FACTORS REGULATING THE INVASION OF TWO MEDITERRANEAN ANURANS
THE ROLE OF NICHE CONSERVATISM, SPECIES INTERACTION AND HABITAT SELECTION

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TESI DOCTORAL

FACTORS REGULATING THE INVASION OF TWO MEDITERRANEAN ANURANS
THE ROLE OF NICHE CONSERVATISM, SPECIES INTERACTIONS AND HABITAT SELECTION

DANIEL ESCORIZA BOJ

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Factors regulating the invasion of two Mediterranean anurans

The role of niche conservatism, species interactions and habitat selection

Daniel Escoriza Boj

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El Dr. Dani Boix i Masafret, de l'Institut d'Ecologia Aquàtica de la Universitat de Girona,

DECLARO:

Que el treball titulat *FACTORS REGULATING THE INVASION OF TWO MEDITERRANEAN ANURANS THE ROLE OF NICHE CONSERVATISM, SPECIES INTERACTIONS AND HABITAT SELECTION*, que presenta Daniel Escoriza Boj per a l'obtenció del títol de doctor, ha estat realitzat sota la meva direcció.

i, perquè així consti i tingui els efectes oportuns, signo aquest document.

Girona, 6 d'octubre de 2014

Estudiar els amfibis és observar un mostra del passat en l'evolució dels vertebrats. Els amfibis han arribat a sobreviure en els nostres dies tot i la dominància establerta per llinatges més moderns de vertebrats, molt més intel·ligents i competitius, com són els mamífers o les aus. No obstant això, no es pot deixar d'admirar a un grup en què algun dels seus components formen part de llinatges originaris del Triàsic. I una d'aquestes espècies, pertanyent a un llinatge relicte, pràcticament extingit en el Plistocè, s'ha convertit en una espècie invasora. El fet d'una espècie tan aparentment primitiva i ineficient s'hagi transformat en un invasor d'èxit, colonitzant un paisatge summament alterat i sota unes noves condicions ambientals, és molt estimulant per a qualsevol científic. Esbrinar la mecànica subjacent en aquesta invasió és l'objectiu d'aquesta tesi.

Em plau dedicar aquesta tesi a la meva família i a la meva dona que han suportat sis anys de dedicació als Discoglossus. Vull agrair el suport que he rebut pels meus companys dins de l'Institut d'Ecologia Aquàtica de la Universitat de Girona, especialment al Dr. Dani Boix, però també al Dr. Albert Ruhí i a en Jordi Sala. Aixi mateix a les persones que d'una manera o altra han ajudat en la consecució final d'aquest treball, al Dr. Ali Bouatour (Institut Pasteur de Tunis), a la Dra. Jihène Ben Hassine (University of Tunis el Manar) i a la Dra. Soumia Fahd (Abdelmalek Esaâdi University). Finalment a tota la gent de Sicília, Tunísia i Marroc que m'han ajudat a explorar els seus meravellosos països.

“I watched a snail crawl along the edge of a straight razor. That's my dream; that's my nightmare. Crawling, slithering, along the edge of a straight razor ... and surviving.”
Colonel Walter E. Kurtz, Apocalypse Now (1979)

“In the beginning, there was darkness. And the darkness was without form, and void. And in addition to the darkness there was also me. And I moved upon the face of the darkness. And I saw that I was alone. Let there be light.”
Bomb #20, Dark Star (1974)

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SUMMARY

The factors that determine the success of an invasive species are still poorly understood. In this thesis, we examined how environmental factors have shaped the invasion of two species of alien frogs and investigated the effect of this invasion on the structure of native larval assemblages. In addition, we studied the ecology of congeneric species to establish whether there is a certain type of adaptive divergence (in the invasive populations) that has favoured the invasion process. The studied region covered several countries of the western Mediterranean basin, namely, Spain, Portugal, France, Italy (Sicily), Tunisia, Algeria and Morocco.

In the first chapter, we examined the niche occupied by *Discoglossus pictus* in north-western Spain. Our interest was to assess with which native species had a greater overlap at two niche scales (regional and local). The regional niche was examined by extracting climatic data based on species occurrence. The local niche was studied by characterising the reproductive habitats and assessing the habitat overlap with sympatric species, including the morphofunctional resemblance. The combined results of these analyses indicated that *D. pictus* is a thermophilous species, which usually uses temporary ponds to breed and belongs to the benthic larval guild. Therefore, a high overlap is expected with the two native anurans: *Epidalea calamita* and *Pelodytes punctatus.*

In the second chapter, we compared the ecology of native and invasive populations of *D. pictus*. Our purpose was to identify mechanisms that may have favoured the invasion process, both biotic and abiotic. We investigated the role of the environmental factors by assessing the resemblance of the climatic niches and characterising their breeding habitats. Additionally, we also investigated the possible existence of adaptive advantages by comparing the larval niche of *D. pictus* with the native frog species. Our findings indicated that niche conservatism can explain most of the invasive range, although both group of populations (invasive and native) did not occupy identical niches. Invasive and native groups also breed in very similar aquatic habitats. Furthermore, *D. pictus* did not show clear adaptive advantages compared to most of the native species.

In the third chapter, we examined the ecology of congeneric species in different Mediterranean regions. Our purpose was to define a baseline composed by the groups of native species (which we expect to be ecologically conservative) and assess if there are or not differences between this group of native populations and the invasive group. These differences could be related to the existence of non-deterministic associations with sympatric species and the use of a sub-optimal niches by invasive populations. Our results indicated that most of the studied populations

are very successful generalists, and as expected, also the invasive group. However, in the invaded region, *D. pictus* appears to be more frequently associated with other anuran species than the populations of the native group, suggesting a loss in the assemblage structure.

Finally, in the fourth chapter, we studied another case of invasion within the same geographical region. Our purpose was to assess the possible existence of common patterns during the invasion phase. To this end, we studied *Hyla meridionalis*, which may have colonised south-west Europe in historical times. Our results indicated that the invasive and native groups show important niche inertia. However *Hyla meridionalis* is not occupying all the suitable niches in southern Europe, possibly due to competitive interactions established with native hylid species.

RESUM

Els factors que determinen l'èxit d'una espècie invasora són encara poc coneguts. En aquesta tesi, s'examina com els factors ambientals han modelat la invasió de dues espècies de granotes invasives i s'investiga l'efecte d'aquesta invasió en l'estructura de les comunitats larvàries natives. A més, s'estudia l'ecologia de les espècies congenèriques per establir l'existència de divergència adaptativa (en les poblacions invasores) que hagi afavorit el procés d'invasió. La regió estudiada abasta diversos països de la conca de la Mediterrània occidental, és a dir, Espanya, Portugal, França, Itàlia (Sicília), Tunísia, Algèria i el Marroc.

En el primer capítol, hem examinat el nínxol ocupat per *Discoglossus pictus* al nord-oest d'Espanya. El nostre interès era avaluar amb quines espècies natives tenien un major solapament en dues escales de nínxol (regional i local). El nínxol regional es va examinar mitjançant l'extracció de les dades climàtiques a partir de la ocurrència de les espècies. El nínxol local va ser estudiat mitjançant la caracterització dels hàbitats reproductius i avaluant el solapament d'hàbitat amb les espècies simpàtriques, incloent la semblança morfofuncional. Els resultats combinats d'aquestes anàlisis van indicar que *D. pictus* és una espècie termòfila, que generalment utilitza basses temporals per reproduir-se i pertany al gremi de larves bentòniques. Per tant, un alt grau de sobreposició es esperable amb dues espècies d'anurs nadius: *Epidalea calamita* i *Pelodytes punctatus*.

En el segon capítol, es va comparar l'ecologia de les poblacions natives i invasores de *D. pictus*. El nostre objectiu va ser identificar els mecanismes que podrien haver afavorit el procés d'invasió, tant biòtics com abiòtics. Hem investigat el paper dels factors ambientals a partir de l'avaluació de la similitud dels nínxols climàtics i la caracterització dels hàbitats de reproducció. A més, també es va investigar la possible existència de superioritat adaptativa, comparant el nínxol larvari de *D. pictus* amb les espècies de granotes natives. Les nostres troballes indiquen que el conservadorisme de nínxol pot explicar la major part de la distribució invasiva, encara que tots dos grups de poblacions (invasores i natives) no ocupaven nínxols idèntics. Grups invasors i natius també es reprodueixen en hàbitats aquàtics molt similars. A més, *D. pictus* no va mostrar una clara superioritat adaptativa en comparació amb la majoria de les espècies natives.

En el tercer capítol, hem examinat l'ecologia de les espècies congenèriques en diferents regions del Mediterrani. El nostre propòsit va ser definir una línia de base composta pels grups d'espècies natives (que esperem siguin ecològicament conservadors) i avaluar si hi ha o no

diferències entre aquest grup de poblacions natives i el grup invasiu. Aquestes diferències podrien estar relacionades amb l'existència d'associacions no deterministes amb les espècies simpàtriques i l'ús de nínxols subòptims per les poblacions invasores. Els nostres resultats indiquen que la majoria de les poblacions estudiades són generalistes de gran èxit, i com era d'esperar, també el grup invasiu. No obstant això, a la regió envaïda, *D. pictus* sembla estar associat més freqüentment amb altres espècies d'anurs que les poblacions del grup natiu, el que suggereix una pèrdua en l'estructura de la comunitat larvària.

Finalment, en el quart capítol, es va estudiar un altre cas d'invasió dins de la mateixa regió geogràfica. El nostre objectiu va ser avaluar la possible existència de patrons comuns durant la fase d'invasió i per assolir-lo estudiem *Hyla meridionalis*, que pot haver colonitzat el sud-oest d'Europa en temps històrics. Els nostres resultats indiquen que els grups invasors i nadius mostren important inèrcia en l’ús del nínxol. No obstant això *Hyla meridionalis* no està ocupant tots els nínxols adequats al sud d'Europa, possiblement a causa de les interaccions competitives establertes amb espècies congenèriques.

RESUMEN

Los factores que determinan el éxito de una especie invasora son aún poco conocidos. En esta tesis, se examina cómo los factores ambientales han modelado la invasión de dos especies de ranas invasivas y se investiga el efecto de esta invasión en la estructura de las comunidades larvarias nativas. Además, se estudia la ecología de las especies congenéricas para establecer la existencia de divergencia adaptativa (en las poblaciones invasoras) que haya favorecido el proceso de invasión. La región estudiada abarca varios países de la cuenca del Mediterráneo occidental, es decir, España, Portugal, Francia, Italia (Sicilia), Túnez, Argelia y Marruecos.

En el primer capítulo, hemos examinado el nicho ocupado por *Discoglossus pictus* en el noroeste de España. Nuestro interés era evaluar con qué especies nativas tenían un mayor solapamiento en dos escalas de nicho (regional y local). El nicho regional se examinó mediante la extracción de los datos climáticos a partir de la ocurrencia de las especies. El nicho local fue estudiado mediante la caracterización de los hábitats reproductivos y evaluando el solapamiento de hábitat con las especies simpátricas, incluyendo la semejanza morfofuncional. Los resultados combinados de estos análisis indicaron que *D. pictus* es una especie termófila, que generalmente utiliza estanques temporales para reproducirse y pertenece al gremio de larvas bentónicas. Por lo tanto, un alto grado de superposición es esperable con dos especies de anuros nativos: *Epidalea calamita* y *Pelodytes punctatus*.

En el segundo capítulo, se comparó la ecología de las poblaciones nativas e invasoras de *D. pictus*. Nuestro objetivo fue identificar los mecanismos que podrían haber favorecido el proceso de invasión, tanto bióticos como abióticos. Hemos investigado el papel de los factores ambientales a partir de la evaluación de la similitud de los nichos climáticos y la caracterización de los hábitats de reproducción. Además, también se investigó la posible existencia de superioridad adaptativa, comparando el nicho larvario de *D. pictus* con las especies de ranas nativas. Nuestros hallazgos indican que el conservadurismo de nicho puede explicar la mayor parte de la distribución invasiva, aunque ambos grupos de poblaciones (invasoras y nativas) no ocupaban nichos idénticos. Grupos invasores y nativos también se reproducen en hábitats acuáticos muy similares. Además, *D. pictus* no mostró una clara superioridad adaptativa en comparación con la mayoría de las especies nativas.

En el tercer capítulo, hemos examinado la ecología de las especies congenéricas en diferentes regiones del Mediterráneo. Nuestro propósito fue definir una línea de base compuesta por los grupos de especies nativas (que esperamos sean ecológicamente conservadoras) y evaluar si hay

o no diferencias entre este grupo de poblaciones nativas y el grupo invasivo. Estas diferencias podrían estar relacionadas con la existencia de asociaciones no deterministas con las especies simpátricas y el uso de nichos subóptimos para las poblaciones invasoras. Nuestros resultados indican que la mayoría de las poblaciones estudiadas son generalistas de gran éxito, y como era de esperar, también el grupo invasivo. Sin embargo, en la región invadida, *D. pictus* parece estar asociado más frecuentemente con otras especies de anuros que las poblaciones del grupo nativo, lo que sugiere una pérdida en la estructura de la comunidad larvaria.

Finalmente, en el cuarto capítulo, se estudió otro caso de invasión dentro de la misma región geográfica. Nuestro objetivo fue evaluar la posible existencia de patrones comunes durante la fase de invasión. Para ello, estudiamos *Hyla meridionalis*, que puede haber colonizado el sudoeste de Europa en tiempos históricos. Nuestros resultados indican que los grupos invasores y nativos muestran importante inercia en el uso del nicho. Sin embargo *Hyla meridionalis* no está ocupando todos los nichos adecuados en el sur de Europa, posiblemente debido a las interacciones competitivas establecidas con especies congenéricas.

I. INTRODUCTION

I. 1. The worldwide decline of amphibians

Amphibians are an ancient lineage of tetrapods with a great evolutionary success that extend over most of the continents. This lineage include 7364 species, most of which (85%) belong to the order Anura (Amphibiaweb 2014). Amphibians are characterised by their relative dependence on environmental humidity and stenothermality (Feder 1982; Gifford and Kozak 2012). For these reasons, this group showed a maximum diversification in non-seasonal rainforest climates, where 50–100 species can coexist (Daniels 1992; Hero et al. 2001; Andreone et al. 2008). Nonetheless, some species have been able to colonise semi-arid and sub-arctic regions, developing unique adaptations that enable them to complete their life cycles in these extreme environments (Storey and Storey 1996; Cai and Storey 1997).

In recent decades, amphibians have shown an important worldwide decline, and at least 168 species have become extinct (Houlahan et al. 2000). This decline is not occurring at random and has a well-defined geographic and ecological pattern, which is more pronounced in tropical rainforest assemblages (Laurance et al. 1996; Stuart et al. 2004). The causes are not well understood, but the hypotheses explaining this decline can be summarised in two groups (Collins and Storfer 2003). The first set of hypotheses includes the causes in which the underlying ecological mechanisms are well known and have been acting for an extended period time, such as the overexploitation of amphibians as a food resource and the massive disturbance of habitats, including the spread of alien species (Towns and Daugherty 1994; Cushman 2006; Cruz et al. 2008; Jenkins et al. 2009). The second group includes the hypotheses related to subtle alterations in biosphere dynamics (e.g., increase of UV radiation and global warming) or the widespread action of chemical pollutants and epidemic diseases. The impact of these factors is less understood, but they have been repeatedly invoked to explain the decline of local amphibian populations, even in pristine habitats (e.g., Kiesecker and Blaustein 1995; Daszak et al. 1999; Bosch et al. 2001; Davidson et al. 2002; La Marca et al. 2005; Skerrath et al. 2007; Raxworthy et al. 2008).

In this thesis, I will focus on one of the worldwide extinction-driving factors: the spread of alien species. My interest is to study the ecological space occupied by alien frogs relative to the native species as a first step to assess their putative impact on native assemblages.

I. 2. The concept of biological invasion

Biological invasion is the process that mediates the establishment of an alien species (i.e., "an organism occurring outside its natural past or present range and dispersal potential, whose presence and dispersal is due to intentional or unintentional human action"; Walther et al. 2009). These alien species are considered invasive when they show a rapid increase in their range in the invaded region without necessarily implying a negative impact on the native assemblages (Richardson et al. 2000). The phenomenon of biological invasions is global and involves most of the taxonomic groups (Lodge 1993). The impact of an alien species usually relates to the trophic interactions established within the new community, but in some cases their impact could extend to the entire ecosystem (Mooney and Cleland 2001; Snyder and Evans 2006; Ehrenfeld 2010). These effects can be catastrophic, causing the extinction of entire groups of native species, especially in naïve communities such as insular habitats (Smith and Quin 1996; Towns and Daugherty 1999; Wiles et al. 2003). However, there are also examples where there is not an observable impact in native diversity (Brown and Gurevitch 2004; Gurevitch and Padilla 2004; Sax et al. 2007). These alien species became integrated into local ecosystems, sometimes associated synergistically with other aliens (O'Dowd et al. 2003; Beard and Pitt 2005). In any case, determine the factors that regulate the process of invasion of alien species is a conservation priority for two main reasons: (1) invasive species are a major negative force shaping worldwide ecosystems (Zavaleta et al. 2001; Bax et al. 2003), and (2) the application of management and eradication measures depend on thorough knowledge of their ecology (Clout and Veitch 2002).

Not all introduced species are successful invaders (Table 1). The process of invasion is structured in a continuum of stages, from the translocation (introduction), until the alien species is able to reproduce (naturalization) and extend its range (invasion; Richardson et al. 2000; Catford et al. 2009). However only a small subset of introduced species become naturalized (Williamson and Fitter 1996; Levine et al. 2004), due to the presence of ecological filters that hinder their spread (Theoharides and Dukes, 2007). The way that these filters are regulating successful invasions are not entirely ascertained, possibly because they vary stochastically (Kolar and Lodge 2001; Hayes and Barry 2008). However, there are some attributes that make a species a potentially successful invader, such as the ability to exploit a broad range of habitats and to display behavioural plasticity (Marchetti et al. 2004).

Table 1. Examples of failed introductions in amphibians. Data provided by Non-Indigeneous Aquatic species (a), NOBANIS (b), GB non-native species (c), New Zealand frogs (d), Guam Environment (e) databases and Fa et al. 2010 (f). ?: date of introduction unknown. Legend: W: west; NW: north-western; E: east; C: central; SE: south-estern; SW: south-western; UK: United Kingdom; USA: United States of America.

Family	Species	Origin	Alien range	First record
Ambystomatidae	*Ambystoma gracile*	USA	Guam	? (e)
Proteidae	*Proteus anguinus*	Adriatic region	Austria Germany	1933 (b)
Salamandridae	*Cynops pyrrhogaster*	Japan	USA	1932 (a)
			W Europe	1900 (b)
	Lissotriton montandoni	E Europe	Germany	1901 (b)
	Pachytriton labiatus	China	USA	2010 (a)
	Triturus marmoratus	SW Europe	Germany	1936 (b)
Alytidae	*Alytes obstetricans*	SW Europe	Poland	? (b)
Bombinatoridae	*Bombina variegata*	W Europe	UK	1965 (c)
Bufonidae	*Atelopus zeteki*	Panama	USA	1963 (a)
	Bufo bufo	UK	New Zealand	1860 (d)
	Duttaphrynus melanostictus	SE Asia	USA	2010 (a)
	Rhaebo blombergi	Ecuador, Colombia	USA	1963 (a)
Eleutherodactylidae	*Eleutherodactylus coqui*	Caribbean Islands	Guam	? (e)
Hylidae	*Hyla arborea*	W Europe	UK	1900 (c)
	Hyla meridionalis	NW Africa	Germany	1950 (b)
	Pseudacris regilla	NW USA	Germany	? (b)
			Guam	? (e)
	Litoria adeiladensis	Australia	New Zealand	1981 (d)
	Litoria caerulea	Australia	New Zealand	1897 (d)
	Litoria gracilenta	Australia	New Zealand	1972 (d)
	Pachymedusa dacnicolor	Mexico	USA	1964 (a)
Hyperoliidae	*Afrixalus fornasini*	E Africa	USA	2010 (a)
Leptodactylidae	*Leptodactylus fallax*	Dominica	Jamaica Puerto Rico	? (f)
Microhylidae	*Gastrophryne carolinensis*	USA	Guam	? (e)
Pipidae	*Hymenochirus boettgeri*	C Africa	USA	1964 (a)
Ranidae	*Pelophylax lessonae*	UK	New Zealand	1860 (d)
	Rana temporaria	UK	New Zealand	1860 (d)

There may also be external factors in addition to species qualities that affect the invasion success of a species. Elton (1958) suggested that the composition of the assemblages could play an important role in determining their invasibility. The effect of the species interactions in the assemblage structure is a key issue in ecology and has also important implications for understanding the processes of invasion. MacArthur and Levins (1967) proposed that there is a limit to the degree of resemblance of those species that coexist and share similar resources (limiting similarity hypothesis). Diamond (1975) argued that in the bird assemblages from oceanic islands species associations were competitively organised, with some combinations being less frequent than expected. Subsequently, other authors corroborated the existence of non-random patterns in the assembly of several groups of biota (e.g., Fox and Brown 1993; Gotelli and McCabe 2002). Moreover, the existence of assembly rules is of great importance to understand the "invasibility" of an assemblage (i.e., its resistance to invasion) and species "invasiveness" (i.e., the ability of a species to become established under new ecological conditions). In this sense, alien species frequently become established in a community by occupying an empty niche, finding minimal resistance to the colonization (Houlahan and Findlay 2004). However, alien species can also become established in a niche previously occupied by one or more native species, usually causing a loss of the species' association structure. This invaded assemblage tends to reach a new equilibrium, which may involve the extinction or depletion of native species (Holway 1999; Gotelli and Arnett 2000). The mechanisms that enable aliens to outcompete native species are diverse and sometimes indirect (e.g., related to the transmission of pathogens, Crowl et al. 2008), although they commonly depend on their capacity to exploit a wide range of resources (Rahel and Olden 2008).

I. 3. Amphibians as invasive species

Amphibians are a special group of invasive species, as they theoretically lack some of the attributes that favour invasiveness. Most amphibians have permeable skin, making them very prone to dehydration (Toledo and Jared 1993); for this reason, many amphibians depend on thermal buffered corridors for their migratory movements (Gibbs 1998; Escoriza and Ben Hassine 2014). Moreover, many amphibians show a strong dependence on the existence of aquatic habitats to complete their life cycles, requiring specific conditions for successful breeding (Wilbur 1987; Hecnar and M'Closkey 1997). Nevertheless, in some cases, anurans have shown remarkable success as invaders (Kark et al. 2009). More than 40 species have been introduced far away of their native ranges and have been able to maintain stable populations over time (Table 2). Most of the examples belong to a few derived lineages that include species with exotrophic larval development and with high dispersal capacity (van Boxclaer et al. 2009; Pramuk et al. 2010; Tingley and Shine 2011).

Table 2. World-wide examples of alien amphibians. Data provided by Invasive Species Database (a), Alien Species of Japan (b), Non-indigenous Aquatic Species (c), NOBANIS (d) and GB non-native species databases (e), and Pyke et al. 2002 (f), Wycherley et al. 2003 (g), Recuero et al. 2007 (h), Diesmos et al. 2008 (i), Brown et al. 2010 (j), Ng and Yeo 2012 (k), Xu et al. 2012 (l), Domeneghetti et al. 2013 (m), Pearson 2014 (n), Tingley et al. in press (o), Amphibiaweb 2014 (p), de Carvalho et al. 2013 (q), and Vences et al. 2004 (r). Legend: W: west; NW: north-western; E: east; S: south; C: central; SE: south-estern; SW: south-western; UK: United Kingdom; USA: United States of America.

Family	Species	Origin	Alien range	Effect
Cryptobranchidae	*Andrias davidianus*	China	Japan	Hybridization with native species (b)
Salamandridae	*Ichthyosaura alpestris*	W Europe	UK	Competition with native species (d)
	Lissotriton vulgaris	W Europe	Australia	Not assessed (o)
	Triturus carnifex	Italy	UK, Portugal, France	Competition and hybridization with native species (e)
Alytidae	*Alytes obstetricans*	W Europe	UK	Not assessed (e)
	Discoglossus pictus	NW Africa	SW Europe	Not assessed
Bombinatoridae	*Bombina bombina*	E Europe	Sweden	Not assessed (d)
Bufonidae	*Amietophrynus gutturalis*	C and S Africa	Mauritius	Not assessed (p)
	Bufotes viridis	E Europe	Sweden	Not assessed (d)
	Duttaphrynus melanostictus	S Asia	Magadascar Papua New Guinea	Competition and predation of native species (n)
	Rhinella marina	C and S America	Caribbean islands Pacific islands Australia	Decline of native lizards, snakes and mammals (c)
Dendrobatidae	*Dendrobates auratus* *Dendrobates leucomelas*	C America	Hawaii	Not assessed (c)
Dicroglossidae	*Hoplobatrachus chinensis*	China	Philippines	Competition with native anurans (i)
	Hoplobatrachus tigerinus	SE Asia	Magadascar Maldives	Not assessed (p)
Eleutherodactylidae	*Eleutherodactylus coqui*	Puerto Rico	Caribbean islands SE USA Hawaii	Disturbances in the ecosystem nutrient cycling (a, c); Competition with native anurans (a, c)
	Eleutherodactylus johnstonei *Eleutherodactylus planirostris* *Eleutherodactylus cystignathoides*	Caribbean basin	Caribbean islands C and S America SE USA Bermuda	Competition with native anurans (a, c)
Hylidae	*Hyla meridionalis*	NW Africa	SW Europe Canary islands Minorca	Competition and hybridization with native species (h)
	Hyla squirella	SE USA	Bahamas	Not assessed (p)
	Litoria aurea *Litoria raniformis* *Litoria ewingii*	Australia	New Zealand New Caledonia Vanuatu	Predation of native species (f)
	Litoria fallax	Australia	Guam	Not assessed (c)
	Osteopilus septentrionalis	Cuba, Bahamas	Caribbean islands SE USA	Competition and predation of native anurans (a, c)
	Pseudacris clarkii	Texas	Montana	Not assessed (p)

Family	**Species**	**Origin**	**Alien range**	**Effect**
Hylidae	*Scinax quinquefasciatus*	Ecuador	Galapagos Islands	Not assessed (p)
	Scinax ruber *Scinax x-signatus*	S America	Puerto Rico Martinique Guadaloupe	Competition with native anurans (a, c)
Leptodactylidae	*Leptodactylus labyrinthicus*	S Brazil	Central Amazonia	Competition and predation of native anurans (q)
	Leptodactylus validus	Trinidad and Tobago	Lesser Antilles	Not assessed (p)
Microhylidae	*Gastrophryne carolinensis*	SE USA	Caribbean islands	Not assessed (a, c)
	Kaloula pulchra	SE Asia	Philippines Guam Indonesia	Competition with native anurans (c, i, k)
Microhylidae	*Microhyla fissipes*	SE Asia	Singapore	Competition with native anurans (k)
Pipidae	*Xenopus laevis*	S Africa	America, Europe, Asia	Competition with native anurans (a, c); vector of Chytridiomycosis (a,c)
Ptychadenidae	*Ptychadena mascariensis*	Magadascar	Seychelles Mascarene islands	Not assessed (r)
Pyxicephalidae	*Strongylopus grayii*	S Africa	Saint Helena	Not assessed (p)
Ranidae	*Glandirana rugosa*	Japan	Hawaii	Not assessed (c)
	Hylarana erythraea	SE Asia	Philippines Sulawesi	Competition with native anurans (i)
	Hylarana guentheri	SE Asia	Singapore Guam	Competition with native anurans (k)
	Lithobates catesbeianus	E USA, Canada	NW and S America, Europe, Asia	Competition of native anurans; vector of Chytridiomycosis (a, c)
	Lithobates grylio	E USA	China Bahamas Puerto Rico	Competition with native anurans (c, l)
	Pelophylax bedriagae *Pelophylax kurtmuelleri* *Pelophylax perezi* *Pelophylax ridibundus* *Pelophylax shqipericus*	S and E Europe	W Europe C and E Asia Malta	Hybridogenesis with native green frogs; competition with native amphibians (e, g, m)
Rhacophoridae	*Polypedates leucomystax*	SE Asia	Japan Philippines Sulawesi	Competition with native anurans (b, k)
	Polypedates megacephalus	SE Asia	Guam	Not assessed (c)

Three of these species stand out due to the profound disturbance that they have caused in the local ecosystems and are classified among the 100 worst invasive species (Lowe et al. 2000):

a) The cane toad *Rhinella marina* was introduced in Australia in 1935 from South America for pest control (Philips et al. 2007). This species has rapidly colonised much of tropical Australia, including inland semiarid regions (Clarke et al. 2000). This process has been facilitated by the development of an invasive phenotype that allowed it to spread faster in the boundaries of its range (Llewelyn et al. 2010). The cane toad invasion has affected many species of amphibian predators (such as crocodiles, snakes, varanid lizards and dasyurid marsupials; Smith and Philips 2006; Shine 2010). These animals die when they attempt to eat these toads due to the high toxicity of their cutaneous secretions (Philips et al. 2003).

b) The Caribbean tree frog *Eleutherodactylus coqui* was accidentally introduced to Hawaii from Puerto Rico in the 1980s. On the Hawaiian Islands, it has reached very high densities, with up to 90,000 specimens$\cdot$ha^{-1} (Beard et al. 2008). The expansion of this species has reduced insect biomass, subsequently altering the nutrient cycle in the native forests. These disturbances have also favoured the establishment of alien plants in these forests (Sin et al. 2008).

c) The bullfrog *Lithobates catesbeianus* has been introduced in many temperate and tropical regions of Asia, America and Europe throughout the 20th century (Nori et al. 2011). The bullfrog has caused the depletion of some species of aquatic frogs, especially by out-competing native species in disturbed habitats (Pearl et al. 2004; Fuller et al. 2011).

The effect of these species on the native amphibian communities is diverse. For instance, the local existence of congeneric species in the invaded region can produce hybridisation, diluting the native genotype and producing, in some cases, the rapid extinction of non-hybridogenetic forms (Holsbeek and Jooris 2010). The alien species can also be epizootic vectors, introducing pathogens to which they are resistant. In at least two species, *Xenopus laevis* and *Lithobates catesbeianus,* these species act as reservoirs of the fungus *Batrachochytrium dendrobatidis*, which has a lethal effect on other frogs (Schloegel et al. 2010; Solís et al. 2010). In the case of *Rhinella marina,* the toxicity of its eggs causes mass mortalities in the larvae of other frogs (Crossland 2000). However, the most common effect is the appearance of competitive interactions and the subsequent displacement of the native species from their optimal niches.

I. 4. The study of the community interactions of invasive amphibians

Most anuran larvae are trophic generalists that feed on detritus, algae and macrophytes (Díaz-Paniagua 1985; Kupferberg et al. 1994; Petranka and Kennedy 1999; Altig et al. 2007). This produces some degree of overlap within natural larvae assemblages, even among those species that exploit different microhabitats (Prado et al. 2009; Schiesari et al. 2009). In order to reduce negative interactions, associations among species are usually structured (i.e., some species combinations are uncommon or “forbidden”), especially when considering species that occupy similar functional niches (Both et al. 2011a). For this reason, the addition of a new species can cause the appearance of trophic overlap, likely reducing larvae fitness and survival (Kupferberg 1997; Kiesecker et al. 2001; Pearl et al. 2004; Smith 2005).

The existence of niche overlap can be assessed by different methods, such as examining the spatial partitioning in the use of habitat (Cabral-Eterovick and Souza-Barros 2003) or studying the tadpole diet (Rossa-Feres et al. 2004). The strongest interactions are expected to appear between species that belong to the same eco-morphological guild, as these species are expected to use a similar proportion of resources (McDiarmid and Altig 1999).

Larvae body plan show important variability, depending on the use of the water column (benthic, pelagic and neustonic morphs), flow systems (lotic, lentic and phytotelmata guilds) and foraging habits (macrophagous, raspers, suspension filters or endotrophic morphs; Altig 1970; Liu et al. 1996; Vera-Candioti 2006, 2007; Wells 2010). The different anuran lineages show a high degree of convergence when occupying the same larval niche, allowing to define functional groups which can be indentified in larval communities at global scale (McDiarmid and Altig 1999). Anuran larvae are also a special case among the vertebrates, as some phenotypic traits are inducible by environmental factors. This fine adjustment of tadpole morphology (i.e., within the same species) can improve larval survival under stressful conditions. For example, variations in the depth of the tail produce differences in swimming performance, decreasing the vulnerability of the larvae in predator-rich environments (McCollum and van Burskirk 1996; Arendt 2010). For these reasons, the examination of morphological characters of tadpoles can provide valuable information on their ecology by comparing species or conspecific populations (Altig and Johnston 1989; van Buskirk and Relyea 1998).

The analysis of larval morphology can based on the description certain categorical traits, linear measurements, or geometrical analysis (Altig 2007; Hossie et al. 2010; Escoriza and Ben

Hassine 2014). This latter method provides a very flexible approach that allows to define the species' average phenotype and analyze the relationships established in the morphospace. This can be useful in the study of the ecology of invasive species. It is expected that sympatric species which have co-evolved together do not share exactly the same position in the morphospace, showing a relatively low overlap comparing their phenotypic clouds (which are defined by the variation of the habitat-induced phenotypes). The existence of ecological character displacement among species that compete for similar resources is common in many vertebrate assemblages (Bowers and Brown 1982). Therefore we can expect that an invasive species could occupy a random position in the morphospace, e.g., being functionally redundant. In this case, the analysis of the morphospace relationships could be very helpful in order to detect the most vulnerable species to the alien.

I. 5. Invasive frogs in south-western Europe

In the south-west of Europe were recorded several species of alien amphibians (see Table 1), but we will focus our study on two of them: *Discoglossus pictus* and *Hyla meridionalis.* Both species are from North African origin and have spread to regions with Mediterranean conditions (Gasc et al. 1997). However, *H. meridionalis* could be a historical introduction (much older than *D. pictus,* introduced a century ago) and during this period it spread over a wide geographical region, where it contacts other hylid species. Therefore, although both colonization processes show certain similarities, also are sufficiently different to reveal distinct factors which could act facilitating the invasion.

I. 5. 1. *Discoglossus pictus* in south-western Europe: an example of a recent invasion

The genus *Discoglossus* belongs to the family Alytidae, a basal lineage within the anurans which is endemic to the western Palearctic region (Roelants et al. 2007). The genus *Discoglossus* and the sister genus, *Latonia*, were spread throughout most of the Mediterranean region until the late Pleistocene (Holman 1998); however, after the last glacial maximum these genera only survived in a few southern refugia (Delfino 2003; Zangari et al. 2006). Most species of the genus *Discoglossus* appear in highly seasonal subtropical climates and are associated with aquatic habitats with hydrological instability (Knoeppfler 1962; Capula 2007). However, one species, *Discoglossus montalentii*, which is the most basal species of the genus (Pabijan et al. 2012), is a rheophilic specialist (Salvidio et al. 1999).

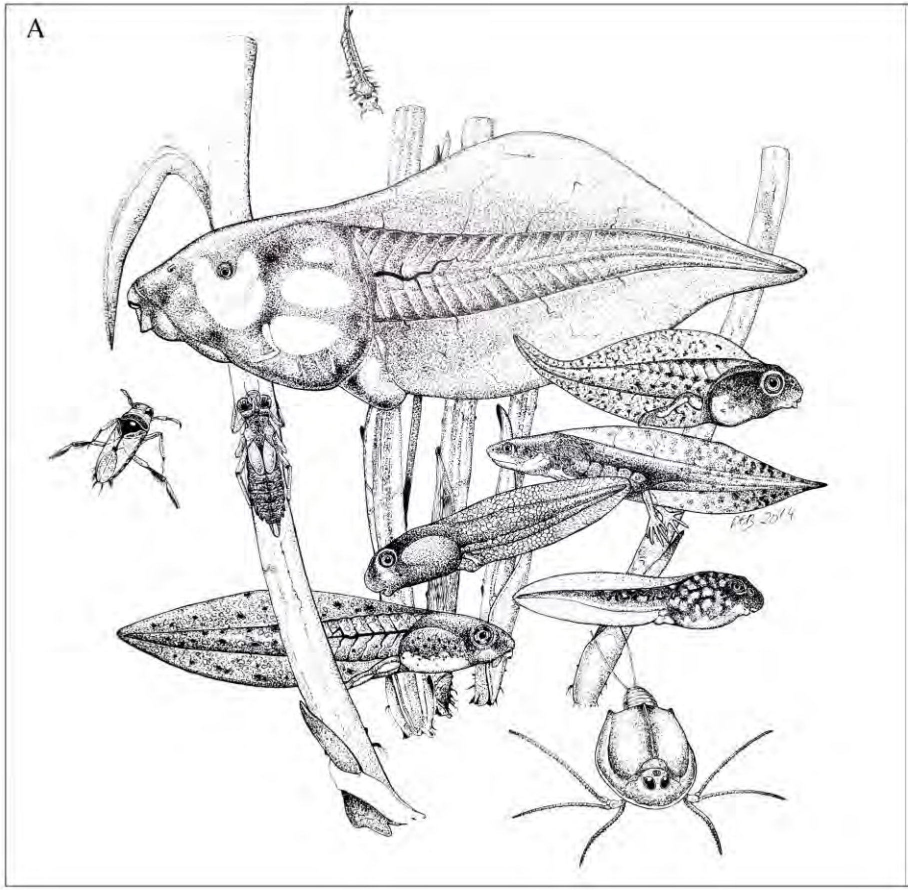
Discoglossus pictus is the species of the genus which occupies a wider geographical range (from north-eastern Morocco to Tunisia, also appearing in some central Mediterranean islands, such as Sicily, La Galite, Gozo and Malta and in south-western mainland Europe; Lanza et al. 1986). In this region, it occurs from the margins of the temperate belt in the Etna and eastern Pyrenees to the arid climates in southern Tunisia (Escoriza et al. 2007; Turrisi et al. 2007; Ben Hassine and Nouira 2012). In most of its native range, *D. pictus* is one of the most abundant amphibian species and occupies a large part of the available water bodies, with the exception of lotic habitats, like rivers and fast-flowing streams (Ben Hassine and Nouira 2012; Samraoui et al. 2012).

Discoglossus pictus was introduced into the southern tip France from Algeria in the early 20th century (Wintrebrert 1908). The allochthonous character of these populations was confirmed using molecular analysis, proving that its origin can be traced to northern Algeria (Martínez-Solano 2004). This species has rapidly extended its range, and at the present time it occupies an area of approximately 2000 km² between southern France and north-eastern Spain, with some examples of a "jumping distribution" (Fradet and Geniez 2004; Franch et al. 2007). In north-eastern Spain, *D. pictus* is a thermophilous species that appears to be mainly confined to the lowlands within the coastal mesothermal belt (Llorente et al. 1995). The painted frog occupies a wide range of habitats, even highly disturbed anthropic ecosystems (Knoepffler 1962; Geniez and Fradet 2013). The impact of this species is unknown but could be important given the range of habitats occupied by this species (Montori et al. 2007), where it can interact with many of the native frog species (Fig.1).

I. 5. 2. *Hyla meridionalis*: an historical invasion

The family Hylidae is part of the most recently derived lineages of frogs (Roelants et al. 2007) and is also one of the most successful, comprising approximately 945 species. This family is distributed throughout most of the temperate and tropical regions of the world, except in the Afrotropical region (AmphiaWeb 2014). The Mediterranean region is an important region of speciation for the genus *Hyla*, where between 8 and 10 species occur. These species are parapatrically distributed, and each genotype corresponds to the use of different refugia during the successive glacial maxima (Canestrelli et al. 2007; Stöck et al. 2008, 2012). In the Palearctic ecoregion, the genus *Hyla* is mainly restricted to the mesothermal belt, but some populations of *H. meridionalis* extend to edge of the arid zone in marginal habitats (Bons and Geniez 1996; Escoriza and Ruhí 2014). *Hyla* species are generalists in the selection of habitats (García-París et al. 2004) and usually breed in lentic water bodies (Díaz-Paniagua et al. 2005; Lanza et al. 2007).

The populations of the Iberian Peninsula are clustered into two distinct lineages, one within the Eurasian clade "*arborea*" and the other belonging to the African clade "*meridionalis*", which split 9.8 MYA (Stöck et al. 2012). All European populations of *H. meridionalis* belong to the same haplotypes found in Morocco, suggesting a relatively recent holocenic invasion, which could be mediated by humans (Recuero et al. 2007). In Europe, this species is distributed in the Canary Islands, Minorca and mainland Spain, Portugal, southern France and north-eastern Italy. In most of this European range, *H. meridionalis* is the only species of hylid, but there is overlap with native species west of the Iberian Peninsula and more locally in south-western France (Gasc et al. 1997; Lescure and De Massary 2013). Competition and hybridisation occur in these contact zones, as *Hyla molleri* (the native species) and *H. meridionalis* are synchronous breeders (Malkmus 2004).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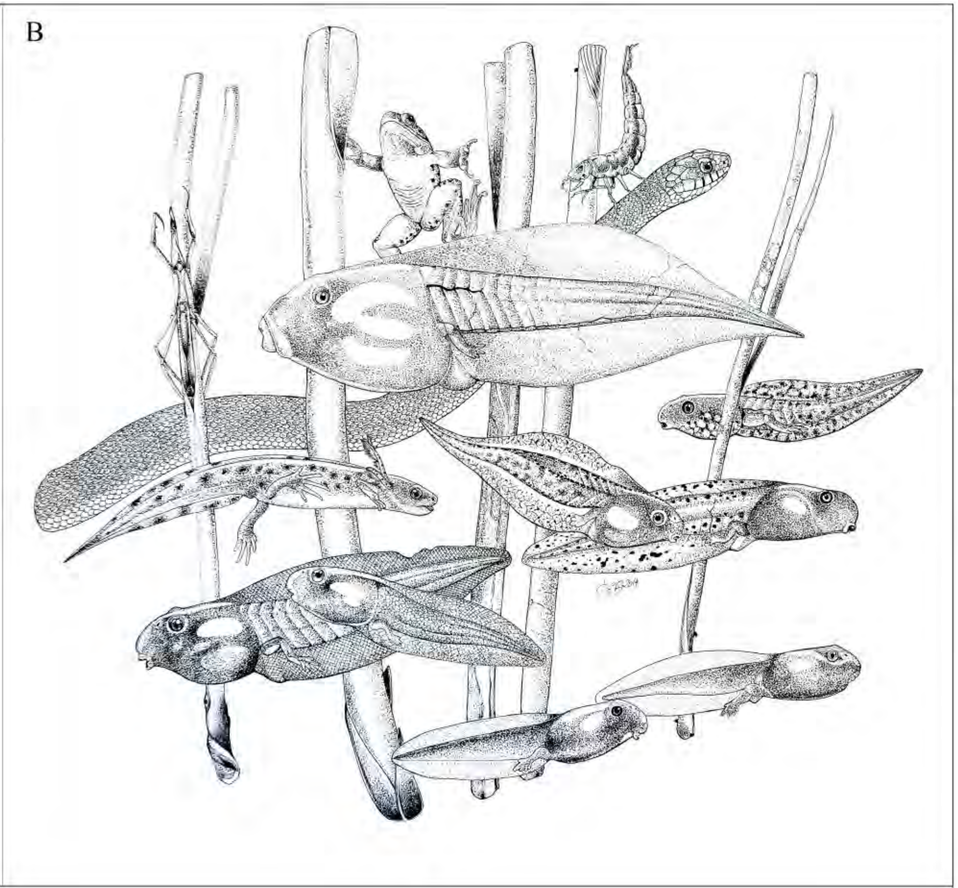
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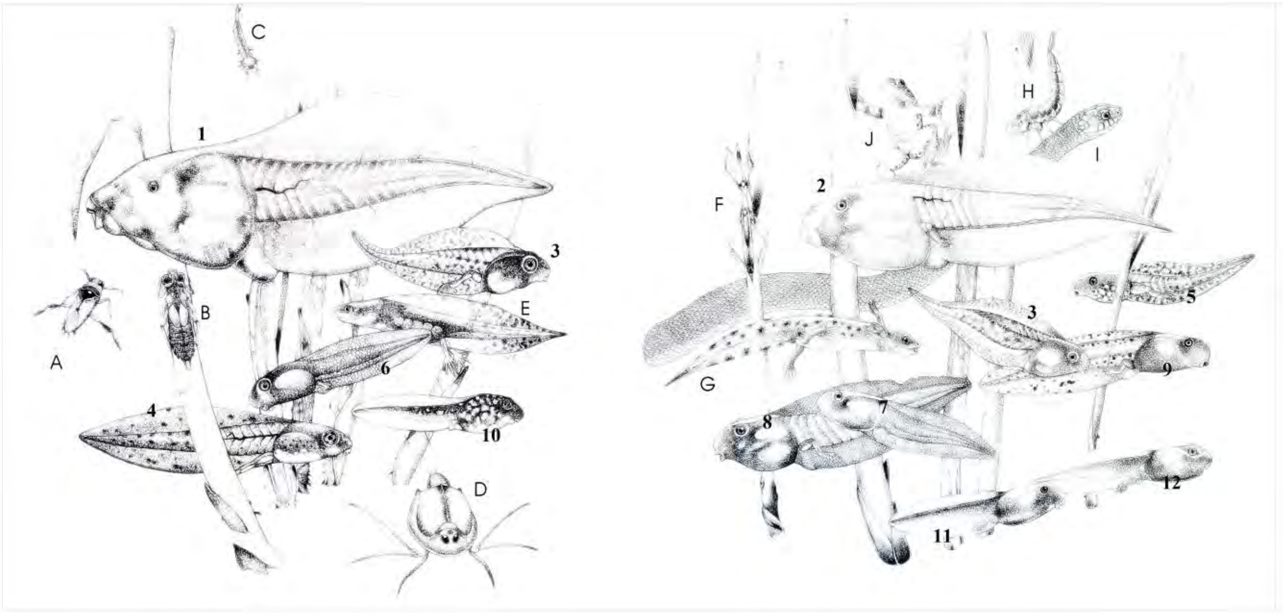


Figure 1. Examples of tadpole morphofunctional diversity of two anuran assemblages in the western Mediterranean region. A: North-western Morocco (Maâmora forest), where two nektonic species occur, but with very different body sizes, including a “gigantic” morph reaching 150 mm of total length (1, *Pelobates varaldii;* 3, *Hyla meridionalis*). There is also two small-sized benthic species (6, *Discoglossus scovazzi*; 7, *Amietophrynus mauritanicus*), but both show different phenology and co-occur over a short period of time. A fifth species, a great sized pseudonektonic morph, *Pelophylax saharicus* (4) is a later breeder, which usually breeds in permanent ponds. B: North-eastern Spain (Albera plain). In this assemblage appears an invasive anuran (7, *Discoglossus pictu*s) occupying similar niche that two native species, small-sized benthic guild (8, *Pelodytes punctatus*; 11, *Epidalea calamita*). *Pelobates cultripes* (2) and *Hyla meridionalis* (3), belong to the nektonic guild and use different microhabitats than the invasive species. Similarly to the moroccan assemblage both nektonic species show a great divergence in body sizes. Other sympatric anurans (9, *Alytes obstetricans*; 12, *Bufo spinosus;* both also classified in the benthic guild), and the pseudonektonic *Pelophylax perezi* (5) show null or low spatial overlap with *D. pictus*. Additional characteristic fauna from each region, A: *Notonecta meridionalis*; B: *Anax imperator* (larva); C: *Culex pipiens* (larva); D: *Triops mauritanicus*; E: *Pleurodeles waltl* (larva); F: *Ranatra linearis*; G: *Triturus marmoratus* (larva); H: *Dytiscus circumflexus*; I: *Natrix natrix astreptophora*; J: *Pelophylax perezi* (juvenile).

II. AIM AND THESIS OUTLINE

The purpose of this thesis is to study the factors that have regulated the establishment of two alien frogs in south-western Europe. In particular, we aim to test the following hypotheses:

1) The addition of alien species causes functional redundancy and the loss of structure in species associations (**Chapters 1, 2 & 3**).
2) Niche conservatism is a main ecological driver determining the invasive range of *Discoglossus pictus* (**Chapter 2**) and *Hyla meridionalis* (**Chapter 4**).
3) Both species show wide ranges of ecophysiological tolerance (**Chapters 2, 3 & 4**).
4) Both species are generalists and can exploit marginal or underused habitats for native species (**Chapters 2 & 4**).
5) Alien species are competitively superior to native species (**Chapter 2**).
6) Habitat selection is identical in native and invasive populations of *Discoglossus pictus* (**Chapter 2**) and *Hyla meridionalis* (**Chapter 4**) because amphibians are morpho-functionally conservative.
7) The congeneric species occupy the same morpho-functional niches. The genera constitute well-defined clusters in the larval morphospace (**Chapter 3**).
8) Invasibility is promoted by vacant niches and less functional diversity (**Chapters 2 & 3**).
9) The external morphology of tadpoles is linked to the characteristics of aquatic habitats (**Chapter 1**).

III. MATERIALS AND METHODS

III. 1. Study area and species

The study area comprised the distribution of three species of the genus *Discoglossus* and *Hyla meridionalis* in the western Mediterranean region (Fig. 2, 3; Lanza et al. 1986; Schleich et al. 1996). The species of the genus *Discoglossus* occur in northern Morocco (*D. scovazzi*); the south-western Iberian Peninsula (*D. galganoi*); north-eastern Iberian Peninsula and southern France (alien *D. pictus*); northern Tunisia (native *D. pictus*); and Sicily (native *D. pictus*). *Hyla meridionalis* also occurs in north-west Africa and south-west Europe, between 28°N and 46°N latitude (Schleich et al. 1996; Gasc et al. 1997). The area was within the dry-summer subtropical climate belt (Köppen-Geiger climate classification; Kottek et al. 2006), with annual precipitation ranging between 340 and 750 mm, mainly occurring in late autumn, winter and early spring (Hong Kong Observatory 2012). The study examined the two native subspecies of Iberian *Discoglossus* together (*D. g. galganoi* and *D. g. jeanneae*), because they are closely related, show similar habitat preferences, and coexist with the same species (García-París et al. 2004; Pabijan et al. 2012). The population groups of *D. pictus* were analyzed separately because the composition of their respective assemblages was clearly different (Turrisi et al. 2007; Ben Hassine and Nouira 2012; Escoriza and Boix 2012).

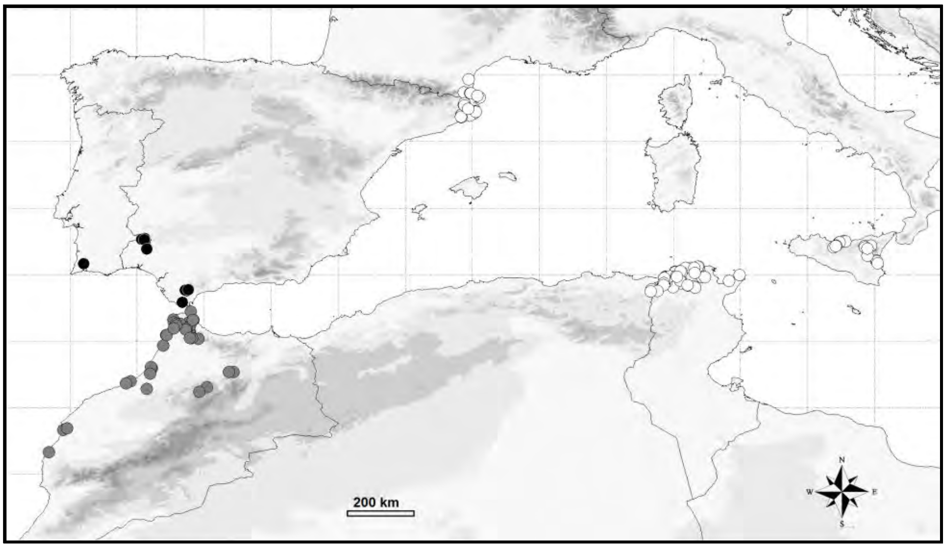


Figure 2. Study area of *Discoglossus* species (**Chapters 1, 2** & **3**). Black circles: *D. galganoi*, gray circles: *D. scovazzi,* white circles: *D. pictus.*

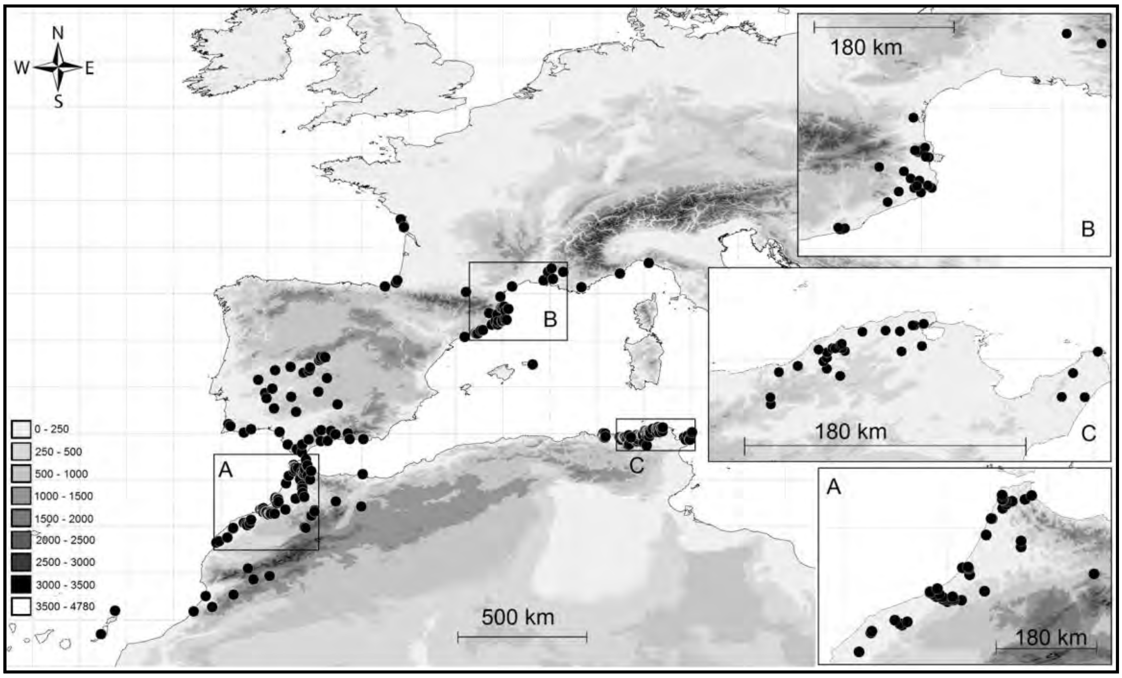


Figure 3. *Hyla meridionalis* study area (**Chapter 4**). The black points show the locations included in the climate models. The thumbnails display the locations where aquatic habitats were sampled. A: Morocco, B: north-eastern Spain and southern France; C: northern Tunisia.

III. 2. Larvae sampling and habitat characterization

Sampling was conducted during the period 2010–2012. This sampling was carried out between February and May, depending on the region, because previous surveys showed that prometamorphic cohorts (Gosner's 36–39; Gosner, 1960) of *D. pictus* and *H. meridionalis* can be found in these periods. Surveyed habitats included temporary ponds, permanent ponds, and stream pools. Tadpoles were captured using a 250 µm mesh-size net by performing a minimum of one sweep with a dipnet per 10 m^2 per water body, totalling up to 60 sweeps in larger water bodies and covering both shallow and deep areas.

The characterization of aquatic habitats was performed in all chapters. The physical and chemical variables measured in each water body were the average depth (cm), surface area (m^2), slope, water temperature (ºC), dissolved oxygen (mg$\cdot$L^{-1}), pH, and conductivity (µS$\cdot$cm^{-1}). Average depth was defined as the mean value of five successive measurements from the shore to the centre. The surface area was obtained by measuring the maximum length of the longitudinal axis and the length of the transverse axis and assuming an elliptical shape for the water body. In water bodies larger than 100 m^2, the surface area was estimated with a Garmin Dakota 100. The slope was calculated with the following equation:

slope = maximum depth / {sqrt (surface area / 2)}

Chemical and physical parameters were measured in situ with a Crison 524 conductivity meter (for conductivity), an EcoScan ph6 (for pH), and a Hach HQ10 Portable LDO meter (for dissolved oxygen). Water bodies were sampled between 12:00 h and 15:00 h (local time) to maintain maximum homogeneity of measured parameters.

III. 3. Morphological analyses

We compare similarities in larval morphology through the analysis of digital images. This method allows us to measure minor shape variability and to calculate areas and linear measurements from photographs. The photographs were taken from specimens previously captured and fixed in formalin, using a Nikon D–80 camera mounted at a fixed distance. The tadpoles were kept in suspension in water in a horizontal position on a bottom covered with graph paper used as a reference to measure their lengths. The longitudinal axis of the tadpole was kept parallel to one of the horizontal lines of the graph paper. The photographs were taken in a plane perpendicular to the tadpole. We used the ImageJ 1.42 program and the FracLac 2.5 plugin (Rasband 2009) and Sigma Scan Pro v. 5.0.0 (1987–1999 SPSS Inc.) to obtain linear measurements and areas, and to perform shape analysis from digital images. The variables measured from digital images describe the shape of the body and tail, and the position of eye and oral cavity. In **Chapter 1**, 14 morphological variables were measured, and their description is detailed below (see also Fig. 4). (1) Body span ratio (BS): ratio between major axis and minor axis of body length (2) body circularity (BC); (3) tail span ratio (TaS): ratio between major axis and minor axis of tail; (4) circularity of the tail (TaC); (5) total length (TL): measured from the tip of the snout to the tip of the tail; (6) body length (BL): measured from the tip of the snout to the beginning of the muscular portion of the tail; (7) tail length (TaL): measured from the onset of the muscular portion of the tail to the tip of the tail; (8) eye diameter (ED): antero-posterior eye diameter; (9) eye-mouth distance (EMD): measured from the angle of the mouth to the nearest point on the eye; (10) muscular tail diameter (MTaD): dorsal ventral diameter portion of the tail muscle at its insertion; eye-body ratio (E / B): ratio between eye area and body area; (11) tail body ratio (Ta / B): ratio between tail area and body area; (12) fin tail ratio (F / Ta): ratio between fin area and tail area; (13) mouth position (ME): angle from line originated at the rear of the mouth and crosses with the central axis of the eye; and (14) eye circularity (EC).

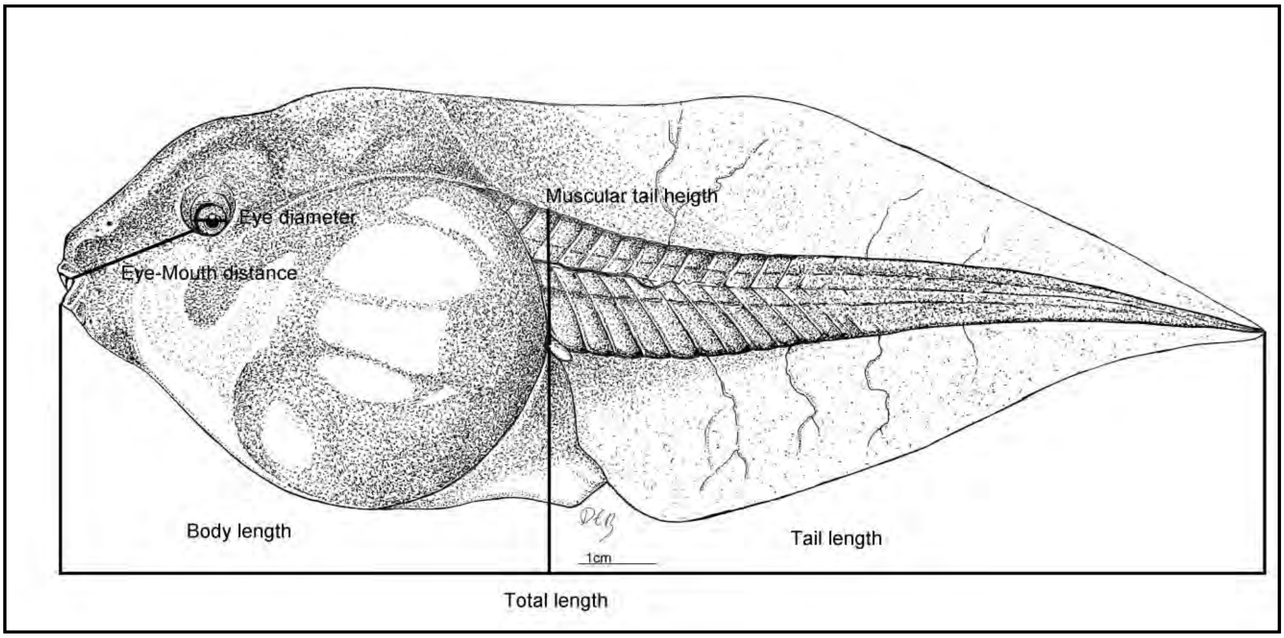


Figure 4. Linear measures included in the morphological analysis performed in **Chapter 1**. *Pelobates varaldii* tadpole (nektonic ecomorph) at Gosner's stage 30 (Kenitra, Morocco).

In the **Chapter 2** we examined larvae belonging to eight species in the invaded assemblage (north-eastern Spain) and four species in the native assemblage (Tunisia). To reduce the allometric effects of larval development, we selected only prometamorphic larvae (Gosner's 36–39; Gosner 1960). With the exception of *Amietophrynus mauritanicus,* which were not found above Gosner's stage 28, specimens of all anuran species at the sampled sites were included in this analysis. The morphological data were obtained by digital analysis of photographs, following the procedure detailed previously. We measured three morpho-functional traits that relate to habitat use strategy: body size, body shape, and colouration of the larvae (Altig 2007; see Fig. 5, 6, 7). Colouration was included in this analysis because the larvae of certain anuran species can adjust their body colouration depending on background conditions (McCollum and Leimberger 1997; see Fig. 8), whereas other species, such as the larvae of Bufonidae, display unchanging colouration (Díaz-Paniagua et al. 2005). The morphological variables measured were as follows: (1) total body area (TA), measured from the tip of the snout to the tip of the tail; (2) mean grey value (MG), calculated as the sum of the grey values for all pixels, divided by the total number of pixels, values close to 0 indicate darker colors while values close to 255 indicate lighter colors; and (3) span ratio (SR) i.e., the ratio between the longitudinal and transversal axes.

In **Chapter 3** we measured total area (Fig. 9), and span ratio (Fig. 10) of several species and genus of western Mediterranean anurans. Both morphological variables were used to display the position in the morphological space of each family. Families were considered morphologically similar if they showed a large overlap of the ellipses generated by the 95% confidence interval.

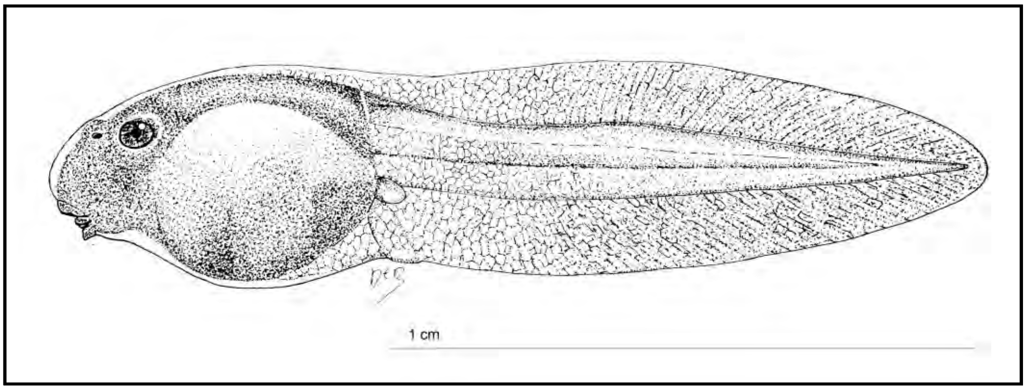


Figure 5. Example of anuran larva belonging to the benthic guild: *Discoglossus pictus* at Gosner's stage 28 (Tafoughalt, Morocco). It can be appreciated the small size, the depressed body morphology (i.e., high span ratio) and the small eyes, located dorsally.

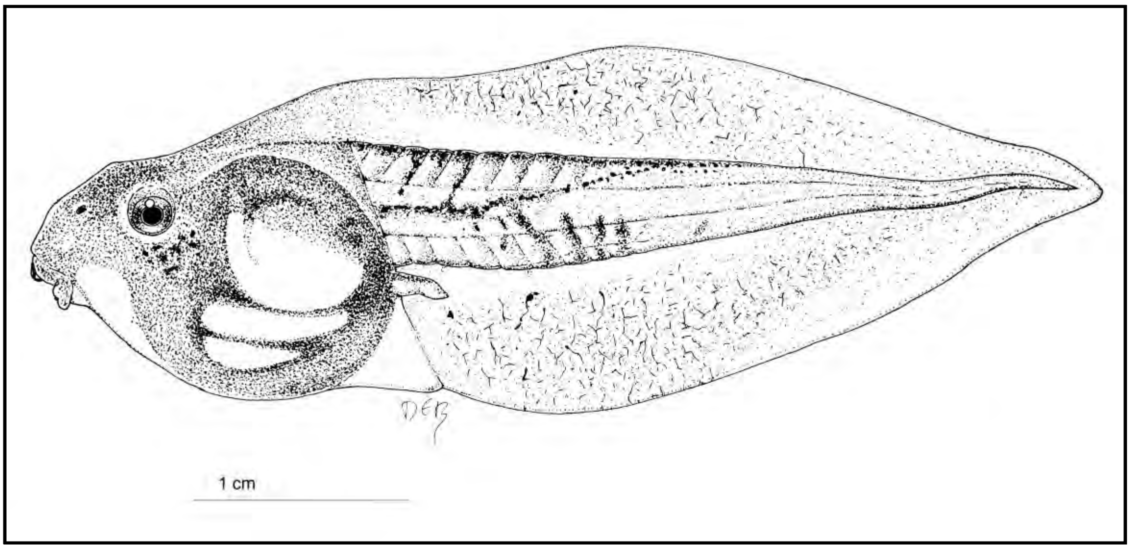


Figure 6. Example of anuran larva belonging to the nektonic guild: *Hyla meridionalis* at Gosner's stage 33 (Mohammedia, Morocco). It can be appreciated the medium size, the compressed body morphology (i.e., low span ratio) and the large eyes, located laterally.

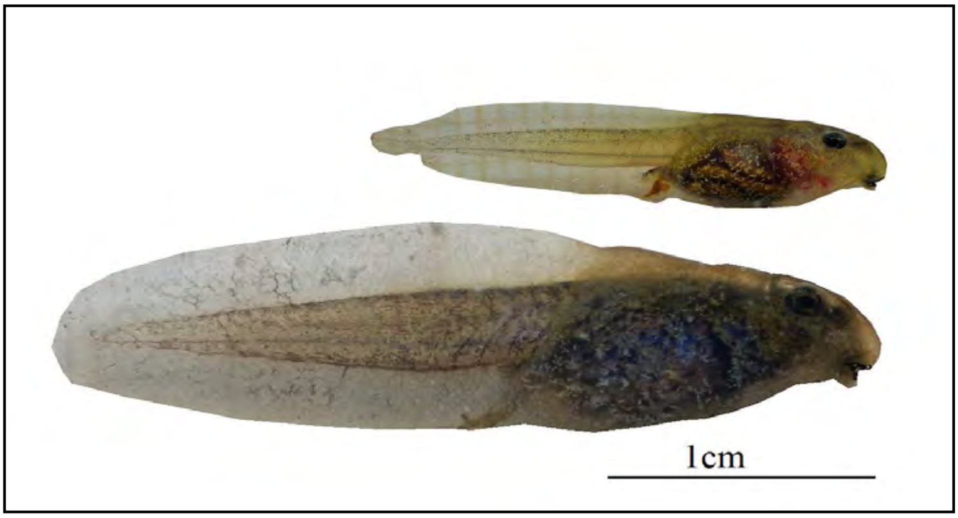


Figure 7. Intraspecific (*Discoglossus pictus*) variation in size, at the same development stage, Gosner's 36 (Catania, Sicily).

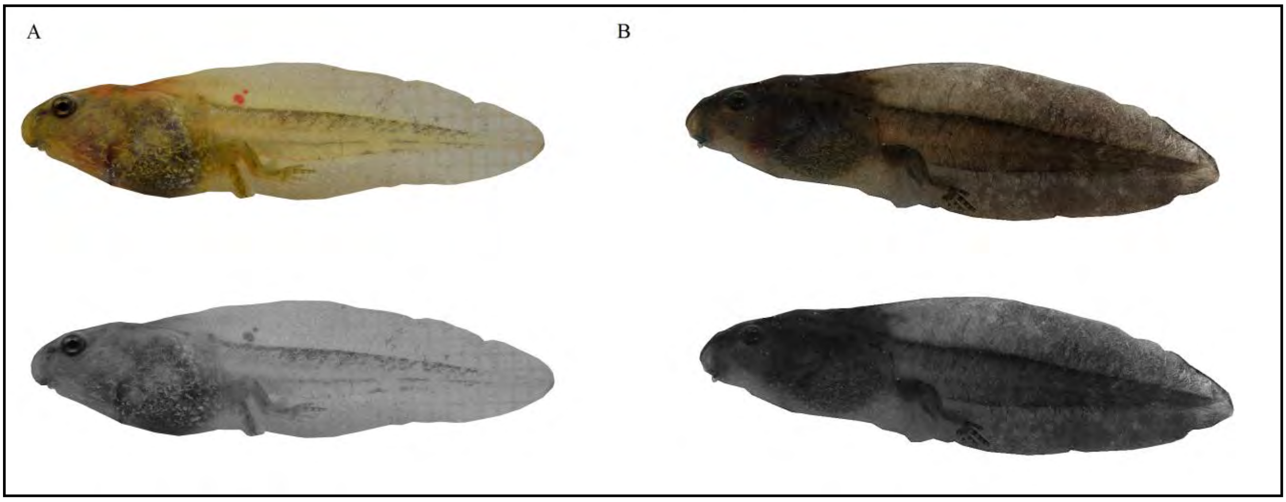


Figure 8. Intraspecific (*Discoglossus pictus*) variation in coloration, depending on water transparency. a) turbid water, mean gray value = 107 (Bizerte, Tunisia) ; b) transparent water, mean gray value = 48 (Siracusa, Sicily).

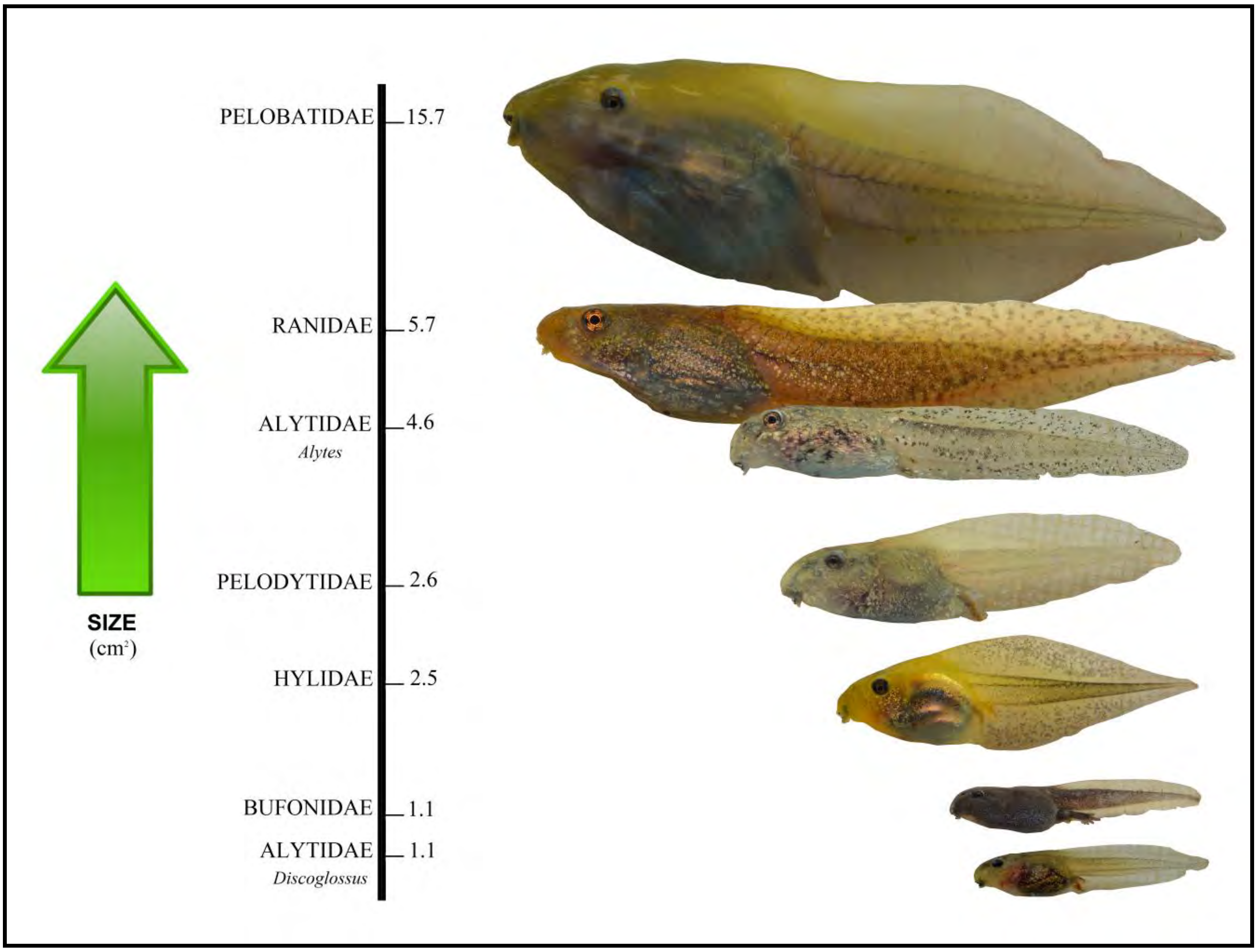


Figure 9. Variability in average larval size (cm^2) at prometamorphic stages (36–39) of six anuran families occurring in the western Mediterranean basin. The genus *Discoglossus* shows the lowest values, along with the species of the family Bufonidae.

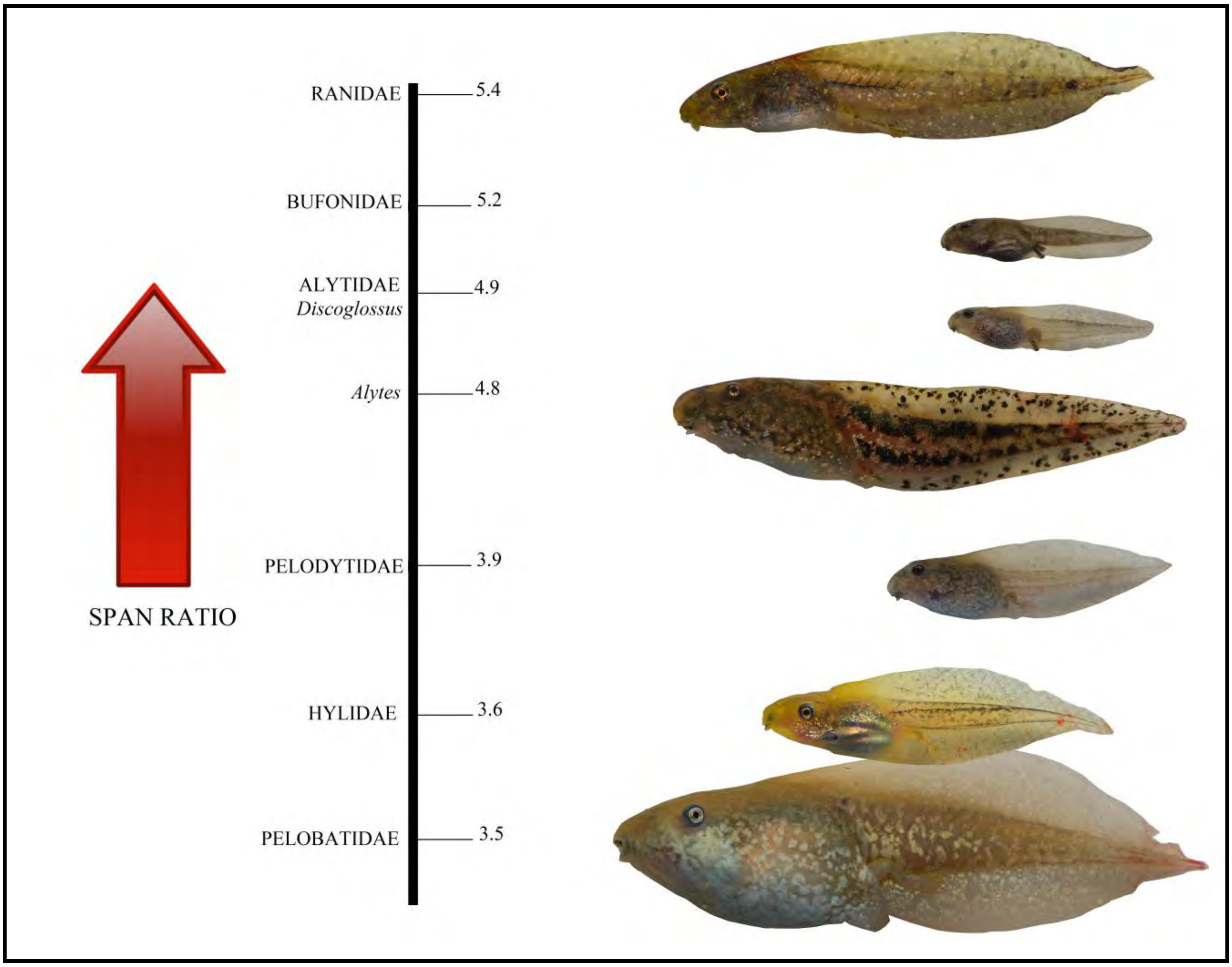


Figure 10. Variability in average larval span ratio (ratio between the longitudinal and transversal axes) at prometamorphic stages of six anuran families occurring in the western Mediterranean basin. The genus *Discoglossus* shows high values, along with the species of the family Bufonidae and Ranidae.

III. 4. Landscape and climate data

The study of niche at regional scale was performed in **Chapters 1, 2,** and **4**. The regional niche was quantified on the basis of presence localities, and the data extracted from environmental layers were used to characterize these localities. In the **Chapter 1** we described the regional niche including topographic (altitude) and 19 climate variables describing the thermal and precipitation ranges and their annual variation (Hijmans et al. 2005).

In **Chapter 2** and **4** we included only those variables that describe some aspects of the climate space that are assumed to be ecologically relevant to the species; in amphibians these factors are related to the water-energy balance and temperature extremes (Rodríguez et al. 2005; Smith et al. 2005; Escoriza and Ruhí 2014). We characterized the environmental water-energy balance using an aridity index (AIT): mean annual precipitation / mean annual potential evapotranspiration (Trabucco and Zomer 2009). Thermal ranges were assessed based on mean temperature of warmest quarter (i.e., 3–month period) and mean temperature of coldest quarter. These climatic data was obtained from WorldClim database (Hijmans et al. 2005) and the Consortium for Spatial Information (Trabucco and Zomer 2009). Climate data were obtained from records were collected in situ using a Garmin Dakota 100 navigator, but in the **Chapter 1** we also include data provided in the literature. These environmental data was extracted with a spatial resolution of 30 arc-seconds.

In the **Chapter 4** we also examined the type of terrestrial habitat surrounding the aquatic habitats, as this also determines the amphibian composition in the aquatic habitats (Houlahan and Findlay 2003). The land cover types were obtained from the GlobCover 2009 database (Bontemps et al. 2011). The categories were extracted from the coordinates of each aquatic habitat using the QGIS package (Quantum GIS Development Team 2012), and describe the terrestrial habitat surrounding the aquatic habitats. This database classifies the land cover into 23 categories from forests to urban areas, and is based on satellite photographs (ENVISAT) having a spatial resolution of 300m.

III. 5. Data analyses

III. 5. 1. Reproductive habitat selection

In **Chapter 1** the differences observed in habitat selection while comparing *D. pictus* with the other species were tested by a Mann-Whitney U test. We also calculated Pianka's index from the same presence-absence matrix to estimate the degree of habitat overlap. Pianka's index varies from 0 (no resource overlap) to 1 (complete overlap in resource use) (Pianka 1974). Habitat selection by the benthic and nektonic guild was assessed by a MANOVA test, and significant differences among guilds were identified using a Tukey HSD test.

In **Chapter 2** the data obtained from the characterization of habitat were used to assess differences in habitat selection between the two groups of populations of *D. pictus*. To detect and calculate the statistical significance of any such differences, we used a permutational MANOVA (PERMANOVA, one fixed factor: population group) based on a resemblance matrix of Euclidean distances. The statistical significance was obtained after 999 unrestricted permutations (Anderson 2005). Comparisons of amplitude in habitat use among *D. pictus* and other anuran species were performed computing the Bayesian posterior estimate of the Standard Ellipse Area (SEA). This type of analysis provides a metric that is not biased by differences in the sample size (Jackson et al.

2011), and is widely used as a method of estimation of bivariate niche breadth (Layman and Allgeier 2011; Thomson et al. 2012). The physical and chemical variables were subjected to a principal component analysis (PCA), and the first two components were used to generate the SEA. The statistical significance was obtained after 10000 iterations, calculating the mean and 95% credible limits of the posterior distribution. These analyses were performed using the packages Primer-E 6, Statistica vs 7 and siar (Parnell and Jackson 2013) for R (R development Core Team 2011).

In **Chapter 3** the explanatory power of the physical and chemical parameters of the aquatic habitats was assessed using a distance-based linear modelling (DistLM) routine implemented in PRIMER v 6.1.11 package (PRIMER Ltd., Plymouth). This analysis was performed using the presence / absence matrix as the dependent variable, its similarity being defined using Sørensen's coefficient (Vellend 2001). The contribution of each environmental variable to the variation in species occurrence was assessed separately by marginal tests, and significance was assessed after 9999 permutations of the normalized predictor data (Anderson 2004).

In **Chapter 4** resemblance in the selection of aquatic habitats among groups, and relative to other anuran species, was assessed by performing outlying mean index (OMI) analysis. This method explores the tendency of a species to occupy a specific habitat, determining the position of a species in the available niche space and its niche breadth (Dóledec et al. 2000). OMI measures the marginality of the niche (those species that show high values of OMI) and the tolerance of a species (high tolerance values indicate that a given species has a broad niche). These indices were computed for the three groups of populations and for an out-group (absences), on the basis of the characteristics of aquatic habitats (see Fig. 11). Habitats with species absence were defined as those water bodies within the range of *H. meridionalis* where we could find no larvae of this species, but in which at least one other anuran species was observed. The statistical significance of the OMI indices was obtained after 999 Monte-Carlo randomizations. The relative contribution of environmental factors (aquatic habitat descriptors and the type of land cover) in explaining the occurrence of *H. meridionalis* was established using binomial generalized linear models (GLM). The best explanatory models were selected based on Akaike's Information Criterion (AIC) (Burnham and Anderson 2002), by comparing all possible combinations of variables. The variables included land cover type, the first axis of the principal component analysis (PCA) obtained for the variables surface area and average depth (water body size), and the first axis obtained by PCA for the physical and chemical water parameters (water parameters). The models were compared using

AIC values and two associated measures: delta AIC (Δ_i) and Akaike weights (w_i). In general, a Δ_i value < 2 is strong evidence for the model, while the best model among the various candidates has the w_i value closest to 1. The relative contribution of each variable was estimated by the sum of w_i for the models, where this variable was included (Burnham and Anderson 2002). These statistics were calculated using the package AICcmodavg (Mazerolle 2013) for R (R Development Core Team 2011).

Figure 11. Occurrence of anuran larvae depending on the pond characteristics (in this case, on the hydroperiod). A) Hydroperiod 30–60 days: Alytidae, Bufonidae and Pelodytidae. B) Hydroperiod 60–90 days: Pelodytidae and Hylidae. C) Hydroperiod 90–150 days: Hylidae, Pelobatidae and Ranidae.

III. 5. 2. Species associations

In **Chapter 1** we studied co-occurrence patterns by means of C-score metric (Gotelli and Entsminger 2009), calculated from a presence-absence matrix including all eight species in 135 aquatic habitats. C-score analysis was performed comparing the species and the morphological guilds, as the inclusion of guilds facilitates ecological interpretation of this type of analysis (Heino 2009; Gotelli and Ulrich 2010). We used a sequential swap algorithm with fixed row and column constraints (Gotelli 2000) and contrasted the observed pattern with 5000 randomized matrices. Species pairs' significant associations were tested using an approach for empirical Bayes confidence intervals (Gotelli and Ulrich 2010). C-score analysis was performed by Ecosim v. 7.72 software (Gotelli and Entsminger 2009), and pairwise co-occurrences were calculated using PAIRS (Ulrich 2008).

In **Chapter 3** the pairwise associations between *Discoglossus* species and other anuran species were examined by calculating the Dice's index (Dice 1945), providing a measure of habitat overlap based on the presence / absence matrix (Vilisics and Hornung 2009). Habitat overlap was correlated with morphological distance, obtained from the distance between the centroid of *Discoglossus* species and the centroid of other sympatric species. Normalized morphological variables were used to produce a Euclidean distance matrix for calculating centroid distances in principal coordinates space. This analysis was performed using PRIMERv 6.1.11 package (PRIMER Ltd., Plymouth). The correlation between species distances and habitat overlap was assessed by Spearman's test, obtaining statistical significance after 9999 Monte Carlo resampling procedures. We also compared the matrices of *Discoglossus* presence / absence with anuran species richness, controlling for water body size, using a partial Mantel test (Parra et al. 2006). Matrices for species richness per water body and water body size were constructed based on Euclidean distances, while those for presence / absence were based on Sørensen's similarity coefficient (Gotelli and Ellison 2012). Water body size was computed from surface area × average depth. The significance of correlation was assessed after 9999 permutations, and these analyses were performed using the package spaa (Zhang et al. 2013), coin (Hothorn et al. 2013), and vegan (Oksanen et al. 2012) for R (R Development Core Team 2011).

III. 5. 3. Larval morphospace

In **Chapter 1** we examined how ecology, phylogeny and morphology are correlated. We assessed the putative interaction among them by correlating distance matrices. We calculated principal component analysis (PCA) factor scores from the morphological variables and selected the first five meaningful axes (those with an eigenvalue > 1), to include them in a discriminant analysis and thereby obtain a morphological distance matrix based on squared Mahalanobis distances (SMD). We obtained phylogenetic distance matrix from pairwise divergence times for genera as established in TimeTree (Hedges et al. 2006), using a mixed average of nuclear and mitochondrial genes. Finally, we obtained the ecological distance matrix calculating the Euclidean distances from median values of the variables PS and PD. The median was chosen over the mean because of the presence of outliers in most cases (Table 1). We calculated the Euclidean distances using the PRIMER–6.1.6 program (PRIMER-E Ltd. 2006, Plymouth), and conducted Mantel tests using the PASSaGE software package (Rossenberg 2009) to determine significant correlations between the distance matrices. The Mantel test has been widely used to test covariation between phylogeny and ecology (Marroig and Cheverud 2001; Rezende et al. 2007). We tested the significance of correlations after 9999 Monte Carlo randomizations (Manly 1991), holding the phylogenetic distance constant when correlating morphology and pond characteristics. We included only pairwise distances to *Epidalea calamita* when calculating the correlations among distance matrices as the phylogenetic distances have been obtained to the genus level. Before statistical analyses were performed, quantitative variables were transformed logarithmically while the proportions were transformed by arcsin. Linear measures, except TL, have been entered as residuals obtained from linear regression (log EMD and log ED regressed against log BL, and log MTaD regressed against log TaL). All Statistical analyses (unless otherwise indicated) were carried out with Statistica v. 8 (StatSoft Inc. 2007). A PCA including morphological variables was initially performed to visualize possible clusters formed by the studied species. Once these clusters were identified, we applied Principal Component Analysis Regression (PCAR), because this method allowed us to identify which were the best explanatory variables defining the groups. PCAR detects significant variables from an array of correlated variables using all PCA axes (Jolliffe 2002).

In **Chapter 2** we analysed the variability in morphological traits in *D. pictus* compared with the variability shown by sympatric anuran species. These differences were assessed comparing the Bayesian posterior estimate of SEA obtained from the first two PCA components, using the same procedure described previously. Morphological overlap was calculated by generating an x, y space from the orthogonal variables yielded by PCA while omitting mean grey value. The overlap values were then obtained using co-inertia analysis, obtaining significance after 9999 Monte Carlo permutations. In an inertia analysis, the shape of the cluster of points generated for one species is examined to determine whether the species position in the available space differs from that expected from by the simulated values. Therefore, this analysis provides an estimate of the niche overlap between species, the “inter-inertia ratio” (Dolédec et al. 2000). These analyses were performed using the packages Statistica 7, ade4 (Chessel et al. 2012) and siar (Parnell and Jackson 2013) for R (R Development Core Team 2011).

In **Chapter 3** we assessed the position in morphological space of each of the anuran species in the different assemblages, based on the total area and span ratio. This enables identification of groups of species that are similar morphologically and measurement of the diversity within the genus in this morphological space. The morphological diversity within the genus *Discoglossus* was measured by a permutational MANOVA (PERMANOVA, one fixed effect: species). This analysis was carried out on the resemblance matrix obtained from the Euclidean distances of the normalized predictor data. The statistical significance was obtained after 999 unrestricted permutations (Anderson 2005), and the analysis was performed using PRIMER v 6.1.11 package (PRIMER Ltd., Plymouth). The functional richness of each assemblage (the volume of the functional space generated for an assemblage; Villéger et al. 2008) was estimated from the same morphological variables. This index depends on the species richness (Gerisch et al. 2012) and permits estimation of the functional complexity of the assemblage. The functional richness was obtained from the average values per species for the variables total area and span ratio, using the package FD (Laliberté and Shipley 2013) for R (R Development Core Team 2011).

III. 5. 4. Climate niche analysis

In **Chapter 1** we analysed the bioclimatic data obtained from 2232 presence localities (at a resolution of 1 km²) of the eight species, including all of Catalonia (Atlas dels Amfibis i Rèptils de Catalunya i Andorra database; Llorente et al. 1995). The dataset obtained from the localities of the presence and untransformed bioclimatic variables had been reduced by PCA, obtaining the PCA factor scores of the first three axes (those with an eigenvalue > 1) and included them in a discriminant analysis to obtain Squared Mahalanobis Distances between pairs of species.

In **Chapter 2** the three bioclimatic variables (aridity index, mean temperature of the warmest quarter and mean temperature of coldest quarter) were included in a PCA, with the purpose of estimating the differences between the native and alien *D. pictus* populations. The divergence in the climate space revealed by the first two PCA axes was assessed using co-inertia analysis with statistical significance assessed after 9999 Monte Carlo permutations. These analyses were run using Statistica 7 and ade4 (Chessel et al. 2012) for R (R Development Core Team 2011). We also tested if niche models could predict the existence of suitable conditions for native *D. pictus* in the invasive range. We used MaxEnt version 3.3.3k (Phillips et al. 2012), calibrating this model with only native localities. The MaxEnt models were run with 75% of the locations allocated to training and with a regularisation value of 1, allowing linear, quadratic and hinge features based on the number of presence records for the species (Anderson and Gonzalez 2011). The projection displayed the 10th percentile of the predicted training values as species presence (Pearson et al. 2007), with the sensitivity-specificity sum maximisation (SSM) threshold (Liu et al. 2005) based on inclusion of species absences. The accuracy of the model was calculated based on the area under the ROC curve (AUC) and the rate of correct classifications. The sensitivity-specificity sum maximisation threshold and threshold statistics were calculated using the PresenceAbsence package (Freeman 2012) for R (R Development Core Team 2011).

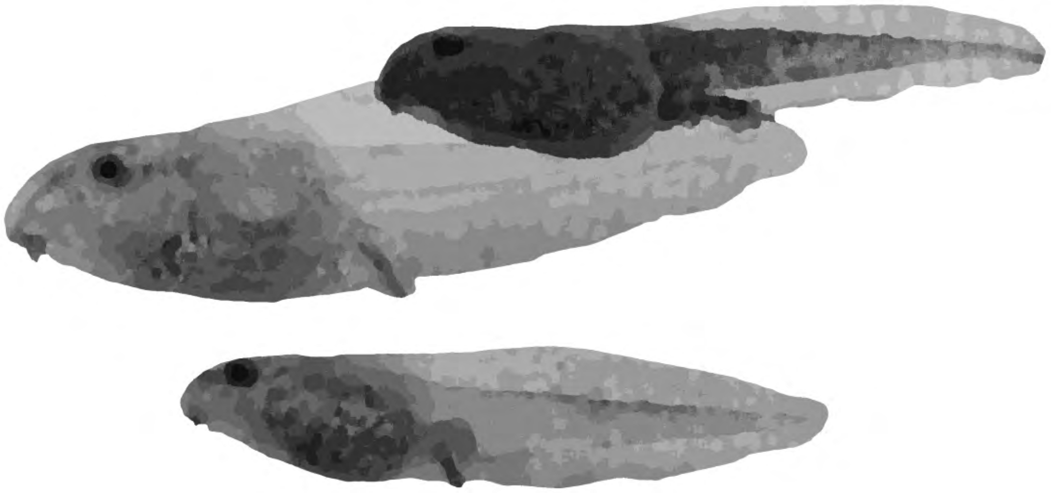
In **Chapter 4** we evaluate niche divergence by means of niche equivalency and background similarity tests, comparing the actual ecological niche model (ENM) with random models generated from pseudoreplicate datasets (Warren et al. 2008). The study units were the main lineages (eastern and western clades), as the boundaries among the three Moroccan haplotypes of *H. meridionalis* are not well known (Recuero et al. 2007). The niche equivalency test (NET) assesses whether the

ENMs drawn from allopatric populations of two species are more similar than expected for the null hypothesis that their ENMs are not equivalent. The background similarity test (BST) determines whether ENMs based on the distribution of two geographically separated species resemble more than expected in relation to the local environmental background available for each population (Anderson and Gonzalez 2011). These models were carried out using the climatic variables AIT, MTWQ and MTCQ. The statistical significance of the values for niche overlap (based on Schoener's D and modified Hellinger distances) (Warren et al. 2008) was assessed from 99 permutations. The ENMs for the various population groups (native for the western lineage, native and alien for the western and eastern lineages) were run with 75% of the locations allocated to training and with a regularization value of 1, enabling linear, quadratic and hinge features based on the number of presence records for the species (Anderson and Gonzalez 2011). In the projection, the 10th percentile of the predicted training values indicated species presence (Pearson et al. 2007). The accuracy of the model was calculated based on the area under the ROC curve (AUC). These analyses were performed using the package phyloclim (Heibl and Calenge 2013) for R (R Development Core Team 2011) and MaxEnt 3.3.3k (Phillips et al. 2012).

IV. RESULTS AND DISCUSSION

IV. 1. Chapter 1

Assessing the potential impact of an invasive species on a Mediterranean amphibian assemblage: a morphological and ecological approach



IV. 1. 1. Abstract

The introduction of exotic species is a major cause of ecological disturbance and has recently been shown to promote the decline of some amphibian populations. In Western Europe, several amphibian species have been introduced in recent decades, among them *Discoglossus pictus*, impact of which in native amphibian communities is still unknown. We studied the potential impact of *D. pictus* by analysing the degree of niche overlap, assuming the possible existence of competitive interactions with native species. We have studied the structure of the anuran assemblage at local level, defining the morphospace occupied by native species and the habitat occupied by the different ecomorphs. The analysis of distance matrices suggested that there was a covariation between morphological characters and habitat selection. We determined the position of *D. pictus* within the morphospace of the native anuran community, patterns of co-occurrence among alien, and native species and habitat selection. The potential effect of climate on local assemblages had been controlled based on data obtained from climate models. These analyses showed that *D. pictus* was clustered with the benthic guild, selected positively small ephemeral ponds and was a thermophilic species. Based on these results, a greater degree of niche overlap was expected with *Epidalea calamita* and *Pelodytes punctatus*. The definition of morphological groups can be useful to understand the invaded assemblage structure and the potential effect of an alien frog on native communities.

IV. 1. 2. State of the art

Introduced exotic species are a major cause of biodiversity loss, either as predators, or competitors, by toxicity or being vectors of pathogens (Adams 2000; Crossland 2000; Daszak et al. 2004; Beard and Pitt 2005; Savidge et al. 2007; Ujvari and Madsen 2009). In Europe, alien herptile species have probably been introduced since historical times (Fritz et al. 2006; Guicking et al. 2006; Harris et al. 2009), and some have had devastating effects on island endemics (Moore et al. 2004; Luiselli 2006). Throughout the 20th century, several new species of amphibians and reptiles were introduced in Europe. Some of them established breeding populations (e.g., Frazer 1964) and have had negative effects (such as competition or predation) on native reptiles and amphibians, especially on those species that occupy a similar ecological niche (Neveu 1997; Cadi and Joly 2004). *Discoglossus pictus* is native to the north-eastern Morocco, Algeria, Tunisia and Sicily (Zangari et al. 2006) and was introduced in southern France (Wintrebert 1908), where it has spread to the Mediterranean regions of France and north-eastern Spain (Lanza 1989; Llorente et al. 1995).

The impact that *D. pictus* may have on native anuran communities has not been established, but it may be important given the variety of habitats it occupies (e.g., riparian and mediterranean forests, marshes, etc.), its dispersal capacity (estimated at 1.53 $km \cdot year^{-1}$) and the high densities it reaches in favourable habitats (Llorente et al. 2004; Montori et al. 2007). Interspecific competition has been described as a decisive factor in the survival of anuran larvae (Morin 1983; Dayton and Fitzgerald, 2001), especially because as most anuran larvae are nonselective omnivores and filter-feeders (Alford 1999; Petranka and Kennedy 1999). In this sense, competitive interactions among tadpoles of native and alien species may decrease the survival rates of the native species (Kupferberg 1997; Kiesecker et al. 2001; Smith 2005).

One possible approach to the study of the potential impact of an alien species on native species is to analyse the degree of niche overlap among them (Wauters et al. 2002; Phillips et al. 2007b). Morphological variability has been linked as a response to the use of a particular ecological niche and different evolutionary lineages tend to converge on the same morphological design (Østbye et al. 2006). Morphological characters have been used to predict the degree of niche overlap in several vertebrate communities (Carrascal et al. 1990; Losos 1990). In amphibians, it has been suggested that phenotypical variability in larvae may depend on the type of aquatic habitat and the presence of certain predators, independent of the phylogeny (Richardson 2001). McDiarmid and Altig (1999) described several ecomorphological guilds, such as a benthic guild, tadpoles of which rasp food from submerged areas and live at the bottom of lentic water bodies. The benthic guild is morphologically characterized by depressed bodies, dorsal eyes and low fins. Nektonic species also rasp submerged surfaces but they live in the water column and have compressed bodies, lateral eyes and high fins. Stronger competitive interactions can be expected to occur among morphologically similar, but not necessarily related, species that show greater niche overlap (Schoener 1974; Bardsley and Beebee 2001; Richardson 2001; Barnett and Richardson 2002; Relyea 2002) and among species that breed in a limited number of ponds (e.g., in deserts, Dayton and Fitzgerald 2005). In larger ponds the greater complexity of habitat promotes species richness by the differential microhabitat selection (Nudds 1982). However, in larger ponds some degree of competition may exist among species belonging to different guilds because these ponds host more complex assemblages (Wilbur 1987) and larger tadpoles exploit resources more successfully than smaller ones (Steinwascher 1979; Woodward 1982; Richter-Boix et al. 2007).

We also study interactions at local scale among *D. pictus* and native species. Null-model analysis based on a presence-absence matrix was performed with the purpose to detect aggregation patterns among species and morphological groups, by comparing observed cooccurrences with those randomly generated (Gotelli 2000). We calculated Pianka's index from the same co-occurrence matrix. This index provides an estimate of the degree of niche overlap between a pair of species for a given dimension, such as diet, habitat or activity patterns (Pianka 1974). Likewise, pond characteristics related to hydroperiod length have a strong determining effect on the type of interactions established among aquatic species (Wellborn et al. 1996). In ephemeral ponds, the competition is more intense, possibly because of a lower presence of predators (Wilbur 1980), and so determining whether an invasive frog species selects specific ponds characteristics could help us to understand the way this species is going to interact with the native species. Finally, we analyse the effect of climate on species ranges at regional scale. Climatic divergence was determined based on GIS-supported data and regional presence records for each species, which allowed us to compare the degree of overlap among species ranges of which can be limited by competition (Arif et al. 2007).

The aim of this study is to determine the potential impact of *D. pictus* on native larval anuran assemblages.This impact is studied by (1) defining the degree of niche overlap by analysing the morphological similarities of larvae, (2) detecting co-occurrence structure, (3) identifying the pond size (PS) and pond depth (PD) selected as breeding points for each species, and (4) analysing the degree of bioclimatic divergence.

IV. 1. 3. Results

IV. 1. 3. 1. Sampling and habitat characterization

The tadpoles we collected belong to all eight species of Anura that occur in the area below 1000 m a.s.l.: *Alytes obstetricans* (Laurenti) (31 specimens), *Discoglossus pictus* Otth (33 specimens), *Bufo spinosus* Daudin (19 specimens), *Epidalea calamita* (Laurenti) (32 specimens), *Hyla meridionalis* Boettger (30 specimens), *Pelobates cultripes* (Cuvier) (36 specimens), *Pelodytes punctatus* (Daudin) (31 specimens) and *Pelophylax perezi* Seoane (31 specimens). Physical characteristics (i.e., PS and PD) of the ponds along with the significance values obtained from pairwise comparisons by Mann-Whitney U test are shown in Table 3. Location, number of ponds measured per site and the composition of species per site are shown in Table 4. Species co-occurrences per sampled pond in the presence-absence dataset are summarised in Table 5.

Table 3. Median and mean and SD of PS, expressed in m^2, and maximum PD, expressed in cm. Test statistic and P-values (in brackets) obtained by comparing each species with *D. pictus* by Mann-Whitney U test (M-W U test) are included. Significant values are marked in bold. *n* shows the number of ponds in which size and depth were measured

Species	***n***	**PS median**	**PS mean ± SD**	**M-W U test**	**PD median**	**PD mean ± SD**	**M-W U test**
A. obstetricans	4	8.6	11.71 ± 2	5 (0.06)	19.5	21 ± 16	12 (0.68)
B. spinosus	3	16	2004 ± 3455	8.5 (0.47)	30	82 ± 102	9 (0.54)
B. calamita	20	75	644 ± 1471	72 (0.83)	18	30 ± 34	67 (0.63)
D. pictus	8	152	3028 ± 7485		22	66 ± 121	
H. meridionalis	21	820	4403 ± 7227	58.5 (0.21)	50	52 ± 35	53 (0.13)
P. cultripes	17	820	3961 ± 7097	39 (0.09)	57	84 ± 91	**34 (0.05)**
P. punctatus	11	76	2014 ± 6103	41.5 (0.83)	25	60 ± 99	42.5 (0.9)
P. perezi	10	61	3361 ± 8073	39 (0.93)	40	42 ± 41	21.5 (0.1)

Table 4. Sites of sampling. Lat Latitude; Long longitude, both expressed in decimal degrees; Ponds: number of ponds used to characterize (depth and size); Species: species found. Legend: AO: *A. obstetricans*; BB: *B. spinosus*; EC: *E. calamita*; DP: *D. pictus*; HM: *H. meridionalis*; PC: *P. cultripes*; PP: *P. punctatus*; PZ: *P. perezi.*

Site	**Lat**	**Long**	**Ponds**	**Species**
1	42.39ºN	2.76ºE	3	AO
2	42.38ºN	2.97ºE	14	DP, EC, HM, PC, PP, PZ
3	42.37ºN	3.14ºE	1	DP, EC, PP
4	42.30ºN	3.28ºE	3	EC, PP
5	42.28ºN	3.16ºE	6	DP, BB, EC, HM
6	42.18ºN	2.79ºE	1	DP, EC, HM, PC, PP
7	42.03ºN	3.17ºE	5	DP, EC, PZ
8	42.00ºN	2.76ºE	3	DP, PZ
9	42.00ºN	2.82ºE	13	AO, DP, EC, HM, PZ
10	41.89ºN	3.00ºE	3	DP, BB
11	41.87ºN	3.13ºE	1	DP, EC, HM, PC
12	41.83ºN	3.00ºE	2	DP, EC, PC
13	41.78ºN	2.95ºE	16	AO, DP, BB, EC, HM, PZ
14	41.82ºN	2.68ºE	11	DP, EC, PC, PP, PZ
15	41.76ºN	2.68ºE	3	EC
16	41.66ºN	2.77ºE	9	DP, EC, HM, PZ

Table 5. First row of the columns: number of species recorded per pond. The number in each row indicates the times that this species has appeared as unique species (column 1) or associated with other species (subsequent columns). The last column shows the total number (*n*) of ponds in which was each species present. Legend: AO: *A. obstetricans*; BB: *B. spinosus*; EC: *E. calamita*; DP: *D. pictus*; HM: *H. meridionalis*; PC: *P. cultripes*; PP: *P. punctatus*; PZ: *P. perezi.*

Species	**1**	**2**	**3**	**4**	**5**	***n***
AO	12	3	0	0	0	15
BB	4	1	0	0	0	5
EC	15	12	9	2	1	39
DP	27	8	7	2	1	45
HM	9	17	5	2	1	34
PC	7	6	3	2	1	19
PP	5	7	6	0	1	19
PZ	14	4	0	0	0	18

IV. 1. 3. 2. Phenotypic constraints

The results obtained by correlating phylogenetic and morphological distance matrices through performing a Mantel test suggested a significant covariation between phylogenetic history and phenotype (Z = 196.784, one tailed P = 0.04). This result was affected by the presence of morphological similarities among phylogenetically distant lineages and the weakness of phylogenetic signal produced by the presence of clades that diverged early in the evolution of anurans. In this sense, the suborder Archeobatrachia (*Alytes* and *Discoglossus*) presents an estimated divergence time of 213 Mya compared to other genera (Hedges et al. 2006). Partial Mantel result showed that the correlation between habitat conditions and morphological distances was significant (Z = 123.582, one tailed P = 0.04), while controlling the effect for phylogenetic distances. This result indicated that morphology covariates with pond characteristics.

IV. 1. 3. 3. Morphological guilds

The PCA based on morphological characters (Fig. 12) and the morphological distance matrix (Table 6) showed that the species are grouped into two main morphs, with the exception of *P. perezi*, which showed intermediate characters. One group was formed by *H. meridionalis* and *P. cultripes* and a second group by the *B. spinosus, E. calamita, A. obstetricans, D. pictus* and *P. punctatus*. A comparison of both groups by PCAR (Table 7) indicated that the second group was characterized by the oral disc in the ventral position and a more slender structure while the first group had larger and more circular eyes, greater fin area and greater size. After obtaining these results, and based on previously described ecomorphs (McDiarmid and Altig 1999), we labelled the first group nektonic, the second one benthic and studied *P. perezi* separately. *Pelophylax perezi* differed from the benthic group by having a greater eye, a proportionally larger tail and bigger size, and from nektonic species by having a more slender structure, the oral disc located in more ventral position and a proportionally larger tail (Table 7).

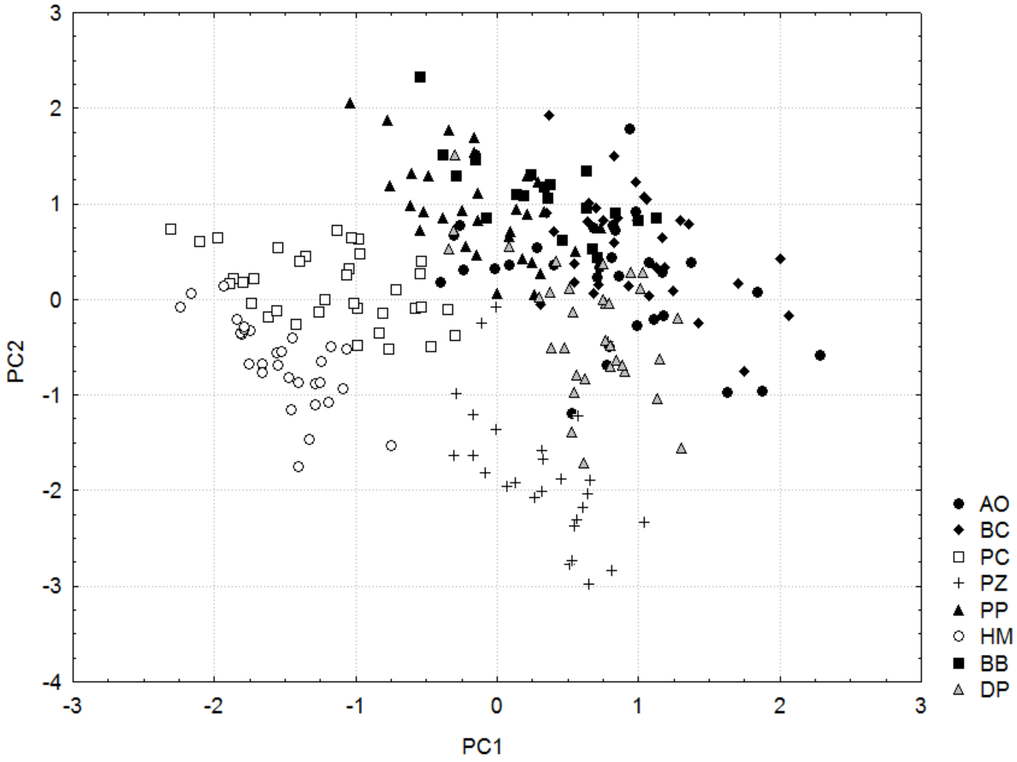


Figure 12. PCA scatter plot obtained from morphological variables. Legend: AO: *A. obstetricans,* BB: *B. spinosus,* EC: *E. calamita,* DP: *D. pictus,* HM: *H. meridionalis,* PC: *P. cultripes,* PP: *P. punctatus,* PZ: *P. perezi.* Explained variance PC1: 29.2%, PC2: 24.3%. PC1: positive values are correlated with the position of the mouth and elongated morphology, negative values are correlated with greater area of the fin and circular morphology. PC2: positive values are correlated with the circular morphology of the tail and the position of the mouth and negative values with the relative area of the eye, relative area of the tail and the total length.

Table 6. Squared Mahalanobis Distances (SMD) obtained from morphological variables. Legend: AO: *A. obstetricians*; BB: *B. spinosus*; EC: *E. Calamita*; DP: *D. pictus*; HM: *H. meridionalis*; PC: *P. cultripes*; PP: *P. punctatus*; PZ: *P. perezi.*

	AO	**BB**	**EC**	**DP**	**HM**	**PC**	**PP**
AO							
BB	8						
EC	10	4					
DP	12	8	7				
HM	46	51	65	41			
PC	28	39	53	43	12		
PP	6	5	14	11	36	26	
PZ	23	37	37	21	18	20	31

Table 7. Sum of loadings (SL) obtained from the PCAR of morphometric variables comparing different guilds excluding *P. perezi*. Benthic (B) vs. Nektonic (N): negative values characterize benthic species and positive values nektonic species. Benthic vs. *P. perezi* (Pz): negative values characterize benthic species. Nektonic vs. *P. perezi*: negative values characterize *P. perezi.* Legend: ME: Mouth eye angle; TaS: Tail span ratio; BS: Body span ratio; CB: Circularity of the body; Ta / T: Tail area / total area ratio; MTa: Muscular tail residual; EMD: Eye mouth distance residual; CTa: Circularity of the tail; ED: Eye diameter residual; TL: Total length; EC: Eye circularity; F / Ta: Fin area / tail area ratio; E / B: Eye area / body area ratio. 0 = no difference; ± 1 = maximun difference.

	B vs N	**B vs Pz**	**N vs Pz**
ME	-0.82	-0.58	-0.83
TaS	-0.67	-0.38	-0.78
BS	-0.52	-0.24	-0.66
CB	0.11	-0.22	-0.51
Ta / T	0.22	-0.14	-0.50
MTa	0.23	-0.06	-0.36
EMD	0.24	0.19	-0.08
CTa	0.36	0.26	0.23
ED	0.49	0.40	0.29
TL	0.51	0.52	0.55
EC	0.55	0.53	0.69
F / Ta	0.57	0.56	0.71
E / B	0.58	0.76	0.82

IV. 1. 3. 4. Niche overlap analysis at local scale

Co-occurrences analysis based on of C-score metric for the entire set of species indicated a random distribution across aquatic habitats (P = 0.98). Comparing intraguild co-occurrences, the results suggested random co-occurrence among the benthic species (P = 0.82), and aggregation between the nektonic species (P = 0.02). Studying pairwise aggregation patterns, we found that *D. pictus* is significantly segregated from *E. calamita* (P = 0.04) and *P. perezi* (P = 0.007) but was not associated with any species. From the same matrix of co-occurrences, Pianka's index revealed that *D. pictus* had the greatest habitat overlap with *E. calamita* (Table 8). This discrepancy was explained because of 39 presences of *E. calamita* and 45 presences of *D. pictus*, both species cooccurring only 13 times. The MANOVA test showed significant differences in the selection of the pond characteristics comparing the benthic with the nektonic guild and *P. perezi* ($n = 94$, $F = 6.02$, $P = 0.0001$). The Tukey HSD test indicated that significant differences existed between the benthic guild and the nektonic guild depending on PS and PD, with the benthic group having lower mean values for both variables (benthic PS mean = 1,408 m^2 and PD mean = 45 cm, nektonic PS mean = 4205 m^2 and PD mean = 66 cm, $df = 91$, both $P > 0.01$). *P. perezi* was not differentiated from either of these two guilds as a function of these variables (PS mean = 3361 m^2 and PD mean = 59 cm, $df = 91$, all $P < 0.05$).

Table 8. Pianka's niche overlap index calculated from the presence / absence matrix including 135 aquatic habitats. Legend: AO: *A. obstetricians;* BB: *B. spinosus;* EC: *E. Calamita;* DP: *D. pictus;* HM: *H. meridionalis*; PC: *P. cultripes*; PP: *P. punctatus*; PZ: *P. perezi* .0 = no overlap; 1 = complete overlap.

	AO	**BB**	**EC**	**DP**	**HM**	**PC**	**PP**
AO							
BB	0						
EC	0.04	0					
DP	0.04	0.07	0.31				
HM	0.04	0	0.36	0.18			
PC	0	0	0.18	0.14	0.31		
PP	0	0	0.29	0.2	0.16	0.26	
PZ	0	0	0	0	0.16	0	0

IV. 1. 3. 5. Niche overlap analysis at regional scale

The pairwise distances obtained by the climatic analysis (Table 9) showed that *D. pictus* had higher overlap with other thermophilous species, especially with *H. meridionalis*, an alien tree frog native of north-western Maghreb and to a minor degree with *E. calamita*. The divergence increased when *D. pictus* was compared with the species that occur in more temperate conditions, such as *A. obstetricans* and *B. spinosus*, suggesting that patterns of local co-occurrence observed among *D. pictus* and these species can be affected by environmental constraints.

Table 9. Squared Mahalanobis distances (SMD) obtained from the analysis of 20 environmental variables. Legend: AO: *A. obstetricans;* BB: *B. spinosus;* EC: *E. calamita;* DP: *D. pictus;* HM: *H. meridionalis;* PC: *P. cultripes;* PP: *P. punctatus;* PZ: *P. perezi.*

	AO	**BB**	**EC**	**DP**	**HM**	**PC**	**PP**
AO							
BB	13.73						
EC	12.46	1.57					
DP	10.2	2.15	0.75				
HM	10.88	0.91	1.00	0.36			
PC	13.97	1.68	0.08	1.29	1.49		
PP	13.1	0.55	0.4	1.54	1.06	0.37	
PZ	12.81	0.77	0.15	1.01	0.79	0.19	0.07

IV. 1. 4. Discussion

The morphological analysis allowed us to define two main ecomorphs in the region: the benthic guild, composed of *A. obstetricans*, *D. pictus*, *P. punctatus* and both Bufonidae species; and the nektonic guild, which includes *H. meridionalis* and *P. cultripes*. Analysis of the covariation between morphology and presence in a particular habitat indicated a relationship between two variables. This relationship persisted after controlling for the effect of phylogeny in the sample. The phylogenetic history has proved to have a significant paper in the phenotypic expression (Pérez-Barbería and Gordon 1999), although in the studied species the phylogenetic signal was affected by the presence of clades with high genetic divergence. The fact that distantly related genera such as *Discoglossus* and *Bufo* (estimated divergence time: 213 Mya, Hedges et al. 2006) and *Pelobates* and *Hyla* (estimated divergence time: 191 Mya, Hedges et al. 2006) share morphological traits and occupy similar type of ponds reinforces the hypothesis that the phenotypic expression is correlated with the habitat use. The benthic guild appears in small ephemeral ponds (e.g., García-París et al. 1989; Diaz-Panigua 1990; Montori 1997), but they may occasionally occur in larger ponds (e.g., Boix et al. 2004). The exception is *B. spinosus*, which mainly breeds in streams and permanent ponds (Campeny and Montori 1988), possibly because it is less palatable to vertebrate predators (Denton and Beebee 1991). Nektonic species appear in larger temporary ponds (Diaz-Panigua 1990; Cancela da Fonseca et al. 2008), but with important differences: *P. cultripes* seem to be restricted by a relatively long hydroperiod and by the absence of exotic predators (Lizana et al. 1994), whereas *H. meridionalis* can breed from ephemeral pools (Esteban 1984) to permanent ponds where exotic predators are present (Beja and Alcazar 2003). In our sampling, the presence of predators has not been considered, and future studies will need to assess whether, in the area of study, it is possible to observe similar patterns.

Co-occurrence results suggest that there is a structured pattern in the distribution of *D. pictus* compared with *E. calamita* and *P. perezi*. Accordingly, *P. perezi* seems to be limited by the persistence of water at the end of spring (Diaz-Panigua 1990), and it is likely that the difference in the length of the hydroperiod segregates *P. perezi* from *D. pictus*. In the case of *E. calamita*, the use of similar types of ponds, similar breeding periods (Boix et al. 2004; Montori et al. 2007) and the high degree of similarity in their bioclimatic requirements suggest that competition is a possible explanation for the observed pattern. Pianka's index indicates that *P. punctatus* also has great habitat overlap with *D. pictus*, although the obtained C-score indicates a lack of structure in their mutual cooccurrences. It is likely that some degree of competition may exist between the two species, as they breed in very similar ponds, but *Pelodytes* tadpoles feed more frequently in the water column than *E. calamita* or *Discoglossus* (Diaz-Panigua 1985,1987). For this reason, the diet overlap may be incomplete, reducing the competition between these species. In the case of the remaining benthic species, *A. obstetricans* and *B. spinosus*, Pianka's index indicates a very low habitat overlap with *D. pictus*, which cannot be explained by the use of aquatic habitats of different sizes, either in *A. obstetricans* or in *B. spinosus*. In both cases, the segregation observed could be partly determined by different environmental preferences, as suggested by the distances obtained from bioclimate data. Both species are morphologically similar to *D. pictus* and in contact areas competitive interactions may occur. In the case of amphibian assemblages, small ephemeral ponds support less species richness than larger ephemeral ponds (Werner et al. 2007). This is especially relevant given *D. pictus*, *E. calamita* and *P. punctatus*, that breed in such ephemeral ponds, depending on unpredictable rainfall, tend to reproduce synchronously (Diaz-Panigua 1990). Competition has been proposed as a key factor in determining the species structure in such ephemeral ponds (Wilbur 1987). This lower species richness might be caused by opportunistic oophagy by more developed tadpoles (Banks and Beebee 1987; Tejedo 1991; Petranka and Kennedy 1999) or by changes in food resource availability caused by pioneer species (Alford and Wilbur 1985; Lawler and Morin 1993). In north-eastern Catalonia, *D. pictus* has well established populations and is expanding its range (Franch et al. 2007) possibly helped by its ability to occupy a wide range of aquatic habitats. Its expansion may primarily affect *E. calamita*, but may also have a diffuse effect on other species belonging to the benthic guild, reducing their access to recruitment points. However, the expansion of *D. pictus* apparently has not led a general decline in other species of anurans. *Discoglossus pictus* possible effects on native anurans may be more severe in areas that are particularly favourable for the species, such as floodplains or small ponds, as adults strongly depend on the proximity of water (Capula 2007). This would explain why the effect on other species of anurans belonging to the benthic guild is not widespread, as both adults of *P. punctatus* and *E. calamita* appear in drier habitats (García-París et al. 2004). The definition of morphological groups provides a way to determine the potential threat which could be an alien species on native anuran communities. Our results indicate that the existence of native species occupying similar ecological niche, at least during part of their life cycle, does not preclude the possibility that this community can be invaded. Special attention should be paid to these native species which present greater niche overlap with the alien species.

IV. 2. Chapter 2. Factors regulating the invasive success of an alien frog: a comparison of the ecology of the native and alien populations

IV. 2. 1. Abstract

We examined niche occupancy of *Discoglossus pictus*, an anuran recently established in Europe, comparing the niches of native (north Africa) and alien populations (south-western Europe) at two spatial scales to determine whether adaptive divergence had occurred between these two populations. Additionally, we determine whether the alien species showed a wider larvae niche and higher phenotypic variability compared with co-occurring anurans. We characterized the breeding habitats and the climatic space occupied by native and alien groups of populations of *D. pictus* and examined morphological traits of *D. pictus* and sympatric anuran larvae. Our results revealed no divergence in breeding habitat use between native and alien populations. A shift was observed between the realized niches occupied by the native and alien populations, but this shift might only reflect cryptic niche conservatism. The range of reproductive habitats selected by *D. pictus* was not wider than those of most native species. In the invaded range, *D. pictus* showed morphological overlap with some native species and broader phenotypic variability, but the adaptive advantages of this latter attribute were uncertain. Our results suggest that the invasive capacity of this species depends on favourable abiotic conditions rather than on its adaptive advantages over native anurans.

IV. 2. 2. State of the art

Alien species are a major cause of the imbalance of native communities, playing a dominant role in species extinction worldwide (Simberloff et al. 2013). The eradication of these species requires considerable effort and one of the first steps is to determine which factors regulate their invasion phase (Sakai et al. 2001). The success of an alien species in becoming established in a new region depends on favourable abiotic conditions and the adaptive capacity of the species to environmental variation (Sax et al. 2007). Niche conservatism, especially in ectothermic vertebrates, means that the requirement for environmental similarity between the invaded and the native regions constrains the ranges of numerous alien species (Wiens and Graham 2005). However, some species can modify their behaviour or develop invasive phenotypes that allow them to occupy new niches (Rödder and Lötters 2009; Phillips et al. 2010).

Here we examine the potential roles of environmental conditions in enabling invasion by *Discoglossus pictus* Otth, 1837, an alien anuran that has recently become established in southern France and northern Spain, introduced from northern Africa (Martínez-Solano 2004). In its native range, *D. pictus* displays high predatory opportunism (Ben Hassine and Nouira 2009) and occupies a wide variety of habitats, from montane mesic forests to pre-Saharan oases (Capula 2007; Ben Hassine and Nouira 2012). This species was discovered in the extreme south of France in 1906 (Wintrebert 1908), and since then, it has expanded its range at a dispersal rate of 1.53 km per year (Montori et al. 2007). In south-western Europe, it is a generalist species that is well adapted to anthropic landscapes (Geniez and Fradet 2013), occurring in very diverse types of aquatic habitats, although most frequently in seasonal ponds (García-París et al. 2004). The larval development of *D. pictus* is rapid, usually within less than two months, allowing it to breed in ephemeral rainwater pools (Capula 2007).

We compared the patterns of use of aquatic habitat of native and alien populations of *D. pictus* by characterizing their occupied habitats in terms of pond size and physical and chemical variables that, in such habitats, typically determine species composition (Skelly 2001). The investigation focused on breeding habitat selection because availability of suitable aquatic habitats is a key factor underlying the local distribution of anurans (Hazell et al. 2001). Such divergence could occur due to availability of suitable habitats or by competitive exclusion mediated by native species. This variation in the niche may also extend into the climatic envelope occupied by the alien populations (Broennimann et al. 2007). However, in amphibians the climate tolerance is strongly restricted by physiological limits, mainly, the water availability and thermal extremes (Ródriguez et al. 2005; Kozak and Wiens 2006). It is likely that the distribution of *D. pictus* has been limited by conservatism in the climatic niche, as is true for other species of alien anurans (Wiens et al. 2010), although there are examples of relatively rapid niche shifts (Tingley et al. 2012).

We also examined the influence of interspecific interactions on the invasion process. Because most anuran larvae are generalist feeders that face high levels of competition (Dutra and Callisto 2005), mechanisms of temporal and spatial segregation are important in reducing the competition (Lawler and Morin 1993). The absence of these structured patterns of segregation is associated with an overlap in the larval habitat between native and invasive species, causing a significant decrease in the survival and fitness of native larvae (Smith 2005). The establishment of an alien species may be assisted by use of a broader range of available habitats than native species, which could otherwise displace the alien (Mooney and Cleland 2001). Alien species may show varying degrees of functional overlap with native species. Some species can occupy similar niches to those of native species (Wu et al. 2005), but for others invasion may be assisted by high functional divergence (Cleland et al. 2011). In anuran larvae, morphological convergence occurs between species within the same trophic guild, enabling analysis of certain morphological characters to be used for comparison of functional niches (Altig and Johnston 1989; Altig 2007). The analysis of larval morphology also enables to determine whether an invasive species shows greater phenotypic variability than native species. Anuran larvae show a high ability to adjust their phenotypes in response to changes in environmental conditions (van Buskirk and Relyea 1998). A greater phenotypic plasticity might involve an adaptive advantage, as has been demonstrated in other invasive species, such as several invasive plants and the sunfish *Lepomis gibbosus* (Fox et al. 2007; Davidson et al. 2011).

We studied the habitat and organismal attributes that have probably influenced the invasiveness of *D. pictus*. First, we assessed the divergence, if any, in the breeding habitats used by the alien and native populations of *D. pictus,* and compared its amplitude of habitat use to those of co-occurring anurans. We expected that: (i) there would be no differences in the habitat selection of the native and alien population groups; and (ii) *D. pictus* would show a greater niche breadth than most or all of the native anurans. We then analysed both its phenotypic variability and its morphological overlap with sympatric species, predicting that: (iii) alien populations of *D. pictus* would display greater phenotypic variability than the other anuran species present in the invaded range; and (iv) the alien populations would show no morphological overlap with the native anurans, suggesting that they had become established in Europe by occupying an empty functional niche. Finally, we investigated whether the invasive range of *D. pictus* can be explained by niche conservatism.

IV. 2. 3. Results

IV. 2. 3. 1. Reproductive habitat selection

Discoglossus pictus was found in 34 aquatic habitats in the invaded region and 83 in the native region of the species (Fig. 13). Our results revealed that the native and alien groups of populations occurred in aquatic habitats with similar water body size, physical and chemical characteristics except for conductivity, which was higher in Tunisia (Table 10). Both groups of populations breed mainly in temporary ponds, 97.6% (native) and 82.4% (alien), and less frequently in permanent ponds 1.2% (native) and 5.9% (alien), and stream pools 11.8% (alien) and 1.2% (native). The values of the parameters characterizing the aquatic habitats selected by other anurans from the invaded assemblage are shown in Appendix 1a, and indicated that most of native species bred in habitats which showed high variability in these parameters. The variance explained by the first two axes and the factors loadings of the parameters measured in aquatic habitats are shown in Appendix 1b, revealing that the surface of the pond, dissolved oxygen and pH were the variables that most accounted to the observed variance. Contrary to the expected, the results of the SEA indicated that alien *D. pictus* populations did not show broader habitat use than co-occurring anurans except for *Bufo spinosus* (Table 11).

Table 10. Descriptive statistics of physical and chemical parameters measured in the characterization of aquatic habitats PERMANOVA statistics (Pseudo-F and *P* values) comparing habitat selection by *D. pictus* in its native and alien ranges. Significant results are marked in bold. Legend: *n*: sample size.

		Alien	**Native**	**PERMANOVA**
n		34	83	
Surface area (m^2)	Mean	226	943	1.81
	range	2–5,040	1–18,656	0.17
Depth (cm)	Mean	18	27	3.03
	range	5–95	4–121	0.08
Slope	Mean	5.85	5.34	0.13
	range	0.71–15.32	0.08–42.25	0.74
Temperature (ºC)	Mean	19	19	0.03
	range	12–28	10–28	0.87
Dissolved oxygen (m·L^{-1})	Mean	6.50	8.17	3.24
	range	1.26–20.00	1.56–20.00	0.07
pH	Mean	8.06	7.94	0.73
	range	6.90–9.70	6.20–9.40	0.38
Conductivity (μS·cm^{-1})	Mean	395	1308	4.96
	range	74–1,875	44–11,140	**0.03**

Table 11. Niche breadth in aquatic habitat selection in the invaded assemblage. Legend: *n*: sample size; CI: Bayesian confidence interval.

	n		
Alytes obstetricans	30	Mean	1.77
		CI	1.24–2.50
Epidalea calamita	11	Mean	3.91
		CI	2.18–6.86
Bufo spinosus	13	Mean	**1.07**
		CI	**0.63–1.81**
Discoglossus pictus	34	Mean	2.44
		CI	1.72–3.47
Hyla meridionalis	14	Mean	2.29
		CI	1.36–3.83
Pelobates cultripes	18	Mean	3.63
		CI	2.29–5.72
Pelodytes punctatus	22	Mean	4.63
		CI	3.01–7.02
Pelophylax perezi	12	Mean	2.55
		CI	1.44–4.43

IV. 2. 3. 2. Morphological variability

The results of the morphological analysis showed a great variability in tadpole body area, span ratio and coloration, from large species with depressed bodies (e.g., *Pelobates cultripes* (Cuvier, 1829)), to small species with compressed bodies (e.g., *Epidalea calamita* (Laurenti, 1768)) and species with dark homogeneous coloration (e.g., *B. spinosus*) in the assemblages from both regions (Tables 12, 13). The PCA showed that the first axis was correlated with the size and shape of the tadpole while the second axis was correlated with coloration. The variance explained by the first two axes and the factors loadings of the morphological variables are showed in Appendix 1b, showing that the three variables highly accounted to the observed variance. In the assemblage from the invaded region, *D. pictus* showed greater morphological variability than the *B. spinosus* and *H. meridionalis* Boettger, 1874 but lower than *P. cultripes* and *Alytes obstetricans* (Laurenti, 1768) (Table 12). In the invaded assemblage several species showed a high morphological overlap with *D. pictus*, similar to that observed in the native assemblage (Tables 12, 13).

Table 12. Morphological results for the invaded assemblage. The columns show the mean values for the morphological variables. Phenotypical variability: amplitude of morphological variation as shown by SEA analysis (mean and 95% credible intervals). Morphological overlap: two values are showed, the inter-inertia ratio and P values. Inter-inertia ratio values close to 1 indicate higher overlap. Comparisons are established between *D. pictus* and other species. Species which differ from *D. pictus* are shown in bold. Legend: *n*: sample size.

	n	**Total area**	**Span ratio**	**Mean gray value**	**Phenotypical variability**	**Morphological overlap**
Discoglossus pictus	38	153	4.6	113	0.94 (0.68–1.29)	
Alytes obstetricans	28	461	4.8	102	**1.53** **(1.05–2.19)**	0.36 **0.02**
Epidalea calamita	22	113	5.3	93	0.80 (0.53–1.21)	0.34 0.86
Bufo spinosus	34	108	5.3	58	**0.68** **(0.48–0.94)**	0.06 0.55
Hyla meridionalis	35	300	3.6	114	**0.55** **(0.40–0.76)**	0.19 0.92
Pelobates cultripes	23	1310	3.6	90	**2.71** **(1.80–4.05)**	0.01 **0.02**
Pelodytes punctatus	20	261	4.0	107	1.03 (0.66–1.58)	0.72 0.47
Pelophylax perezi	17	464	4.2	111	1.03 (0.65–1.67)	0.11 0.78

Table 13. Morphological results for the native assemblage. The columns show the mean values for the morphological variables. Morphological overlap: values close to 0 indicate higher overlap. Comparisons are established between *D. pictus* and other species. Significant results are marked in bold. Legend: *n*: sample size.

	n	**Total area**	**Span ratio**	**Morphological overlap**
Discoglossus pictus	46	168	4.5	
Bufotes boulengeri	27	211	5.2	**0.04**
Hyla meridionalis	12	201	3.4	0.13
Pelophylax saharicus	12	703	5.4	0.19

IV. 2. 3. 3. Climate niche

The climate data showed that the tolerance ranges of the alien *D. pictus* populations (Table 14) were within the ranges observed for the native populations (i.e., below the maximum and above the minimum temperatures and above the minimum value of the AI). In the climate space, both groups of populations differed in the first component (Fig. 13), in which the three variables showed similar factor loadings (MWQ = -0.93, MCQ = -0.81, AI = 0.80). The alien populations differed from the native populations by showing higher values of the aridity index (i.e., lower aridity) and lower temperatures (Fig. 13). Co-inertia analysis indicated that there were significant differences between the alien and native populations in the position occupied in the climate space (inter-inertia ratio of 7%, $P = 0.02$). The MaxEnt model produced an AUC of 0.86, indicating a high predictive value. The 10th percentile had a sensitivity of 0.92 (high proportion of observed positives predicted) and a specificity of 0.45 (low proportion of observed negatives predicted). The SSM had a sensitivity of 0.72 (high proportion of observed positives predicted) and a specificity of 0.80 (high proportion of observed negatives predicted). Only the first threshold, with higher sensitivity, indicated the existence of suitable conditions for *D. pictus* in the north-eastern Iberian Peninsula and southern France (Fig. 14).

Table 14. Climate descriptive statistics, comparing the locations in the invaded range (Europe) with those from the native range (northern Africa).

Climate variables		**Invaded range**	**Native range**
Temperature of warmest quarter (ºC)	Mean	22.0	24.9
	range	19.0–23.4	19.4–32.3
Temperature of coldest quarter (ºC)	Mean	7.9	9.8
	range	5.0–10.5	3.6–13.3
Aridity index	Mean	0.72	0.43
	range	0.58–1.05	0.05–0.95

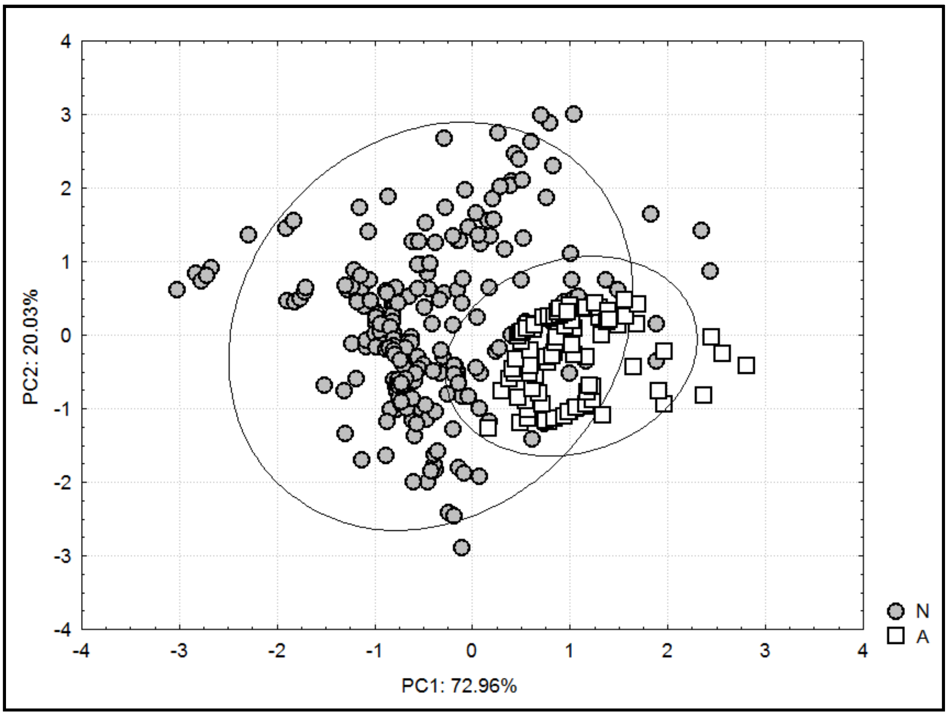


Figure 13. Principal component analysis (PCA) plot representing the realized niches occupied by native (N) and alien (A) populations of *D. pictus*. The ellipses represent the 95% confidence intervals.

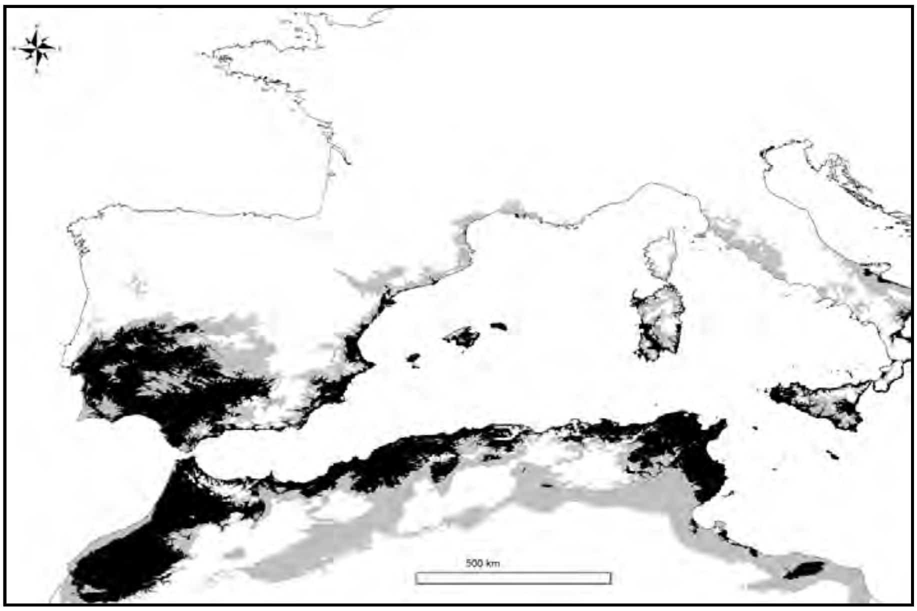


Figure 14. Ecological niche model generated by MaxEnt, using the variables temperature of the warmest quarter, temperature of the coldest quarter and aridity index. The grey layer indicates the 10th percentile threshold (more flexible in not only predicting potentially suitable habitats, but also predicting a higher proportion of not suitable habitat), and the black layer shows the sensitivity-specificity sum maximisation threshold (minimising the error rate in the prediction of suitable and not suitable habitats, miss a higher proportion of potentially suitable habitat)

IV. 2. 4. Discussion

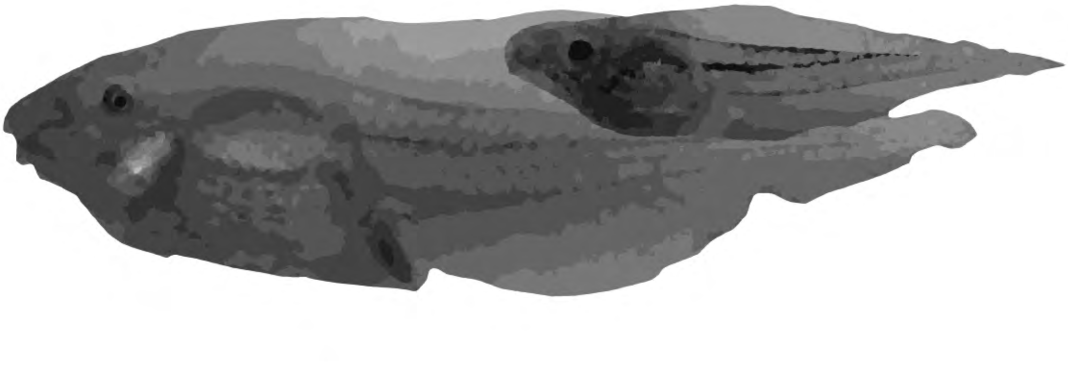
Discoglossus pictus has become established in southern Europe, occurring in similar aquatic habitats to those occupied in Tunisia. This species occupied ponds of variable sizes and hydroperiods, including ephemeral pools where few anuran species can complete their larval development (García-París et al. 2004). The ranges we found for some of the physical and chemical characteristics of the reproductive habitats were relatively large, but did not exceed the variability observed for other anurans present in the invaded range, except in the case of *B. spinosus*. These wide ranges in habitat use are typical of several species of Mediterranean anurans (Jakob et al. 2003; Gómez-Rodriguez et al. 2009) and could be related to irregularity in precipitation and consequent fluctuations in physical and chemical parameters of aquatic habitats, especially at the end of the hydroperiod (Boix et al. 2004). Only *B. spinosus* had a narrower range, but this species usually breeds in permanent water bodies (Bosch and Martínez-Solano 2003), which fluctuate less in the chemical parameters (Friday 1987).

Discoglossus pictus showed intermediate morphological variability compared with the sympatric anuran species, showing greater values than *H. meridionalis* and *B. spinosus*. However both species are very successful in the anuran assemblages of north-eastern Spain (Llorente et al. 1995). Although morphological variability may promote survival of larvae confronted with stressors such as predatory pressure or competition (Relyea 2004), it may not be critical to reproductive success. These species display other mechanisms to reduce vulnerability to predation, such as low palatability, in the case of *B. spinosus* (as described for other species of the *B. bufo* complex; Lardner 2000), or evasive behavior, in the case of *H. meridionalis* (as the other *Hyla* species; Chovanec 1992). Similarly, part of the observed variability may only indicate plasticity of morphological traits in response to pond permanence, because the larval size and body shape can covariate with pond size (Denver et al. 1998; Vences et al. 2002). This would explain the lower variability in species such as *B. spinosus*, which breed in water bodies with stable hydroperiods (Díaz-Paniagua et al. 2005). Our analysis of phenotypic traits also revealed that *D. pictus* had high morphological overlap with some species in the invaded assemblage, similar than with those in the native assemblage. This could imply that the establishment of *D. pictus* has not been assisted by greater divergence in morphological traits. This is in contrast to the high morpho-functional divergence that has contributed to the establishment of numerous alien species, possibly helping them to occupy empty niches (Ordonez et al. 2010). This finding supports the hypothesis that there are communities that are not species-saturated and therefore can be invaded without necessarily causing the extinction of the original pool of species (Sax et al. 2007).

The analysis of climate data revealed a shift in the realized niches occupied by both groups of populations. The MaxEnt model predicted quite accurately the distribution of *D. pictus* in south-western Europe, but with a low specificity. These apparent shifts in the realized niche may reflect that the attributes of the native range of *D. pictus* describe only part of the fundamental niche of the species (cryptic niche conservation hypothesis; Schulte et al. 2012). The MaxEnt model also revealed potentially suitable niches on the Mediterranean coast of the southern and south-western Iberian Peninsula. This result suggested that the invasion by *D. pictus* has not yet reached equilibrium and that this species could continue to expand to the south in the Iberian Peninsula. This region is inhabited by the endemic species, *Discoglossus galganoi* (Martínez-Solano and García-París 2002; Pabijan et al. 2012), and the eastern populations occur in fragmented populations of a few individuals (Sancho 2000). The potential impact of *D. pictus* on this species is unknown, but given the colonizing ability of *D. pictus*, it is advisable to closely monitor the dynamics of its expansion in the Iberian Peninsula.

The existence of similar environmental conditions and the generalist character of the species, which shows a great capacity to exploit marginal habitats, possibly favoured the establishment of *D. pictus* in south-western Europe. Our findings demonstrate that alien species can become established successfully within a relatively diverse species assemblage (theoretically less invasible; Elton 1958), even when the native species appear to belong to a similar functional guild and show similar niche breadths. This suggests that the invaded community was not previously saturated with species, as has been described for other invaded communities in which the introduction of new species produced an overall increase in species richness (Sax and Gaines 2003). This is because invasibility depends not only on species diversity (or interspecific competition), but also on other factors such as productivity and rate of disturbance (Davis et al. 2005). The latter factor favours opportunistic species that can exploit the resources available in disturbed environments (e.g., anthropogenic habitats), where many alien species become established (Hansen and Clevenger 2005). Finally, our results have implications for the management of these alien populations. The opportunism involved in the selection of habitat makes their management particularly complex, because *D. pictus* can breed in small rainwater pools, which are difficult to control because their appearance is erratic. Moreover, its high overlap in habitat use with other anurans requires that the appropriate control measures do not disturb the reproduction of the native species.

IV. 3. Chapter 3. Reproductive habitat selection in alien and native populations of the genus *Discoglossus*



IV. 3. 1. Abstract

The existence of suitable breeding habitats is an important factor explaining the regional presence of an anuran species. This study examined patterns of habitat selection in populations of three species of the genus *Discoglossus*: *D. galganoi* (south-western Iberian Peninsula), *D. scovazzi* (Morocco) and *D. pictus* (three different areas were included in the study: Sicily, Tunisia and north-eastern Iberian Peninsula). The populations of *D. pictus* on the Iberian Peninsula are allochthonous, and analysis of these patterns may provide insights into the processes that regulate the invasion phase. The hypotheses tested were: (i) congeneric species show the same patterns of habitat selection, and alien species have been established following these patterns; (ii) there are differences in species associations between assemblages structured deterministically and by chance, i.e., native versus invaded assemblages. The larval habitats of three species of this genus were characterized by measuring physical and chemical parameters of the water bodies. We examined the covariation between the presence of *Discoglossus* species and the species richness of sympatric anurans, and investigated a possible relationship between morphological similarity (as a proxy of functional group) and overlap in habitat use. The results showed that congeneric species are morphologically conservative and select very similar types of aquatic habitat. The alien population and other sympatric species showed a high degree of overlap in habitat use, which was greater than that observed in the native assemblage with a similar functional richness. Species associations were not structured on the basis of morphological similarity in any of the assemblages. Among native populations, the presence of *Discoglossus* was either negatively correlated or not significantly correlated with species richness. Only the alien population showed a positive correlation between its presence and species richness, which suggests a loss of assemblage structure.

IV. 3. 2. State of the art

The processes that influence invasion by alien species are not fully understood (Arim et al. 2006; Simberloff et al. 2013). Several factors may explain the successful establishment of alien species in a new assemblage, including a favourable environment and advantageous interactions with native species (Lawler et al. 1999; Theorharides and Dukes 2007; Willis et al. 2010). Environmental conditions may be similar to those in the original range of the alien species, making them pre-adapted for colonization of the region (Kats and Ferrer 2003). Alien species may also have a wide adaptive range, and can become established by occupying new niches in the invaded region (Alexander and Edwards 2010). Adaptive divergence in the niche of an invasive species can be assessed by comparing the ecology of native and non-native populations to determine the extent to which they respond differently to the same environmental variables (Govindarajulu et al. 2006; Rödder and Weinsheimer 2009).

In this paper we have focused on the use of aquatic habitats during invasion by an anuran species. We examined the selection of these types of habitat because the availability of suitable aquatic habitats is a key factor underlying the local distribution of anurans (Hazell et al. 2001). Given that most anuran larvae are generalist feeders that face high levels of competition (Dutra and Callisto 2005), mechanisms of temporal and spatial segregation are important in reducing the interactions among controphic species (Lawler and Morin 1993; Prado et al. 2009). Invasive frogs can greatly reduce the reproductive success of native species (Kupferberg 1997), and this may be related to the extent of niche overlap (Semeniuk et al. 2007; Escoriza et al. 2014).

This study examined the variation in the morphological and functional overlap between co-occurring species. It was expected that differences between native and invaded assemblages would cause the invaded assemblages to show high functional redundancy (Reise et al. 2006). Larval assemblages are generally organized following the rules of assembly, i.e., interspecies interactions determine the species composition of these assemblages (Vignoli et al. 2010; Moreira and Maltchik 2012). However, in anuran assemblages, structured co-occurrences may appear only among functionally analogous species (Both et al. 2011b; but see Vasconcelos et al. 2011). The patterns of association among species must be examined in relation to habitat features. Some attributes of a water body, such as its size, can explain important variations in the species composition (Hazell et al. 2004; Peltzer and Lajmanovich 2004; Both et al. 2011a); this affects the observed species associations when comparing assemblages from distinct areas.

This study examined how these factors influence aquatic habitat selection by different species of the genus *Discoglossus* Otth, 1837. This genus is native to the Mediterranean region, but one species includes an alien population in the north-eastern Iberian Peninsula (Martínez-Solano 2004). We compared the selection of breeding habitats by the non-native population and native populations of congeneric species to detect generalized patterns that occurred in spite of possible differences in their ecological backgrounds. We expected these species to be ecologically similar, because amphibian lineages can show an important morphological and functional inertia during diversification (Kozak et al. 2005). This approach can reveal differences in the ecology of alien populations compared with native populations of the same or closely related species, which has been little studied in other invasive frogs. Specifically, we wanted to evaluate the following hypotheses: (i) Congeneric species are functionally conservative and show similar patterns of habitat selection. Similarly, alien populations have become established by following the patterns of habitat selection used by native populations of the same species and closely related species; (ii) Differences occur in the associations among sympatric anurans when assemblages structured deterministically and by chance (native versus invaded assemblages) are compared.

IV. 3. 3. Results

IV. 3. 3. 1. Reproductive habitat selection

A total of 368 water bodies were sampled (Table 15), and larvae of 17 anuran species were found. The *Discoglossus* species studied showed wide variability in their ranges of habitat use, especially in some parameters such as water body surface area (Table 15). The water parameters of their reproductive habitats (i.e., water temperature, dissolved oxygen, pH and conductivity) did not explain the patterns of selection of most *Discoglossus* species (Table 16). However, most of the *Discoglossus* species selected smaller and shallower water bodies than expected on the basis of the characteristics of the available water bodies. The only exceptions were the Tunisian populations of *D. pictus*, which occupied most available bodies of water (82% of bodies of water examined) (Table 15).

Table 15. Physical and chemical characteristics (mean and range) and species richness (mean and range) of water bodies. Legend: *n*: number of water bodies; T: water temperature; O_2: dissolved oxygen; Cond: conductivity; S: species richness / pond; SWIP: south-western Iberian Peninsula; NEIP-SF: north-eastern Iberian Peninsula and southern France.

		n	**Surface area (m^2)**	**Depth (cm)**	**T (ºC)**	**O_2 ($mg \cdot L^{-1}$)**	**pH**	**Cond ($\mu S \cdot cm^{-1}$)**	**S**
Morocco	presence	39							
D. scovazzi	mean		3127	28	17	9.5	7.9	724	1.6
	range		1.5–40828	7–103	11–24	3.6–13.7	6.0–10.0	26–5420	1–2
	absence	37							
	mean		27531	28	18	7.8	7.9	578	1.4
	range		175–515599	17–49	12–23	1.9–12.5	6.7–9.6	53–4930	1–3
SWIP	presence	17							
D. galganoi	mean		17	8	15	10.3	7.5	275	1.1
	range		1.2–130	2–17	9–21	5.5–19.7	6.6–8.5	57–594	1–2
	absence	61							
	mean		427	40	14	9.8	7.8	447	1.4
	range		1.2–7233	4–121	8–22	2.7–18.5	5.8–9.5	25–4370	1–3
NEIP-SF	presence	33							
D. pictus	mean		199	23	19	5.9	7.9	325	1.9
(alien)	range		1.6–1920	5–95	13–28	0.7–20.0	6.2–9.7	32–1875	1–5
	absence	34							
	mean		1101	45	19	5.7	8.1	325	1.5
	range		4.9–15410	7–225	13–35	0.3–18.2	6.6–10.1	33–1182	1–3
Tunisia	presence	83							
D. pictus	mean		943	27	18	8.2	7.9	1308	1.7
	range		1.4–18656	4–121	10–28	1.6–20.0	6.2–9.4	44–11140	1–4
	absence	18							
	mean		679	30	19	7.7	7.9	811	1.1
	range		5.9–3199	3–127	15–28	2.0–14.2	7.0–9.6	61–2960	1–2
Sicily	presence	29							
D. pictus	mean		349	28	18	9.3	7.9	2679	1.4
	range		0.7–2929	5–94	10–27	2.8–19.5	6.8–8.6	120–18290	1–4
	absence	17							
	mean		1715	97	17	9.2	8.1	861	1.6
	range		65–4753	21–165	9–24	6.4–16.6	6.8–8.2	115–2390	1–3

Table 16. Relationship between occurrence of the genus *Discoglossus* and water body parameters. The results show *p* values and the proportion (prop) of the variance in *Discoglossus* occurrence explained by each variable, obtained by DistLM. Legend: T: water temperature; O_2: dissolved oxygen; Cond: conductivity; SWIP: south-western Iberian Peninsula; NEIP-SF: north-eastern Iberian Peninsula and southern France; *p* values < 0.05 are in bold.

			Surface area (m^2)	**Depth (cm)**	**T (ºC)**	**O_2 ($mg·L^{-1}$)**	**pH**	**Cond ($\mu S·cm^{-1}$)**
D. scovazzi	Morocco	*Pseudo-F*	2.58	0.00	1.66	1.89	0.04	0.08
		p	**0.03**	0.94	0.10	0.06	0.89	0.79
		prop	0.03	0.00	0.01	0.01	0.00	0.00
D. galganoi	SWIP	*Pseudo-F*	3.86	28.40	9.94	0.99	0.90	1.31
		p	**0.05**	**0.001**	**0.004**	0.34	0.34	0.23
		prop	0.04	0.25	0.11	0.01	0.01	0.02
D. pictus	NEIP-SF	*Pseudo-F*	3.20	8.65	0.31	0.05	1.31	0.00
	(alien)	*p*	**0.03**	**0.001**	0.60	0.83	0.26	0.99
		prop	0.05	0.12	0.00	0.00	0.02	0.00
D. pictus	Tunisia	*Pseudo-F*	0.17	0.24	0.16	0.24	0.05	1.03
		p	0.73	0.62	0.70	0.62	0.83	0.33
		prop	0.00	0.00	0.00	0.00	0.00	0.01
D. pictus	Sicily	*Pseudo-F*	57.66	16.07	0.22	0.01	1.51	3.75
		p	**0.001**	**0.001**	0.67	0.92	0.22	**0.03**
		prop	0.57	0.27	0.00	0.00	0.03	0.08

IV. 3. 3. 2. Morpho-functional analysis

A total of 315 tadpoles were examined belonging to seven families occurring in the region, Alytidae (2 species; 24 specimens), Bufonidae (6 species; 90 specimens), Discoglossidae (3 species; 79 specimens), Hylidae (2 species; 38 specimens), Pelobatidae (2 species; 38 specimens), Pelodytidae (2 species; 20 specimens) and Ranidae (3 species; 26 specimens). Comparison of the morphology within the genus *Discoglossus* showed that no species differed significantly from the rest: all showed body areas below 325 mm^2 (mean = 152 mm^2) and depressed morphology, with span ratios above 3.5 (mean = 4.6) (Table 17). Our analysis indicated that different species of *Discoglossus* occupied a similar position in morphological space, close to species of the families Bufonidae and Pelodytidae (Fig. 15 and Table 18). This analysis also revealed differences in functional richness among assemblages: the two assemblages of the Iberian Peninsula had higher values for functional richness than the Sicilian and north African assemblages (Table 18).

Table 17. Morphological variability of *Discoglossus* species at Gosner's stages 36–39, comparing one species with the rest. The values are the mean and range (minimum-maximum). Legend: *n*: sample size; Area: tadpole body surface area in mm²; Span ratio: ratio between the total length and the body depth. PERMANOVA: pseudo-F statistic and *p* values respectively.

	n	**Area**	**Span ratio**	**PERMANOVA**
D. scovazzi	18	139 (80–216)	4.7 (3.5–5.4)	0.65 0.39
D. galganoi	28	146 (111–173)	4.8 (3.8–5.8)	0.02 0.89
D. pictus	33	151 (66–323)	4.7 (3.7–5.7)	0.68 0.39

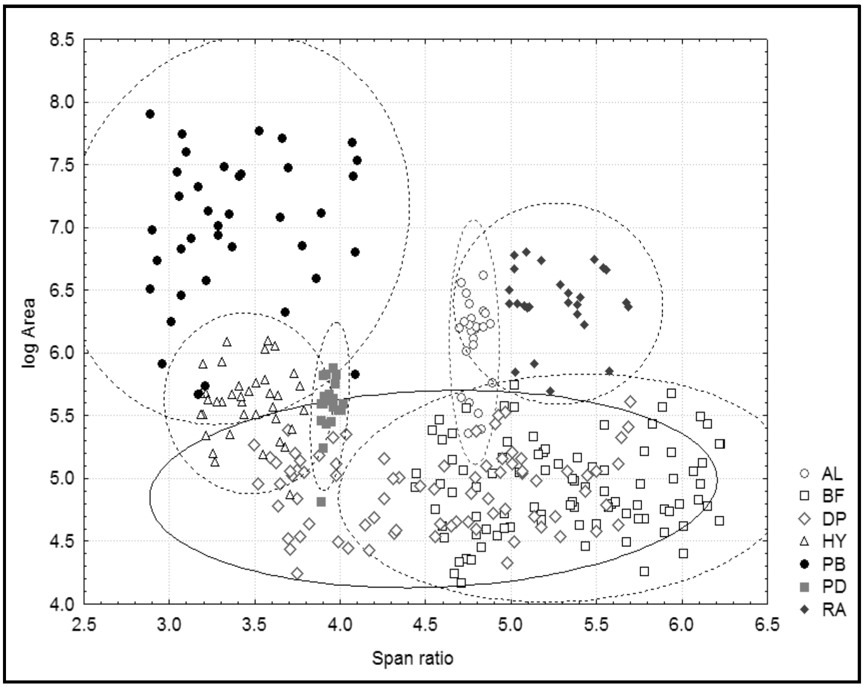


Figure 15. Plot of the morphological space, including all families. Span ratio: ratio between the total length and body depth. The ellipses represent the 95% confidence intervals. Legend: AL: Alytidae; BF: Bufonidae; DP: Discoglossidae; HY: Hylidae; PB: Pelobatidae; PD: Pelodytidae; RA: Ranidae.

Table 18. Functional richness of the assemblages, species habitat overlap and morphological distances. In habitat overlap (Dice's index), values close to 0 indicate minimum overlap in habitat use with local species of *Discoglossus*. Centroid distance: distance between principal coordinate centroids, obtained from the variables total body area and body shape. Legend: SWIP: south-western Iberian Peninsula; NEIP-SF: north-eastern Iberian Peninsula and southern France.

	Functional richness	**Species**	**Habitat overlap**	**Centroid distance**
Morocco	2.7	*Discoglossus scovazzi*		
		Amietophrynus mauritanicus	0	1.58
		Bufotes boulengeri	0.08	0.75
		Hyla meridionalis	0.32	1.66
		Pelobates varaldii	0.07	4.07
		*Pelophylax saharicu*s	0.14	1.52
SWIP	3.7	*Discoglossus galganoi*		
		Alytes cisternasii	0	1.19
		Bufo spinosus	0	0.42
		Epidalea calamita	0.12	0.42
		Hyla meridionalis	0	2.04
		Pelobates cultripes	0	2.85
		Pelodytes ibericus	0	1.30
		Pelophylax perezi	0.17	0.99
NEIP-SF	3.6	*Discoglossus pictus*		
(alien)		*Alytes obstetricans*	0.24	0.77
		Epidalea calamita	0.38	0.98
		Bufo spinosus	0.12	0.98
		Hyla meridionalis	0.23	1.48
		Pelobates cultripes	0.27	2.48
		Pelodytes punctatus	0.28	0.75
		Pelophylax perezi	0.06	0.96
Tunisia	3.1	*Discoglossus pictus*		
		Amietophrynus mauritanicus	0.28	1.75
		Bufotes boulengeri	0.21	0.88
		Hyla meridionalis	0.51	1.48
		Pelophylax saharicus	0.06	1.50
Sicily	2.3	*Discoglossus pictus*		
		Bufo bufo	0.05	0.67
		Bufotes siculus	0	0.68
		Hyla intermedia	0.19	1.38
		Pelophylax bergeri	0.10	0.78

IV. 3. 3. 3. Species associations

The Dice's index indicated a high degree of habitat overlap between *Discoglossus* and other species of anuran in the invaded assemblage. Although the index was also high in the Tunisian assemblage, the others showed much lower values, especially the SWIP population (Table 18).

This association between species was not structured on the basis of morphological resemblance in any of the studied assemblages (Table 19). In all assemblages, the average values of species richness per pond were lower than expected from local species richness, in both presence and absence habitats (Table 15). The results of the partial Mantel test revealed that species richness was negatively correlated with the occurrence of *D. galganoi*, and non-significantly associated with the occurrence of *D. scovazzi* and native populations of *D. pictus* (Table 19). In alien populations of *D. pictus*, the Mantel test revealed a weak positive correlation with the presence of this species (Table 19).

Table 19. Associations among *Discoglossus* species and other anurans. Legend: A: Spearman's correlation between species habitat overlap (Dice's index) and morphological similarity (centroid distance). B: Partial Mantel's test correlation between *Discoglossus* occurrence and species richness, holding pond size constant.

	Region	**A**		**B**	
D. scovazzi	Morocco	z	-0.20	R	0.08
		p	0.95	*p*	0.07
D. galganoi	SWIP	z	-1.3	R	-0.07
		p	0.26	*p*	**0.05**
*D. pictu*s	NEIP-SF	z	-0.13	R	0.07
	(alien)	*p*	0.92	*p*	**0.0002**
D. pictus	Tunisia	z	0.69	R	0.02
		p	0.75	*p*	0.45
D. pictus	Sicily	z	1.39	R	-0.01
		p	0.33	*p*	0.75

IV. 3. 4. Discussion

The *Discoglossus* species showed some common patterns in habitat selection, despite the existence of regional differences in habitat variables. All the species could reproduce in very small water bodies, and to some extent without dependence on the physical and chemical characteristics of the water bodies. Use of this type of aquatic habitat, which is usually ephemeral, is favoured by larval development occurring within 21 to 60 days, a shorter period than that of other sympatric anurans (García-París et al. 2004; Capula 2007). The range of tolerance to variability in water chemistry parameters was very broad in most of the species studied. In particular, *D. pictus* and *D. scovazzi* tolerated very high conductivity. Water salinity (or its proxy, conductivity) is an important factor limiting the occurrence of anurans; it affects the survival and fitness of the larvae (Christy and Dickman 2002), particularly at values greater than 6000 $\mu S \cdot cm^{-1}$ (Smith et al. 2007). However, this threshold is far below the maximum values measured for *D. pictus* in Tunisia and Sicily. This tolerance may favour the colonizing ability of these species, which can complete larval development even under severe fluctuations in water chemistry such as those that occur in temporary ponds at the end of the hydroperiod (Boix et al. 2004).

The detection of common patterns in habitat selection was complemented by the results of the morphological space analysis. All of the species of *Discoglossus* examined showed high span ratios (i.e., depressed morphology) and small body size. This indicates that these species correspond to the benthic ecomorph (McDiarmid and Altig 1999), and shows the persistence of common functional traits during diversification of the genus, similarly to other amphibian lineages (Adams et al. 2009).

The analysis of associations in habitat use revealed that these were not dependent on morphological resemblance. This could be explained by the fact that *Discoglossus* species were associated both with highly divergent species (e.g., *Pelobates* and *Hyla*) and with those showing low levels of divergence (e.g., *Bufo*). This analysis also revealed that, in the invaded assemblage, *D. pictus* showed high habitat overlap with other anuran species; it was the only species whose occurrence was positively associated with species richness. This contrasts with the associations established by *D. galganoi* from the SWIP assemblage, which shows little overlap with other species. The variability in functional richness among assemblages could explain some of the differences in habitat use and species associations. The presence of *D. galganoi* only in small water bodies (up to 130 m^2) could be related to high functional richness of the SWIP assemblage, in contrast to the broad habitat ranges observed in other groups of *Discoglossus* that inhabit regions such as Tunisia, Morocco and Sicily, which have function-poor assemblages. It has been described that larval anuran assemblages with high functional richness display important segregation in habitat use, thereby reducing species interactions (Heyer 1973; Inger et al. 1986). This segregation in habitat use may be mediated by mechanisms such as differences in reproductive timing (Gottsberger and Gruber 2004), the presence of predators (Hecnar and M'Closkey 1997) or physiological tolerance (Skelly 2001), or in oophagy by previous larval cohorts (Drewes and Altig 1996). In any case, these mechanisms did not completely prevent association among species, because *Discoglossus* species were found with most of the anurans that appeared in the local assemblages. However, many of these combinations should be rare, given the observed overlap values.

The assemblage from NEIP-SF had a species composition and functional richness similar to the SWIP assemblage. However, these groups of *Discoglossus* species showed important differences in their patterns of association. It is unlikely that these differences were due to diversification in their functional niches, because the populations displayed very similar phenotypes. Thus, these differences could be explained by altered patterns of co-occurrence in the NEIP-SF assemblage. This is similar to findings in other invaded communities (Gotelli and Arnett 2000; Sanders et al. 2007), where the presence of an alien species promotes a loss of assemblage structure (i.e., there is less segregation among species). These assemblages may evolve towards the integration of alien species, i.e., coexistence (Macneil et al. 2001), or towards depletion or extinction of the native species (Mooney and Cleland 2001).

IV. 4. Chapter 4. Niche conservatism determines the occurrence of allopatric populations of *Hyla meridionalis* under different biogeographical scenarios

IV. 4. 1. Abstract

Niche conservatism is a process that regulates the distribution of many amphibians, during both speciation processes and the phase of invasion by alien species. However, in a few species it is possible to investigate the ecological divergence between populations that have been isolated by different evolutionary and environmental processes. *Hyla meridionalis* is an anuran for which there is one group of alien (i.e., recently established) populations in south-western Europe and two parapatric groups of populations in northern Africa, with an estimated divergence time between 2 and 12 mya. In this study we tested the hypothesis that niche conservatism determines the occurrence of a species in two distinct niche scales and in separate geographical regions, using *H. meridionalis* as a model. To do this we examined the niche occupied by three isolated groups of populations (in Tunisia, Morocco and south-western Europe) to assess factors that may act at the local and regional levels. Our results indicate that *H. meridionalis* is partly occupying an aquatic habitat that is suboptimal for other anuran species. This fact may have favored its persistence in this niche and the establishment of this species in regions where no equivalent species occur. The two lineages of *H. meridionalis* overlap extensively in the climate space, although the western lineage has spread into more marginal niches. Overall, our results support the hypotheses that niche conservatism regulates the distribution of alien populations, and that the species niche can be stable at an evolutionary time scale.

IV. 4. 2. State of art

It has recently been observed that although some genera of vertebrates are highly diverse, this diversity is not reflected in their niche radiation, and some related species share the same ecological space (Wiens 2004; Hawkins et al. 2006; Cooper et al. 2011). The ranges of these conservative species are maintained by interspecific competition, or environmental barriers including steep thermal or aridity gradients that species are unable to overcome, which promote further genetic isolation (Kozak and Wiens 2006; Lovette and Hochachka 2006). This niche conservatism also regulates the invasion phase of several alien species (Wiens and Graham 2005). Most aliens spread into niches that are similar to those they occupy in their native ranges, provided that the conditions are within their eco-physiological ranges (Rodda et al. 2011). In the case of amphibians there is also evidence of phylogenetic patterns in the worldwide niche space (Hof et al. 2010), possibly determined by their poor capacity for dispersal and their relatively strict physiological requirements (Welsh and Lind 1996; Araújo and Pearson 2005). For these reasons, many alien amphibians occupy similar or identical niches to those in their native range (Govindarajulu et al. 2006; Escoriza et al. 2014).

In this study we investigated the niches of an anuran species, the Mediterranean tree frog *Hyla meridionalis* Boettger, 1874, with the aim of determining whether niche conservatism occurs among allo-parapatric populations of this species. In North Africa, *H. meridionalis* occurs from north of Tunisia to the Atlantic coast of Morocco (Salvador 1996; Ben Hassine and Nouira 2012). It also occurs in Europe, where it occupies a discontinuous range: one group of populations occurs in southern France, north-eastern Italy and the northern Iberian Peninsula, and the other group occurs in the south-west of the Iberian Peninsula (Gasc et al. 1997). Moreover these two groups of populations are composed by two distinct haplotypes originating from northern Morocco (populations from northern Iberian peninsula and southern France) and southern Morocco (populations from south-western Iberian Peninsula; Recuero et al. 2007). These European populations may be the result of a relatively recent invasion from Morocco, probably associated with human activities (Recuero et al. 2007), maintaining a nearly parapatric range with native hylids (Gasc et al. 1997). The African populations show a high degree of genetic divergence, with the populations of Tunisia and north-eastern Algeria having separated from the Moroccan populations at 2–12 mya ago (Recuero et al. 2007; Stöck et al. 2008). This biogeographic pattern allows test some hypotheses about whether niche conservatism determined the distribution of *H. meridionalis.* We expected that these populations occur in relatively similar niches, because amphibian lineages show an important conservatism in several niche levels, even on time scales of million of years (Kozak et al. 2005; Kozak and Wiens 2010). However, the northernmost range of *H. meridionalis* cannot be predicted from ecological models based on the native range of the species (Sillero 2010). Similarly, populations of *H. meridionalis* from Tunisia are mainly restricted to meso-thermal climates, while in Morocco they reach the boundaries of the Saharan region (Ben Hassine and Nouira 2012; Bons and Geniez 1996). These differences could indicate niche shifts or cryptic niche conservatism, whereby the native distribution does not include the entire fundamental niche of the species (Schulte et al. 2012).

We analysed the niche occupied by *H. meridionalis* at two spatial scales (local and regional), because the occupancy of niches at these scales is regulated by different factors (Huston 1999). At the local level (spatial extent below 25 km^2; Cowley et al. 2001) we studied the selection of aquatic habitats, as they are a key factor explaining the local occurrence of anuran species (Parris and McCarthy 1999). We also considered the type of terrestrial habitat surrounding these habitats, as this also determines the amphibian composition in the aquatic habitats (Houlahan and Findlay 2003). Furthermore, the investigation of habitat use has relevance not only to an understanding of the process of niche diversification. The existence of broad ranges in the habitat selection or the occurrence in a suboptimal niche for native species may reveal some of the factors that could favor the success in the establishment of an alien species (Kiesecker et al. 2001; Korsu et al. 2012). The regional niche was quantified on the basis of presence in each locality, and the data extracted from environmental layers were used to characterize these localities. These layers describe some aspects of the climate space that are assumed to be ecologically relevant to the species (Rödder et al. 2009); in amphibians these factors are related to the water-energy balance and temperature extremes (Ródriguez et al. 2005; Smith et al. 2005; Buckley and Jetz 2007). This quantification of the climate space occupied by species belonging to the same lineage can provide important insights into the environmental drivers that promote species diversification (Rissler and Apodaca 2007).

In summary, in this study we used *H. meridionalis* to investigate niche stability under different evolutionary and environmental scenarios. We first assessed the selected aquatic habitat on the basis of characterization of the water bodies and the land cover types of adjacent terrestrial habitats, expecting to find similar preferences in the three groups (hypothesis i). Additionally, we compared the habitat use of *H. meridionalis* to that of other sympatric species. This study may provide some insights into the factors active during the establishment of *H. meridionalis* in south-western Europe. In this sense, and given the local absence of other hylid species, we expected *H. meridionalis* to occur in an empty niche, or a niche that is underused by other anuran species (hypothesis ii). Finally, we described the climate niches quantitatively, and determined whether there was niche divergence by comparing the population groups, predicting that niche conservatism determines the species range (hypothesis iii).

IV. 4. 3. Results

IV. 4. 3. 1. Aquatic and terrestrial habitat selection

The descriptive statistics obtained from the characterization of larval habitats are shown in Appendix 2a The three groups of populations showed a wide range in terms of the selection of water body morphology (i.e., surface area and depth) and water parameters. The results of the OMI analysis revealed that the first axis was related to the water body morphology, while the second axis was related to water parameters (Fig. 16). Depth was the most important factor explaining the occurrence of *H. meridionalis*, when compared with other anuran species (Fig. 16). The position on the first factorial plane indicated that the European populations occupy a more marginal niche, differentiated from native groups by the water body depth (Table 20 and Fig. 16). However, the three groups showed an important overlap in the niche space (Fig. 17).

Table 20. Habitat selection of the studied groups of populations of *H. meridionalis*. The variability of species niche (Inertia), the group centroid (OMI), the group tolerance (Tol), the residual tolerance (Rtol), the percentages of variability (%) and the OMI significances obtained after 999 Monte-Carlo randomizations (*P*) are shown. Significant values marked in bold.

	Inertia		**OMI**	**Tol**	**Rtol**	***P***
Europe	5.17	index	0.43	0.81	3.92	0.02
		%	8.5	15.6	75.9	
Morocco	4.28	index	0.23	1.03	3.02	0.014
		%	5.3	24.1	70.50	
Tunisia	5.40	index	0.26	1.27	3.87	0.07
		%	4.8	23.5	71.7	
Absence	7.63	index	0.16	1.19	6.28	0.004
		%	2.1	15.6	82.2	

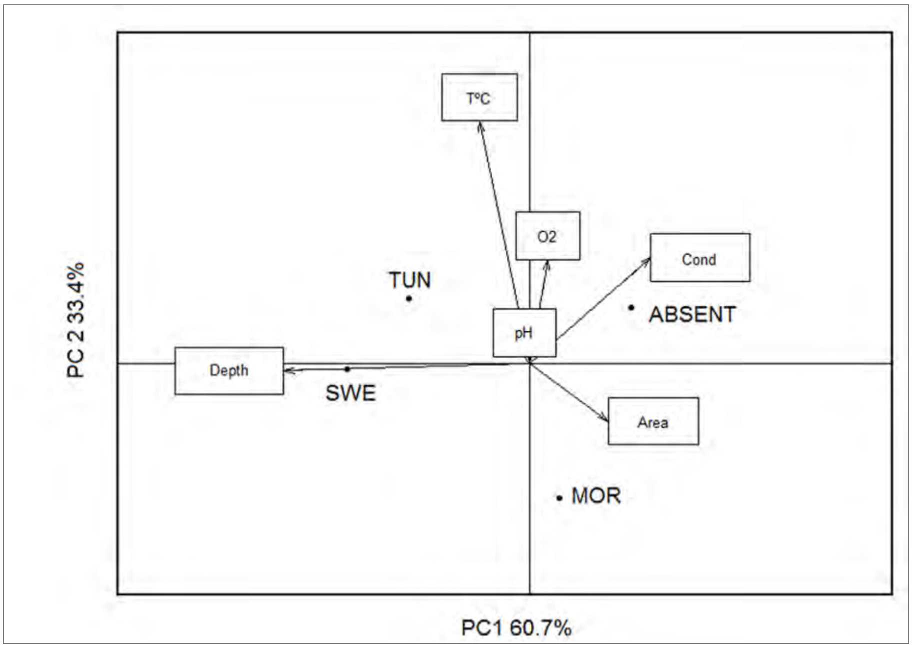


Figure 16. Aquatic habitat selection. Position of the *H. meridionalis* groups and environmental vectors on the first two axes of OMI analysis. Legend: Groups of populations: south-western Europe (SWE); Morocco (MOR); Tunisia (TUN) and ABSENT (water bodies with absence of *H. meridionalis*); T ºC: water temperature; O_2: dissolved oxygen; Cond: conductivity; Depth: water body average depth; Area: surface area of the water body.

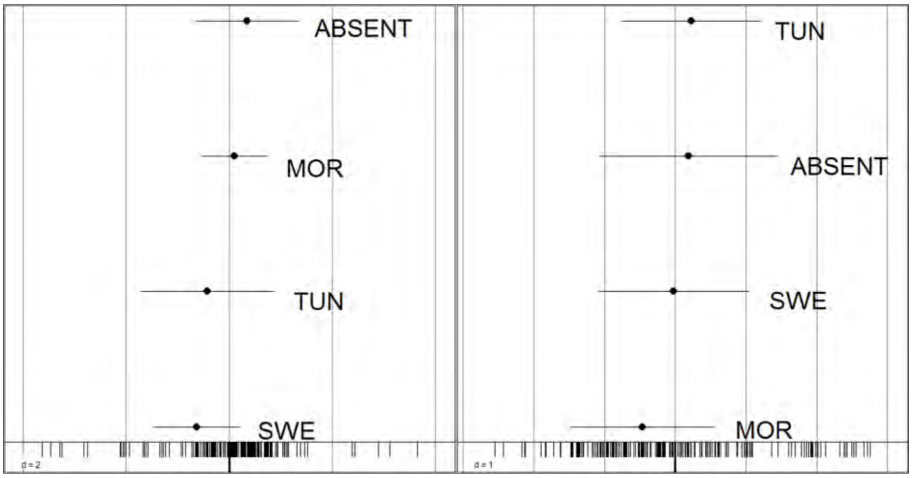


Figure 17. Aquatic habitat selection. Niche position and niche breadth on the first two axes of OMI analysis. Legend: Groups of populations: south-western Europe (SWE), Morocco (MOR), Tunisia (TUN) and ABSENT (water bodies with absence of *H. meridionalis*).

With respect to land cover, the data indicated that all three groups were associated with open terrestrial habitats: mosaic cropland (59% of total habitat area) and rain-fed cropland (32%) in Tunisia; mosaic cropland (38%), rain-fed cropland (23%) and mosaic vegetation (17%) in Morocco; and rain-fed cropland (21%), mosaic cropland (17%), mosaic vegetation (17%) and closed broadleaved deciduous forest (10%) in Europe. In the three study regions, the variables water body size and water parameters made similar contributions to both PCAs (Appendix 2b). Only one model for Tunisia was supported (Table 21). The variable that most explained the occurrence of *H. meridionalis* was water body size (w_i = 0.96), with the contributions of the variables land cover (w_i = 0.26) and water parameters (w_i = 0.26) being less. Two models for Morocco were supported, although the w_i values suggested that the first model was better (Table 21). The variable that most explained the occurrence of *H. meridionalis* was water body size (w_i = 0.79), whereas the contributions of land cover (w_i = 0.28) and water parameters (w_i = 0.26) were less. Two models for Europe were well supported, although the w_i values suggested that the first model was better (Table 21). The variable that most explained the occurrence of *H. meridionalis* was water body size (w_i = 0.99), whereas the contributions of land cover (w_i = 0.27) and water parameters (w_i = 0.34) were less.

IV. 4. 3. 2. Climate space

The PCA results showed an important overlap in the climate space among the three population groups. The eastern lineage was fully contained within the range of the western lineage (Fig. 18). The results of NET indicated that the ENMs for the Tunisian and Moroccan lineages were not ecologically equivalent for the two similarity metrics (similarity *P* values = 1). The BST revealed that the ENMs for the Tunisian group were more similar than expected, given the available climate space for the Moroccan populations, but the Moroccan ENMs were less similar than expected, given the available background of the Tunisian populations (Table 22). NET comparison of the alien and native populations within the western lineage also revealed that both ENMs were not ecologically equivalent (similarity *P* value = 1). The BST indicated that the ENMs for the alien populations were more similar to the Moroccan ENMs than expected by chance, whereas the ENMs for the Moroccan group were more similar to the alien ENMs than expected by chance (Table 22). The ENMs produced an AUC of 0.93 for the native group in the western lineage (Fig. 19A), 0.92 for the western lineage (alien and native localities; Fig. 19B), and 0.96 for the eastern lineage (Fig. 19 C), indicating a high predictive value for all these models. These results also indicated that aridity contributed similarly in all the models, whereas the contribution of temperature was more variable (Table 23). The model only involving the native populations of the western group failed to predict part of the invasive range, while the model involving both native and alien populations overestimated the range of the species, specifically in the west and south-east of the Iberian Peninsula. The projections for both lineages in North African were similar, but the suitable conditions for the western lineage extended farther south in Morocco, near the margins of the Sahara.

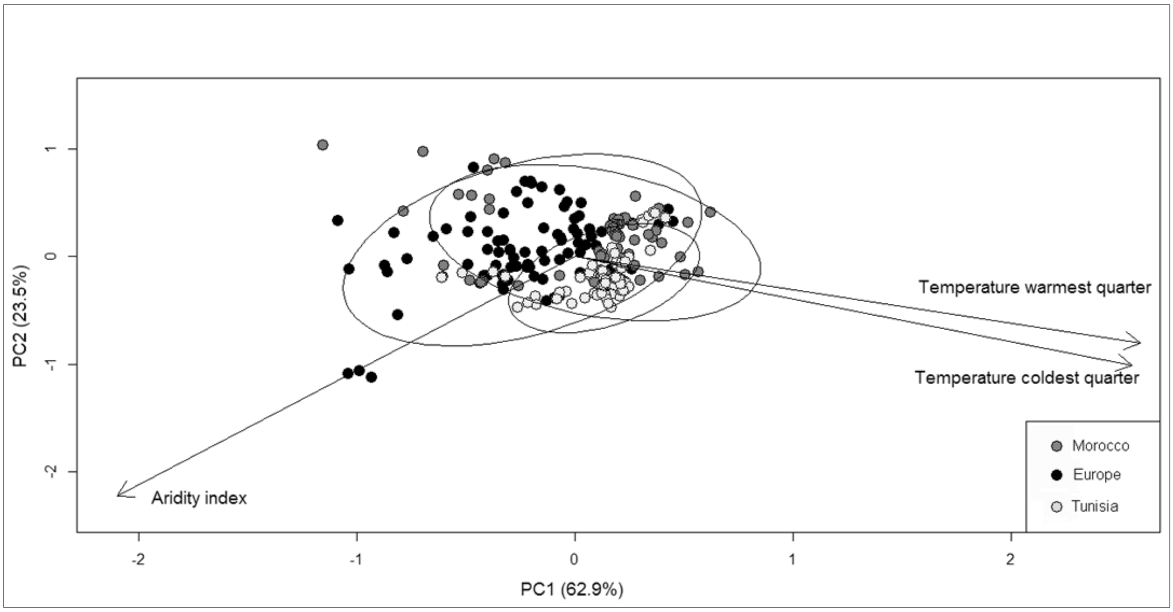


Figure 18. Climate space obtained from three bioclimatic layers (*aridity index*, *mean temperature of the warmest quarter* and *mean temperature of the coldest quarter*). PCA ordination diagram with populations (convex hull and standard error ellipses) and environmental variables (arrows).

Table 21. GLM of *H. meridionalis* ocurrence using best subsets of variables selected by AIC. Size, values of axis 1 of PCA based on water body size variables; water chem, values of axis 1 of PCA based on water physical and chemical parameters; land cover, vegetation categories. Most supported models are those which showed $\Delta_i < 2$. Legend: AIC: Akaike Information Criterion; Δ_i : Delta AIC ; w_i: Akaike weights.

	Model		AIC	Δ_i	w_i
Tunisia	1	size	131.50	0.00	0.54
	2	size + water chem	133.60	2.10	0.19
Morocco	1	size	64.68	0.00	0.47
	2	size + land cover	66.53	1.86	0.19
	3	size + water chem	66.76	2.08	0.17
Europe	1	size	100.55	0.00	0.48
	2	size + water chem	101.88	1.33	0.25
	3	size + land cover	102.60	2.05	0.17

Table 22. Results of background similarity test, based on three climatic variables: *mean temperature of warmest quarter, mean temperature of coldest quarter* and *aridity index*. 95% CI: confidence intervals obtained after 99 permutations.

Observed range	Background range	Similarity statistics	Observed value	95% CI
Tunisia	Morocco	Schoener's D	0.23	0.12–0.13
		I	0.48	0.34–0.35
Morocco	Tunisia	Schoener's D	0.23	0.45–0.47
		I	0.48	0.70–0.72
Europe	Morocco	Schoener's D	0.49	0.35–0.36
		I	0.80	0.60–0.62

Table 23. Relative contributions of the climate variables to the ENMs.

	Variables	Percent contribution	Permutation importance
Model 1	Aridity index	43.3	40.0
(Morocco)	Temperature warmest quarter	15.5	13.7
	Temperature coldest quarter	41.2	46.3
Model 2	Aridity index	38.2	50.2
(Morocco + Europe)	Temperature warmest quarter	34.4	22.7
	Temperature coldest quarter	27.4	27.1
Model 3	Aridity index	36.6	44.8
(Tunisia)	Temperature warmest quarter	56.0	48.6
	Temperature coldest quarter	7.4	6.5

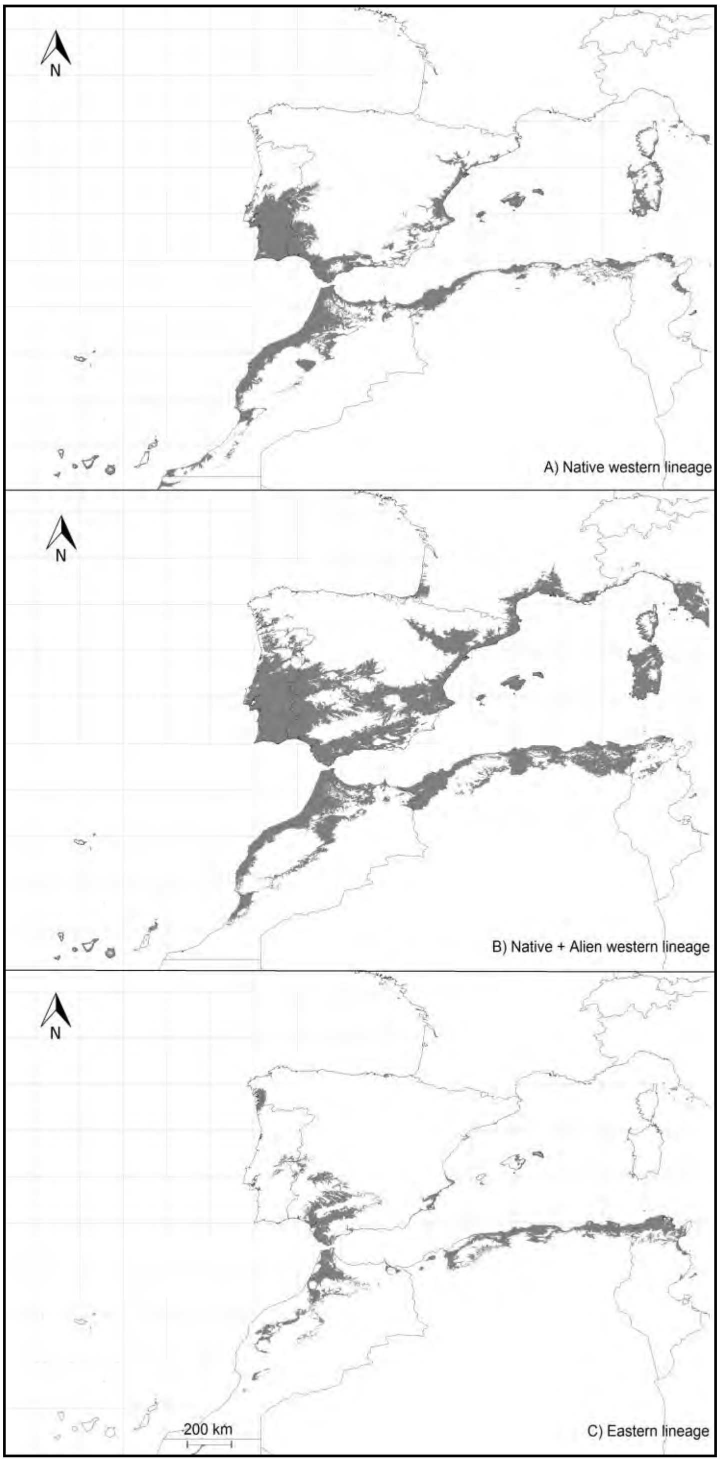


Figure 19. Ecological niche models for lineages in *H. meridionalis* using three bioclimatic layers layers (*aridity index*, me*an temperature of the warmest quarter* and *mean temperature of the coldest quarter*). The gray layer showed the 10th percentile of the predicted training values. Panel A: Western lineage (only Native populations); panel B: Western lineage (Alien and Native populations); and panel C: Eastern lineage.

IV. 4. 4. Discussion

IV. 4. 4. 1. Habitat selection and species invasiveness

Our results show that the three groups of populations of *H. meridionalis* occupied similar aquatic habitats, and that their local presence was mainly determined by water body size (or the hydroperiod, as both variables are related; Brooks and Hayashi 2002). This is probably related to the need of this species for a minimum aquatic period of 2–4 months, to enable larval development to be completed (Díaz-Paniagua 1986). Although this species can breed in a large range of water bodies (Díaz-Paniagua et al. 2005), our results indicate that it tends to occupy deeper water bodies than other anuran species. This suggests that *H. meridionalis* uses a suboptimally occupied niche, corresponding to medium-sized nektonic larvae (Díaz-Paniagua 1985). Several factors could regulate this segregation, including the greater diversity of predators in this type of water bodies, which greatly reduces the presence of other amphibian species (Babbitt et al. 2003). In this sense, species of the genus *Hyla* display some strategies to reduce predation (e.g., active swimming behaviour; Chovanec 1992). Moreover, the analysis of land cover data indicated that the terrestrial habitat did not appear to contribute to the occurrence of the species, which contrasts with observations for other anuran species that are sensitive to changes in vegetation structure related to anthropic disturbance (Guerry and Hunter 2002; Porej et al. 2004). This may also have favoured the invasiveness of *H. meridionalis*, which can successfully colonize agricultural and urban landscapes in south-western Europe (Beja and Alcazar 2003; Thirion and Geniez 2013).

IV. 4. 4. 2. Divergence in the realized niches

The result of the analysis of climate data indicate that the allo-parapatric groups of populations occupied different realized niches. The differences in climate space were mainly caused by the occurrence of the populations in separate geographic areas. This interpretation is supported by the results of the BST, which revealed a greater similarity between population groups than was expected by chance. The exception was for the lineages in North Africa, where the native group of the western lineage occupied a wider niche than the eastern group. The two lineages present in North Africa occur in humid, sub-humid, and semiarid regions (Ben Hassine and Nouira 2012; Bons and Geniez 1996). However, *H. meridionalis* extended its niche to arid region in southern Morocco, but not in Tunisia, although regions with similar climate conditions are present in Tunisia. This difference can be explained by a greater tolerance of the western native populations to aridity, suggesting a niche shift between these lineages. Alternatively, the penetration into the arid regions of south-western Morocco may be favoured by the local orography. *Hyla meridionalis* occurs up to the alpine areas in the Atlas Mountains (Bons and Geniez 1996), which extend in a north-south direction. The relatively humid conditions in these mountains have favoured the southern dispersal of several mesic species (Courtney and Chew 1987; Alaoui-Haroni et al. 2009) that have followed river systems originating in the Atlas and anti-Atlas mountains (oued Masa, oued Souss, and oued Draa), where most of the records of *H. meridionalis* in the arid region of Morocco are concentrated. This explanation is supported by the ENM results, which indicate that aridity contributed similarly in the eastern / native-western models.

The European populations occur mainly in Mediterranean-type climates, and are favoured by similar conditions to those occurring in the native range of the species. The boundaries of this invasion are probably determined by two factors: competition, mediated by the occurrence of other hylid species (Gasc et al. 1997); and climatic ecotones. Interspecific competition is a mechanism that defines evolutionarily stable ranges among species that are not segregated in habitat use (Case et al. 2005). *Hyla meridionalis* maintains a nearly parapatric distribution with other hylid species (*H. molleri*, *H. arborea* and *H. intermedia*) throughout most of its European range, although some overlap occurs locally in south-western Portugal, western and central Spain, and south-western France (Patón 1989; Malkmus 2004; Lescure and De Massary 2013). In Europe, *H. meridionalis* is more tolerant to Mediterranean conditions than are native hylids, which are absent under these climate conditions (Malkmus 2004; Morand 2013). The ENMs indicate that the suitable niche in the west of the Iberian Peninsula is larger than the actual range of the species. This can be explained by the presence of *H. molleri* in this region, which may prevent colonization by *H. meridionalis.* This is supported by the fact that these species are ecologically equivalent, having similar larval and adult habitats (García-París 1985; Malkmus 2004).This convergence in the use of habitats produces occasional hybridization events between *H. molleri* and *H. meridionalis* (Oliveira et al. 1991). The ENMs also indicate that suitable conditions for *H. meridionalis* occur in the east of the Iberian Peninsula, where only small isolated populations were present (Egea-Serrano et al. 2005). In this region there are no native hylid species; the semiarid climatic conditions may have hindered the penetration of *H. meridionalis*.

The ENMs indicate that much of the invasive range of *H. meridionalis* cannot be predicted solely from the native locations of the species, as reported by Sillero (2010). This is a common finding in the analysis of the niches of alien species (Mack 1996; Urban et al. 2007), as their native ranges do not always describe their fundamental niches (cryptic niche conservatism hypothesis; Schulte et al. 2012). This is because the range limits of the native populations can also be determined by interactions with other species, or the presence of biogeographic barriers.

In its northern limits, the progressive temperature decrease restricted the expansion of *H. meridionalis* to regions with a maritime influence (Thirion and Geniez 2013). This expansion following the Atlantic plains is possibly related to the thermal buffering effect of the ocean, which acts to reduce the incidence of extreme climatic events (e.g., very cold winters), which are related to rapid population turnover and the extinction of marginal populations (Mehlman 1997). The range limits of *H. meridionalis* may also be determined by other factors, including a loss of genetic and phenotypic diversity in populations at the periphery. These factors may have acted to gradually shrink the niche of *H. meridionalis*, limiting the size and connectivity of its populations and making them more sensitive to local extinction processes (including perturbation of aquatic habitats), and ultimately preventing further expansion of the species in Europe.

IV. 4. 4. 3. Conclusions

The analysis presented herein suggests the presence of evolutionary stability in the two niche dimensions examined for *H. meridionalis*. This stability could be related to the existence of a homogeneous niche in the region that has not promoted ecological divergence between the two lineages. Our study indicates that populations of *H. meridionalis* in Europe are restricted mainly to sub-Mediterranean and Mediterranean climates, and that the replacement of native hylid species is unlikely. However the ability shown by this species to colonize disturbed habitats, in conjunction with the global rise in temperature, could favour the expansion of *H. meridionalis* towards the range of native hylids, promoting negative interactions (i.e., hybridization and competition) between these species. For this reason we consider it important to monitor the contact zones between *H. meridionalis* and other hylids, with particular emphasis on aquatic habitats, in order to assess whether there appears to be a decrease in the reproductive success of native hylid species.

V. GENERAL DISCUSSION

V. 1. Factors regulating invasion success

Our findings revealed some of the factors that have favoured the anuran invasion of south-western Europe by two species of north African frogs: a similar environmental background and the species' highly opportunistic nature. These results have important implications when assessing which introduced species can become invasive, by examining the availability of suitable niches in the recipient region (at regional and local scale) and the breadth of habitat selection of the alien species in its native range.

V. 1. 1. Niche conservatism hypothesis

Previous studies noted the role of niche conservatism (i.e., the tendency of allopatric populations to occupy a similar climate space despite variation in the local conditions; Peterson and Vieglais 2001) in delimiting species distributions, especially in ectothermal vertebrates (Wiens and Graham 2005; Escoriza and Ben Hassine in press). In the case of amphibians, the invasive species were almost entirely confined to regions that showed conditions comparable to those found in their native ranges (Duarte et al. 2012). Nonetheless, there are several examples of amphibians that showed a remarkable invasion success and have been established in areas far outside their geographical origin (Brown et al. 2006; Christy et al. 2007; Ficetola et al. 2007; Kuraishi et al. 2009). This ability to colonise regions under exotic regimes suggests that species can quickly adapt to new environmental conditions under some circumstances (Broenniman et al. 2007; Gallagher et al. 2010). This fact apparently contradicts the hypothesis that the fundamental niche is stable at an evolutionary time scale (Peterson et al. 1999; Martínez-Meyer et al. 2004; Hawkins et al. 2007).

To determine the appearance of niche shifts, we must first define the species' fundamental niche. This aspect of the niche sometimes cannot be described based only on the actual distribution of the species (i.e., building a model based in the observed occurrences). This is because a species' niche is not only contained by its physiology but also by the landscape features or interactions exerted with sympatric species (Loo et al. 2007; Sexton et al. 2009; Schulte et al. 2012). However, these models performed well if they capture most of the species'niche heterogeneity, that is, if the data included in the model come from several combinations of environmental variables (Soberón and Peterson 2005). Once the mathematical model representing the fundamental niche of a species is defined, some authors consider that niche shifts appeared when there were divergence in the positions occupied in the climate space, comparing populations which are usually allopatric

(Graham et al. 2004; Beaumont et al. 2009; Lozier and Mills 2009). However, this result can only describe spatial variation on the climate continuum, thus it does not reflect true niche shifts (Soberón and Peterson 2011). Another possible approach is to compare whether the niche is more similar than would be expected comparing both environmental backgrounds (Warren et al. 2008; Nakazato et al. 2010; Warren et al. 2010). This idea postulated that the observed niche dissimilarities can be justified by spatial climate anisotropy, but if two species share the same fundamental niche, they would probably select an environment as similar as possible within the available climate conditions. This also incorporates the concept that variations in the climate niche are not necessarily linked to changes on species' natural history, since there are environmental gradients which are more ecologically relevant than others (Rödder and Lötters 2009).

In any case, the application of both approaches in our study provides a straightforward conclusion: the climate space occupied by invasive and native populations of *D. pictus* and *H. meridionalis* were not identical, although they were more similar than was expected if the alien species occupied this space randomly. These findings support the initial hypothesis that both populations (native and alien) share the same fundamental niche i.e., that the invasive ranges in amphibians are mainly explained by niche conservatism (Wiens and Graham 2005; Pyron et al. 2008).

V. 1. 2. Invasive species and habitat selection: opportunistic generalists

Another key aspect to understand the invasion process is the capacity of aliens to exploit efficiently most of the available habitats (Marchetti et al. 2004). In the case of amphibians, aquatic habitats are ecological bottlenecks because most species require of these habitats to complete their complex biphasic life cycles (Semlistch 2002). Some features of the habitat such as the hydrological stability of the water body and the composition of the vegetation cover of the surrounding terrestrial habitat, become strong environmental filters regulating the assemblage composition (Pechmann et al. 1989; Wellborn et al. 1997; Knutson et al. 1999). It is expected that invasive amphibians to be generalists in their habitat selection, exploiting underused habitats for native species. In this sense, there are several examples of invasive frogs that favour marginal environments (such as anthropic ecosystems) being more successful in this type of environments than native species, which they displace (Diesmos et al. 2008; Fuller et al. 2011). This is because invasive species show great adaptive qualities such as the ability to tolerate the presence of predators, high dispersal ability or broad physiological tolerance (Moyle 1973; Llewelyn et al. 2010; Escoriza and Boix 2014). In the present study, our results indicated that: (i) both species were wide-range generalists in their habitat use, showing great tolerance to variations in the size and water chemistry of the ponds; (ii) the occurrence of *H. meridionalis* was independent of the vegetation structure, and this species can occur in highly disturbed ecosystems where some native anurans are absent. These findings are consistent with those described in other invasive species, which also show very broad niches (Latini and Petrere 2004; Geng et al. 2007; Schweiger et al. 2010) and positively select highly disturbed habitats (Sax and Brown 2000).

V. 1. 3. The myth of saturated niches

The wide range in the use of aquatic habitats causes *D. pictus* and *H. meridionalis* to coexist with most of the sympatric species and to establish some degree of interaction. It is expected that the intensity of these interactions determines the assemblage resistance to the invasion, following the Eltonian concept of assemblage invasibility (Naeem et al. 2000; Dukes 2002). Our results suggested that in the case of *H. meridionalis,* the assemblage resistance is likely to be low because it is occupying an empty or underused functional niche (in the regions where there is not native hylids). However, *D. pictus* became established by sharing a similar functional niche (small-sized benthic larva) with two native species. This fact suggests that this niche was not naturally saturated i.e., it supports the integration of new species without causing the removal of native species. The increase in species diversity at large spatial scales has been described to occur several times after invasions (Sax et al. 2003, 2007; Stohlgren et al. 2008) because interactions at the same trophic level rarely cause the complete extirpation of native species, especially if there is high environmental heterogeneity (Davis 2003; Gurevitch and Padilla 2004; Fridley et al. 2007). This apparent lack of saturation can be favored by the fact that *D. pictus* is not phylogenetically related to any of the local species. In the case of *H. meridionalis*, the presence of closely related species possibly act to limit its distribution in the Iberian Peninsula. In other groups of biota, resistance to invasion is determined by phylogenetic identity, which also usually implies some level of functional overlap (Darwin's naturalization hypothesis; Darwin 1859; Procheş et al. 2008; Davies et al. 2011). This can be especially important in amphibians, which experience biphasic cycles where the overlap is usually limited to a specific period of development. However the species belonging to the same lineages can overlap in both life phases (larvae and postmetamorphical stages), since these species share a similar natural history.

The establishment of alien species is favoured by the ability to exploit most of the available resources, which may be promoted by their greater adaptive capacity (Sexton et al. 2002; Daehler 2003; Davidson et al. 2011), although not necessarily (Peperkorn et al. 2005; Palacio-López and

Gianoli 2011). Our findings were more close to those described by the latter authors and revealed that *D. pictus* does not display a broader ecological range than most of native species, also generalist species (García-París et al. 2004; Díaz-Panigua et al. 2005). Moreover, invasive populations use the ecological space very similarly to native conspecifics or congeneric populations. Most of the congeneric species also use a wide range of aquatic habitats. This suggests that the species of the genus *Discoglossus* were highly opportunistic breeders, possibly as an adaptation to the unpredictability of the Mediterranean regimes (Jakob et al. 2003; Fortuna et al. 2006; Gómez-Ródriguez et al. 2009).

V. 2. Analysis of the impact of invasive frogs

The negative impact of aliens usually involves those species that share the same trophic level (Savidge 1987; Salo et al. 2007). Amphibians are a particular case in vertebrates, as they display important ontogenic shifts in the use of trophic resources (from sediment or planktonic microphages to terrestrial carnivores; McDiarmid and Altig 1999). For this reason, invasive amphibians can overlap with native species during some or all stages of their life cycle (Kiesecker et al. 2001; Smith 2005). We applied a novel approach, not previously used in the analysis of the impact of invasive species, with the aim to assess the functional overlap in the larval niche. This approach, based on larval phenotypic resemblance, enables us to identify the most vulnerable species within the recipient assemblage (Appendix 3). In the north-east of the Iberian Peninsula, two species of Anura (*Epidalea calamita* and *Pelodytes punctatus*), share a position similar in the larval morphospace to that of *D. pictus*, suggesting that can interact competitively.

The presence of the larvae of alien species can strongly reduce the survival and fitness of native anuran larvae, mainly by competitive displacement (Smith 2005; Wu et al. 2005; Lillo et al. 2011). However, they can also have a minimal impact due to the absence of any spatial overlap (Williamson 1999; Crossland et al. 2008; Ng and Yeo 2012). Our findings are consistent with the former authors and, similarly to the morphological analysis, revealed the existence of high habitat overlap in the case of *D. pictus* and *E. calamita* and *P. punctatus*. This overlap appears both in the preference for the pond typology (ephemeral ponds; Appendix 4) and reproductive timing (from February to April). Moreover, under controlled conditions, it is demonstrated that negative interactions appear between these species (Richter-Boix et al. 2013). For these reasons is expected that strong interactions will arise, as the assemblages are competitively structured in ephemeral ponds (Wellborn et al. 1996; Skelly 1997). However, the impact of *D. pictus* on these two species is not apparent, possibly because the spatial overlap is greatly reduced in the postmetamorphic phase, as *E. calamita* and *P. punctatus* are more terrestrial species which can tolerate drier conditions (García-París et al. 2004). With respect to other anurans, the interactions are most likely less significant, mainly because these species do not show important spatial overlap and belong to distinct functional groups, implying some degree of trophic segregation.

The previous scenario described the expected interactions when considering the current range of *D. pictus*. However, the result of niche models indicated that *D. pictus* does not likely occupy its entire potential range, which in the Iberian Peninsula comprises most of the Mediterranean region. If its expansion continues, *D. pictus* could contact with *Discoglossus galganoi jeanneae*, an endemic and locally endangered species (Martínez-Solano and García-Paris 2002; Martínez-Solano 2014). The contact between these two species could cause hybridisation and displacement, as described in other invasions comprising congeneric species (Pagano et al. 2003; Largiadèr 2007). This can trigger the disappearance of some eastern populations of *D. galganoi*, which are very vulnerable to extinction (Sancho 2000).

The effect of *D. pictus* on the entire ecosystem is most likely not extremely important. This species preys on a wide range of small invertebrates (Bea et al. 1994), which are the usual prey of other species of frogs and are most likely well adapted to support this predatory pressure. Likewise, the impact on the prey biomass likely has little relevance; although this species can locally reach moderately high densities, the regulatory mechanisms (e.g., interaction with predators such as ardeid birds, natricine snakes and cannibalism; Knoeppfler 1962; Martínez-Solano 2009) that act on other anurans are also acting on populations of *D. pictus*.

In the case of *H. meridionalis*, our results indicated that it is also a generalist species; however, it occurs with some frequency in deep ponds that are used less frequently by other anurans. The existence of congeneric species is limiting the expansion of this species, particularly west of the Iberian Peninsula, where appear suitable conditions for this species. In this region, the synchronic use of the same aquatic habitats causes the appearance of hybridisation with the native hylid *Hyla molleri* (Rosa and Oliveira 1994; Malkmus 2004). The presence of this species in Mediterranean ecosystems of south-west Europe can be dated to historical times (Recuero et al. 2007). This species is well integrated into the local trophic webs, and its populations are regulated by similar factors as the native frogs (Gómez-Mestre and Keller 2003; García-París et al. 2004; Clavero et al. 2005).

VI. CONCLUSIONS

- *Discoglossus pictus* is a thermophilous species that mainly breeds in temporary ponds, belonging to the benthic larval guild. These findings suggested the existence of high niche overlap with the native *Epidalea calamita* and *Pelodytes punctatus.* However, this overlap decreases after metamorphosis, as the latter species are more terrestrial.
- The analysis of larval morphology allows us to recognise two main morpho-functional groups (benthic and nektonic) within the anuran assemblage from the north-eastern Iberian Peninsula. The benthic group (characterised by its elongated morphology, small eyes in a dorsal position, low caudal fin and ventral oral disc) includes *Alytes obstetricans, Discoglossus pictus, Pelodytes punctatus, Epidalea calamita* and *Bufo spinosus*. The nektonic group (characterised by its compressed morphology, large lateral eyes, high caudal fin and terminal oral disc) includes *Hyla meridionalis* and *Pelobates cultripes*. *Pelophylax perezi* displays some intermediate characters (elongated morphology but large lateral eyes) that do not clearly allow its classification into one of these groups (pseudonektonic group).
- The larvae of the species of the genus *Discoglossus* display high morpho-functional resemblance, suggesting that, as in other amphibian lineages, genotypic divergence is not necessarily linked with morphological diversification. This observation can be extended to other genera occurring in the western Mediterranean region: *Alytes, Bufo, Bufotes-Epidalea, Hyla, Pelobates, Pelodytes* and *Pelophylax*. All of them, including *Discoglossus*, form well defined clusters in larval morphospace formed by major western Mediterreanean lineages.
- Niche conservatism determines the invasion range of *D. pictus*, but it is possible that this species has not yet reached the equilibrium in the invasion phase. Suitable niches exist in most of the Mediterranean region of the Iberian Peninsula, suggesting that it could continue expanding its range.
- *Discoglossus pictus* shows an intermediate adaptive capacity compared to sympatric species from the invaded region. Only *Bufo spinosus* has a clearly smaller range of habitat use and lower phenotypic plasticity, but the huge ecological success of Bufonidae species relativises the importance of these factors.
- *Discoglossus pictus* shows significant morphological overlap with some of the native species, suggesting the existence of functional redundancy, as these morphological

characters are related to the habitat use. This suggests that *D. pictus*, unlike many other invasive species, is not established in a fully vacant niche.

- Invasive populations of *D. pictus* show similar ecology to the conspecific and congeneric native populations. Most species of this genus are generalists in habitat selection, tolerating broad variations in the physical and chemical parameters of water. However, invasive populations tend to be associated more frequently with other sympatric species than native species. This indicates a lack of deterministic species associations, which is a characteristic of assemblages that are colonised by alien species.
- The ecological drivers that acted during the colonisation of *H. meridionalis* are similar to those observed for *D. pictus*, i.e., niche conservatism and wide ranges of habitat use. The limits of the distribution of *H. meridionalis* are established by the presence of ecologically analogous species and by the ecotonal transition between Mediterranean and temperate climates.
- Niche conservatism also appears between two allopatric and genetically divergent populations of *H. meridionalis* in northern Africa. This suggests that the niche is stable at evolutionary time scales.
- The analysis of external morphological traits is a useful tool to identify the most vulnerable species within the native assemblages.

VII. LITERATURE

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APPENDICES

Appendix 1. Additional data of the Chapter 2.

Appendix 1a. Descriptive statistics (mean and range values) of physical and chemical parameters measured in the characterization of aquatic habitats, of native species from the invaded assemblage. Legend: *n*: sample size; x: mean; r: range; T: water temperature; Oxygen: dissolved oxygen in water; Cond: water conductivity; AO: *Alytes obstetricans*; EC: *Epidalea calamita*; BS: *Bufo spinosus*; HM: *Hyla meridionalis*; PC: *Pelobates cultripes*; PP: *Pelodytes punctatus*; PZ: *Pelophylax perezi*.

	n		**Surface area (m^{-2})**	**Depth (cm)**	**Slope**	**T (ºC)**	**Oxygen ($mg\cdot L^{-1}$)**	**pH**	**Cond ($\mu S\cdot cm^{-1}$)**
AO	30	x	69	36	11.57	18	5.26	8.41	299
		r	2–705	3–121	0.77–44.91	14–28	0.28–15.60	7.20–9.01	33–1182
EC	11	x	1130	7	6.70	21	7.91	8.14	359
		r	27–10337	2–16	1.50–16.33	17–25	3.01–16.10	7.11–9.70	55–1532
BS	13	x	349	41	8.44	17	6.32	8.22	240
		r	2–4032	6–225	3.00–23.98	13–21	0.91–8.64	7.50–9.10	42–568
HM	14	x	895	49	8.68	21	4.99	8.34	251
		r	3–5765	12–109	0.84–22.20	16–35	0.28–10.30	7.50–9.01	108–419
PC	18	x	2765	38	2.53	19	8.16	7.86	379
		r	24–10337	12–88	0.79–9.81	14–29	2.14–18.51	6.60–9.57	80–1872
PP	22	x	1813	31	5.54	21	10.60	8.05	539
		r	8–15410	4–82	0.81–17.19	11–26	0.68–19.24	6.00–9.57	114–1532
PZ	12	x	913	66	9.68	25	5.57	8.37	262
		r	5–5765	13–225	0.84–13.95	16–35	0.58–14.60	7.44–9.06	108–419

Appendix 1b. Explained variance and factor loadings of the PCA axes included in SEA analysis.

Variables		**PCA1**	**PCA2**
Pond parameters	%Explained variance	25.84	19.95
	Surface area	0.15	0.79
	Depth	-0.34	0.14
	Slope	-0.59	-0.41
	Temperature	0.52	-0.04
	Dissolved oxygen	0.80	-0.19
	pH	0.47	-0.63
	Conductivity	0.44	0.38
Larval morphology	%Explained variance	51.13	31.51
	Area	0.75	0.48
	Span ratio	-0.85	-0.06
	Mean gray value	-0.49	-0.84

Appendix 2. Additional data of the Chapter 4.

Appendix 2a. Descriptive statistics (mean and range values) of water body size, and physical and chemical water parameters obtained from the characterization of aquatic habitats. Aquatic habitats where we detected the presence of *H. meridionalis* were included in "presence", while aquatic habitats where we did not detect the presence of *H. meridionalis* were included in "absence". Legend: n: number of water bodies; T: water temperature; O_2: dissolved oxygen; Cond: conductivity.

	n	**Area (m^2)**	**Depth (cm)**	**T (ºC)**	**O_2 ($mg \cdot L^{-1}$)**	**pH**	**Cond ($\mu S \cdot cm^{-1}$)**
Tunisia							
presence	41	797 (19–9842)	39 (10–121)	19.0 (13.0–26.2)	7.7 (1.8–17.1)	7.9 (6.2–9.6)	789 (44–4650)
absence	60	963 (1–18656)	20 (3–126)	18.9 (10.0–27.8)	8.3 (1.6–20.0)	7.9 (6.6–9.4)	1514 (86–11140)
Morocco							
presence	53	21422 (13–515599)	29 (12–74)	16.9 (9.8–23.0)	6.9 (1.9–13.7)	7.9 (6.7–9.6)	676 (53–4930)
absence	13	1222 (2–7125)	23 (9–43)	15.7 (10.9–24.0)	8.1 (3.2–14.1)	8.2 (6.3–10.2)	653 (26–3500)
Europe							
presence	31	1526 (3–8715)	41 (9–95)	18.9 (12.0–27.7)	7.4 (0.3–18.5)	8.1 (5.8–10.3)	294 (60–1040)
absence	40	372 (2–4032)	33 (5–225)	18.7 (12.8–29.9)	6.2 (0.4–18.2)	8.0 (6.6–9.8)	343 (30–1532)

Appendix 2b. First component of the variables included in the GLM. The table displayed the explained variance (in %) and the factor loadings.

Dimension	**First axis**	**Tunisia**	**Morocco**
Water body size	Explained variance	50.4%	51.3%
	Surface area	0.71	0.72
	Average depth	-0.71	0.72
Water parameters	Explained variance	43.9%	45.3%
	Temperature	0.67	0.49
	O_2	0.82	0.89
	pH	0.68	0.77
	Conductivity	0.42	0.41

Appendix 3. List of pictures of anuran larvae occurring in the studied region.

GENUS *ALYTES*

A. obstetricans (Girona)

1.- *Alytes cisternasii*. Huelva, south-western Spain. Large benthic type.

2.- *Alytes obstetricans almogavarii.* Girona, north-eastern Spain. Medium to large benthic type.

D. pictus (Sicily)

3.- *Discoglossus galganoi galganoi.* Algarve, south-western Portugal. Small benthic type.

4.- *Discoglossus galganoi jeanneae.* Cádiz, south-western Spain. Small benthic type.

5.- *Discoglossus pictus.* Catania, Sicily. Small benthic type.

6.- *Discoglossus scovazzi*. Tetouan, northern Morocco. Small benthic type.

P. varaldii (Morocco)

9.- *Pelobates cultripes*. Girona, north-eastern Spain. Large to very large nektonic type.

10.- *Pelobates varaldii.* Beni Slimane, north-western Morocco. Large to very large nektonic type.

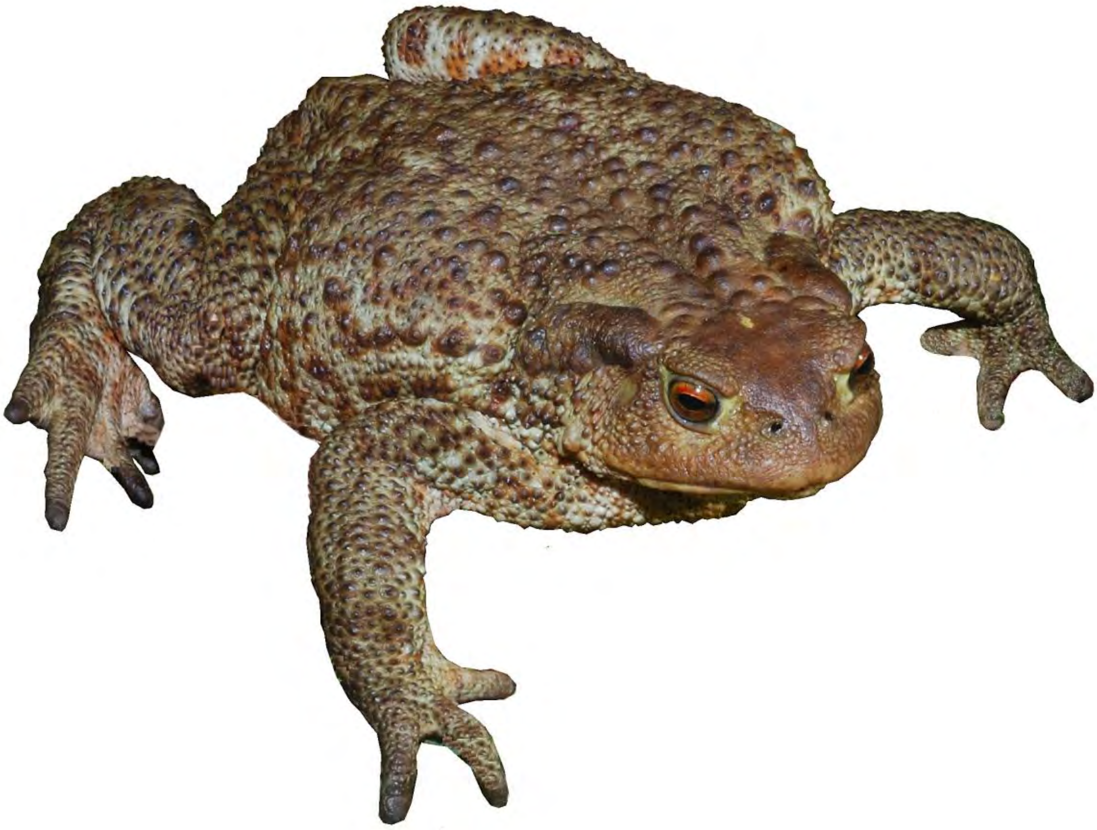
P. punctatus (Girona)

7.- *Pelodytes ibericus*. Cádiz, south-western Spain. Small to medium benthic type.

8.- *Pelodytes punctatus*. Girona, north-eastern Spain. Small to medium benthic type.

A. mauritanicus (Tunisia)

11.- *Amietophrynus mauritanicus*. Casablanca, western Morocco. Small benthic type.



B. bufo (Sicily)

12.- *Bufo bufo*. Palermo, Sicily. Small benthic type.

13.- *Bufo spinosus*. Girona, north-eastern Spain. Small benthic type.

B. boulengeri (Morocco)

14.- *Bufotes boulengeri*. Cap Bon, Tunisia. Small benthic type.

15.- *Bufotes siculus*. Catania, Sicily. Small benthic type.

E. calamita (Málaga)

16.- *Epidalea calamita*. Málaga, south-western Spain. Small benthic type.

GENUS *HYLA*

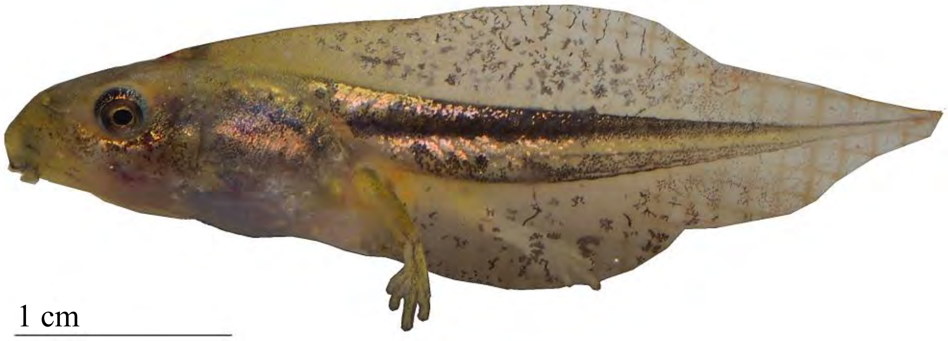
H. intermedia (Sicily)

17.- *Hyla intermedia*. Catania, Sicily. Medium nektonic type.

18.- *Hyla meridionalis*, western lineage. La Provence, south-eastern France. Medium nektonic type.

19.- *Hyla meridionalis*, eastern lineage. Numidie, north-eastern Algeria. Medium nektonic type.

20.- *Hyla molleri*. Madrid, central Spain. Medium nektonic type.



P. bergeri (Sicily)

21.- *Pelophylax bergeri*. Catania, Sicily. Medium nektonic type

22.- *Pelophylax perezi*. Girona, north-eastern Spain. Medium to large nektonic type.

23.- *Pelophylax saharicus*. Kroumiria, north-western Tunisia. Medium to large nektonic type.

Appendix 4. Pictures of studied breeding hábitats.

1.- Temporary pond in southern France. Breeding habitat of *Hyla meridionalis, Discoglossus pictus, Pelodytes punctatus* and *Triturus marmoratus.*

2.- Temporary pond in north-eastern Catalonia. Breeding habitat of *Discoglossus pictus, Epidalea calamita, Pelobates cultripes, Hyla meridionalis, Triturus marmoratus* and *Salamandra salamandra terrestris*.

3.- Permanent pond in north-eastern Catalonia. Breeding habitat of *Alytes obstetricans,Salamandra salamandra terrestris* and *Lissotriton helveticus*.

4.- Temporary pond in southern France. Breeding habitat of *Bufo spinosus, Hyla meridionalis*, *Pelophylax perezi, Lissotriton helveticus* and *Triturus marmoratus.*

5.- Ephemeral pool in Huelva, south-western Spain. Breeding habitat of *Discoglossus galganoi galganoi*.

6.- Cattle-water trough in southern Portugal. Breeding habitat of *Discoglossus galganoi galganoi*.

7.- Temporary pond in Cadiz. Breeding habitat of *Hyla meridionalis, Pelobates cultripes, Pelodytes ibericus* and *Pleurodeles waltl*.

8.- Temporary pond in Huelva. Breeding habitat of *Alytes cisternasii, Pelobates cultripes*, *Pleurodeles waltl* and *Salamandra salamandra morenica*.

9.- Temporary pond in north-western Morocco. Breeding habitat of *Pelobates varaldii, Hyla meridionalis* and *Pleurodeles waltl*.

10.- Temporary pond in north-western Morocco. Breeding habitat of *Hyla meridionalis, Bufo spinosus* and *Salamandra algira splendens*.

11.- Temporary pond in north-western Tunisia. Breeding habitat of *Hyla meridionalis, Discoglossus pictus* and *Pleurodeles nebulosus*.

12.- Temporary pond in northern Sicily. Breeding habitat of *Discoglossus pictus.*