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Patterns of biological invasion in the herpetofauna of the Balearic Islands: Determining the origin and predicting the expansion as conservation tools.

Mestrado em Biodiversidade, Genética e Evolução

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Patterns of biological invasion in the herpetofauna of the Balearic Islands: Determining the origin and predicting the expansion as conservation tools.

Thesis submitted in order to obtain the Master's degree in Biodiversity, Genetics and Evolution

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CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos

Para ser grande, sê inteiro: nada

Teu exagera ou exclui.

Sê todo em cada coisa. Põe quanto és

No mínimo que fazes.

Assim em cada lago a lua toda

Brilha, porque alta vive.

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Abstract

Dispersal movements of fauna occur naturally; however the human culture and its expansion, leading to several translocations of animals in a high rate, are causing a virtual epidemic of biological invasions. These biological invasions are very problematic and currently are a major cause of biodiversity loss. Thus, it becomes important to understand how the process of introduction happens, which are the statuses of the populations introduced, and which are the better measures to prevent and minimize the effects. The herpetofauna of Balearic Islands constitute the most outstanding case in the Mediterranean Basin, with more alien amphibians and reptiles, some recently detected, than native.

The principal goals of this thesis are: (1) to confirm the allochthonous status of the putative alien populations; (2) to determine the origin(s) of the introduced populations; (3) to infer the most probable pathway of introductions; (4) to model the habitat suitability of the Balearic Islands for snake species; and (5) to evaluate how the suitability will evolve throughout time.

The species that were analysed in this work are five snakes (*Hemorrhois hippocrepis, Malpolon monspessulanus, Macroprotodon sp., Natrix maura* and *Rhinechis scalaris*), two lizards (*Timon lepidus* and *Podarcis sicula*) and one amphibian (*Hyla meridionalis*).

The methodology used to achieve the origin comprises the comparison of sequences of introduced populations with those from phylogeographic studies of the native range. To model the suitability, a projection to Balearic Islands using occurrence data from the autochthonous distribution and bioclimatic variables is performed for the present and for the future (2020, 2050 and 2080).

The timing of introduction is different between the species and populations: *Macroprotodon* sp., *N. maura*, *R. scalaris* (Menorca), *P. sicula* and *H. meridionalis* took place during historical times; *H. hippocrepis*, *M. monspessulanus*, *R. scalaris* (other islands) and *T. lepidus* in XXI century. The results show a Tunisian origin for *Macroprotodon (mauritanicus)*, a potential Iberian origin for *H. hippocrepis* and *M. monspessulanus*, an Iberian/SE French origin for *N. maura*, a SE Iberian origin for *T. lepidus*, a Sicilian/Sardinian origin for *P. sicula* and a SW Iberian origin for *H. meridionalis*. The results of *R. scalaris* do not allow the identification of the putative origin of the population within its range in Iberia and SE France. The main pathway of introduction for the most recent introductions is the ornamental plants trade (mainly old olive trees).

Regarding to the suitability of the islands to snakes species, the obtained results predict significant but limited suitability in the present, which will become, with exception of *M. mauritanicus*, higher in the future for all the three consecutive scenarios of climate change analysed.

This multidisciplinary approach, joining molecular data with ecological modelling together, reveals a promising tool for understanding of the complex invasion process and, hence, supporting conservation planning.

Resumo

Os movimentos de dispersão de fauna ocorrem naturalmente. No entanto, o ser humano com a sua cultura e expansão acelerou a sua taxa de translocação causando uma virtual epidemia de invasões biológicas. Atualmente, as invasões representam um grave problema e uma das principais causas que ameaçam a biodiversidade. Assim, perceber como o processo de introdução acontece, qual é o estado das populações introduzidas e como podemos atuar para prevenir e minimizar os efeitos, tornou-se essencial. As Ilhas Baleares representam um dos mais notáveis casos do Mediterrâneo com um maior número de espécies de répteis e anfíbios introduzidos (alguns de detecção recente) do que nativos.

Os principais objetivos desta tese são: (1) confirmar o estado alóctone das populações sob análise; (2) identificar qual (quais) a(s) origem(s); (3) inferir qual o mais provável meio pelo qual as espécies são introduzidas; (4) modelar a adequabilidade do habitat das Ilhas Baleares para as espécies de cobras; e (5) predizer como a adequabilidade do habitat vai evoluir ao longo do tempo.

As espécies em que se vai focar o trabalho compreendem cinco cobras (*Hemorrohis hippocrepis*, *Malpolon monspessulanus*, *Macroprotodon sp.*, *Natrix maura* e *Rhinechis scalaris*), dois sáurios (*Timon lepidus* e *Podarcis sícula*) e um anfíbio (*H. meridionalis*).

A metodologia usada para identificar a origem de cada população consiste na comparação de sequências das populações introduzidas com sequências geradas por estudos filogeográficos da distribuição nativa. Para a avaliação se o arquipélago é adequado ou não para as espécies de cobras foram projectados modelos para as Baleares, recorrendo à combinação de pontos de ocorrência na área nativa e variáveis bioclimáticas. Os modelos foram projetados para o presente e para o futuro (2020, 2050 e 2080).

A época da introdução difere entre as várias populações: *Macroprotodon.*sp., *N. maura*, *R. scalaris* (Menorca), *P. sicula* e *H. meridionalis* ocorreram durante tempos históricos; *H. hippocrepis*, *M. monspessulanus*, *R. scalaris* (outras ilhas) e *T. lepidus* já no século XXI. Os resultados mostram uma origem Tunisina para *Macroporptodon (mauritanicus)*, uma potencial origem Ibérica para *H. hippocrepis* e *M. monspessulanus*, uma origem Ibérica/Francesa para *N. maura*, uma origem no SE Ibérico para *T. lepidus*, uma origem da Ilha de Sardenha para *P. sicula* e uma origem no SW Ibérico para *H. meridionalis*. Os resultados de *R. scalaris* não permitem concluir acerca da origem geográfica das populações. O principal meio pelo qual os animais mais recentes é o tráfico de plantas ornamentais (oliveiras velhas).

No que respeita à adequação das ilhas às espécies de cobras, os resultados obtidos revelam uma adequabilidade do habitat limitada no presente e que incrementar-se-á nas três diferentes épocas projetadas no futuro (com excepção de *M. mauritanicus*).

A abordagem multidisciplinar, combinando dados moleculares com modelação ecológica, revela-se uma promissora ferramenta no conhecimento do complexo processo de invasão e, consequentemente, para suporte ao delineamento de estratégias de conservação.

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1. Introduction: Biological Invasions

Long distance dispersal movements of fauna occur naturally, however the human culture and its expansion, are leading to several translocations of animals in a high rate, causing a virtual epidemic of biological invasions. These phenomena are very problematic in conservation terms and are currently a major cause of biodiversity loss (Brown *et al.* 2007; Ficetola *et al.* 2009). Thus, it becomes important to understand how the process of introduction happens, what are the status of the population introduced and how can we act to prevent and minimize the effects.

The biological invasion process is characterized by three main steps:

1. Transport/Initial dispersal
2. Establishment
3. Spread and invasion of new range

The first step of transport can occur either intentionally by humans or not. The transportation can be by simply ride unintentional or by intentional movement of the species due to its value or to provide a service. Examples of the first category could be the transportation of brown tree snakes (*Boiga irregularis*) in wheels wells of aircraft (Fritts *et al.* 1999), sessile invertebrates riding on the hulls of ships and disease-causing agents moving about on infected humans, their domestic animals or other vectors. In the second category are included introductions for using as pets, human or livestock food and biocontrol of pests. In general, marine invertebrates, insects and land snails are dispersed in an unintentional way. In contrast, plants, birds and mammals tend to be dispersed in a human intentional pathway. Reptiles and amphibians are dispersed by both pathways (Kraus 2009).

The establishment represents the step in which the population introduced is able to reproduce with success. It is a process not clearly understood yet and the generalization is difficult to make due to the unique features of each species. However it is known that several conditions are necessary for an alien species to establish. The climate must be similar (but not necessarily the same) to that in the native range (Blackburn & Duncan 2001; Bomford & Glover 2004) and the resources should be sufficient to complete its life circle, which means sufficient food, living space, habitat for growth and reproduction, and other biotic factors. The lower presence of predators, competitors, parasites and disease organisms in the non-indigenous area could give alien species an advantage over native species, widening its ecological niche (see below) and potentiating its expansion and success. The propagule pressure (number of individuals released) is also a factor determinant for the establishment, since if species are released more often at more sites or in a great number the probability of success is higher (Williamson 1996; Kolar & Lodge 2001; Ficetola *et al.* 2008; Hayes & Barry 2008). Life-history and behavioural characteristics of each species, as well as the 'invasional meltdown' (earlier invaders facilitating later-arriving aliens) can help also to the survival and reproduction on the new area (Reichard & Hamilton 1997; Simberloff & von

Holle 1999; Thuiller *et al.* 2006). The success of the invasion can be explained based on several hypotheses from ecological to genetic ones (reviewed in Hufbauer & Tourchin 2007). Ecological theories include empty-niche hypothesis (invasive species are able to use resources that are ignored or underutilized by native species) (Elton 1958; Hierro *et al.* 2005), biotic-resistance hypothesis (natives that are close related to introduced species can limit its expansion via competition) (Elton 1958; Levine *et al.* 2004) and the pre-adaptation of the invaders to the novel environment hypothesis which make them a better competitor (Baker & Stebbins 1965; Sax & Brown 2000). Genetic changes, like founder effects (Mayr 1954) and hybridization (Ellstrand & Schierenbeck 2000) both with closely related natives or among populations of the introduced species, may increase genetic variation and allow the emergence of new genotypes that potentiate a better relation with the new environment. In summary, the establishment success of a species (i.e. its survival and reproduction) depends on the interaction between the biotic and abiotic conditions at the invaded region and the species' needs (Kraus 2009).

Finally, the spread can be explosive or not, depending on the species' attributes. In some cases, a 'lag-phase', lasting from years to decades, occurs in which population sizes are doubling but appear dormant due to the low overall size of the population, which limits the human power to detect its presence (Crooks & Soulé 1999). Hassol & Katzenberg (1995) suggest four mechanisms that can be underlying this period. One of the mechanisms is genetic: the invader keeps a stable population at relatively low levels for a number of years, various genotypes being tested against the environmental conditions, until one 'breaks through' and begins the spread actively, causing an explosive growth. A second mechanismt is related to suitability of the environmental conditions to the species that begin the exponential growth only when conditions are optimal. A third mechanism regards the difficulty in finding mates which could also be responsible to a slower growth in the beginning, but once the threshold population size is reached a normal exponential growth begins. Finally, a fourth mechanism arises from detection probability. Even the normal growth of the population can be interpreted as containing a lag phase in which the population could grow more a less continuously but without being noticed until the population reaches the double of the size. As a consequence of this lag-phase, usually the invasion only is detected too late, when the management is almost impossible and the populations are already widely distributed.

1.1 Why reptiles and amphibians are good invaders?

Reptiles and amphibians have several characteristics that make them good invaders, depending on each species biological traits. First, they are very susceptible to be transported by human networks, due to their small size (in some species) or secretive behaviour which make difficult to detect them and also due to the use of human manufactures and ornamental plants as a refuge (Pitt *et al.* 2005; Reynolds & Niemiller 2010). Second, some species are resistant to starvation during transportation and able to withstand until two years without feeding (Grably & Peiery 1981; de Vosjoli *et al.* 1995; McCue 2010).

Third, they have cryptic behaviours, allowing the development of populations in an undetectable way until is readily established. Forth, generalized diets enable many reptiles and amphibians to adapt to the resources available on the novel environment (Meshaka 2004; Pitt *et al.* 2005). Fifth, they generally have high reproductive rates which facilitate the rapid growth of population and the recovering of stochastic events. Finally, some species are very mobile, dispersing rapidly in the new area (Pitt *et al.* 2005)

Some species examples can be presented, like Burmese pythons (*Python molurus bivittatus*) which were introduced in the US by pet trade, which is now a great competitor and predator, with high reproductive rates, that already feeds on endangered species of Florida (Pough *et al.* 1998). Another classical example is that of the bullfrog (*Lithobates catesbeianus*, Frost *et al.* 2006) that, besides the high mobility and high reproductive capacity, have generalized diet habits endangering some native species (Moyle 1973; Pitt *et al.* 2005). However, despite several case have been studied (Kraus 2009), amphibians and reptiles species are so diverse that in each case of invasion unique properties and features make the alien species to be succeed.

1.2 Impacts by introduced herpetofauna

The effects of an introduction are difficult to predict. However several general impacts are well established like biotic homogenization, disruptions to food-webs, alterations of community structure, damage to human structure, among others. In the particular case of amphibians and reptiles there are evidences for ecological effects, evolutionary effects and social effects (Kraus 2009).

Figure 1: *Natrix maura*

The ecological effects include, for example, negative impact in both native prey and predator species. The snake *Natrix maura* (Figure 1) was introduced in Balearic Islands about 2000 years ago, possibly by the Romans, and it is said to be responsible for the decline of the midwife toad (*Alytes muletensis,* Figure 2), endemic of Mallorca, through the predation of larvae and adults. It is suspected that this snake also contributed for the extinction of the endemic *Alytes talaioticus* during the Holocene (Guicking *et al.* 2005; Kraus 2009; Álvarez *et al.* 2010). The cane toad (*Rhinella marina,* Frost *et al.* 2006) was intentionally translocated to several countries in attempts to control agricultural pests. However, the effect of the introduction of this species went beyond of the biocontrol purpose, since it secretes a skin toxin, causing the dead to the native predators. It is considered one of the most notorious invasive species of the world (Brown *et al.* 2007; Faraone *et al.* 2008; Kraus 2009).

Figure 2: *Alytes muletensis*

Another ecological effect is the wider changes in ecosystem dynamics. An example of that is the introduction of brown tree snake (*Boiga irregularis*, Figure 3) in Guam in the early 1950s, probably by ship transportation from a small island in Papua New Guinea. This specie has spread throughout Guam and has affected native birds, small reptiles and mammals, including the fruit bat (*Pteropus mariannus*) (Reaser *et al.* 2007). Other vertebrates were independently introduced in Guam too, like the green anole (*Anolis carolinensis*) and the black dongo (*Dicrurus macrocercus*). These introductions, both of the snake and the other vertebrates, caused a change in the ecosystem dynamics since even with low abundance of native preys, the snake keep its abundance due to the presence of the alien preys. In other words, the Guam's food-web is now composed by alien species, both predators and prey (Fritts & Rodda 1998).


Figure 3: *Boiga irregularis*

The competition with native species can also be an impact of herpetofauna, which is the case of the bullfrog (*Lithobates catesbeianus*, Frost *et al.* 2006), which was introduced in North America, Mexico, Caribbean, Hawaii, Japan and Europe. This amphibian caused the declining of autochthonous amphibian populations, like the foothill yellow-legged frog (*Rana boylii*) and the northern red-legged frog (*Rana aurora*), through the competition for resources and by passive exclusion under condition in which food resources are clumped (Kiesecker *et al.* 2001; Faraone *et al.* 2008).

Finally, other ecological effects include the community homogenization and the introduction of new parasites and diseases that are harmful for the resident species. An example is the case of the African clawed frog (*Xenopus laevis,* Figure 4) which spread the chytrid fungus (*Bratrachochytrium dendrobatidis*), a pathogen that cause decline and extinction of many species of amphibians worldwide (Faraone *et al.* 2008; Kraus 2009).


Figure 4: *Xenopus laevis*

The evolutionary consequences include genetic effects like hybridization and outbreeding depression, and morphological, physiological, and behavioural changes on native species (Kraus 2009). An example of a morphological and behavioural changes induced by alien on native species occurred in the United States between the introduced fire ant and the native fence lizard (*Sceloporus undulates*). The study by Langkilde (2009) confirms that lizards adapted to the invasive ant with antipredator strategies, such as the body-twitching upon initial attack and the fast escape from the mound


Figure 5: *Rhinella marina*

and source of attack, and by alteration of morphologies, i.e. longer limbs, to mitigate potentially lethal attack by the ants. Another example is represented by populations of Australian snakes (*Pseudechis porphyriacus*) exposed to the toxin of cane toads (*Rhinella marina,* Figure 5) for several decades developed a certain degree of resistance to that toxin, which represents a physiological and evolutionary adaptation (Phillips & Shine 2006).

However, the amphibians and reptiles do not only impact the native species of the new region, but also have social impacts that have indirect or direct effect on humans' life. For example, the cane toad caused economic losses for apiarists and reduction crop-pollination services, due to their predation upon honey bees (*Apis mellifera*). Moreover, some introductions may pose risks to human health such as that one of the brown treesnakes (*Boiga irregularis*) in Guam, which increased the susceptibility of humans to diseases (including lethal diseases) as a consequence of snakes' bites.

Finally, the introduction of alien species cause losses on scientific knowledge like evolutionary status of populations, ecological relationships and dynamics, original ranges for a species, and so on. This kind of knowledge is absolutely necessary for setting conservation strategies (Hassol & Katzenberger 1994; Kraus 2009).

In summary, a vast of negative impacts was reported along of the last decades and many of them are still being reported. So it is necessary to look closely for the introduction problems and look for each case with unique eyes, in order to detect precious information to fight against this epidemic problem.

1.3 Biological Invasions on Islands: the case of Balearic Islands

Islands are geographically isolated from continents, which make them to have special properties. The number and organismal taxa that can naturally reach and colonize this ecosystem is limited, the number of populations is fewer and the total population size is smaller. These factors added to the isolation and the endemism create a higher sensitivity to disturbances and a higher vulnerability to biodiversity extinction, with upper rates than on continental ecosystem (Vitousek *et al.* 1996; Reaser *et al.* 2007; Ficetola & Padoa-Schippa 2009).

The theory of island biogeography states that species richness on islands results from the equilibrium between species extinction, species colonization and speciation (MacArthur & Wilson 1967). However, humans have disturbed this balance by increasing colonisation rates and extinction risk on insular ecosystems. Thus, islands that are subject to major human impact have more alien species than native. In the islands, biological invasions are even more dangerous when the presence of predators is lower, the competitors are absent and the native prey are vulnerable to the new organism introduced or to its parasites, which could cause harmful for the indigenous fauna (Whittaker & Fernández-Palacios 2007; Álvarez *et al.* 2010). Besides that, islands have normally a more benign environment and lower diversity of habitat types, which facilitate the establishment of the invasive species (Gimeno *et al.* 2006).

The Mediterranean basin is considered one of the world biodiversity hotspots (Myers *et al.* 2000) and is distinctive in the way that interaction between biota and human activities unfold since the ancient times. Because of the ancient presence of human activities including organisms transport, it is often difficult the distinction between allochthonous from autochthonous species (Blondel *et al.* 2010).

Mediterranean islands share reptiles and amphibian species with the mainland Europe, Africa and, in the west, also with Asia, but also have endemic species constantly threatened by biological invasions from these continents through cargo, pet trade and the intentional release (Ficetola & Padoa-Schioppa 2009; Kraus 2009).

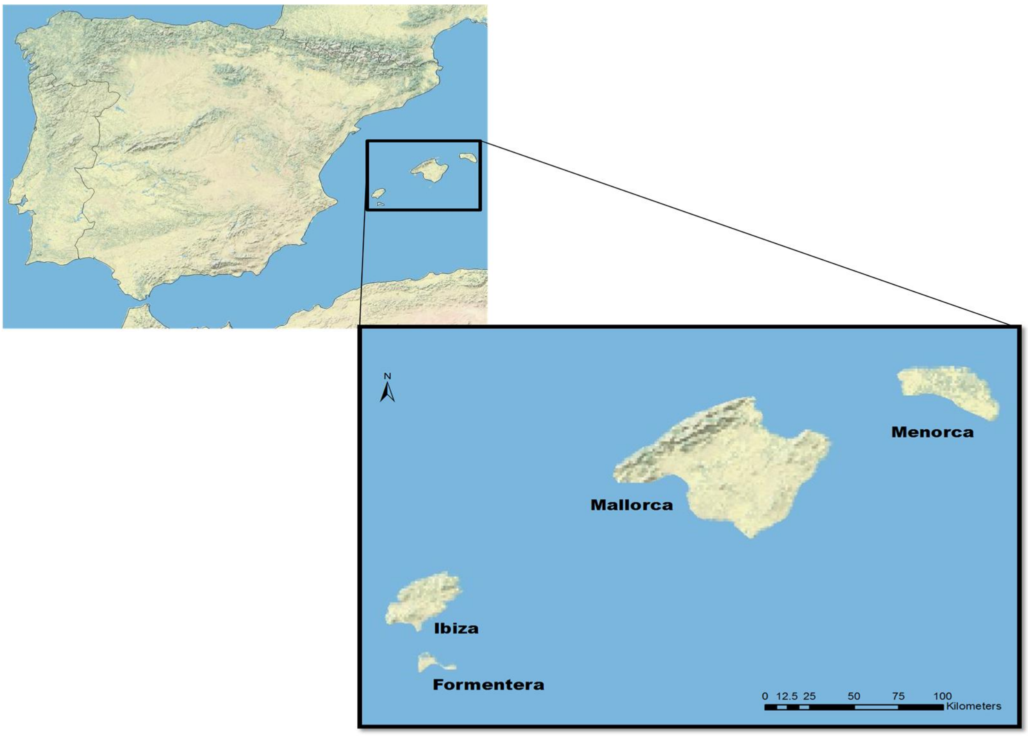

Figure 6: Map of the Balearic Islands

In this respect, Balearic Islands (Figure 6) represent the most exceptional case within the Mediterranean and one of the most outstanding in the world, with a higher number of alien amphibians and reptiles than native. Before the human arrival in the Neolithic, Balearic Islands harboured a rich endemic fauna and flora (Pons & Palmer 1996; Alomar *et al* 1997). For the earliest faunas (pre-Messinian crisis) there are records of a ochotonid lagomorph (*Gymnesicolagus gelaberti,* Mein & Adrover 1982), three glirid rodents (*Carbomys sacaresi,* Mein & Adrover 1982; *Margaritamys llulli,* Mein & Adrover 1982; and *Peridyromys ordinasi,* Mein & Adrover 1982), one large terrestrial tortoises (*Geochelone* sp) and a sea turtle (*Trionyx* sp) for Mallorca Islands (Mein and Adrover, 1982; Adrover *et al.*, 1983, 1984; Gràcia *et al.* 2005). In Menorca, there are records of *Gymnesicolagus*, a glirid, similar to *M. llulli* from the Mallorca, and a large tortoise (Bover *et al.* 2008).

After that period, the Balearic Islands became isolated from the mainland, but also the Pityusic (Ibiza and Formentera) and the Gimnesic (Mallorca and Menorca) sub-archipelagos split and never contacted again. The climate change and the connection between islands within the same sub-archipelago during the glaciations had a great influence in the evolution of different fauna in the islands. In a general way, the Pytiusic Islands are separated by a narrow and shallow channel; allowing a connection during the glaciations. As a consequence, Formentera and Ibiza shared the fauna throughout their paleogeographical history. On the other hand, Gimnesic Islands have deeper channels among them, preventing the connection between islands, although the presence in Menorca of three common mammals, suggest a conection during the glaciations on Late Pliocene/Early Pleistocene (Bover 2004). Thus Mallorca and Menorca had different paleogeographical histories and in each island a different faunal evolution occurred.

Three faunal assemblages evolved respectively in Mallorca, Menorca and Pityusic Islands (Figure 7). For all the islands, different mammals (bovids, insectivores, glirid, rabbit, bats) are recorded, as well as several species of birds. Regarding the herpetofauna, fossil records of Mallorca archaeological deposits report the existence of one lizard (*P. lilfordi*, Gunther 1874) and two amphibians (*Discoglossus* sp. and *Alytes muletensis*, Sanchiz & Androver 1977). In Menorca, two post-Messinian faunas occurred, one during the Early and Middle Pliocene (Quintana 1998; Alcover *et al.* 1999) and the other during Late Pliocene or Early Pleistocene (Bover *et al.* 2008). The Early/Middle Pliocene fauna was composed, namely, by a giant tortoise (Bate 1914), a lizard *Podarcis* sp. (likely the ancestor of *P. lilfordi*, Bailón 2004), geckos (Gekkonidae indet.), a amphisbaenid (*Blanus* sp., Garcia-Porta *et al.* 2002, Bailón *et al.* 2005), a colubrid (*Coluber* sp., Bailón *et al.* 2005) and two viperids (*Vipera natiensis* and *Vipera sp.* Bailón *et al.* 2002 and Vipera sp). The Late Pliocene/Early Pleistocene records report a lizard (*Podarcis sp.*) and two amphibians (*A. Muletensis*, Barbadillo 1987 and *Discoglossus* sp., Alcover *et al.* 1981, Quintana 1998). Finally, for the Pytiusic Islands there are different fossil records to three different periods. During the Late Miocene/Early Pliocene a lizard and a tortoise were recorded (Moyà-Solà *et al.* 1984, 1999). During the Late Pliocene, a middle sized tortoise (*Cheirogaster* sp., Bour, 1985) and a lizard (*Podarcis* sp, Kotsakis 1981) were found. In the deposits of the Late Pleistocene lizard species (*Podarcis pityusensis,* Boscá 1883) were recorded.

The first records of the humans at the Islands are from the third millennium BCE (Alcover *et al.* 2001; Ramis *et al.* 2002) and the human settlement occurred probably in the last third of that millennium (Bover *et al.* 2008). A long history of colonization, consequence of several phenomena in the western Mediterranean, like the demographic growth, discovering of new uninhabited regions and the development of maritime contacts, set the Balearic Islands in the route of several civilizations (Iberians, Phoenicians, Greeks, Carthaginians, Romans, Byzantines and Berbers) that start to bring the first aliens species, namely for domestication, ornamentation and pet (Lewthwaite 1985; Chapman 1990).

An intensification of human activity occurred during the last century in the Balearic Islands, especially on Mallorca, due to tourism development and to pet and nursery trade such as the trade of live plants for ornamental purposes. These activities have been provoking new extinctions and range retractions in the native herpetofauna which is nowadays composed by only one amphibian, the Mallorcan midwife toad (*Alytes muletensis)* and two reptiles, Lilford's wall lizard and Ibiza wall lizards (*P. lilfordi* and *P. ptyusensis,* respectively) (Pynia & Carretero 2011). On the other hand, a striking number of species has been introduced in the main islands, as reported in the Table 1.

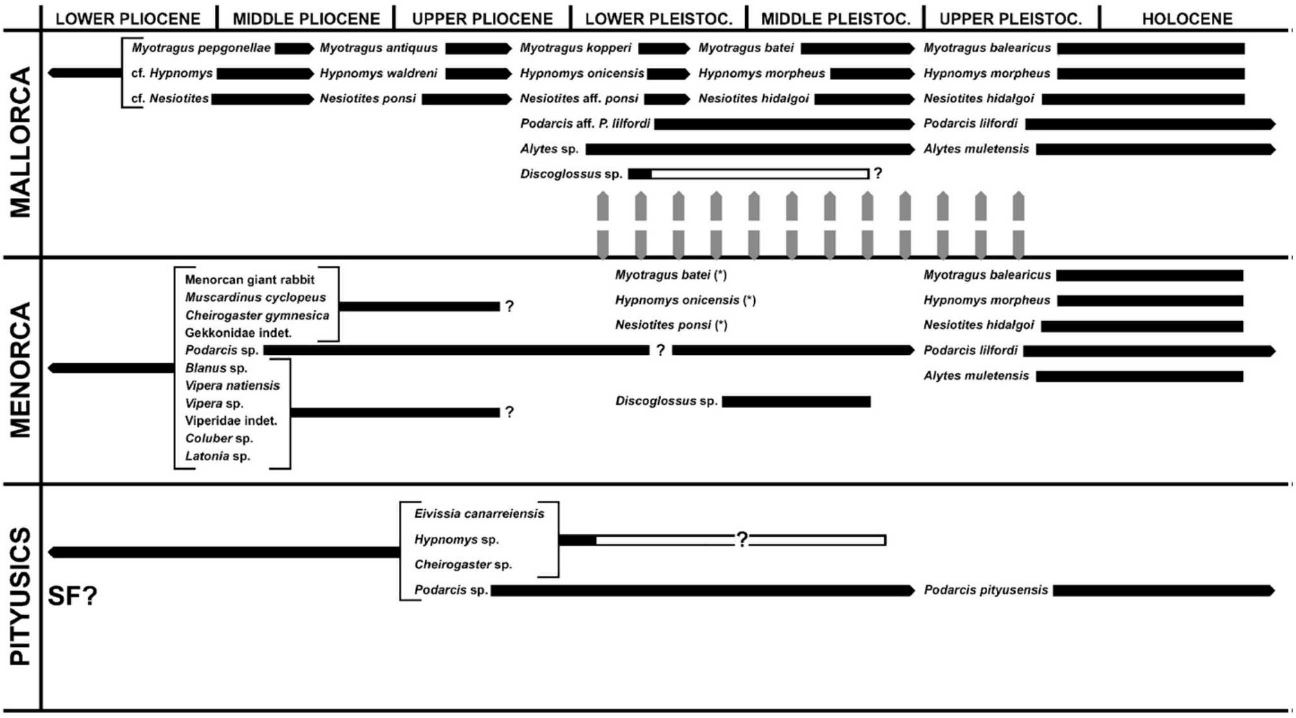

Figure 7: Diagram of the different faunal sets (only terrestrial vertebrates are figured) that lived in the Balearic Islands during the Plio-Quaternary.(SF?) = Ses Fontanelles fauna, with unknown chronology (Late Miocene–Early Pliocene?). (*)= Considered as endemic fauna (*M. binigausensis*,*H. Eliomyoides* and *N. meloussae*, respectively) by Pons-Moyà *et al.* (1981). The gray arrows show the contact between Menorca and Mallorca during Quaternary glaciations. (Bover *et al.* 2008)

Mallorca is the island with the highest number of putative aliens, 21 species, followed by Menorca with 15 species introduced, both Formentera and Ibiza with 8 species and Cabrera with 2 introduced species. Some of the introduced species are already naturalized, like the the ladder snake (*R. scalaris*), the Italian wall lizard (*P. sicula*) and the Mediterranean tree frog (*Hyla meridionalis*) in Menorca. Some other species have been recorded only in recent times, like the Montpellier snake (*M. monspessulanus*) in Mallorca and Formentera, and the ocellated lizard (*T.lepidus*) in Mallorca Island (Table 1).

Among the alien species, the snakes represent the most concern group for the conservation of native species, since they are strong predators. In Mallorca, the false smooth snake (*Macroprotodon* sp.) and the Montpellier snake (*M. monspessulanus*) can feed on geckos and lizards, while the introduced large Psamodrommus (*Psammodromus algirus*) has a generelised invertebrate diet and the ladder snake

(*Rhinechis scalaris*) mainly feed on mammals and birds. In the case of Lilford's wall lizard (*Podarcis lilfordi*) in Mallorca and Menorca, it has been hypothesized that the introduction of the false smooth snake (*Macroprotodon* sp.) caused their extinction (Mayol 1985). Since Ibiza and Formentera were snakes-free until recently, the impacts of the introduction of snakes in these islands can be very catastrophic as it has been for the endemic lizard of Mallorca and Menorca.

The number of observations per year has been increasing for all the alien species in the Baleatic Islands (Figure 8), which suggests their expansion both in terms of range and population size. Thus, monitoring the populations' trend of these alien species as well as forecasting their expansion are urgent actions. The use of modelling techniques will allow the understanding of how the population will expand in space and time, which is essential to better define conservation strategies. A further discussion on this topic will be present on the sub-chapter 1.6 and in the chapter 4.

Table 1: List of amphibians and reptiles of the Balearic Islands as reported by Mateo et al. (2011) and Pinya & Carretero (2011). Ar: Alien species without evidence of recent reproduction; Aa: Acclimatized Alien species without evidence of reproduction; Ex: Extinct; Na: Naturalized Alien Species; Nv: Native species; S: Population is stable; I: Population is increasing; D – Population is decreasing; - Absent.

Species	**Mallorca**	**Menorca**	**Ibiza**	**Formentera**	**Cabrera**
Pelophylax perezi	Na S	Ar	Na S	Na S	-
Bufo balearicus	Na	Na	Na	-	-
Alytes muletensis	Nv	Ex	-	-	-
Alytes obstetricans	-	Ar	-	-	-
Hyla meridionalis	Ar D	Na S	-	-	-
Testudo hermanni	Na S	Na S	-	-	-
Testudo graeca	Na D	-	Ex	Na	-
Emys orbicularis	Na S	Na S	-	-	-
Trachemys scripta	Na I	Aa I	Aa	-	-
Chrysemys picta	Na	-	-	-	-
Mauremys leprosa	Na S	Aa	-	-	-
Chelydra serpentina	Aa	-	-	-	-
Chamaeleo chamaelon	Aa	-	-	-	-
Tarentola mauritanica	Na S	Na S	Na S	Na S	Na S
Hemidactylus turcicus	Na S	Na S	Na S	Na S	Na S
Podarcis lilfordi	Nv S	Nv	-	-	Nv
Podarcis pytiusensis	Na S	-	Nv S	Nv	-
Podarcis sicula	-	Na S	-	-	-
Psammodromus algirus	Na I	-	-	-	-
Scelarcis perspicillata	-	Na S	-	-	-
Timon lepidus	Aa I	-	-	-	-
Macroprotodon mauritanicus	Na S	Na S	-	-	-
Natrix maura	Na S	Na S	-	-	-
Malpolon monspessulanus	Aa I	-	Na I	Aa	-
Hemorrhois hippocrepis	Na I	-	Na I	Na I	
Rhinechis scalaris	Aa I	Na S	Aa I	Aa I	

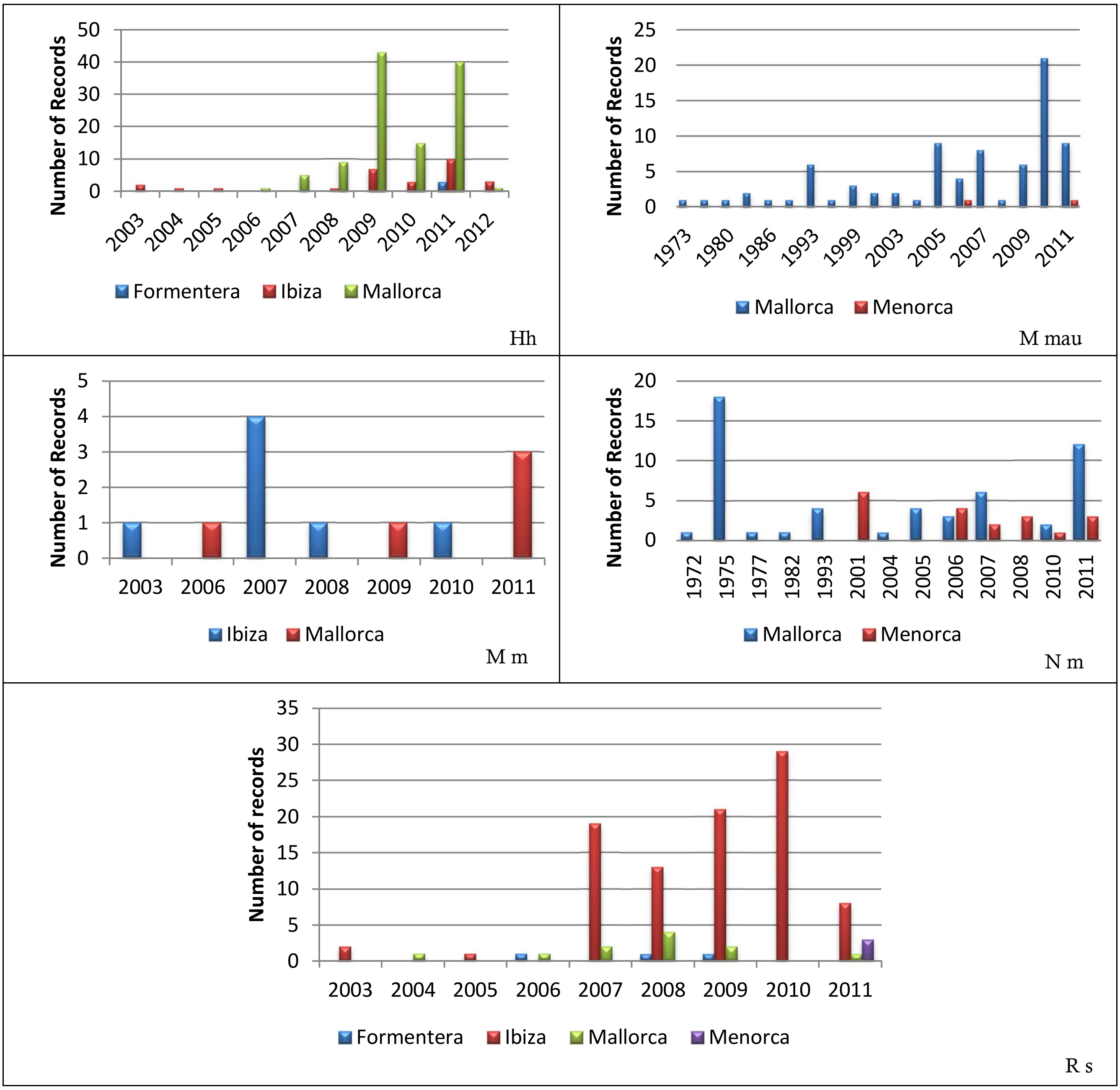

Figure 8: Number of records through the years for each species of Balearic snakes. Information provided by Jose A. Mateo and COFIB. Hh: *Hemorrhois hippocrepis*; M. mau: *Macroprotodon mauritanicus*; M m: *Malpolon monspessulanus*; N m: *Natrix maura*; R s: *Rhinechis scalaris.*

1.4 Study Area

The Balearic Islands have a total area of 5040 km^2 and 1428 km of coastline, laying 80 to 300 km east of the Iberian mainland. This archipelago is composed by two islands groups: the Gymnesics (eastern) and the Pitiusics (western). The Gymnesic group is constituted by the main islands of Mallorca and Menorca, the small island of Cabrera and 30 surrounding islets. The Pitiusic group includes the Ibiza and Formentera islands and 60 smaller surrounding islets.

Mallorca is the biggest island with very diverse ecosystems and very different landscapes. In fact, it is possible to recognize three distinct areas. The Serra Tramuntana is an elevated mountain range that shapes the northern coast of the island; this Serra has the highest peak of the island with 1445m, which is named Puig Major. Other distinctive areas are the Serres de Llevant composed by small rounded hills that mark the landscape creating white-sand coves and green pine forest, and the flatland area called *Pla*, which is located between two mountain ranges and characterized by rural landscapes.

Menorca Island is predominantly flat, with subtle rolling hill and sheltered by the small mountain range of the northern coast with a maximum of 347 m at Sa Muntanya del Toro. So, in the north the island is more rugged and with a jagged coastline and in the south is more flat with cliffs, water carved gullies and white-sand coves nestled amid pine trees.

Ibiza has a quite uniform landscape and moderate relief, being the highest point Sa Talaiassa (475m). A variety of habitats can be found since coastal plains, lagoons, marshes, saltflats (*salines)*, fixed and shifting dunes, cliffs and beaches. The flat alluvial plain of Ibiza is formed by Quaternary calcareous deposits and two enclosing wooded hills, which rise from the sea, Puig d'es Corb Mari (160m) and Puig d'es Falcó (144m). This island is part of a Special Protection Area for Birds and EEC's Natura 2000 Network, and it is listed on the Ramsar List of Wetlands of International Importance.

Formentera is the smallest inhabited island in the Balearics complex, with 19 km long and located 6 km south of Ibiza. It has a narrow shape that gives it a long stretch of coast (82 km), in proportion to its surface area. This island has a particular landscape composed by a combination of Mediterranean vegetation dune areas with pine forests, savin groves and conifers.

Cabrera is the unaltered island ecosystem of the Balearic Archipelago and it was officially declared Marine and Land National Park in 1991. It is an area with a great wealth of biodiversity, featuring countless endemic invertebrates and 150 migratory bird species which stop in the island during both their spring and autumn routes.

The climate on the Balearic Islands is thermomediterranean type, which means that the summers are hot and dry and the winters are mild with some rain in autumn. During the winter the minimum temperature is 10ºC and in July temperatures can reach 35ºC, on average (ICA 2011).

1.5 Study Species

In this thesis, we will study the biological invasions of amphibians and reptiles of Balearic Islands, with a special focus on snakes due to the predator characteristics that make them an alien potentially more harmful for the native biota. In addition, two lizards and one amphibian will be also analysed, in order to understand whether common pattern and pathway of introduction occur.

Snakes

Horseshoe whip snake, *Hemorrhois hippocrepis*

The distribution of this species comprises central and southern of Portugal, southern, central and eastern of Spain, Morocco, northern Tunisia and northern Algeria. In the Iberian Peninsula, the species did not occur in high and cold zones, in steppe regions with rigorous winter and in zones with temperate-Atlantic climate. It is suspicious to be introduced by humans in Zembra Islands (Tunisia) and in the Italian Islands of Sardinia and Pantellaria in historical times (Carranza *et al.* 2006; Miras *et al.* 2009a). The closest relative of *H. hippocrepis* is *H. algirus*, which occurs in drier areas of the Maghreb (Utiger 2002).

The horseshoe whip snake (Figure 9) is very thermophile and rupicolous, being found in a variety of arid, dry and rocky Mediterranean habitats. However, it is also possible to occur in modified habitats like scrubland, coastal plains, vineyards, olive groves, rural gardens and urban areas (Miras *et al.* 2009). Indeed, this species is considered the most anthropophile Iberian snake, using the human manufactures as habitat and refuge. Its distribution is influenced by physiological features, since the males need a temperate spring to complete the spermatogenesis (Pleguezelos & Feriche 1999).

This snake is diurnal, but it can be active also at dusk and at night in warm weather. On the whole, it is active between March and November, although in some climatically favourable areas it can be semi-active during the winter when is normally refugee between rocks (Feriche 2004). Its reproductive season occurs from middle of May until middle of July, with the copulation occurring from middle May until end of June, the ovulation during the June and the first postures of 4-11 eggs in the first half of July. The number of eggs depends on the size of the females. Newborns with 237-308 mm begin to be observed in August and September; almost all the mature females are able to reproduce every year.

The diet spectrum is quite wide including mammals, reptiles and birds. Sporadically, it feeds on amphibians and on invertebrates such as oligochaetes and hexapods. The main predators are the ocellated lizard (*T. lepidus*), the Montpellier snake (*M. monspessulanus*), the eastern Imperial eagle (*Aquila heliaca*), the common buzzard (*Buteo buteo*), the short-toed snake eagle (*Circaetus gallicus*) and the domestic cat (Feriche 2004).

Figure 9: *Hemorrhois hippocrepis* (pictures taken from the internet)

False smooth snakes, *Macroprotodon sp.*

The false smooth snakes are distributed in the Mediterranean areas of Iberian Peninsula and North Africa, since Morocco until Egypt, south Israel and Argelia. It is also present in Mallorca, Menorca, Galita, Lampedusa, Djerba, Zembra, Zembretta, and Habibas Islands, where is probably introduced (Doumergue1901; Lanza & Bruzzone 1959; Marinkelle 1962; Busack & McCoy 1990; Joger 1999; Wade 2001). Recently, a revision of the genus was made by Wade (2001), using morphological features, in which four species were identified: *M. cucullatus* (arid areas of North Africa), *M. mauritanicus* (before, *M. cucullatus mauritanicus,* North Algeria and Tunisia, Balearic Islands), *M. abubakeri* (North Algeria) and *M. brevis* (before *M. cucullatus brevis,* Iberian Peninsula) (Figure 10).

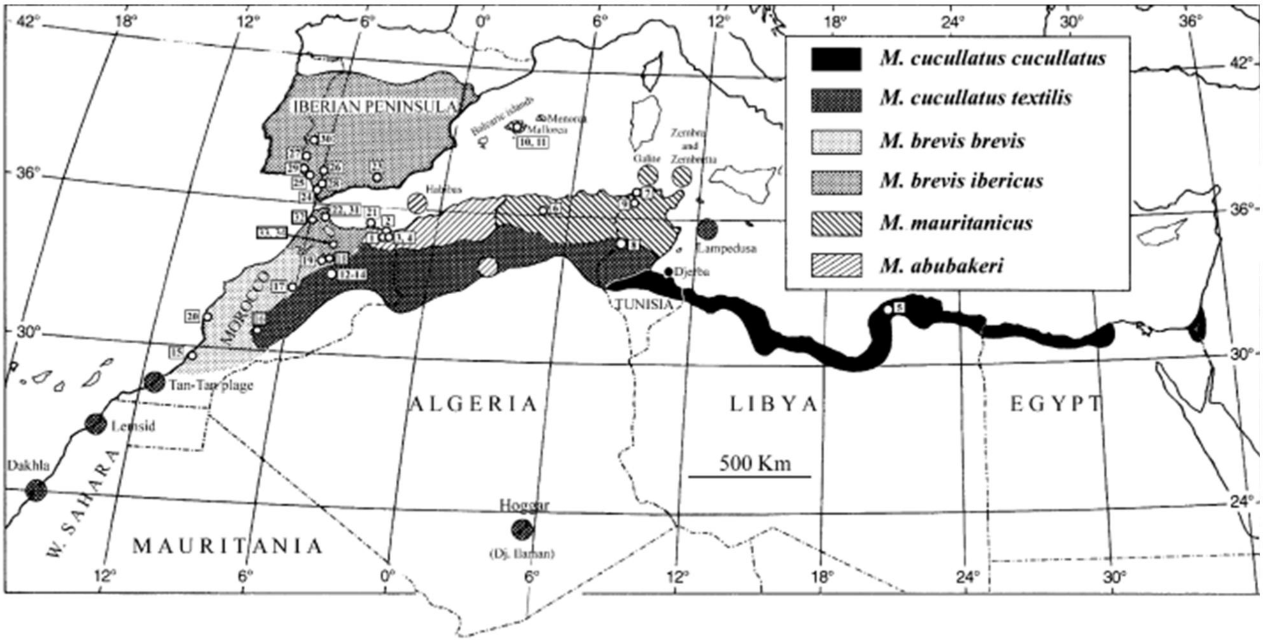

Figure 10: Present taxonomy of *Macroprotodon sp.* (Wade 2001) showing the geographical distribution of species and subspecies.

The false smooth snake (Figure 11) occurs in a wide spectrum of ecological habitats, from sub-arid habitats to sub-humid/humid ones including sandy areas, scrubland, woodland, oasis, plantations, cultivated land, stone walls and ruins and urban areas (Corti *et al.* 2009). A requirement in the habitat is the existence of refuge sites, namely rocks. This species also uses the the human manufactures as refuge, making it more susceptible to be transported (Carranza *et al* 2004; Pleguezelos & Férnandez-Cardenete 2002).

This snake has mainly nocturnal, semifossorial habits, emerging at twilight and remaining active throughout the night (Carranza *et al.* 2004). The reproductive behaviour is not well known due to its secretive habits. However, captive experiments demonstrated that aggression between males begun in mid-April, suggesting that this could be the beginning of the breeding season (Capula & Luiselli 1997). The breeding occurs every two years with females laying two to six eggs (Filipe & Luiselli 2000).

Figure 11: *Macroprotodon sp.* (pictures taken from the internet)

The diet of this snake is different from region to region. In the mainland, the snakes feed mainly on reptiles, while on the islands it feeds on reptiles and small mammals depending on the population (Pleguezelos 1994). A case of cannibalism was reported for Mallorca (Capellà *et al.* 2010).

Montpellier snake, *Malpolon monspessulanus*

The distribution of the Montpellier snake (Figure 12) includes Portugal (absent in some mountains in the north, Oliveira & Crespo 1989), Spain (generally absent from the Cantabrian Mountains and Asturias), southeastern France, northeastern Italy (Liguria) and all North Africa through Algeria, Morocco and coastal areas of Western Sahara (Martínez-Solano *et al.* 2009). It also occurs on Corfu, Cefalonia, Lesbos, Zante, Thasos, Eubea, Escopelos, Skiathos and Lampedusa Islands (Kasapidis *et al.* 1996; Augusto 2001; Corti *et al.* 2001). There are three subspecies described: *M. m. monspessulanus* (Southwest Europe and Western Maghreb), *M. m. insignitus* (from east Morocco through Algeria and Tunisia eastwards to western Syria) and *M. m. fuscus* (Turkey, Southeast Europe, North Iraq and Western Iran) (De Haan 1999). Recently, Carranza *et al.* (2006) proposed the separation of the units *M. m. monspessulanus* and the *M. m. insignitus* and *M. m. fuscus*, since they show clear morphological differences, different ranges of colour patterns and different mitochondrial gene fragments.

This snake is very thermophile and it is able to occupy almost every kind of Mediterranean habitats, from coastal dunes, scrubland with low cover and open spaces to cultivated land and even urban areas, from sea level to 2200 m (Blázquez & Pleguezuelos 2002; Martínez-Solano *et al.* 2009). The proximity to human areas makes this species susceptible to be transported. Moreover, due to the size it can reach and to its predatory behaviour to game birds, it is chased by humans in some parts of its range (Pleguezuelos 2003).

The Montpellier snake is diurnal, although in warmer days of summer it can be active during the crepuscule and less active during the day. In general, it is active from May to November, with an increment of males' activity between May and June. Its reproductive period begins on spring, with the copulation occurring between May and June, the postures of 4-11 eggs in July and the observation of newborns in August. The males are territorial and fight during the mating period (Pleguezelos 2003).

Figure 12: *Malpolon monspessulanus* (pictures taken from the internet)

The diet of this snake is very generalist with more than 30 prey species identified. The diet varies through the development of the snake: the newborns feed on orthoptera and other insects whereas the individuals with biggest sizes may predate on mammals (rabbits), large reptiles and birds. Some cases of cannibalism were already observed (Recuero *et al.* 2010). The main predator of the Montpellier snake is the short-toed snake eagle (*Circaetus gallicus*).

Viperine snake, *Natrix maura*

The distribution comprises the northwest of North Africa, Tunisia, Algeria and Morocco, and the south-west Europe, Portugal, Spain (absent in Lugo, Asturias and Cantabria), almost all France, south-west Switzerland and northwest Italy. It is also present on several Mediterranean islands, namely Mallorca, Menorca, Sardinia and Corsica, where it is assumed to be introduced by human (Miras *et al.* 2009b). Its range is delimited by the availability of water, so its absence on climatic favourable areas is due to their arid character (Santos *et al.* 2002).

The viperine snake (Figure 13) is typically found in aquatic habitats, both natural and artificial ones, like rivers and lagoons or pools and decorative fountains, respectively. It can also occur in aquatic environments with brackish and salt waters. This snake can be found out of water, but always close to it (Santos 2004; Miras *et al.* 2009b).

It has diurnal habits during the spring and the autumn and nocturnal habits during the summer. Its activity period is from March to October, with some interruptions in the hotter areas and finishing earlier in the colder zones. The corporal temperature range is wide and it is rapidly adaptable to the water temperature. Indeed, if the water temperature is optimal, the viperine snake is able to remain for long periods in the water, capturing preys. The reproductive period begins on spring and the newborns are observed on August (the time can change depending on the region). Each female can do a posture of 2-32 eggs, depending on its size (Santos 2004).

The diet of *N. maura* is composed by fishes, amphibians (larvae and adults), invertebrates (oligochaeta, leeches, gastropods, insects); occasionally, it can feed on reptiles and small mammals. Normally, it has an opportunist habits, choosing the most frequent prey. The size of the prey is positively related to the size of the snake. The main predators of the viperine snake are birds of prey, birds of the family Ardeidae, the otter, some carnivores and the Montpellier snake (*M. monspessulanus*) (Santos 2004).

Figure 13: *Natrix maura* (pictures were taken from internet)

Ladder snake, *Rhinechis scalaris*

The ladder snake (Figure 14) is distributed in Portugal, Spain (absent from northernmost Spain), southern France and in the north-west of Italy. It is present also in Ons and Arousa islands off Galicia (Spain), Balearic Islands (Spain) and Illes d'Hyères (France) (Pleguezelos & Honrubia 2002; Pleguezelos *et al.* 2009a).

This species can occur in a variety of Mediterranean habitats, like woodland and scrubland, with a certain degree of vegetation cover and humidity. It can also be found in more arid areas and agricultural regions, olive groves, vineyards, stone walls and ruins (Pleguezelos *et al.* 2009a). Normally, the habitat should be rich on small mammals' communities (Pleguezelos 2006).

Its habits are crepuscular and nocturnal, being possible to found active individuals during the winter in the most southern parts of its range. The mating period occurs during the spring and the posture in July with the females depositing 4-15 eggs under ground cover (Pleguezelos 2006; Pleguezelos *et al.* 2009a) and the first newborns begin to appear in October.

The diet consists mainly on endotherms vertebrates, namely small mammals which represent 95% of its diet. It is able to climb trees, so occasionally, it predates nests of birds. The main predator of the ladder snake is the short-toed snake eagle (*Circaetus gallicus*) (Pleguezelos 2006).

Figure 14: *Rhinechis scalaris* (pictures taken from internet)

Other species

Ocellated Lizard, *Timon lepidus*

The distribution of this lizard comprises the majority of the Iberian Peninsula (absent from the humid areas of Cantabria and in the highest parts of mountains), southern France and north-western Italy (Mateo & Cheylan 1997), as well as some Iberian islands (Pleguezelos *et al.* 2009b). Currently four subspecies are recognized: *T. l. lepidus* (from coastal Portugal to the Atlantica and Mediterranean coast of France), *T. l. ibericus* (northern Spain), *T. l. oteroi* (Island of Salvora, Spain) and *T. l. nevadensis* (Baetic area, Spain) (Paulo *et al.* 2008).

The ocellated lizard (Figure 15) can be found on a diversity of Mediterranean habitats, since sandy areas in the coast and mountain areas, uncultivated and man-made habitats, to open areas with abundant refuge sites (Castilla & Bauwens 1992; Mateo 2002).

This lizard is heliothermic and diurnal and remains inactive during the winter months. The reproductive season varies with the region and the altitude, however in general the mating ritual starts in April and the mating in the second half of May. The female deposits its 7-25 eggs (depends on the female's size) in June and the first newborns are observed in end of August until the beginning of October. The reproduction is annual.

The diet is composed mainly by arthropods (beetles), snails, small mammals and plant matter (e.g. fruits) (Castilla *et al.* 1991; Hódar *et al.* 1996). The *T. lepidus* is predated by several predators like the shor-toed eagle, the Montpellier snake and the mongoose (Mateo 2004).

Figure 15: *Timon lepidus* (pictures taken from the internet)

Italian wall lizard, *Podarcis sicula*

The Italian wall lizard's (Figure 16) native range is restricted to the Italian Peninsula and Sicily. However, nowadays is introduced worldwide from United States to Turkey, representing an outstanding case of reptile species largely introduced. In the specific case of Iberian Peninsula are known five populations: Noja, Cantabria (Meijide 1981), Almería (Mertens & Wermuth 1960), Lisbon (González de la Vega *et al.* 2001), La Rioja (Valdeón *et al.* 2010) and Sant Celoni, Barcelona (Rivera *et al.* 2011) (see Silva-Rocha *et al.* 2012 for further details – Appendix IV, Figure 1). Of the multiple subspecies described, and three of them are widely accepted: *P. s. campestris* (northern half of Corsica and Italy), *P. s. cetti* (southern Corsica and Sardinia), *P. s. sicula* (Italy and Sicily).

This lizard is very eclectic in habitat use being found in natural grassy areas, roadside verges, scrublands meadows and costal dunes, but also in agro environments, inside pine plantations, and associated to parkland urban areas, stone walls and buildings (Capula 1994; Oliverio *et al.* 2001; Biaggini *et al.* 2006; Corti 2006). The use of trees and human manufactures as refuges enhances its accidental transportation.

It is diurnal and active all year, although during the winter the activity decreases and only some individuals are seen in sunny days. The reproductive season is annual and occurs during March and July with females depositing 2-12 eggs (Salvador 2006). In the native range, the species is active from February-March to October-November, although it can be observed in activity during winter on sunny days, in the southernmost portion of its range. The reproduction occurs up to two times a year, with females laying 2-5 eggs (Corti 2006).

The Italian wall lizard has an aggressive behavior and show competitive interaction with other *Podarcis* species and may cause even the extinction of their populations (Nevo *et al.* 1972; Downes & Bauwens 2002).

The diet of this lizard is omnivore, feeding on vegetables and animals, mainly insects. In Menorca Island, is predated by marten, domestic cat and the false smooth snakes *Macroprotodon* sp. (Salvador 2006).

Figure 16: *Podarcis sícula* (pictures taken by Daniele Salvi, Paolo Mazzei and puffinbytes (flikr username))

Mediterranean tree frog, *Hyla meridionalis*

The distribution of *Hyla meridionalis* comprises the Atlantic coasts of Morocco to Northern Tunisia, south-western and north-eastern Iberian Peninsula (but nor rest), South France and Nortwest Italy. It is considered introduced in Canary Islands, Menorca, Madeira and Iberian Peninsula (Recuero *et al.* 2007; Donaire-Barroso *et al.* 2009). Its distribution is fragmented in the drier parts of its range due to limited available habitat.

The Mediterranean tree frog (Figure 17) can be found on trees, shrubs, orchads, vineyards and grasses generally near to freshwater habitats. During the breeding and larvae developments, this species inhabits in ponds, irrigation ditches, temporary pools, flooded meadows, lagoons, cattle pools, wells and even swimming pools (Tejedo & Reques 2002; Donaire-Barroso *et al.* 2009). In some areas it has a sympatric distribution with *H. arborea*, producing infertile hybrids with intermediate coloration (Rosa & Oliveira 1994).

The reproductive season begins on autumn in the South and on spring in Centre and North of the Iberian Peninsula. The males initiate its approximation to the ponds, spending the days in bushes, in the nightfall they begin to sing and at night they go to the water and continue to sing. Some opportunist males do not sing, but are near the singers, in order to intercept the females attracted by the song. Generally, females are able to deposit 141-678 eggs in submerged plants which hatch 8-15 days after the posture. The metamorphosis occur 2.5-3 months later (Sillero 2008).

The diet of *H. meridionalis* depends on the development status. The larvae feed by absorption of detritus on the bottom, scraping algae attached to macrophytes, aspiring the water columns, biting the plants and pumping to the surface. The adults feed on insects. The *H. meriodinalis* is predated by several vertebrates, from which try to defend using the camouflage strategy (Sillero 2008).

Figure 17: *Hyla meridionalis* (pictures taken from internet)

1.6 Ecological Niche Modelling – Invasive Species

Prediction of species distribution through modelling has becoming an important tool in the investigation of several issues. It allows the understanding of species requirements (Austin & Meyers 1996; Hirzel et al 2002) and its biogeography (Anderson *et al.* 2002; Graham *et al.* 2004), the discovering of unknown populations and species (Raxworthy *et al.* 2003; Bourg *et al.* 2005), the identification of appropriate sites for translocation and reintroductions (Danks & Klein 2002; Peterson *et al.* 2006), the selection of areas for conservation (Williams *et al.* 1996; Pressey 1994), the test of environmental change effect on species (Sánchez-Cordero *et al.* 2005; Peterson *et al.* 2006), between other applications. In fact, Ecological Niche Models (ENM) represent a complementary approach to the genetics analysis which permit a deeper understanding on the complex dynamic of a biological invasion. Currently, the ENM are also being applied to investigate the niche conservatism or the niche shift with evolutionary purposes (Figure 18). The niche conservatism is the tendency of species to maintain ancestral ecological requirements (Wiens & Graham 2005), which is an assumption needed to researchers being able to predict invasion ranges with data from the native range (Peterson & Vieglais 2001).

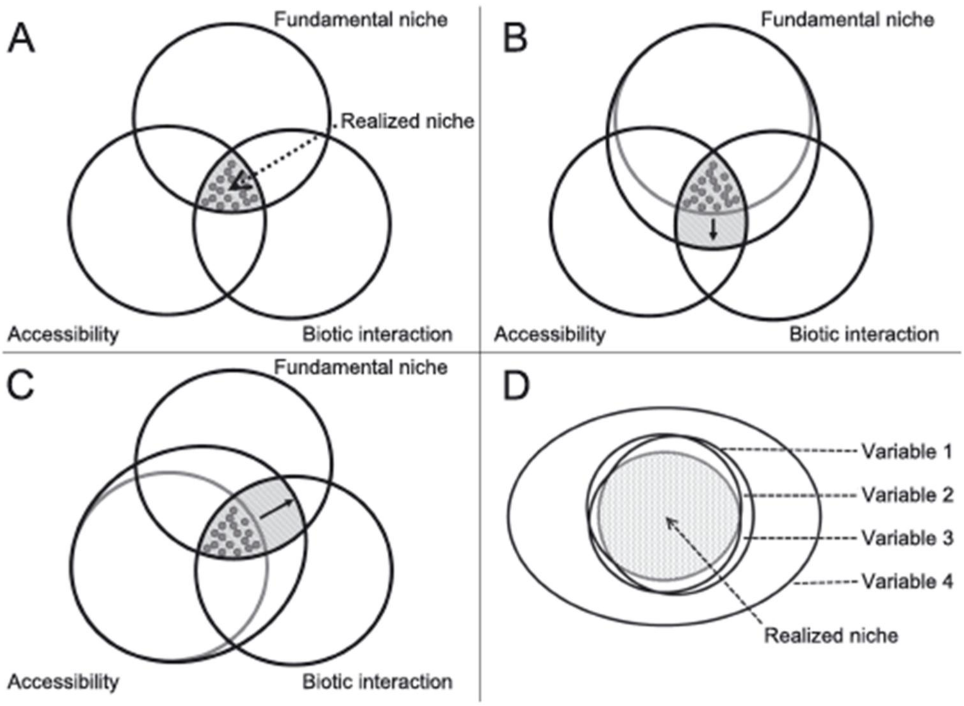

Figure 18: (A) Relationships between fundamental niche, biotic interaction and accessibility in ecological space (after Hutchinson 1957 and Soberón & Peterson 2005); the realized niche (= conditions as observed within the realized distribution) is indicated in grey and native species records as dots. (B) Increase of fundamental niche space resulting from a shift in the realized niche. (C) Better exploitation of the fundamental niche after access into new areas. (D) Different degrees of limitation of the realized niche between variables: variables 1–3 are actually limiting the realized niche, whereby the limits of the 'relaxed' variable 4 is not reached within the realized niche (Rödder & Lötters 2009). The situations B and C can be observed in the case of the invasive species.

There are four principal factors that can influence a success in an invasion: colonization opportunity which allow the species to arrive new areas (depends on human activity, wind, ocean currents and other vectors), avoidance of demographic problems of small population size (depends on stochastic events), extension of the distribution (depends on dispersal ability, movements of other species) and the suitability of the new environment to the invasive species (Peterson 2003). It is on the last factor referred that ENM are useful and applied. More specifically, they are being used to assess the areas with higher risk of invasion (Le Maitre *et al.* 2008), to target management actions to control invasive species (Peterson & Robins 2003), to evaluate if the distribution of the established species will increase in the future (Martin 1996; Zambrano *et al.* 2006) and to anticipate critical routes, arrival sites and initiation points for successful invasions (Peterson 2003).

In general, the models are based on the *realized niche* of a species' native range and can provide useful information to achieve the aims presented previously. Normally, the climate is the main variable used, since that climate is considered one of the most important drivers of environmental suitability for the alien species (Ficetola *et al.* 2010), being useful to the identification of a species potential invasive distribution (Welk *et al.* 2002; Peterson *et al.* 2003; Mau-Crimmin *et al.* 2006). Studies with the Argentine ant (*Linepithema humile*) and American bullfrog (*Lithobates catesbaianus*) demonstrate a good prediction of the suitable area in the present and in the future of the invasive ranges (Roura-Pascual *et al.* 2004; Ficetola *et al.* 2007; Giovanelli *et al.* 2008). Conversely, models based on allochthonous ranges may fail to predict the original range, indicating shifts in the original *realized niche* (Fizpatrick *et al.* 2007). However, other studies demonstrate that suitable areas based only on climate are normally very large, which make difficult to implement conservation strategies. Ficetola *et al.* (2007, 2010) proposed that integration of other features recorded at finer scale, like hunting pressure and land use, improve the performance of the predictions. These results reflect the necessity of having in accounted not only one factor (climate), but also other abiotic and biotic factors that can lead to the invasion success, in order to better understand the biological invasion complex. Besides, it is very important to select the correct predictors and variable data sets, since the bad choice can lead to wrong answers. Rödder & Lötters (2009) tested several predictors and variables for the Mediterranean house gecko (*Hemidactylus turcicus*) and achieved that a different degree of conservatism of the climatic niches is obtained for each of the data sets. It is important to notice, that ENMs *per se* are not able to distinguish between a shift on the realized niche and a shift on the fundamental niche (Schulte *et al.* 2012). Moreover, other authors suggest that reciprocal comparisons between predicted native and invaded ranges will facilitate a better understanding of the biogeography and native species (Fitzpatrick *et al.* 2007).

Furthermore, it is needed to realize which the assumptions the ENMs are based on. It is assumed that the current distribution of the species in the native range is the best indicator of its environmental requirements (Beaumont *et al.* 2009) and that genetic and phenotypic composition of the species is constant over time and space (Jeschke & Strayer 2008). However, environmental features change in time and space and some species are able to adapt and evolve in the new environment (Urban *et al.* 2008). For example, models based only on climate are assuming that the realized climatic niche will be maintained in both space and time which it was already suggested that is not happening. Broennimann *et al.* (2007) and Fitzpatrick *et al.* (2007) support the idea that invasive species are able to grow and reproduce in climates that are different from those recorded in the native range, which is not completely surprising since founder effects are expected. Another assumption is the no dispersal limitation, which means that the species can inhabit all suitable regions and nowhere else (Guisan & Zimmermann 2000; Peterson 2003; Guisan & Thuiller 2005; Jeschke & Strayer 2008), however normally species have limitations on dispersal abilities which make them to occupy only a part of the suitable regions. It is needed, then, be careful when interpreting the models.

2. Main Objectives

The main objective of this thesis is to understand the process of biological invasion of reptiles and amphibians on the Balearic Islands. To attain this general aim, we investigated the origin of the alien species by using molecular markers and we predicted their expansion processes by means of species distribution models (SDM).

The specific objective of the molecular part of the thesis was to answer these questions: (1) is the species introduced human-mediated or by natural colonization? and if so, (2) which is the origin(s) of the introduction and which was the most probable pathway? To answer these questions, we generated sequences from multiple specimens sampled in the Balearic Islands and we compared them with sequences from the autochthonous range of these species generated by previous phylogeographic studies. This procedure allows assessing whether distinct lineages occur within the islands or if identical haplotypes are found in some continental populations for each species. Besides, we integrate this information with the historical and recent trades to Balearic Islands, in order to identify possible introduction pathways. Information about the origin for the introduced populations and the pathway(s) of dispersal are crucial for developing conservation and management policies aimed at preventing negative effect on native species.

The specific objective of the modelling part of the thesis was to answer the questions (1) Are the Balearic Islands climatically suitable for the introduced species? (2) How this suitability will evolve throughout time? (3) What are the most important variables that have influence on the species distribution? To assess these issues, it was built a SDM predicting which areas are more susceptible to a successful invasion on the Balearic Islands, through the analysis of the relationships between the known species' occurrence records and the environmental variables which are correlated to the species physiology and their persistence. Specifically, models of the *realized niche* were first constructed for all the species from their records in their respective native ranges and such models were projected for the allochthonous range (the Balearic islands) to the current and to the future (years 2020, 2050 and 2080) scenarios. The current models allow representing how the species can be distributed in the islands at a short-term according to their suitable areas, while the future models report how the forecasted climate change will affect to the distributions at mid-term. Both analyses are, hence, complementary and essential for understanding of the invasion process and for developing effective conservation measures when feasible.

3. Inferring the Origin of Allochthonous Herpetofauna

3.1 Methodology

3.1.1 Sampling

The sampling area comprises four main islands of the Balearic Archipelago, namely Ibiza, Formentera, Mallorca and Menorca. Before the sampling, a survey was conducted in search of the distribution records already available in the literature and at CIBIO database in order to prioritise the areas where for which a higher sampling effort was necessary. An intensive sampling was necessary in all the islands to obtain an exhaustive tissue and occurrence data collection for allochthonous herpetofauna, and in particular in Ibiza from which were no samples and few distribution records were previously available

The sampling strategy was designed to cover all the geographic districts of each island, in order to collect individuals of all the known populations, as well as to certify that species were not present elsewhere. Tissue samples were collected both from live animals (most of lizards and amphibians) and road kills (most of snakes). For lizards and snakes a small portion of the tail tip was collected, for amphibians either a small portions of the tail or toes were collected from larvae and adults respectively.

Tissue samples and distribution records were also obtained by the collaboration with local environmental authorities and researchers, namely the Conselleria de Medio Ambiente (Ibiza), José A. Mateo (Consellería de Medi Ambient, Mallorca) , the Consorci por el la Recuperació de la Fauna de les Illes Balears (COFIB) and the Grup Balear d'Ornitologia i Defenda de la Naturalesa (GOB). The collaboration with these institutions allowed the establishment of collaboration networks across the Balearic Islands which is functional for monitoring the distribution of allochthonous species and to obtain more data and samples from future captures.

For the lizard *P. sicula*, not only the Menorcan populations were sampled, but also four populations introduced in the Iberian Peninsula in order to assess whether multiple introduction occurred in Menorca either from native range or from recently introduced populations from the Iberian Peninsula. In particular specimens of *P. sicula* from Lisbon (western Portugal, González de la Vega *et al.* 2001), Noja (Cantabria, northern Spain, Meijide 1981), Almería (southern Spain, Mertens & Wermuth 1960) and La Rioja (northern Spain, Valdeón *et al.* 2010) were collected. All Iberian localities, but La Rioja and Sant Celoni, are coastal, Lisbon and Almería being strictly urban whereas Noja is a sand dune area surrounded by urban habitat. La Rioja and Sant Celoni populations, located 100 km and 15 km inland respectively, are associated to old olive trees on sale in a plant nursery (Valdeón *et al.* 2010; Rivera *et al.* 2011).

3.1.2 Molecular Methods

DNA Extraction and Amplification

All tissues for genetic analyses were collected and then stored in pure ethanol. Total genomic DNA was extracted from tissues samples following the standard saline method (Sambrook et al. 1989). In summary, the saline method consists of adding 600 µL of lysis buffer (0.5M tris, 0.1M EDTA, 2% SDS, pH 8.0) and 15 µL of proteinase K to a small amount of tissue previously cut into small pieces and placed into a 2 mL eppendorf tubes. This permits tissue lysis and is conducted overnight at 56°C with agitation. As soon as the tissue is digested, the eppendorf tubes stay 30 minutes in the freezer. After this time, 300 µL of ammonium acetate are added, followed by centrifugation at 14000 rotations per minute (rpm) for 15 minutes, at -4°C. The resulting supernatant is transferred to a new eppendorf tube and 600 µL of cold isopropanol are added, followed by a briefly agitation and overnight in the freezer. After this time, the eppendorf tubes are centrifuged at 14000 rpm for 30 minutes at -4°C and the supernatant is despised. 1000 µL of cold ethanol are added to the pellet and the eppendorf tubes are centrifuged again at 14000 rpm for 15 minutes at -4°C. The ethanol must evaporate for a few hours at room temperature. The final step is the addition of 50 to 200 µL of ultrapure water, after what the eppendorf tubes stay hydrating overnight.

One or two fragments of mitochondrial gene were amplified for all the species. The gene fragments were selected according to those used by previous phylogeographic studies in order to compare the sequence generated for Balearic specimens with those generated in these previous studies for specimens sampled across the entire species' range. The description of the primers used, as well as the conditions used and fragment size obtained are indicated in Table 2. The target gene fragments were amplified using the Polymerase Chain Reaction method (PCR). This technique consists of 3 major steps: denaturing, annealing and extension. The first step denatures the double-strand DNA template so that the entire DNA became single-strand. This allows the oligonucleotide primers to anneal to the single-strand DNA template at specific locations (i.e. the primers' complements). The second step of the cycle involves the actual annealing of the primers to the DNA template. The third step involves the extension of the nucleotide chains in which the polymerase adds nucleotides to the 3' end of the primer sequence annealed to the template DNA. The primers are necessary for the initiation of the reaction and the DNA template acts as a reference strand for the polymerase which adds the complementary nucleotide bases starting at the position just after the 3' end of the primer sequence. The primers are incorporated into all subsequently amplified DNA templates insuring perfect priming sites in subsequent PCR cycles (Palumbi *et al.* 2002). The PCR products were purified and sequenced by an external service (by the company Macrogen® Korea).

Table 2: List of primers used for amplification and sequencing of selected gene fragments for each study species. Amplification conditions and fragment size are also reported. Cytb: Cytochrome b.

Species	**Gene**	**Primer**	**Conditions**	**Fragment size (bp)**
Rhinechis scalaris	Cytb	GluDG and Cytb2 (Palumbi 1991)	3 min 94°C; (30 s 94°C+30 s 50°C+50 s 72°C) x 35 cycles; 5 min 72°C	353
Hemorrhois hippocrepis	Cytb	Cytb1 and Cytb2 (Palumbi 1996)	3 min 94°C; (30 s 94°C+45 s 50°C+1 min 72°C) x 35 cycles; 10 min 72°C	241
	12S	12Sa and 12Sb (Kocher *et al* 1989)	90 s 94°C; (30 s 94°C+45 s 45°C+1 min 72°C) x 35 cycles; 10 min 72°C	219
Malpolon monspessulanus	Cytb	Cytb1 and Cytb2 (Palumbi 1996)	3 min 94°C; (30 s 94°C+45 s 50°C+1 min 72°C) x 35 cycles; 10 min 72°C	254
Macroprotodon sp.	Cytb	GluDG and Cytb2 (Palumbi 1991)	3 min 94°C; (30 s 94°C+30 s 51°C+50 s 72°C) x 35 cycles; 5 min 72°C	250
	12S	12Sa and 12Sb (Kocher *et al* 1989)	3 min 94°C; (30 s 94°C+30 s 50°C+30 s 72°C) x 35 cycles	311
Natrix maura	Cytb	L14724 (Guicking *et al* 2002) and H16064 (Brubrink *et al* 2000)	3 min 94°C; (30 s 94°C+30 s 50°C+50 s 72°C) x 35 cycles; 5 min 72°C	961
Timon lepidus	Cytb	CytbF (Kocher *et al.* 1989) and CytbR (Moritz *et al.* 1992)	3 min 94°C-, (30 s 94°C+30 s 50°C+50 s 72°C) x 35 cycles; 5 min 72°C	547
Podarcis sicula	Cytb	GluDG-A and cb3H (Palumbi 1991)	3 min 94°C; (30 s 94°C+30 s 51°C+50 s 72°C) x 35 cycles; 5 min 72°C	684
Hyla meridionalis	COI	Amp-P3F and Amp-P3R (San Mauro *et al.* 2004)	3 min 94°C; (1 min 94°C+1 min 42°C+1 min 72°C) x 35 cycles; 5 min 72°C	785

Sequence data and Phylogenetic analysis

We obtained from GenBank the mitochondrial sequences generated from native range of each species, as well as the outgroup sequences, by the following studies:

- *Hemorrhois hippocrepis*: Carranza *et al*. (2006)
- *Malpolon monspessulanus*: Carranza *et al*. (2006)
- *Macroprotodon* sp.: Carranza *et al*. (2004); Vasconcelos & Harris (2006)
- *Natrix maura*: Guicking *et al*. (2008)
- *Rhinechis scalaris*: Nulchis *et al*. (2008)
- *Timon lepidus*: Paulo *et al*. (2008), Miraldo *et al*. (2011), Miraldo *et al*. (2012)
- *Podarcis sicula*: Podnar *et al*. (2005)
- *Hyla meridionalis*: Recuero *et al*. (2007)

For the snake *R. scalaris,* other than analysing introduce populations we also performed a range-wide phylogeographic assessment (Localities in Appendix I), since the assessment of the genetic diversity by Nulchis *et al.* (2008) was based on only 12 samples.

In the table 3, each gene fragment the accession number of the sequences used are reported for each species.

Table 3: Accession number of sequences used for each species and each fragment gene.

Species	Gene fragment	Accession number
Hemorrhois hippocrepis	Cytb	DQ451952-DQ451987/AY643392
	12S	DQ451988-.DQ452006/AY643308
Malpolon monspessulanus	Cyb	DQ451880-DQ451925/AY643396
Macroprotodon sp.	Cytb	AY643358-AY643398/DQ324857-DQ324862
	12S	AY643278-AY643314
Natrix maura	Cytb	AY487681-AY487722
Rhinechis scalaris	Cytb	EU497634
Timon lepidus	Cytb	AF379000/AF379003/AF379006-AF379012/GQ142119/DQ902142/DQ902140/DQ902139/JN860936-JN860973/JN860975-JN860995/AF3789968-AF3789984/AF378991-AF378999
Podarcis sicula	Cytb	AY185095/AY185094/AY770869-AY770905
Hyla meridionalis	COI	DQ996400-DQ996457

The sequences generated in this study were checked by analyzing the electropherograms and then aligned with those gathered from GenBank using ClustalW (Larkin *et al.* 2007). For the species for which two markers were sequenced, we generated three different alignments: one for each marker separately and another with the information concatenated. All the sequences generated in this thesis have been deposited in GenBank and accession numbers release is under process.

Multiple sequences alignments were analysed using the Maximum Likelihood (ML) method to infer the relationships between the Balearic samples and those from the native range of each species (also including other allochthonous populations in the case of *Podarcis sicula*). Maximum likelihood method calculates the 'most likely tree' as the tree that has the highest probability of producing the observed data according to a specific model of nucleotide change (model of evolution) (Strimmer 1997; Felsenstein 2004).

Table 4: Model of sequence evolution selected for each dataset under the Bayesian Information Criterion . K2: Kimura-2 parameter; HKY: Hasegawa- Kishino-Yano; Tn93: Tamura-Nei; GTR: Generalized time-reversible; +G: Gamma distributed

Species	**Gene**	**Best Model**
Hemorrhois hippocrepis	12S	K2 (Kamura 1980)
	Cytb	HKY (Hasegawa *et al.* 1985)
	12S+Cytb	HKY (Hasegawa *et al.* 1985)
Malpolon monpessulanus	Cytb	HKY+G (Hasegawa *et al.* 1985)
***Macroprotodon* sp.**	12S	TN93 (Tamura & Nei 1993)
	Cytb	HKY+G (Hasegawa *et al.* 1985)
	12S+Cytb	GTR+G (Tavaré 1986)
Natrix maura	Cytb	TN93+G (Tamura & Nei 1993)
Timon lepidus	Cytb	HKY+G (Hasegawa *et al.* 1985)
Podarcis sicula	Cytb	HKY+G (Hasegawa *et al.* 1985)

The best model of sequence evolution was estimated by Mega 5 (Tamura *et al.* 2011) under the Bayesian Information Criterion (BIC) (Table 4). The ML analyses were performed in Mega 5 with the heuristic search mode and the node support was calculated over 1000 bootstrap replicates. In addition to the tree-building method, we analysed the genealogical relationships among native and non-native haplotypes by means of a statistical parsimony network using the program TCS 1.21 (Clement *et al.* 2000). This program collapses sequences into haplotypes and calculates the frequencies of the haplotypes in the sample. These frequencies are used to estimate haplotype outgroup probabilities which correlate with haplotype age. After that, the program calculates an absolute distance matrix for all pairwise comparisons of haplotypes. The probability of parsimony is calculated for pairwise differences until the probability exceeds 0.95, which means that the number of mutational differences associated with the probability just before this 95% cut-off is the maximum number of mutational connections between pairs of sequences justified by the parsimony criterion. These justified connections are then made resulting in a 95% set of plausible solution (Clement *et al.* 2000).Parsimony-based methods seek the topology that minimizes the number of inferred evolutionary changes.

3.2 Results

In this section, firstly is presented an overview of the samples obtained for each species and used in the molecular analyses, followed by two sections where the results of the molecular analyses are reported species by species. These results are presented in two subsections: the 'Snakes' section, including results for the four snake species, and the 'Other species' section including results for the two lizards and the amphibian.

3.2.1 Sampling data

A total of 30 samples of *H. hippocrepis* were collected, 25 from Mallorca and five from Ibiza (Table 5). For *M. monspessulanus* and *T. lepidus,* single samples were collected in Mallorca. For *Macroprotodon sp.,* five samples were collected, four from Mallorca and one from Menorca. The five samples of the viperine snake were all collected from Mallorca. For *R. scalaris*, a total of 47 samples were analysed, 43 from the native range (Appendix I) and four from the Balearic Islands. A total of 23 samples of *P. sicula* were collected, 16 from four Iberian localities and seven from Menorca. The number of specimens analysed for each species and the locality of the samples are reported in Table 5. All samples from the Balearic Islands sampled are with the code DB in the ML trees and stastitical parsimony networks.

Table 5: Locality and sample size for each species of this study.

Species	**Locality**	**Province State**	**Country**	**Sample size**	
Hemorrhois hippocrepis	Calvia (Magaluf)	Mallorca	Spain	2	25
	Capdepera			16	
	Magaluf			1	
	Sineu			1	
	Unknown			3	
	Artà			2	
	Can Toni Sa Rota	Ibiza	Spain	1	5
	Sant Joan de Labritja			2	
	Santa Eulària			2	
Malpolon monspessulanus	Near Acudia	Mallorca	Spain	1	
***Macroprotodon* sp.**	Carrer de la Cala Antena, Manacor	Mallorca	Spain	1	5
	Santa Eugènia			1	
	Palma			1	
	Unknown			1	
	Cabo de Cavalleria	Menorca	Spain	1	
Natrix maura	Coco d'es Bov	Mallorca	Spain	1	5
	Pollença			4	
Rhinechis scalaris	Sant Antoni de Portmany	Ibiza	Spain	1	4
	San Joan	Mallorca	Spain	1	
	Unknown	Menorca	Spain	2	
Timon lepidus	Palma de Mallorca	Mallorca	Spain	1	
Podarcis sicula	Parque de Nicolás Salméron	Almería	Spain	2	23
	Playa del Ris (Noja)	Cantrabria		7	
	Alfaro	La Rioja		2	
	Gardens of Expo	Lisboa	Portugal	5	
	Es Mercadal	Menorca	Spain	2	
	Ferreries			3	
	Ciutadella			2	
Hyla meridionalis	Lithica quarry	Menorca	Spain	3	9
	Curniola			6	

3.2.2 Snakes

Horseshoe whip snake, *Hemorrhois hippocrepis*

The cyt b data set includes 61 sequences with 241 base pairs. Two different haplotypes were identified among the Balearic samples, corresponding to those previously found in the native range by Carranza *et al.* (2006). The 12S alignment includes 37 sequences with 219 base pairs. One single haplotype was identified among the Balearic samples, corresponding to those previously found in the native range by Carranza *et al.* (2006). The combined cytb+12S data set includes 31 sequences of 459 base pairs (241 bp of cytb and 219 bp of 12S). Similarly to the cyt b data set, two different haplotypes were identified among the Balearic samples, corresponding to those already found in the native range by Carranza *et al.* (2006). Distribution of the haplotypes from the native and introduced snakes are depicted on Figure 19.

In all the ML trees (based on cyt b, 12S and on the combined data set, Figures A1 and A2 from Appendix II, and Figure 20, respectively), snakes from Mallorca and Ibiza are related to those from Spain and Morocco. In the trees based on cyt b and on the combined data set, Mallorcan haplotypes, cluster in two different haploclades, one composed by samples from Spain and the other by samples from Spain and Morocco.

The network analysis of the combined data set shows one distinct network (Figure 21). The maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 9. The relationships between haplotypes as depicted by the network corroborate the results from the ML analysis. Mallorcan snakes cluster in two different groups, one with only Spanish samples and the other one including Spanish and Moroccan samples. Ibiza snakes cluster with the group of Iberian and Moroccan samples. Phylogenetic trees and networks based on the 12S and the cytb data sets are reported in the Appendix II.

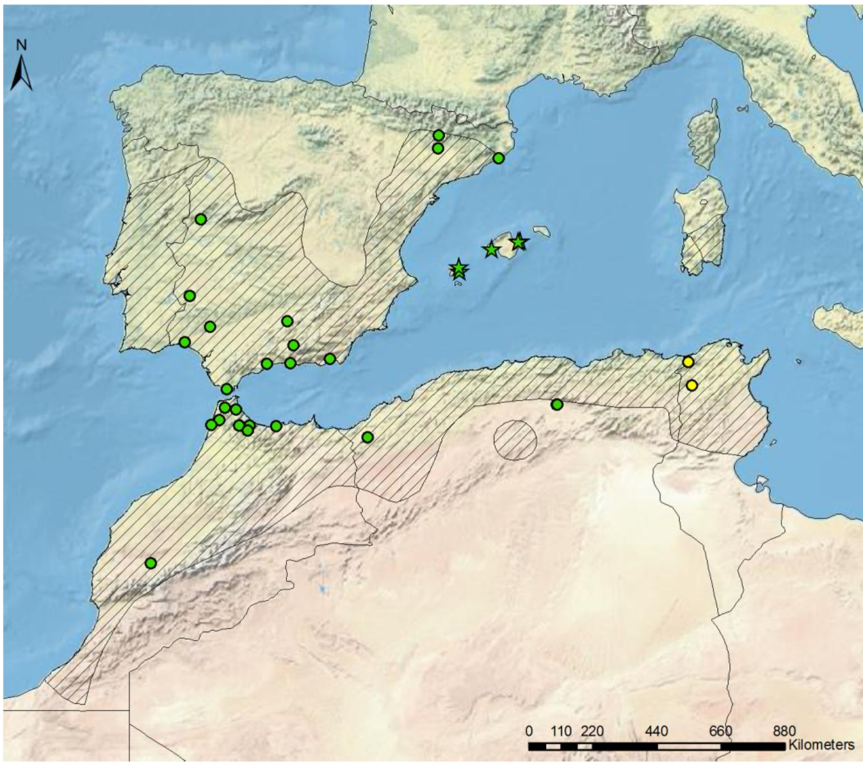

Figure 19: Haplotypes distribution in the native and introduced populations of *H. hippocrepis*. Circles: samples from native range (Carranza *et al.* 2006); stars: samples from introduced populations. Native range is indicated by the shade area. Circle are coloured according to the clades found in the parsimony network (Figure 21) and in the ML tree (Figure 20).

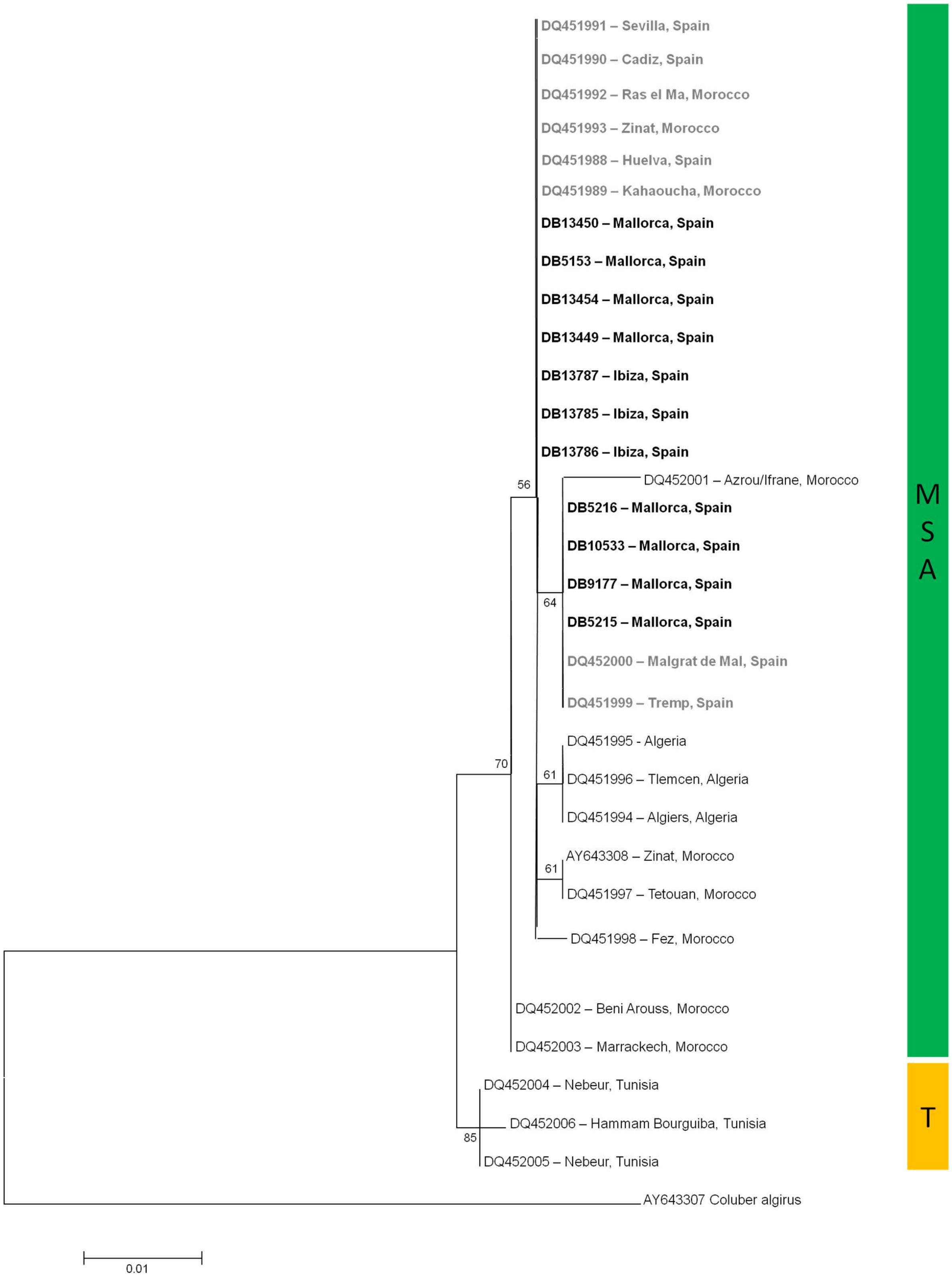

Figure 20: ML tree based on of the combined data set (cytb+12S) depicting the relationships between haplotypes of the native range of *H. hippocrepis* from Carranza *et al.* (2006) and those from the introduced populations from Mallorca and Ibiza Islands. Numbers on branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported). MSA: Morocco+Iberia+Algeria; T: Tunisia.

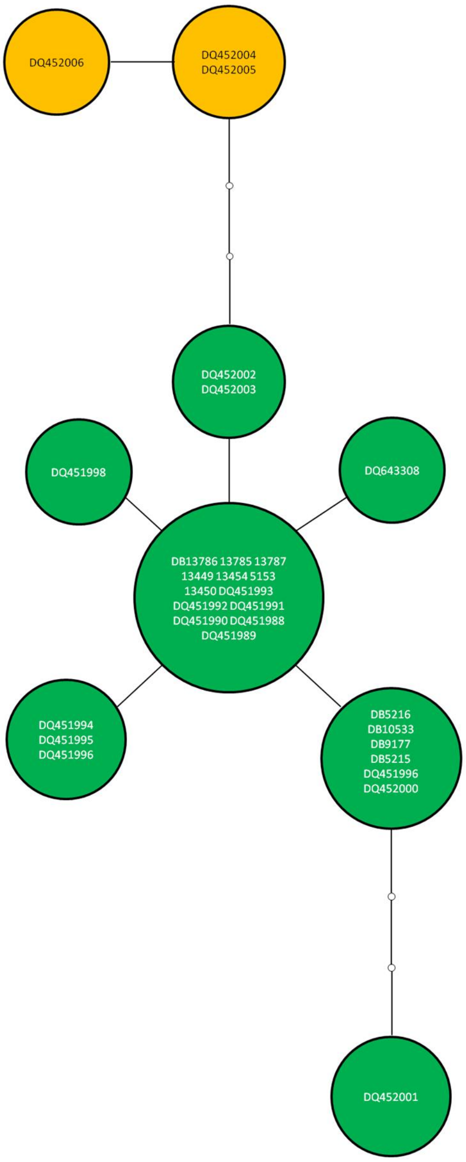

Figure 21: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *H. hippocrepis* populations. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes.

Montpellier snake, *Malpolon monspessulanus*

The cyt b alignment includes 48 sequences with 254 base pairs. A total of 14 haplotypes were identified, 13 of them corresponding to haplotypes previously found in samples from Carranza *et al.* (2006) and one corresponding to the Mallorcan sample. Distribution of the haplotypes from the native and introduced snakes is depicted on Figure 22.

The ML analysis (Figure 23) shows two main clades: one that includes Moroccon and Iberian Peninsula samples; other that includes Tunisian, Cyprian and Egyptian and Greek samples. The sample from the Balearic Island clusters together with those from Morocco and Iberian Peninsula group, but it is slightly different from them. The partition between haploclades correspondent to the two subspecies *M. m. insignitus* and *M. m. fuscus* is not well supported in the tree; nevertheless, the haploclade correspondent to *M. m. monspessulanus,* in which Balearic sample is included, has good bootstrap value (89). The network analysis (Figure 24) shows three distinct networks and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be six. The relationships between haplotypes as revealed by the networks corroborate the results from ML analysis. Each network corresponds to a currently recognised subspecies with Mallorca samples clustering with those from *M. m. monspessulanus* distributed in Morocco and Spain.

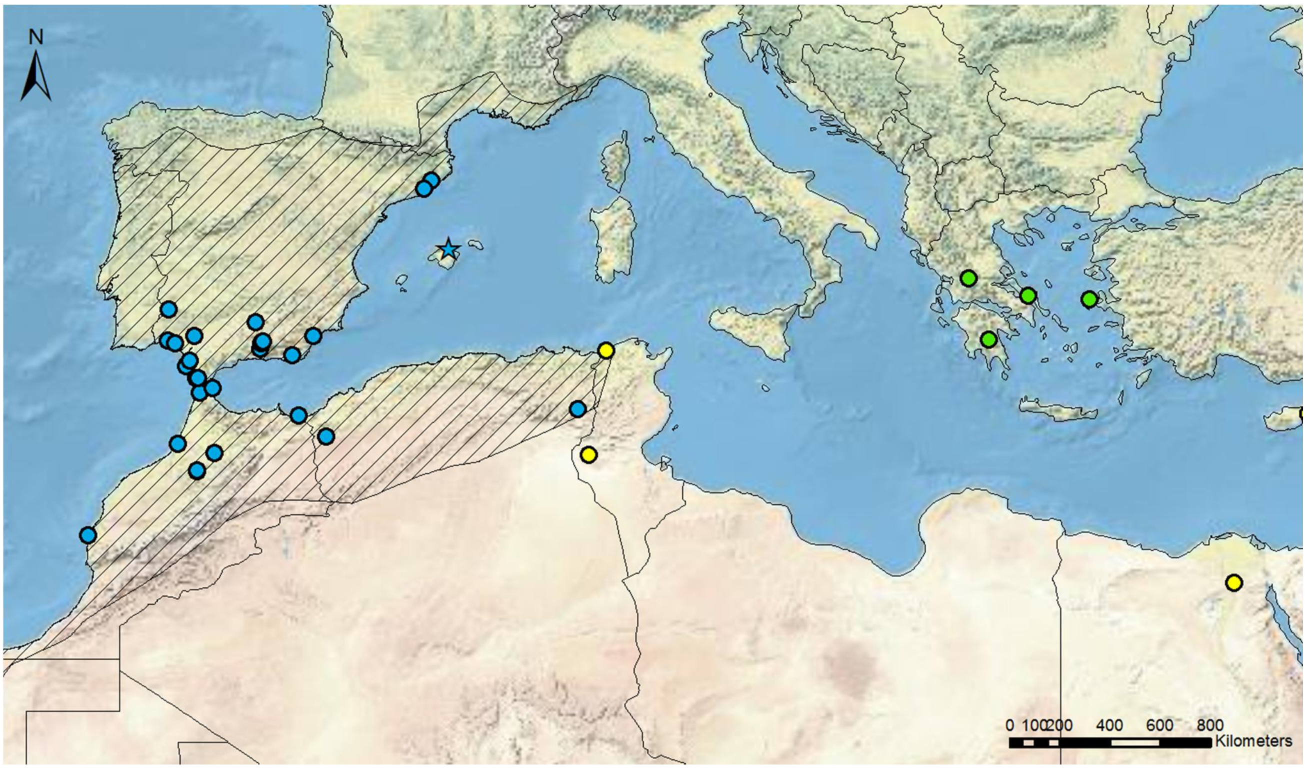

Figure 22: Haplotypes distribution in the native and introduced populations of *M. monspessulanus*. Circles: samples from native range (Carranza *et al.* 2006); stars: samples from introduced populations. Native range is indicated by the shade area. Circle are coloured according to the clades found in the parsimony network (Figure 24) and in the ML tree (Figure 23).

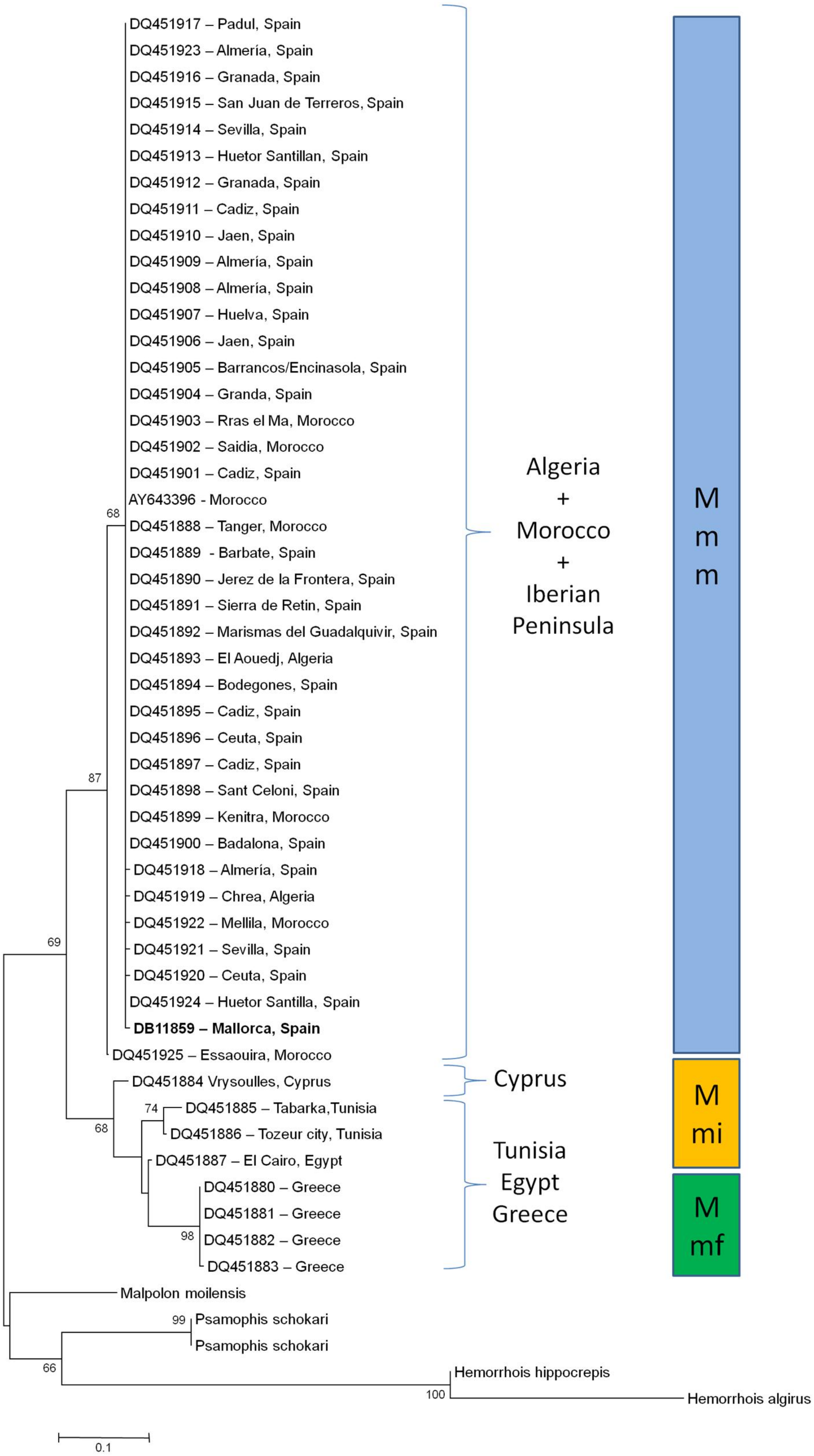

Figure 23: ML tree based on of the cytochrome b data set depicting the relationships between haplotypes of native range of *M. monspessulanus* from Carranza *et al.* (2006) and those from the introduced populations from Mallorca Island. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported). Mmm: *M. m. monspessulanus*; Mmi: *M. m. insignatus*; Mmf: *M. m. fuscus*.

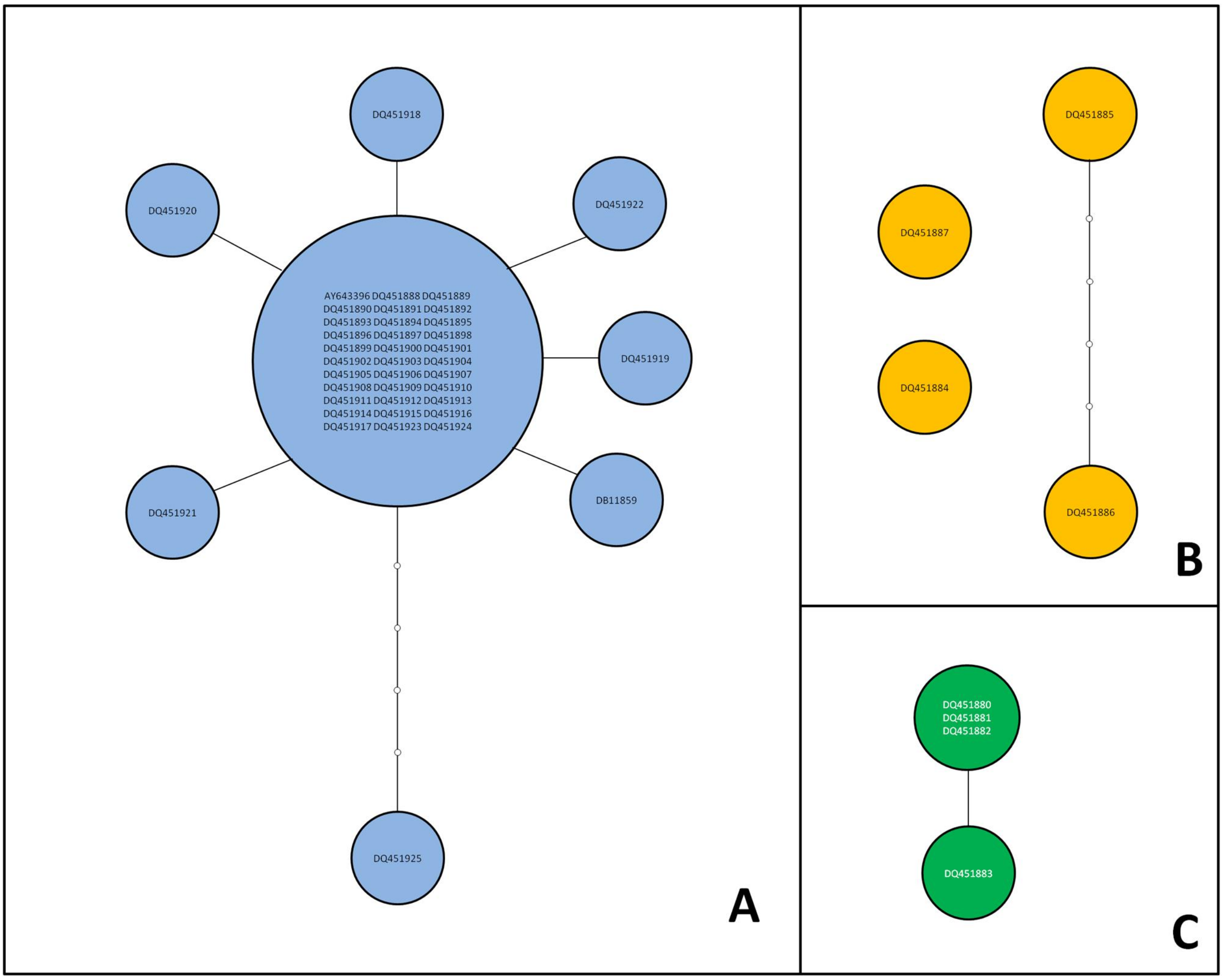

Figure 24: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *M. monspessulanus*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes. A: *M. m. monspessulanus*; B: *M. m. insignitus*; C: *M. m. fuscus*

False smooth snake, *Macroprotodon sp.*

The data set for cyt b gene includes 38 sequences with 250 base pairs. One single haplotype was identified among the Balearic samples, corresponding to those previously found in the native range by Carranza *et al*. (2004). The 12S gene alignment includes 40 sequences with 311 base pairs. A single haplotype was identified among the Balearic samples, corresponding to the Mallorcan haplotype previously found by Carranza *et al.* (2004). The final alignment of the combined data set includes 34 sequences of 561 base pairs (250 bp of cyt b and 311 bp of 12S). One single haplotype was identified among the Mallorcan and Menorcan samples, corresponding to the Mallorcan haplotype already found by Carranza *et al.* (2004). Distribution of the haplotypes from the native and introduced snakes is depicted on Figure 25.

The ML trees based on the combined data set and on the 12S data set show five distinct clades, corresponding to the five previously described for the genus *Macroprotodon* (Carranza *et al.* 2004). The analysis of cyt b data set (Figure 26) allows the distinction of one more clade that includes the Libyan sample. In all the analyses, samples from Mallorca and Menorca Islands cluster within the *Macroprotodon mauritanicus* clade. The network analysis based on cyt b (Figure 27) haplotypes shows five distinct sub-networks and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 6. The relationships between haplotypes as revealed by the networks corroborate the results from ML analysis.

ML trees and networks from 12S data set and combined data set are at the Appendix III.

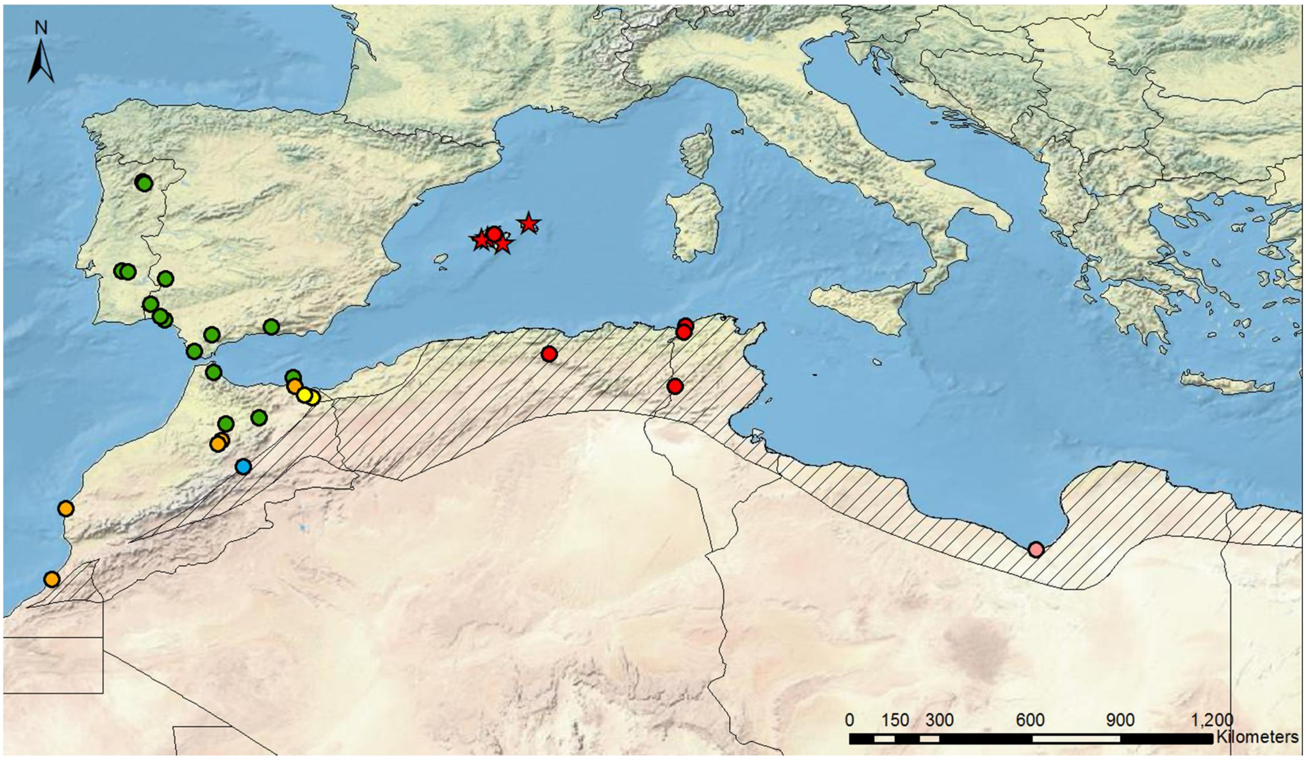

Figure 25: Haplotypes distribution in the native and introduced populations of *Macroprotodon* sp. Circles: samples from native range (Carranza *et al.* 2004); stars: samples from introduced populations. Native range is indicated by the shade area, according to Carranza *et al.* (2004) and Wade (2001). Circle are coloured according to the clades found in the parsimony network (Figure 27) and in the ML tree (Figure 26).

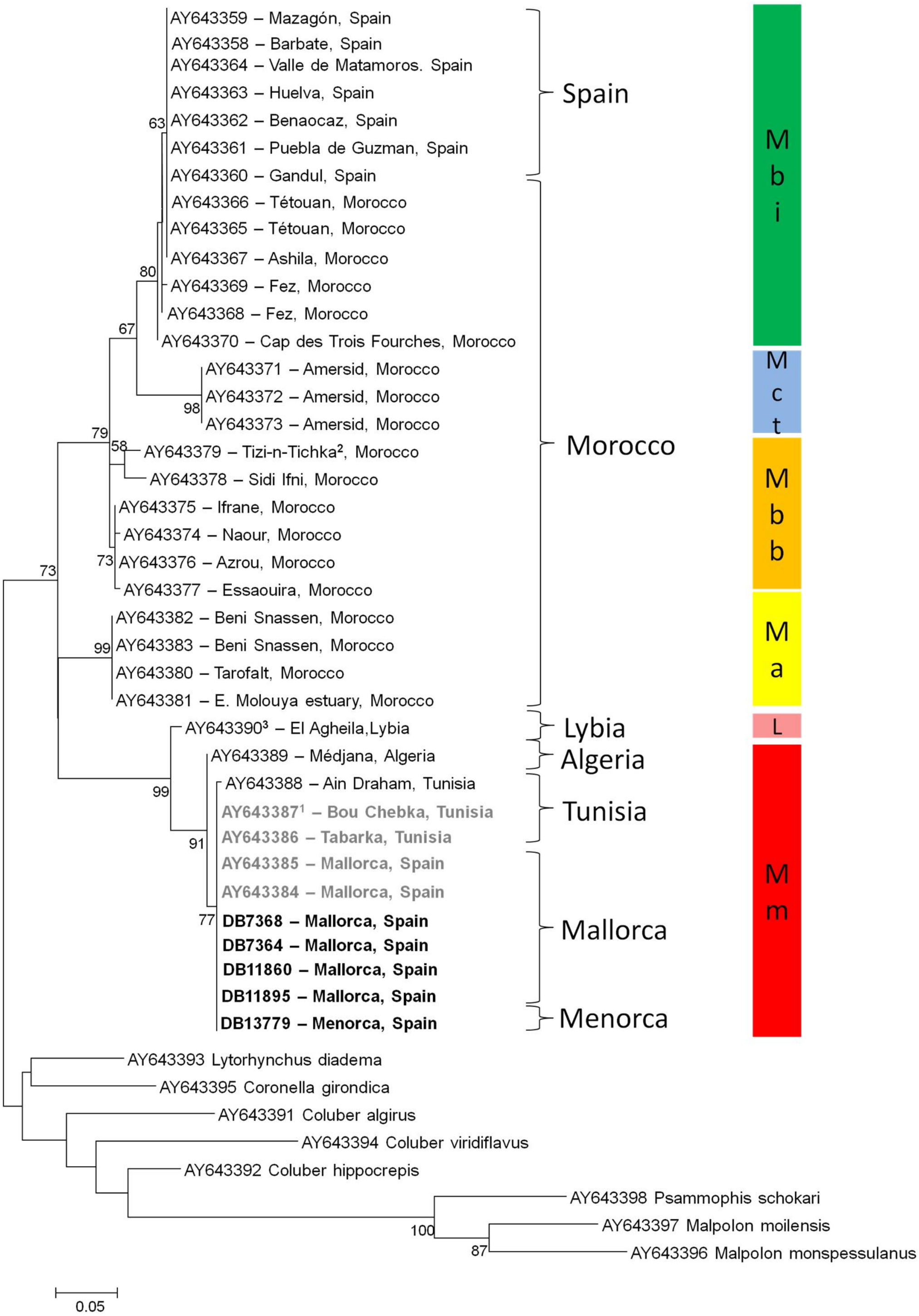

Figure 26: ML tree based on of the cytochrome b data set depicting the relationships between haplotypes of native range of *Macroprotodon sp.* from Carranza *et al.* (2004) and those from the introduced populations from Mallorca and Menorca Islands. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported). Mbi: *M. brevis ibericus*; Mct: *M. cucullatus textilis*; Mbb: *M. brevis brevis*; Ma: *M. abubakeri*; L – Lybian clade; Mm: *M. mauritanicus.* [1] Classified as *Macroprotodon cucullatus textilis* at Natural History Museum of London, however its distribution suggest that it belongs to *Macroprotodon mauritanicus* species (Wade, 2001); [2] Classified as *Macroprotodon cucullatus textilis* at Département de Biologie of Université Abdelmalek Essadi (Tétouan, Morocco), however its distribution suggest that it belongs to *Macroprotodon brevis brevis* species (Wade, 2001); [3] Classified as *Macroprotodon cucullatus cucullatus* at Natural History Museum of London, however its distribution suggest that it belongs to *Macroprotodon mauritanicus* species (Wade, 2001).

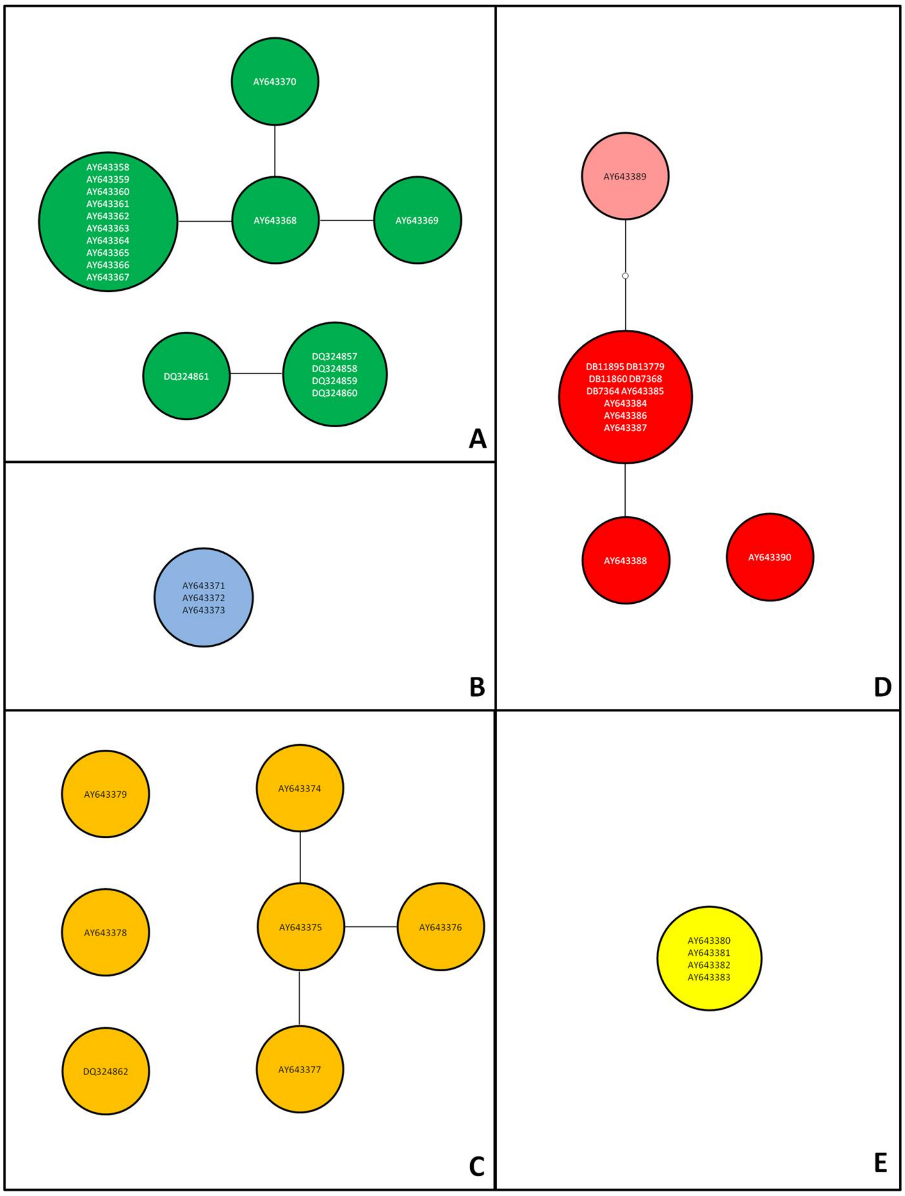

Figure 27: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *Macroprotodon sp.*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes. A: *M. brevis ibericus*; B: *M. cucullatus textilis*; C: *M. brevis brevis*; D: *M. mauritanicus*; E: *M. abubekeri.*

Viperine snake, *Natrix maura*

The alignment of COI gene includes 49 sequences with 961 base pairs. A single haplotype was found among the Balearic samples, corresponding to haplotypes previously found in samples from native range by Guicking *et al.* (2008). Distribution of the haplotypes from the native and introduced snakes is depicted on Figure 28.

ML tree (Figure 29) reveals five distinct haploclades: three 'European' (E1, E2, and E3), one 'Tunisian' and one 'Moroccan'. Mallorcan samples cluster in the 'E1' (European 1) clade represented with samples from Spain, Portugal and France. The network analysis shows three distinct networks and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 6 (Figures 30-31). The relationships between haplotypes as depicted by the networks corroborate the results from ML analysis.

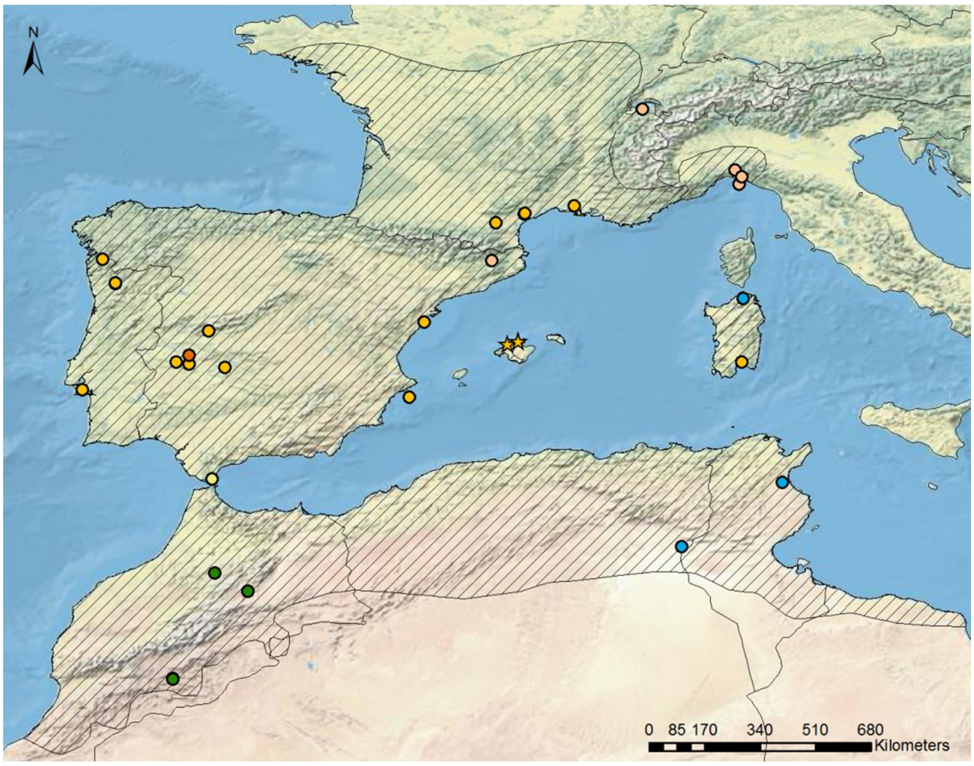

Figure 28: Haplotypes distribution in the native and introduced populations of *N. maura*. Circles: samples from native range (Guicking *et al.* 2008); stars: samples from introduced populations. Native range is indicated by the shade area. Circle are coloured according to the clades found in the parsimony network (Figures 30-31) and in the ML tree (Figure 29).

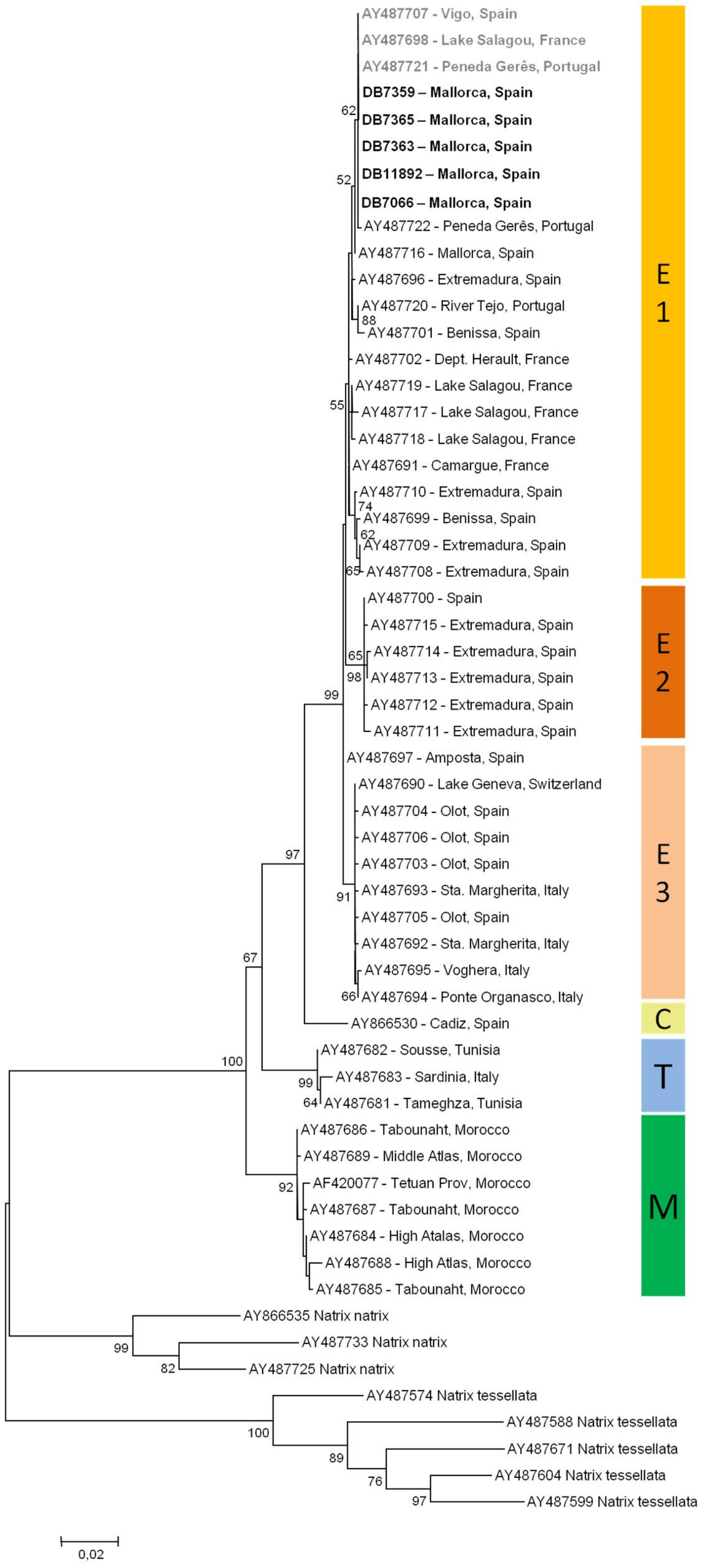

Figure 29: ML tree based on of the cytochrome b data set depicting the relationships between haplotypes of native range of *N. maura.* From Guicking *et al.* (2008) and those from the introduced populations from Mallorca Island.. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported). E1: European 1; E2: European 2; E3: European 3; C: Cádiz ; T: Tunisia; M: Morocco.

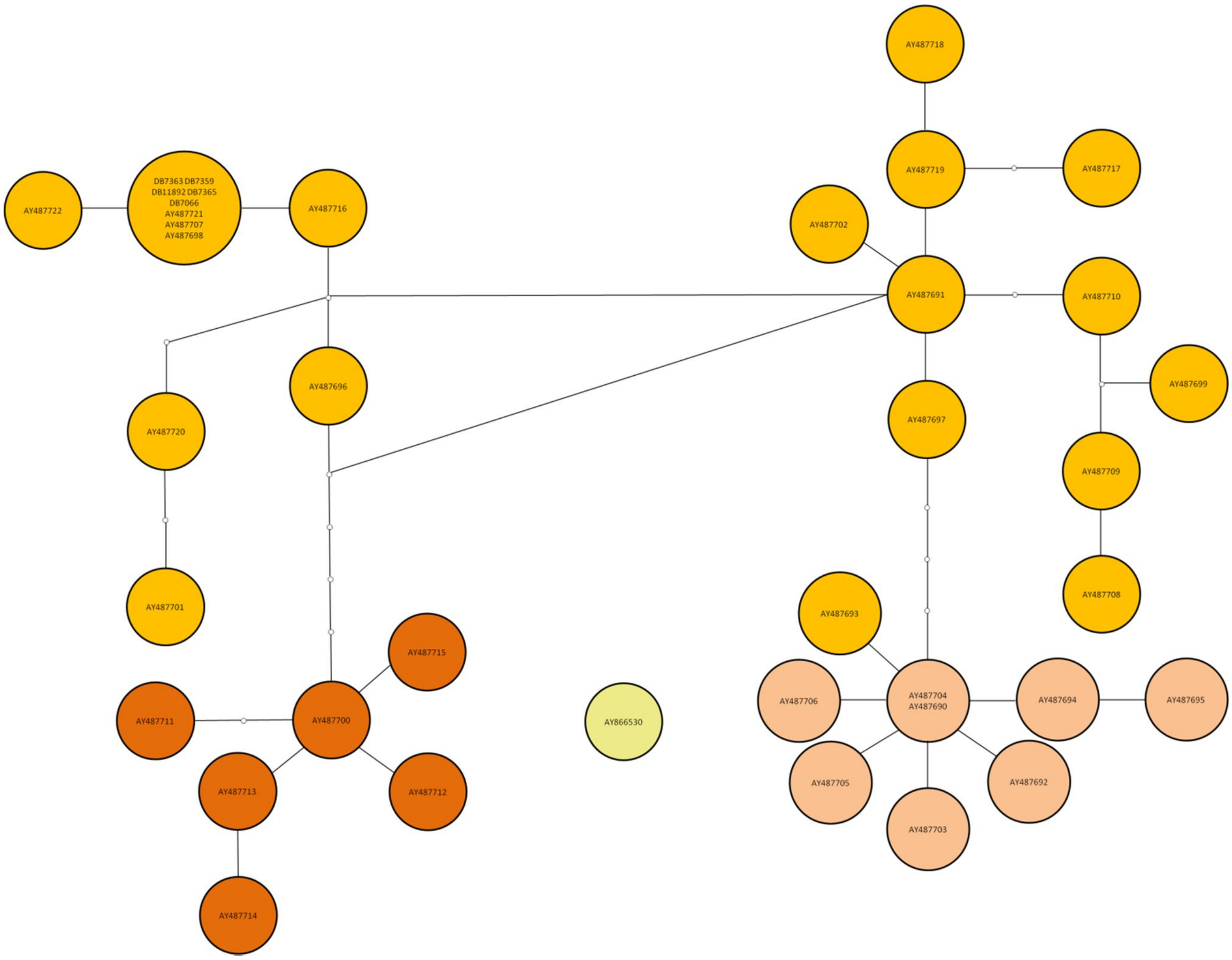

Figure 30: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *N. maura*. Represented only European and Cádiz haploclades. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes.

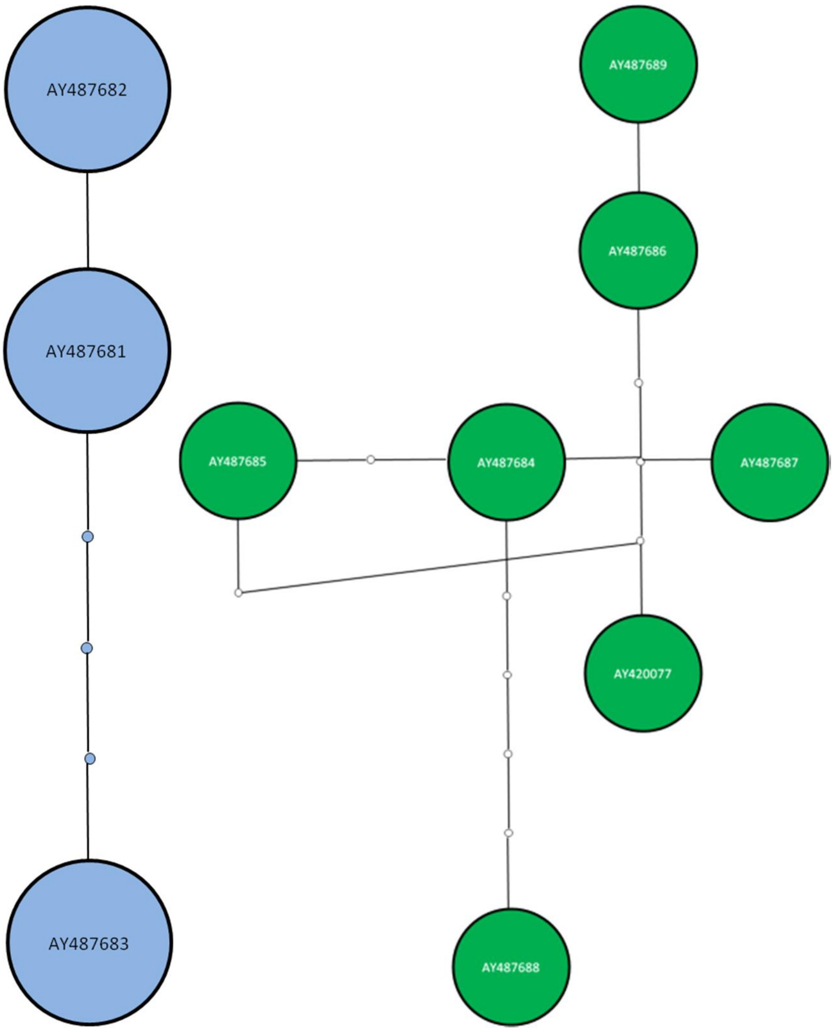

Figure 31: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *N. maura*. Represented only Tunisian (blue) and Moroccan (green) haploclades. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes.

Ladder snake, *Rhinechis scalaris*

The alignment of cyt b gene comprises 52 sequences with 353 base pairs. Three haplotypes were found, one haplotype corresponding to the commonest haplotype widespred across the species' range identified by Nulchis et al (2008) and two haplotypes occurring in southern Spain. The haplotype found in the Balearic samples is the commonest haplotype occurring in the native range. Distribution of the haplotypes from the native and introduced snakes is depicted on Figure 32.

A ML analysis was not performed for this species, due the low genetic differentiation between the three haplotypes found (≤ 2 nucleotide positions) in the species' range. The network analysis of cyt b shows one distinct network and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 8. The network obtained revealed a very low differentiation within the native range and no differentiation between native and introduced populations (Figure 33).

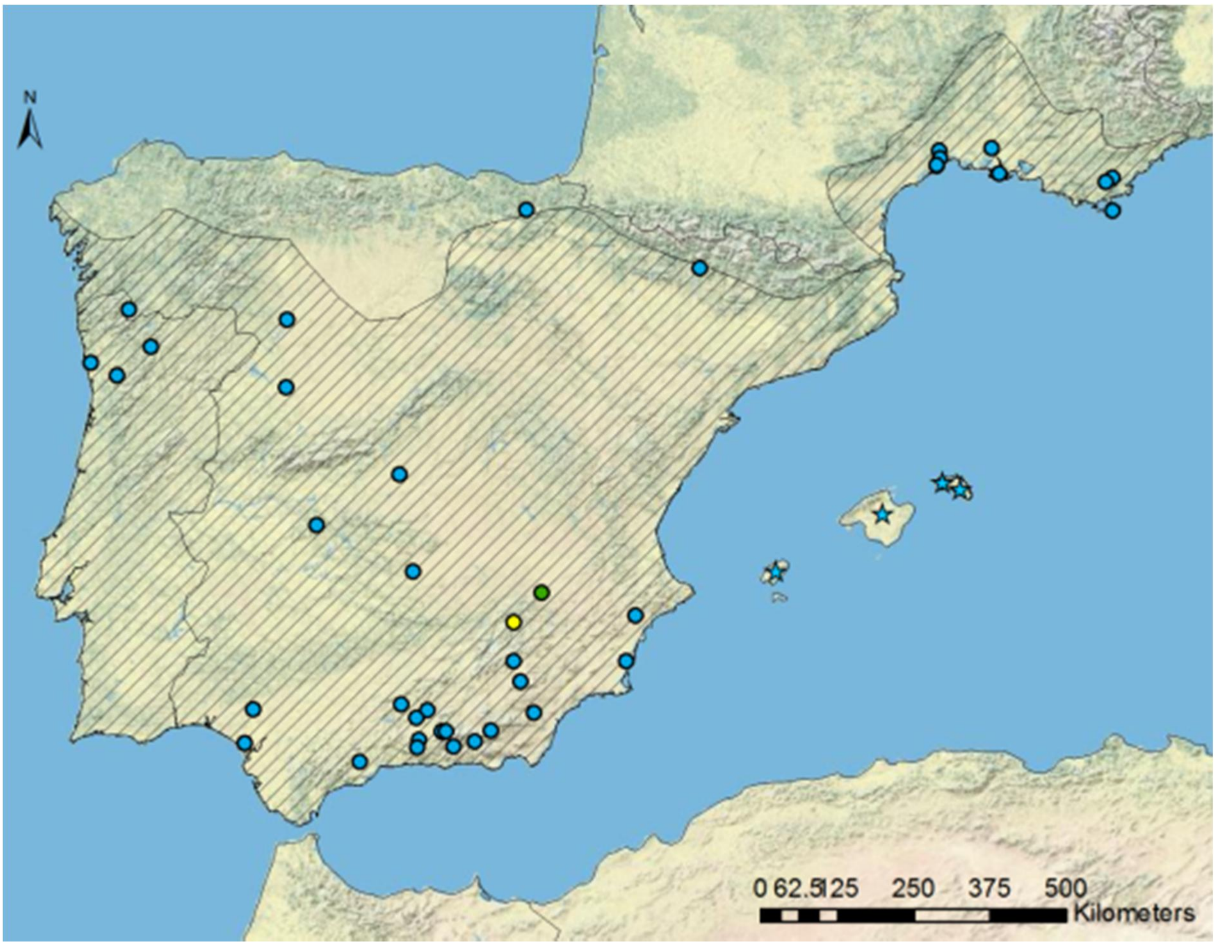

Figure 32: Cytochrome b haplotypes distribution in the native and introduced populations of *R. scalaris*. Circles: samples from native range; stars: samples from introduced populations. Native range is indicated by the shade area. Circle are coloured according to the clades found in the parsimony network (Figure 33).

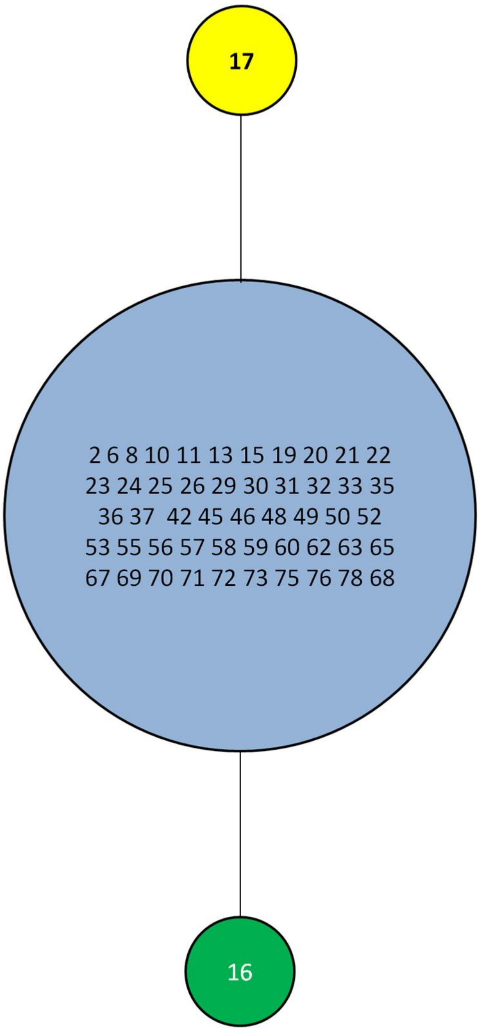

Figure 33: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *R.scalaris*. See Appendix I and Table 5 for localites codes and geographic details.

3.2.3. Other species

Ocellated lizard, *Timon lepidus*

The alignment of cytochrome b gene comprises 101 sequences with 547 base pairs. The haplotype found in the Mallorcan samples correspond to one haplotype previously found in samples from the native range by Paulo *et al.* (2008). Distribution of the haplotypes from the native and introduced snakes is depicted on Figure 34.

The ML tree from cyt b (Figure 35) analysis does not allow the distinction between the subspecies *T. l. lepidus* and *T. l. ibericus*, while the distinction between these two and *T. l. nevadensis* was clear. The sample from Mallorca belongs to the *T. l. nevadensis* clade. The network analysis shows 2 distinct networks and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 10. The relationships between haplotypes as revealed by the networks corroborate the results from ML analysis. In the fig. 36, the network of the *T. l. nevadensis* haploclade, where the sample from Mallorca is included, is presented.

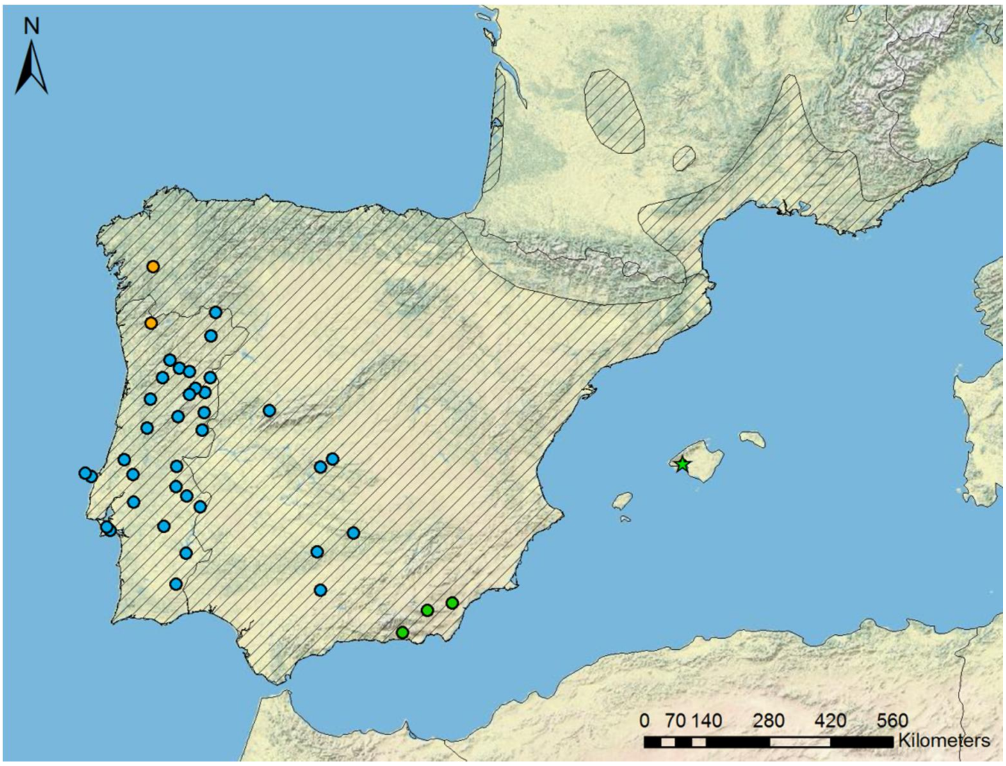

Figure 34: Haplotypes distribution in the native and introduced population of *T. lepidus.* Circles: samples from native range; stars: samples from introduced population. Native range is indicated by the shade area. Circle are coloured according to the clades found in the parsimony network (Figure 36) and in the ML tree (Figure 35).

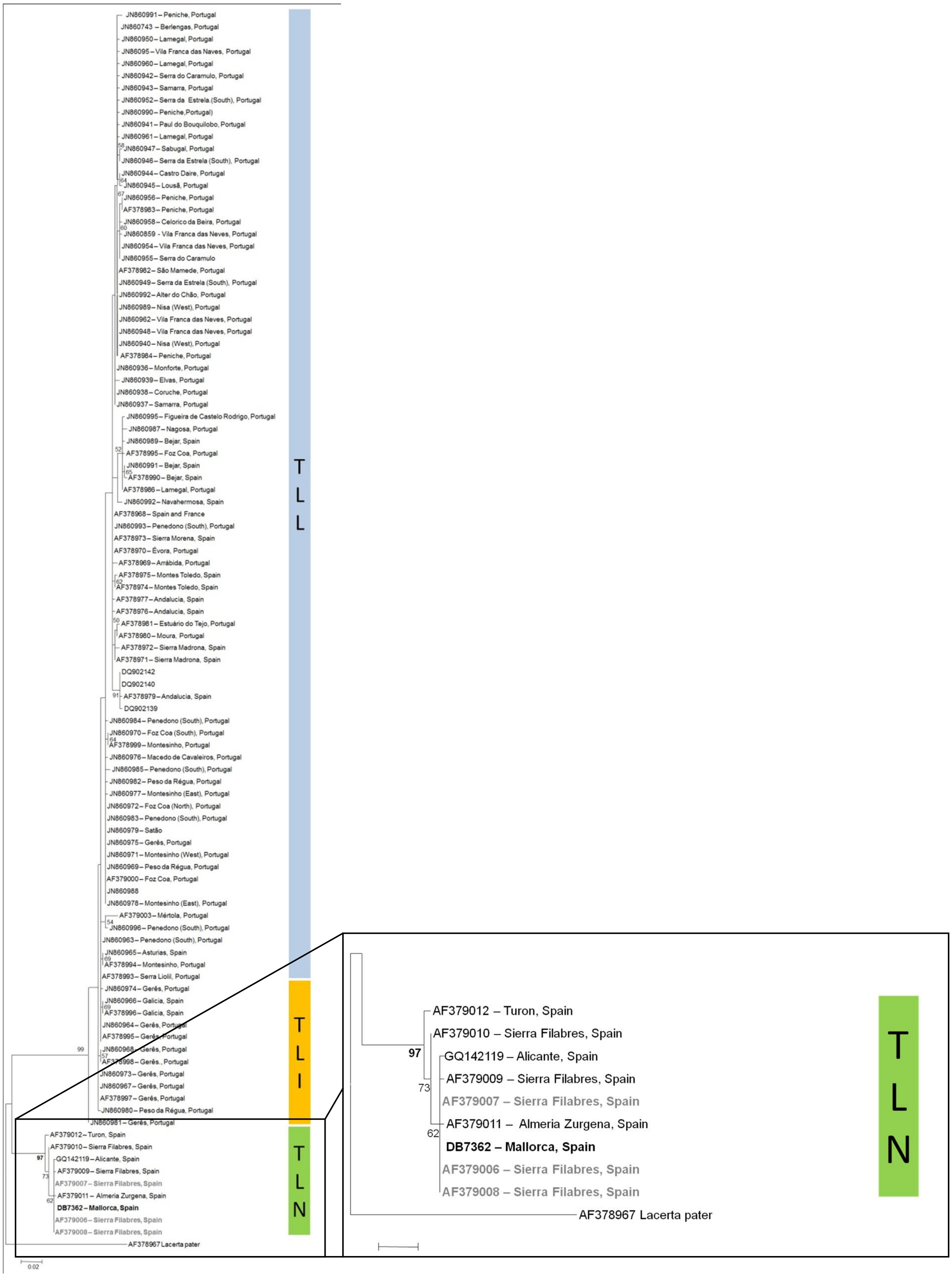

Figure 35: ML tree based on of the cytochrome b data set depicting the relationships between haplotypes of native range of *T. lepidus.* Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported). TLL: *T. l.lepidus*, TLI: *T. l. ibericus*; TLN: *T. l. nevadensis*.

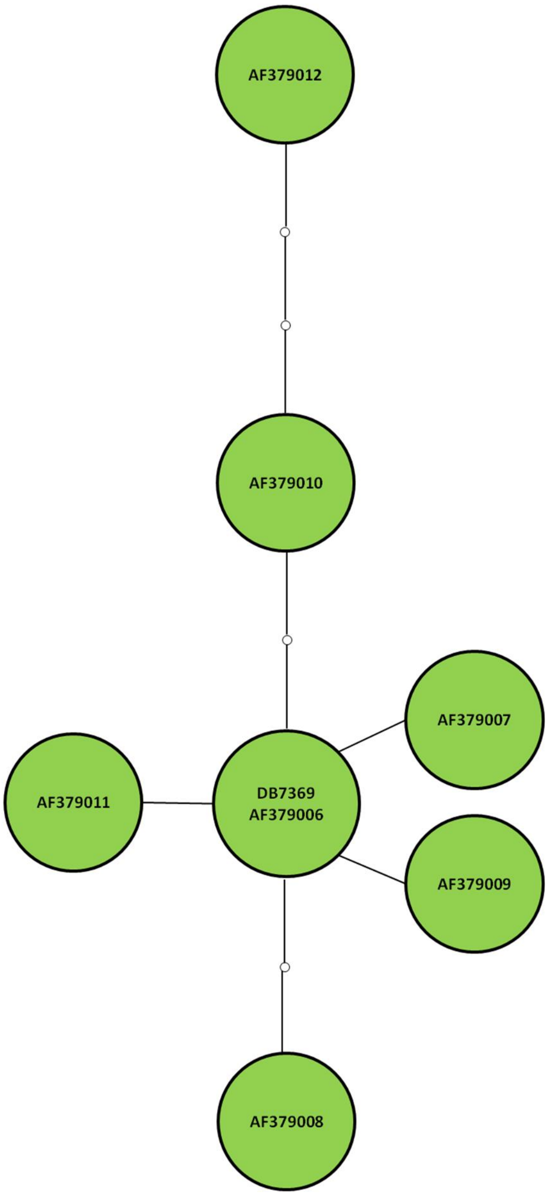

Figure 36: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *T. l. nevadensis*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes.

Italian wall lizard, *Podarcis sicula*

The final alignment includes 65 cytochrome b sequences and 687 base pairs. A total of 42 haplotypes were identified among which 35 from the sequences from Podnar *et al.* (2005) and eight from the five introduced populations from Iberian Peninsula and Menorca. In particular, in Almería, La Rioja and Lisbon populations, two different haplotypes each were found, while in Cantabria and Menorca one haplotype occurred.

The ML tree shows that lizards from Noja (Cantabria) are closely related to those from the "Tuscany" clade in Podnar et al (2005). Also, the lizards from Lisbon cluster together with Tuscanian samples, yet being fairly differentiated from them. Haplotypes from Almería and Menorca are related with those from Sicily and Sardinia and included within the "Sicula" haploclade. Moreover, the five Menorcan samples analyzed in this study showed the same haplotype as the single Menorcan sample analysed by Podnar *et al.* (2005). The two haplotypes found in the specimens from La Rioja cluster in a distinct clade, which is related to the "Monasterace" (Southeast Calabria) and "Sicula" haploclades. All the relationships above reported were highly supported by bootstrap analysis with BP values ranging from 86 to 99.

The network analysis shows 11 distinct networks and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 11. The relationships between haplotypes as depicted by the networks corroborate the results from ML analysis. Cantabria lizards cluster together with those from Tuscany, Almería and Menorca samples group with samples from the Sicula clade, and La Rioja and Lisbon samples constitute two independent groups.

ML tree and stastical parsimony networks are in the Appendix IV.

Mediterranean tree frog, *Hyla meridionalis*

The final alignment of COI gene includes 40 sequences with 785 base pairs. One single haplotype was identified among the Balearic samples, corresponding to two haplotypes previously found in samples from the native range by Recuero *et al.* (2007). Distribution of the haplotypes from the native and introduced snakes is depicted on Figure 37.

A ML analysis was not performed for this species, due to the low genetic differentiation between the 21 haplotypes found in the species' range. The network analysis (Figure 38) shows two distinct networks and the maximum number of mutational steps allowing for a 95% parsimonious connection between haplotypes was estimated to be 12. The network 1 corresponds to the 'Western' group by Recuero *et al.* (2007) with samples from France, Spain, Portugal and Morocco. This group is clearly subdivided in three sub-groups also found by the previous authors: 'Southwestern' (Western Morocco + Southwestern Iberia); 'Central' (Central Morocco); and, 'Northern' (Northern Morocco + Northeastern Iberia +

Canary Islands). The network 2 corresponds to the ‘Tunisian’ group with samples only from Tunisian populations. Menorcan samples are within the ‘Western group’, more precisely, in the ‘South-western sub-group’.

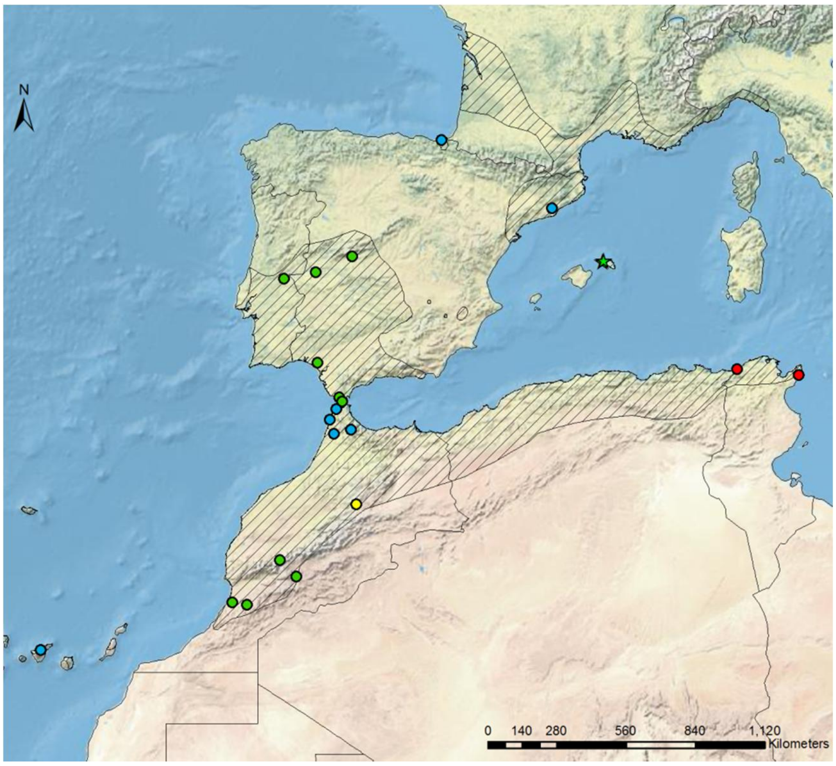

Figure 37: Haplotypes distribution in the native and introduced population of *H. meridionalis.* Circles: samples from native range (Recuero *et al.* 2007); stars: samples from introduced population. Native range is indicated by the shade area. Circle are coloured according to the clades found in the parsimony network (Figure 38).

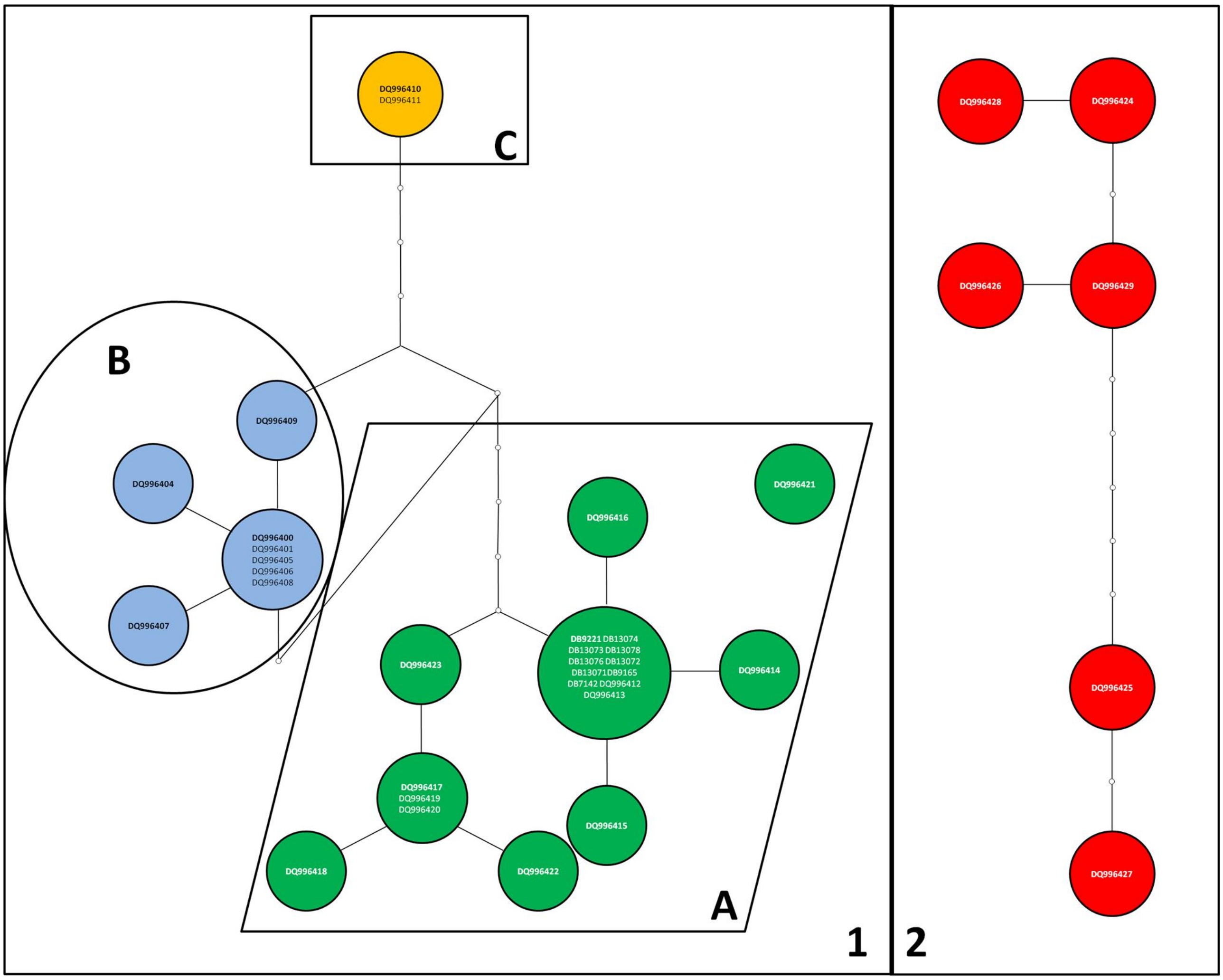

Figure 38: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *H. meridionalis*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes. 1: Western Group; 1A: Southwestern sub-group; 1B: Northern subgroup; 1C: Central subgroup; 2: Tunisian Group, as classified by Recuero *et al.* 2007.

3.3 Discussion

The results from molecular analyses allowed assessing the allochthonous status of the study species in the Balearic Islands and also provided insights about the probable pathways through which these species arrived to the Archipelago. These findings and their conservation implications are discussed in the following sections.

Timing and Origin of the Balearic introductions

The occurrence records of the study species in the Balearic Islands regard different time periods from species to species, being possible to distinguish between species historically present in the islands and species which arrived only very recently.

The first group includes the snakes *M. mauritanicus* from Mallorca and Menorca, *N. maura* from Mallorca, and *R. scalaris* from Menorca, the lizard *P. sicula* and the tree frog *H. meridionalis* from Menorca. Previous studies suggest an introduction during Roman times for the snakes *M. mauritanicus*, *N. maura* and *R. scalaris* (Vigne and Alcover 1985; Gosàlbez 1987; Busack & MacCoy, 1990; Pleguezuelos & Fernández-Cardenete, 2002), during the Middle Age for *P. sicula* (Mertens & Wermuth 1960; Meijide 1981; Mayol 1985; Rivera & Arribas 1993) and during II and III bC for *H. meridionalis* (Rivera & Arribas 1993; Esteban *et al.* 1994).

The second group includes the snakes *H. hippocrepis* and *M. monspessulanus* from Ibiza, and *R. scalaris* from Ibiza, Mallorca and Formentera, and the lizard *T. lepidus* from Mallorca. Previous studies support an introduction during the last decade for all the species. For *H. hippocrepis* and *M. monspessulanus* the first observations in Ibiza date from 2003 (Álvarez *et al.* 2010), for *R. scalaris* are from 2003 in Ibiza, 2004 in Mallorca and 2006 in Formentera (Álvarez *et al.* 2020; Oliver & Álvarez 2010; Mateo 2011); for *T. lepidus* the exact year of the first observations is unknown, but observations become more frequent after 2005 (J. A. Mateo pers. comm.).

Phylogenetic analyses of all the species sampled in the Balearic Islands corroborate their allochthonous status, yet for most of them it was not possible to unambiguously identify their origin. The main sources of introduction can be identified either in North Africa or in Europe although in some cases it was not possible to disentangle between these two areas of origin. The ML analysis supports a Tunisian origin for Mallorcan and Menorcan population of *Macroprotodon sp.* (Figure 39) which therefore are assigned to *M. mauritanicus* following Wade (2001). This result corroborates the origin already proposed for Mallorcan snakes by Busack & McCoy (1990), Wade (2001), and Carranza *et al.* (2004), on the basis of the morphological and genetic similarity between Balearic and Tunisian haplotypes.

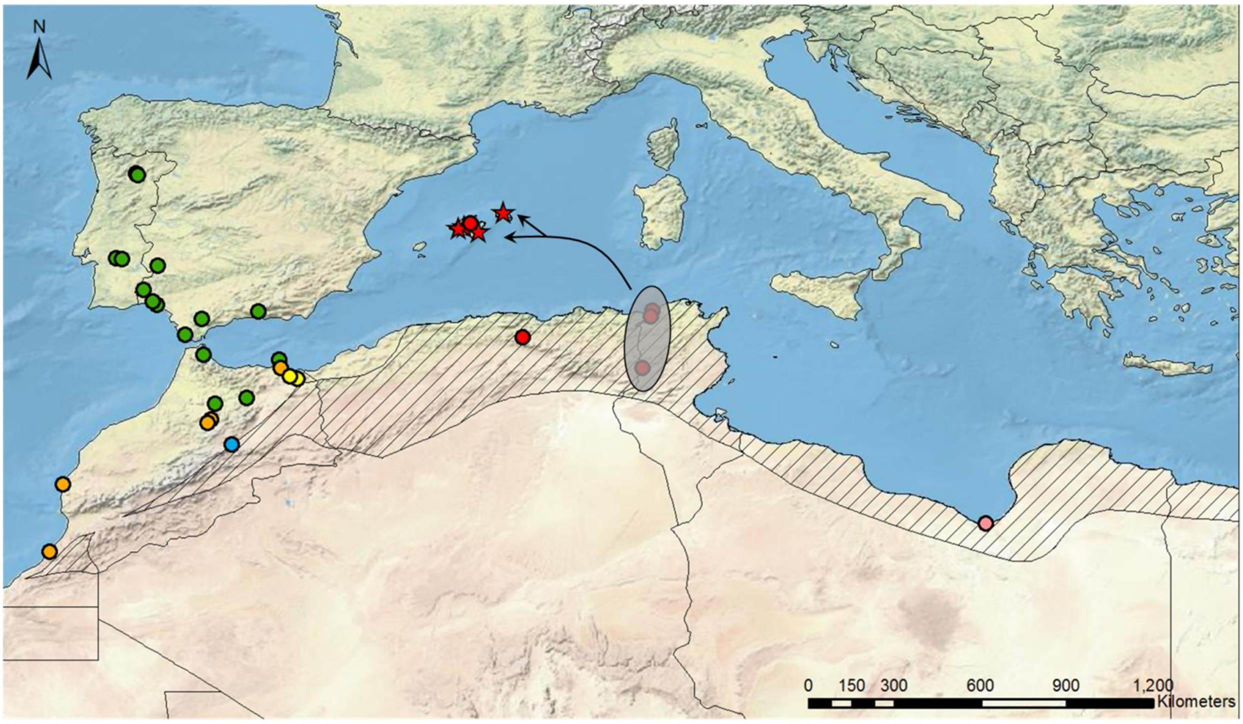

Figure 39: Geographic origin of *Macroprotodon mauritanicus.*in Mallorca and Menorca as inferred by genetic analysis Native range is indicated by the shade area, being this species restricted to north Tunisia and northeastern Algeria (see Figure 10).

The results from ML analyses support either a Northeastern African or a European origin for *H. hippocrepis* and *M. monspessulanus* (Figure 40 and Figure 41, respectively). For both the species, a recent expansion from Northeastern Africa to Iberia has been suggested to explain the low differentiation on their phylogenies (Carranza *et al.* 2006). Several evidences support the Northeastern African origin, such as the higher nucleotide diversity found in the Maghreb region compared to the Iberian one and the occurrence of the commonest haplotype of Maghreb in Iberia Peninsula (see Carranza et al., 2006 for further details). The low phylogeographic structure, characterized by North African and Iberian haplotypes being mixed in the same clades, did not allow the clear inference of the origin for the both introduced species. However, in *H. hippocrepis*, Mallorcan samples clustered in a sub-haploclade including haploclades found only in the Iberian Peninsula. This suggests a Spanish origin for *H. hippocrepis* from Mallorca, yet a North African origin cannot be completely discarded. Besides the phylogenetic evidence, it is also documented the presence of these snakes in Mallorca near 'nurseries' of olives trees (Álvarez *et al.* 2010). Although determining the source of every olive tree importation is problematic, one *H. hippocrepis* individual was found inside an olive tree trunk arrived in Mallorca from Córdoba Province (Andalusia, Southern Spain). Thus, at least Spanish companies are involved in the ornamental plants trade. Therefore, the association of these snakes to the olive trade from Spain would further support the Spanish origin of the Mallorcan population of *H. hippocrepis*.

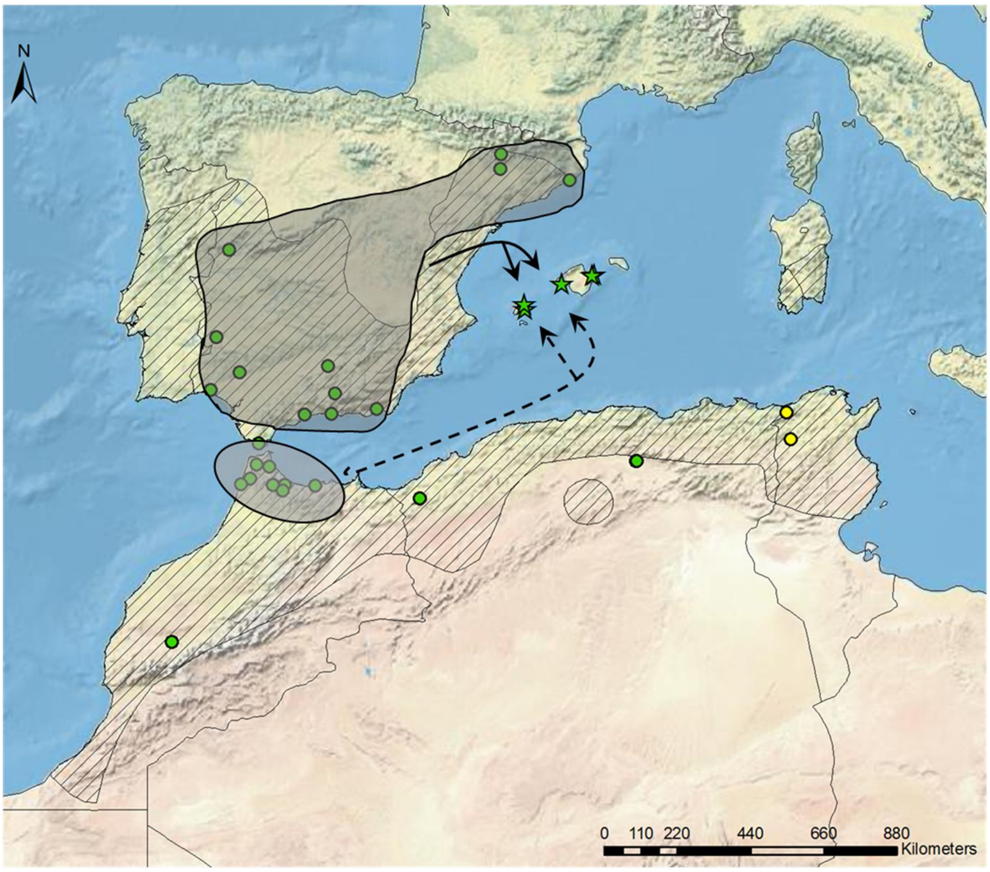

Figure 40: Geographic origin of the introduced populations of *H. hippocrepis* in Mallorca and Ibiza as inferred by genetic analysis. Native range is indicated by the shade area.

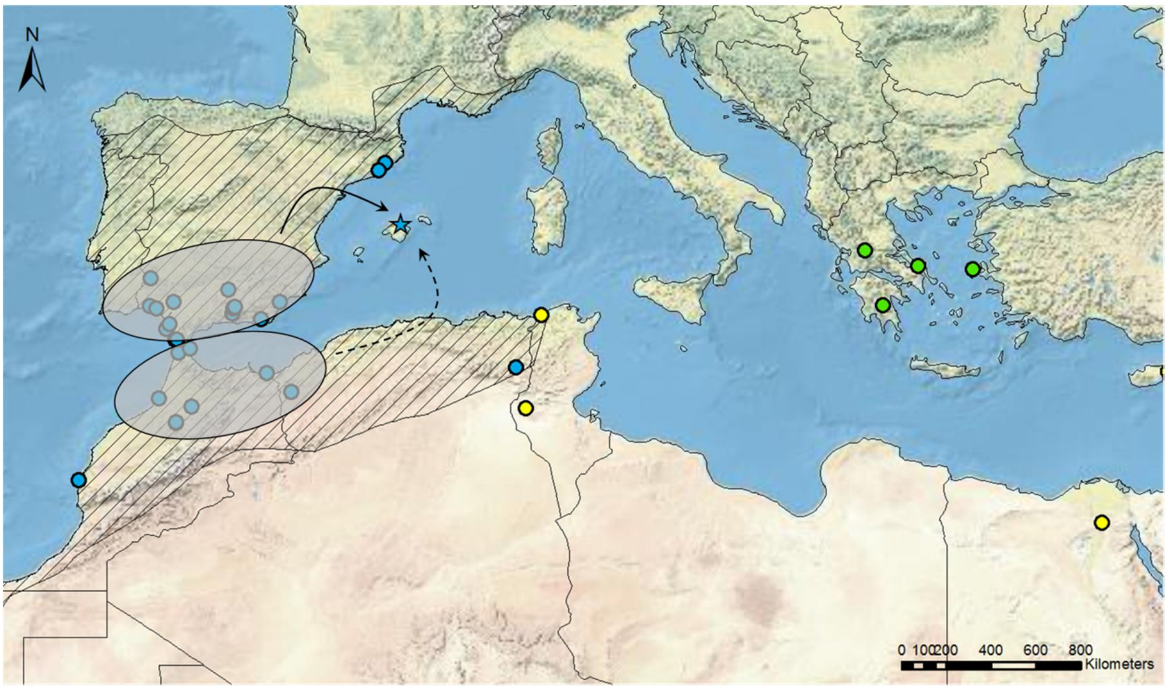

Figure 41: Geographic origin of the introduced populations of *M. monspessulanus* in Mallorca as revealed by genetic analysis. Native range is indicated by the shade area.

For the remaining species, a European origin is supported by our results. As regard to *N. maura,* phylogenetic analysis support the European origin proposed by Guicking *et al.* (2008). In fact, snakes samples from the introduced range carry the same haplotype of snakes from Spain, France and Portugal, suggesting an Iberian or French origin for the Mallorcan population (Figure 42). Moreover, an origin from the southernmost Iberian Peninsula (Cádiz) can be excluded since here a distinct lineages occurs (Barata *et al.* 2007).

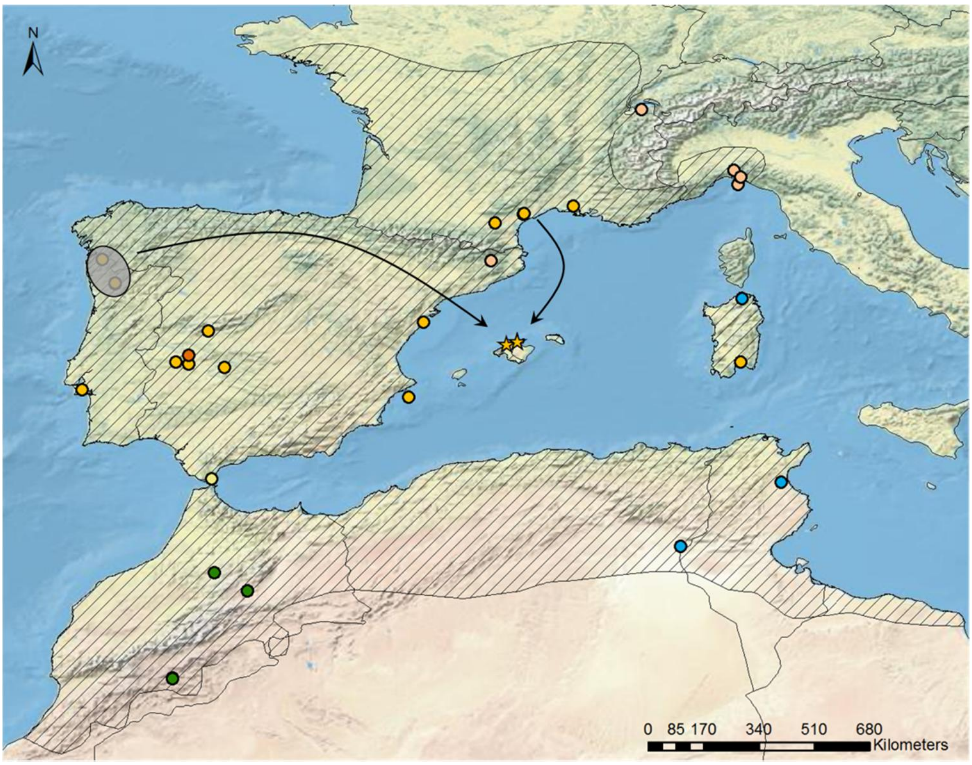

Figure 42: Geographic origin of the introduced populations of *N. maura* in Mallorca as revealed by genetic analysis, Native range is indicated by the shade area.

The origin for *R. scalaris* is difficult to determine, given its extremely low genetic differentiation across the whole native populations, with the three haplotypes found differing only by 1-2 nucleotide positions. The haplotype found in Mallorca, Menorca and Ibiza is identical to the commonest haplotype found across the species'range suggesting a recent arrival to the Balearic archipelago from the mainland, yet it is not possible to point a source area. Similarly to *H. hippocrepis* and *M. monspessulanus*, the ladder snake, can be found near olive trees from the importation industry (Álvarez *et al.* 2010). As reported by the same authors, at least one company from Spain is involved in olive trees trade, while no Portuguese or French importations are known. Thus also for the Mallorcan and Ibizan populations of *R. scalaris* a Spanish origin can be suggested,

For the lizard *T. lepidus,* the phylogenetic analysis supports a southeastern Iberian origin. Indeed, the sample analysed from Mallorca population carry the same haplotype of lizards from Sierra Filabres in Southern Spain (Figure 43). It also indicates that our sample belong to the subspecies *T. l. nevadensis*, which only occurs in a restricted area in the Baetic Region (Paulo *et al.* 2008). These results are in agreement with the taxonomic assessment of the Mallorca samples performed by Mateo & López-Jurado

(1994), based on morphological features such as the colour pattern (*nevadensis* subspecies being more greyish than the others subspecies which are more greenish). The same authors observed the presence in Mallorca also of individuals showing a colour pattern of the subspecies *T. l.lepidus* from Central Iberian Peninsula, thus suggesting multiple introductions of the ocellated lizard in Mallorca from different localities of the Iberian Peninsula. However, further investigation is needed to clarify these possible multiple sources of introduction.

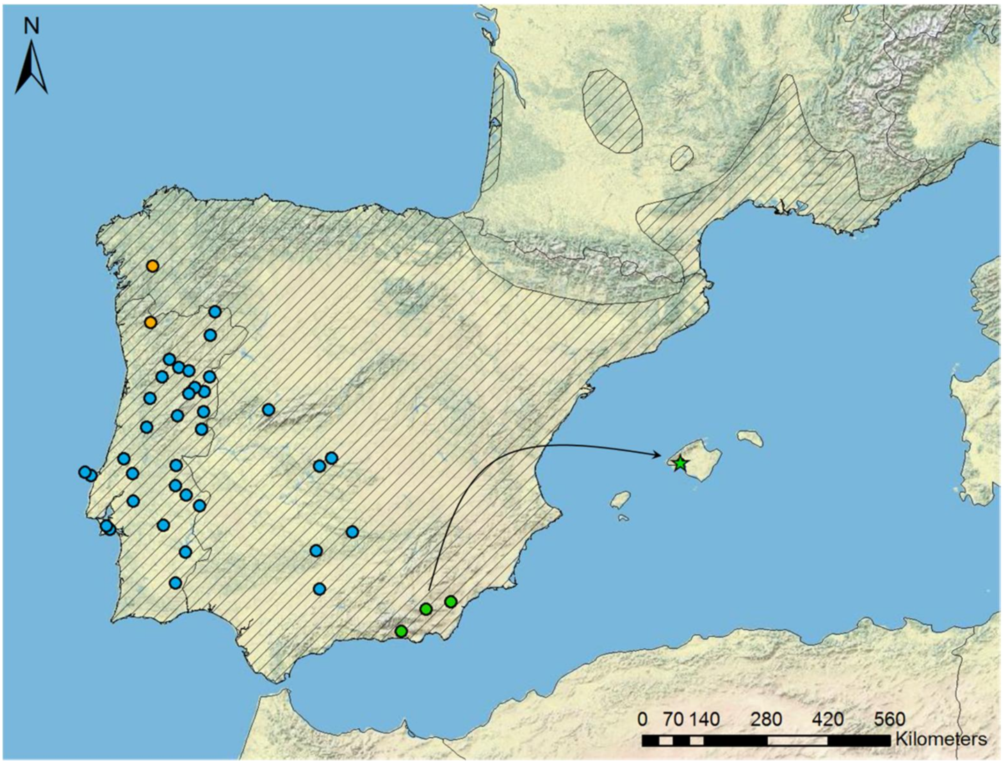

Figure 43: Geographic origin of introduced populations of *T. lepidus* in Mallorca as revealed by genetic analysis. Native range is indicated by the shade area.

The genetic analysis of the Italian wall lizards sampled in Menorca suggests a Sicilian or a Sardinian origin for the Menorca population and excludes the hypothesis of multiple introductions from (recently introduced) Iberian populations. However, it is difficult to firmly disentangle between a Sicilian and a Sardinian origin for the Menorcan population of *P. sicula*. Indeed, according to Podnar *et al.* (2005), the Sardinian samples (assigned to the subspecies *P. s. cetti*) only differ from the Sicilian ones (assigned to the subspecies *P. s. sicula*) by three substitutions in a gene fragment of 867 nucleotides, which point to a historical colonization of Sardinia from Sicily (Lanza 1982). Thus, Menorcan samples could have been originated either from Sicily as suggested by Greca and Sacchi (1957) or from Sardinia as suggested by Müller (1905) and Eisentraut (1950). Although Podnar *et al.* (2005) hypothesise an introduction from Sicily since Menorcan haplotypes are slightly closer to the Sicilian than to the Sardinian haplotypes (differences Menorca and Sicily/Sardinia are 2/3 respectively), the gene fragment used lacks enough resolution for supporting this statement. Moreover, the haplotype comparison is only based on single specimens from Menorca, Sicily and Sardinia. Additionally, the morphological inspections of Menorcan lizards show a closer match with the *cettii* specimens occurring in Sardinia rather than with the *sicula* morphotypes observed in Sicily. Thus, taking this evidence into account, we would tentatively suggest a Sardinian origin for the Menorcan population of *P. sicula*, although the large spectrum of morphological variation within *P. sicula* advices for caution in the use of morphological diagnosis when inferring the origin of populations. The general result for the other introduced populations suggests multiple and independent introductions from different parts of Italy, its native range (Figure A11, Appendix IV).

Finally, for *H. meridionalis* all the Menorcan samples share the same haplotype with samples from Cáceres and other localities from central-western Spain and central-eastern Portugal (Figure 44). Thus, despite the low phylogeographic structure showed by this species across its range (likely due to the recent colonization of the south-western Europe from Morocco, Recuero et al. 2007), our results suggest an origin of the Menorcan population of *H. meridionalis* from the south-western quadrant of the Iberian Peninsula.

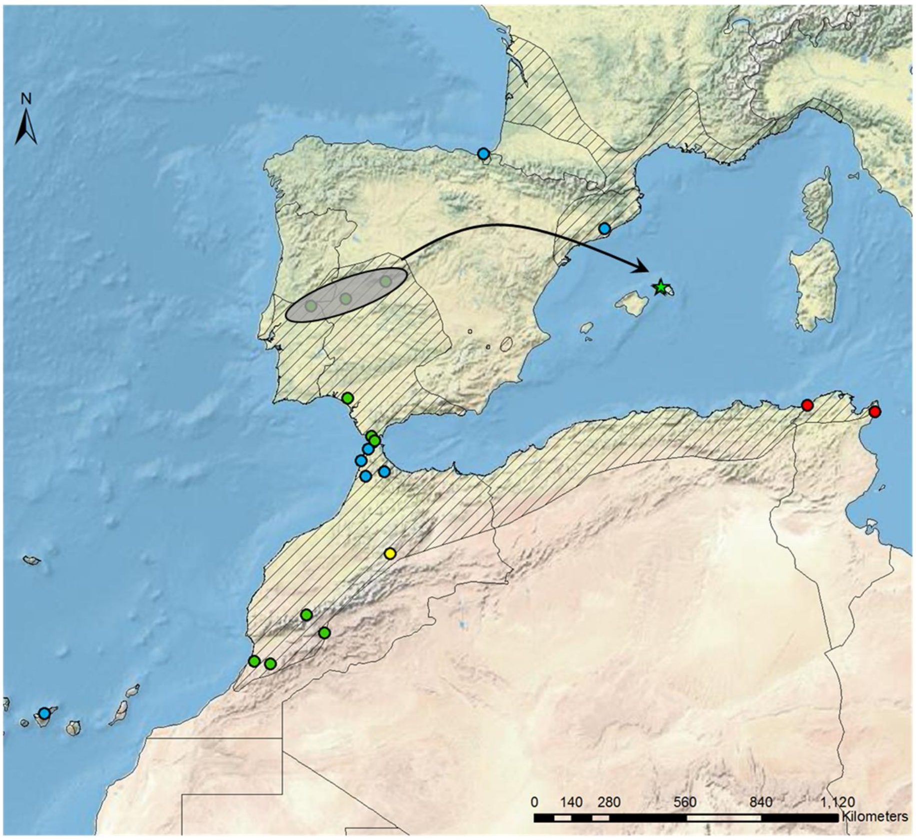

Figure 44: Geographic origin of introduced populations of *H. meridionalis* in Menorca as revealed by genetic analysis. Native range is indicated by the shade area.

Pathways of introduction

The pathway of introduction for all the species is human-mediated.

In the case of the older introductions, such as the Menorcan population of *R. scalaris*, the Mallorcan and Menorcan populations of *M. mauritanicus,* the Mallorcan population of *N. maura* and the Menorcan population of *P. sicula,* the anthropogenic origin is supported by multiple evidence such as the absence of fossil record, the low genetic differentiation between the islands and the mainland populations and also the similarity of morphological traits among native and introduced populations (Kotsakis 1981; Carranza *et al.* 2004; Guicking *et al.* 2008; Mateo *et al.* 2011; Pynia & Carretero 2011). For *H. meridionalis*, different sources of data also support the human-mediated introduction hypothesis. First, the archaeological deposits from II and III bC (Riviera & Arribas 1993) support the assumption of the introduction by Romans (Guicking *et al.* 2008). Second, the low genetic differentiation between the native and the introduced populations, together with the poor dispersal abilities of amphibians (Smith & Green 2005), make improbable a natural arrival of *H. meridionalis* to the Balearic Islands, especially from the genetically closest populations of NW Iberia or Morocco.

The main pathways by which these species arrived to the Balearic Islands in historical times can be either voluntary or accidental. As regards snake, among the voluntary factors that can lead to an introduction, Roman religious purposes cannot be discarded, since snakes appear to have a wide range of religious associations for the Romans, namely as guardians of a place, to give good luck and to demonstrate if a place is sacred or not (Stell 2006). Moreover, snakes were also used by Romans to frighten enemies during assaults (Bruno & Maugeri 1990). Therefore, a probable voluntary introduction is favoured, since the humans obtained direct benefits from the translocation. Among the accidental factors, the cargo (refers to accidental transport in packaged or unpackaged goods for human use) of several commercial products during an intense maritime trade and commercial routes established between Balearic Island and the mainland can be one of the factors. Many species use the human-manufactures as a refuge, making more probable its accidental translocation from the native range toward the introduced range. For example, in the case of *H. meridionalis*, it is suggested that the first settlers transported with them cargos such as cuttle and seeds in the Canary Islands, and in these way they could have carried other species also, intentionally or passively (Recuero *et al.* 2007). Nowadays, the banana plantations and movements among islands are assumed to be the main factors responsible for the tree frog expansion (Pleguezelos 2002).

Regarding the more recent introductions, like *M. monspessulanus*, *H, hippocrepis* and *R. scalaris*, a specific pathway of introduction is suggested: the 'nursery trade' which refers to the trade in live plants for ornamental purposes and food trees for tropical gardening (Kraus 2009; Álvarez *et al.* 2010). Individuals from all the three snakes' species were found primarily in trunks of olive trees deposited in the nursery centres of Capdepera (Mallorca) and Sant Llorenç de Baláfia (Ibiza) and the first occurrence records are concentrated in these areas. For the lizard *T. lepidus,* an introduction with olive trees can also be considered, since this species is commonly found inside olive trees (Rivera *et al.* 2011). The olive trade business has increasing through the years in the Balearic Islands, as a consequence of a mode in the garden decorations. Reptiles use the olives as a refuge and there are evidences of several species living together in old trees (Rivera *et al.* 2011), which increases the probability of introductions of multiple species in multiple sites. Thus, olive trade seems an important pathway of reptiles' introduction through this region.

Finally, an introduction by terrarium keepers can also be hypothesized in the case of *T. lepidus* as suggested by Mateo *et al.* 2011, due to the spectacular colour pattern and large body size of this species. These animals can either be deliberately released by their owners or escape since they are extremely fast and easily escape when handled. Several sites on the internet, concerning the pet trade, promote the adoption of this species, as well as present a care sheet with all the information needed to maintain the animal. The introductions due to pet trade have been growing since the 70's almost exponentially, indeed, pet trade is the main pathway by which amphibians and reptiles are being introduced.(Kraus 2009).

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4. Predicting the Expansion of Balearic Snakes

4.1 Methods

Study species and its distribution

In this part of the work, only snakes are used since they are the only species which we have enough data for modeling and they represent a predator potentially very harmful for the remained native biota. Then, the focus species are *Hemorrhois hippocrepis, Malpolon monspessulanus, Macroprotodon mauritinacus* (*Macroprotodon* sp) and *Rhinechis scalaris.*

The occurrence data from the native distribution of each species was compiled from GBIF (Global Biodiversity Information Facility, http://www.gbif.org/). For the species with North African distribution (all except *Rhinechis scalaris*), more occurrence points were needed to do a proper analysis, which were collected from literature review and from other collectors from CIBIO whose kindly ceded the data. A total of 2953 records were collected for *Hemorrhois hippocrepis*, 7164 records for *Malpolon monspessulanus*, 796 for *Macroprotodon mauritanicus* and 6152 for *Rhinechis scalaris.* For *M. mauritanicus*, the presence records from the whole genus from Morocco, Algeria and Tunisia of North Africa had to be considered since for the distribution of *M. mauritanicus* as proposed by Wade (2001), very few records were available.

Environmental data

Climatic predictors were downloaded from WorldClim online data (http://www.worldclim.org/, Hijmans *et al.* 2005) at a resolution of 30 arc-seconds (about 1km). A total of seven Bioclimatic variables from the 19 available were used to calculate the models, chosen by a correlation lower than 0.7 criteria (Spearman's correlation rank: close to +1 means perfect correlation and close to 0 means weak correlation; Sillero 2009; BFSC 2012) and by the biological importance for the species:

- Bio 2: Mean Diurnal Range (Mean of monthly (max temp – min temp))
- Bio 3: Isothermality (BIO2/BIO7) (* 100)
- Bio 4: Temperature Seasonality (standard deviation *100)
- Bio 8: Mean Temperature of Wettest Quarter
- Bio 15: Precipitation Seasonality (Coefficient of Variation)
- Bio 18: Precipitation of Warmest Quarter
- Bio 19: Precipitation of Coldest Quarter

For the future climate, three coupled atmosphere-ocean general circulation models (CCCMA, HadCM3 and CSIRO) with three socio-economic emission scenarios (A1b, A2a and B2a) were used, for three future periods: 2020, 2050 and 2080. Scenarios are an important tool to address the complexity and uncertainty of future challenges, relating the response of the Earth system to atmospheric composition, the effectiveness of mitigation and the changes in demographics, economies, technologies and policies.

Rather than a forecast, each scenario is one alternative image of how the future can unfold (Arnell *et al.* 2004). The three scenarios illustrate the relationships among the forces driving greenhouse gas and aerosol emissions, and their evolution on a regionalization perspective (A2a and B2a) and on a globalization perspective (A1b) (Nakicenovic *et al.* 2000). The scenario A1b describe a future world with a very rapid economic growth, a rapid introduction of new and more efficient technologies, a balance across fossil intensive and non-fossil energy sources, and a global population that until mid-century increase and then decrease. The scenario A2A consists of a highly heterogeneous future world in which the driving forces are a high rate of population growth, increased energy use, land-use changes and slow technological change. The B2a scenario reflects a general evolution towards environmental protection and social equity, having a lower population growth, more diverse technological changes and slower land-use changes. Comparing the three scenarios, A2a is the most drastic one and the other two have intermediate to low impacts (IPCC 2007).

Ecological Niche Model

The ENM chosen to achieve the goals of this work was the Maxent, since it works with presence-only data (Philips *et al.* 2006), its performance is comparable to several traditional tools using presence/absence data (Elith *et al.* 2006; Hernandez *et al.* 2006), it has high quality performance with both small and large sample size (Elith *et al.* 2006; Wisz *et al.* 2008) and it is capable of dealing with continuous and categorical ecogeographical variables (EGV) at the same time (Philips *et al.* 2006). Some drawbacks are also found, like the fewer guidelines for its use in general and fewer methods for estimation the amount of error in a prediction, the requirement of further studies applied to the amount of regularization and the use of an exponential model for probability, which is not inherently bounded above and can give very large predicted values for environmental conditions outside the range present in the study area (over-fitting) (Phillips *et al.* 2006). The over-fitting of the produced model can be avoided by using regularization which relaxes the constraint such that the expected value of each environmental variable may fall within a predefined margin around the empirical average (Dudík *et al.* 2004).

Maxent is a machine learning algorithm which estimates the distribution of the species by finding the probability distribution of maximum entropy (i.e. the closest uniform as possible) subject to constraints representing our incomplete information about the distribution. The constraints are that the expected value of each environmental variable should match its average over sampling locations from environmental layers (Philips *et al.* 2006). Maxent searches for the statistical model that produces the most uniform distribution but still infers as accurately as possible the observed data. To do that, it compares the presence-only records with random data extracted automatically from all the background (including the species records; see Phillips *et al.* 2009). Therefore, Maxent calculates a set of ten models for each datasets through an iterative process and performed an average ensemble model (Araújo & New 2007; Sillero 2009, 2010). Maxent weights each EVG by a constant and the estimated probability distribution is exponential in the sum of the weighed features, divided by a scaling constant to ensure that the probability values range from 0.0 to 1.0 and sum to 1.0. The program starts with a uniform probability distribution (gain = 0) and iteratively alters one weight at a time to maximize the likelihood of the occurrence dataset. The algorithm converges to the optimum probable distribution, and thus, the gain can be interpreted as representing how much better the distribution fits the sample points than the uniform distribution (Phillips *et al.* 2004, 2006; Phillips and Dudik 2008). The suitability is evaluated by each grid cell as a function of environmental variables and can range from 0 (unsuitable) to 1 (optimal).

The model was developed on the basis of distribution records within the native range of each species and projected to evaluate the environmental suitability at each grid cell to the area of Balearic Islands, namely Ibiza, Formentera, Mallorca and Menorca Islands.

Maxent was run with randomly selection of 70% of the presence records as training data and 30% as test data for all the species locations on 10 replicate models. Response curves were graphed to understand how predicted relative probability of occurrence depends on the value of each environmental variable. Maxent is an exponential model, so the probability assigned to a location is proportional to the exponential of the selected combination of variables, thus allowing construction of response curves to illustrate the effect of selected variables on probability of use. The curves show how the logistic prediction changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. The y-axis represents the predicted probability of suitable conditions, as given by the logistic output format. Increasing trends for variables indicate a positive association, descending movements represent a negative relationship and the magnitude of these movements indicates the strength of these relationships (Baldwin 2008, 2009; Phillips 2012). Here it will be analysed the response curves of each environmental variable isolated, since although we used a criteria of less of 0.70 of correlation, the variables have a degree of correlation.

The area under the curve (AUC) of the receiver operated characteristics (ROC) was used to test the agreement between observed species presence and projected distribution (Manel *et al.* 2001). The ROC plot relates the Sensitivity (proportion of observed presences correctly predicted) with 1-Specificity (proportion of observed absences/pseudo-absences incorrectly predicted). To develop a ROC plot, a certain percentage of the data is selected for training data; the other portion is used for test data. A good model is defined by a curve that maximizes sensitivity for low values of the false-positive fraction. The significance of this curve is quantified by the area under curve (AUC) and has values that typically range from 0.5 (no better than the expected by random) and 1.0 (perfect fit). Values less than 0.5 indicate that a model fits worse than random (Fielding & Bell 1997; Engler *et al.* 2004; Hernandez *et al.* 2006; Baldwin 2009).

A jackknife test was used to measure the importance of each environmental variable to explain the species distribution, by training with each environmental variable first omitted and then used singly. Like this, this approach provides information on the performance of each variable in the model in terms of how important each variable is at explaining the species distribution and how much unique information each variable provides (Yost *et al.* 2008; Baldwin 2009; Phillips 2012).

Projections to the three future coupled atmosphere-ocean general circulation models were reclassified in habitat suitability models according to the maximum training sensitivity plus specificity logarithmic thresholds given by Maxent. Cells with values higher than the threshold were considered as suitable for the presence of the species; cells with values lower than the threshold, were considered unsuitable for the species, i.e. the species was considered to be absent from these cells.

4.2 Results

Firstly, the statistical results from the models will be presented in order to demonstrate the good predictive value of the models obtained. Then, the projected models of the present and of the future for the Balearic Islands will be described, taking into account the three emission scenarios (A1b, A2a, B2a).

Statistical results

In respect to the omission (known areas of presence are predicted as absences) and predicted area variation with the choice of the cumulative threshold, all the species' models show a good match with the predicted omission rate, as it is possible to see on Figure 45.

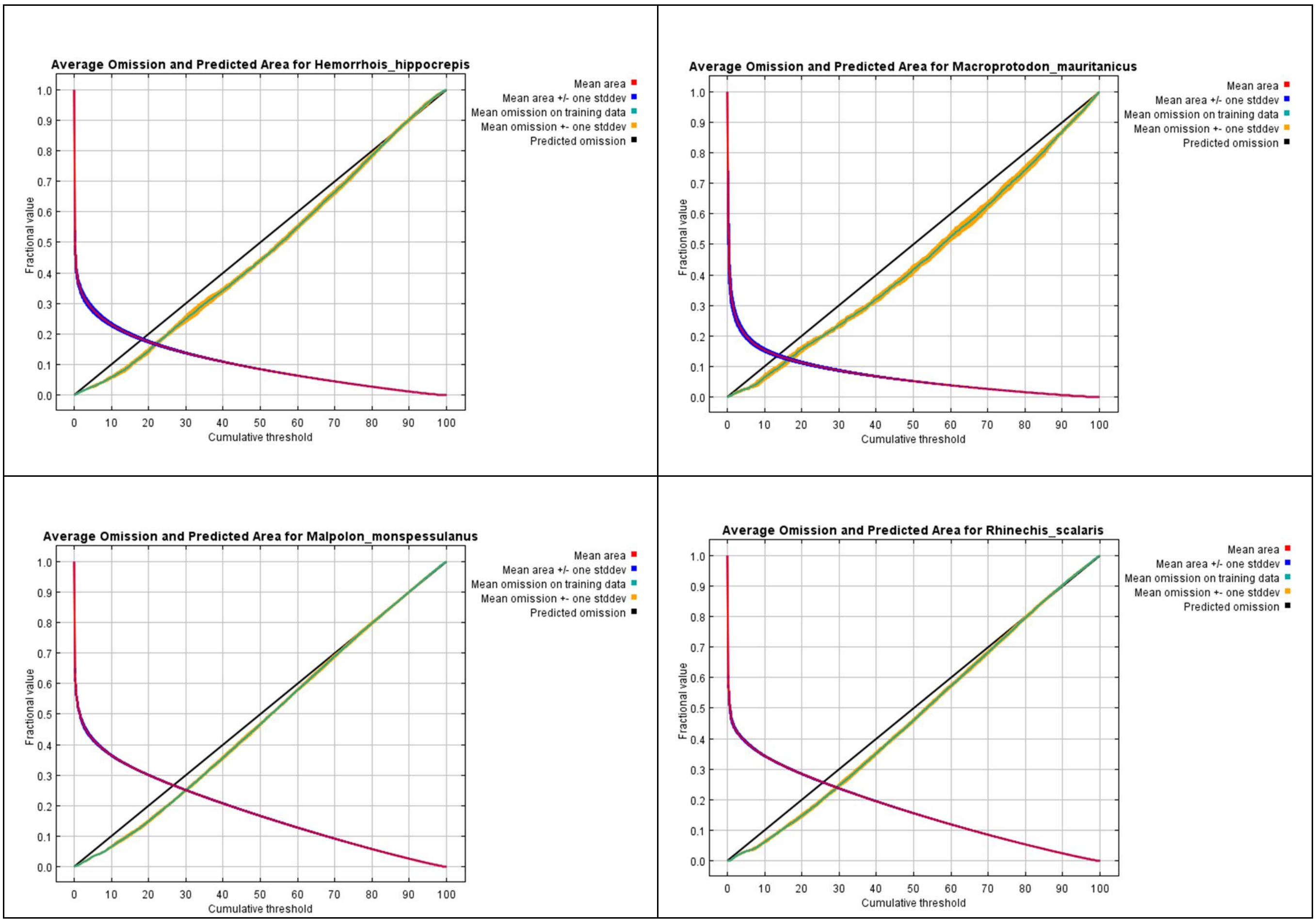


Figure 45: Omission and Predicted Area Plots for the species analysed in this study.

The AUC values support a good predictive ability for all the species analysed, with values higher than 0.90 to *H. hippocrepis* and *M. mauritanicus* and values higher than 0.80 to *M. monspessulanus* and *R. scalaris* (Table 6).

Table: 6 Mean values of AUC of the models for all the species.

	H. hippocrepis	***M. mauritanicus.***	***M. monspessulanus***	***Rhinechis scalaris***
Mean AUC	0.906 ± 0.002	0.940 ± 0.002	0.828 ± 0.002	0.840 ± 0.001

For all the species is possible to observe that the precipitation of warmest quarter (bio18) is the variable which more contributes to fitting the model, i.e. that more explain the variation in the models. The second more explanatory variable varies with the species, being the precipitation seasonality (bio15) to *H. hippocrepis*, the temperature seasonality (bio 4) to *M. monspessulanus* and the mean diurnal range (bio 2) to both *M. mauritanicus* and *R. scalaris*. The less explanatory variable common to all species is the mean temperature of wettest month (bio8) (Table 7).

Table 7: Contribution of each variable for each species' models. Two main explanatory variables for each species are in bold. (PC) Percentage contribution (PI) Permutation Importance

Variable	***H. hippocrepis***		***M. mauritanicus***		***M. monspessulanus***		***R. scalaris***	
	PC	PI	PC	PI	PC	PI	PC	PI
Bio2	13.6	12.2	**21.1**	**19.8**	21.1	15.1	**28.7**	**20.9**
Bio3	5.8	7.7	5.4	8.6	5	7.2	5.1	7.2
Bio4	12.1	10.7	4.3	2.2	**21.8**	**20.2**	16.4	16.1
Bio8	2.3	5.9	0.6	1.3	0.9	4.4	1.1	5.2
Bio15	**22.6**	**22.2**	14.7	11	4.8	7.9	6.1	10.2
Bio18	**28.6**	**22.2**	**40.5**	**50.1**	**35**	**28.5**	**38.3**	**28**
Bio19	15	17.4	13.3	6.9	11.4	16.7	4.3	12.4

These patterns are more a less concordant to jackknife analysis, with the precipitation of warmest quarter (bio 18) being the environmental variable with highest gain when used in isolation and with more decrease gain when it is omitted for all the species. This means that this variable appears to have most useful information by itself and the most that isn't present in the other variables. It is important to notice that these results are accordant among training gain (Figure 46), test gain and AUC plots (Appendix V).

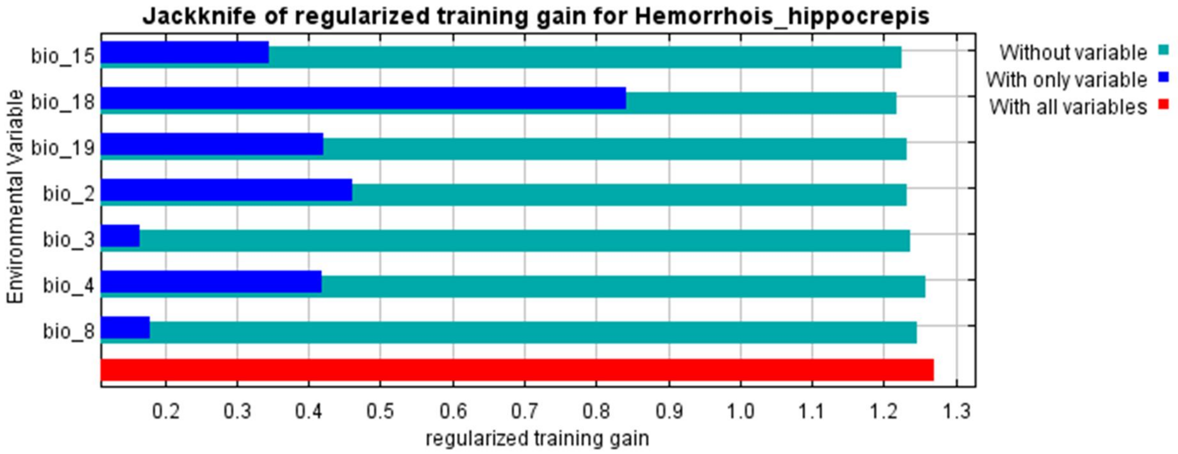


Figure 46: Jackknife of regularized training gain results for all the four species in study.

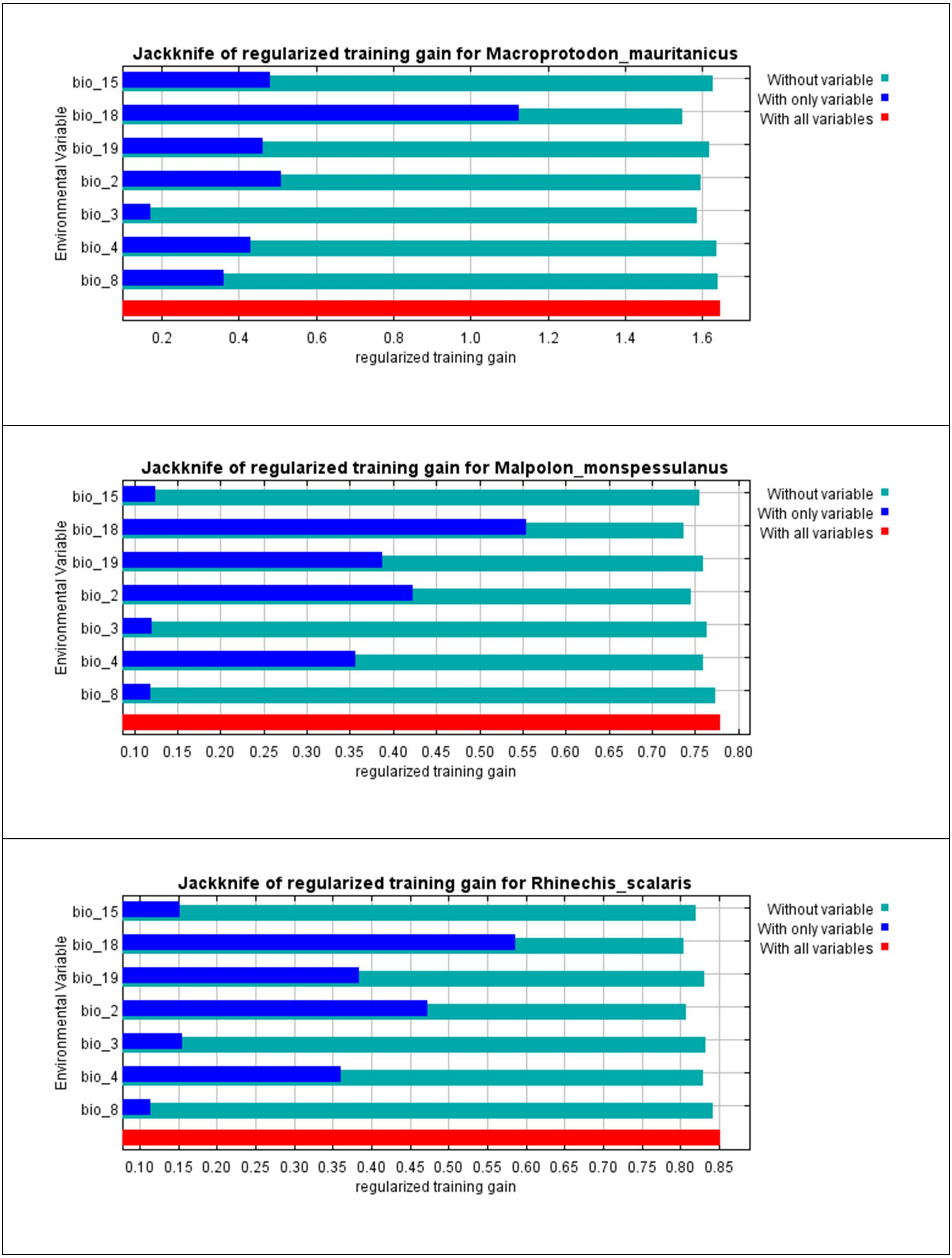


Figure 46 (continuation): Jackknife of regularized training gain results for all the four species in study.

The response curves that Maxent produced reveals that the probability of occurrence for *H. hippocrepis* is maximum for precipitation of coldest quarter (bio 19), between 600 and 800 mm and for the precipitation of warmest quarter (bio 18), suggested by previous methods as the most explanatory variable. Bio18 has a positive relationship only on the lowest values of precipitation (0-25/30 mm) (Figure 47).

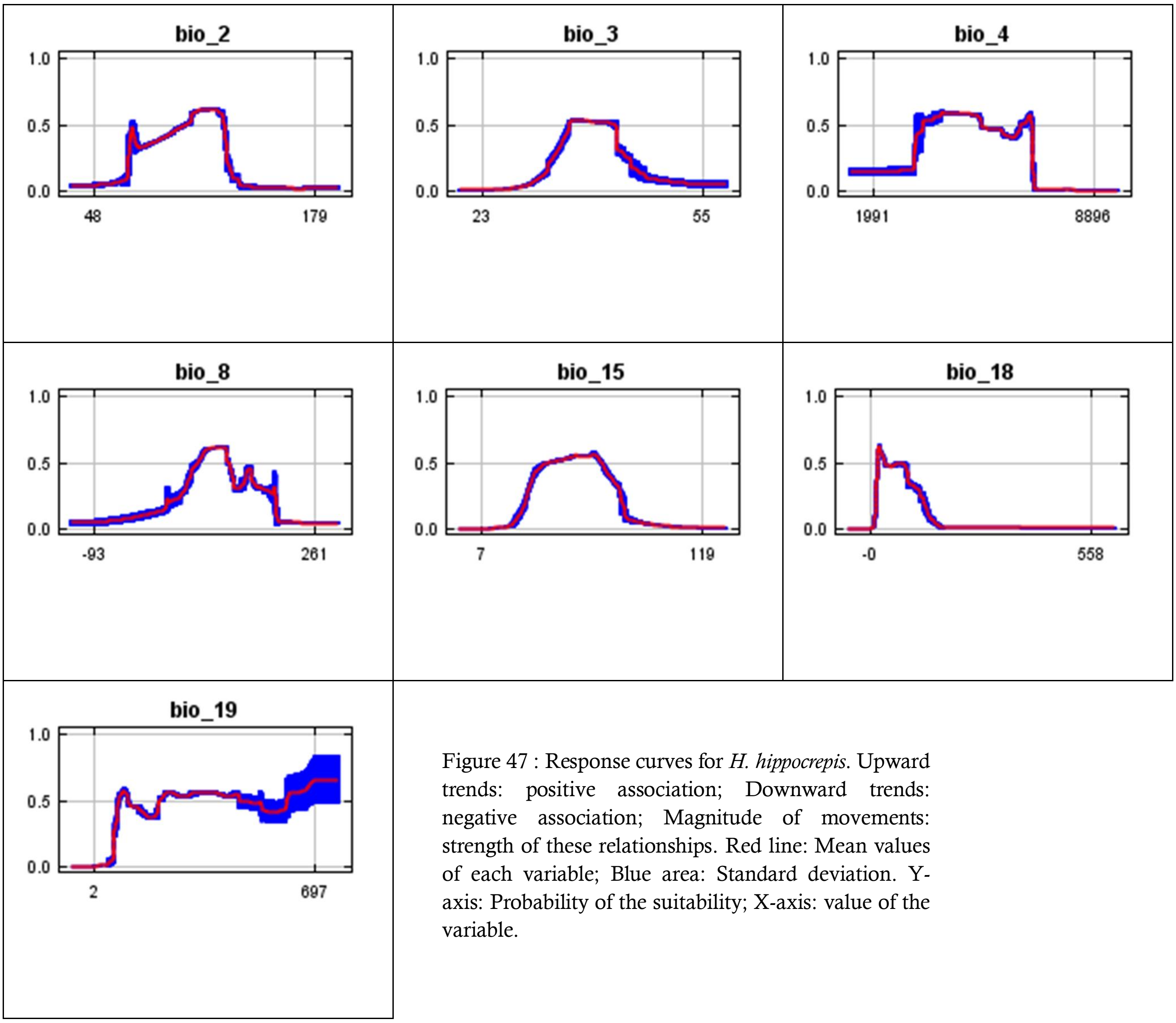


Figure 47 : Response curves for *H. hippocrepis*. Upward trends: positive association; Downward trends: negative association; Magnitude of movements: strength of these relationships. Red line: Mean values of each variable; Blue area: Standard deviation. Y-axis: Probability of the suitability; X-axis: value of the variable.

For *M. mauritanicus*, the probability of occurrence is maximal for temperature seasonality (bio 4), between 3000 and 4000, and for precipitation of warmest quarter (bio 18) at lowest values and then quickly drops toward 0 (Figure 48).

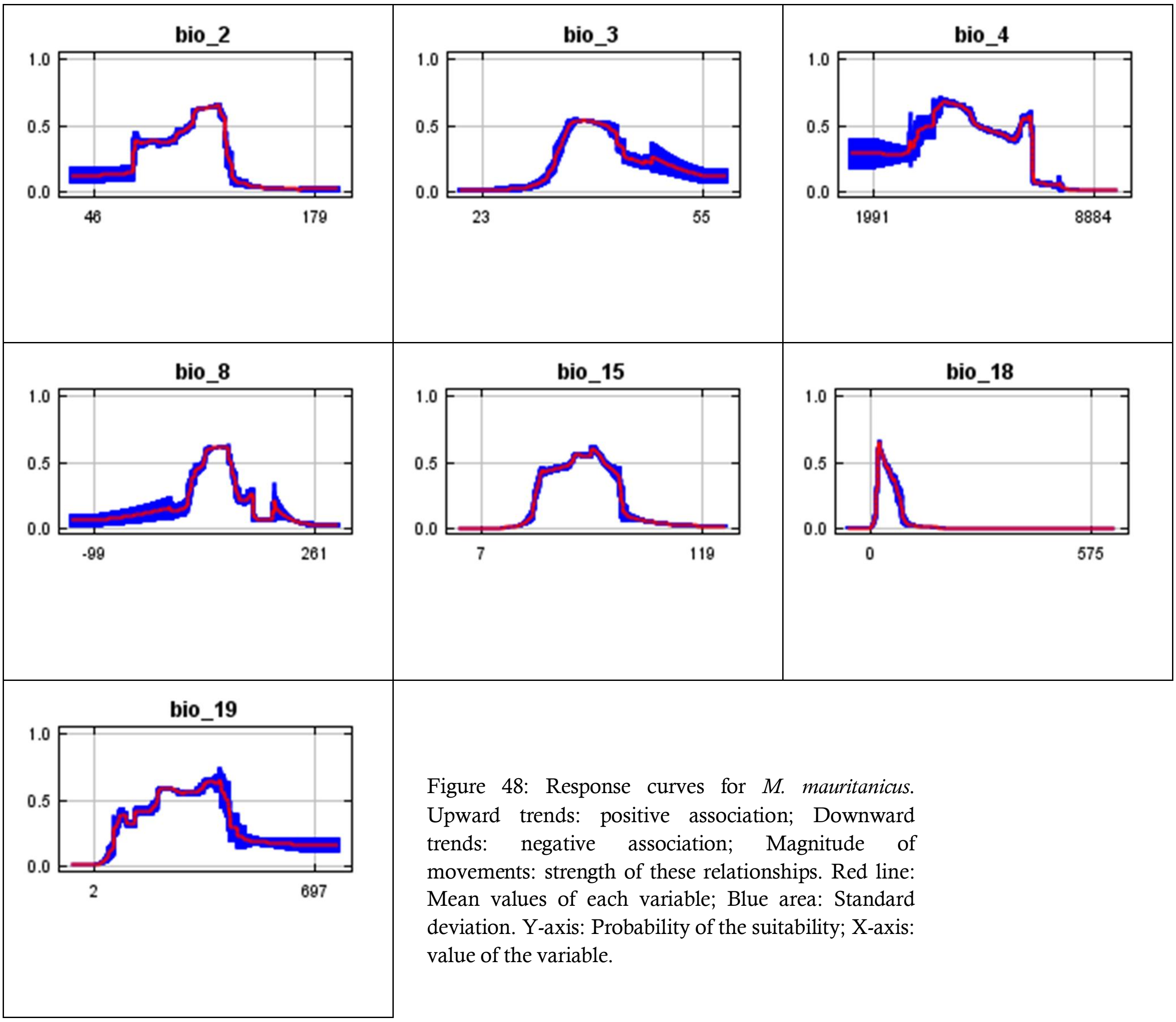


Figure 48: Response curves for *M. mauritanicus*. Upward trends: positive association; Downward trends: negative association; Magnitude of movements: strength of these relationships. Red line: Mean values of each variable; Blue area: Standard deviation. Y-axis: Probability of the suitability; X-axis: value of the variable.

For *M. monspessulanus*, the response curves support the precipitation of warmest quarter (bio18) and the precipitation of coldest quarter (bio 19) as the variables with the maximum predicted probability of suitable conditions (Figure 49). For bio18, the highest response occurs at lower values until 70mm; and for bio19, the highest response occurs from middle values to higher values (until about 800mm).

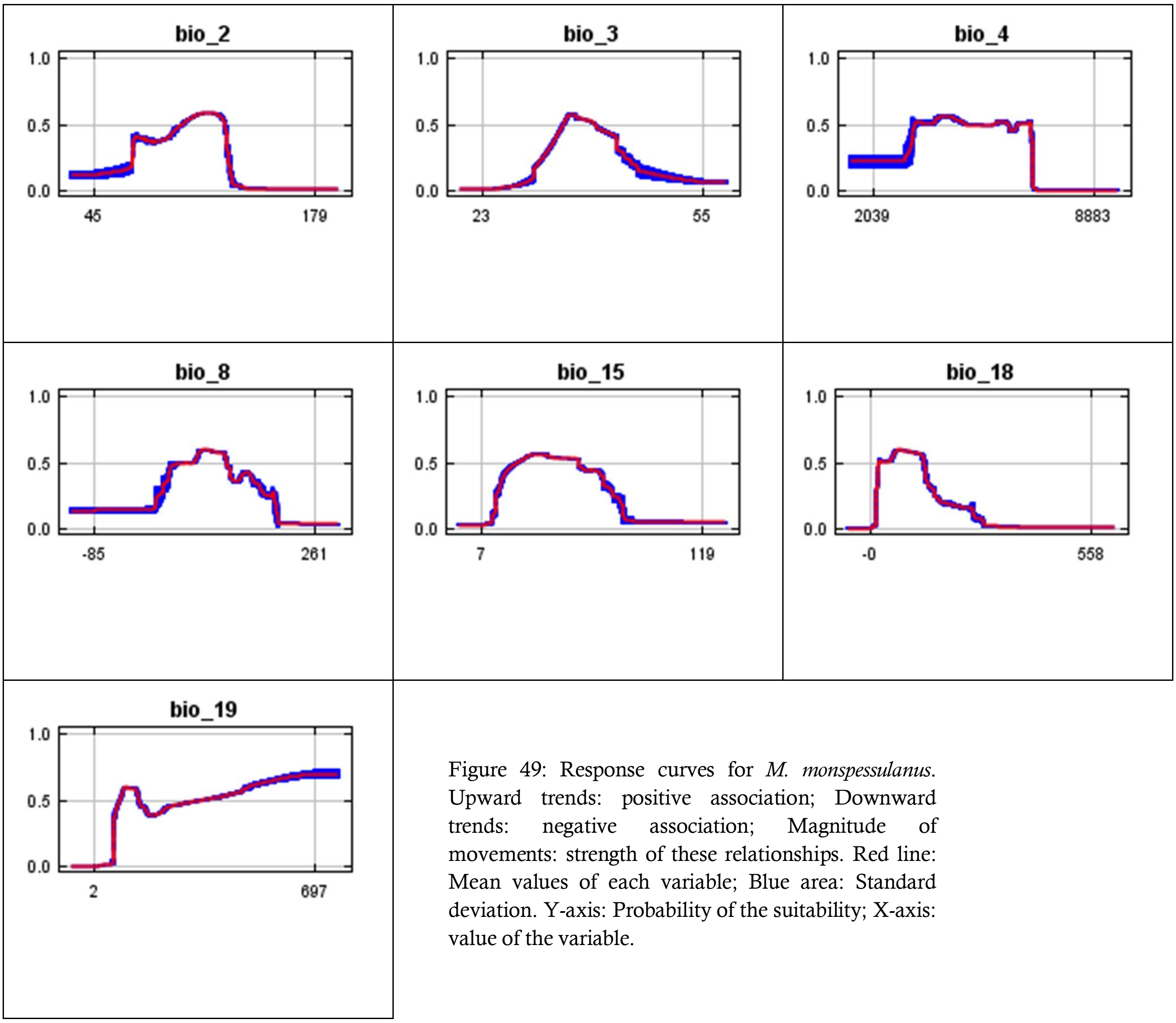


Figure 49: Response curves for *M. monspessulanus*. Upward trends: positive association; Downward trends: negative association; Magnitude of movements: strength of these relationships. Red line: Mean values of each variable; Blue area: Standard deviation. Y-axis: Probability of the suitability; X-axis: value of the variable.

Finally, the response curves of *R. scalaris* depict the precipitation of coldest quarter as the variable with the maximum predictive probability of suitable conditions, between 200mm and 800mm (Figure 50). The precipitation of warmest quarter (bio 18) and the mean diurnal range (bio 2) also have high values of probability, in lower values and between 80 and 120, respectively.

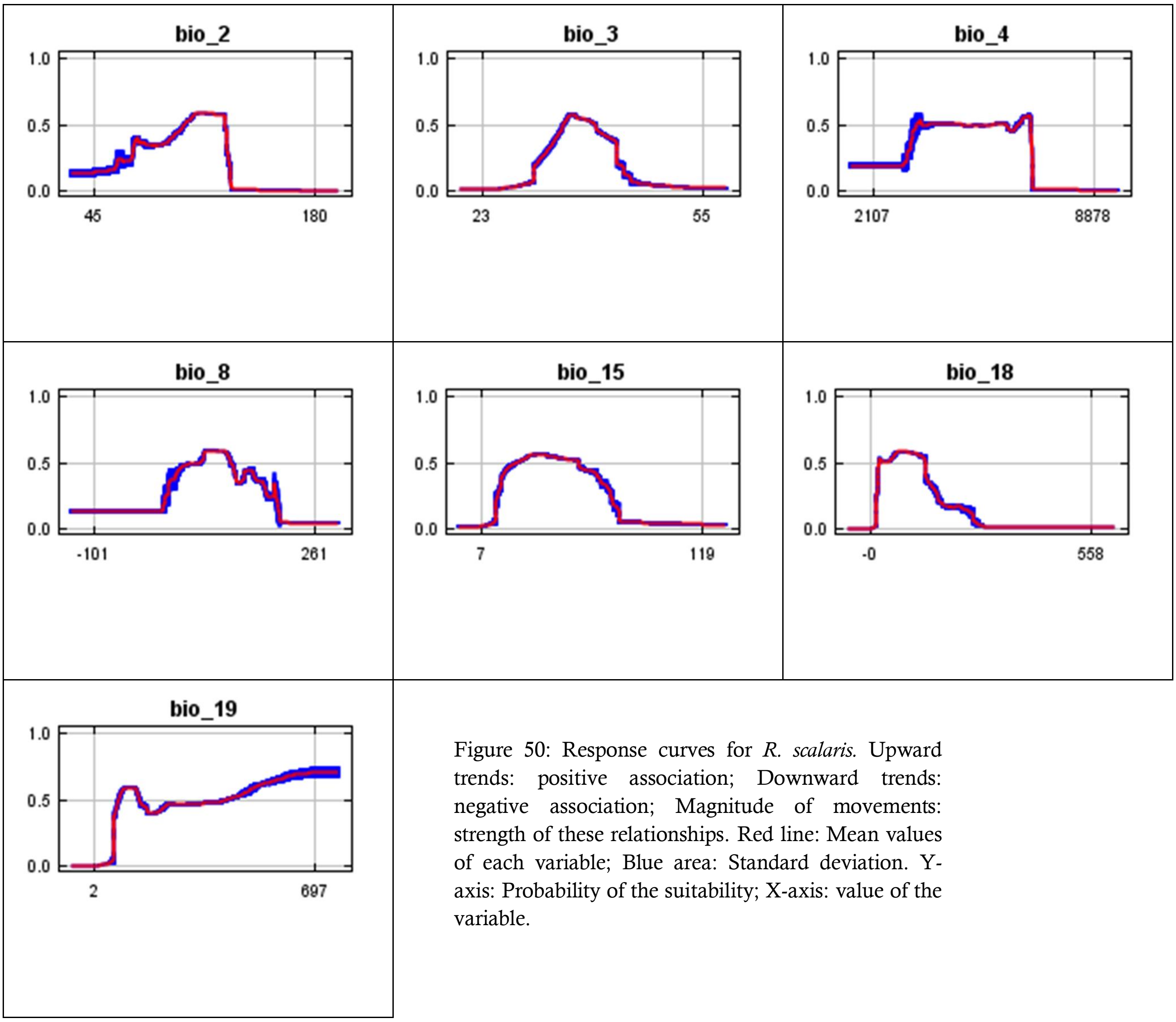


Figure 50: Response curves for *R. scalaris.* Upward trends: positive association; Downward trends: negative association; Magnitude of movements: strength of these relationships. Red line: Mean values of each variable; Blue area: Standard deviation. Y-axis: Probability of the suitability; X-axis: value of the variable.

In general, the results of response curves are concordant to the previous jackknife results, supporting the importance of precipitation of warmest quarter (bio18) to the explanation of the species distribution, more specifically being the lower values of precipitation the more responsible for that influence.

Suitability of Balearic Islands

In general, the Balearic Islands seem of limited suitability for the snake species, with the exception of Ibiza and some parts of Mallorca. Significantly, some known occurrence points from each species are found both in predicted suitable and unsuitable areas (Figure 51 and Figure 52).

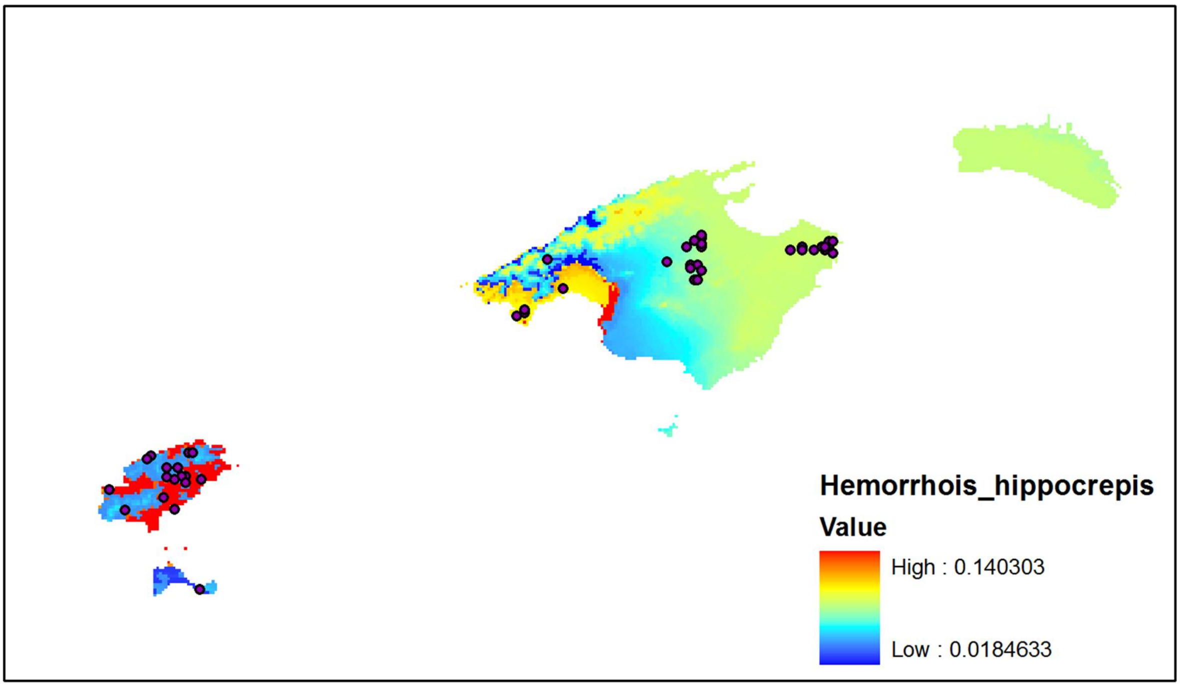
In the projections for the future, Balearic Islands become, gradually, more suitable for the all the species, with exception of the smooth false snake (*M. mauritanicus*) which only might occupy Formentera, south of Mallorca and Cabrera (Figures 53 to 76). Both *R. scalaris* and *M. monspessulanus* have the higher suitability probabilities and might occupy almost all the extension of all the islands. The horseshoe whip snake (*H. hippocrepis*) might be widespread on the island with exception of the Serra Tramuntana mounting range.

When comparing the emission scenarios (A1b, A2a, B2a), the A2a is that with more drastic scenarios, revealing in general a lower suitable area for all the four species and a major impact of climate change. Within the models generated with this scenario, all of them respect an interesting pattern, a decrease of suitability from 2020 to 2050 with a posterior increase to 2080. The A1b and B2a models reveal, in general, appreciable values of suitability for all the species but with slight differences between them (Table 8).

Table 8: Percentage of suitable habitat for each species in Balearic Islands, each scenario and all the years adopted for the study.

Scenario	Year	*H. hippocrepis*	*M. mauritanicus*	*M. monspessulanus*	*R. scalaris*
A1b	Present	0	0	0	0
	2020	88.35	18.31	99.87	99.84
	2050	89.85	21.71	98.87	98.26
	2080	63.38	11.27	97.18	97.18
A2a	Present	0	0	0	0
	2020	76.67	0.13	97.29	99.38
	2050	74.69	0.00	91.34	88.74
	2080	96.39	14.87	99.23	99.23
B2a	Present	0	0	0	0
	2020	87.54	23.92	99.85	99.85
	2050	88.96	23.99	99.80	99.82
	2080	91.56	28.10	98.95	97.17

For *H. hippocrepis*, the model using the A1b scenario predicts a suitable area relatively stable through the years of 2020 and 2050 with a posterior loss of 26.5% of it in 2080, becoming Ibiza an unsuitable island for the species. The A2a model shows a different result with the loss of 2% of suitable area from 2020 to 2050 and a gain of 21.7% until 2080. Lastly, the model using the B2a scenario predicts a continuous increase of suitable area since 2020 (87.5%) to 2080 (91.6%) (Table 8). In all the scenarios, the species is able to occupy most of the islands, except the Serra Tramuntana in Mallorca (Figures 53-58).



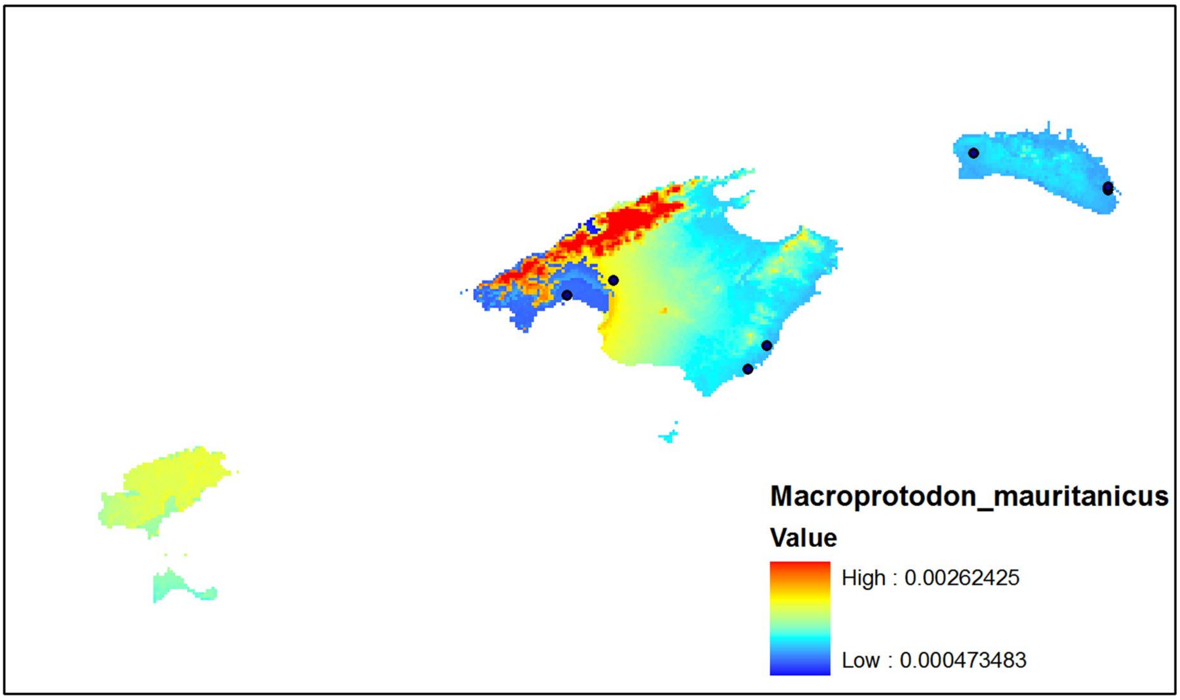
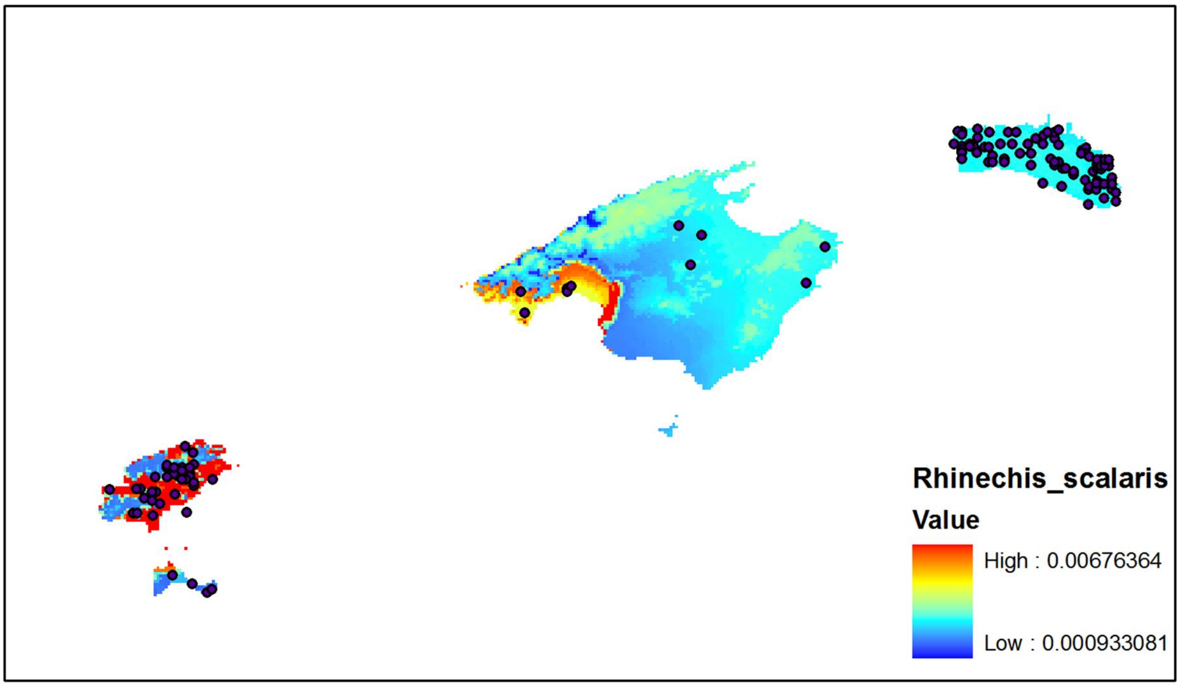


Figure 51: Prediction of the probability for the present of the species *H. hipprocrepis* and *M. mauritanicus.* Presence records are also presented. Values of probability are probabilities between 0 and 1.



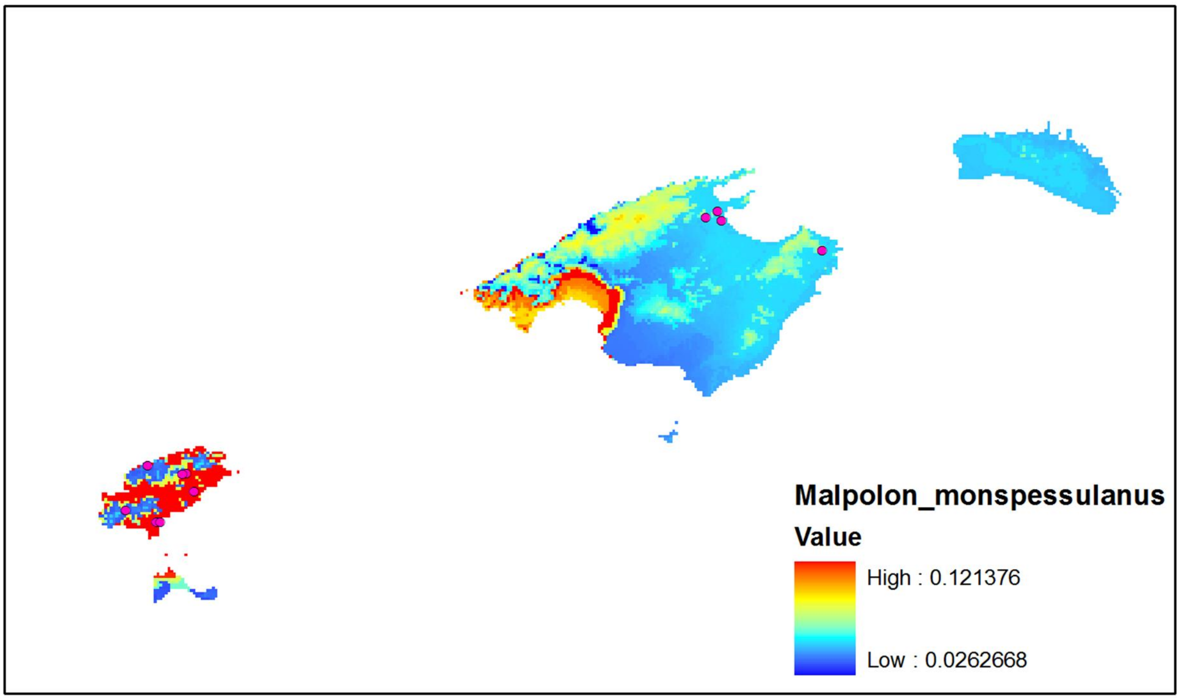


Figure 52: Prediction of the probability for the present of the species *R. scalaris* and *M. monspessulanus.* Presence records are also presented. Values of probabilities are probabilities between 0 and 1.

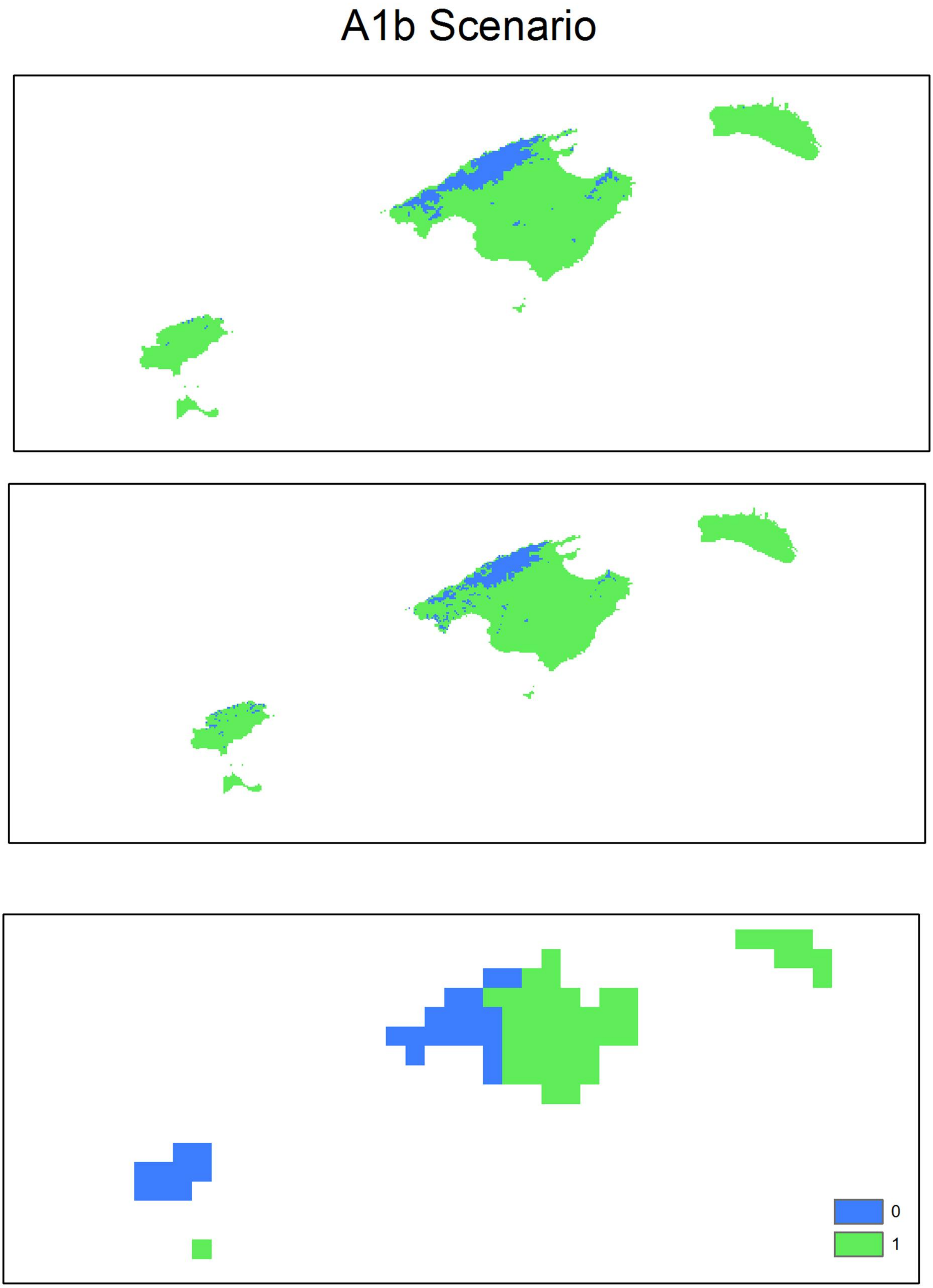


Figure 53: A1b scenario habitat suitability model for the species *H. hippocrepis*. Top: 2020; Middle: 2050; Bottom: 2080.

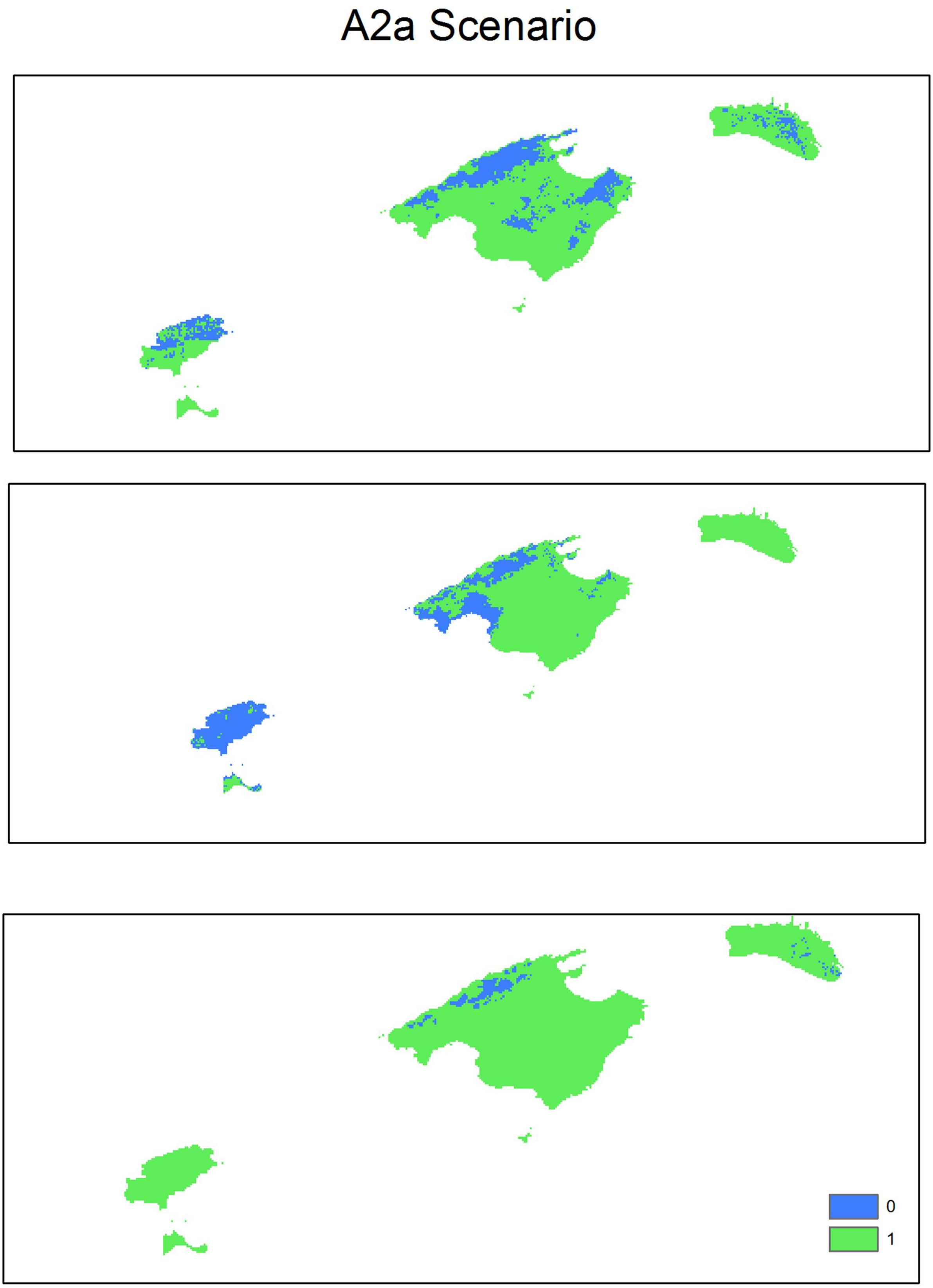


Figure 54: A2a scenario habitat suitability model for *H. hipprocrepis.* Top: 2020; Middle: 2050; Bottom: 2080.

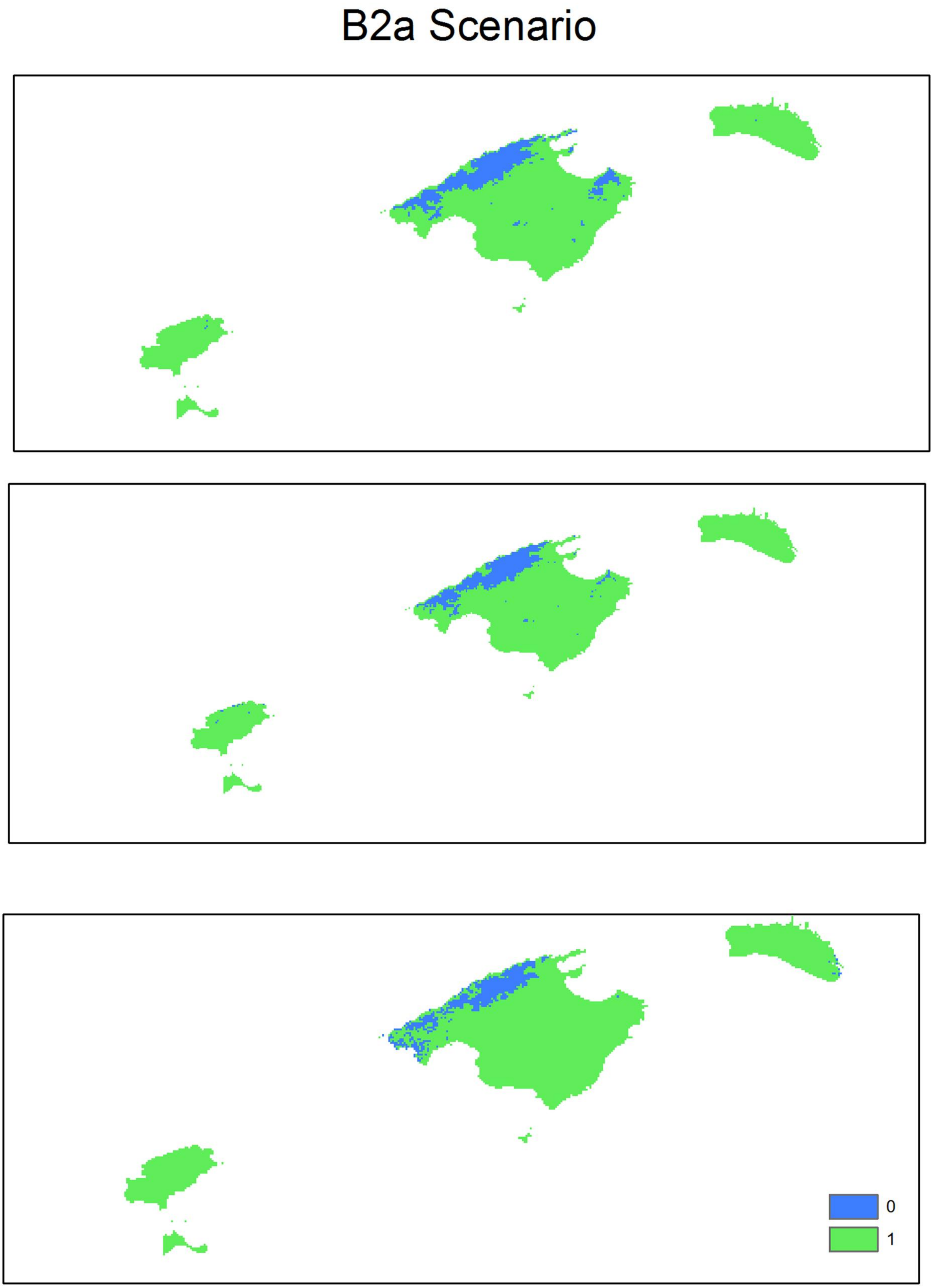


Figure 55: B2a scenario habitat suitability model for *H. hipprocrepis.* Top: 2020; Middle: 2050; Bottom: 2080.

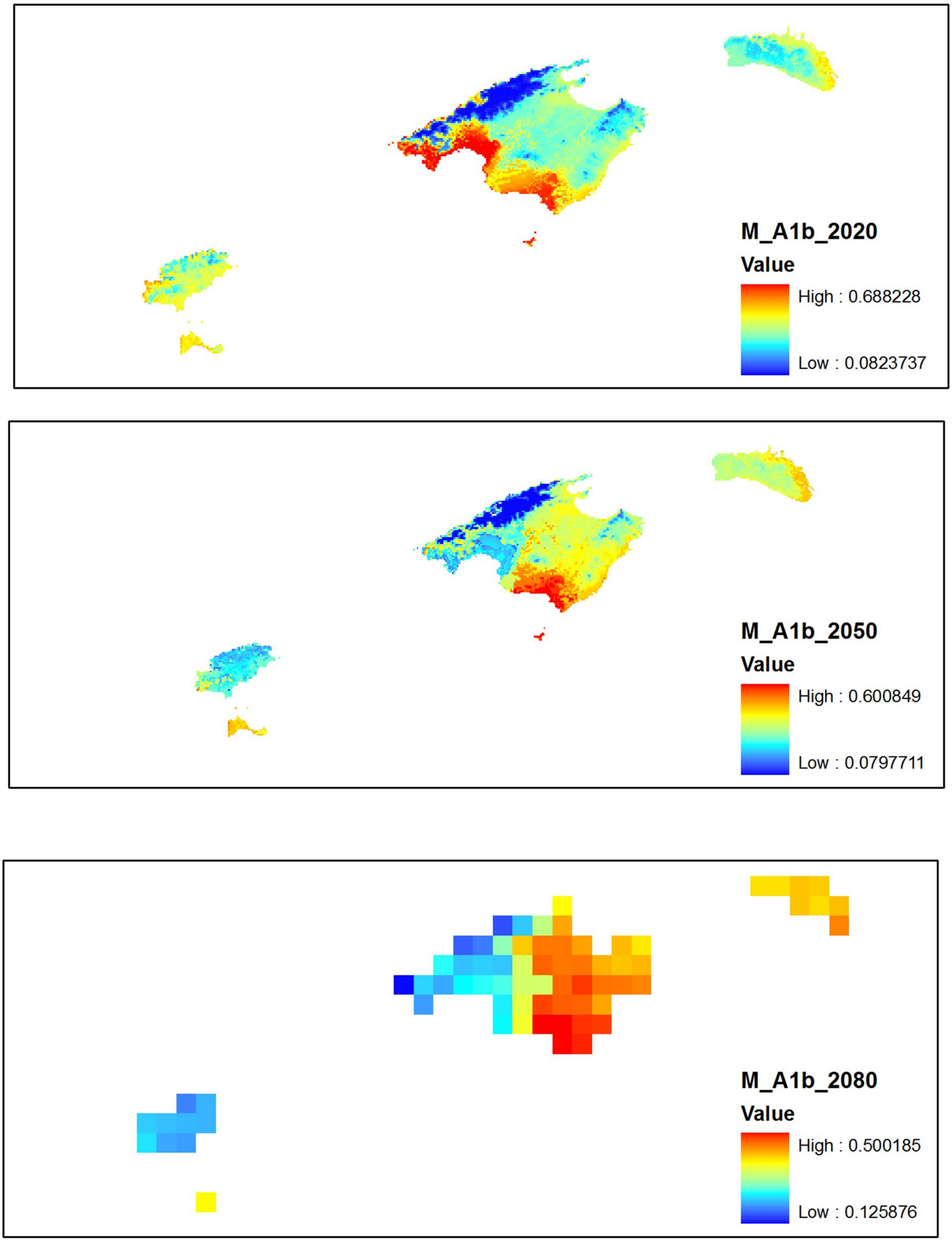
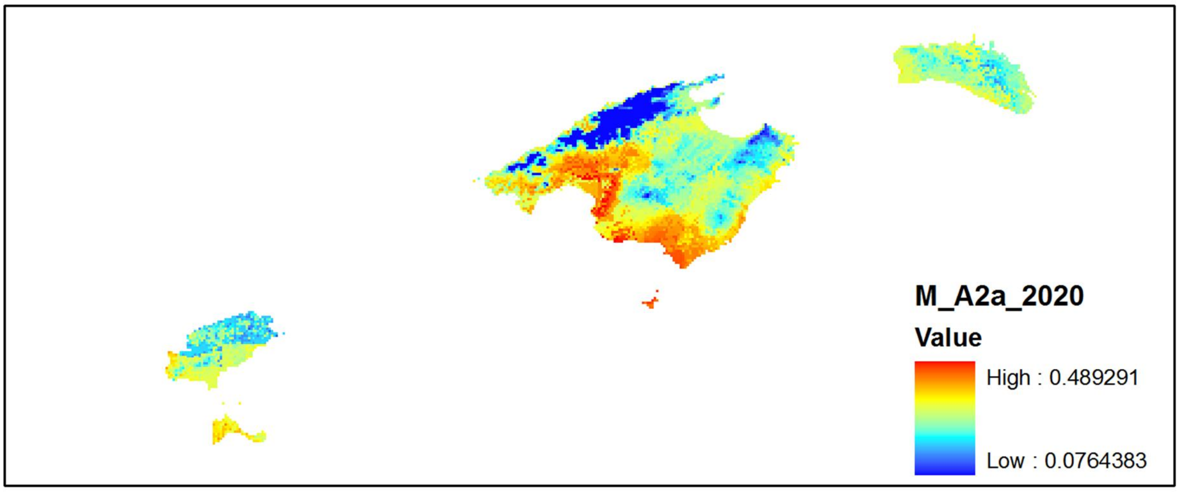
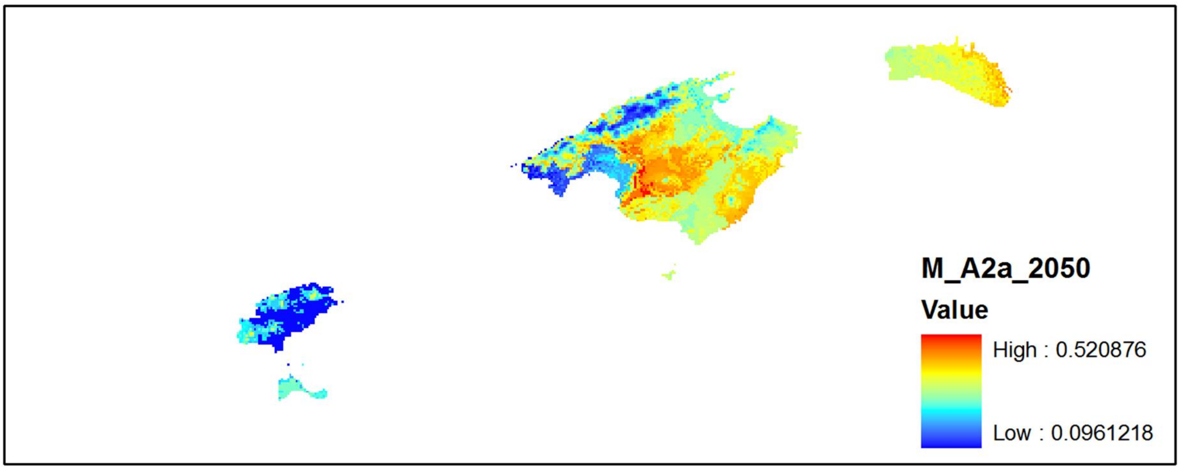


Figure 56: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A1b and for *H. hippocrepis.*





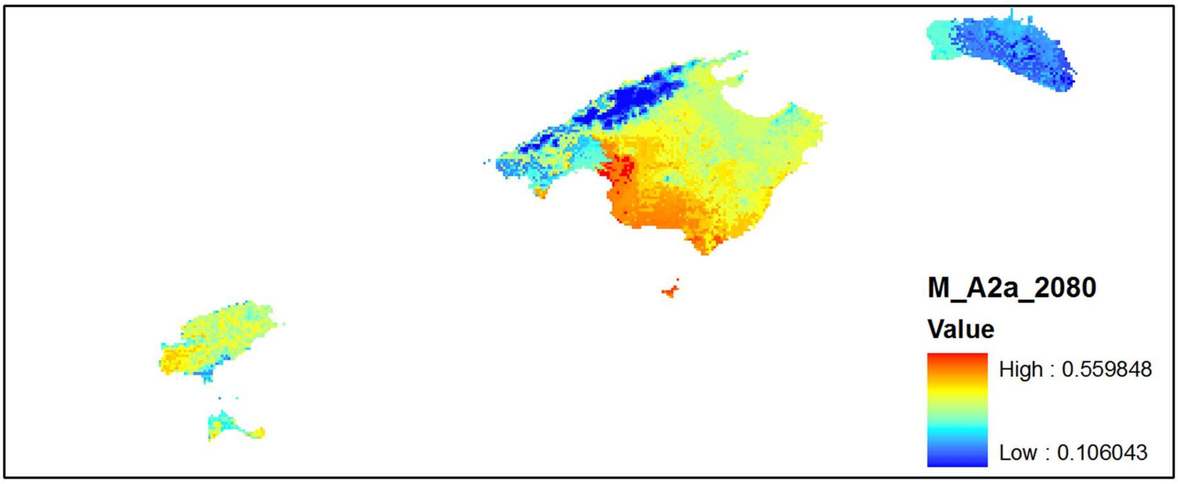


Figure 57: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A2a and for *H. hippocrepis.*

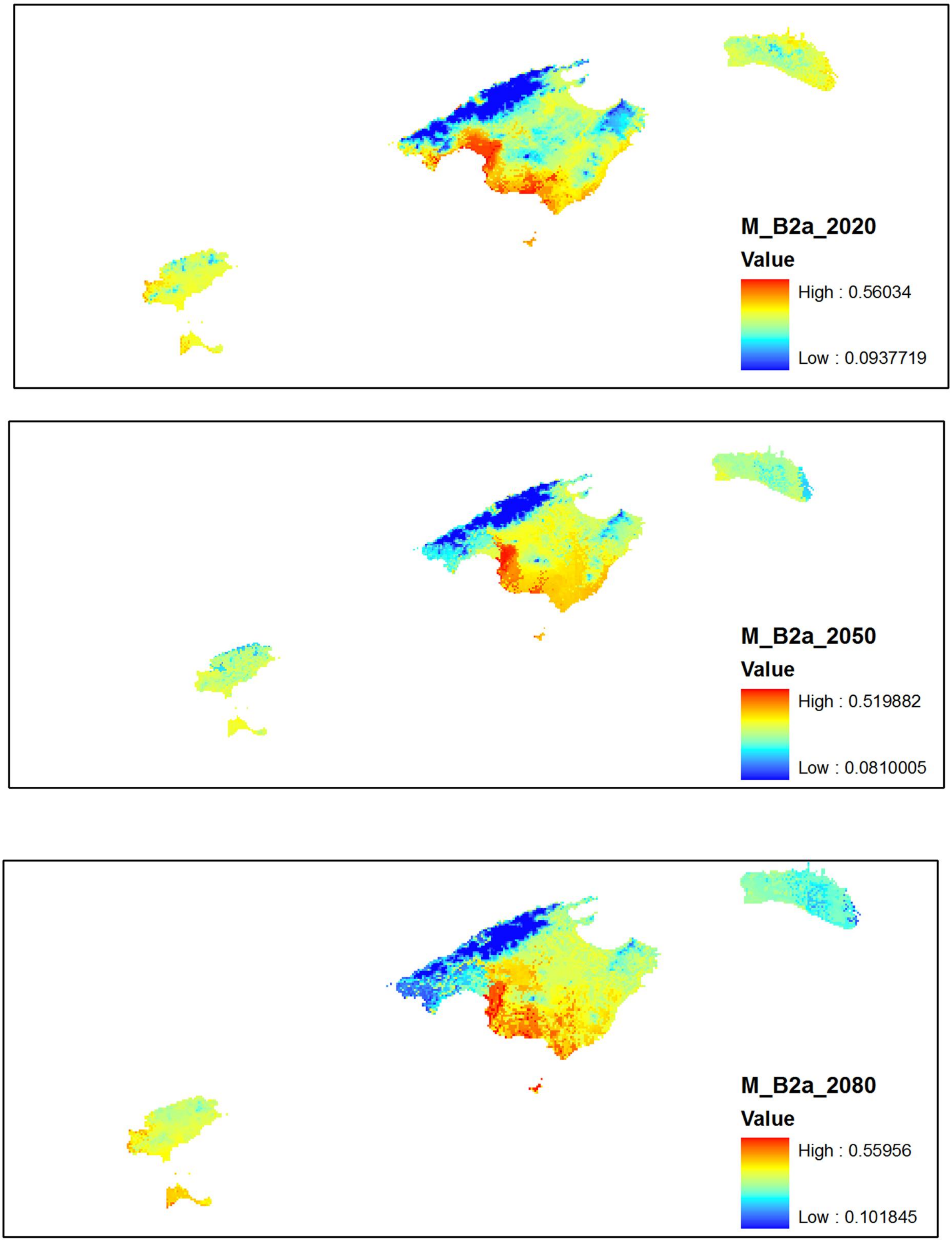


Figure 58: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario B2a and for *H. hippocrepis.*

For *M. monspessulanus*, all the scenarios predict a large suitable area along the time scale. Both the A1b and B2a model scenarios show a decreasing of suitable habitat through the years, with a 97%/98% of area in 2080. Again, the A2a model scenario project a different result from the previous ones with a loss of 6% of suitable area in 2050 and a posterior recuperation of that loss in 2080 (Table 8). In all the scenarios, the species can occupy almost all the extension of all the islands, except Ibiza Island in the A2a-2050 model (Figures 59 to 64).

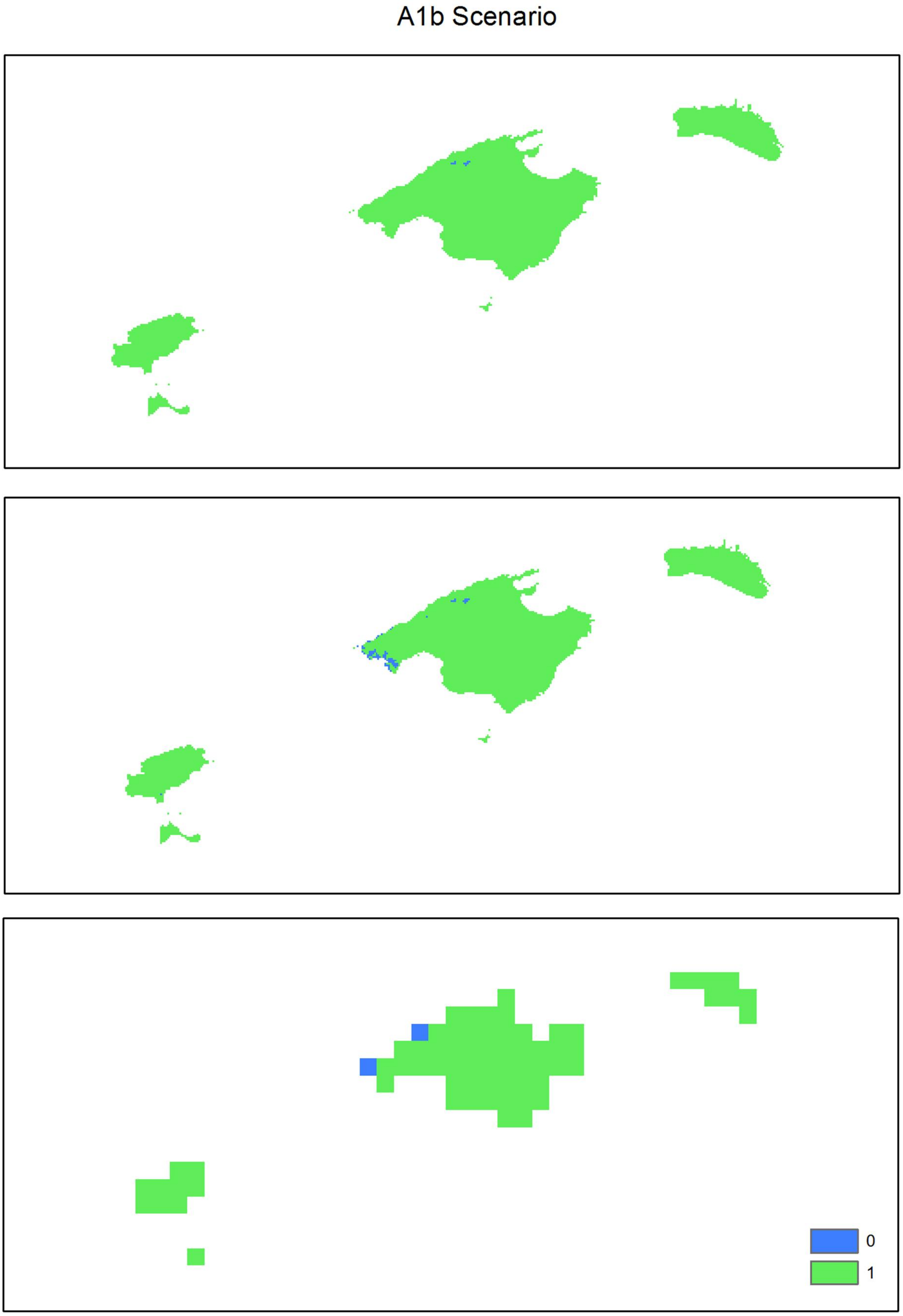


Figure 59: A1b scenario habitat suitability model for the species *M. monspessulanus.* Top: 2020; Middle: 2050; Bottom: 2080.

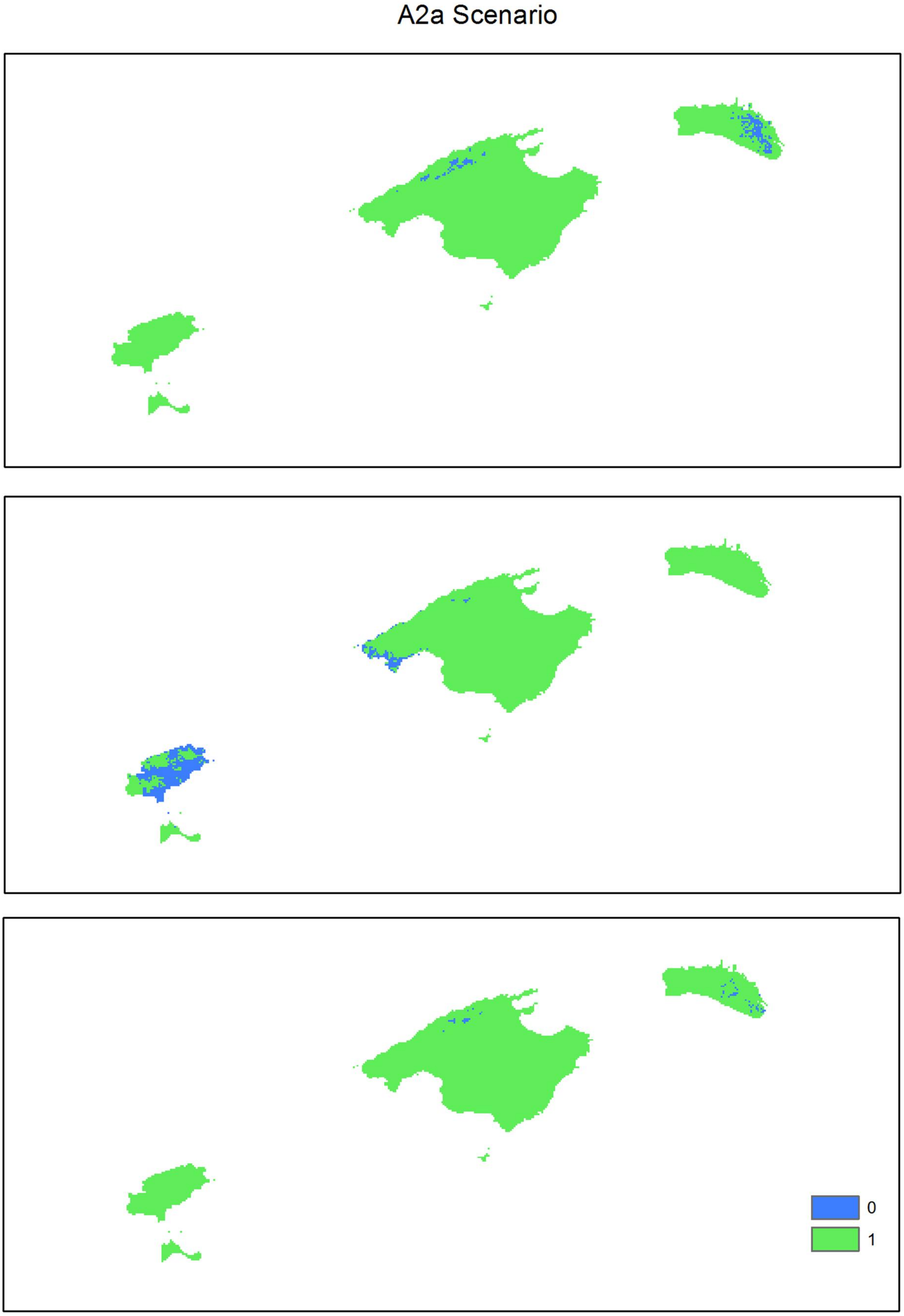


Figure 60: A2a scenario habitat suitability model for *M. monspessulanus*. Top: 2020; Middle: 2050; Bottom: 2080.

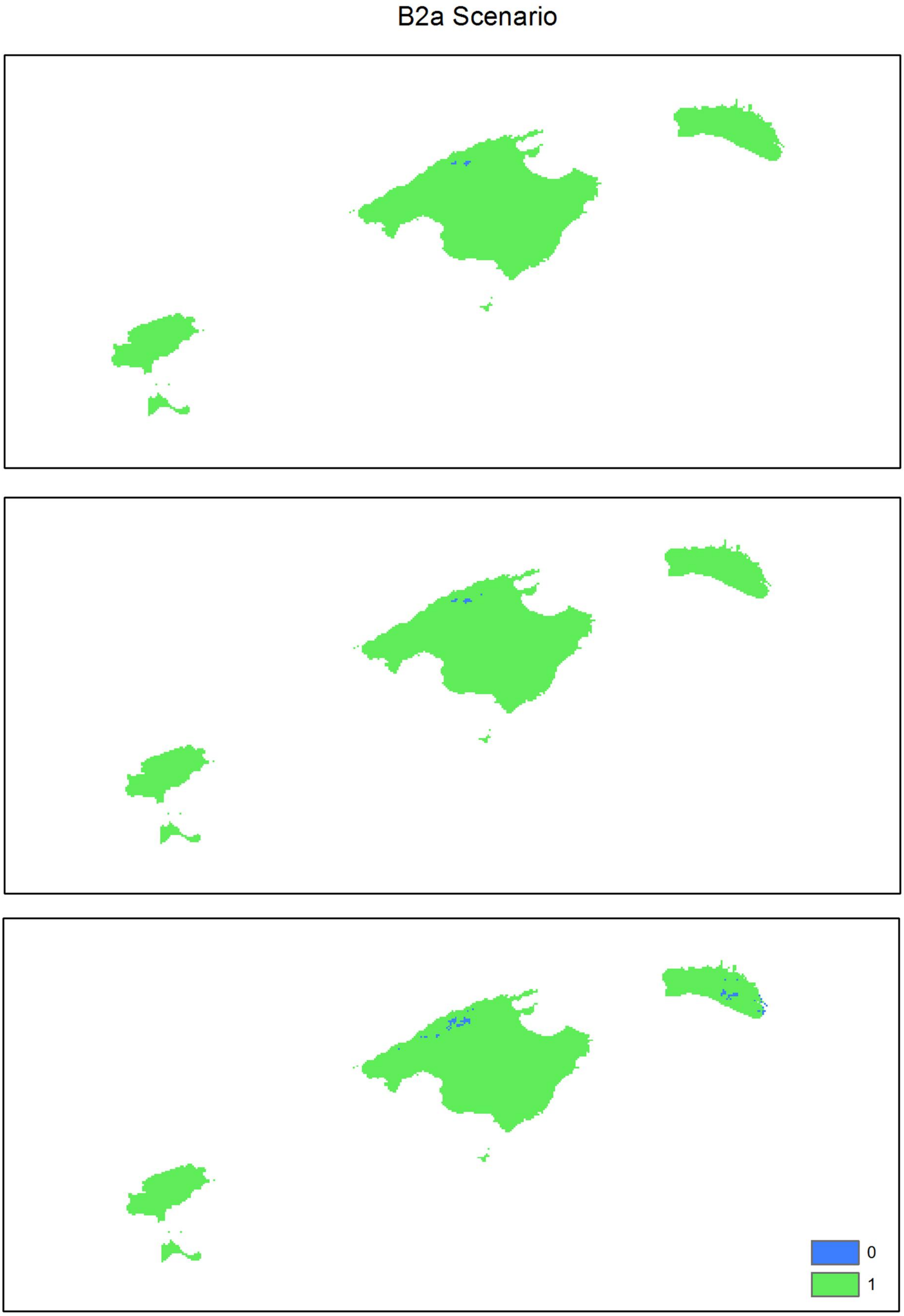


Figure 61: B2a scenario habitat suitability model for *M. monspessulanus.* Top: 2020; Middle: 2050; Bottom: 2080.

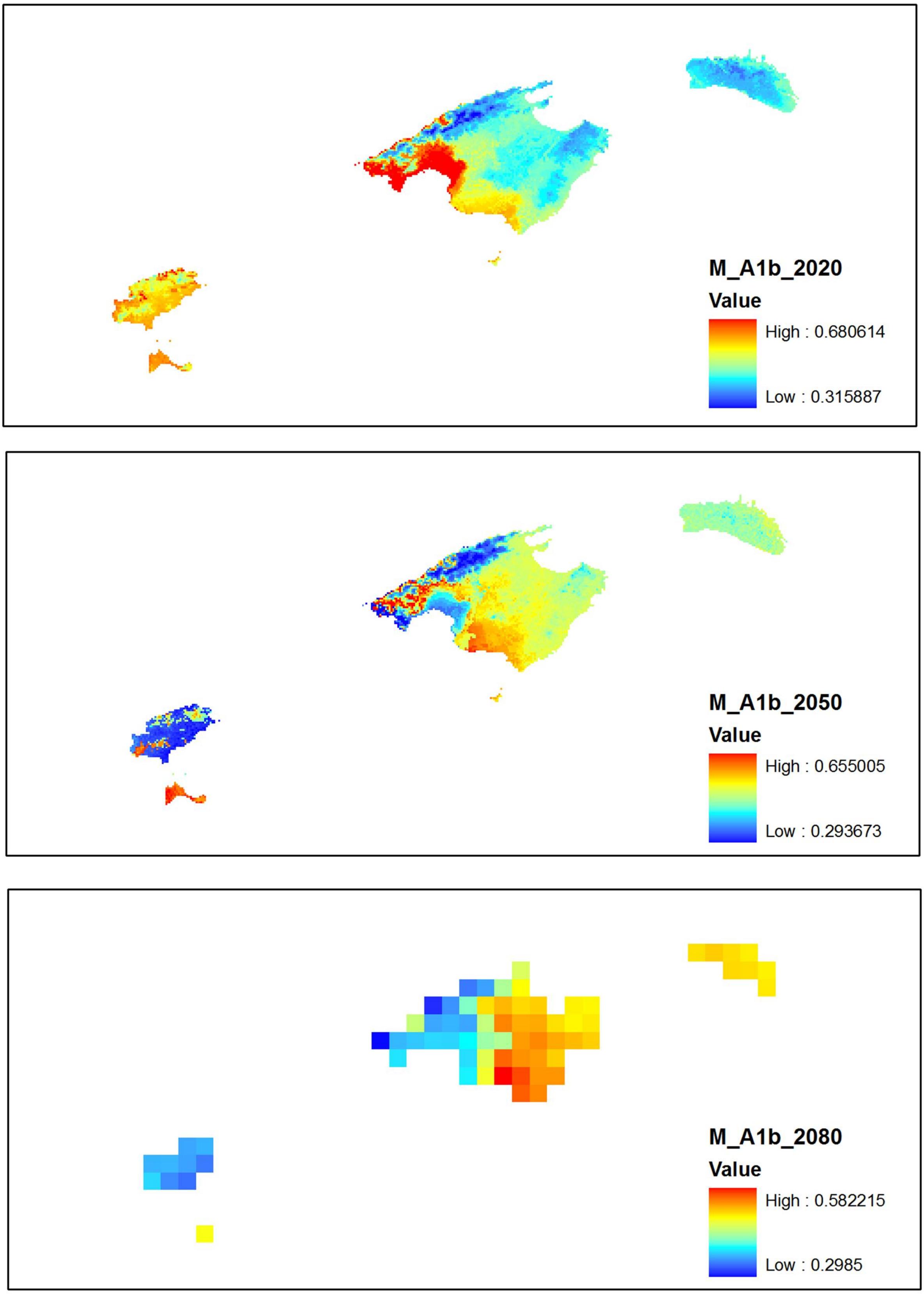


Figure 62: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A1b and for *M. monspessulanus*.

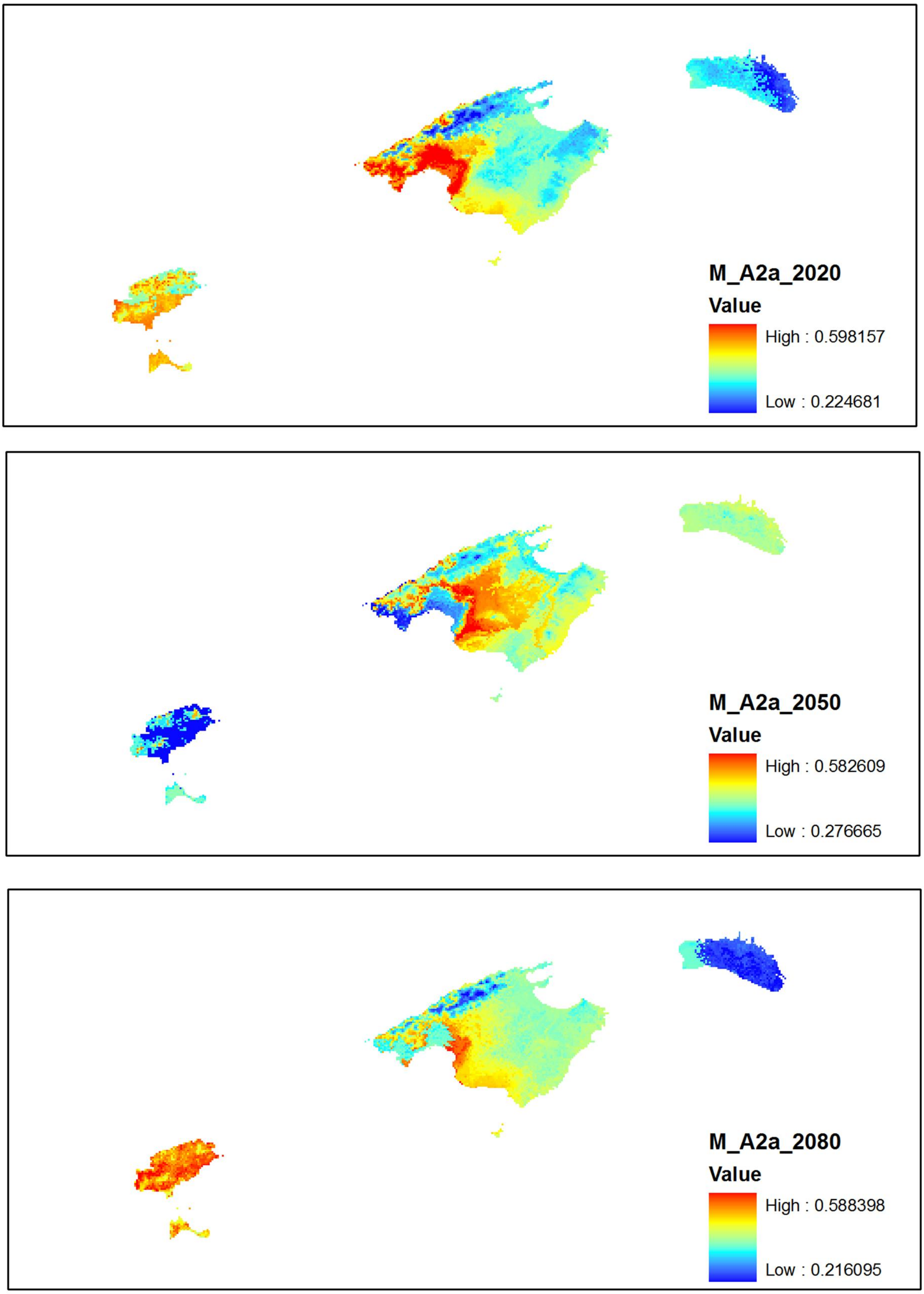


Figure 63: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A2a and for *M. monspessulanus*.

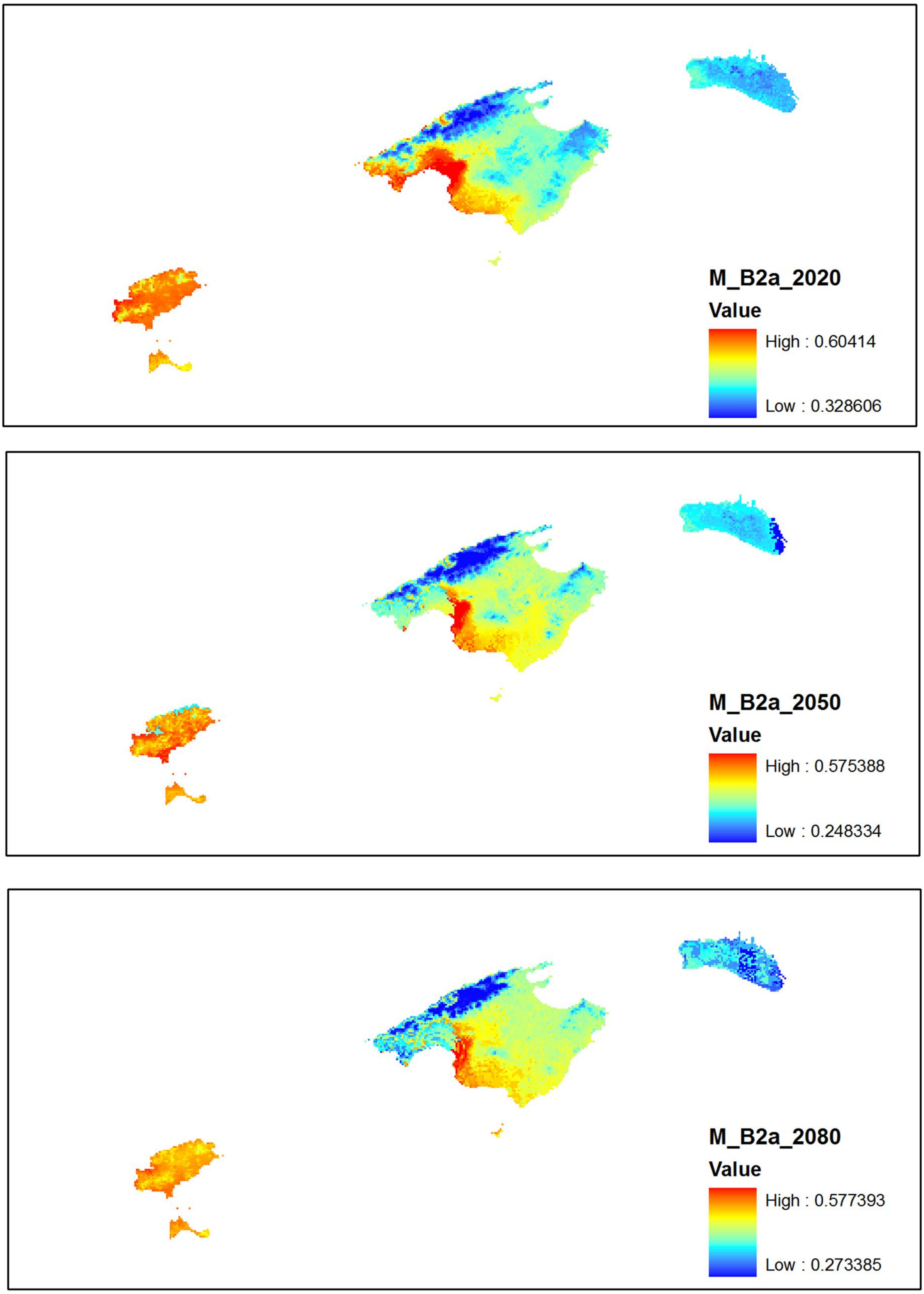
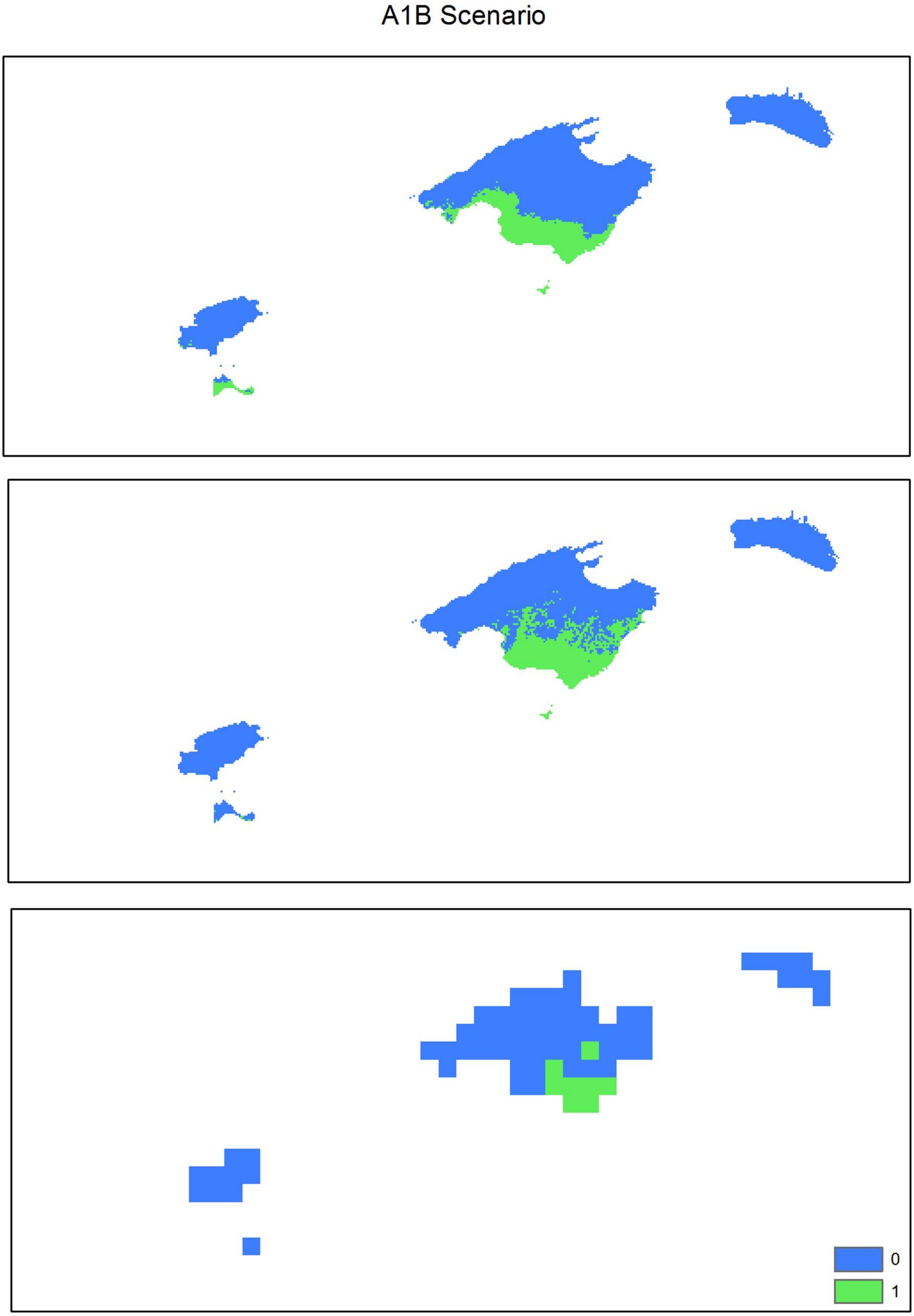


Figure 64: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario B2a and for *M. monspessulanus*.

For *M. mauritanicus*, all the scenarios predict a restricted distribution. The A2a model scenario is the one that reveals the lower suitable area, being close to 0% in 2020 and 2050 and increasing to 14% in 2080. The models using A1b and B2a scenarios show opposite results. Indeed, in A1b model, it is possible to observe an increasing of suitable area from 2020 to 2050 with a posterior decrease until 2080, whereas in the B2a model a decrease is clear during the same time with a posterior increase (Table 8). The suitable area projected is very restricted to some parts of Mallorca Island, Cabrera and Ibiza (Figures 65 to 70).



A1B Scenario

Figure 65: A1b scenario habitat suitability model for *Macroprotodon sp.* Top: 2020; Middle: 2050; Bottom: 2080.

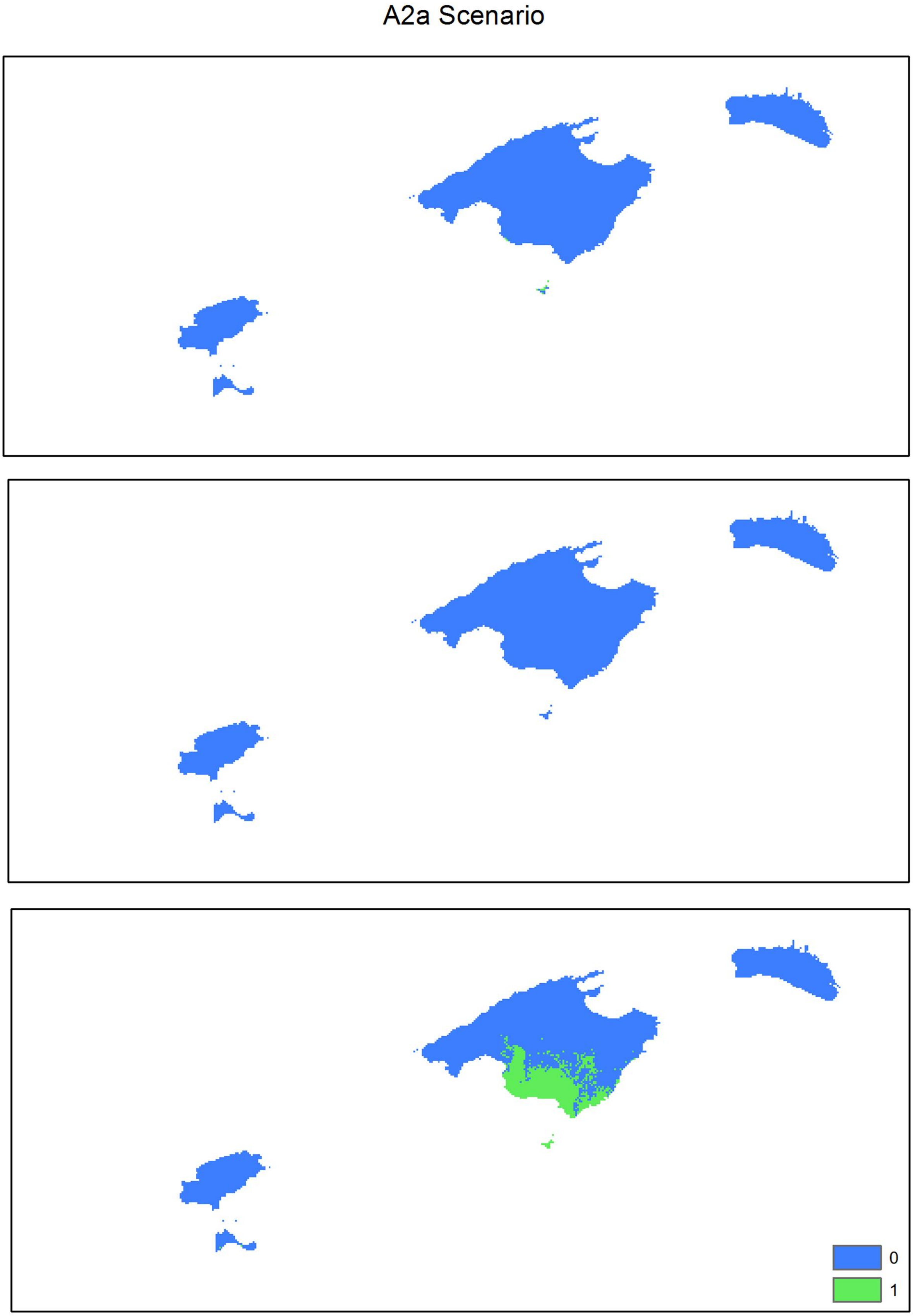


Figure 66: A2a scenario habitat suitability model for *Macroprotodon sp.* Top: 2020; Middle: 2050; Bottom: 2080.

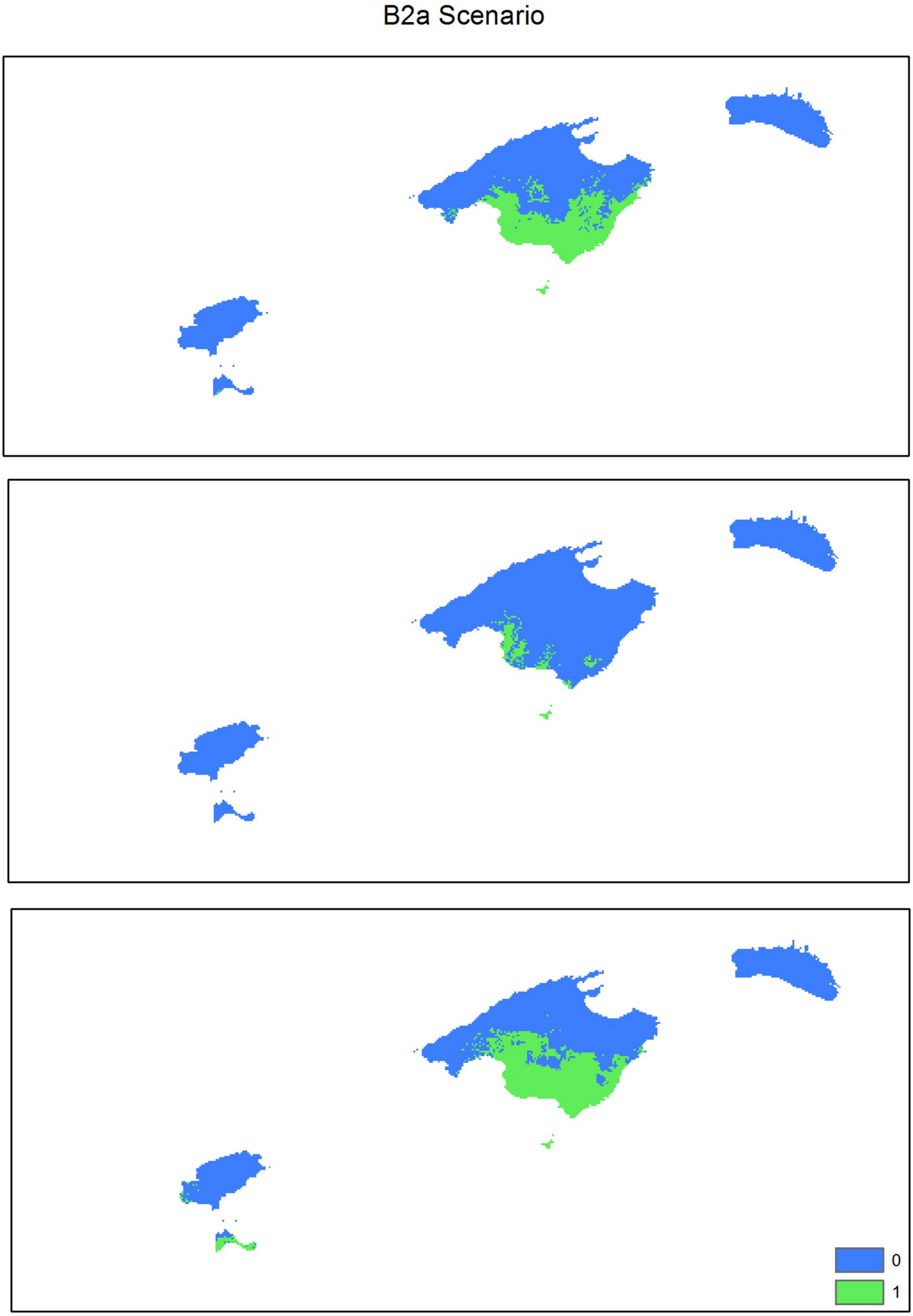


Figure 67: B2a scenario habitat suitability model for *Macroprodon sp.* Top: 2020; Middle: 2050; Bottom: 2080.

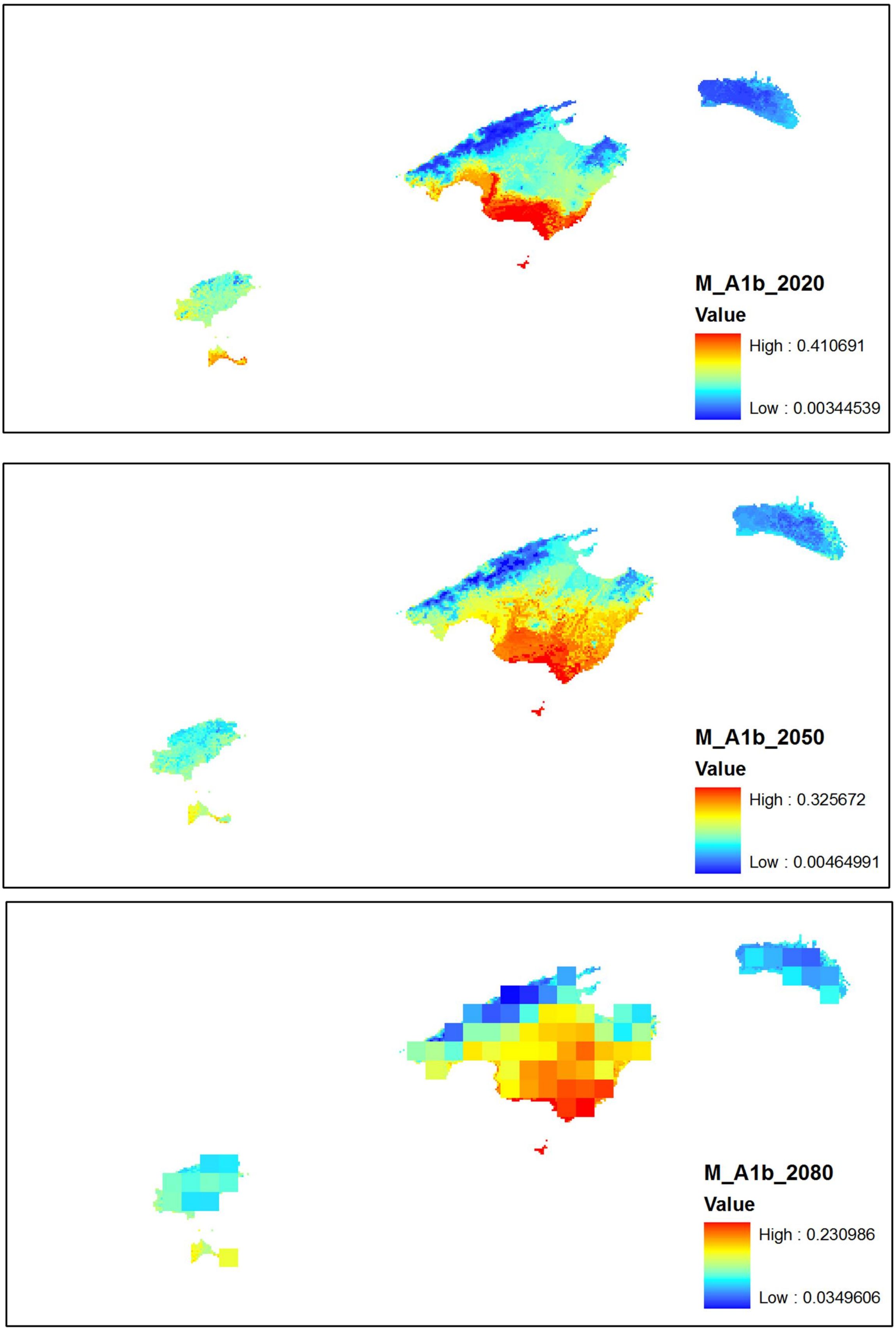


Figure 68: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A1b and for *Macroprotodon sp.*

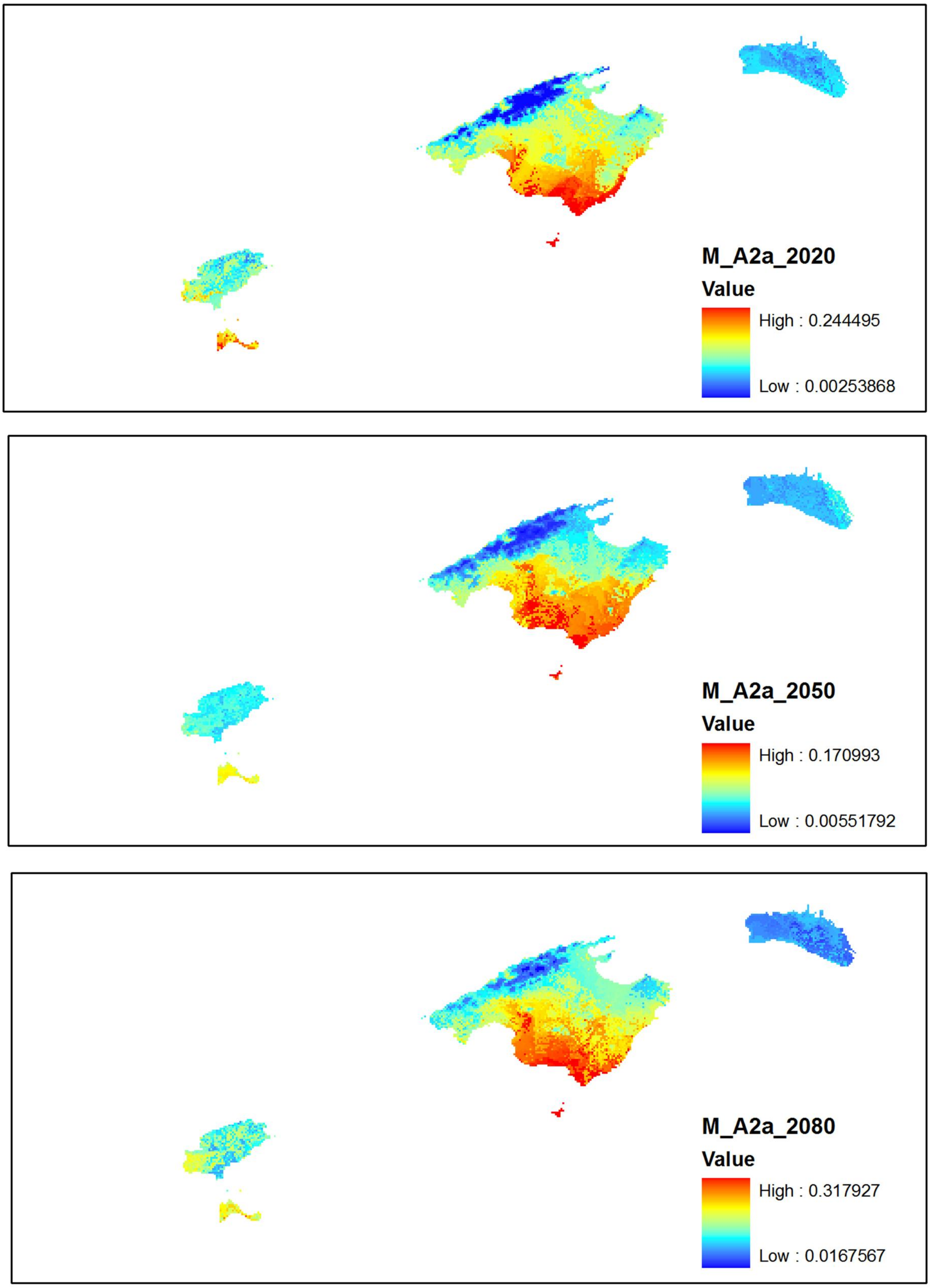


Figure 69: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A2a and for *Macroprotodon sp.*

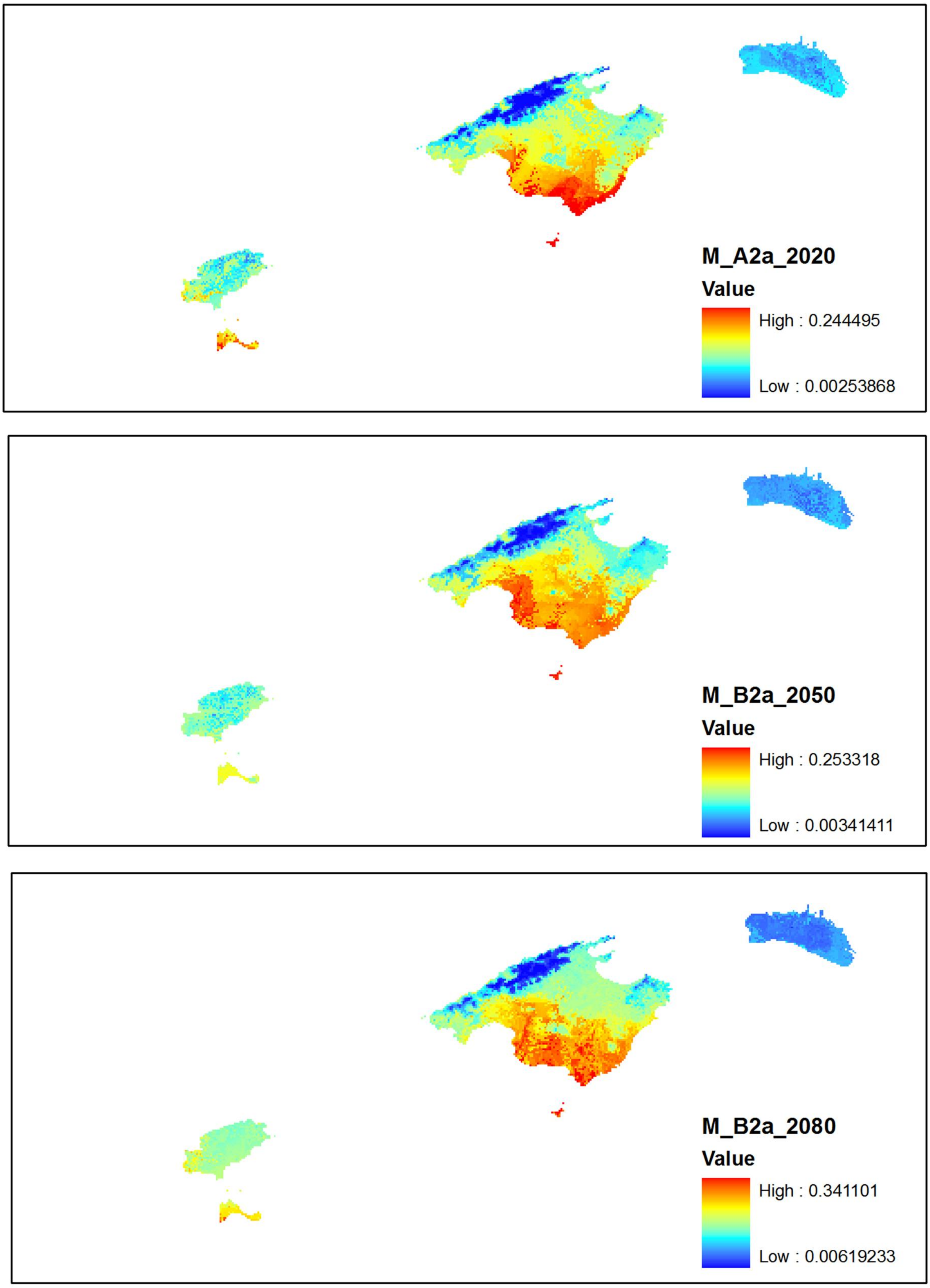


Figure 70: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario B2a and for *Macroprotodon sp*.

For *R. scalaris*, all the scenarios predict a large suitable area for the future. Indeed, the results obtained for this species follow the same pattern of the ones obtained for the Montpellier snake. The A1b and the B2a project a slight decrease of suitable habitat from 2020 to 2080, with a percentage of 97% in that last year. The A2a model predict a loss of habitat from 2020 to 2050 (10%) and a gain from 2050 to 2080 (10%) (Table8). Again, only in the model A2a-2050, the species is not able to occupy all the Balearic area (Figures 71 to 76).

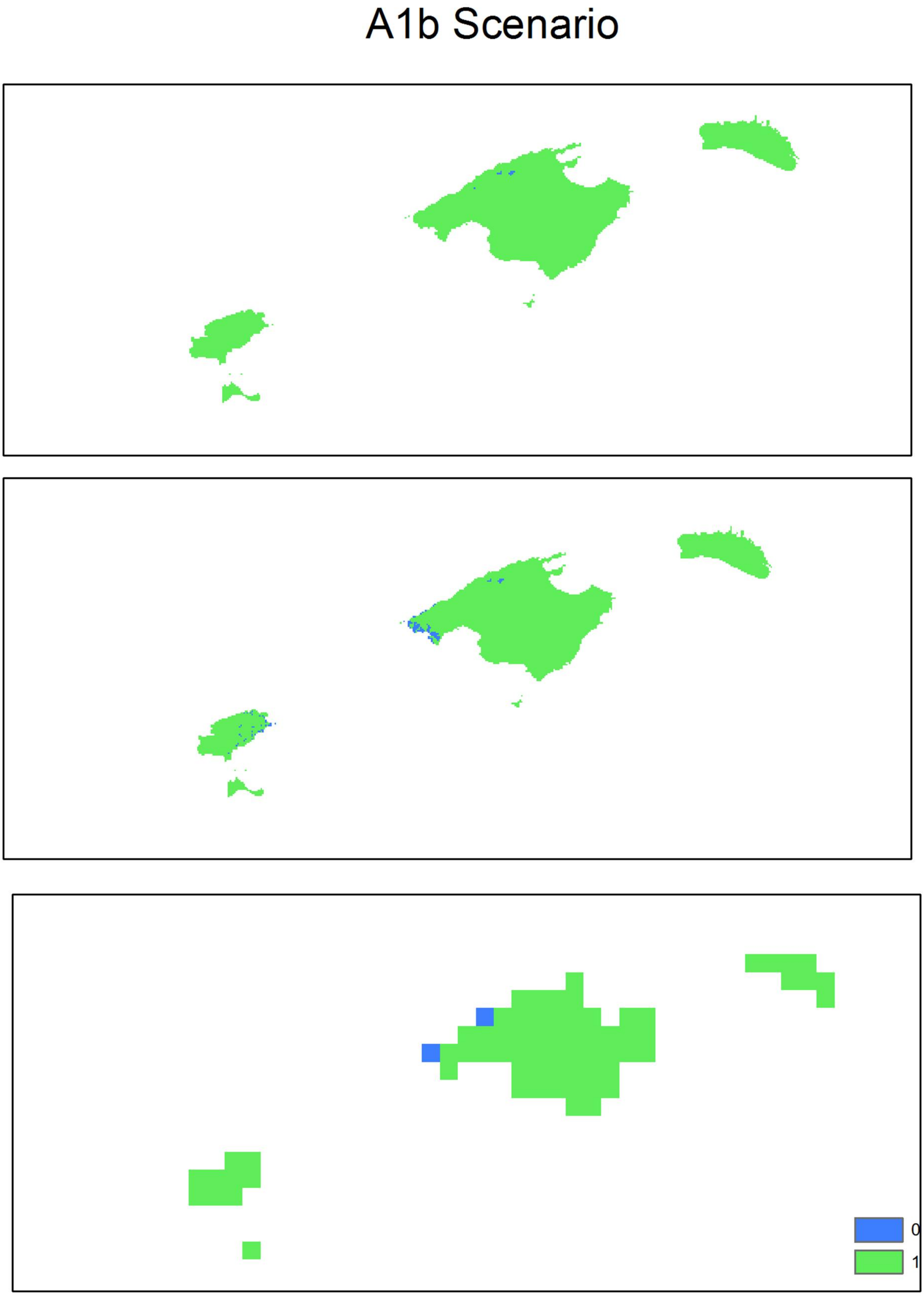


Figure 71: A1b scenario habitat suitability model for the species *R. scalaris.* Top: 2020; Middle: 2050; Bottom: 2080.

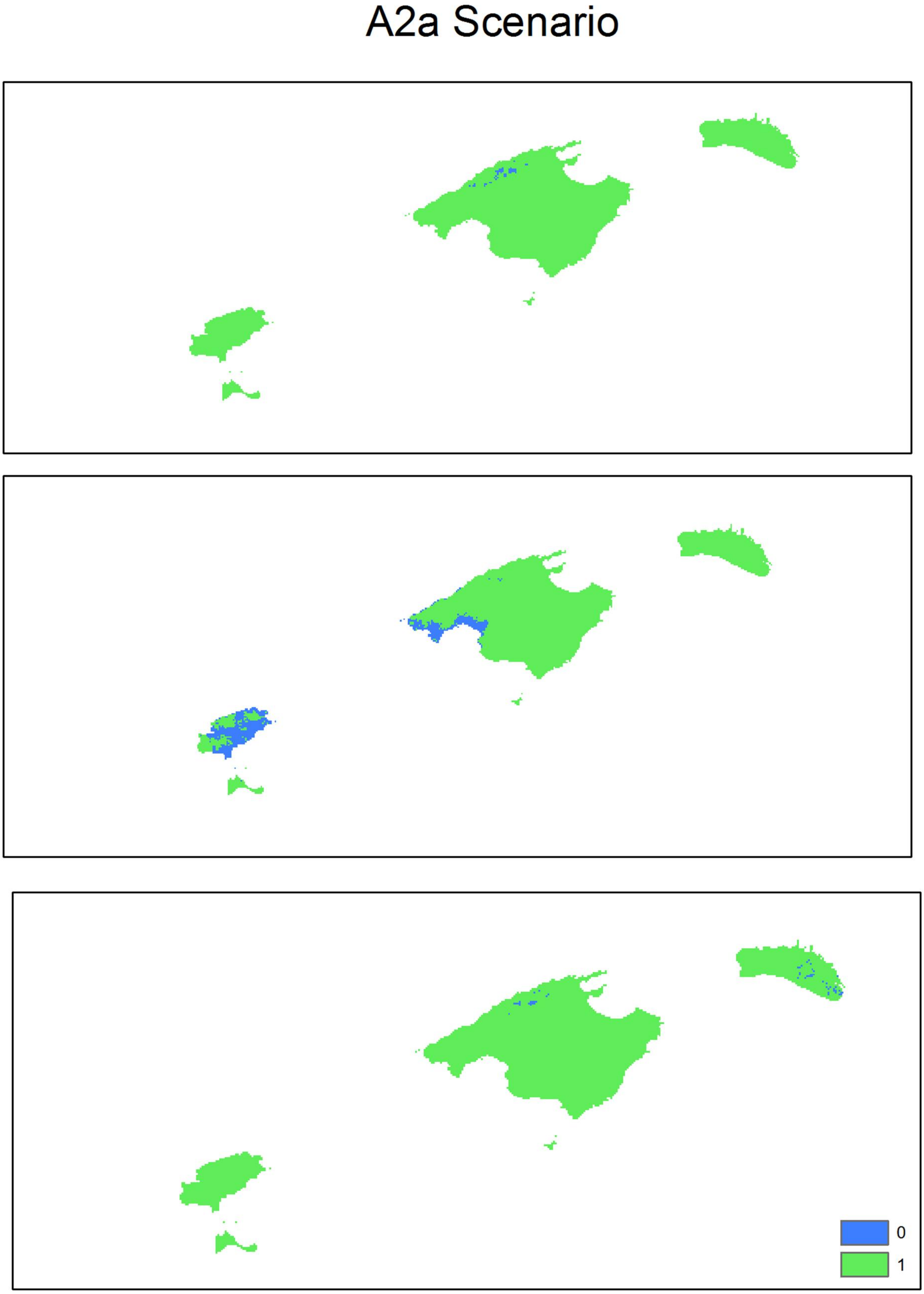


Figure 72: A2a scenario habitat suitability model for *R. scalaris.* Top: 2020; Middle: 2050; Bottom: 2080.

Figure 73: B2a scenario habitat suitability model for *R. scalaris.* Top: 2020; Middle: 2050; Bottom: 2080.

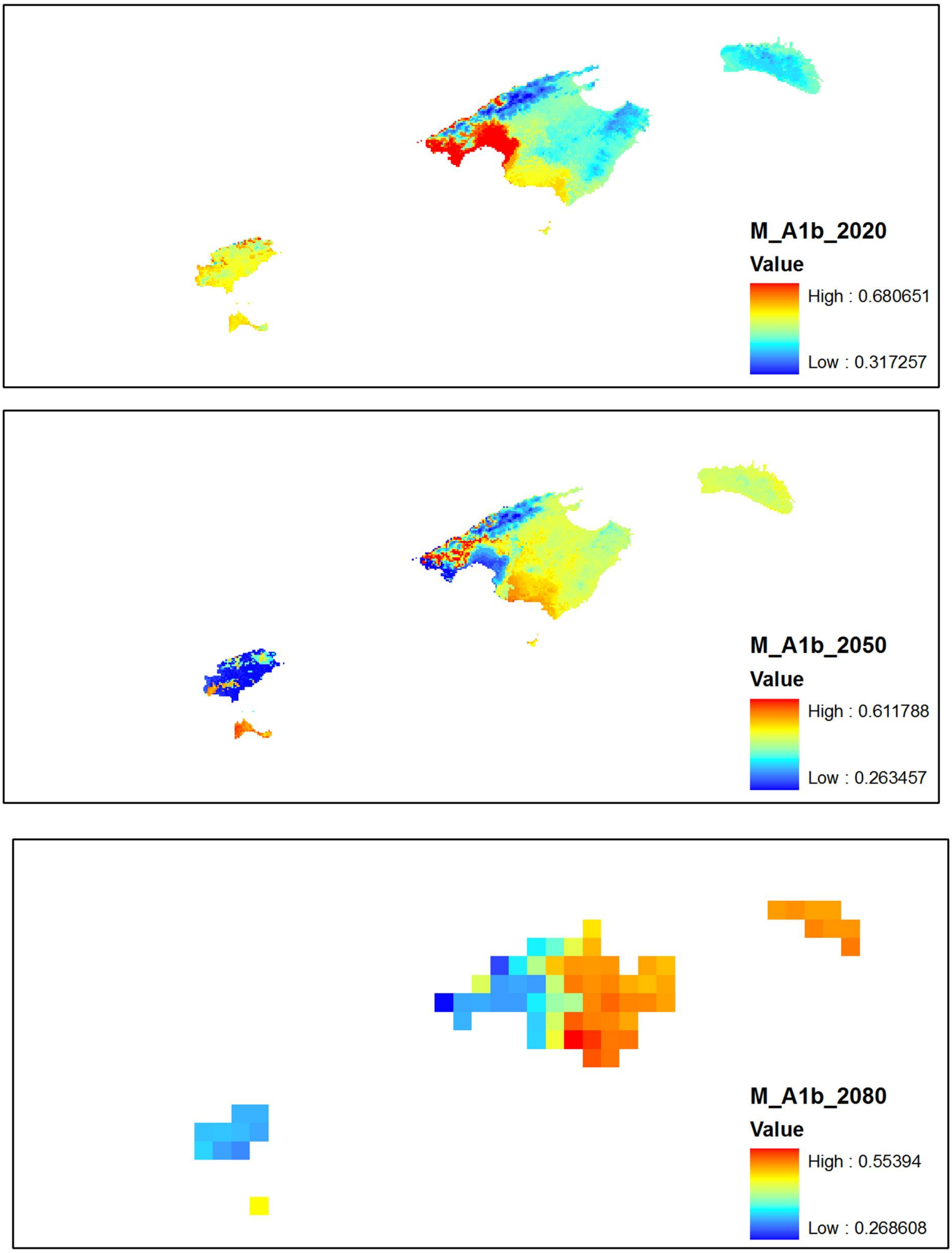


Figure 74: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A1b and for *R. scalaris.*

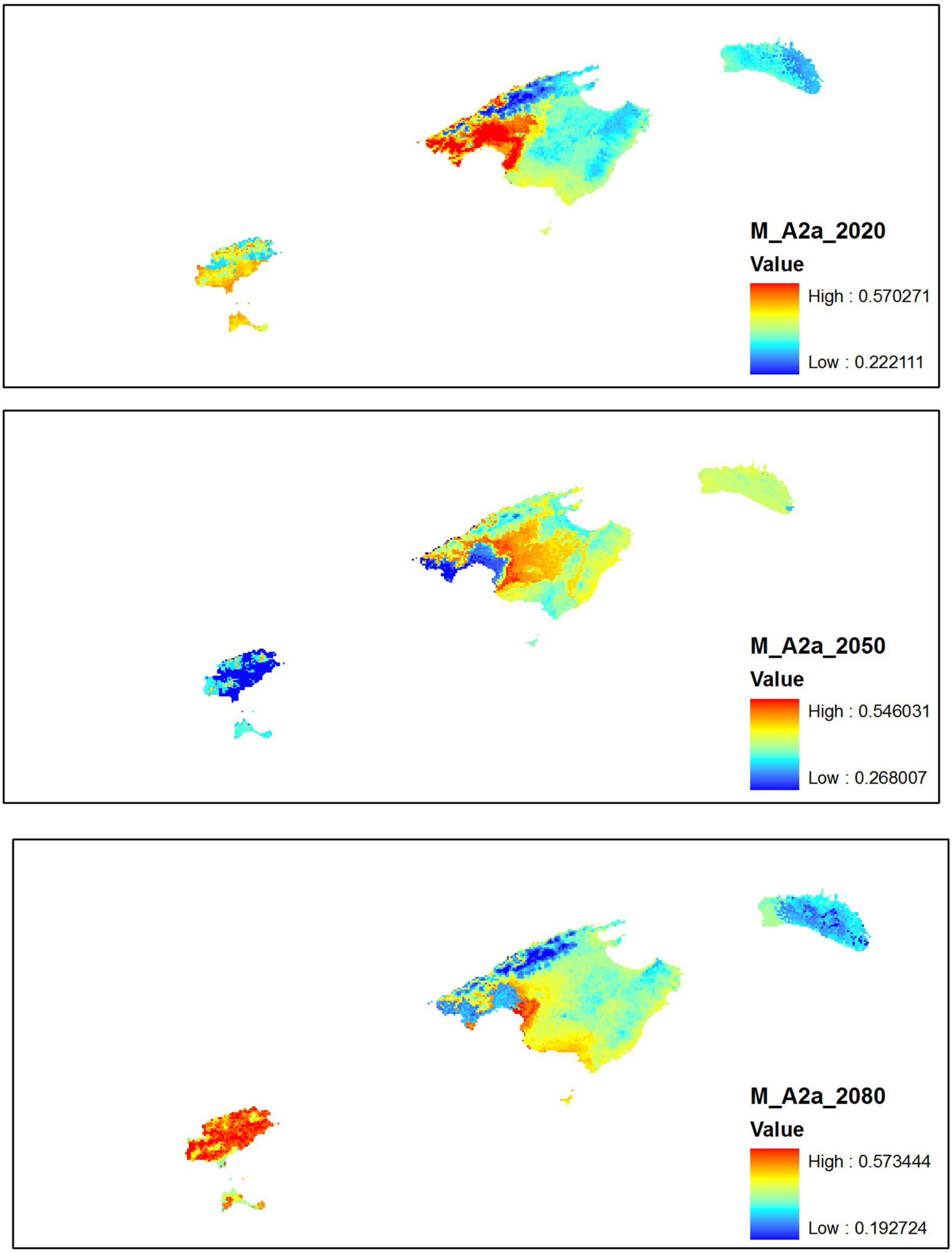


Figure 75: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario A2a and for *R. scalaris.*

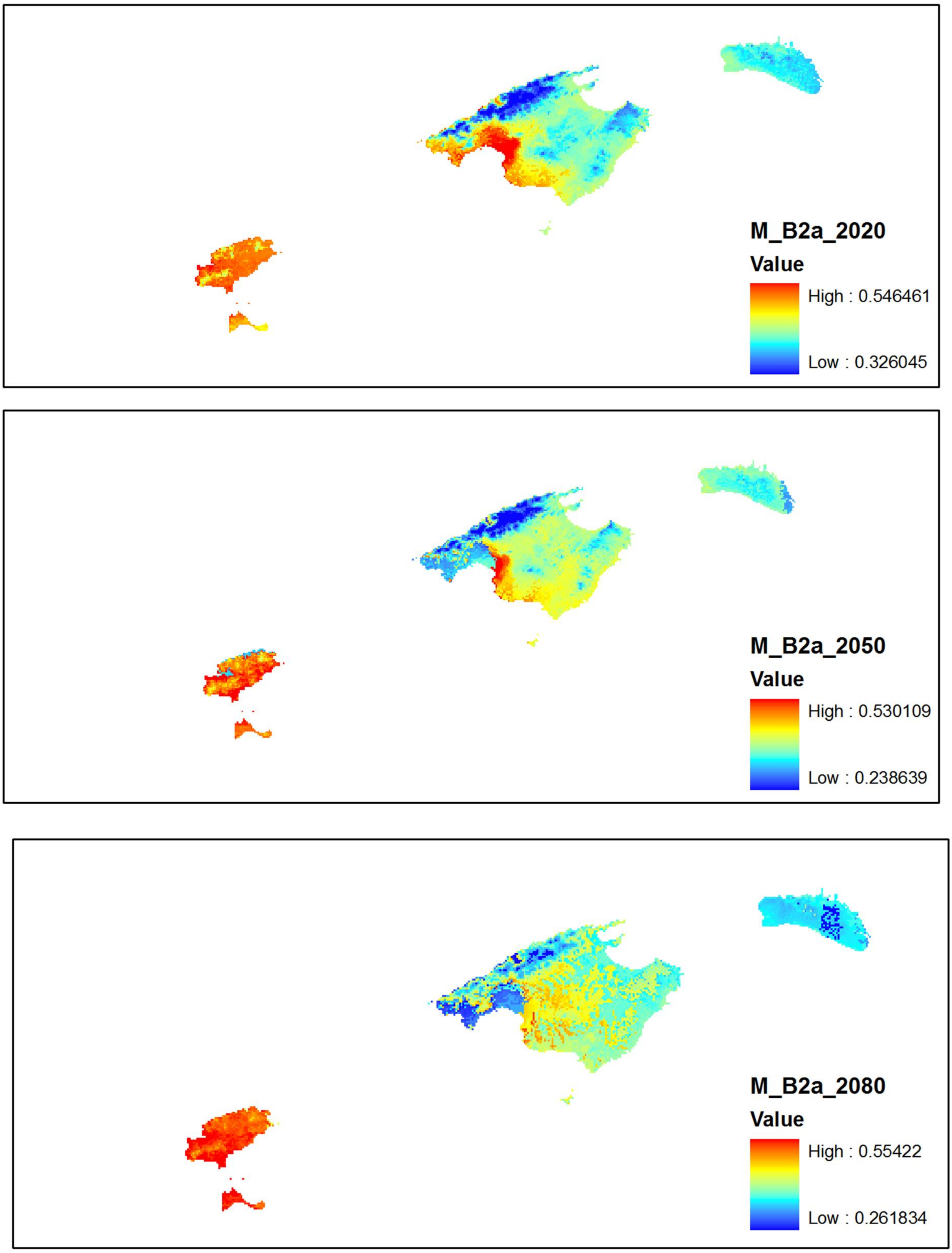


Figure 76: Mean of climate change scenarios (Hadcm3, CCCMA, CSIRO) models for the emission scenario B2a and for *R. scalaris*

4.3 Discussion

Are the Balearic Islands suitable?

As inferred from the occupation of their original ranges, in the present day, the global range of Balearic Islands seems generally of limited suitability for all the species. Indeed, only some areas of Mallorca and Ibiza currently harbour the approximate bioclimatic conditions that are adequate for the species.

Nevertheless, when comparing the models' projection with known presence points, it immediately become evident that some points are within unsuitable areas. For the false smooth snake (*M. mauritanicus*), introduced in historical times, a wide distribution both in Mallorca and Menorca Islands is detected. The other snake species were introduced more recently, but also are spreading in the Archipelago.

This failure to predict the current invasion range can be due to several causes. The models obtained are based on the bioclimatic *realized niche* where the species is assumed to be only able to establish in areas with similar climatic conditions as their native range (Kearney 2006). This is not always the case, since biotic and abiotic interactions can modify the dimensions of the *realized niche* (Broennimann *et al.* 2007). Thus the niche projected in the new localities (i.e. introduced areas) should be considered as a coarse approximation. Then, the lack of the same bioclimatic conditions in Balearics compared to those in their native range can led the models underestimate real suitability, like that obtained here. That bioclimatic difference seems to be related with driest summers, since precipitation of warmest quarter is the main explanatory variable of all the species distribution. The precipitation seasonality and the temperature seasonality appear to be also important, regarding *H. hippocrepis* and *M. monspessulans*, which means that these species. Balearic Islands are driest and hotter during the year than the native range, which may support the low suitability of the models.

The niche shift hypothesis can be an alternative explanation to this results, since clearly the species were able to establish and spread on islands, although the models failed to predict it. The expansion beyond their predicted climate envelope in their invasive range can be caused by two main factors. One of them is the release from biotic and abiotic constraints, i.e. absence of competitors, predators, like raptors (the main predators on continental areas), or pathogens (Mitchell & Power 2003; Torchin *et al.* 2003; Callaway & Maron 2006), and the availability of empty niches (Hierro *et al.* 2005). The other is the evolution of the alien species in the new range allowing it to expand into new niches, which can occur by genetic drift or through selection in the native range (Müller-Schärer *et al.* 2004; Müller-Schärer & Steinger 2004). For example, *Macroprotodon mauritanicus* has apparently. changed its diet habits, feeding mainly on mammals instead of reptiles like the mainland snakes of this genus (Pleguezelos *et al.* 1994). The first factor affects the *realized niche* of the species and the second factor the *fundamental niche*. In the particular case of Balearic snakes', we suggest that the first one it is the main responsible, principally for the most recent introduced ones. However, the simple use of correlative ENM does not allow the distinction between them.

Still, the niche shift can only be an artefact. The native *realized niche* can represent only a part of the *fundamental niche*, thus the presence of individuals on the unsuitable areas can just correspond to an exploration of other part of the *fundamental niche*. As previously demonstrated, a wrong choice of the predictors and variables can also lead to a result like the one obtained. Rödder *et al.* (2009) used three sets of variables to predict the invasive range of the slider turtle (*Trachemys scripta*) and the model incorporating natural history traits was the one which better predict the range. Thus, the mechanistic models based on physiological variables may improve the prediction ability, comparable to correlative models.

Besides the physiological predictors, other variables can also be important in the specific case of the Balearics. The interaction between introduced species through competition or predation may not be considered, since the species inhabits the same areas in the native range, Iberian Peninsula and North Africa. However, the human-mediated introductions are well evidenced for Balearic Archipelago, like was possible to observe in the main pathways of the previous chapter. The constant importation of trees and, consequently, a potential constant of introduction of new individuals, can also play an important role in the species distribution. This current new arriving can enhance a faster expansion and also create opportunities of species explore all the possibilities of the niche.

How will the suitability evolve with time?

In the future, globally, all the scenarios predict a good suitability of the Islands for all the species, with the exception of *M. mauritanicus*.

As expected, the A2a models scenario reveals the more drastic situation for all the species, since it is the one with more impact in biodiversity communities, producing high degree of emission gas and a rapid modification in land-uses which difficult the adaptation of the species. Nevertheless, the differences between this scenario and the other two are not so significant, predicting all a wide distribution of the species in the Islands for all the three years analysed (2020, 2050, 2080). These results make clear that the climate change can ameliorate even more the environmental conditions for the species. Climate change is, in fact, a potential boost to the establishment of an alien species (Figures 56 to 79). Its influence can be direct by removing physiological constraints, by affecting the dispersal pattern of species in several ways and by increasing the reproductive season of the species; or indirect by making the ecosystems less resistant to invasive species or more resilient to their impacts (Walther *et al.* 2009). Ectotherms, like snakes, are directly influenced by the temperature, since they regulate its body temperature according to the external one (Pough *et al.* 2004). Normally, the increase of the temperature leads to an increase of the activity. Then, the global warming may provide more opportunities for feeding, growth and breeding on temperate reptiles (Gibbons *et al.* 2000), like the species of this study. Indeed, studies with *M. monspessulanus* (Moreno-Rueda & Pleguezelos 2007; Feriche *et al.* 2008; Moreno-

Rueda *et al.* 2009) and *H. hippocrepis* (Zamora-Camacho *et al.* 2010) demonstrated that climate change can benefit the survival and reproduction of these snakes, as well as to increase the activity period, as theoretically assuming. Moreno-Rueda *et al.* (2011) also demonstrate that latitudinal distributions of reptiles may also change in response to climate change. So, the snakes' species appear to be able to change their distribution in order to have more suitable conditions.

Although all the scenarios projected are predicting a high suitability of Balearic Islands compared to present, they are incongruent if the suitable area will increase or decrease long-term. Only for *R. scalaris* and *M. monspessulanus* there are some consistencies between the scenarios with a predicted slightly decrease from 2020 to 2080 in the A1b and B2a models scenarios and an almost stable percentage of area in the A2a model scenario. These reveals the uncertainty in projections associated with the interaction and associated effect of the various pressures involved in global change, like the changes in climatic change, atmospheric composition in terms of CO_2 and nitrogen compounds and changing in land used (Walther 2007; Thuiller 2009). Otherwise, these results may also represent the real trends in which firstly the conditions will improve due to global warming, but then they will come worst due to extreme dissecation.

Usefulness of the models

Both the results from the present and the future show some uncertainties and incongruences associated. The models for present, revealed a weak correspondence with some already known occurrence points; the models for the future, produced different predictions for the suitable area available. However, they are still useful as a first approach to understand the biological invasion history of these species. The present models suggest that more research efforts are needed to completely comprehend the features underlying species distribution. From now on, studies should be addressed to investigate niche shift, as well as to determine which are the most explanatory factors on species distributions then restricting the working hypothesis. On the other hand, the future models show that in the future the environmental conditions in all the islands of Balearic archipelago will be very favourable to the permanency of the snakes in the islands.

5. Conservation implications

The introduction of the species studied in the Balearic Islands is expected to have negative impacts on the native biota. Among the several impacts already discussed in the General Introduction, predation of native lizards by the snakes and competition and potential hybridization of endemic Balearic *Podarcis* with *P. sicula* are the most concerning. However, several other unpredicted effects can come from a biological invasion as reported by previous studies (reported in the the General Introduction).

Conservation measures should, then, be taken with a maximum urgency. These measures depend on the timing and pathways of arrival. Species introduced historically, like *M. mauritanicus*, *N. maura, R. scalaris* (Menorca), *P. sicula* (Menorca) and *H. meridionalis* (Menorca), are already widespread and naturalized with a stable reproductive contingent. Thus, the eradication for these species is unfeasible and probably useless. However, our results support the prevention of the secondary dispersal of these species to new areas of the archipelago where they are still not present is essential. It is especially important to prevent the introduction of the snakes and the Italian wall lizard in sites where endemic species still persist, such as surrounding islets of the main islands and Cabrera.

The trade of olive trees and other ornamental plants is becoming a real concern issue for recent introductions in the Balearic Islands, since ornamental trees are continually being transported from one region to another, translocating with them several species that can be harmful. Several measures can be applied to prevent it: (i) Inspection and Quarantine of arriving goods, containers, baggages and vessels (ii) Control and Monitoring of importation of trees or other plants (iii) Methodic inspection on maritime cargo. If the prevention fails, an eradication and control program should be implemented, paying attention to both biological data, population size and distribution, and a specific educational effort for the general public are needed for such programs to be successful.

The pet trade should be absolutely controlled, if not forbidden, in the islands as it is an important pathway of introduction - potentially of many herp taxa – in the Balearic ecosystems. An educational effort should be done also in order to aware the general public to this problem.

Studies integrating both large-scale environmental layers with data collected at a more local scale, as well as the combination of this climatic data with information on human activities (e.g. land use, ‘nursery’ trade), should be done to evaluate better the risk of invasion (Ficetola *et al.* 2010), in order to restrict and facilitate the decision on conservation strategies.

6. Final Remarks

The development of this thesis allowed the identification of the origin for some of the species, while for others the origin still remains uncertain. The most common origin for the introduced population in Balearic Islands seems to be the Iberian Peninsula. Disregarding the old introductions, the main pathway inferred is the ornamental trees trade, specifically from olive trees. This commercial trade has been increasing through the years, being the responsible for the introduction of many others species than the ones presented here. Therefore, it is urgent to take actions toward the prevention of new introductions, like the implementation of policies to control the olive trade, namely restrict the olive importations from the same general areas and do not allow the importation from other islands or countries. Moreover, the prevention of biota movements between islands within the Balearic Archipelago is also important.

Another conclusion that is possible to take from this work is that the distribution of the species is not predominantly dependent on climatic features, but also in some other characteristics, such capability of adaptation by the species, other abiotic features, biotic interactions with native species or even the constant accidental introduction human-mediated. Furthermore, the suitability of Balearic Island will increase in the future, becoming an even better habitat for the establishment of new invasive species. Hence, it is crucial to take efficient measures that prevent the translocation of snakes' species for the surrounding islets and Cabrera Island. Snakes are a group that can provoke very harmful to endemic lizards present in those areas.

The combination of the information given by the molecular data and the ecological modeling in this work allow having the first knowledge and understanding on the Balearic Islands' alien species, as well as it has an immediate in impact on the conservation strategies applied by the Balearic Government, being that the most important result of this thesis.

7. Future Work

In the future, deeper studies should be carried out, both on the molecular and modelling parts.

From the molecular point of view, other markers should be used to infer the phyleogeography of the species, in order to clarify the origin of those species which origin still uncertain. Microsatellites can be one of the markers to use, since they have higher mutation rates, allowing a better distinction of the relationships in the case of recent introductions and determining bottlenecks and effective population sizes (Freeland 2005).

As to the ecological modelling, several approaches should be tested in order to have a deep understanding on the biological invasion process in the snakes. First, it should be found the real role of human and its activities in the distribution of the species, namely relating the distribution of the species with 'nurseries' of ornamental plants, harbours and population density. Second, it is important to model the invasion process since its beginning until the present, in order to understand how the dispersion occurs, how fast and what the source points. Third, different kind of variables (e.g. plant/tree nurseries, harbours, land use, climate) should be tested through the invasion progress, in order to distinguish which are most important variables in the beginning, during and in the end of an invasion. Finally, studies regarding the niche conservatism will be also interesting to be developed, since the snakes in Balearic Islands show an adaptability that could be inside their realized niche or it can demonstrate a capability of change their niche characteristics.

All these information will certainly clarify our knowledge about the invasion process and will help in the development of better conservation strategies in Balearic Islands.

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APPENDICES

Appendix I: Geographic records of *Rhinechis scalaris*

Table A1: Code number, locality and country of each sample of *R. scalaris* from the native

Code number	Locality	Province state	Country
2	Penafiel	Porto	Portugal
6	Ribeira de Pena	Vila Real	
24	Vila do Conde	Porto	
8	Unkown	Zamora	Spain
16	Peñas de San Pedro		
10	Segura	Jaén	
11	Aliá	Caceres	
13	Algaida	Huelva	
15	Zeneta	Murcia	
19	Ranin	Huesca	
25	Unknown	Ourense	
26	Unknown	Salamanca	
30	Agron	Granada	
33	Monachil		
36	Orce		
37	Arenas del Rey		
42	Colomera		
59	Guadix		
76	Don Fradique		
68	Capileira		
35	Almanzora	Almería	
52	Abla		
50	Sierra Nevada	Almería/Granada	
75			
45	Pizarra	Malaga	
49	Llora	Gerona	
53	Unknown	Ciudad Real	
67	Mestanza		
56	Pradollano	Seville	
57	Camarena	Toledo	
60	San Miguel des Salinas	Alicante	
62	Agost		
17	El Carrascal	Avila	
31	Parc National de Port-Cros	Hyères	France
72			
32	Vic-la-Guardiole	Herault	
63			
55	Saint-Jean-de-Védas		
48	Unkwon	Montpellier	
69	Arles	Bouches-du-Rhône	
73			
70	Le Cannet-des-Maures	Var	
71	Gonfaron		

Appendix II: 12S and Cytb results of *Hemorrhois hippocrepis*

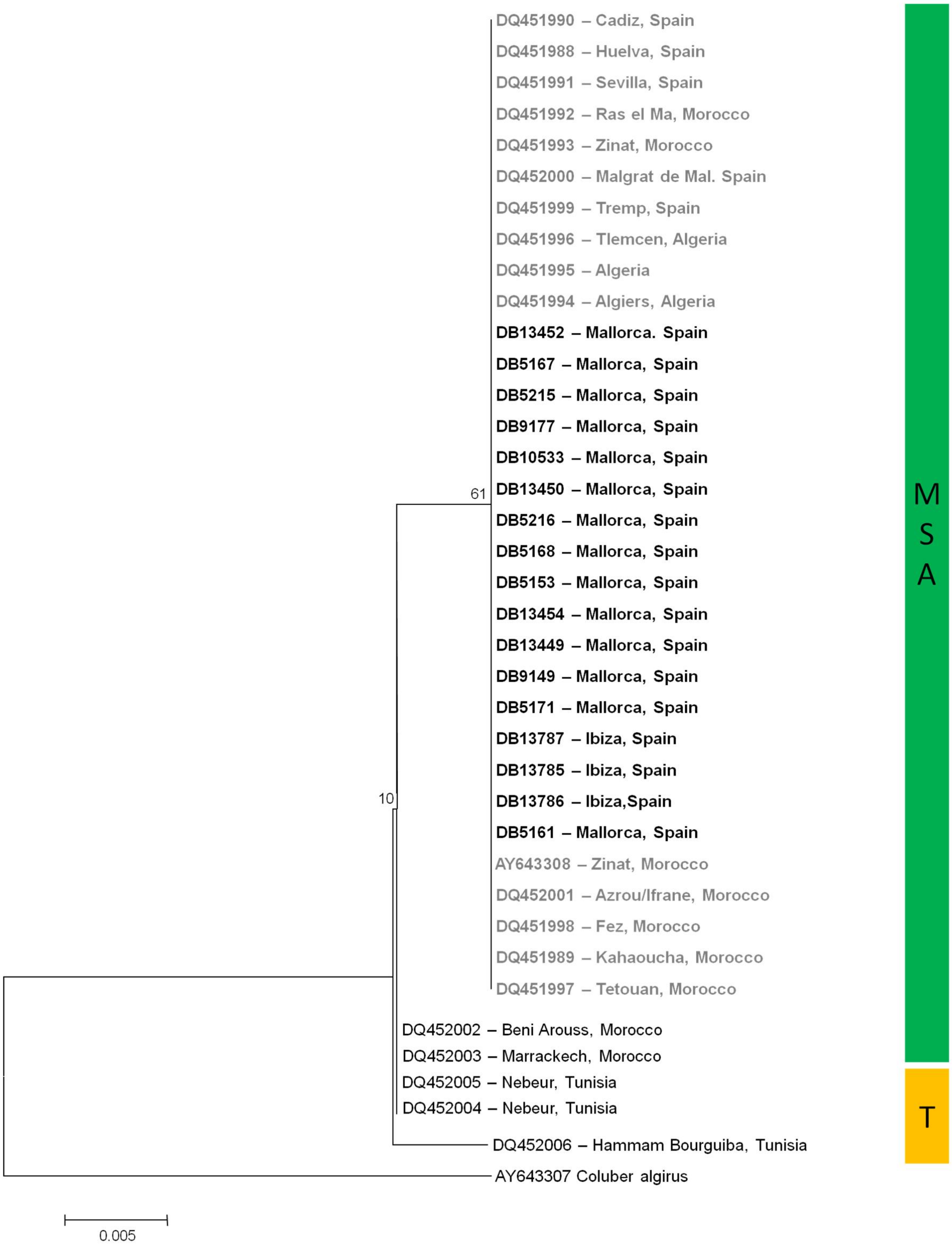

Figure A1: ML tree based on of the 12S data set depicting the relationships between haplotypes of native range from Carranza *et al.* (2006) and those from the introduced populations from Mallorca and Ibiza Islands for *H. hippocrepis*. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates. MSA: Morocco+Iberia+Algeria; T: Tunisia.

Appendix II: 12S and Cytb results of *Hemorrhois hippocrepis*

Figure A2: ML tree based on of the cytochrome b (cytb) depicting the relationships between haplotypes of native range from Carranza *et al*. (2006) and those from the introduced populations from Mallorca and Ibiza Islands for *H. hippocrepis*. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates. MSA: Morocco+Iberia+Algeria; T: Tunisia.

Appendix II: 12S and Cytb results of *Hemorrhois hippocrepis*

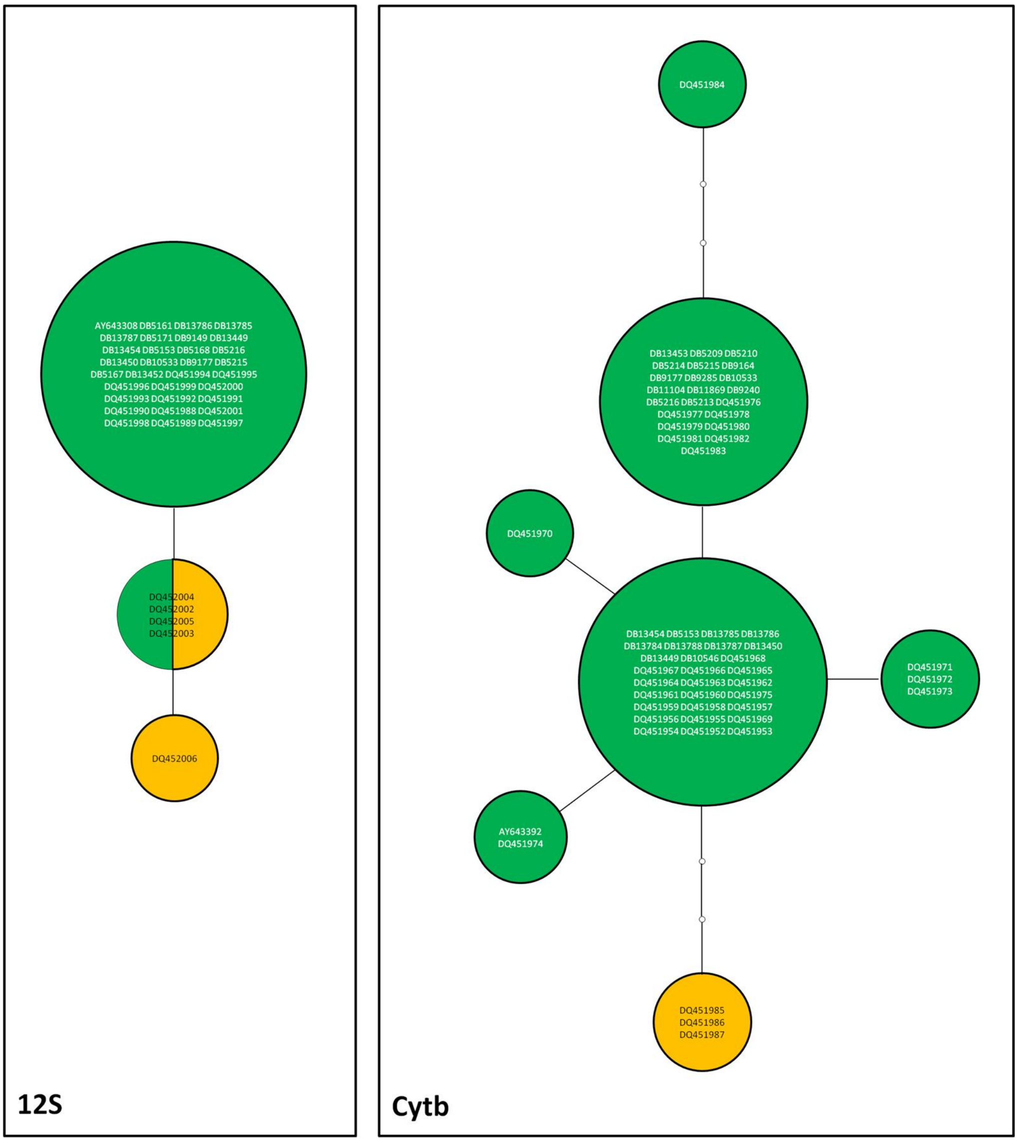

Figure A3: Statistical parsimony network depicting the genealogical relationships between haplotypes from native and non-native *H. hippocrepis.*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes. Grey boxes represent a common haplotype with the sample within the circle correspondent.

Appendix III: 12S and combined data results of *Macroprotodon sp.*

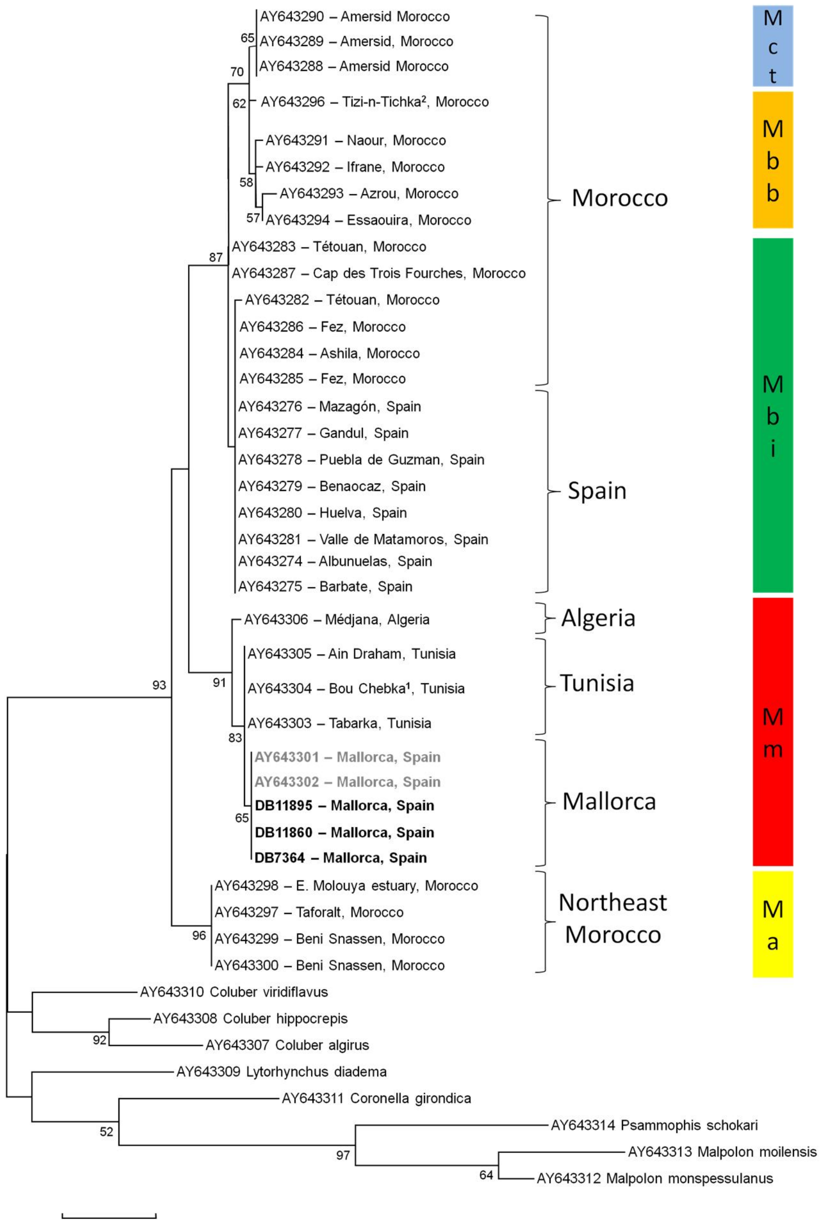

Figure A4: ML tree based on of the 12S data set depicting the relationships between haplotypes of native range from Carranza *et al*. (2004) and those from the introduced populations from Mallorca and Menorca of *Macroprotodon sp*. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported).Mbi: *M. brevis ibericus*; Mct: *M. cucullatus textilis*; Mbb: *M. brevis brevis*; Ma: *M. abubakeri*; Mm: *M. mauritanicus*

Appendix III: 12S and combined data results of *Macroprotodon sp.*

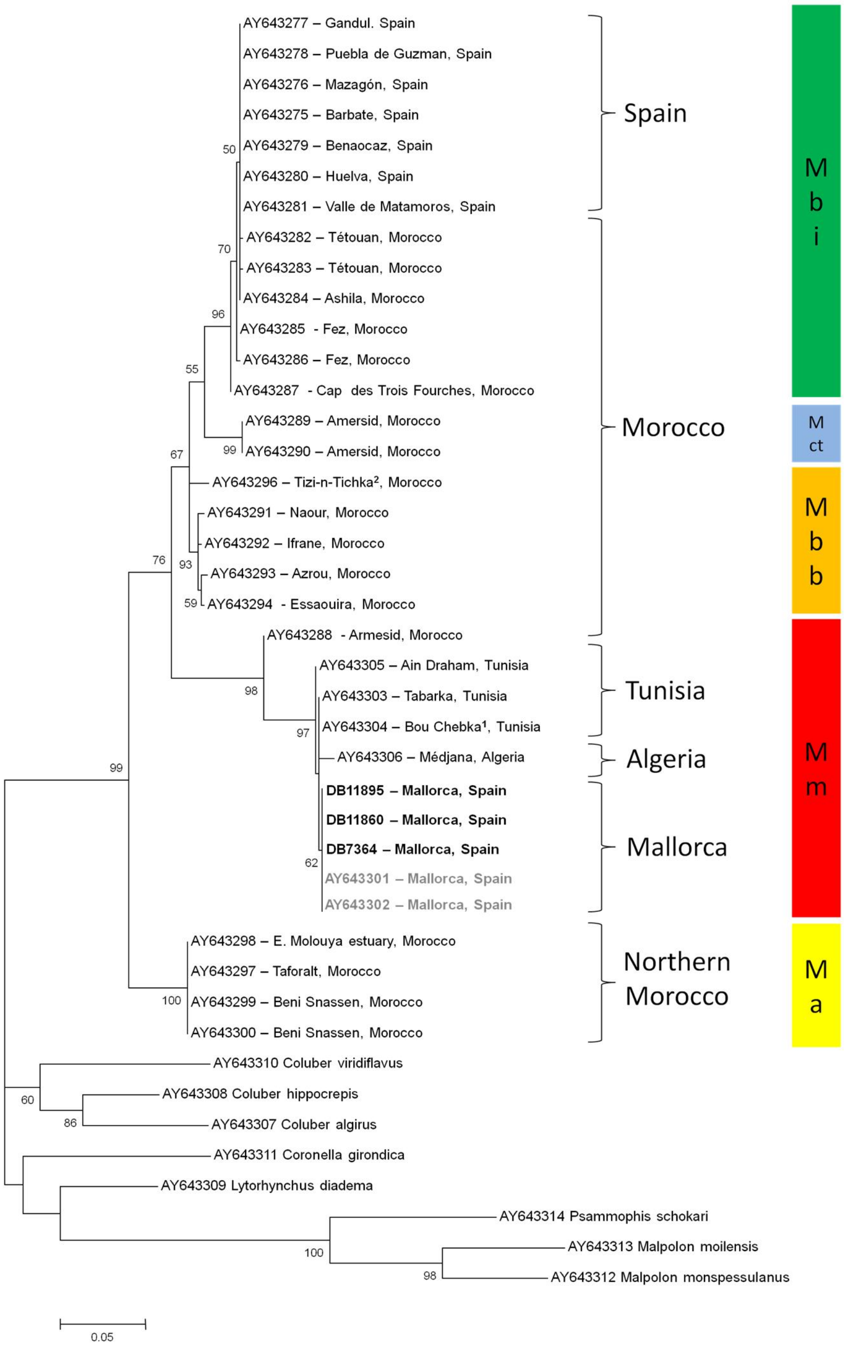

Figure A5: ML tree based on of the combined data set depicting the relationships between haplotypes of native range from Carranza *et al*. (2004) and those from the introduced populations from Mallorca and Menorca of *Macroprotodon sp*. Numbers on the branches indicate ML bootstrap values (BP) over 1000 replicates (BP<50 are not reported).Mbi: *M. brevis ibericus*; Mct: *M. cucullatus textilis*; Mbb: *M. brevis brevis*; Ma: *M. abubakeri*; Mm: *M. mauritanicus*

Appendix III: 12S and combined data results of *Macroprotodon sp.*

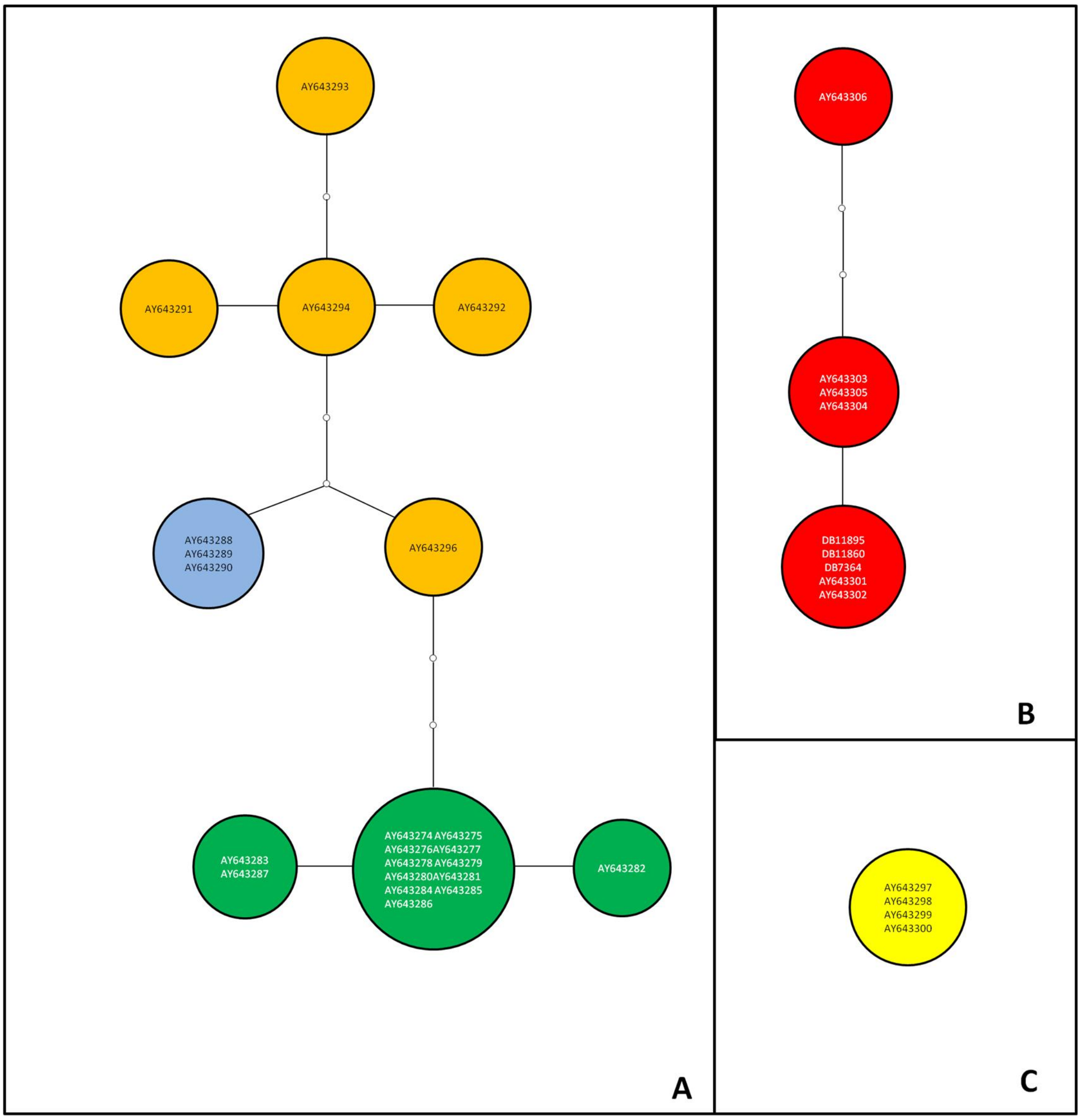

Figure A6: Statistical parsimony network of 12S data set depicting the genealogical relationships between haplotypes from native and non-native *Macroprotodon sp.*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes. A: Mbi + Mct + Mbb; B: Mm; C: Ma. Grey boxes represent a common haplotype with the sample within the circle correspondent.

Appendix III: 12S and combined data results of *Macroprotodon sp.*

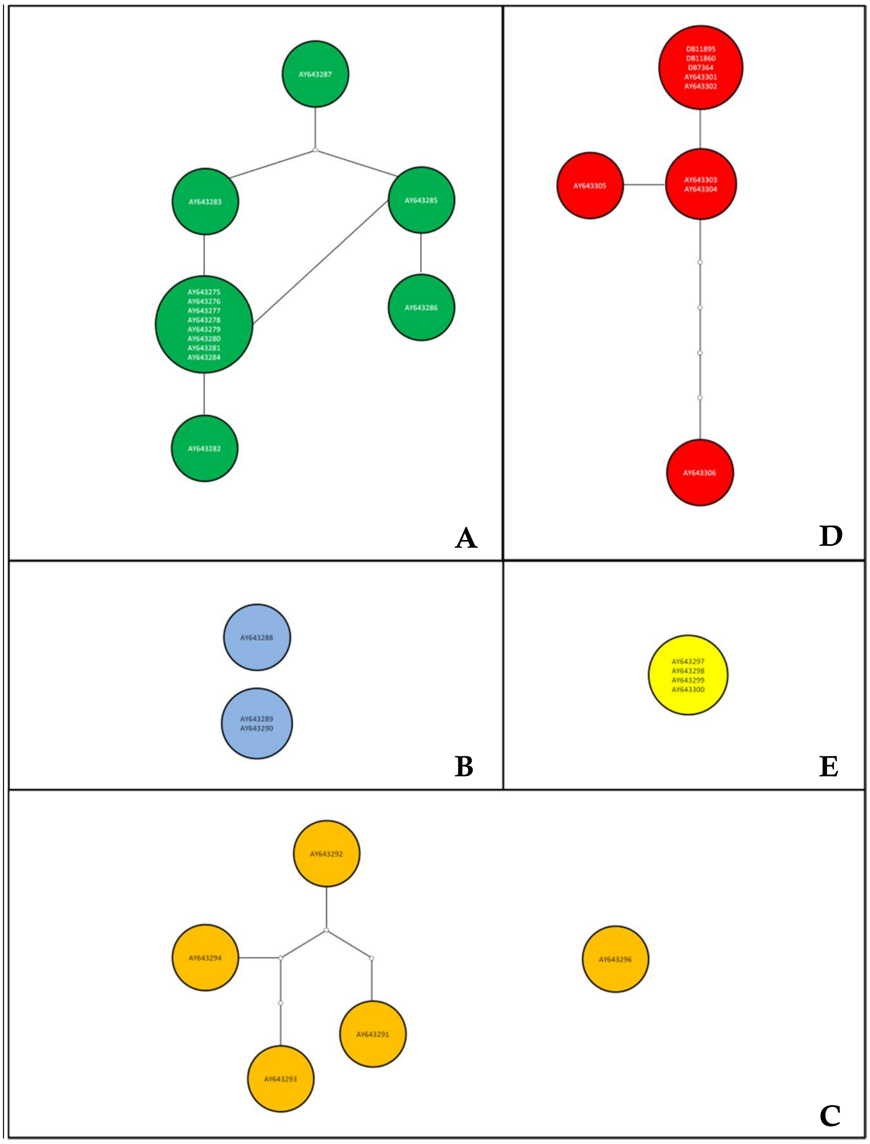

Figure A7: Statistical parsimony network of combined data set depicting the genealogical relationships between haplotypes from native and non-native *Macroprotodon sp.*. Circle size is proportional to the haplotype frequency. White circles represent missing haplotypes. A: Mbi; B: Mct; C: Mbb; D: Mm; E: Ma.

Appendix IV: Additional information on *Podarcis sicula*.

Silva-Rocha I, Salvi D, Carretero MA. 2012. Genetic data reveal a multiple origin for the populations of the Italian Wall lizard *Podarcis sicula* (Squamata: Lacertidae) introduced in the Iberian Peninsula and Balearic Islands. Italian Journal of Zoology. (in press)

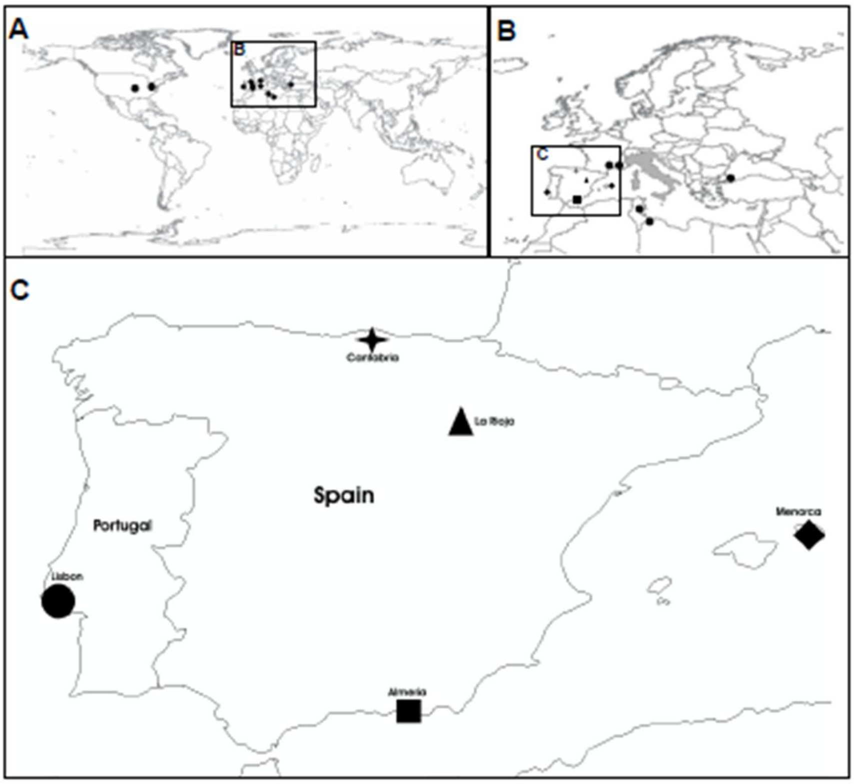

Figure A8: Introduced populations and native range of *Podarcis sicula*. A, introduced populations in the World – United States, North Africa and Europe (black dots); B, introduced populations in Europe (black) and native range (grey); C, introduced populations in the Iberian Peninsula and Menorca.

Appendix IV: Additional information on *Podarcis sicula*.

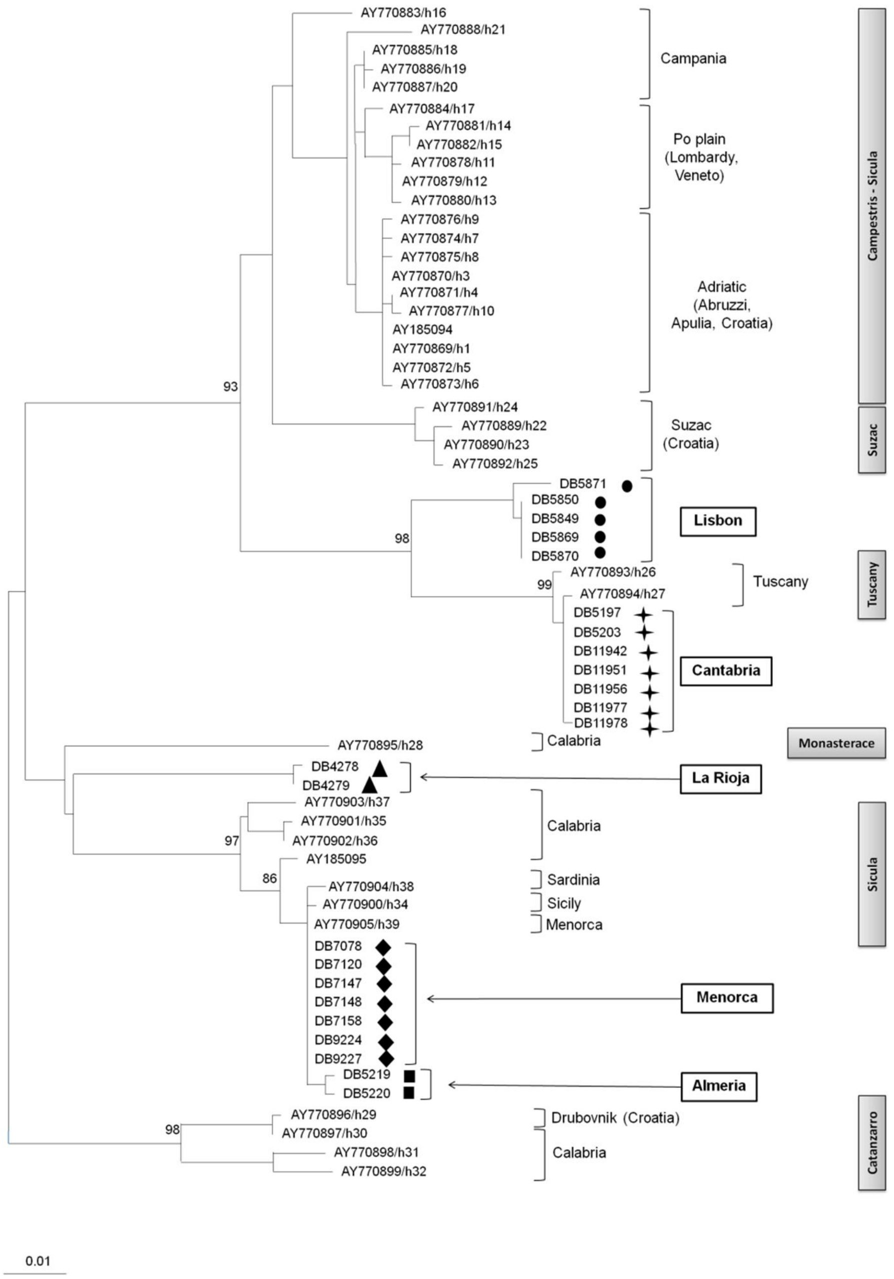

Figure A9: ML phylogenetic tree depicting the relationships between cytochrome b, haplotypes from native *Podarcis sicula* populations (Podnar et al. 2005) and those from the Iberian and Balearic introduced populations: Almeria, Cantabria, La Rioja, Lisbon, and Menorca. Specimens' localities are reported along with *P. sicula* haploclades (grey boxes) as in Podnar et al. (2005). Bootstrap support is indicated above the nodes of interest.

Appendix IV: Additional information on *Podarcis sicula*.

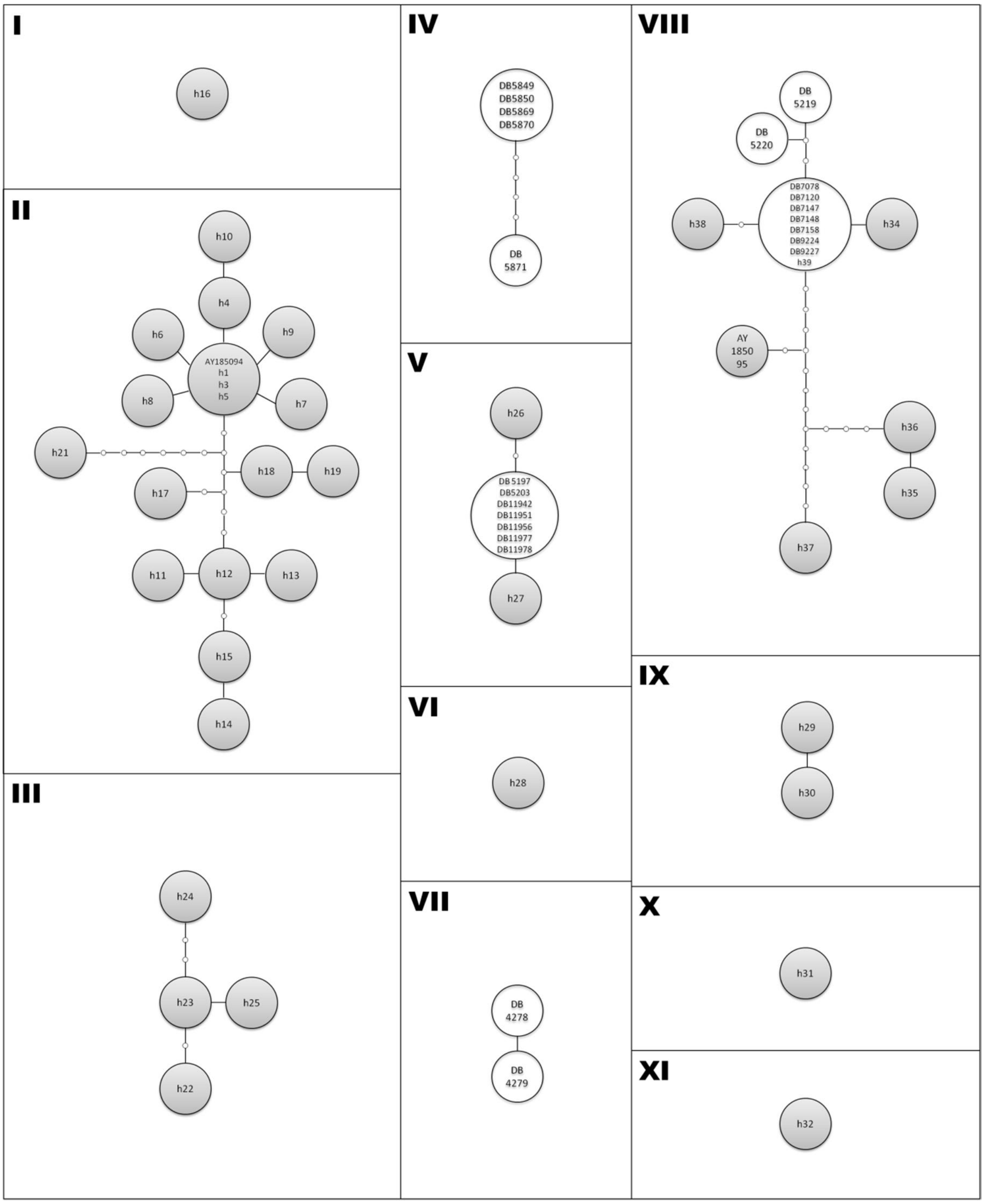

Figure A10: Statistical parsimony haplotype network depicting the genealogical relationships between native and non-native *P. sicula* haplotypes. See Table I and Figure 2 for details on sampling localities and haplotypes codes. Circle size is proportional to the number of samples with the same haplotype. Black circles represent missing haplotypes. The eleven networks include samples from: (I) Campania - *Campestris Sicula clade*; (II) Po Plain + Campania + Adriatic – *Campestris-Sicula clade*; (III) Suzac (Croatia) – *Suzac clade* (IV); Lisbon (non-native); (V) Cantabria (non-native) + Tuscany - Tuscany clade; (VI) Calabria - *Monasterace clade*; (VII) La Rioja (non-native) (VIII) Almería (non-native) + Menorca (non-native) + Sardinia + Sicily + Calabria - *Sicula clade*; (IX) Drubovnik (Croatia) *Catanzaro clade*; (X) Calabria - *Catanzaro clade*; and (XI) Calabria - *Catanzaro clade*.

Appendix IV: Additional information on *Podarcis sicula*.

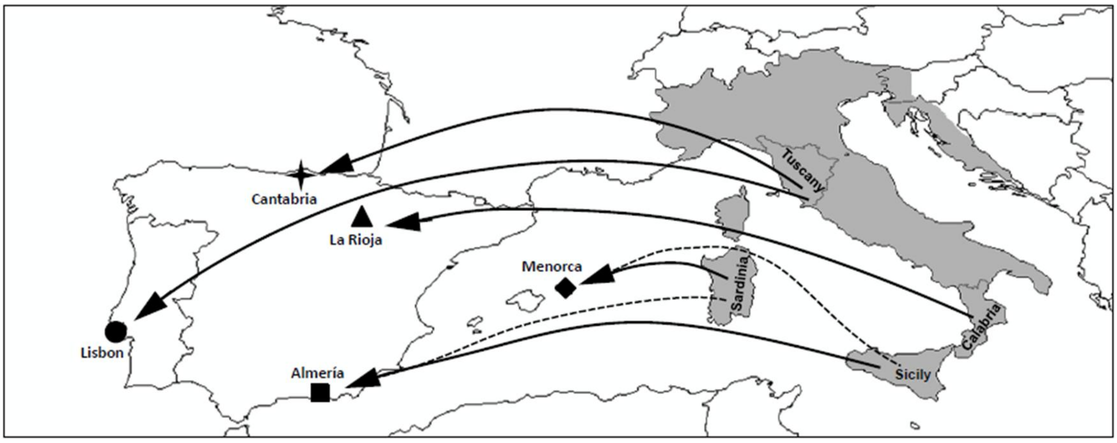

Figure A11. Origin of the Iberian and Balearic populations of *Podarcis sicula* as inferred from genetic analyses.

Appendix V: Jackknife results for test gain data and AUC for all the species.

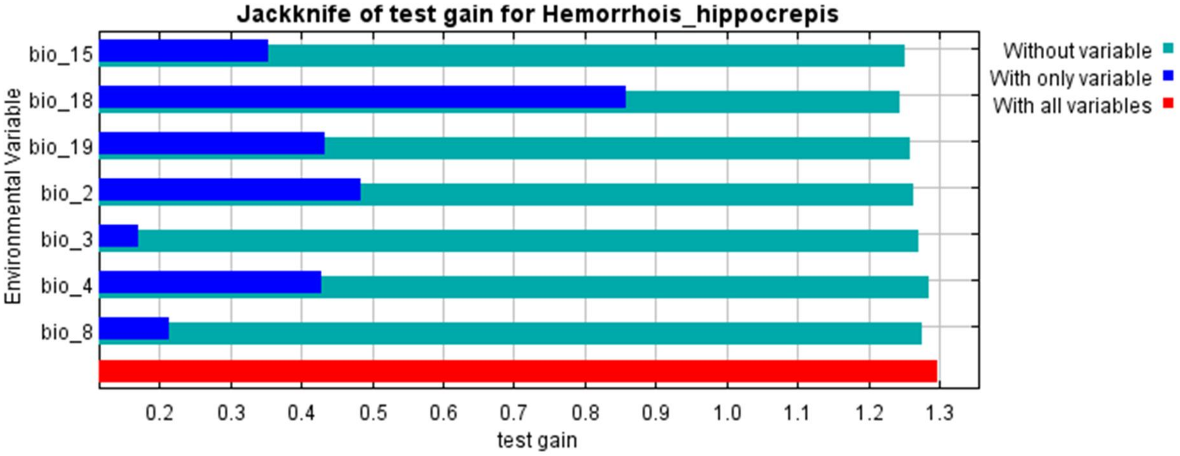

Figure A12: Jackknife results of test gain data for *H. hippocrepis*

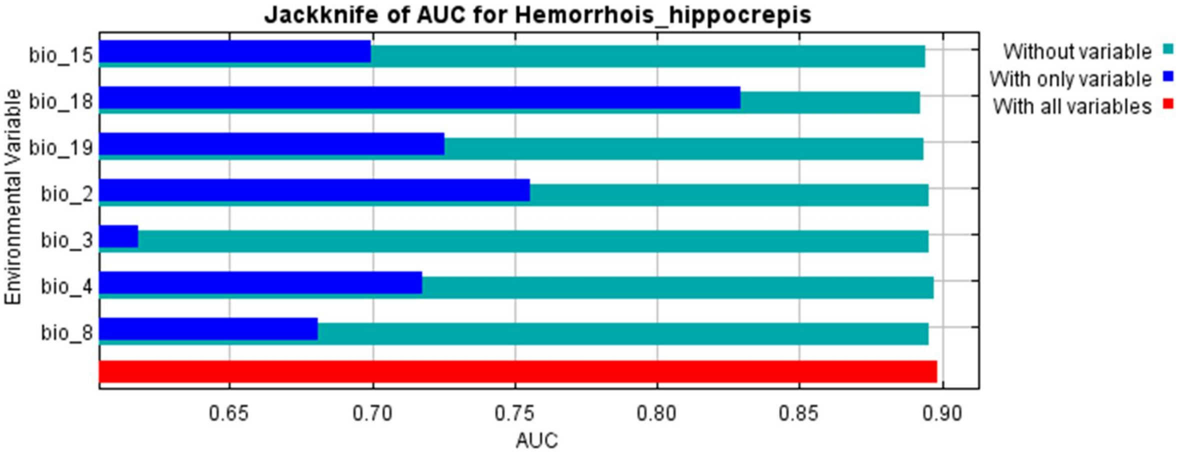

Figure A13: Jackknife results of AUC for *H. hippocrepis*

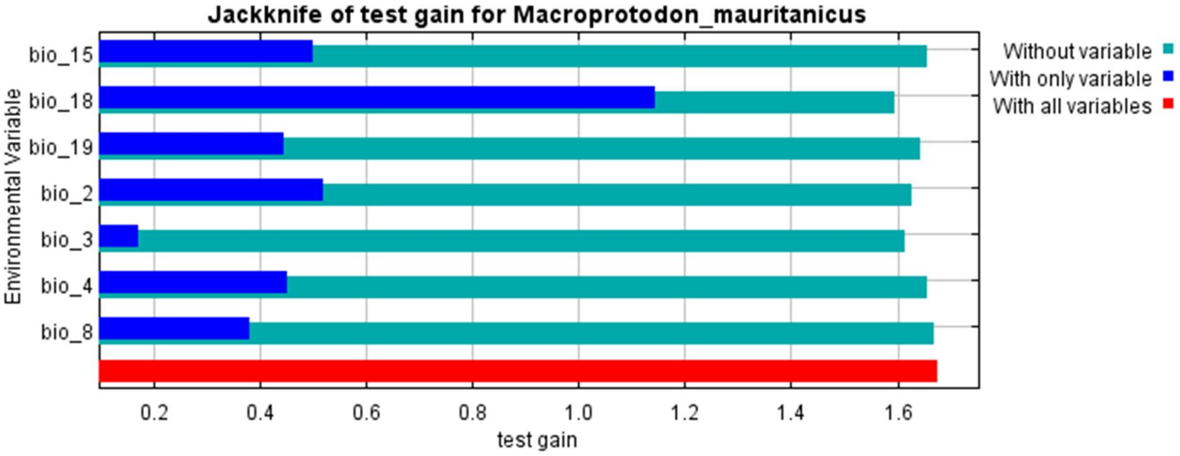

Figure A14: Jackknife results of test gain for *Macroprotodon sp.*

Appendix V: Jackknife results for test gain data and AUC for all the species.

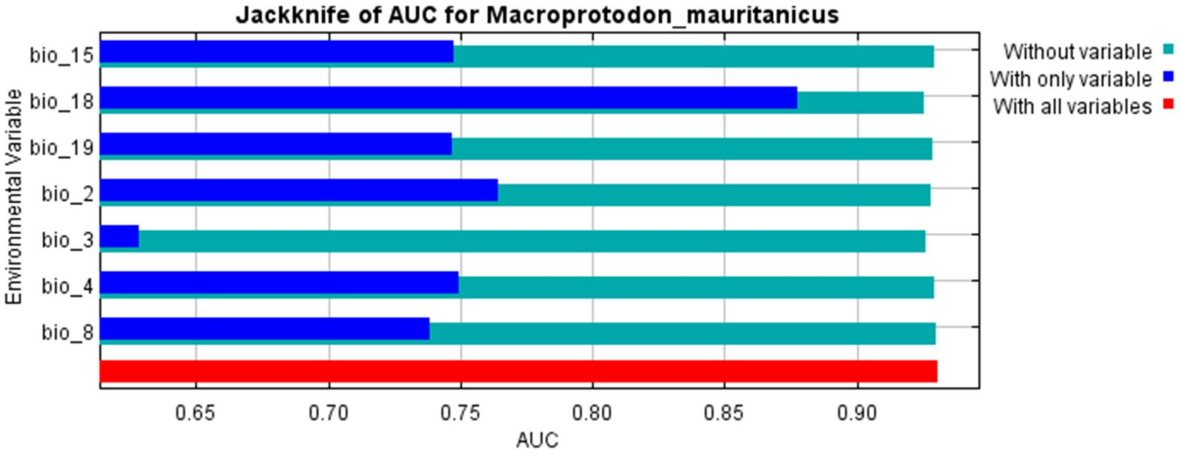

Figure A15: Jackknife results of AUC for *M. mauritanicus.*

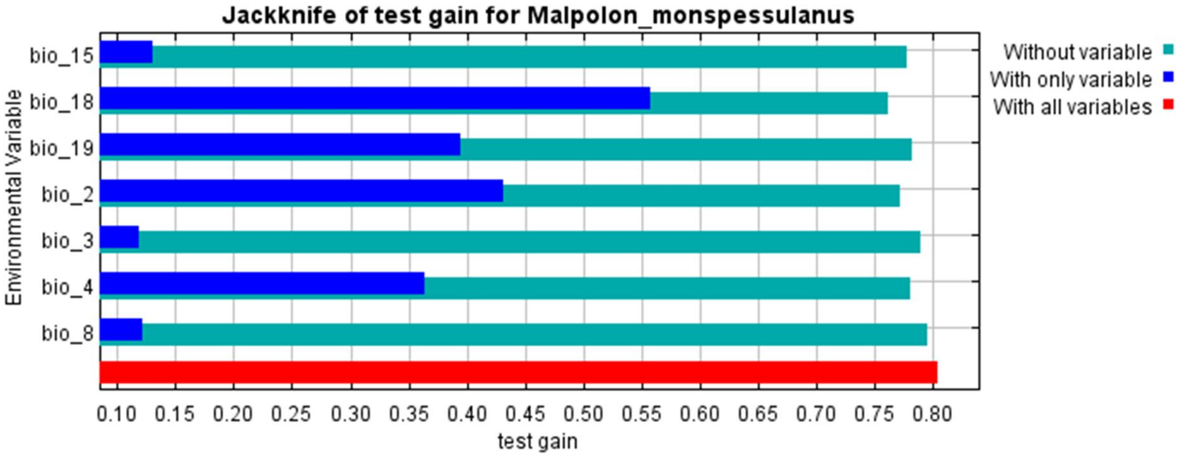

Figure A16: Jackknife results of test gain for *M. monspessulanus.*

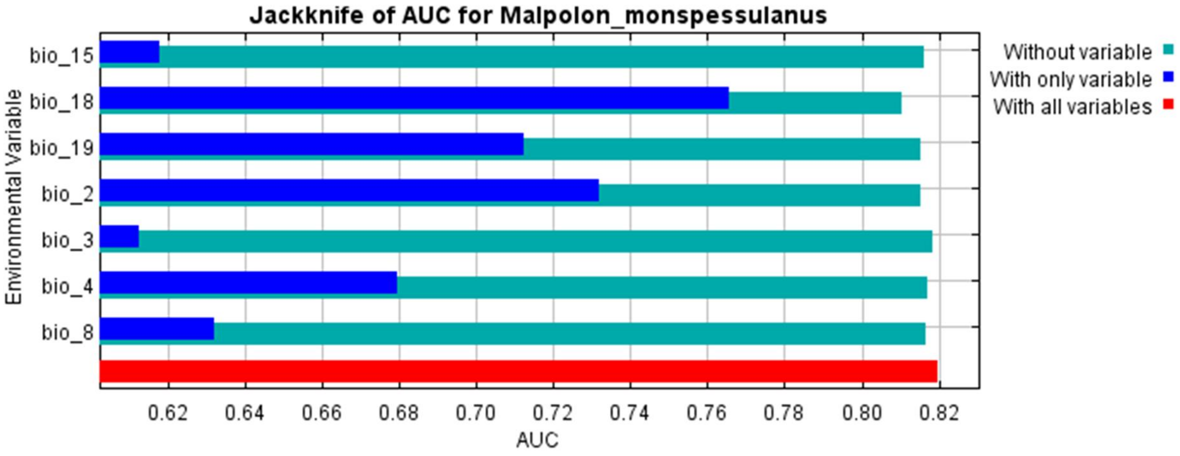

Figure A17: Jackknife results of AUC for *M. monpessulanus*.

Appendix V: Jackknife results for test gain data and AUC for all the species.

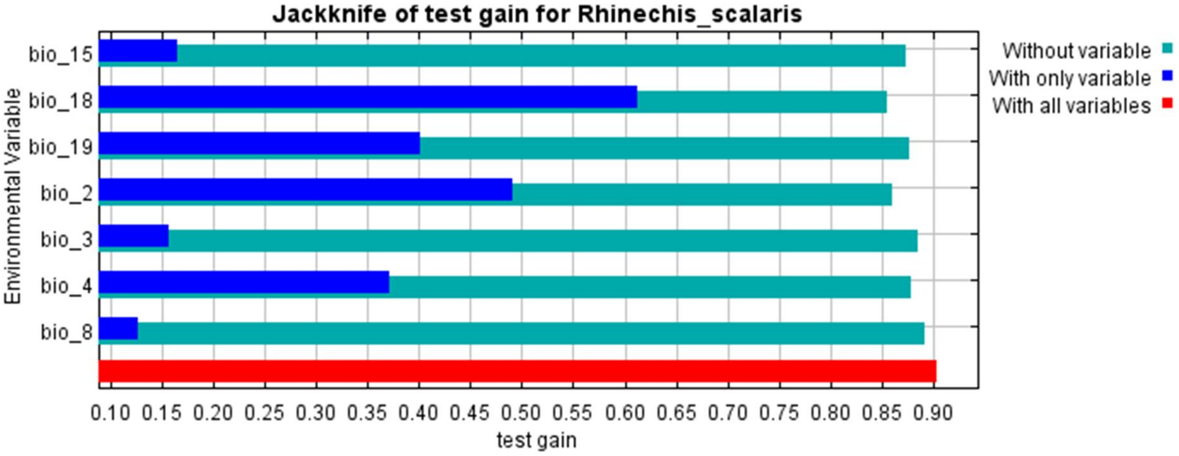

Figure A18: Jackknife results of test gain for *R. scalaris.*

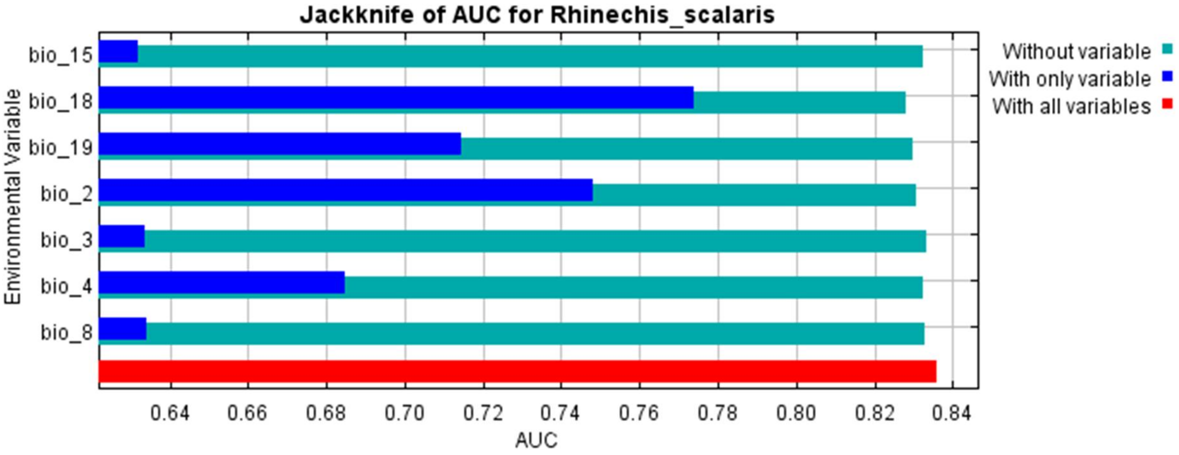

Figure A19: Jackknife results of AUC for *R. scalaris.*