	Average values for the years 1970-2000 (for the 1 km^2 pixel)	-	Associated with a wide range of favourable bioclimatic conditions for occurrence (Duscher *et al.* 2018; Louppe *et al.* 2019)	Fick and Hijmans 2017
	Mean temperature of the coldest quarter (ºC)	TCQ		+		
	Annual mean temperature (ºC)	AMT		+		
	Annual mean precipitation (mm)	AMP		+		
	Mean precipitation of the wettest quarter (mm)	PWQ		+		
	Mean precipitation of the driest quarter (mm)	PDQ		+		

2.5 Field survey to assess model accuracy in Portugal

Based on the areas identified by the models as having a higher probability for raccoon's occurrence and the proximity with confirmed and possible records in Portugal and near its border, we selected two main areas to conduct an intensive field survey to assess for this species presence: northwest Portugal, comprising districts of Porto, Braga, Viana do Castelo and Vila Real, along the rivers Ave, Este, Cávado, Lima, Minho, Coura, Tamente and Salas; and in central Portugal, comprising the district of Castelo Branco, along the rivers Ponsul and Erges (Figure 2.3). Since raccoons show high activity in the summer and autumn to assure enough food to accumulate fat to endure the coming winter (Mech *et al.*, 1968; Hoffman, 1979), between 13th and 17th September 2020, we sampled 24 transects throughout the river margins comprising a total of 40.8 km, in search of presence signs such as scats and footprints. A large part of these surveys, comprising a total of 31.4 km, was conducted with the help of a scent-detection dog, trained specifically to detect presence signs of raccoons (see Table S.2 for further details). Simultaneously, we monitored the areas in Castelo Branco District, near river Erges, in Rosmaninhal, with 18 camera-traps (9 located in the riparian vegetation), to enhance the probability of confirming raccoon presence. A total of 13 camera-traps were placed in July 29th and the other 5 cameras on August 26th, being all monitored until 24/10/2020.

Figure 2.2- Areas sampled on foot and with a scent-detection dog to access raccoon's presence in Portugal. *A: Surveys along the rivers Ave, Este, Cávado, Lima, Minho, Coura, Tamente and Salas. B: Surveys along the rivers Ponsul and Erges, also resorting to camera-trapping.*

3. RESULTS

3.1 Compiled information on raccoon's presence

We compiled a total of 1090 confirmed presence records of the raccoon in Portugal and Spain, which were obtained between 2005 and 2020 by different field methods (Figure 3.1; Table 3.1). 1025 records (94%) refer to locations where there was evidence of breeding populations. The other 65 records (6%) corresponded to isolated individuals with no evidence of belonging to any breeding populations, which were scattered throughout several Iberian regions. Although 824 records from breeding populations (80%) are from the provinces of Madrid and Guadalajara (central Spain), we manage to gather similar data from several other provinces located in northern and southern Spain (e.g. Lugo, Ourense, Cantabria, Biscay, Toledo, Huelva, Seville and Alicante), indicating that raccoons breeding in the wild seem to have a broader, but highly localized, occurrence limited to Spain (Figure 3.1; Table 3.1). Most of the raccoon records in Spain correspond to captured individuals comprising a total of 903 records, many during eradication programs (Table 3.1). In Portugal, we compiled a total of 10 records (Figure 3.1), including 2 confirmed and 2 possible records already reported in previous studies and 6 additional possible records collected in the scope of this study, reported by people who sighted animals in the wild with a morphological description compatible to raccoons, although without a photographic proof to confirm the veracity of the occurrences (Figure 3.1; Table 3.1).

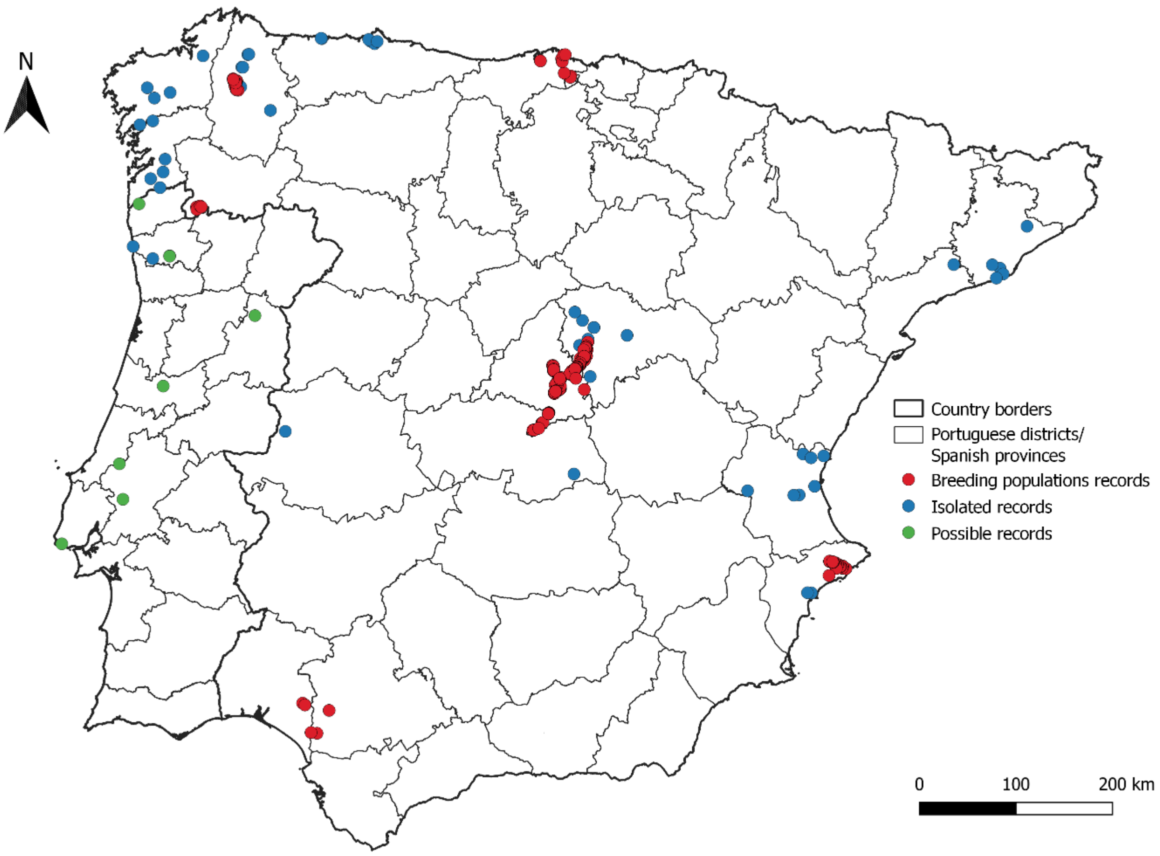

Figure 3.1- Compiled information about raccoon presence in the Iberian Peninsula. *The map includes breeding populations, isolated individuals that were detected, and possible records that could be linked to raccoons but that need to be confirmed.*

Table 3.1- Location, number and detection method of the confirmed raccoon records collected per district/province. **8 possible records in Portugal were obtained in the districts of Viana do Castelo (n=1), Braga (n=2), Guarda (n=1), Leiria (n=1), Santarém (n=2) and Lisbon (n=1). ** Regions where raccoons eradication programs were conducted, involving the capture of individuals. CAP- capture; FTP- footprint; STG- sighting; CAM- camera trap; SDD- scent-detection dog; RK-roadkill; RAD- radio-tracking; SCT- scats; PHT- photograph.*

Country	Portuguese district/ Spanish province	Number of presence records	Number of records with breeding	Detection method								
				CAP	FTP	STG	CAM	SDD	RK	RAD	SCT	PHT
Spain	A Coruña**	6	0	1	0	2	0	0	3	0	0	0
	Alicante**	63	61	43	3	6	11	0	0	0	0	0
	Asturias	7	0	2	0	0	2	3	0	0	0	0
	Barcelona	5	0	0	0	4	0	0	1	0	0	0
	Biscay	2	2	0	0	0	0	0	2	0	0	0
	Cáceres	1	0	1	0	0	0	0	0	0	0	0
	Cantabria	8	8	0	0	3	1	0	4	0	0	0
	Girona	1	0	0	0	0	1	0	0	0	0	0
	Guadalajara**	328	316	291	7	15	11	0	4	0	0	0
	Huelva**	2	2	0	1	0	1	0	0	0	0	0
	Lugo**	109	95	38	35	3	5	22	3	2	1	0
	Madrid**	496	496	496	0	0	0	0	0	0	0	0
	Ourense**	9	9	2	0	0	2	5	0	0	0	0
	Pontevedra	5	0	3	0	1	0	0	1	0	0	0
	Seville**	14	14	11	2	0	1	0	0	0	0	0
	Tarragona	1	0	0	0	1	0	0	0	0	0	0
	Toledo**	24	22	12	10	0	2	0	0	0	0	0
	Valencia**	7	0	2	0	5	0	0	0	0	0	0
Portugal*	Braga	2	0	1	0	0	0	0	0	0	0	1
Total		1090	1025	903	58	40	37	30	18	2	1	1

Considering the temporal variation in the compiled records, a total of 928 raccoon records were collected since 2012 (time of the last update on raccoon's distribution in Spain), from which 876 records (94%) were regarding established breeding populations (Table 3.2). Therefore, the collected data corroborates our first hypothesis that the raccoon's distribution in Iberia has become wider, especially in northwestern Spain, and with new several records registered near the Portuguese borders, including a breeding population in southern Ourense (Lima watershed) and several isolated records in southern Pontevedra (Minho watershed) and western Cáceres (Tagus watershed) (Figure 3.1).

Table 3.2- Number of presence records of raccoons in the Iberian Peninsula, obtained per year since 2012.

	Number of presence records per year									
Year / **District/ Province**	2012	2013	2014	2015	2016	2017	2018	2019	2020	**Total**
A Coruña	0	0	0	3	0	2	0	0	0	5
Alicante	2	22	11	27	0	0	0	0	0	62
Asturias	0	0	0	7	0	0	0	0	2	9
Cantabria	0	1	0	0	0	0	0	0	0	1
Barcelona	1	0	0	1	1	1	1	0	0	5
Biscay	1	0	0	0	0	0	0	0	0	1
Cáceres	0	0	0	0	1	0	0	0	0	1
Girona	0	0	0	0	0	0	0	1	0	1
Guadalajara	5	43	27	46	69	34	46	22	0	292
Lugo	0	36	61	0	0	6	4	0	0	107
Madrid	159	55	15	13	35	19	57	40	0	393
Ourense	0	0	0	0	0	9	0	0	0	9
Pontevedra	0	1	1	0	0	0	0	0	1	3
Seville	0	11	0	0	0	0	0	0	0	11
Tarragona	0	0	0	0	0	1	0	0	0	1
Toledo	0	0	0	12	5	0	7	0	0	24
Valencia	1	0	1	0	0	0	0	0	0	2
Braga	0	0	1	0	0	0	0	0	0	1
Total	169	169	117	109	111	72	115	63	3	928

We identified 15 facilities (e.g. zoos, private animal collections) that could be linked to raccoon presence by keeping raccoons in captivity, in the recent past (last decade) or currently (2020); acting as escape sources to the wild, inducing new introductory events (e.g. leading to animal escapes by not having the necessary measures to ensure their captivity); or functioning as important alternative food sources for raccoons (e.g. facilities with animal feeders providing a constant high food abundance in animal rehabilitation centres or zoos) (Table 3.3; Figure 3.2). The origin of raccoons in captivity was mostly related to delivery or confiscation by public authorities, which had collected them from private owners who kept them not according to the law or could not keep them in their facilities with all the necessary measures (Table 3.3). The capture of wild animals is also an important source of raccoons in, at least, two of those. Escapes of raccoons were confirmed to occur in, at least, two Spanish public zoos ("Zoo Marcelle" in Lugo and "El Arca de Noé" in Alicante, which is currently closed), but in other facilities located in Madrid, Guadalajara, Toledo, Biscay and Asturias similar incidents may have also occurred, but were not confirmed. In Portugal, there are 3 known locations where raccoons are currently kept in captivity (2 public and 1 private) and an additional private facility where this species was present during the last decade. None of these Portuguese facilities had known escape events and all current individuals in captivity are sterilized or prevented to reproduce (Table 3.3; Figure 3.2).

Table 3.3- Facilities that hosted (last decade) or host (2020) raccoons in captivity in Iberia. *Also, their details, animal origin and which ones provide constant and abundant animal food through feeders (anthropic feeding sites).*

Name	Country	Portuguese district/ Spanish province	Current number of individuals (sex)	Escapes	Details	Origin of captive animals
El Arca de Noé	Spain	Alicante	0	Confirmed	Raccoons hosted in the past	70% from private owners; 30% from captures in the wild
El Bosque		Asturias	2 (♂)	No	Animals sterilized	Private owners
El Parque de la Vida			0	Possible	Raccoons hosted in the past	
Karpin Fauna		Biscay	5 (3♂, 2♀)	Possible	Animals sterilized	
Zoo de Guadalajara		Guadalajara	Unknown	No	Animals sterilized; Anthropic feeding site	Captures in the wild
Zoo Marcelle		Lugo		Confirmed	Raccoons in captivity	Unknown
Fauna y Acción		Madrid		Possible	Raccoons hosted in the past	
Madrid Zoo Aquarium				No	Raccoons in captivity	
Safari Madrid				Possible		
Zoo Exóticos Kiko				Possible		
La Herradura		Toledo			Raccoons in captivity; Anthropic feeding site	
Jardim Zoológico de Lisboa	Portugal	Lisbon		No	Raccoons in captivity; No breeding occurring	Portuguese illegal private owners; Other zoos
Monte Selvagem		Évora	2 (♂)		Raccoons in captivity	Private owners
Parque dos Monges		Leiria	0		Raccoons hosted in the past	
Quinta Layla		Castelo Branco	2 (♂)		Animals sterilized	Brought from England

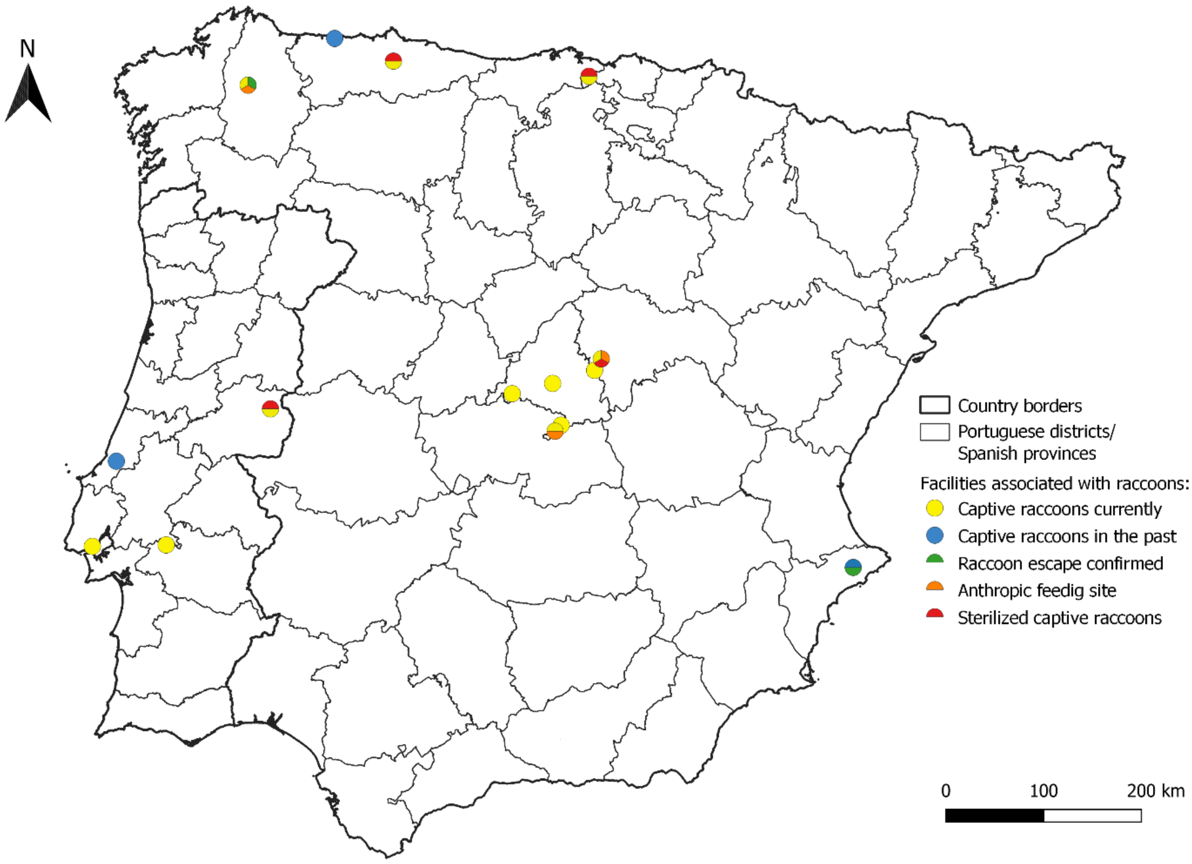

Figure 3.2- Location of facilities in the Iberian Peninsula with captive raccoons currently (2020) or in the past (last decade). *This map includes facilities reported to have sterilized individuals, known escapes of captive individuals, and/or provide constant and abundant food resources near areas with wild raccoon populations (anthropic feeding sites).*

3.2 Environmental predictors of raccoon's occurrence

According to our modelling results, the raccoon's presence is mainly dependent on water, since the most important driver is related to the proximity of this resource or with the ecological conditions it provides (e.g. riparian vegetation). These findings corroborate our second working hypothesis, which hypothesises a strong relation between raccoons and the presence of water bodies. The presence of the raccoon in Iberia is mostly influenced by the proximity to watercourses, as the variable distance to water bodies (DWB) was the one with greater importance in all models, reaching a Permutation Importance always higher than 36% (Table 3.4), and areas closer to water bodies shown to have a higher probability of raccoon's presence (Figure 3.3). Climatic conditions, particularly high precipitation levels, were also important predictors for the occurrence of raccoons. The annual mean precipitation (AMP) and mean precipitation of the driest quarter (PDQ) ranked second and third, respectively, in the cross-validation and bootstrapping models. As for the subsampling model, mean precipitation of the driest quarter (PDQ) seems to have higher importance as a driver of raccoon's presence than the annual mean precipitation (AMP), which is ranked in third place (Table 3.4). The three models generated similar response curves for every variable (see Figure S.1). Areas with annual mean precipitation (AMP) values of 400 and 1450 mm, seem to be more adequate for the species to be present (Figure 3.3). The species' presence is also promoted by the mean precipitation of the driest quarter (PDQ), with values around 40 mm, although there are minor peaks in presence probability between 130 and 200 mm, which although similar in shape, differ in amplitude between the models produced; in the cross-validation models, the probability of presence is similar between both minor peaks, while in the bootstrapping model the higher probability of raccoon presence is achieved at 130mm, and in the subsampling model, it occurs at 200 mm (Figure 3.3).

In all three model types, distance to water bodies (DWB) was the variable with the highest gain, when used in isolation, indicating that it is the most informative variable within the set of candidate variables (Figure 3.4). Furthermore, this variable and annual mean precipitation (AMP) decreased the models' gain the most when omitted (DWB: cross-validation model; AMP: bootstrapping and subsampling models), indicating they are adding information that is not being incorporated into the models by the inclusion of the other variables, thus highlighting their importance in identifying areas with a higher probability of hosting wild breeding populations of raccoons.

The three models reached similar AUC values for training data, although the bootstrapping model reached a slightly higher training AUC value (bootstrapping = 0.970) than the remaining models (cross-validation model = 0.967; subsampling model = 0.962). This pattern indicates that all models were highly robust in identifying high-probability areas for hosting raccoons. Furthermore, using the test samples, the model's AUC had a different ranking order, with the subsampling model (0.954) and cross-validation model (0.951) reaching slightly higher AUC values than the bootstrapping model (0.948), but all equally high. Standard deviation values were lower in the bootstrapping model while the cross-validation model presents the highest value (Table 3.4). This means that the model with bootstrapping replication performed slightly better than the other approaches when using the training data.

Table 3.4- Variable permutation importance, Area Under the Curve (AUC) of the models using the test and training data, and standard deviation (SD). *DWB- distance to water bodies; AMP- annual mean precipitation; PDQ- mean precipitation of the driest quarter; TCQ- mean temperature of the coldest quarter; DUA- distance to urban areas; ALT- altitude; DAF- distance to agricultural fields; RV- riparian vegetation; TC- tree cover.*

		Cross-validation	Bootstrapping	Subsampling
Variable Permutation Importance (%)	**DWB**	36.2	40.2	37.5
	AMP	18.2	18.5	17.1
	PDQ	17.2	14.7	21.8
	TCQ	6.9	8.8	8.5
	DUA	7.7	7	4.7
	ALT	2.4	6.6	6.2
	DAF	0.4	1.9	0.8
	RV	7.2	1.2	2.2
	TC	3.9	1.1	1.1
Training AUC		0.967	0.970	0.962
Test AUC		0.951	0.948	0.954
SD		0.028	0.006	0.016

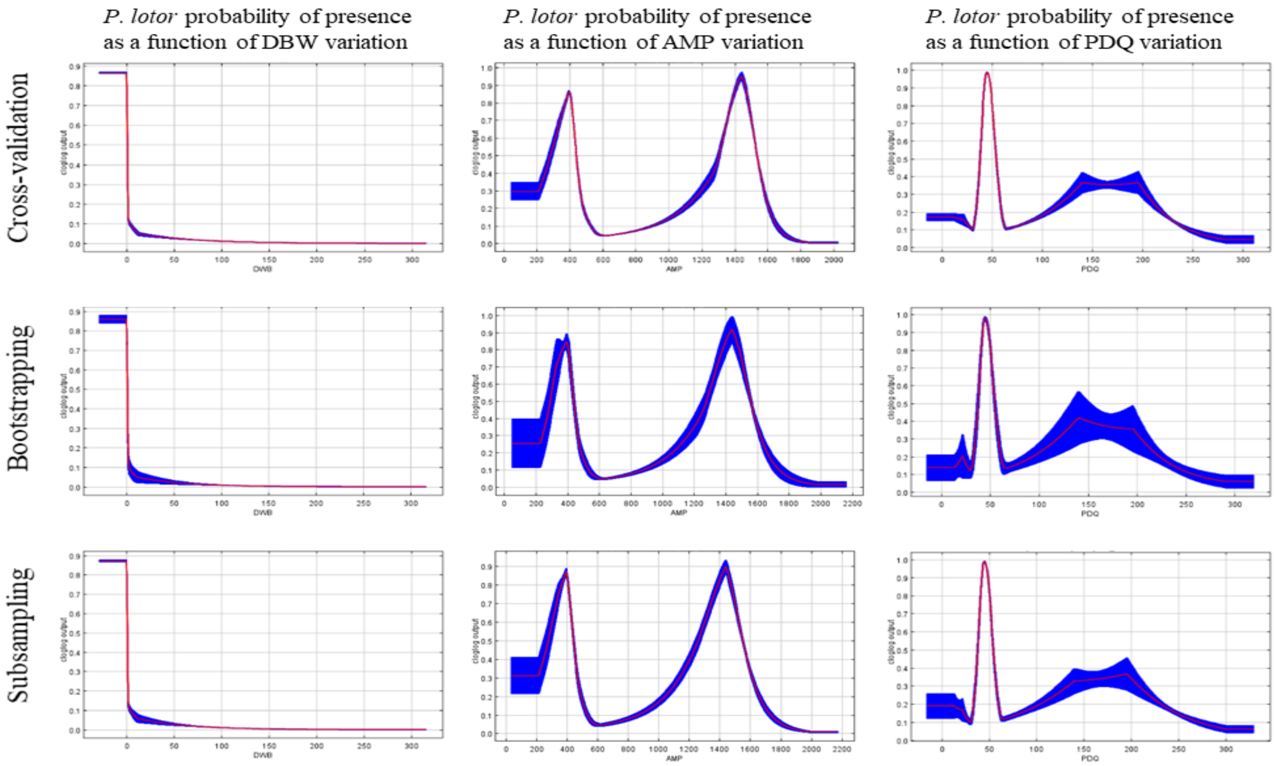

Figure 3.3- Response curves of the three most important variables of each model. *The red line indicates the mean response of the 10 replicates from each model, and the blue one represents their standard deviation. DWB- distance to water bodies (km); AMP- annual mean precipitation; PDQ- mean precipitation of the driest quarter.*

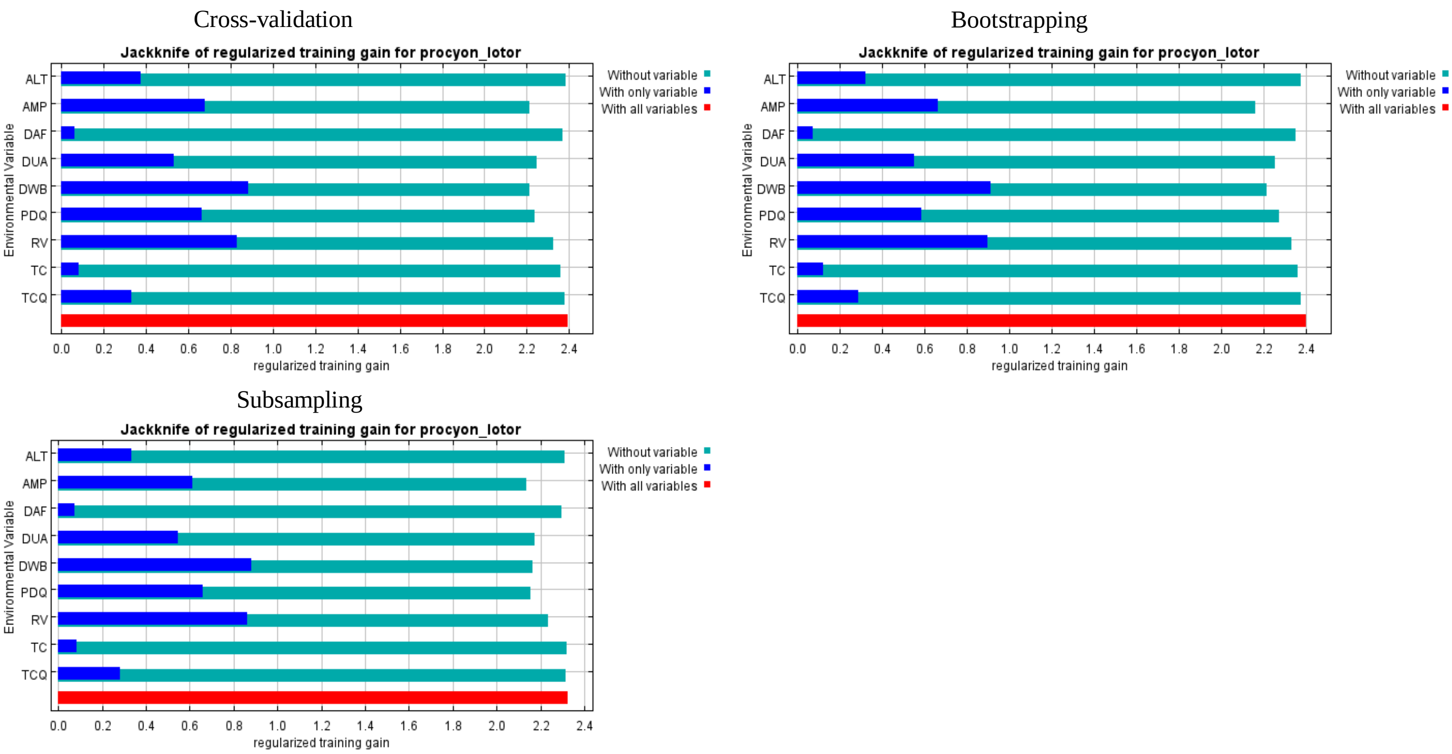

Figure 3.4- Jackknife test results for the three modelling approaches. *The figure shows the training gain for models built without a specific variable, only with that variable or including all variables. ALT-altitude; AMP- annual mean precipitation; DAF- distance to agricultural fields; DUA- distance to urban areas; DWB- distance to water bodies; PDQ- mean precipitation of the driest quarter; RV- riparian vegetation; TC- tree cover, TCQ- mean temperature of the coldest quarter.*

3.3 Raccoon's predicted suitable areas in Iberia

The raccoon's predicted distribution based on the three model approaches (cross-validation, bootstrapping and subsampling) (Figures 3.5-3.7), showed similar patterns. The watersheds in northwestern, central, and southeastern regions of the Iberian Peninsula seem to have a higher probability of hosting breeding populations of raccoons. However, the bootstrapping and subsampling models predicted a wider range of suitable regions, especially in northwestern Portugal. Both models produced very similar outputs, although the later enhanced slightly low and high suitability values, especially in already highlighted areas as northeastern, central, and northwestern Spain, as well as central and northwestern Portugal. In Portugal, the models showed that the main river basins in the northwestern region of the country have a higher probability to host raccoons and thus, are more vulnerable to an invasion from this species, corroborating our third working hypothesis. A comparison between the estimated probability range of raccoons in both Iberian countries highlights that Spain presents more and wider areas with better-predicted conditions for the presence of breeding populations of this invasive species, especially in the central region of the country, which represents the larger suitable area predicted in Iberia (Figures 3.5-3.7).

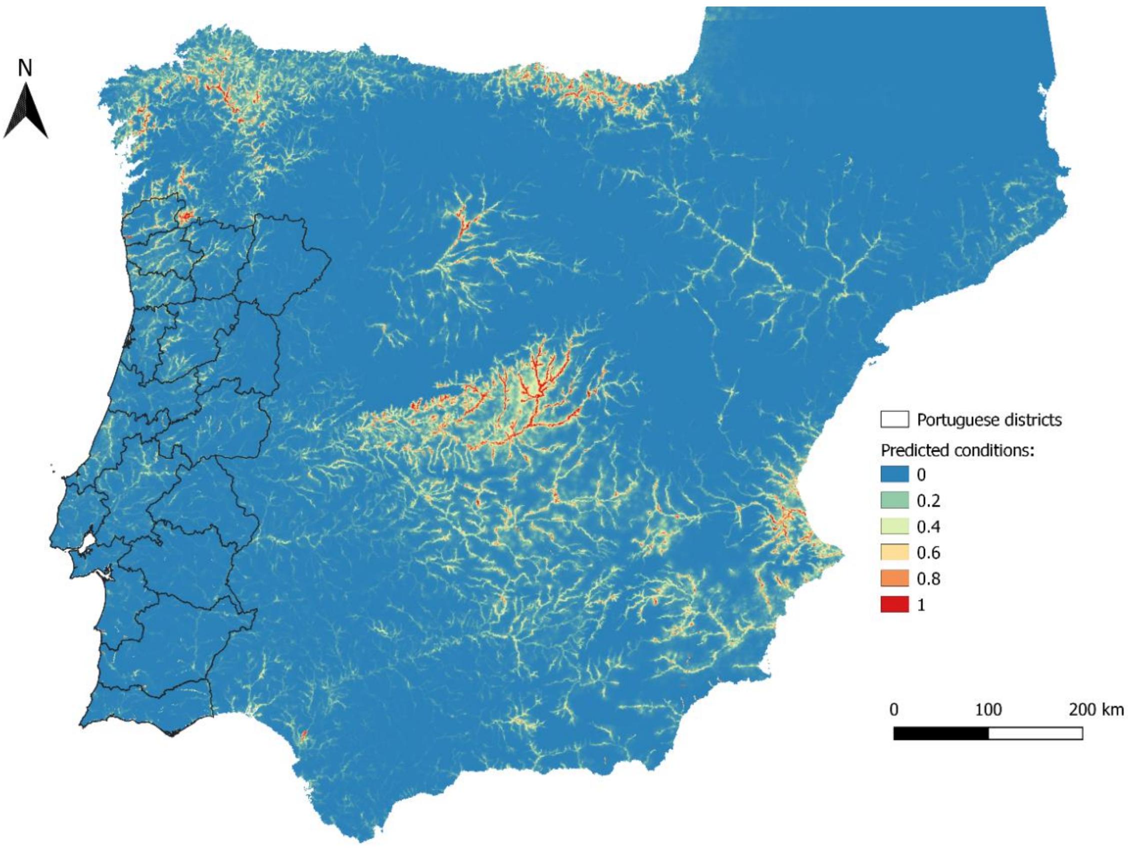

Figure 3.5- Prediction map of raccoon breeding populations' suitable areas, produced using the Maxent cross-validation settings. *Warmer colours mean a higher predicted presence probability (i.e. better conditions) and colder colours the opposite.*

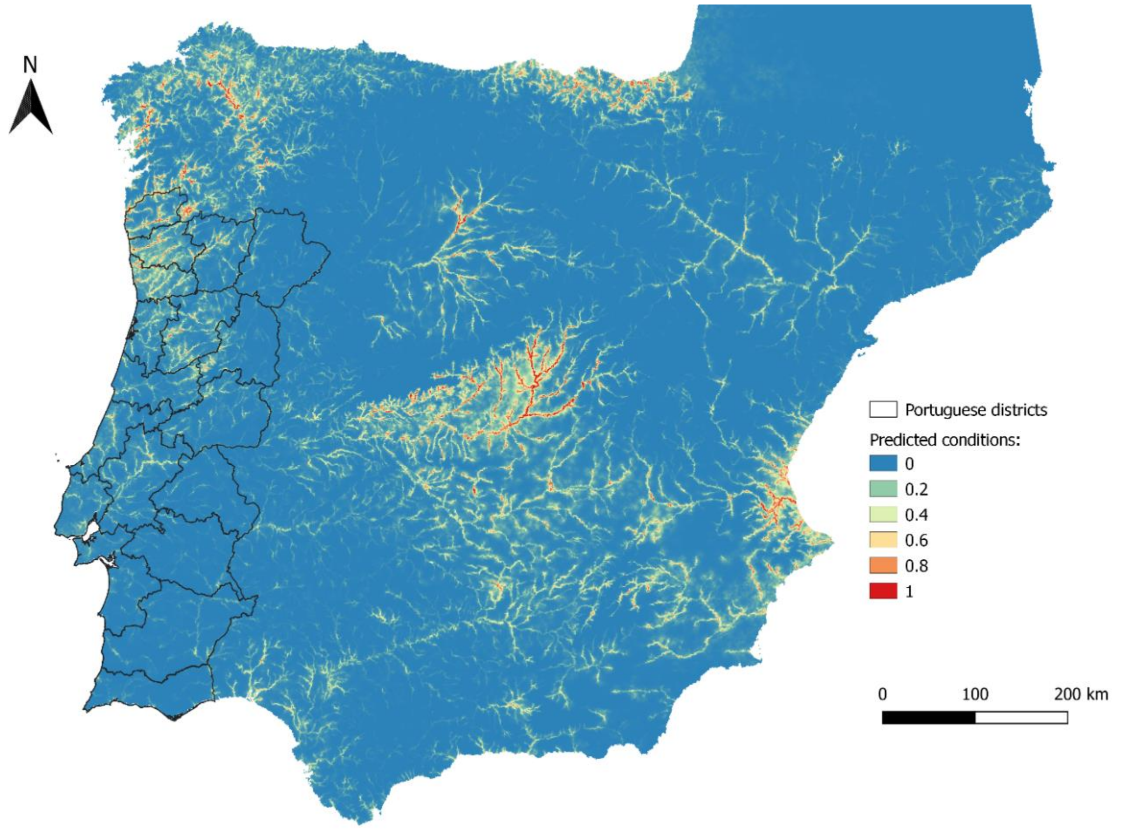

Figure 3.6- Prediction map of raccoon breeding populations' suitable areas, produced using the Maxent bootstrapping settings. *Warmer colours mean a higher predicted presence probability (i.e. better conditions) and colder colours the opposite.*

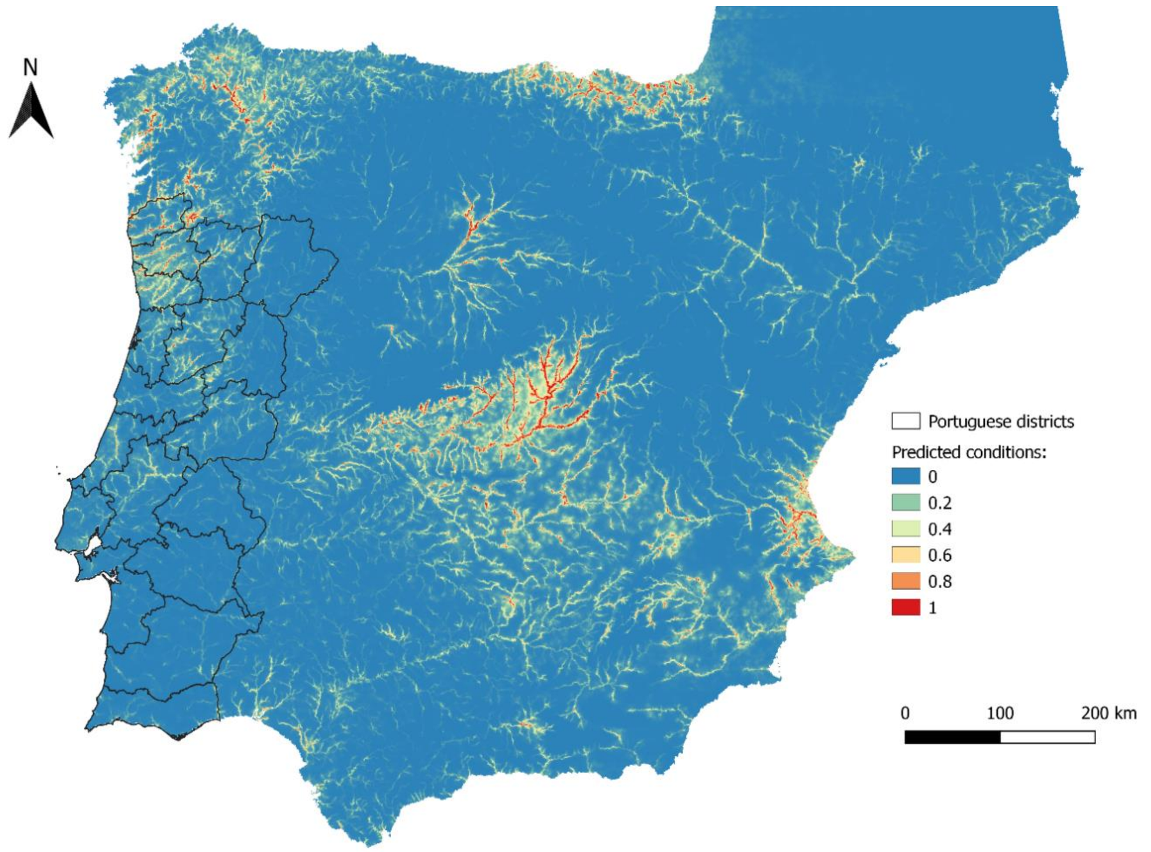

Figure 3.7- Prediction map of raccoon breeding populations' suitable areas, produced using the Maxent subsampling settings. *Warmer colours mean a higher predicted presence probability (i.e. better conditions) and colder colours the opposite.*

3.4 Field survey to assess model accuracy in Portugal

We surveyed 4 watersheds in the northwest region (Minho, Lima, Cávado and Ave,) and 1 watershed in central Portugal (Tagus). Although we detected some mammal species, no clear evidence of presence signs from raccoon was confirmed. However, the scent-detection dog gave a response sign in the Minho river, near Monção, which might be related to the presence of raccoon (see Table S.2). The dog is trained to perform three distinct signals facing a possible presence of raccoons. In the first and the most intense one, the dog shakes its body during several seconds indicating with accuracy a raccoon presence. The second one happens for less time and indicates a possible presence of raccoon in the area. As for the third and last, the signal lasts very little time and usually does not reflect the presence of raccoon, but something else in the area that triggered this slight behaviour by the dog. Likewise, the signal identified by the dog in this area was weak and poorly reliable, suggesting a very unlikely raccoon's presence, despite the proximity of a reported sighting of this carnivore across the river, in Spain.

4. DISCUSSION

Our findings provide a valuable contribution to the knowledge on the population status and ecological requirements of raccoons in the Iberian Peninsula and to identify the areas with a higher risk of expansion in Portugal, topics with strong management implications for this invasive carnivore.

The last assessment reviewing the status of raccoons in the Iberian Peninsula dates to 2012 and only addresses Spain (García *et al.* 2012). Therefore, the information we collected provides a necessary update almost a decade later on the matter. Since then, the number of wild raccoon records increased significantly in Iberia, although the real number of records must be higher, as the collected data resulted from opportunistic records and not from a systematic survey covering all Iberian regions. Most records are still found in central Spain (Guadalajara, Madrid, and Toledo), accounting for 80,4% of the collected established breeding population records, corresponding to the largest population in Iberia. The increasing number of records in this Spanish central region seems to suggest a slow geographical expansion of this population, which managed to increase due to the available resources (and niches), despite several eradication programs during the last few years. Additionally, for Galicia, we collected 124 new records since 2012, contrasting to only one reported by García *et al.* (2012). Here a new breeding population was recorded in southern Ourense, near the border with Portugal. This constitutes a risk to Portugal, since its northwestern region is, according to our modelling results, the most suitable and vulnerable area to a raccoon invasion and expansion due to its environmental characteristics and similarities with the Galician landscapes. Across the Iberian Peninsula, Portugal included, several isolated records of wild raccoons were reported. Even though there is no evidence of any established breeding population in the Portuguese territory, there are some isolated records near the Portuguese border (Cáceres and Pontevedra). The majority of the Spanish records come from capture events in the scope of eradication programs, which indicates that there is some effort being made in trying to prevent further range expansion of this invasive carnivore (Generalitat Valenciana 2013; Vazquez 2013; Morán *et al.* 2015; Layna and Prieto 2017; Suances *et al.* 2018). Additionally, in Spain, areas where the breeding populations were located, seem to be somehow linked to the presence of facilities that kept or keep raccoons in captivity. The largest population was detected in an area where 6 facilities keep raccoons in captivity and some of these might have been the source of this increasing population (García *et al.* 2012; Alda *et al.* 2013). Besides, some of these zoological facilities provide an abundant and permanent source of food to their captive animals, attracting many free-living raccoons and resulting in high densities of this carnivore in the neighbouring areas.

Our results show a clear preference by raccoons to use areas close to water bodies in the Iberian Peninsula. Water is known to be an important predictor of raccoons' presence due to the abundance of food resources (e.g. *P. clarkii*, aquatic birds, etc.) and refuge conditions, such as hollow trees (Stuewer 1943; Lotze and Anderson 1979; Gehrt and Fritzell 1998; Beasley *et al.* 2007; Bartoszewicz *et al.* 2008; García *et al.* 2012). Other studies, in both native and invasive ranges, have found that proximity to watercourses represents a significant and influential variable in predicting raccoon's distribution (Baldwin *et al.* 2006; Farashi *et al.* 2013; Heske and Ahlers 2016; Farashi and Naderi 2017). Additionally, previous studies have already highlighted that the Iberian rivers play a fundamental role in the dispersion of this species (García *et al.* 2012; Alda *et al.* 2013), and therefore might lead to the expansion of its range to the suitable areas identified by our models (Figures 3.5-3.7). Precipitation seems to have also an important role in providing suitable habitats for this species and this pattern is aligned with the findings of previous studies (Farashi *et al.* 2016; Farashi and Naderi 2017; Louppe *et al.* 2019). Since raccoons prefer environments where water is highly available, it seems logical that precipitation correlates to their distribution. However, in Iberia, raccoons seem to adapt to both drier and wetter environments, since their presence is predicted by lower and higher values of annual mean precipitation (AMP) and mean precipitation of the driest quarter (PDQ), with less probability of expansion to areas with intermediate values (Figure 3.3). The preference for areas near water bodies, but with lower precipitation values might be linked to a higher predation success, when aquatic prey species, such as the highly abundant and widespread red swamp crayfish, a primary prey of raccoons in Iberian ecosystems (García *et al.* 2012), are restricted to small stretches of rivers, or too shallow areas, due to a low volume of water in riverine systems. The opposite situation may be linked to less dry environments, in which water is abundant all year, as well as food and refuge.

Other studies identified temperature, altitude, vegetation or urbanization extent as additional important predictors for raccoons occurrence (Farashi *et al.* 2013, 2016; Farashi and Naderi 2017; Duscher *et al.* 2018). However, in the Iberian context, raccoons do not seem to be especially affected by those factors. The lack of a detectable influence of temperature might be linked to the fact that Iberia has a temperate and Mediterranean climate, which might not be a factor important enough to determine their distribution or its importance is overruled by other regional importance variables, as water bodies or precipitation. Regarding altitude, high altitudes may drive raccoons away due to less favourable bioclimatic conditions, like the presence of snow and lower temperatures in harsher winters, as documented in the Alps and Iran (Farashi *et al.* 2013; Mori *et al.* 2015; Duscher *et al.* 2018). However, in Iberia, mountain ranges are smaller and there are higher temperatures and less snow cover, and therefore, possibly, not being an important predictor of its presence in this context. Riverine systems seem to fulfil most of the raccoon's ecological requirements in the Iberia Peninsula, providing both shelter and food (García *et al.* 2012), and therefore other vegetation variables, temperature and altitude, which are important determinants elsewhere, were not influential in this region. Furthermore, most Iberian populations seem to be the result of releases or escapes from captivity (García *et al.* 2012; Alda *et al.* 2013) that often occur in more urbanized areas. This escaped/released individuals might have found free ecological niches and thus expanded their range towards more natural areas, which may lead to a more even distribution between urban and natural environments. Such a pattern may have resulted in a lower influence of urban areas on raccoon's distribution at the expenses of the proximity to water bodies. Although we could not test the effect of any microhabitat variable, we managed to observe during the field surveys (and confirmed by the raccoon's specialist wildlife biologist that join us during the fieldwork) that the areas selected by the models as high presence probability regions contained small steep slope river margins and well-developed riparian vegetation, as also documented in other studies (Stuewer 1943; Baldwin *et al.* 2006; Farashi *et al.* 2013).

According to the models' prediction and the updated distribution range of raccoons in Spain, the northwestern region of Portugal is the most vulnerable area for a raccoon invasion. The Minho river, that separates the two countries and presents, according to our model, a highly suitable habitat, might constitute a major invasion route for raccoons entering in Portugal. There are isolated records in Spain near this river (200 m to 16 km), and a possible, but unconfirmed, record on the Portuguese side. The Lima river might also be an important invasion route since it crosses both countries and still harbours a small breeding population in a major tributary, Salas river, located in southern Ourense (Spain) and close to the Portuguese border (5 km) in Tourém region (Montalegre), despite the attempt to eradicate it (Alberto Gil and Xosé Pardavila, *pers. comm.;* Layna and Prieto 2017). Other northwestern rivers like Cávado, Ave and Este might also become important for the establishment of this species in Portugal, although only records of isolated individuals, mostly unconfirmed or captured, are known in these watersheds. The Tagus river and its tributaries might also be important in the dispersion of this carnivore towards Portugal since the largest established Iberian population (Madrid and Guadalajara) persists alongside this river watershed and an isolated record of a single individual that was captured (eastern Cáceres) close to a tributary near the Portuguese border (8 km). However, our models did not predict the areas along this river in Portugal as highly suitable habitat, with only a few areas with a medium probability of occurrence, especially on Tagus' tributaries (e.g. Erges River, near Rosmaninhal). Studies have indicated that large and wide rivers may act as geographical barriers for raccoons dispersion (Cullingham *et al.* 2009; Fischer *et al.* 2015). However, in central Spain, radio-tracked raccoons have easily crossed the Tagus river (J. F. Layna *pers. comm.*), suggesting that Iberian rivers might not constitute natural barriers for a possible raccoon dispersion. Escapes from captivity might provide other sources for wild living raccoons in Portugal in the future, as we believe our compiled records of captive individuals can be incomplete, particularly for private collections and pets. It is known that this species is widely bred and kept in captivity, leading to many escapes and source of breeding populations, if not sterilized (Biedrzycka *et al.* 2014). In Spain, there are already confirmed escapes from captivity, leading to the establishment of some breeding populations. In Portugal, to our knowledge only 3 facilities (in Lisbon, Lavre and Penamacor) currently keep raccoons in captivity, becoming important to monitor the areas around those structures, especially riparian environments, to prevent and early detect animal escapes.

Other studies have predicted raccoons' suitable habitats around the world based on environmental drivers, but our modelling results showed different patterns for Iberia. Farashi *et al.* (2016), in a world-scale assessment, predicted that raccoons in Iberia would only have favourable occurrence conditions in the north of Spain and the centre of Portugal. However, their sampling design differed from ours, which may induce different range pattern because: 1) they only considered bioclimatic variables in their modelling procedure; 2) the study considered a global scale, and; 3) they did not use as training data the confirmed presence records from any of the Spanish breeding populations. Furthermore, Louppe *et al.* (2019) also modelled the favourable areas for raccoons throughout the world, but their analysis was also based only on bioclimatic variables, although including some raccoon occurrences in Spain. Their results showed that the majority of the Iberian Peninsula, especially the northern regions, constitutes a highly favourable area for raccoons, which is more coincident with our results. Although our study provides a much more spatially detailed prediction for raccoon's occurrence in Iberia. We recognise that there may be other areas within the Iberian Peninsula that can harbour suitable environmental conditions for raccoons, which were not identified by our model (e.g. the eastern part of the Tagus basin, and the Guadiana basin). This sub-estimation of adequate areas for breeding populations of raccoons may be related to the fact that some environmental variables that we wanted to include in the model as candidate drivers were not available for the entire Iberian territory (e.g. riparian vegetation quality index, river margin steepness or prey abundance, especially of the red swamp crayfish). Nevertheless, our study is an important update of the Iberian raccoon distribution, with a huge increase on the number of confirmed records (by compiling dispersed and unpublished records), and therefore, we believe it is an important tool for designing a control action plan targeting this invasive carnivore.

4.1 Conclusions and management implications

We managed to highlight the most vulnerable areas for a raccoon invasion in Portugal, which are crucial to monitor to prevent the expansion of this invasive predator. We also believe that our results are robust enough to act as baseline information in an urgently needed management plan targeting raccoons in Portugal. Firstly, because we modelled the potential distribution area using the environmental characteristics of presence locations associated to the introduced range, instead of using records from the native range, which has shown to narrow the predicted distributions areas (Lamelas-López *et al.* 2020). Also, new environments may present different biotic and abiotic conditions (or niches) that can be explored by generalist species as raccoons (Fitzpatrick *et al.* 2007) and we used breeding populations' data, that indicates that the areas encompass characteristics for individuals to establish their range and breed successfully.

Being generalist carnivores (Kauhala 1996), raccoons may constitute a real threat to Portuguese native species in the future, if they manage to establish a breeding population. Thus, besides their detection and control in the wild, it would be of extreme importance to properly assess raccoons invasion impact on Iberian ecosystems, since there is scarce knowledge on this carnivore negative effects as an invasive species, in Europe (Salgado 2018). Additionally, if this predator expands its invasive range and occupies the watersheds selected by our models, the impacts in native ecosystems might be significant. This can become especially relevant in the northern and central regions of the Iberian Peninsula, by affecting different species such as owls, the European polecat, common genets or Eurasian otters and menacing already threatened species like the European pond turtle, the Mediterranean turtle, the freshwater pearl mussel, or even aquatic birds, especially waterfowl (Ikeda *et al.* 2004; Alvarez 2008; García *et al.* 2012; Simmons *et al.* 2014; Salgado 2018). Although we registered three possible occurrence records in the more adequate and vulnerable area in northwestern Portugal, we did not manage to find any solid evidence of raccoon's presence during our field assessment. However, it is crucial to monitor this area, since it borders Spanish areas presently occupied by raccoons, hosting several rivers with exceptional conditions for raccoon's occurrence. Thereby, we recommend the urgent definition of an action plan by the Portuguese authorities (ICNF), targeting raccoons, especially because we are still in a pre-invasion phase with no evidence of established breeding populations in Portugal. The first step should consider the creation of a prevention plan to regularly monitor the risk areas identified in our study, especially those closest to the border with Spain, following a sampling procedure like the one done in this study. Simultaneously, special attention to the Spanish raccoon situation must be maintained to assure an early-warning system that identifies the presence of individuals in areas near the border and close to possible invasion pathways structure (e.g. riparian systems). Finally, the action plan should also define the regular monitoring of the areas near facilities that keep raccoons in captivity, since they can be a source of escaped animals, later resulting in breeding populations, as occurred in Spain. A fundamental aspect of the action plan should be its integration with all the management actions already in place in the several Spanish communities facing the raccoon problem, and preferably, the establishment of an Iberian management/consulting board to coordinate transnational actions.

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SUPPLEMENTARY MATERIAL

Table S.1- Variance Inflation Factor (VIF) values for each environmental variable used to model raccoon's habitat suitability in the Iberian Peninsula, before and after removing the correlated variables. *VIF=Inf means that there is perfect collinearity. The vifstep function showed which variables were correlated (VIF>5) and removed, resulting in VIF<5 for all the remaining variables. Variable names: AMT- annual mean temperature; TCQ- mean temperature of the coldest quarter; TWQ- temperature of the wettest quarter; AMP- annual mean precipitation; PWQ- mean precipitation of the wettest quarter; PDQ- mean precipitation of the driest quarter; TC-tree cover; DAF- distance to agricultural fields; DUA- distance to urban areas; ALT-altitude; RV- riparian vegetation; DWB- distance to water bodies.*

Environmental variable	VIF	vifstep
AMT	381.285151	removed
TCQ	130.012696	1.942155
TWQ	104.836285	removed
AMP	Inf	2.371864
PWQ	Inf	removed
PDQ	1.171818	3.935469
TC	1.171818	1.137998
DAF	1.439387	1.323848
DUA	1.187804	1.166713
ALT	3.387662	3.202339
RV	1.027856	1.019817
DWB	3.785252	3.670147

Table S.2- Transepts surveyed to assess raccoon's presence in Portugal, including river watershed, locality, distance surveyed on foot and with the scent-detection dog, and the detected mammals in each transect. *A total of 5 watersheds were surveyed between 13th and 18th September 2020: 1) Ave river with Este as a tributary; 2) Minho river with Coura as a tributary; 3) Lima river with Estorãos, Tamente and Salas as tributaries; 4) Cávado river; 5) Tagus river with Erges and Ponsul as tributaries.*

Transept	**Date**	**River**	**Locality**	**Length on foot (m)**	**Length with the scent-detection dog (m)**	**Detected mammals**
1	13/09/2020	Ave	Trofa	2167	1724	*Lutra lutra*
2				1105	1105	*Neovison vison*
3		Este	Rio Mau	60	60	*Lutra lutra*
4				785	785	*Lutra lutra, Neovison vison, Genetta genetta*
5			Cavalões	604	604	*Lutra lutra*
6		Ave	Ponte	1219	659	
7			Silvares	1011	1011	
8	14/09/2020	Coura	Vilar de Mouros	1421	813	*Lutra lutra, Neovison vison, Martes foina, Sus scrofa*
9		Minho	Vila Nova de Cerveira	3470	1240	*Lutra lutra*
10			São Pedro da Torre	2985	2244	None
11			Friestas	893	893	
12				407	200	*Lutra lutra*
13			Monção	2043	1496	Dog's signal, but very unlikely to be due to raccoon's presence
14	15/09/2020	Estorãos	São Pedro d'Arcos	3597	3597	*Lutra lutra, Neovison vison, Genetta genetta, Meles meles, Vulpes vulpes, Sus scrofa*
15		Lima	Bemposta	1733	1462	None
16			Entre Ambos-os-Rios	873	731	
17		Tamente		290	0	
18		Salas	Tourém	2450	1859	*Lutra lutra, Neovison vison, Genetta genetta, Meles meles, Vulpes vulpes, Sus scrofa*
19	16/09/2020	Cávado	Esposende	4568	4568	*Lutra lutra, Neovison vison, Genetta genetta*
20			Fonte Boa	1832	1325	*Lutra lutra*
21			Areias de Vilar	1272	1272	*Lutra lutra, Neovison vison, Sus scrofa, Oryctolagus cuniculus*
22	17/09/2020	Erges	Rosmaninhal	2375	1524	*Lutra lutra, Martes foina, Sus scrofa, Cervus elaphus*
23			Monfortinho	2726	1498	*Lutra lutra, Sus scrofa, Cervus elaphus*
24		Ponsul	Escalos de Baixo	910	802	*Lutra lutra*
Total				40796	31472	

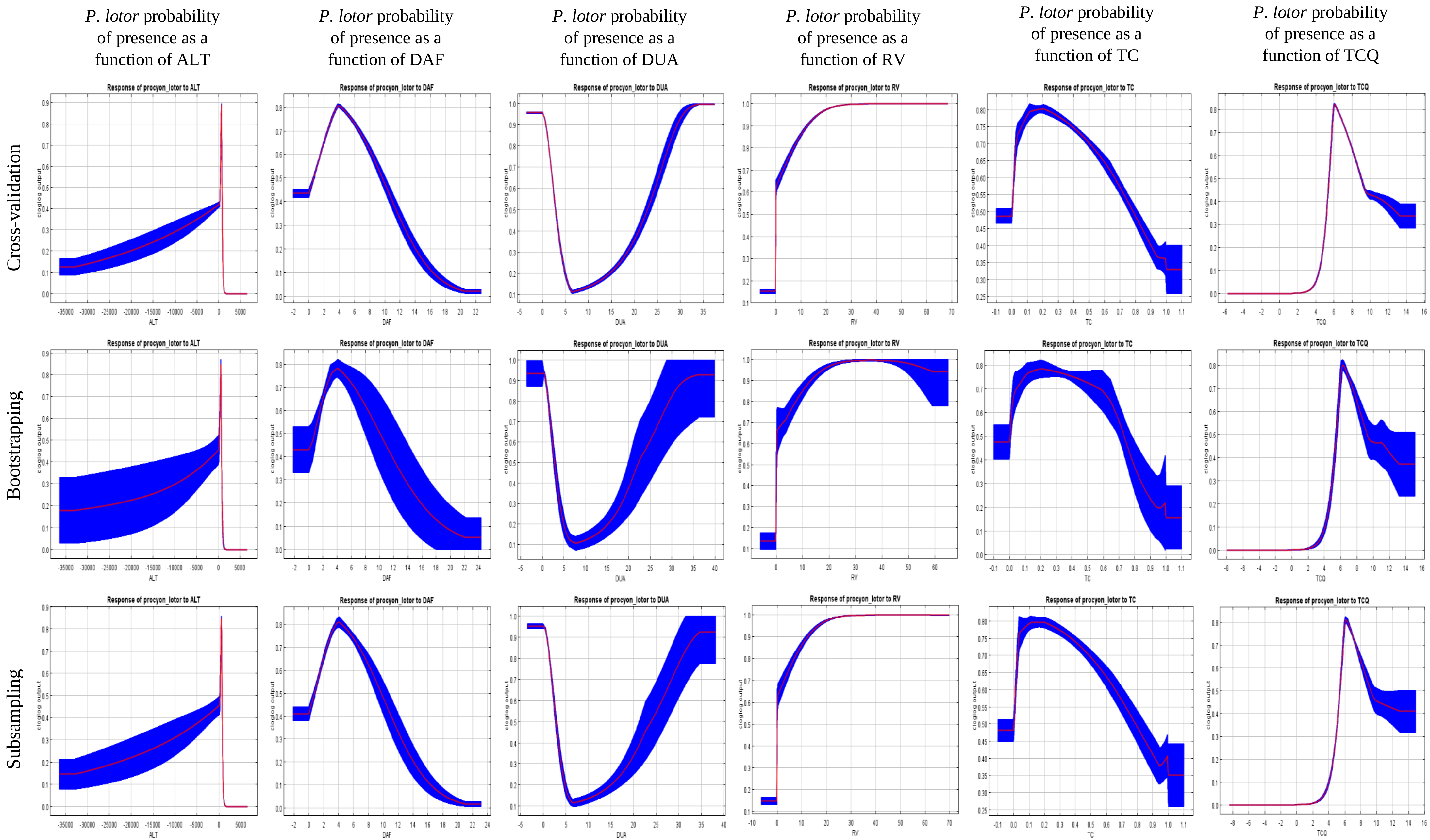

Figure S.1- Response curves for the less influential variables of each modelling fit approach to predict raccoon's suitable habitats in the Iberian Peninsula. *The red line indicates the mean response of the 10 replicates from each model, and the blue shadow represents their standard deviation. Variable names: ALT- altitude; DAF- distance to agricultural fields; DUA- distance to urban areas; RV- riparian vegetation; TC- tree cover, TCQ- mean temperature of the coldest quarter.*