Unravelling Evolutive Histories from the Maghreb:

Two Comprehensive Studies on the Lacertids *Podarcis vaucheri* and *Psammodromus algirus*

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ON THE COVER

Me (centre) and Dr Tahar Slimani (centre left), sampling in the Atlas Mountains during the first field day of my first field trip to Morocco.
Photography by J. Rubert Wilkye, June 2018.

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, _____/_____/_____

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Abstract

Podarcis vaucheri (Boulenger 1905) and *Psammodromus algirus* (Linnaeus 1759) are two lacertid lizards native to the Iberian Peninsula and North Africa. Regarding phylogeographic patterns, although the species have been thoroughly studied within their European ranges, knowledge of their variation South of the Strait of Gibraltar remains limited.

With this thesis we intended to improve the genetic coverage for both species within their Moroccan range, and to address some issues regarding typically considered taxonomic notions for them: the existence of two main lineages within *P. vaucheri* — the "main type" and the "Jebel Sirwah" variant — that could warrant its split into two or more separate species; and the appropriateness of the split of *P. algirus* into two subspecies — *P. a. algirus* and *P. a. nollii* — based on their dorsal striped patterns.

To achieve our aims, we mainly employed molecular tools, estimating the phylogenetic relationships between individuals within the area, with comprehensive samplings across most of their known ranges. Mitochondrial and a nuclear gene sequences — respectively partial ND4 and partial MC1R — were used. For the *P. vaucheri* study, this was complemented with species range modelling analysis, in an attempt to understand interactions with the landscape and range contractions and expansions of the species across time.

Significant levels of genetic diversity were found within *P. vaucheri*, coupled with a clear and strong population structuring across the landscape. Data from species distribution modelling indicates that large areas have been almost continually optimal for the species across the region, although with notable breaks between them. Further evidence for the distinction of a "Jebel Sirwah" variant form — a lineage with an independent origin from the Moroccan *P. vaucheri* clade, estimated to have diverged from the species around 7.06 MYA — was also obtained, and a new population from that lineage was reported for Morocco, in the Mount M'Goun area.

For *P. algirus,* no genetic diversity that could warrant the current subspecific split was identified, and although the typically considered clade divisions for the whole species were evident, no clear phylogeographic patterns were found within Morocco. Such a lack of phylogeographic structure is actually unusual in reptiles from this region, and presumably is associated with the generalist nature of *P. algirus*.

We conclude that at least two species should be recognized within the *P. vaucheri* in North Africa, due to the amount of differentiation within the complex, although a full integrative taxonomic revision is still needed. *P. algirus nollii* should be relegated of its subspecies status, and instead be considered a colour morphotype of *P. algirus*.

Keywords

Podarcis vaucheri, *Psammodromus algirus*, Lacertidae, Morocco, Maghreb, phylogeny, phylogeography, speciation, species distribution.

Resumo

Podarcis vaucheri (Boulenger 1905) e *Psammodromus algirus* (Linnaeus 1759) são dois lacertídeos nativos da Península Ibérica e Norte de África. Apesar dos padrões filogeográficos das espécies terem já sido alvo de estudos minunciosos dentro dos limites da sua distribuição Europeia, estudos da sua variação genética a Sul do Estreito de Gibraltar são ainda relativamente escassos e limitados.

Com esta tese, pretendemos melhorar a cobertura da informação genética disponível para ambas as espécies no território Marroquino, e visar algumas questões relativas à taxonomia para elas tipicamente considerada: a existência de duas linhagens principais em *P. vaucheri* — a "tipo principal" e a variant "Jebel Sirwah" — que podem vir a justificar a separação da espécie atualmente considerada em pelo menos duas distintas; e a adequação da vigente separação de *P. algirus* em duas subspecies — *P. a. algirus* e *P. a. nollii* — atualmente baseada apenas na variação dos seus padrões riscados dorsais.

Para atingir estes objetivos, foram aplicados principalmente ferramentas de análises molecular, estimando-se relações filogenéticas entre os indivíduos da região, através de amostragens compreendendo a quase totalidade da sua distribuição conhecida. Sequências genéticas parcias de origem mitochondrial e nuclear — respetivamente dos genes ND4 e MC1R — foram analisadas. Para o estudo de *P. vaucheri*, métodos de modelação da distribuição de espécies foram usados complementarmente às análises anteriormente referidas, numa tentativa de melhor compreender as interações entre a paisagem e as expansões e contrações na distribuição da espécie ao longo dos tempos.

Níveis significativos de diversidade genética foram encontrados em *P. vaucheri*, juntamente com um padrão claro e demarcado de estruturação populacional ao longo da paisagem. Resultados das análises de modelação da distribuição de espécies indicam que vastas áreas se mantiveram, de forma praticamente continua, ótimas para sustentar a espécie na região, apesar de se observarem quebras notórias entre estas. Evidências adicionais para a distinção da variante "Jebel Sirwah" — uma linhagem com origem independente do clado Marroquino de *P. vaucheri*, cuja divergência estimamos ter acontecido há cerca de 7,06 milhões de anos — foram também recolhidas, e uma nova população dessa linhagem descoberta em Marrocos, na região de montanha de M'Goun.

Para *P. algirus,* não foram identificados níveis de diversidade genética que pudessem justificar a atualmente considerada divisão subespecífica da espécie, e apesar de as divisões cladísticas tipicamente consideradas para espécie no seu todo serem evidentes, não foram encontrados quaisquer padrões filogeográficos em Marrocos. Esta ausência de um padrão filogenético é bastante atípica nas espécies de répteis da região, e poderá estar associada à natureza generalist de *P. algirus.*

Sumariamente, concluímos que pelo menos duas espécies deveriam ser reconhecidas para *P. vaucheri* no Norte de África, devido aos níveis de diferenciação detetados para o complexo específico, apesar de uma revisão taxonomica integrada ser ainda necessária. *P. a. nolli* deveria ser destituído do seu estatuto de subespécie, e considerado como um morfotipo de cor de *P. algirus*

Palavras-Chave

Podarcis vaucheri, *Psammodromus algirus*, Lacertidae, Marrocos, Magrebe, filogenia, filogeografia, especiação, distribuição de espécies.

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List of Abbreviations

12S	Mitochondrially encoded 12S rRNA
95% HPD	95% high posterior density limit
bp	Base pair
ACM4	Cholinergic receptor muscarinic 4
AIC	Akaike information criterion
BI	Bayesian inference
DNA	Deoxyribonucleic acid
GCM	Global climate model
GTR+G+I	Generalised time-reversible model, gamma distributed with invariant sites
JS	“Jebel Sirwah” (referring to the variant form of *P. vaucheri*, not the region)
LGM	Last Glacial Maximum
MC1R	Melanocortin-1 receptor
MCMC	Markov chain Monte Carlo
midH	Middle Holocene
ML	Maximum likelihood
MYA	Million years ago
n/a	not applicable
ND4	NADH dehydrogenase subunit 4
PCR	Polymerase chain reaction
RELN	Reelin
rRNA	Ribosomal ribonucleic acid
tMRCA	Time to most recent common ancestor
TN93+G+I	Tamura-Nei model, 93, gamma distributed with invariant sites

Introduction

Forty-two years ago, in 1977, Carl Woese and George Fox published a rather short paper in the Proceedings of the National Academy of Sciences of the United States. Included was a table of the calculated differences between partial sequences from rRNA genes of thirteen organisms (Woese & Fox, 1977). This modest table would be the very first sequence-based quantitative assessment of phylogenetic relationships between organisms. Though the unfamiliarity of the process and results presented by Woese and Fox at the time caused the paper to, initially, receive little attention and, later, become a target for criticism from the scientific community, it endured (Pace *et al.*, 2012). Since then, however, phylogenetic systematics has become the archetype approach for the study of systematics and the primary methodology for researching evolution.

In more recent years, there has been colossal progress in this field, both theoretical and technical, and a collection of knowledge on the history of living organisms has been amassed. These new insights into evolution eventually led to the awareness that much of the previously considered taxonomic knowledge — that had been acquired mostly through the morphological comparison of living beings — was biased and in need of thorough revision. Scientific names changed; genus, families and orders were reorganized; new species were identified where only one was thought to be, while others were merged into a single species as their morphological differences were revealed to be misleading from an evolutionary standpoint.

The Cartography of Genetics

Moving from phylogeny to phylogeography, one moves from simply identifying and relating the genetic relationships between organisms, to mapping those differences. Results produced from such studies, by interpolating the genetic data from phylogenies with the distribution data of the organisms, enable researchers to answer location-related questions, support conclusions on the spatial behaviour of organisms, and solve spatial problems, thereby providing further insight into the evolution of species.

However, most of the studies that came up with such advances in our understanding of evolution were conducted under the effect a massive geographical bias, with some places of the globe being the target of more research than others. For example, for many of the taxa which are distributed across the Strait of Gibraltar, considerably more is known of their genetic variations in Europe than in Africa (Boakes *et al.*, 2010). Though for taxa that never inhabited both continents or did so in a very distant past — considering evolutionary time —, this information bias is not much of an issue for understanding their evolutive pathway, on the other hand, for those which share much closer ties between the two

continents — like many Iberian and North African taxa, including *Podarcis vaucheri* (Boulenger 1905) and *Psammodromus algirus* (Linnaeus 1759) in which the following studies were focused —, understanding of their European counterparts alone is like solving, at best, only half the puzzle, leaving more than enough space to doubt our interpretation of the final picture.

The Maghreb Ecoregion

Part of the understudied continent of Africa, the Maghreb region is a northern portion of that continent bordering the Mediterranean Sea. In ancient times called Africa Minor, it is typically considered to include the Atlas Mountains and the coastal plains of Morocco, Algeria, Tunisia, and Libya, bordered to the North by the Mediterranean Sea, to the West by the Atlantic Ocean, to the South by the Sahara desert, and to the East by the political border of Libya and Egypt (Jaskula, 2015; Encyclopaedia Britannica, 2019).

From an ecological standpoint, part of the region enjoys a Mediterranean climate and its typical habitats, however, the proximity of the Sahara desert and the high mountain ranges of the Atlas Mountains all contribute for the creation of many unique microclimates and microhabitats throughout the region, providing the conditions for the occurrence of various rare and endemic species (Bons & Geniez, 1996; Le Houérou, 1997), making it an ecoregion of great importance within the larger Mediterranean Basin biodiversity hotspot (Myers *et al.*, 2000).

However, present conditions for the region vastly differ from what they were during various points in the past: as the Maghreb climate changed through time, so did its landscape, forcing upon its inhabiting species many different pressures that shaped its biodiversity to what it is now.

Going back to the beginning of the Cenozoic Era, during the Paleocene and Eocene epochs (66 to 33.9 MYA), Africa was mostly lacking in topographical features and covered in tropical forests, with fossil woods appearing apparently continuously through the Oligocene (33.9 to 23 MYA) records for the Northern part of the continent (Jacobs *et al.*, 2010). Following into the Miocene (23 to 5.3 MYA), North Africa maintained its warm and humid climate and tropical forests; however, as it progressed, grasses began to replace the existing flora, appearing during the middle Miocene and asserting their dominance in the late part of the epoch (Jacobs *et al.*, 2010; Retallack, 2001). As the Pliocene (5.3 to 2.6 MYA) rolled in, global alterations in climate, mainly the glaciations at the poles, brought in a monsoonal climate for North Africa, creating a seasonal alternation of wet and dry climates that continued into the Pleistocene (2.6 MYA to 11,700 years ago) successively

working towards the aridification of the continent, coincidentally with intensifications of the polar glacial cycles (de Menocal, 2004; Jacobs *et al.*, 2010).

Already in the Holocene (11,700 years ago to present), North Africa underwent one of the largest anomalies of the epoch in Earth's biome, as the Sahara desert became "green", with its surface area mostly covered by vegetation and small water bodies (de Noblet-Ducoudré *et al.*, 2000; de Menocal & Tierney, 2012). It would remain in this state until the middle Holocene — around 6,000 years ago —, when the drying out of "green Sahara" started and kept progressing until the region reached its hyperarid desert state of current times (Kröpelin *et al.*, 2008).

In a similar fashion to the climate, the very relief of the region also greatly changed through time until what it is now (Jacobs *et al.*, 2010). The current shapes of the Atlas Mountains and Eastern Moroccan plateaux, around which much of the sampling for this thesis was focused, have only been in existence for a relatively short time (Babault *et al.*, 2008). Although events during as far back as the Triassic (251.9 to 201.3 MYA) have contributed to the later uplifts of the region, the earliest detected orogenic processes are from circa 80 MYA (Teixell *et al.*, 2008), and the thickening of the crust that effectively was associated with the origin of the High and Middle Atlas happened mostly during the Oligocene and extending into the Miocene, it was only during the late Miocene (7.1 to 5.3 MYA) that the mountain belts significantly rose to achieve their current mean heights, as a consequence of a thermal anomaly in the Earth's mantle (Babault *et al.*, 2008).

Equally noteworthy characteristics of the Maghrebian landscape include the Moulouya River valley area, in Eastern Morocco, and the Kabylia region in Eastern Algeria. Both locations have been identified as corresponding to major phylogeographic breaks for regional herpetofauna, delimiting Western and Eastern clades (Beddek *et al.*, 2018). The break at the Moulouya River valley region is suggested to be associated with a 60 km wide marine corridor that opened during the late Miocene and remained until its end (8.0 to 5.6 MYA), with a large marine basin persisting for less than a million years after (Beddek *et al.*, 2018). Unlike with the former location, no events have been identified for the Kabylia region that could explain the observed break there; however, Beddek *et al.* (2018) have observed a correspondence between the break zones and local glacial refugia for plant species, thus suggesting that range contractions and expansions in the area were responsible for the phylogeographic breaks for herpetofauna, which may have shared the glacial refugia with the plant species.

Focal Species

Podarcis vaucheri (Boulenger 1905)

Podarcis Wagler 1830, a wall lizard genus, is a complex of many cryptic species across Europe and North Africa. Until the 1960s, lizards currently belonging to this genus were generally considered part of *Lacerta* Linnaeus 1758, and it was not until the 1970s that *Podarcis* was fully recognized as a genus (Arnold, 1973). Since then many new species have been identified, primarily as a result of analyses of genetic data (Oliverio *et al.*, 2000; Sá-Sousa *et al.*, 2000; Harris & Sá-Sousa, 2002; Pinho *et al.*, 2006; Kaliontzopoulou *et al.*, 2011). Currently, the IUCN Red List of Threatened Species considers 20 species for the *Podarcis* genus (IUCN, 2019), although typically 23 or more are recognized (Table I). This state of flux in the taxonomy is particularly evident in the Iberian Peninsula, where many species have recently been separated from *Podarcis hispanica* "*sensu strictu*"

Table I. Described species for the *Podarcis* genus.

Species according to…		**Author(s)**
…the IUCN Red List	**…published research**	
P. bocagei	*P. bocagei*	(Lopez-Seoane 1885)
P. carbonelli	*P. carbonelli*	Pérez Mellado 1981
P. cretensis	*P. cretensis*	(Wettstein 1952)
P. erhardii	*P. erhardii*	(Bedriaga 1882)
P. filfolensis	*P. filfolensis*	(Bedriaga 1876)
P. gaigeae	*P. gaigeae*	(Werner 1930)
P. hispanica	*P. hispanica*	(Steindachner 1870)
	P. guadarramae	(Boscá 1916)
	P. liolepis	(Boulenger 1905)
	P. virescens	Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet 2014
P. levendis	*P. levendis*	Lymberakis, Poulakakis, Kaliontzopoulou, Valakos & Mylonas 2008
P. lilfordi	*P. lilfordi*	(Günther 1874)
P. melisellensis	*P. melisellensis*	(Braun 1877)
P. milensis	*P. milensis*	(Bedriaga 1882)
P. muralis	*P. muralis*	(Laurenti 1768)
P. peloponnesiaca	*P. peloponnesiaca*	(Bibron & Bory 1833)
P. pityusensis	*P. pityusensis*	(Boscá 1883)
P. raffonei	*P. raffonei*	(Mertens 1952)
P. sicula	*P. sicula*	(Rafinesque-Schmaltz 1810)
P. taurica	*P. taurica*	(Pallas 1814)
P. tiliguerta	*P. tiliguerta*	(Gmelin 1789)
P. vaucheri	*P. vaucheri*	(Boulenger 1905)
P. wagleriana	*P. wagleriana*	Gistel 1868

Steindachner 1870, despite it being still unclear if some are even monophyletic — such as *Podarcis guadarramae* (Boscá 1916) (Pinho *et al.*, 2004, 2007) —, or the status of others — such as the island endemic sometimes considered as a full species, *Podarcis atrata* Boscá 1916 (Castilla *et al.*, 1998).

For the Mahgreb, only a single species, *P. vaucheri* (Figure I), is currently considered, ranging from Morocco to Tunisia. The species was also typically considered as a subspecies of *P. hispanica*, but after early phylogenetic analyses (Oliverio *et al.*, 2000; Harris *et al.*, 2002) it was recognized as a full species (Busack *et al.*, 2005). It is a small lizard sporting either bright and contrasting colours or very pale tones, and a generally white, yellow or orange underside, occasionally presenting black dots around the lower flanks and throat. Males usually have green backs and brown-grey head and neck, and vague or absent yellowish dorsolateral lines, usually broken into irregular spots and bordered by a darker area around the upper flanks. Females can be difficult to distinguish from those of other *Podarcis* species, being mostly brownish and sporting lighter dorsolateral lines than the males, often continuous (Speybroeck *et al.*, 2016).

Podarcis vaucheri was originally described as an African taxa (type locality: Tanger, North Morocco), but is also present in southern Iberia (Harris *et al.*, 2002). Its current distribution includes southern Spain, central and northern Morocco, northern Algeria and northern Tunisia (Figure II), occurring from sea level up to 3,100 meters (Bons & Geniez, 1996). It is also thought to be present in southern Portugal (Malkmus, 1995), and presumably introduced populations have been detected in Greece and the Spanish

Figure I. Adult male *Podarcis vaucheri*. Potography by Matthijs Hollanders.

municipality of Alguaza — outside the considered range in that country —, where it is considered an invasive species (Renoult *et al.*, 2010; Spilani *et al.*, 2018). It is a typically climbing species, often found on rocks, walls, outcrops and even trees, provided there are nooks and crannies where it can hide (Arnold & Burton, 1978), commonly associated to humid and sub-humid regions, being replaced by other lacertids in drier regions. In Morocco, it is restricted to the Mediterranean coast and the region mountain ranges, except for the Anti-Atlas mountain range and the Beni-Snassen Mountain, in the Moulouya River valley region (Bons & Geniez, 1996).

Although *P. vaucheri* is the single species of the genus typically recognised for North Africa, it has been suggested that the Eastern Algerian and Tunisian form might represent a different species (Lima *et al.*, 2009; Kaliontzopoulou *et al.*, 2011; Beddek *et al.*, 2018);

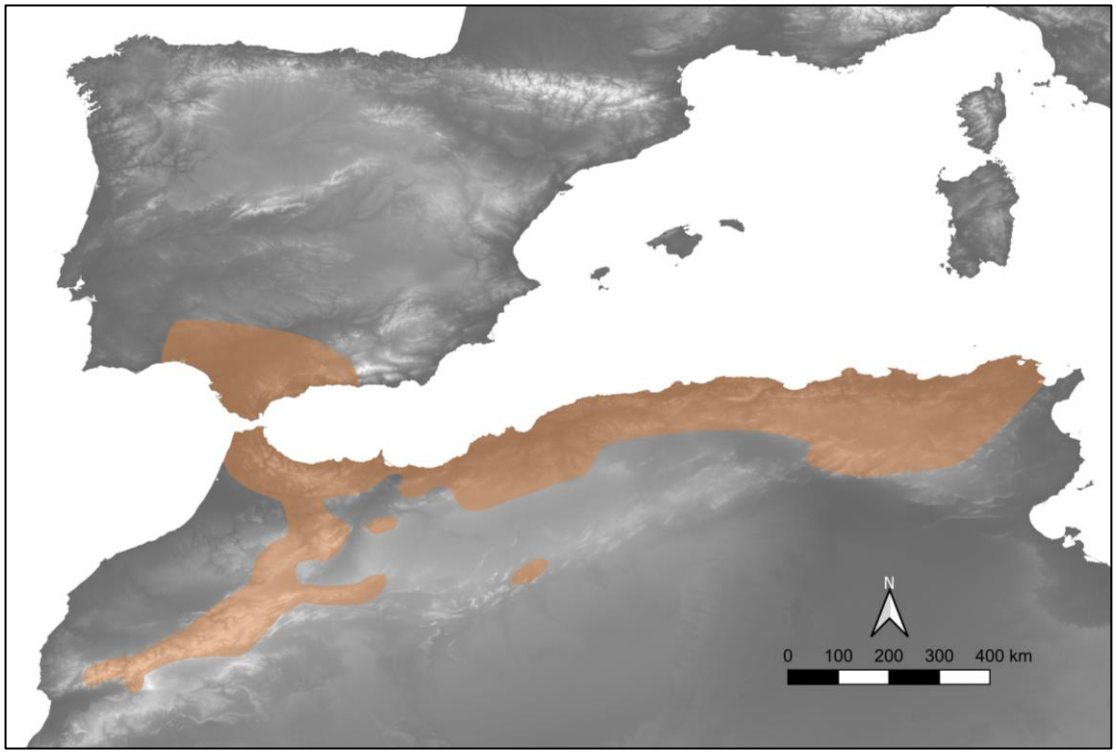


Figure II. *Podarcis vaucheri* species distribution according to IUCN (Mateo *et al.*, 2009a).

indeed, elevation to the species level by (Busack *et al.*, 2005) only included the Moroccan and Spanish populations, leaving the other North African forms as *P. hispanica*, thereby making this species paraphyletic. *Podarcis vaucheri* as a whole — including all North African lineages — forms a sister-taxon to *P. hispanica sensu stricto*, from Southeast Spain (Kaliontzopoulou *et al.*, 2011). Interestingly, as well as the deep separation between the Tunisian lineages and the Moroccan one, the population from Jebel Sirwah, in Southern Morocco, appears rather related to the Eastern lineages instead of the nearby populations (Pinho *et al.*, 2006). Furthermore, this “Jebel Sirwah” (JS) lineage has recently been identified some hundreds of kilometres North of the originally identified population,

in Agoudal, on the Eastern slopes of the Atlas Mountains (Caeiro-Dias *et al.*, 2018). This highlights the ongoing uncertainty regarding the status of populations in the Southeastern part of the range in Morocco.

Psammodromus algirus (Linnaeus 1758)

Psammodromus Fitzinger 1826 is a genus of European and North African sand lizards, with six described species, three of which occur in the Maghreb: *P. algirus*, *P. blanci* (Lataste 1880), and *P. microdactylus* (Boettger 1881), the latter two being endemic to the region and the latter to Morocco only.

Psammodromus algirus (Figure III) was initially described by Linnaeus in 1758. Typically, within *P. algirus* three subspecies are recognized: the nominal *P. algirus algirus, P. algirus doriae* Bedriaga 1886, and *P. algirus nollii* Fischer 1887. Another subspecies was also described, *P. algirus ketamensis* Galan 1931, but this is now generally considered a morphotype of the nominal subspecies (Bons & Geniez, 1996; Pasteur & Bons, 1960).

Psammodromus algirus is a middle-small sized lizard with a short neck and long tail. Adults usually reach up to 7.5 centimetres from snout to vent, with the tail extending to two or three times body length. Colour pattern presents low variation: generally of a brownish shine, darker on the flanks, with a clear lateral pair of white or yellow stripes, or two pairs for *P. algirus nollii* and some individuals from East Spain (Bons & Geniez, 1996), each

Figure III. Adult male and female *Psammodromus algirus*. Photography by Martti Niskanen.

bordered above by a darker one, and a slightly iridescent light underbelly; some individuals may present additional faded dark dorsal stripes or appear almost uniform in colour. Males often sport one or more blue spots over the shoulders and develop an orange colouration around their throat during the breeding season (Arnold & Burton, 1978).

The species ranges across Portugal, Spain, Andorra, Gibraltar, Southern France, Morocco, Northern Algeria, and Northern Tunisia (Figure II). Populations of *P. algirus* are also present in the Italian islet of Conigli, in Lampedusa (Carretero *et al.*, 2009), and the Spanish island of Mallorca (Santos & Carretero, 2015), where they are introduced. The lizards favour low shrubland habitats, but also commonly appear in coastal dunes and pine or oak forest (Arnold & Burton, 1978; Diaz & Carrascal, 1991), generally occurring from sea level to 2600 metres (Bons & Geniez, 1996). Regarding the subspecies distribution across the species range, *P. a. algirus* dominates as the most widespread form, covering almost the totality of its range except for where the other two subspecies are found, *P. a. nollii* inhabits the high plateaux region of Eastern Morocco (Bons & Geniez, 1996), and *P. a. doriae* is restricted to the islets of Galiton and La Fauchelle, off the Tunisian coast (Gasc *et al.* 1997, as cited in Martins, 2007).

The history of how *P. algirus* expanded to its current range has been extensively assessed, although with originally conflicting results. Initially, analyses on mitochondrial DNA and allozymes from Spanish and Moroccan populations lead to the hypothesis that

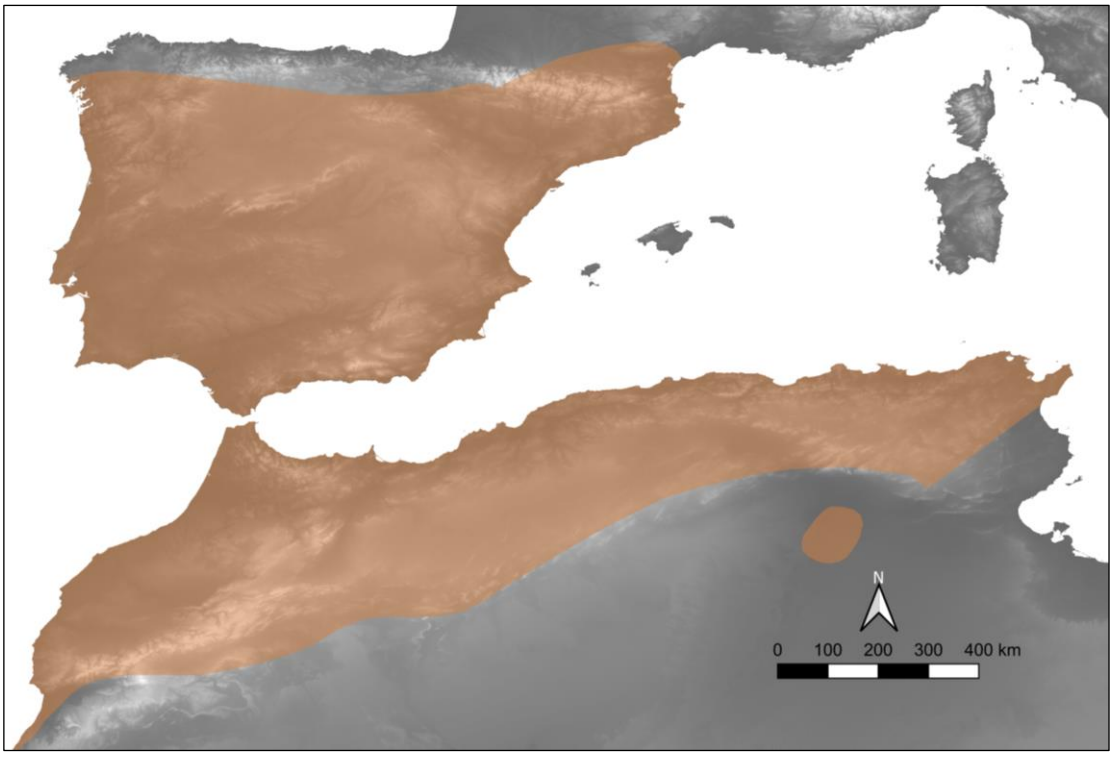


Figure IV. *Psammodromus algirus* species distribution obtained from merging IUCN distributions for *P. algirus* and its junior synonyms *P. jeanneae* and *P. manuelae* (Martínez-Solano, 2009a and 2009b, Mateo *et al.*, 2009b).

the species had emerged in Southern Morocco and from there had expanded North, invading the South of Europe (Busack & Lawson, 2006); still, shortly after, further studies based on mitochondrial gene sequences led to an alternative hypothesis, with the obtained phylogeographic patterns for the species indicating that *P. algirus* had appeared in the Iberian Peninsula and later invaded North Africa (Carranza *et al.*, 2006).

Within the *P. a. algirus* taxa, two main clades are typically considered: the East clade, consisting of the Eastern Iberian populations, and the West clade, consisting of the Western Iberia and African populations (Carranza *et al.*, 2006; Verdú-Ricoy *et al.*, 2010). Within the West clade, three inner clades have also been identified — the African, the Iberian Northwestern, and the Iberian Northeastern —, all with strong statistical support (Verdú-Ricoy *et al.*, 2010; Díaz *et al.*, 2017); while for the East clade, three lineages were observed, more or less geographically distributed across Eastern Spain — a central Spanish one, another covering most of the East, and one restricted to a strip near the South-eastern coast (Díaz *et al.*, 2017).

Concerning the West clade, Busack *et al.* (2006) suggested the lizards from its two Iberian inner clades — Iberian northwestern, and northeastern — should be raised to their own species status, proposing for them the specifics of *P. jeanneae* and *P. manuelae*; however, Carranza *et al.* (2010) later showed that the present level of allozymic and mitochondrial DNA substructuring within the two form was insufficient to justify them as new valid species, and they were demoted into junior synonyms of *P. algirus*.

Aims of the Studies

Morocco has already been demonstrated to be a region where there occur highly diversified but geographically restricted lineages for many reptilian taxa — such as *Podarcis*, *Quedenfeldtia*, or *Saurodactylus* (Rato & Harris, 2008; Kaliontzopoulou *et al.*, 2011; Barata *et al.*, 2012), — alongside with the detection of strong phylogeographic patterns. We intended, with the following studies, to improve the coverage of genetic information for two of its species — the above mentioned *Podarcis vaucheri* and *Psammodromus algirus* — and use this to assess phylogeographic patterns in the country that might help understand their evolutive history. We analysed both mitochondrial and nuclear markers, interpreting our obtained results by comparing them with those published for other Moroccan reptiles.

Specifically for *Podarcis vaucheri*, we intended to determine the relationships both between the main type and the “Jebel Sirwah” variant, and within groups, as although previous studies have already pointed at immense variation within the *P. vaucheri* species in Morocco, possibly enough to warrant specific status (Kaliontzopoulou *et al.*, 2011),

variation within both remains poorly known. If possible, we would also like to provide new insights into the shifts in the species range across time and the potential expansion path of *P. vaucheri* through North Africa and Iberia, with the aid of species range modelling methods.

For *Psammodromus algirus,* unlike in *P. vaucheri*, taxonomic names have been proposed within Morocco, with the two subspecies — *P. a. algirus* (Linnaeus 1759) and *P. a. nollii* (Fischer 1887). However, the "*nollii*" subspecies is based solely on morphological studies and intermediate phenotype of both subspecies have been found (Bons & Geniez, 1996), thus rendering its current status doubtful. To assess the validity — or lack of it — for the "*nolli*" subspecies status, we intended to include samples of individuals from the "*nollii*" range to search for differentiation within the species that could correspond to this taxonomic split.

Material and Methods

Sampling

For both studies, we used samples already stored in CIBIO-InBIO — that had been collected during past expeditions to Morocco — as well as some new samples collected during two expeditions that took place in 2018 and 2019. All lizards were captured under permit from the *Haut Commisariat aux Eaux and Forets of Morocco*.

Both expeditions of 2018 and 2019 were planned to cover many locations throughout the High and Middle Atlas, from the province of Taroudant to that of Agoudal, though neither had as a primary focus the collection of samples for this thesis. Nevertheless, when possible and the location presented itself as favourable, searches were conducted to look for new populations of the taxa of interest, especially for new populations of the *P. vaucheri* Jebel Sirwah variant.

During all sampling efforts, captured individuals were identified to the species level *in situ* and the GPS coordinates of the site registered. Tissue samples were collected — mostly in the form of the lizards' tail tips, though a few were digit samples or whole individuals as vouchers — and stored in tubes filled with 96% ethanol.

DNA Extraction, Marker Choice and Amplification, and Sequencing

All of the laboratory work was conducted at the CIBIO-InBIO facilities of the Campus of Vairão (Vila do Conde, Porto), with the exception of DNA purification and sequencing, which were performed by a commercial company.

Total genomic DNA was extracted from 205 *P. vaucheri* and 33 *P. algirus* muscle tissue samples. The samples were collected during several expeditions by different teams in Morocco, from 2001 to 2019, and were stored in individual tubes in 96% ethanol. All newly extracted samples belonged to Moroccan individuals. The extraction protocol was executed following a standard high-salt method (Sambrook *et al.*, 1989). Briefly, tissue samples are initially submerged in a lysis buffer, proteinase, and ammonium acetate to lyse the cells membrane, and denaturise most of its cellular protein constituents and DNA-bound proteins, which are then precipitated by centrifuging; supernatant is collected and added isopropanol, in which the DNA — due to being less soluble in it that the cellular salts still remaining — will quickly precipitate, allowing collection of a small deposited DNA and salts pellet by removing the supernatant; the pellet is submerged in ethanol to dissolve most of the remaining salts, leaving behind the precipitated DNA after evaporating the supernatant; finally, the pellet is suspended in ultra-pure water and DNA is ready to be used for amplification processes.

Molecular markers were chosen in regard to their expected genetic variability and ease of amplification and sequencing; the choices were made both on personal experience and from consulting previously published studies on reptiles. A region of the NADH dehydrogenase subunit 4 (ND4) mitochondrial gene was selected as the first mitochondrial marker to be tested, and having produced satisfactory amplification and sequencing results, no other mitochondrial markers were further amplified for the full set of samples. The melanocortin-1 receptor (MC1R), cholinergic receptor muscarinic 4 (ACM4) and reelin (RELN) nuclear genes were all tested, but only the first produced satisfactory results; with ACM4 yielding too little genetic variation to be of use, and RELN not amplifying systematically. Although all the markers were tested for the *P. vaucheri* samples, for the *P. algirus* only the ND4 and MC1R were so. In the end, conclusions for both studies were based solely on genetic data from those two markers. In addition, part of the mitochondrially encoded 12S rRNA (12S) mitochondrial gene was also amplified for the *P. vaucheri* samples suspected to be of the JS variant, in order to confirm this identification.

Polymerase chain reactions (PCR) for all markers on both species were performed using published primers and under previously tested conditions (Tables II and III). The economically preferred GoTaq®G2 Flexi DNA Polymerase (Promega) yielded satisfactory results for partial MC1R from *P. vaucheri*, but not for the partial ND4 gene region. 5x HOT FIREPol® Blend Master Mix (Solis BioDyne) yielded satisfactory results when used for the amplification of the partial ND4 region from most *P. vaucheri*. When necessary, Platinum™ *Taq* DNA Polymerase High Fidelity (Invitrogen) was used, giving the best overall results. PCR product quantity was assessed through electrophoresis. 2 µL of PCR product from each sample were ran on a 0.8 % (w/v) agarose gel — previously stained with GelRed (Biotarget) — at 300 V in 0.5x TBE; alongside a standardised ladder and a negative control. The results were visualized in a BioRad Universal Hood II Quantity One 4.4.0. All samples showing PCR product of the intended length and no secondary PCR products were sent to GENEWIZ for standard Sanger sequencing.

Table II. Amplified molecular markers and used primers.

Marker	Primers	Sequence (5'-3')	Source
MC1R	MC1R-F MC1R-R	GGC NGC CAT YGT CAA GAA CCG GAA CC CTC CGR AAG GCR TAG ATG ATG GGG TCC AC	Pinho *et al* (2009)
ND4	ND4 Leu	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC CAT TAC TTT TAC TTG GAA TTT GCA CCA	Arévalo *et al* (1994)
ACM4	TgF TgR	CAA GCC TGC GAG CAA RAA GG ACY TGA CTC CTG GCA ATG CT	Gamble *et al.* (2008)
RELN	RELN 62F RELN 63R	GAG TMA CTG AA TAA ACT GGG AAA C GCCATG TAATYC CAT TAT TTA CAC TG	Pinho *et al.* (2010)
12S	12Sa 12Sb	CTG GGA TTA GAT ACC CCA TAT GAG GGT GAC GGG GCG GTG TGT	Kocher *et al.* (1989)

Table III. PCR conditions per molecular marker and used polymerase.

		Podarcis vaucheri	***Psammodromus algirus***
Marker	**Polymerase**	**PCR conditions (°C(seconds) × number of cycles)**	**PCR conditions (°C(seconds) × number of cycles)**
MC1R	GoTaq	92(180), [92(30), 62↓0.5(30), 72(60)] × 25, [92(30), 50(30), 72(60)] × 15, 72(600)	92(180), [92(30), 62↓0.5(30), 72(60)] × 25, [92(30), 50(30), 72(60)] × 15, 72(600)
ND4	HOT FIRE	95(900), [95(45), 50(45), 72(90)] × 5, [95(45), 47(45), 72(90)] × 30, 72(600)	95(900), [95(45), 50(45), 72(90)] × 5, [95(45), 47(45), 72(90)] × 30, 72(600)
	PlatinTaq	94(240), [94(30), 55(40), 72(30)] × 35, 72(600)	–
ACM4	GoTaq	92(180), [92(30), 62↓(30), 72(60)] × 20, [92(30), 50(30), 72(60)] × 15, 72(600)	–
RELN	GoTaq	92(180), [92(30), 57↓(30), 72(60)] × 15, [92(30), 50(30), 72(60)] × 20, 72(600)	–
12S	GoTaq	94(180), [94(30), 50(30), 72(45)] × 35, 72(600)	–

The economically preferred GoTaq®G2 Flexi DNA Polymerase (Promega) yielded satisfactory results for partial MC1R from *P. vaucheri*, but not for the partial ND4 gene region. 5x HOT FIREPol® Blend Master Mix (Solis BioDyne) yielded satisfactory results when used for the amplification of the partial ND4 region from most *P. vaucheri*. When necessary, Platinum™ *Taq* DNA Polymerase High Fidelity (Invitrogen) was used and gave the best overall results.

PCR product quantity was assessed through electrophoresis. 2 µL of PCR product from each sample were ran on a 0.8 % (w/v) agarose gel — previously stained with GelRed (Biotarget) — at 300 V in 0.5x TBE; alongside a standardised ladder and a negative control. The results were visualized in a BioRad Universal Hood II Quantity One 4.4.0. All samples showing PCR product of the intended length and no secondary PCR products were sent to GENEWIZ for standard Sanger sequencing.

Genetic Data Treatment and Analyses

As mentioned before, only partial ND4 and partial MC1R sequences were analysed to produce the presented results and conclusions made in this thesis. Henceforth, the following information refers only to those two markers.

Sequences were imported to Geneious® v4.8.5 (Biomatters) and aligned using the software's implemented tool — separate alignments were made for each marker —, alongside all published sequences for the intended markers/species retrieved from GenBank. At this point, some sequences were immediately deemed unusable due to very low quality or much shorter length than most others. The remaining new sequences were manually revised for machine and alignment errors, and all sequences in each alignment were trimmed to the same or approximate lengths — for the ND4 phylogenies, a few sequences were up to 30 base pairs (less than 5%) shorter than the alignment's total

length, while for the MC1R the exact same length was maintained for all aligned sequences. Because our sequences were aligned with published ones, the possibility of accidental amplification and inclusion of pseudogenes (Goios *et al.*, 2006) was fairly low, nonetheless a final check on the sequences for the presence of unexpected stop codons and other functional anomalies was performed to ensure the sequence indeed portrayed the functional genes we were interested on. Gblocks (Castresana, 2000) was used on all alignments to determine poorly aligned positions and divergent regions, although none were identified, and thus the total length of the alignments was maintained.

For the mitochondrial data, from partial ND4 sequences, Bayesian inference (BI) and maximum likelihood (ML) approaches were used to estimate phylogenies.

BI phylogenetic analyses were implemented with MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), which uses Markov chain Monte Carlo (MCMC) methods to estimate the posterior distribution of model parameters. Prior to estimating the phylogenies, the most appropriate models of molecular evolution were defined with the Akaike information criterion (AIC), using PartitionFinder2 (Lanfear *et al.*, 2017). Each phylogeny was estimated using random starting trees and ran for 8×10^6 generations, with a sample frequency of 1000 generations. A 25% burn-in was applied to the obtained trees, and remaining data was used to estimate posterior nodal probabilities and produce a summary phylogeny for the analysis. Two separate replicates were performed per dataset and compared to check for local optima (Huelsenbeck & Bollback, 2001); the trees topology was concordant through replicates.

ML analyses were executed with MEGA X v10.0.5 (Kumar *et al.*, 2018), using implemented tools for both defining the most appropriate model of molecular evolution with the AIC and producing the phylogenies. The phylogenetic tree was inferred using a Nearest-Neighbour-Interchange heuristic method and 500 bootstrap test replications were executed to assess its reliability. Once again, two separate replicates were performed per dataset, and the trees topology remained concordant through replicates.

For the nuclear data, from partial MC1R sequences, gene genealogies in the form of haplotype networks were estimated with a statistical parsimony method. Networks were preferred here — as opposed to trees for partial ND4 — because the MC1R alignment displayed much less variation than the ND4 alignment, with many sequences differing in but one nucleotide; this creates a situation in which many equally probable genealogies may be found, a situation that cannot be portrayed in a phylogenetic tree — in which each sample can only be linked to one branch —, but is easily observed in a network (Mardulyn, 2012).

Networks were built with TCS v1.21 (Clement *et al.*, 2000) and visualized in tcsBU (Múrias dos Santos *et al*, 2016). As TCS v1.21 can only work with the main four nucleotide

codes (A, G, C, T) and the alignments included IUPAC nucleotide codes for the presumed heterozygotic positions, prior to the building of the networks, haplotype estimation for the alignments was performed using the built-in Phase tool from DnaSP v5.10 (Librado & Rozas, 2009). This estimated the most probable two haplotype sequences for each individual, which were then used for the building of the haplotype networks.

Molecular Clock Analysis

Molecular clock analysis was performed using BEAST v1.10.4 (Suchard *et al.*, 2018) and its software package tools, to estimate the dates of the major cladogenetic events for the estimated *P. vaucheri* phylogeny from partial ND4 sequences. For calibration purposes, we used the timeframe proposed by Kaliontzopoulou *et al.*, (2011) for the split between *P. hispanica sensu strictu* and *P. vaucheri sensu strictu,* estimated to have happened between 6.56 and 7.61 MYA. We therefore defined a parametric normal distribution calibration point, with the software time to most recent common ancestor (tMRCA) prior, of mean 7.09 and standard deviation 0.27, to include the estimated time frame within 97.5% of the distribution.

All partial ND4 sequences were formatted for use with BEAST through the BEAUti tool. Specifications defined with BEAUti were: TN93+G+I model of molecular evolution; uncorrelated relaxed lognormal clock (estimate); Yule process of speciation with random starting tree; tMRCA normal ($\bar{x}$=7.09; σ=0.27). A Yule process of speciation was chosen as the tree prior since the more basal cladogenic events — such as the *P. vaucheri* "main type" versus the JS variant split, that could actually account to speciation events — were held as of higher interest in this study and this simple one-parameter prior, which is often thought of as a descriptor of net rate of speciation, works well to determine interspecific splits; however it usually performs poorly when evaluating relationships between closely related lineages (Ho & Phillips, 2009). Using a coalescent population tree prior could solve this issue, however, since it considers a constant population size, it would likely end up underperforming for the more basal cladogenetic events (Eriksson *et al.*, 2010) , which we considered as more relevant for this study.

We ran three independent replicates of 10^7 generations each, sampling every 10,000 generations. Since all three replicates yielded high effective sample size (ESS) values — of at least over 300 —, all of them were deemed satisfactory for use in the study and therefore combined, using the LogCombiner tool, to obtain a single sampling of all trees incorporating the molecular clock. Finally, trees were processed with the TreeAnnotator tool with a 10% burn-in applied over the totality of obtained trees, and a final tree for the molecular clock estimates built considering median node heights.

Species Distribution Modelling

For the study on *Podarcis vaucheri*, since one of our objectives included providing new insights into the species colonization of the North African continent, species distribution modelling analyses were performed for the species range and surrounding areas, in order to understand changes in climatic suitability for the species across time. These analyses were executed using Maxent v3.4.1 (Phillips *et al.*, n.d.), which produces models for species niches and distributions by applying a machine-learning algorithm called maximum entropy modelling: from a set of environmental variables grids — for the presented case: climate data — and georeferenced occurrence points for the organisms, the model expresses a probability distribution where each grid cell has a predicted suitability of conditions for the species.

Climate data for the region was retrieved from WorldClim – Global Climate Data version 1.4 (Hijmans *et al.*, 2005), where it was available for the present and for two points in the past — middle Holocene (midH), about 6,000 years ago, and the Last Glacial Maximum (LGM), about 22,000 years ago — under the form of raster layers for 19 bioclimatic variables. For both the midH and LGM, five different global climate models (GCMs) were retrieved — CNRM-CM5, IPSL-CM5A-LR, MIROC-ESM, MPI-ESM-P, and MRI-CGCM3 —, to test different extrapolations of past bioclimatic variables. The selected GCMs were chosen to account for the most possible variation in the simulated conditions for past climate, to avoid biasing our results with the usage of concordant models. Variables were retrieved with a 0’30’’ spatial resolution, representing about 900 meters at the equator. LGM data was not available at that spatial resolution and was downscaled from the original GCM data: the minimum monthly temperature, maximum monthly temperature and monthly precipitation from the five GCMs were downloaded from the Coupled Model Intercomparison Project 5 (Taylor *et al.*, 2012) for the present time and the LGM; units were converted to degrees Celsius and milimetres from the original units of degrees Kelvin and convective precipitation flux ($kg/m^2/s$); absolute and relative anomalies for temperature and precipitation were interpolated with Thin Plate Splines algorithm with function *“fastTPS”* from package *“fields”* in R programming environment (Hijmans *et al.*, 2005; Waltari *et al.*, 2007); reconstructed temperature and precipitation values were obtained using the current Worldclim dataset; the 19 bioclimatic variables for the LGM were generated from the monthly data in R with package *“dismo”.*

To define the study area for modelling, a buffer of 1°36’00’’ — a little less than 200 kilometres at the equator — was applied over the species distribution from IUCN (Mateo *et al.*, 2009), and forced to include the whole northern coast of the Maghreb. Layers for

the bioclimatic variables for both present and past were all clipped to that buffer's area. The resulting clipped layers for the present bioclimatic variables were tested for correlation between them, and from each group of variables presenting a correlation value equal or higher than 0.7 one was semi-arbitrarily selected to use for the study; in the end, 10 bioclimatic variables remained (Table IV).

Table IV. Selected bioclimatic variables for the study and their correlations.

Selected Variables		**Correlated Variables**	
BIO1	Annual Mean Temperature	**BIO8**	Mean Temperature of Wettest Quarter
BIO2	Mean Diurnal Range (mean of monthly max temp - monthly min temp)	*n/a*	
BIO3	Isothermality (BIO2/BIO7 × 100)	*n/a*	
BIO7	Temperature Annual Range (BIO5 - BIO6)	**BIO4**	Temperature Seasonality (standard deviation × 100)
BIO9	Mean Temperature of Driest Quarter	*n/a*	
BIO10	Mean Temperature of Warmest Quarter	**BIO5**	Max Temperature of Warmest Month
BIO11	Mean Temperature of Coldest Quarter	**BIO6**	Min Temperature of Coldest Month
BIO12	Annual Precipitation	**BIO13**	Precipitation of Wettest Month
		BIO16	Precipitation of Wettest Quarter
		BIO17	Precipitation of Coldest Quarter
BIO15	Precipitation Seasonality	*n/a*	
BIO17	Precipitation of Driest Quarter	**BIO14**	Precipitation of Driest Month
		BIO18	Precipitation of Warmest Quarter

Georeferenced occurrence points to use as presences for the analysis consisted of all *P. vaucheri* observations from our database and various others from published papers on the species, from those, samples with longitudes East of 4°50'60''E were excluded; this boundary was selected to exclude the JS variant form from Tunisia, which we suggest to have a distinct origin from *P. vaucheri* "main type", and was based on the geographical split for the *P. vaucheri* lineages proposed by Beddek *et al.* (2018).

10 replicates were run in Maxent. To obtain a projection for each time period in the past, respective obtained layers treated as follows: (1) for each replicate, the respective minimum training threshold was applied to the continual probability layer, producing a discrete suitable/unsuitable layer — respectively above/equal or under the threshold —, (2) the 10 resulting replicates for each GCM were summed, producing 5 discrete layers of suitability evaluated between 0 and 10, (3) a threshold of 9 was applied over these, producing suitable/unsuitable layers — respectively above/equal or under the threshold — , where suitable pixels corresponded to those maintained throughout all replicates, and (4) the 5 resulting layers from each GCM were summed producing one discrete layer of suitability evaluated between 0 and 5. For the present prediction, only steps (1) and (2) were executed on its obtained layers from Maxent. To obtain an "all-time optimum"

estimate, steps (1), (2), and (3) to all obtained layers from Maxent, the resulting layers summed, and step (3) repeated with a threshold of 10, producing a discrete suitable/unsuitable layer where suitable pixels corresponded to those maintained throughout all time periods, GCMs, and replicates.

Manuscript I

Competing Cousins: Phylogeography and Contact Zones of *Podarcis vaucheri* Lineages Accross Morocco

Abstract

The *Podarcis* genus has already been shown to comprise an immense amount of variation within itself, with more and more species being added to its ranks as more speciation events are identified through molecular approaches; nevertheless, for its African range, only the *P. vaucheri* remains considered. At least two highly differentiated lineages have already been accounted for the species in the region: the “main type” and the “Jebel Sirwah” variant form. However, no comprehensive studies for its Moroccan range have been conducted, and a sound phylogeny remains undone. Intending on clarifying the relationships between the *Podarcis* lizards in Morocco, we analysed mitochondrial and nuclear sequences from samples across its whole Moroccan range, estimating a phylogeny and the divergence times for its most relevant cladogenic events, and further complementing our results with species distribution modelling for present and past conditions. We identified great mitochondrial genetic diversity within *P. vaucheri*, alongside clear phylogeographic patterns that were coherent with both recent and ancient past climatic conditions and events. We also obtained additional support for suggesting the elevation of the “Jebel Sirwah” variant into full species status.

Introduction

Evolutionary inferences derived from molecular phylogenetic approaches have profoundly modified the way we understand biological diversity, often identifying l much higher levels of diversity than previously perceived. A clear example of this is the group of Iberian and North African *Podarcis* wall lizards, the *Podarcis hispanica* species complex (Kaliontzopoulou *et al.*, 2011). Prior to the employment of molecular tools, *Podarcis hispanica* (Steindachner 1870) *sensu lato* was considered a single taxonomic unit spreading across the Iberian Peninsula and North Africa. Phylogeographic assessments indicated both high diversity and paraphyly (Busack *et al.*, 2005; Geniez *et al.*, 2014; Harris & Sá-Sousa, 2002; Pinho *et al.*, 2004, 2007), so that multiple lineages are now accepted as distinct species, including *P. guadarramae* (Boscá 1916), *P. liolepis* (Boulenger 1905), and *P. virescens* Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet 2014. Many of these species remain difficult to identify in the field, while some, such as *P. guadarramae,* are

not identified as monophyletic in all assessments (Pinho *et al.*, 2004, 2007), highlighting the complexity of the situation.

The Andalusian wall lizard, *Podarcis vaucheri* (Boulenger 1905), was considered as part of this complex as subspecies under the nomina of *Podarcis hispanica vaucheri* until the turning of the 21st century. Oliverio *et al.* (2000) considered it as a full species on account of the genetic distance between the lizards in North-West Africa and *P. hispanica* from the Iberian Peninsula, and this was formally proposed by Busack *et al.* (2005). The range of *P. vaucheri* was established as southern Spain and the North African countries of Algeria, Morocco, and Tunisia, being the sole species of the genus considered for this continent (Bons & Geniez, 1996). Various studies highlighted the distinction between the Iberian populations and the North African ones, which could be differentiated based on both molecular (Harris *et al.*, 2002; Kaliontzopoulou *et al.*, 2011) and morphological approaches (Busack *et al.*, 2005), which also indicated that a population from Assilah in Northwestern Morocco belonged to the Iberian form.

More recent studies have suggested the existence of at least one other variant — and possible candidate to be elevated to the species level — within the *P. vaucheri* African range. Initially detected in the Jebel Sirwah region, in Morocco, from mitochondrial DNA (Harris *et al.*, 2002), the variant was later confirmed for that region through allozymes studies (Pinho *et al.*, 2004). The variant individuals also seemingly exhibit a distinct coloration from the "main type", especially the males, which sport a dull dark brown dorsal coloration, highly contrasting with the bright green usually seen in the *P. vaucheri* "main type" males (Speybroeck *et al.*, 2016). Further assessment indicated the variant was more closely related to Tunisian individuals (Pinho *et al.*, 2006), and the discovery divergent lineages within this group in Eastern Algeria (Lima *et al.*, 2009) demonstrated the complexity of the situation. Much more recently, the variant was identified in another Moroccan location, in the region of Agoudal (Caeiro-Dias *et al.*, 2018). Nevertheless, *P. vaucheri sensu strictu* remains confirmed as a monophyletic group, with support for two major lineages within the species — the Moroccan variant populations and the Tunisian and East Algerian lineages versus the remaining Western lineages of *P. vaucheri* (Kaliontzopoulou *et al.*, 2011; Beddek *et al.*, 2018). Surprisingly, however, there have been no studies including extensive sampling across *P. vaucheri* in Morocco, so its phylogeographic patterns across this region remain unknown and the true range of the variant lineage — nicknamed the "Jebel Sirwah" (JS) variant — is still unaccounted for.

Although the two lineages appear to be sister-taxa, the oldest lineages of the "main type" are from Southern Spain, and the African clade originated from a rather recent invasion, posterior to the Messinian Salinity Crisis, around 2.69 MYA (Kaliontzopoulou *et al.*, 2011). This implies that the oldest lineage in Africa would be the JS variant, having

split from Iberian *P. vaucheri* around 6.56 MYA, long before its current "main type" African lineages dispersed to the continent. The JS variant presumably was once widespread across the region east of the Atlas Mountains, all the way to Tunisia, but shifts in climate and a decrease in habitat suitability would have isolated its populations. One explanation for the current distribution in Morocco could be that *P. vaucheri "*main type" may outcompete the JS variant west of the Atlas Mountains. If the species are indeed competing, the variant populations may be finding themselves stuck between unsuitable habitat to the East and the encroaching *P. vaucheri* "main type" to the West.

As with many other species, distribution and ecological affinities of *Podarcis* in North Africa are less well known relative to European populations. To better assess this, Kaliontzopoulou *et al.* (2008) performed species distribution modelling for the *P. vaucheri sensu strictu* in North Africa, considering individuals from all lineages. These authors identified the species favoured coastal areas and mountain ranges, further extending to locations were the species had not been previously recorded but which exhibited suitable conditions. Humidity, habitat type, and temperature were the variables identified being critical regarding distribution. After execution of the modelling analysis, some of the predicted locations lacking presence reports for the species were confirmed through fieldwork, demonstrating the validity of the approach.

With the present study, we intended to (1) improve the current coverage of genetic data for the species across Morocco, in order to (1a) build a well-supported estimate of relationships derived from partial NADH dehydrogenase subunit 4 (ND4) mitochondrial gene sequences within this potential species-complex and (1b) determine phylogeographic patterns in the country, (2) assess possible differences between the *P. vaucheri* "main type" and the JS variant using a nuclear marker, partial melanocortin-1 receptor (MC1R) gene sequences, (3) further assess the distribution of known and suspected populations of the JS variant through fieldwork in the region, and (4) develop and project a habitat suitability model to past environmental conditions, to relate the obtained phylogeographic structure with recent shifts in habitat suitability.

Material and Methods

Phylogenetics

A total of 273 sequences for partial ND4 — 156 newly sequenced and 117 retrieved from GenBank — and of 167 sequences for partial MC1R — all new — were used for the analyses. 34 sequences were also obtained for part of the ACM4 gene, but a lack of variation meant that we discontinued assessment of this nuclear gene. Partial ND4

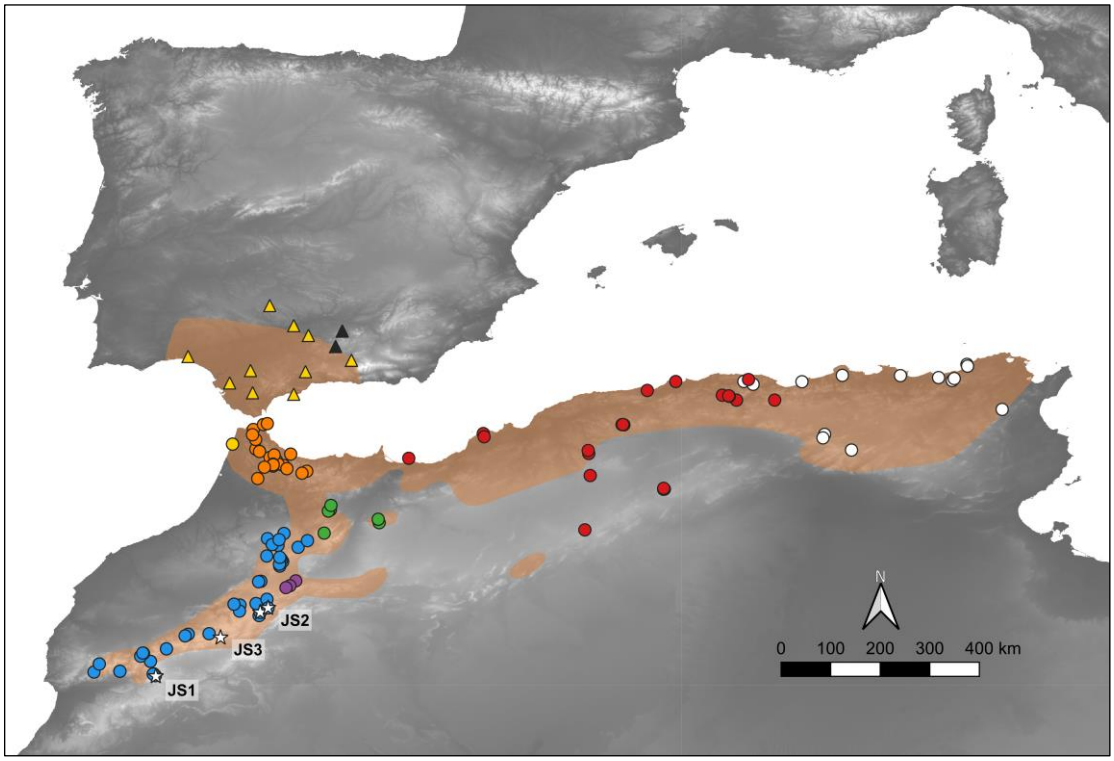


Figure 1. Map of sampling locations for *P. vaucheri*. Light brown shade represents the species distribution according to IUCN. Colours relate to lineages identified from partial ND4 (Figure 2). *P. vaucheri* samples from North Africa are represented as circles and from Iberia as triangles, JS variant type samples from Morocco are represented as stars.

sequences included samples from Morocco, Algeria, Tunisia, and Spain, while partial MC1R sequences were only obtained for Moroccan samples (Figure 1).

Total genomic DNA was extracted from small samples of tissue preserved in 96% ethanol, following a standard high-salt method of extraction (Sambrook *et al.*, 1989). Amplification of partial ND4, partial MC1R, and partial ACM4 followed primers and conditions by Arévalo *et al.* (1994), Pinho *et al.* (2010), and (Gamble *et al.*, 2008), respectively, and reagent concentrations for the use of 5x HOT FIREPol® Blend Master Mix (Solis BioDyne) for the first marker and GoTaq®G2 Flexi DNA Polymerase (Promega) for the other two. PCR products were sent to a commercial company (GENEWIZ) for purification and sequencing.

Sequences from each marker were aligned and manually trimmed — to 660 bp for partial ND4, and to 534 bp for partial MC1R — and checked for inconsistencies using Geneious® v4.8.5 (Biomatters). Gblocks (Castresana, 2000) was used to determine poorly aligned positions and divergent regions but identified none for both alignments.

For the mitochondrial DNA data from partial ND4, Bayesian inference (BI) and maximum likelihood (ML) approaches were used to estimate a phylogeny. For the BI approach, the most appropriate model of molecular evolution was defined with the AIC criteria, using PartitionFinder2 (Lanfear *et al.*, 2017), and the phylogenetic analyses

implemented with MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The selected model was the TN93+G+I. Bayesian analyses were done using random starting trees, ran for 10^6 generations, and sampled every 1000 generations; a 25% burn-in was applied and remaining data was used to estimate posterior nodal probabilities and produce a summary phylogeny. Two separate replicates were performed and compared to check for local optima (Huelsenbeck & Bollback, 2001). For the ML approach, MEGA X v10.0.5 was used for both defining the most appropriate model of molecular evolution with the AIC and producing a phylogeny. The selected model was also the TN93+G+I. A phylogenetic tree was inferred through a Nearest-Neighbour-Interchange heuristic method and 500 bootstrap test replications were executed to assess its reliability.

For the MC1R data, haplotype estimation was performed using the built-in Phase tool from DnaSP v5.10 (Librado & Rozas, 2009). A phylogenetic network using statistical parsimony was built with TCS v1.21 (Clement *et al.*, 2000), and visualised in tcsBU (Múrias Dos Santos *et al.*, 2016).

Molecular Clock

Molecular clock analysis was performed using BEAST v1.10.4 (Suchard *et al.*, 2018) and its software package tools, to estimate the dates of the major cladogenetic events for the obtained *P. vaucheri* phylogeny for partial ND4. For calibration purposes, we used the timeframe proposed by Kaliontzopoulou *et al.*, (2011) for the split between *P. hispanica sensu strictu* and *P. vaucheri sensu strictu*, which falls between 6.56 and 7.61 MYA. We therefore defined the time to most recent common ancestor (tMRCA) as falling within a normal distribution of mean 7.09 and standard deviation 0.27.

All partial ND4 sequences were formatted for use with BEAST through the BEAUti tool. Specifications defined with BEAUti were: TN93+G+I model of molecular evolution; uncorrelated relaxed lognormal clock (estimate); Yule process of speciation with random starting tree; tMRCA normal ($\bar{x}$=7.09; σ=0.27). A Yule process of speciation was chosen as the tree prior since the more basal cladogenic events — such as the *P. vaucheri* "main type" versus the JS variant split, that could correspond to speciation events — were considered of higher interest in this study and this simple one-parameter prior, which is often regarded as a descriptor of net speciation rate, works well to determine interspecific splits, although less so when evaluating relationships between closely related lineages (Ho & Phillips, 2009).

Three replicates of 10^7 generations, sampling every 10,000 generations were ran. As the three replicates yielded high effective sample size (ESS) values — of at least over 300 —, all of them were deemed satisfactory and combined, using the LogCombiner tool, to

obtain a sampling of all trees incorporating the molecular clock. Finally, the trees were processed with the TreeAnnotator tool with a 10% burn-in applied over the totality of obtained trees, and a final tree for the molecular clock estimates built considering median node heights.

Species Distribution Modelling

Species distribution modelling was performed with Maxent v3.4.1 (Phillips *et al.*, n.d.). World-wide layers for 19 bioclimatic variables were retrieved from WorldClim–Global Climate Data version 1.4 (Hijmans *et al.*, 2005) for the present, middle Holocene (midH, about 6,000 years ago), and the Last Glacial Maximum (LGM, about 22,000 years ago); for historical assessments, 5 different global climate models (GCMs) were considered to capture the most variation in the simulations. Layers were used at a 0'30'' spatial resolution (900 meters at the equator); the LGM data was not available at that spatial resolution and was downscaled from the original GCM data. To define the study area, all layers were clipped to a buffer of 1°36'00'' (about 200 kilometres at the equator) and forced to include the whole northern coast of the Maghreb. Resulting present layers were tested for correlation between them, and from each group of variables with a correlation value equal or higher to 0.7, one was selected for use in this study: in the end, 10 bioclimatic variables remained.

As presences, we used all georeferenced *P. vaucheri* observations from our database and various others from published papers on the species, removing those with longitudes East of 4°50'60''E — this boundary was selected to exclude the JS variant form from Tunisia, which we suggest to have a distinct origin from *P. vaucheri* "main type", and was based on the geographical split for the two forms proposed by Beddek *et al.* (2018); however, sample points from the localities were Moroccan JS variant has been identified were mostly kept, disregarding only those confirmed as belonging to JS variant individuals.

Ten replicates were run in Maxent. To obtain a projection for each time period in the past, respective obtained layers were treated as follows: (1) for each replicate, the respective minimum training threshold was applied to the continual probability layer, producing a discrete suitable/unsuitable layer — respectively above/equal or under the threshold —, (2) the 10 resulting replicates for each GCM were summed, producing 5 discrete layers of suitability evaluated between 0 and 10, (3) a threshold of 9 was applied over these, producing suitable/unsuitable layers — respectively above/equal or under the threshold —, where suitable pixels corresponded to those maintained throughout all replicates, and (4) the 5 resulting layers from each GCM were summed producing one discrete layer of suitability evaluated between 0 and 5. For the present prediction, only steps (1) and (2) were executed on the obtained layers from Maxent. To obtain an "all-time

optimum" estimate, steps (1), (2), and (3) were executed for to all obtained layers from Maxent, the resulting layers summed, and step (3) repeated with a threshold of 10, producing a discrete suitable/unsuitable layer where suitable pixels corresponded to those maintained throughout all time periods, GCMs, and replicates.

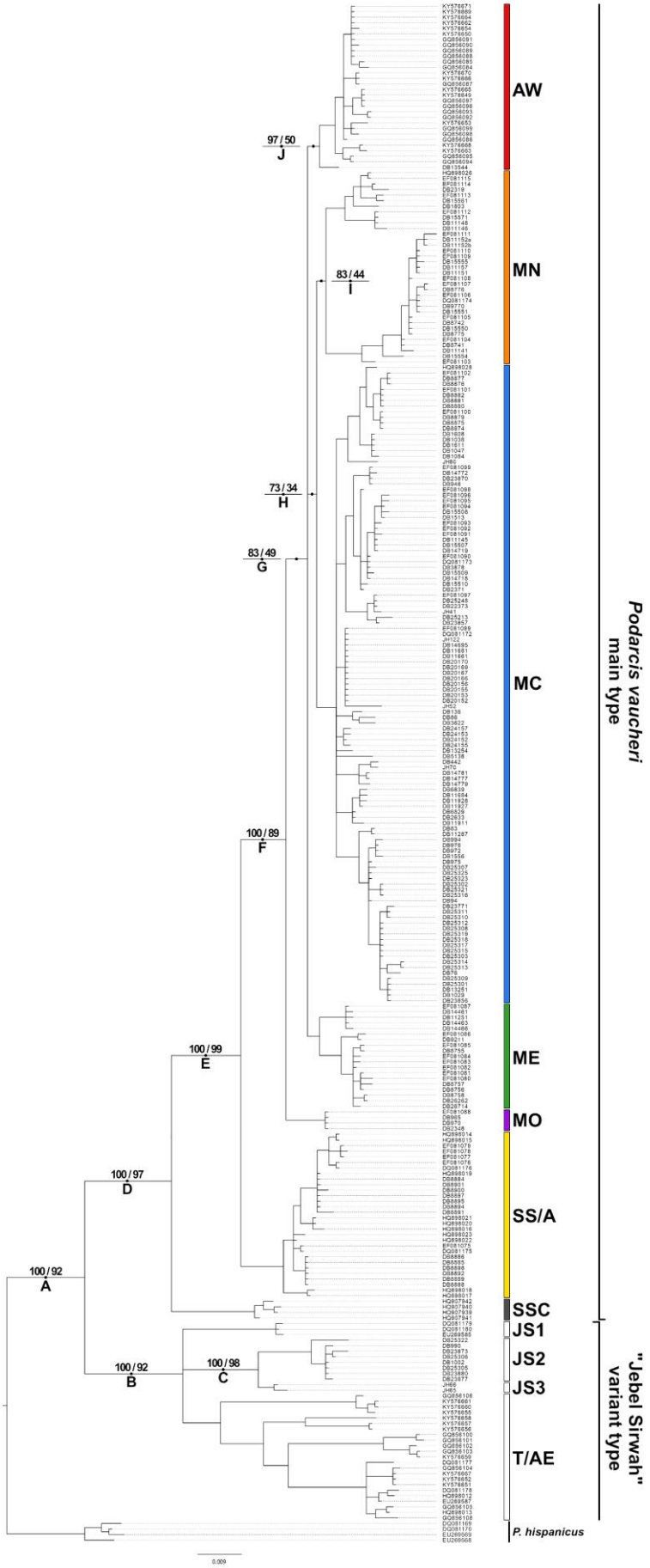
Results

Phylogenetics

The estimate of phylogeny based on partial ND4 sequences (Figure 2) clearly shows the existence of two major clades within *P. vaucheri*: one including the Moroccan — excepting the JS variant populations —, West Algerian, and Spanish populations, and another with East Algerian, Tunisian, and the three identified Moroccan JS variant populations, with the breaking point between them in the Kabylia region (Figure 1), as identified in previous studies (Beddek *et al.*, 2018; Kaliontzopoulou *et al.*, 2011; Lima *et al.*, 2009) these clades respectively correspond to *P. vaucheri* "main type", and the JS variant form.

For the *P. vaucheri* "main type" clade, obtained data indicates relatively high levels of mitochondrial DNA genetic diversity coupled with strong geographical structuring. Samples can be grouped in three smaller clades: Spanish South-central lineage (SSC); Spanish South lineage plus Moroccan from the city of Assilah (SS/A); and remaining Moroccan plus West Algerian. Within the latter, five mitochondrial lineages can be observed, geographically structured across the landscape (Figures 1, 2, and 3): a diverse and widespread lineage covering most of the High and Middle Atlas, from Taroudant to the Ifrane province (MC, blue); a small and restricted one, from western High Atlas, in the Khenifra province, near the city of Midelt (MO, purple); a northern lineage in the Riff area, in the provinces of Tetouan and Chefchaouen (MN, orange); an East Moroccan lineage spread in the provinces of Boulemane, Taza, and Sefrou (ME, green); and a West Algerian lineage with Algerian samples from the Tizi Ouzou province eastwards to the border plus a single sample from the Chafarinas Islands (AW, red).

Within the JS variant clade, two smaller clades can be defined: one with the samples from Tunisia and East Algeria (T/AE), and another with the Moroccan populations. Regarding the latter, an individual lineage was identified for each of the populations: the namesake population from Jebel Sirwah (JS1); from Agoudal (JS2); and from Mount M'Goun (JS3). Despite being approximately geographically equidistant, the populations from Agoudal and M'Goun are genetically more closely related between themselves — pairwise distance of 2.9% — than to the one from Jebel Sirwah — respectively 7.3% and 5.8%. As previously reported, East Algerian and Tunisian populations also exhibit significant amounts of genetic diversity between them.


97 / 50
J
83 / 44
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73 / 34
H
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G
100 / 89
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100 / 97
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A
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B
100 / 98
C
AW
MN
MC
ME
MO
SS/A
SSC
JS1
JS2
JS3
T/AE
P. hispanicus
Podarcis vaucheri
main type
"Jebel Sirwah"
variant type
0.009

Figure 2. ***(from previous page)*** Obtained estimate of a phylogeny for *P. vaucheri,* derived from BI with partial ND4 sequences. BI posterior probabilities and ML Bootstrap values are indicated above branches for the most relevant nodes. The tree was rooted with *P. hispanica*.

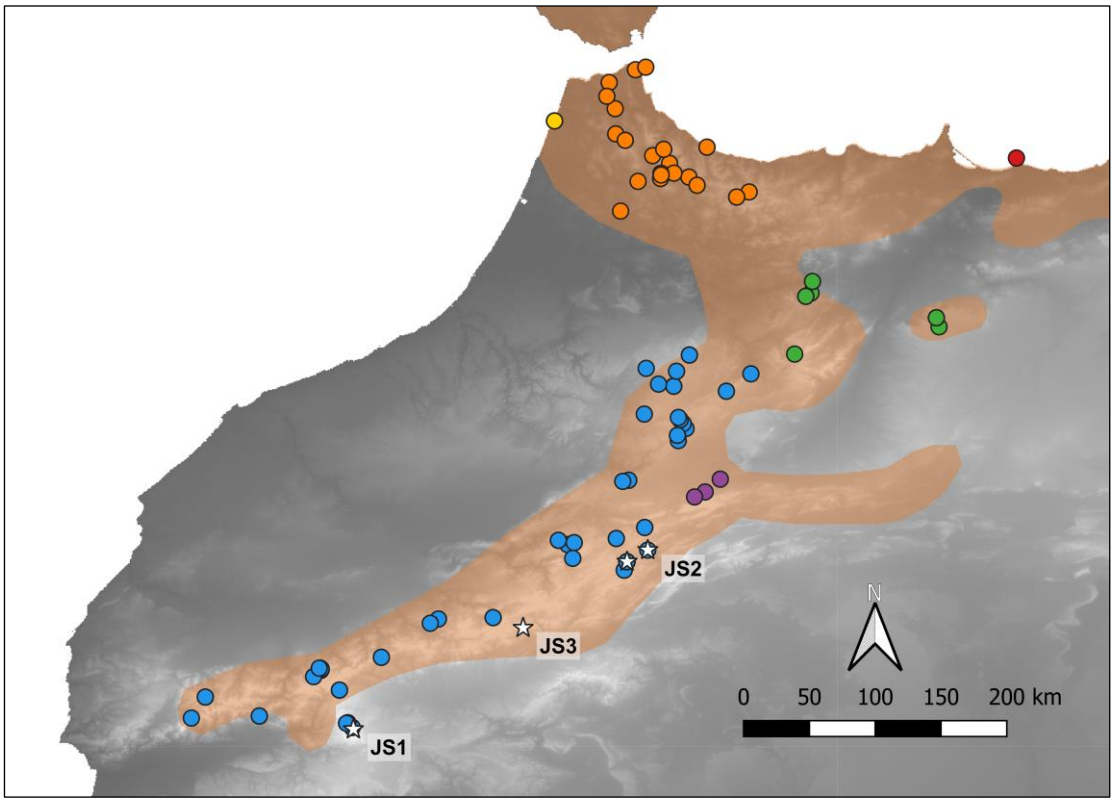

Figure 3. Map of sampling locations for *P. vaucheri,* detail of Morocco. Light brown shade represents the species distribution according to IUCN (Mateo *et al.,* 2009). *P. vaucheri* "main type" samples are represented as circles and JS variant type samples as stars. Colours relate to partial ND4 lineages (Figure 2).

The data obtained from partial MC1R (Figure 4, Figure 5) shows much less diversity than that observed for the partial ND4 mitochondrial gene sequences. The haplotype network is dominated by one haplotype which comprises more than half of the sequences. In total 26 haplotypes were identified, 4 main ones, with 22 smaller others orbiting or linking them — 11 of which are composed of one single sample. The observed haplotypes do not differ notably from each other, with the most divergent ones differing by 7 nucleotide changes. Although some semblance of organization can be seen around the haplotypes for the MN and JS populations — with each clustering in more separate branches — there is not enough differentiation within the network to establish clear haplogroups. Notably, samples from Assilah appear scattered through the network.

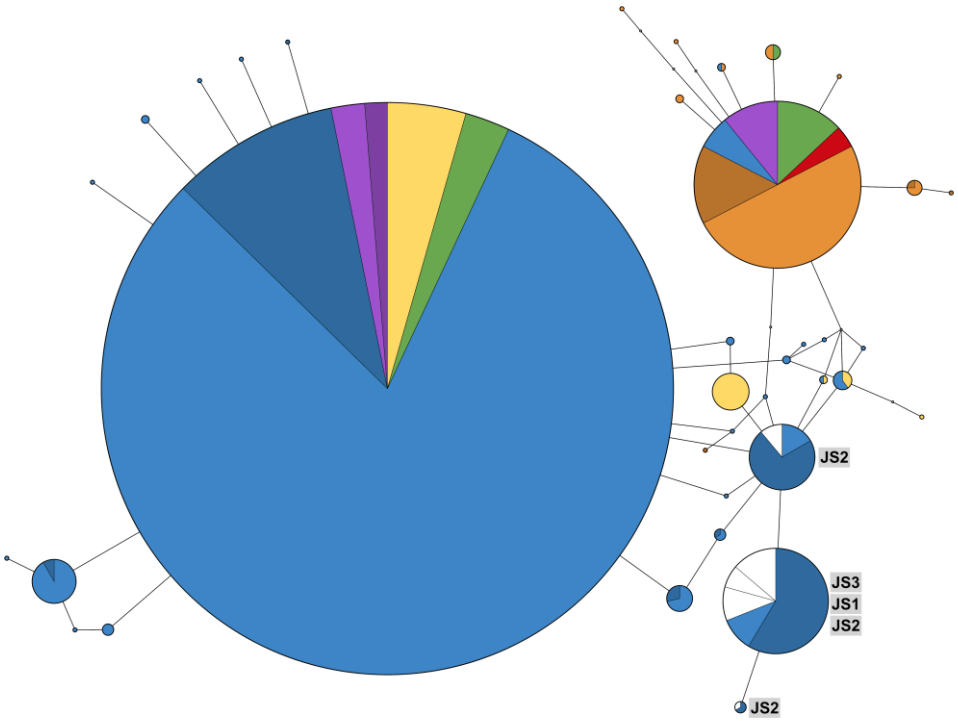

Figure 4. Haplotype network for *P. vaucheri* from partial MC1R. Colours relate to lineages identified from partial ND4 (Figure 2); darker patches represent samples for which partial ND4 couldn't be successfully amplified or sequenced but were assigned to the lineages as per sampling location. Dotted line indicates the two suggested haplogroups. JS variant populations are identified next to the respective haplotypes.

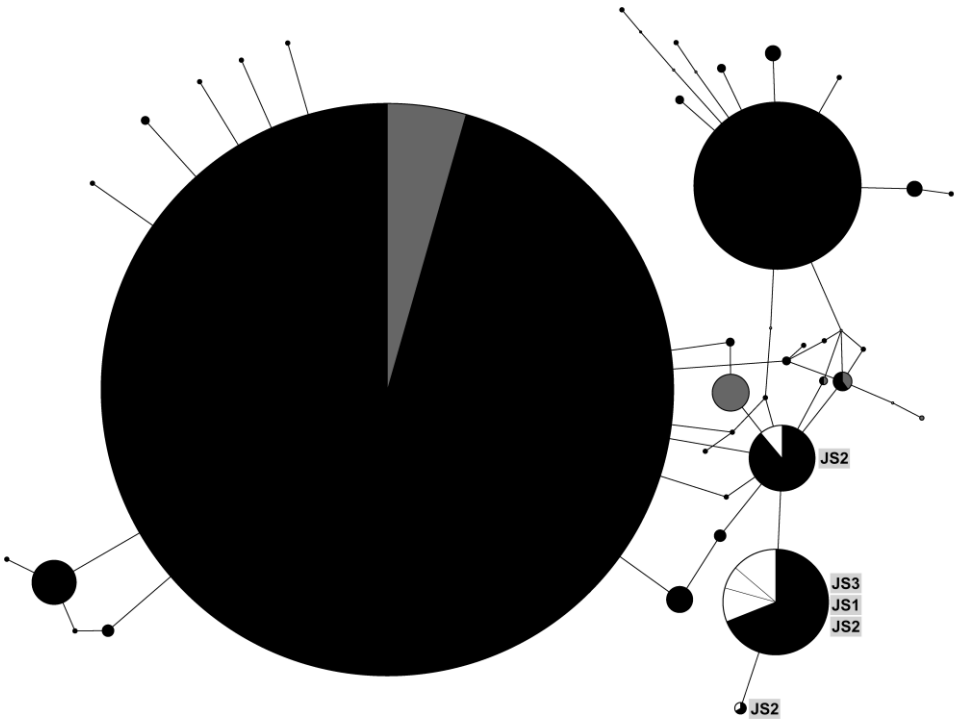

Figure 5. Haplotype network for *P. vaucheri* from partial MC1R, simplified version. Colours relate to *P. vaucheri* "main type" Moroccan clade (black), the population from Assilah (grey), and the JS variant (white). JS variant populations are identified next to the respective haplotypes.

Molecular Clock

Estimates of relationships derived from this analysis presented similar topologies and yielded the same lineages as those obtained from BI and ML, except for within the *P. vaucheri* "main type" Moroccan clade, where some sub-lineages were inconsistent.

Ten different relevant cladogenic events were inferred from the results (Table 1). Considering *P. hispanica* versus all *P. vaucheri sensu strictu* occurred around 6.56 to 7.61 MYA depending on the calibration used (Kaliontzopoulou *et al.*, 2011), the divergent event that followed, between *P. vaucheri* "main type" versus the JS variant type clade (A), would have happened soon after it, around 7.0. MYA. Moroccan *P. vaucheri* "main type" split from the Iberian lineages (E) around 4.34 MYA, when it would have presumably dispersed into North Africa for the first time. Divergence within the JS variant clade appears to be considerable, with the split occurring between the Moroccan versus the Tunisian and East Algerian forms (B) around 4.59 MYA; in line with the splits of the Iberian forms of *P. vaucheri* "main type" versus its North African forms (D, E), which would have been around 5.35 MYA and 4.34 MYA. Further splits within the Moroccan JS variant followed shortly, the first happening around 3.56 MYA.

Table 1. Time to most recent common ancestor (tMRCA) and respective 95% high posterior density limits (95% HPD) for the major splits in *P. vaucheri* phylogeny.

Major Splits	**Clade (Figure 2)**	**tMRCA (MYA) 95% HPD**
Min type *P. vaucheri* — JS clade	A	7.06 [6.53, 7.60]
JS1 + JS2 + JS3 — T/EA	B	4.59 [3.18, 6.13]
JS1 — JS2 + JS3	—	3.32 [1.90, 4.89]
JS2 — JS3	C	1.87 [0.97, 3.02]
SSC — Other *P. vaucheri* "main type"	D	5.35 [3.92, 6.92]
SS/A — Other North African *P. vaucheri* "main type"	E	4.34 [3.02, 5.98]
First split for Moroccan *P. vaucheri* "main type" lineages	—	3.56 [2.47, 4.89]
Split within MN lineage	I	2.76 [1.80, 3.87]
Chafarinas Islands — AW	J	2.12 [1.91, 3.26]

Species Distribution Modelling

For the present (Figure 6, A), the predictive model overestimated the suitable areas when compared to the known distribution or the predictions obtained by Kaliontzopoulou *et al.* (2008). The model identified as the most contributing bioclimatic variables annual precipitation (39.9%) and annual mean temperature (31%), for defining conditions suitability for the species in the region, in concordance with Kaliontzopoulou *et al.* (2008) results, whom had identified as such humidity, temperature, and habitat type. We did not include habitat type in our analysis as information of this is not available for the past. The

isolated suitable regions in Algeria identified by Kaliontzopoulou *et al.* (2008), where the species was confirmed as present, were also identified, although as much larger.

When comparing our middle Holocene (Figure 6, C) projection to the present prediction, suitable area is considerably more extensive in Morocco — considering almost all of the area North of the Atlas Mountains as highly suitable — and West Algeria, although it contracts in East Algeria and Tunisia. The break in range suitability around the Moulouya River valley can still be observed for this time, albeit reduced, and a narrow but abrupt break appears for the Kabylia region, in East Algeria. The Last Glacial Maximum (Figure 6, D) projection presents high range suitability across almost the totality of projection area, presenting a rather homogeneous distribution of probabilities. The “all-time optimal range” (Figure 6, B) across the three time points is more or less in accord to present day distributions, however, since the predictive model for the present was the most restrictive, it was expected that it would have had the greatest impact on shaping this estimation.

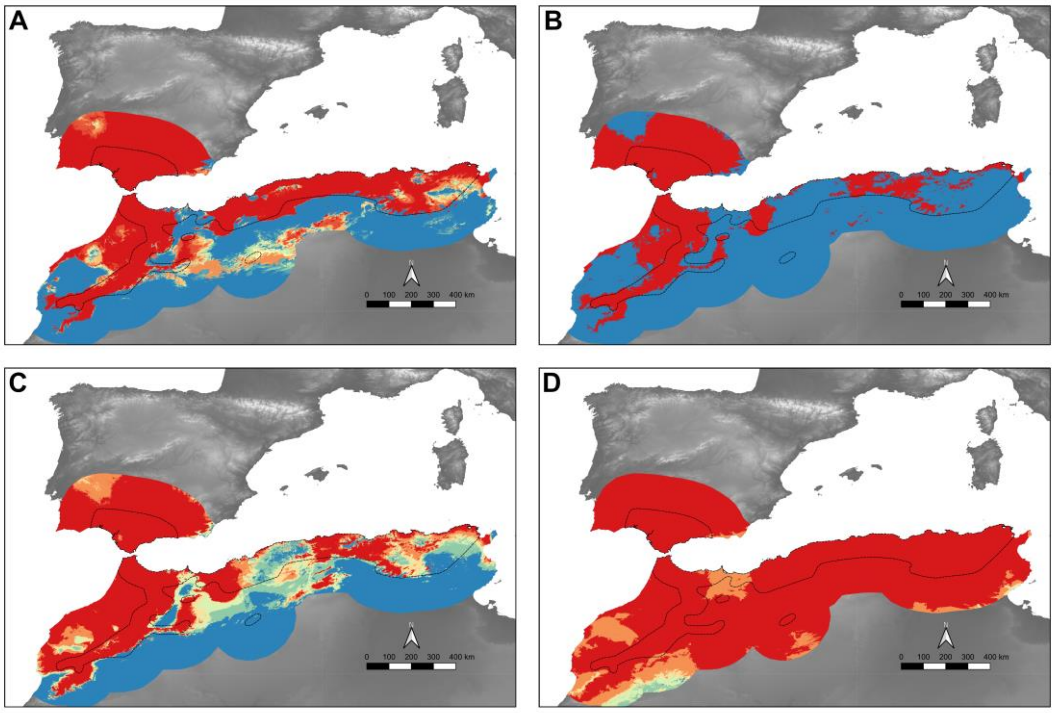

Figure 6. Results from species distribution modelling for *P. vaucheri* “main type” habitat suitability across time — present (A), “all-time optima” (B), middle Holocene (C), and Last Glacial Maximum (D) — for an area of approximately 200 km radius around the current IUCN distribution (dotted black line). Warmer colours represent higher model concordance for habitat suitability (red for maximum suitability) and cold colours lower habitat suitability (blue for no suitability).

Discussion

The observed clear phylogeographic patterns for *P. vaucheri* were expected to be found, as other Moroccan lacertids also exhibit the same kind of clear patterns of population

structuring (Barata, Carranza, *et al.*, 2012; Barata, Perera, *et al.*, 2012; Perera & Harris, 2010; Tamar *et al.*, 2016).

Moroccan *P. vaucheri* from the city of Assilah by grouping together with the Spanish South lineage samples in the partial ND4, show strong signs of having their origin in an additional, more recent, dispersal event from Iberia. Busack *et al.* (2005), had already identified this pattern, although due to the limited number of populations sampled it was generally overlooked. Since Assilah is a port city and our samples were collected on the old city walls, it would be easy to assume an anthropogenic introduction of individuals brought in ships from Spain — similarly to the situation in Greece and in Alguaza, Spain (Renoult *et al.*, 2010; Spilani *et al.*, 2018). However, the notable diversity of the samples, forming two different subgroups for the partial ND4 phylogeny and appearing scattered through the haplotype network for partial MC1R imply that, if it was an introduction, it would have to be of multiple individuals. Considering how other reptile species have crossed the Strait of Gibraltar during the Last Glacial Maximum (Carranza, Arnold, *et al.*, 2006), when sea levels were much lower, it is not improbable that this might be another example of that kind of dispersal, which would be compatible with the existence of notable genetic diversity within the Assilah population. More data is needed to confirm this, in particular assessment of variation within Iberian populations of *P. vaucheri* using the MC1R marker. Despite carrying out fieldwork around Assilah, nearby populations of *P. vaucheri* where not found (Pers. Obs). This seems to indicate it is unlikely that diversity is due to extensive gene-flow with nearby native *P. vaucheri* populations, at least currently. Still, models imply considerably more suitable habitat in the region for the near past, further complicating the situation. Based on the present data we therefore cannot separate between a potential recent anthropogenic introduction or a natural colonization associated with sea level declines during the last glacial maxima; under the latter hypothesis, it implies *P. vaucheri* naturally crossed the Strait of Gibraltar multiple times at very different time periods.

From the data obtained from partial ND4 phylogeny, the first separation within Moroccan *P. vaucheri* “main type” lineage — disregarding the one from Assilah — seems to be the MO lineage, from western High Atlas, which forms a sister-taxa with the common ancestor for all other Moroccan lineages. Further splits within the Moroccan *P. vaucheri* “main clade” show no pattern of directional expansion, presumably because since the African colonization would have happened so long ago (4.34 MYA) and, according to our mdoels, habitat suitability changed massively even in only the last few thousand years, currently observable mitochondrial DNA patterns only managed to retain signs of past fragmentation, but not clues regarding its colonization route.

Obtained date estimations for these latter splits within the Moroccan clade were not taken into account as the Yule tree prior, used for the molecular clock analysis, is not the

best choice to use with closely related lineages (Ho & Phillips, 2009). This resulted in a lack of topologic consistence between the BI/ML phylogeny and the obtained tree from the molecular clock analysis for the Moroccan clade sub-lineages. The choice of coalescent population tree prior could have solved this issue, however, since it considers a constant population size, it is not be the most appropriate for analysis between different taxa, and would likely underperform for the more basal cladogenic events (Eriksson *et al.*, 2010) — such as the *P. vaucheri* "main type" versus the JS variant split, which were of higher interest in the present study.

Concerning the JS variant, from the partial ND4 data the lineage appears to have split from *P. vaucheri* "main type" — long before even the Iberian-African split of the latter (4.34 MYA) —, around the same time (7.06 MYA), or even earlier, than other typically considered species within the genus have split from each other, for example *P. guadarramae* versus *P. bocagei* (Seoane, 1885), or *P. virescens* versus *P. carbonelli* (Pérez-Mellado, 1981) (Kaliontzopoulou *et al.*, 2011). This could be taken to support the hypothesis that the JS variant as a whole — including both the Moroccan and Tunisian forms — should be elevated to a distinct species. However, notable divergence for the JS variant does not end here, with the Moroccan and Tunisian lineages exhibiting high diversity between — and within — themselves and a rather ancient divergence time (4.59 MYA) as well. Both these divergence times are also comparable to those used to propose specific and subspecific status to lineages of *Timon*, another lacertid, from the Eastern part of the Mediterranean Basin (Ahmadzadeh *et al.*, 2012), further supporting the re-evaluation of the Moroccan *Podarcis* taxonomy for the description of at least a different species — the JS variant — or potentially as two additional species — its Moroccan and Tunisian lineages —, so that three named forms would occur in North Africa.

Regarding the two typically identified major phylogeographic break points for North African herpetofauna: the Moulouya River valley area, in East Morocco, and the Kabylia region, in East Algeria (Beddek *et al.*, 2018), phylogeographic analysis for *P. vaucheri sensu strictu* could appear to be associated with the latter, with the Kabylia region corresponding to the breaking point between *P. vaucheri* "main type" and the Tunisian JS variant. However, concerning the Moroccan JS variant populations, the High and Middle Atlas mountains now appear as the major barrier; between them and *P. vaucheri* "main type"; a pattern similar to that identified for *Agama impalearis* (Brown *et al.,* 2012). On the other hand, here the situation is particularly complicated, given the known contact in Agoudal, and that Jebel Sirwah is generally considered isolated from the other mountains in the Atlas range.

Results from our species distribution modelling analysis fail to offer any immediate explanation for the deeper breaks in the phylogeography of *P. vaucheri sensu strictu,*

presumably due to the greater age of these relative to the time-frame modelled. Still our results suggest that in the recent past much larger areas where suitable for the species while the “all-time optima” estimation shows that many regions weren’t always optimal. Even though these are from a very recent time frame —from the LGM, 22,000 years ago, to present — we can contend that the extremes of suitable habitat of the last few thousand years may reflect the extremes of the last few million years, which would justify how, during periods of wide suitability, some populations managed to reach places where they are now isolated by unsuitable habitat — such as the idolated Algeria populations previously identified (Kaliontzopoulou *et al.*, 2008), and explain the development, during periods of limited suitability, of the deep phylogeographic patterns seen.

Nonetheless, results from this analysis should be approached with care, as our present prediction appears to overestimate habitat suitability — both when compared to the known distribution of the species and the results from Kaliontzopoulou *et al.* (2008) —, which would likewise imply an overestimation for past projections. This is probably due to our analysis considering only climatic variables for developing the model, leaving out other environmental factors such as habitat type — because of the unavailability and unreliability of that data for the past —, which heavily contributes to defining location suitability for the species (Kaliontzopoulou *et al.*, 2008). Furthermore, for the predictions in the Iberian Peninsula, even though other environmental factors could indeed be favourable in the area as predicted, the model disregards the presence of other species from the genus that might outcompete *P. vaucheri* in those areas — especially considering *P. vaucheri* seems to exist almost totally in allopatry with other *Podarcis* species in Iberia (Kaliontzopoulou *et al.*, 2011).

Conclusion

The practice of defining a new species from within a previously defined species is always a controversial venture, with two main “factions” forming within researchers: “lumpers” who avoid the definition of new species from already established putative species-complexes, and “splitters” who tend to suggest more species, often using the Phylogenetic Species Concept (Zachos, 2018). Nevertheless, based on the already identified morphological differences and mitochondrial DNA divergence between the JS variant and *P. vaucheri* “main type”, the variant lineage does seem to be a candidate for receiving full species status.

However, since further research is required on aspects such as potential hybrid occurrance in Agoudal, analyses of additional nuclear markers, and morphological

comparisons including Algerian and Tunisian forms, we suggest for now that it is appropriate to refer to a *Podarcis vaucheri species complex* until the situation is clarified.

The evidence does seem to support the hypothesis that, as *P. vaucheri* “main type” dispersed into Africa after the Messinian Salinity Crysis, it would have replaced the JS variant — likely to have been widespread across North Africa prior to *P. vaucheri* “main type” expansion — across most of its Moroccan and West Algerian range. Our models suggest habitat suitability for the LGM is extremely high across most of the area: a situation that could have been similar to the one at the time of *P. vaucheri* “main type” dispersal into Africa, particularly if we assume that colonisations are more likely at periods of lower sea levels. If this expansion really was the case, the notion that the “main type” outcompetes the JS variant seems plausible and could be experimentally tested under controlled conditions (e.g. Carazo *et al.*, 2007). In the case competition with *P. vaucheri* “main type” is found not to be relevant for defining the JS variant range, we could presume external pressures would have caused the latter range contraction, and the former simply took over the available ecological niche as conditions for its dispersal happened to be favourable.

As more comprehensive studies on African taxa are conducted, more potentially overlooked taxa become evident, and evolutive histories are rewritten (Brown *et al.*, 2002; Carranza, Harris, *et al.*, 2006; Fonseca *et al.*, 2008; Kapli *et al.*, 2015). For *P. vaucheri,* its evolutive history is far from having been told, and more sampling around the main lineages breaks in North Africa are still needed for further understanding. The combination of the now standard molecular-based phylogenetic analyses with species distribution models can facilitate this type of work, allowing researchers to identify beforehand possible locations that may have defined the species history and where to look for isolated populations that might complement sampling (Kaliontzopoulou *et al.*, 2008), allowing them to focus their field efforts in those areas for a more efficient application of resources and a faster comprehension of how past processes shaped the biodiversity of today.

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Manuscript II

From Morphology to Genetics: A Phylogeographic Take on the Taxonomy of the Algerian Sand Racer, *Psammodromus algirus*

Abstract

Currently, two subspecies of *P. algirus* are typically considered in Morocco — *P. algirus algirus* and *P. algirus nollii* –, while a third form "*ketamensis*" is generally identified as a morphotype. However, this classification is based on colouration differences, and has not been assessed using genetic data, as the previously conducted genetic studies included only specimens from the range of P*. algirus algirus*. In order to better assess phylogeographic patterns within the species we sequenced a nuclear and a mitochondrial marker from Moroccan individuals, including samples from within the "*nollii*" range, and combined this with previously published data, predominantly from the Iberian Peninsula. No genetic evidence to support the separation of the Moroccan *P. algirus* in two distinct subspecies was found. Unlike most other studies of reptiles from this region, the identified diversity appears to have no corresponding geographical structuring, possibly due to the generalist nature of the species.

Introduction

Psammodromus Fitzinger 1826 is a genus of European and North African lacertids, with six described species, three of which are known to inhabit Morocco: *P. algirus* (Linnaeus 1759), widespread through the country, *P. blanci* (Lattaste 1880), present only to the East, and *P. microdactylus* (Boettger 1881), an elusive species endemic to Morocco (Bons & Geniez, 1996; Mendes *et al.*, 2017).

Regarding the former, *Psammodromus algirus* inhabits most of Iberia and characteristically Mediterranean regions of the Maghreb. In Morocco, besides covering the Mediterranean coast, it is also found on the northern slopes of the High and Middle Atlas — where it can reach up to 2600 meters —, the Rif region, in locations dotting the area between the Atlas Mountains and the Atlantic coast, and in eastern Morocco, between the Middle Atlas and the Algerian border (Bons & Geniez, 1996). Within its Moroccan range, two subspecies are typically considered: *P. algirus algirus* (Linnaeus 1759) and *P. algirus nollii* Fischer 1887*.* The former covers most of the range, while the latter is found in its easternmost interior part, across the Hauts-Plateaux, mainly in the province of Taourirt and on the southern slopes of the Middle Atlas, between the provinces of Khenifra and Errachidia. Distinction between the two is based on *P. algirus nollii* presenting an extra

pair of dorsal lines. Individuals of the two subspecies with intermediate phenotypes have also been reported (Bons & Geniez, 1996). A third monochromatic subspecies was also described, *P. algirus ketamensis* (Galán 1931), from the Rif mountains, but this is now generally considered a morphotype of *P. algirus algirus* (Pasteur & Bons, 1960, Bons & Geniez, 1996).

Various studies have assessed phylogeographic patterns within *P. algirus*. Busack and Lawson (2006) analysed mitochondrial DNA and allozyme data from *P. algirus* from Spain and five Moroccan regions, three in the Rif, one in the Middle Atlas, and another in the High Atlas, and proposed that the lizards had evolved in Southern Morocco and expanded northward. In the same year, however, Carranza *et al.* (2006) suggested the species had instead emerged in Iberia and invaded North Africa after the opening of the Strait of Gibraltar. They analysed data from partial sequences from the mitochondrial cytochrome b, 12S rRNA and 16S rRNA from Iberian and North African *P. algirus*, reporting the existence of two lineages within Iberia — Eastern and Western — that had split around 3.6 Ma, and that North African samples weakly formed a group within the Western lineage — with Tunisian samples distinct from the Moroccan ones — having split from their Iberian counterparts only 1.9 Ma. Verdú-Ricoy *et al.* (2010) later revisited the phylogeography of *P. algirus*, corroborating the existence of the two lineages proposed and further splitting the western one into three clades: the African, the Iberian Northwestern, and the Iberian Southwestern, obtaining strong statistical support for these groupings. Mendes *et al.* (2017) assessed relationships across the Strait of Gibraltar within the genus performing ancestral range analysis to confirm the origin each lineage. Regarding *P. algirus*, they identified the species as having its origin in the Iberian Peninsula, and that a dispersion to North Africa occurred around 1.4 MYA. The other African taxa, *P. microdactylus* and *P. blanci*, both have an African origin from a now extinct common ancestor that would have come to North Africa from the Iberian Peninsula around 10 MYA

Despite these earlier studies, within *P. algirus*, patterns of genetic diversity across Morocco remained largely unassessed, and in particular the "*nollii*" range was essentially unsampled. This is critical, since many reptile species show a pattern of deep divergence between specimens in Eastern Morocco and those to the West of the Atlas Mountains, including species within the genera *Saurodactylus*, *Podarcis* and *Trogonophis* (Kaliontzopoulou *et al.*, 2011; Rosado *et al.*, 2017; Salvi *et al.*, 2018).

With the present study, we therefore intended to improve the current coverage of genetic data for the species across Morocco to determine phylogeographic patterns for the species in the country, using both mitochondrial and nuclear DNA sequences, and to compare our results to patterns recovered from other reptiles across this region. At the

same time, by including individuals from the *P. algirus nollii* range, we plan to re-evaluate support for this subspecies.

Material and Methods

A total of 31 individuals were sequenced, with 4 belonging to *P. algirus nollii* (samples 15, 16, 17 and 29) — based on distribution and visual identification in the field — 2 found in an area where both subspecies and intermediates are recorded (samples 11 and 12), and the remainder to *P. algirus algirus* from across the range in Morocco (Figure 1). A sample was included from the "*ketamensis*" type locality of Ketama (sample 10), but no confirmation of morphotype was available. Samples consisted of tail-tip muscle, stored in 96% ethanol.

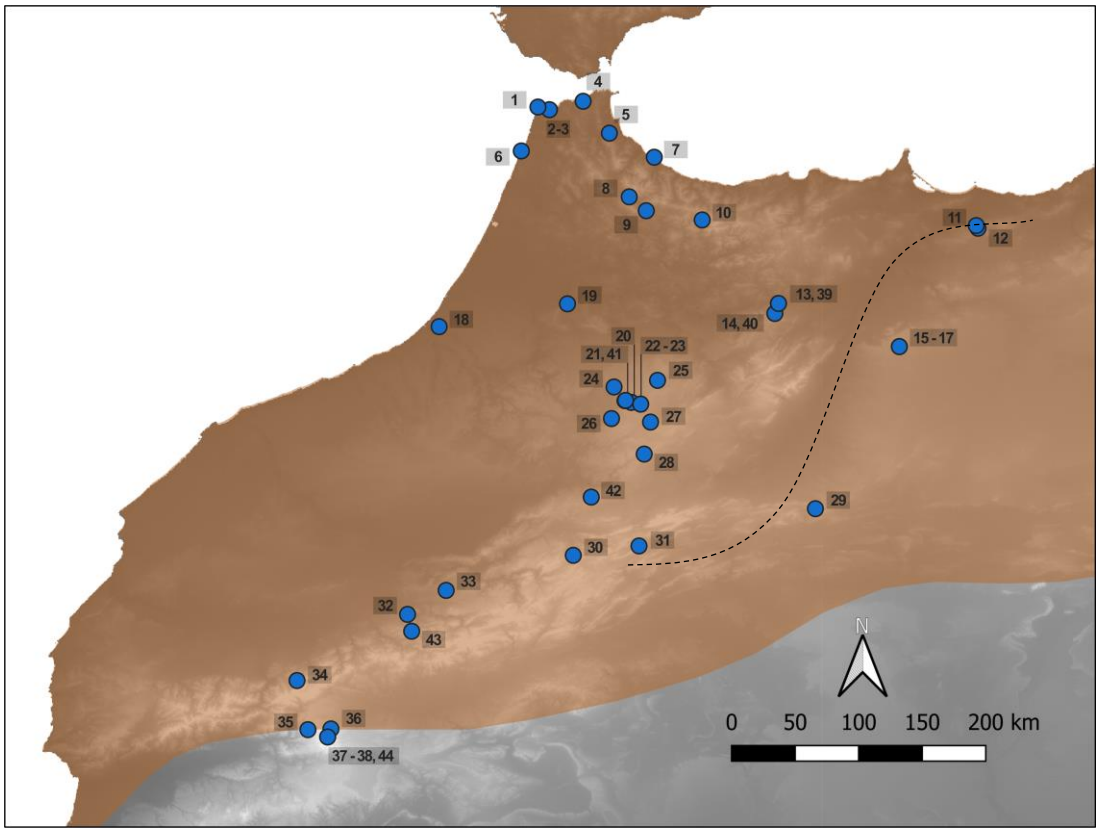

Figure 1. Map of sampling locations for *P. algirus*. Light brown shade represents the species distribution according to IUCN (Mateo *et al.*, 2009). Dotted line marks the putative border between *P. a. algirus* and *P. a. nollii* distribution ranges, approximately following the Moulouva river valley area. Numbers relate to partial ND4 phylogeny (1-38; Figure 2) and partial MC1R haplotype network (6-11, 14-17, 19, 20, 22-25, 27-31, 33, 35-37, 39-44; Figure 3).

A standard high-salt method was employed for total DNA extraction (Sambrook *et al.*, 1989). Two molecular markers were selected for analysis: partial NADH dehydrogenase subunit 4 (ND4) mitochondrial gene, and partial melanocortin 1 receptor (MC1R) nuclear gene. Amplification conditions followed Arévalo *et al.* (1994) for the ND4 partial gene, and Pinho *et al.*, (2010) for the MC1R gene region, with reagent concentrations following

manufacturer indications for the polymerases — 5x HOT FIREPol® Blend Master Mix (Solis BioDyne) for the ND4, GoTaq®G2 Flexi DNA Polymerase (Promega) for the MC1R. PCR products were sent to a commercial company (GENEWIZ) for purification and sequencing.

Obtained sequences were complemented with sequences retrieved from GenBank and provided by other authors upon request, bringing the total of analysed samples to 392 for partial ND4 and 44 (88 sequences) for partial MC1R; GenBank sequences from *Psammodromus hispanicus* Fitzinger 1826 were used as outgroup. For both markers, sequences were aligned, manually revised and trimmed to the same length — to 652 bp for partial ND4, and to 562 bp for partial MC1R — using Geneious® v4.8.5 (Biomatters). Gblocks (Castresana, 2000) was used to determine poorly aligned positions and divergent regions, though none were identified.

For the ND4 data, Bayesian inference (BI) and maximum likelihood (ML) approaches were used to estimate a phylogeny. For the BI, the most appropriate model of molecular evolution was defined with the AIC, using PartitionFinder2 (Lanfear *et al.*, 2017) and the phylogenetic analyses implemented with MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). BI was performed with the GTR+G+I model, using random starting trees, run for 8×10^6 generations, and sampled every 1000 generations. A 25% burn-in was applied to the obtained trees. Remaining data was used to estimate posterior nodal probabilities and produce a summary phylogeny. Two separate replicates were performed and compared to check for local optima (Huelsenbeck & Bollback, 2001). The ML approach was executed with MEGA X v10.0.5, both for defining the most appropriate model of molecular evolution with the AIC and producing a phylogeny. The chosen model was the TN93+G+I and a phylogenetic tree was inferred using a Nearest-Neighbor-Interchange heuristic method, with 500 bootstrap replications used to assess reliability. Again, two replicas were performed.

For the MC1R data, haplotype estimation was performed using the built-in Phase tool from DnaSP v5.10 (Librado & Rozas, 2009). A phylogenetic network using statistical parsimony was built with TCS v1.21 (Clement *et al.*, 2000) and visualized in tcsBU (Múrias dos Santos *et al*, 2016).

Results & Discussion

Our estimate of a phylogeny based on partial ND4 (Figure 2) is congruent with the eastern and western lineage division for *P. algirus*, with North African samples falling in the western lineage, as expected. Within this lineage, the three subdivisions identified by Verdú-Ricoy *et al.* (2010) are also observed, clustering the African clade and the Iberian northwestern

and southwestern clades. Within the African clade, the specimen from Tunisia is sister-taxon to the remaining Moroccan samples. Within Morocco there is considerable diversity, up to 2.34%. However, there is no clear phylogeographic structure, and in particular samples from the *P. algirus nollii* range do not form a clade.

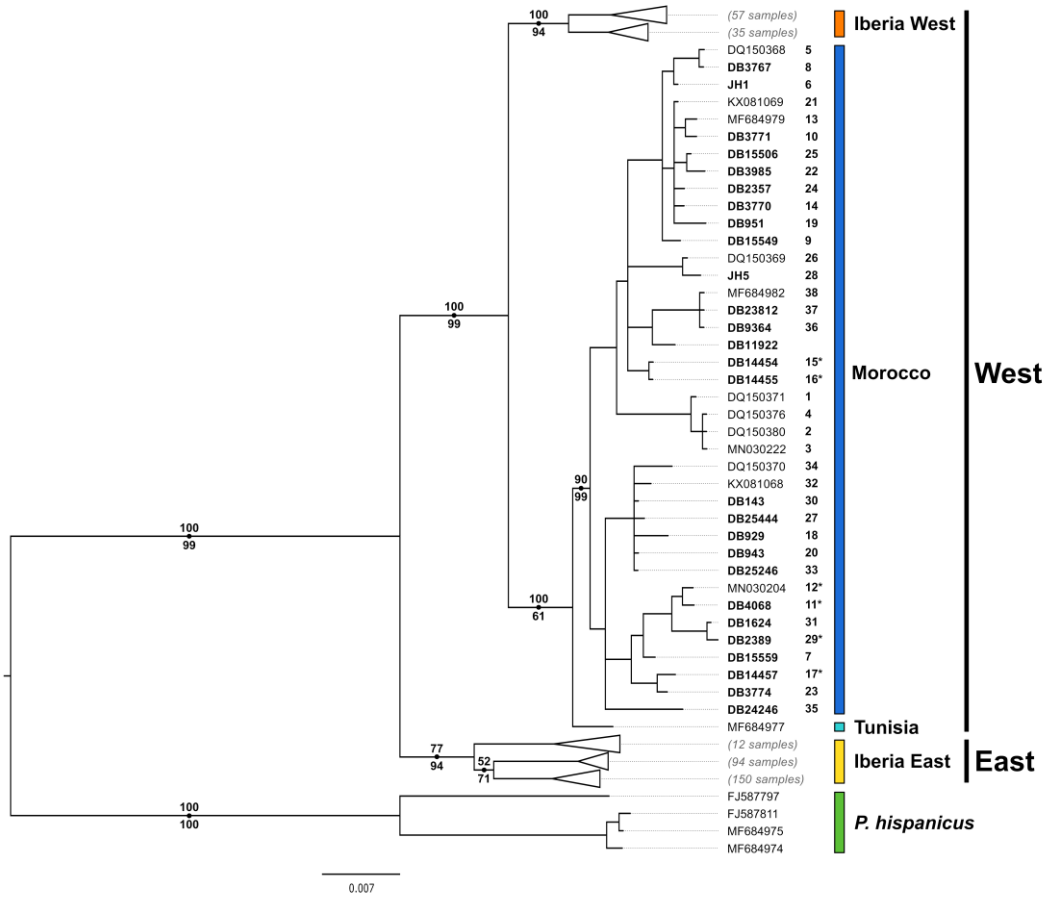

Figure 2. Obtained estimate of a phylogeny for *P.algirus*, derived from BI with partial ND4 sequences. BI posterior probabilities and ML Bootstrap values are indicated respectively above and under relevant nodes. Samples in bold correspond to samples sequenced on purpose for this study. Numbering relates the map of sampling locations (Figure 1), with an asterisk marking samples from within the "*nollii*" range. Colours relate to partial MC1R haplotype network (Figure 3). Iberian clades are collapsed according to the lineages defined by Diaz *et al.* (2017) for ease of representation, with the number of samples within each indicated next to it. The tree was rooted with *P. hispanicus*.

For the haplotype network from partial MC1R (Figure 3), 15 haplotypes were identified for Morocco, with two common haplotypes. Iberian individuals clearly demonstrated the eastern and western clade division based on the MC1R marker. Only 5 haplotypes were identified, although sampling was much more limited. The most divergent haplotypes within Iberian populations were separated by 19 nucleotide changes — for comparative purposes, the closest *P. algirus* haplotypes to *P. hispanicus* are separated by only 5 nucleotide changes to the East and West Iberian clades, respectively.

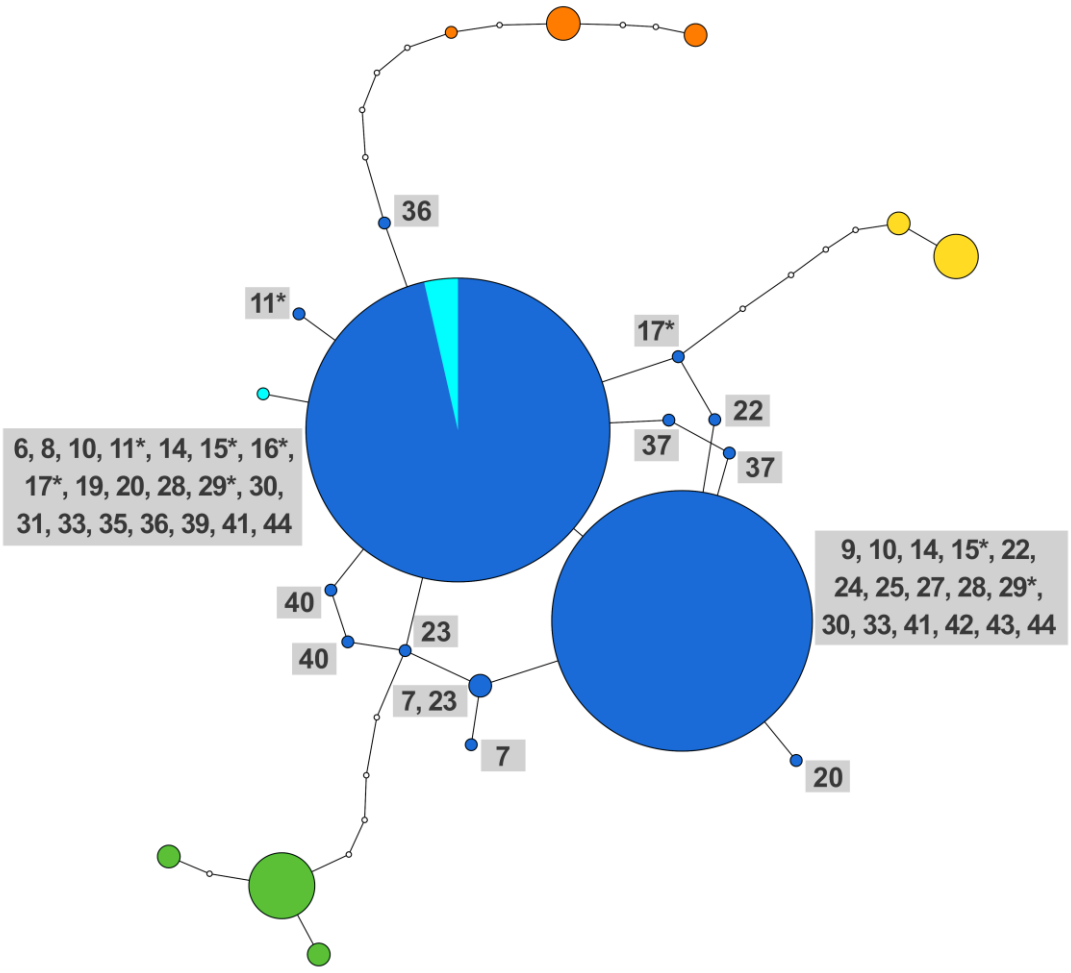

Figure 3. Haplotype network for *P. algirus* from partial MC1R sequences. Four haplogroups can be identified, three for *P. algirus*: North African — Moroccan (blue) and Tunisian (cyan) — Iberian West (orange), Iberian East (yellow); and one for *P. hispanicus* (green). Numbering relates the map of sampling locations (Figure 1), with an asterisk marking samples from within the "*nollii*" range; colours relate to partial ND4 phylogeny (Figure 2).

For Moroccan individuals, no strong association was found between their genetic diversity and geographical distribution. The lack of coherency between phylogeny and geographical distribution for Moroccan *P. algirus* was unexpected, as many other widespread reptiles across this region exhibit clear patterns of population structuring (Kaliontzopoulou *et al.*, 2011; Barata, Carranza, *et al.*, 2012; Barata, Perera, *et al.*, 2012). Indeed, the Moulouya River valley region, which approximately separates the two Moroccan subspecies, often coincides with phylogeographic breaks in reptiles (Beddek *et al.*, 2018). In the case of *P. algirus*, not only is there no break at this point, but there is no clear phylogeographic structure at all. For *P. algirus*, the observed situation could be due to the species being a generalist (Moreno-Rueda *et al.*, 2018), thriving on low shrub habitats independently of their actual floristic composition (Diaz & Carrascal, 1991) and capable of shifting between habitats during their seasonal availability (Martín & López, 1998), not having its distribution highly affected by many of the mesoclimates within the

Moroccan landscape nor by constrains from competition with other lacertid species (Diaz & Carrascal, 1991). Juveniles are also able to survive in inappropriate habitats for the species, improving their mobility between populations (Martín *et al.*, 1998). In this way, there may be even greater movement of individuals across the species range, limiting breaks in gene-flow that over time could lead to phylogeographic structuring.

Samples from the "*nollii*" range (samples 11, 12, 15, 16, 17, 29) show no evidence of being distinct based on either marker, nor did the specimen from the type locality of "*ketamensis*" (sample 10). Similar instances where identified colour morphotypes did not correspond with distinct genetic lineages have already been observed in this region, for example, the snake *Psammophis schokari* (Forskal 1775) exhibits three colour morphotypes throughout its North African range although these do not reflect the species' evolutionary history, but rather local adaptations to the environment (Rato *et al.*, 2007). Within Iberian *P. algirus*, Díaz *et al.* (2017) also suggested that phenomena of adaptation for crypsis could be masking their phylogeographic structure, working both to promote divergence within clades, but also admixture between them. It is not clear if the two forms come into contact in Morocco, but given the complex patterns observed in the Iberian Peninsula, this would be interesting to assess if possible.

Conclusions

The lack of observed genetic differentiation for the supposed *P. algirus nollii* individuals for the ND4 gene, alongside the evidence regarding striped versus unstriped forms of *P. algirus* from the Iberian Peninsula, indicate that these phenotypes do not reflect evolutionary history, and therefore do not warrant subspecific status. Therefore, the individuals previously considered as *P. algirus nollii* are here considered morphotypes of *P. algirus*. We suggest the same is likely for the considered for the "*ketamensis*" form, although we note that this still requires confirmation using individuals identified as monochromatic.

Although this work provides greatly improved phylogenetic coverage for the Moroccan region, the countries of Algeria and Tunisia are still poorly sampled. The single Tunisian sample formed a sister-taxon to the Moroccan clade using the mitochondrial DNA marker as previously found (Carranza *et al.*, 2006), albeit with a shallow divergence, and without evidence of differentiation based on the nuclear marker assessed for the first time in this study. In a comparative study of the region, Beddek *et al.* (2018) identified two major biogeographic breaks: one along the Moulouya river valley and another in Kabylia in Central Algeria. Sampling in this second region might help identify the distribution limits of Moroccan and Tunisian lineages.

Within Morocco, no phylogeographic structure was observed, unlike in almost all other studies of lacertid lizards from the region to date. We hypothesize that this is associated with habitat usage and dispersal ability, which enables greater levels of gene-flow between populations relative to most other similar species, however additional studies of more generalist species across the region are needed to further assess this hypothesis.

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Discussion and Final Remarks

Overall, results from both studies were mostly in line with the initial hypotheses for the studied taxa.

Podarcis vaucheri “main type” and the JS variant were sister-taxa in our estimate of a phylogeny derived from partial ND4 sequences, forming two well supported clades, which were estimated to have split apart around 7.06 MYA. Results also suggested high levels of structuring for populations of the species across North Africa, with a clear-cut geographical pattern to account for the lineage diversity, and virtually no sympatry between populations from different lineages. Indeed, the exceptions were between the two major clades: two of the Moroccan JS variant populations with the southernmost Moroccan *P. vaucheri* “main type”, and on the Eastern limit for the two forms in Algeria. Thus, while different “populations” were never found in sympatry, the highly divergent, potential species were. This in itself may be considered as further evidence warranting specific status to these lineages. On the other hand, despite the deep divergences identified in the mitochondrial DNA, the haplotype network from partial MC1R sequences revealed little discrimination. Regarding the evolutive history for the *Podarcis vaucheri sensu strictu* and the colonization of the African continent by the genus, we suggest that three different dispersal events took place. The first would have been around 7.06 MYA, corresponding to the cladogenic event that separated the “main type” and JS variant lineages, as the latter dispersed into North Africa at a time when extensive geological events were taking place in the region that is now the Alboran Sea, in the lead up to the Messinian Salinity Crisis. A second over-seas dispersal of *P. vaucheri* “main type” from Iberia to North Africa took place around 4.34 MYA. Finally, a much more recent one ocurred, corresponding to a second coming of Iberian *P. vaucheri* individuals of the South Spanish lineage into the city of Assilah. It is unclear from the current data if this was due to an anthropogenic introduction or not.

Psammodromus algirus, in its turn, yielded no evidence that could support the current systematics for its Moroccan subspecies, as samples from the “*nollii*” range were not differentiated from the remaining populations. Contrary to expectations, no phylogeographic structuring was observed in the country. Network haplotype from partial MC1R sequences once again revealed no pattern for the Moroccan populations, though intercontinental and clade splits — East versus West and, within the West clade, Iberian West versus African — were extremely evident.

Phylogeographic Patterns

Regarding general phylogeographic patterns for North African reptiles, when taking a look at the bigger picture, there seems to be a common pattern of East and West split across taxa in the region. These splits tend to occur in association with one of the two major biogeographic breaks identified for the region: along the Moulouya river valley or in Kabylia in Central Algeria (Beddek *et al.*, 2018). For *P. vaucheri sensu strictu*, the latter seems to be the case, as it is around the Kabylia region in Algeria that the populations almost suddenly change from exclusively "main type" *P. vaucheri* to the JS variant — disregarding the isolated pockets for the variant in Southern Morocco. For *P. algirus*, the former was not observed, since no genetic differentiation was detected between samples from both sides of the Moulouya river valley — which approximately corresponds to the typically considered separation between *P. a. algirus* and *P. a. nollii*. Still, since no Algerian specimens were analysed, and only one from Tunisia was available to us, the existence of such a break to the East, in the Kabylia region, remains as an unassessed possibility.

Going into detail on the phylogeographic structuring within the studied taxa populations, *Podarcis vaucheri* did, as mentioned before, present a very clear structure for its mitochondrially defined lineages, coherent to their distribution across the Moroccan landscape — this was to be expected, as such patterns are common for North African herpes (Rato & Harris, 2008; Kaliontzopoulou *et al.*, 2011; Barata *et al.*, 2012; Beddek *et al.*, 2018). Results from the species distribution modelling results also suggests that this structuring may be currently maintained by patches of unsuitable habitat between the populations, hampering the dispersal capabilities of the lizards and impeding gene flow between populations across North Africa.

Psammodromus algirus, however, did not show such organization for its phylogeography, going against the generally observed trend. This does not, nonetheless, represent a completely new instance, as a similar situation has been identified for another Ibero-North African reptiles: within the *Acanthodactylus* Wiegmann 1834 genus, *A. erythrurus* (Schinz 1833), *A. lineomaculatus* (Schinz 1833), and *A. blanci* (Doumerge 1901) all present high patterns of genetic diversity across its range — even higher than for Moroccan *P. algirus* —, with very little coherence between their phylogeny and geographic distribution (Harris *et al.*, 2004; Fonseca *et al.*, 2009). This result could be because *P. algirus* is a highly generalist as mobile species, capable of adapting to a wide range of mesoclimates, and not to impacted by competition with other lacertids, nor requiring any specific floristic composition for its survival (Diaz & Carrascal, 1991; Martín & López, 1998; Moreno-Rueda *et al.*, 2018), but could also be simply because the species is too young in the continent, the dispersal event that brought it there having happened at around a mere

1.40 MYA (Mendes *et al.*, 2017), thus not yet giving the local populations time to genetically differentiate between themselves.

Defining Species and Subspecies

Although results seem to corroborate our suspicions for incoherencies in the current systematics of both taxa, it is still true that both works meddle with a very dynamic and debated concept: the definition of species and, subsequently, of subspecies (Zachos, 2016). We are long past the classical definition of species, where they were defined as "a group of organisms in which any two healthy individuals of the appropriate genders can produce fertile offspring", and genetic tools now dominate (Küpper & Remedios, 2019). The phylogenetic species concept of "an irreducible group whose members are descended from a common ancestor and who all possess a combination of certain defining, or derived, traits and a shared and unique evolutionary history", proposed by Joel Cracraft in 1983, is often considered the current sound approach to deal with taxonomic classification (Zachos, 2018). However, the lack of a "threshold line" for this concept brings up many discussions between authors more prone to lumping species together and those more prone to split them (Zachos, 2018). To overcome some of these issues, many authors now recommend an "integrative taxonomy" approach, whereby genetic, morphological and ecological data are combined (Dayrat, 2005; Will *et al.*, 2005).

Regarding the *Psammodromus algirus* case, as we are dealing with taxonomic changes at a subspecific level, agreeance on the baseline concept may be even harder than for discussing species, as no genuine consensus of a definition was ever achieved for subspecies, and the term is many times freely applied to describe completely different evolutionary processes across taxa (Fitzpatrick, 2010). Still the fact that no differentiation at all was observed between the two forms, intermediate individuals of both subspecies have been found, and that populations of the striped phenotype used as the main characteristic to justify *P. a. nolli* also exists in Eastern Spain (Bons & Geniez, 1996) without having received any subspecific status for it should altogether pose a strong case to relegate its typically considered status.

For *P. vaucheri* and its JS variant, our suggested alterations to the latter taxonomic status could too find some hindrance before being recognized, as even though the analysis to partial ND4 strongly suggests the existence of at least two species, with differentiation level comparable to those between other taxa from the same genus (Kaliontzopoulou *et al.*, 2011), that alone should hardly be taken as enough to justify the description of a new species, particularly considering that no differentiation was found at a nuclear DNA level, for the partial MC1R sequences. However, more arguments besides those mount to build

the case for JS as an independent species, such as its most probable different origin from *P. vaucheri* “main type”, their striking coloration differences, and the fact that populations of the two lineages seem to interact differently — sometimes occurring in sympatry — than those within the “main type” — always recorded in strict allopatry. Although all this may not still be enough to warrant elevating the variant to its own specific status, it surely creates a sound case for it to be thus considered.

While most of the current data does seem to indicate at least two species could be defined within North Africa, it could be debated whether even more should be considered. For example, within the JS variant lineage, estimated divergence times from mitochondrial DNA are rather old among some populations — around 4.59 MYA between the Tunisian and Moroccan populations, and 3.32 MYA between JS1 and the other two Moroccan populations —, and, given how isolated these populations are, they may have been evolving separately and without gene flow for considerable periods of time. This would, however, require further study of the lineage genetic markers before could be proposed.

Research Prospects for *Podarcis vaucheri* and *Psammodromus algirus*

Regardless of the knowledge about the two species added by this thesis much remains unknown and open for further research.

For one, a taxonomic work describing the *Podarcis* JS variant form should be in order, as considerable support has been gathered to propose raising its status to a full species, distinct from the remaining *P. vaucheri*. This should include a detailed morphological study of the variant individuals — which at least appear to exhibit different dorsal coloration —, a thorough assessing of the variant’s populations range — particularly at the border with *P. vaucheri* range in the Kabylia region in East Algeria (Beddek *et al.*, 2018) —, and further genetic studies on both Moroccan *P. vaucheri sensu strictu* forms to understand current and past interactions between them — mainly focused on the study of nuclear DNA, to assess factors like hybridization between the forms and further corroborate — or argue against — the evolutive history inferred from mitochondrial DNA for the genus (Moore, 1995).

Psammodromus algirus will require an expansion of sampling going eastwards across the species range, as although good phylogenetic coverage in Morocco has been provided, what might be going on in Algeria and Tunisia remains unassessed and — considering that no significant phylogeographic breaks have been detected for it in the Moulouya river valley area — what lies further East, in the Kabylia region, might be of great importance to understand the species history; assuming it may exhibit one of the two

commonly identified patterns for North African herpes (Beddek *et al.*, 2018). Further genetic studies on *P. algirus* as a whole could also be worthwhile, as — even if local variations such as *P. jeanneae* and *P. manuelae* have been revoked as their own species (Verdú-Ricoy *et al.*, 2010) —, the West and East clades appear to present significant amounts of diversity that may warrant the description of, at least, a different subspecies for *P. algirus* for each of them. By comparison, since we defend the taxonomic split of the JS variant from *P. vaucheri* and its raising to full species status, which are in a situation where both of them still present shared haplotypes for partial MC1R, then evidence to support multiple species within *P. algirus*, where partial MC1R is clearly differentiated between the Iberian clades and even within the West clade, between the Iberian and African clades. Results obtained by Díaz *et al.* (2017) do, however, argue against such suggesting, showing that nuclear DNA from microsatellites clutch together populations from the two Iberian clades, away from others of their typically considered clade. This brings back up the issue that defining species is not an easy feat, and thus reinforces notion that “integrative taxonomy” approaches are required to fully — or at least, in the best way possible — discern variant lineages from actual full species.

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Supplementary Material

For Manuscript I

Supp. 1 Sample codes, country, sampling coordinates, GenBank accession codes, and author for *Podarcis vaucheri* sequences used in this study. Samples in bold were sequenced specifically for this study. pg. 70

For Manuscript II

Supp. 2 Sample codes, country, sampling coordinates, GenBank accession codes, and author for *Psammodromus algirus* sequences used in this study. Samples in bold were sequenced specifically for this study pg. 76

Supp. 1. Sample codes, country, sampling coordinates, GenBank accession codes, and author for *Podarcis vaucheri* sequences used in this study. Samples in bold were sequenced specifically for this study.

Sample Code	Country	Sampling Coordinates		GenBank Accession Codes		Author(s)
				MC1R	ND4	
DB76	Morocco	32.2165	-5.5497	—	*n/a*	*(present study)*
DB83	Morocco	32.2165	-5.5497	*n/a*	*n/a*	*(present study)*
DB86	Morocco	32.6620	-5.4994	—	*n/a*	*(present study)*
DB94	Morocco	32.2165	-5.5497	*n/a*	*n/a*	*(present study)*
DB136	Morocco	32.6708	-5.4531	*n/a*	*n/a*	*(present study)*
DB442	Morocco	31.5892	-6.9369	*n/a*	*n/a*	*(present study)*
DB874	Morocco	28.0277	-11.3568	*n/a*	—	*(present study)*
DB946	Morocco	33.1599	-5.0655	*n/a*	*n/a*	*(present study)*
DB965	Morocco	32.5795	-4.8569	*n/a*	*n/a*	*(present study)*
DB966	Morocco	32.5396	-4.9384	*n/a*	—	*(present study)*
DB969	Morocco	32.5406	-4.9393	*n/a*	—	*(present study)*
DB970	Morocco	32.5406	-4.9393	*n/a*	*n/a*	*(present study)*
DB972	Morocco	32.3027	-5.3278	*n/a*	*n/a*	*(present study)*
DB975	Morocco	32.3023	-5.3281	*n/a*	*n/a*	*(present study)*
DB976	Morocco	32.3023	-5.3281	*n/a*	*n/a*	*(present study)*
DB980	Morocco	32.1265	-5.3040	*n/a*	—	*(present study)*
DB990	Morocco	32.1261	-5.3042	*n/a*	*n/a*	*(present study)*
DB994	Morocco	32.1261	-5.3042	—	*n/a*	*(present study)*
DB1002	Morocco	32.1265	-5.3041	*n/a*	*n/a*	*(present study)*
DB1029	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB1038	Morocco	30.7786	-7.6460	*n/a*	*n/a*	*(present study)*
DB1047	Morocco	30.7809	-7.6435	*n/a*	*n/a*	*(present study)*
DB1084	Morocco	30.7809	-7.6435	*n/a*	*n/a*	*(present study)*
DB1513	Morocco	33.0742	-5.0058	*n/a*	*n/a*	*(present study)*
DB1556	Morocco	31.9697	-5.4880	*n/a*	*n/a*	*(present study)*
DB1593	Morocco	31.9697	-5.4880	*n/a*	—	*(present study)*
DB1608	Morocco	30.7767	-7.6530	*n/a*	*n/a*	*(present study)*
DB1611	Morocco	30.7767	-7.6530	*n/a*	*n/a*	*(present study)*
DB1802	Morocco	35.2500	-5.1800	*n/a*	—	*(present study)*
DB1803	Morocco	35.2500	-5.1800	*n/a*	*n/a*	*(present study)*
DB2319	Morocco	34.7684	-5.5162	—	*n/a*	*(present study)*
DB2324	Morocco	34.7684	-5.5162	*n/a*	—	*(present study)*
DB2348	Morocco	32.6791	-4.7389	—	*n/a*	*(present study)*
DB2371	Morocco	33.5427	-5.3169	—	*n/a*	*(present study)*
DB2603	Morocco	32.1842	-5.8782	*n/a*	—	*(present study)*
DB2633	Morocco	32.1842	-5.8782	*n/a*	*n/a*	*(present study)*
DB3632	Morocco	32.6708	-5.4531	—	*n/a*	*(present study)*
DB3878	Morocco	33.1863	-5.3313	*n/a*	*n/a*	*(present study)*
DB5138	Morocco	30.8171	-8.8630	*n/a*	*n/a*	*(present study)*
DB6829	Morocco	32.0621	-5.8890	*n/a*	*n/a*	*(present study)*
DB6839	Morocco	32.2025	-6.0010	*n/a*	*n/a*	*(present study)*
DB8740	Morocco	34.8782	-4.6109	*n/a*	—	*(present study)*
DB8741	Morocco	34.8782	-4.6109	—	*n/a*	*(present study)*
DB8742	Morocco	34.8782	-4.6109	*n/a*	*n/a*	*(present study)*
DB8755	Morocco	34.1042	-4.0725	—	*n/a*	*(present study)*
DB8756	Morocco	34.1042	-4.0725	—	*n/a*	*(present study)*
DB8757	Morocco	34.1042	-4.0725	—	*n/a*	*(present study)*
DB8758	Morocco	34.1042	-4.0725	*n/a*	*n/a*	*(present study)*
DB8775	Morocco	35.0226	-5.2045	*n/a*	*n/a*	*(present study)*
DB8776	Morocco	35.0226	-5.2045	*n/a*	*n/a*	*(present study)*
DB8874	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8875	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8876	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*

DB8877	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8879	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8880	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8881	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8882	Morocco	30.8333	-8.3333	*n/a*	*n/a*	*(present study)*
DB8884	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8885	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8886	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8888	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8889	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8891	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8892	Morocco	35.4711	-6.0312	—	*n/a*	*(present study)*
DB8894	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8895	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8897	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8898	Morocco	35.4711	-6.0312	—	*n/a*	*(present study)*
DB8900	Morocco	35.4711	-6.0312	*n/a*	*n/a*	*(present study)*
DB8901	Morocco	35.4711	-6.0312	—	*n/a*	*(present study)*
DB9211	Morocco	33.6541	-4.1587	—	*n/a*	*(present study)*
DB9770	Morocco	35.0630	-5.2020	*n/a*	*n/a*	*(present study)*
DB11141	Morocco	34.9174	-4.5137	*n/a*	*n/a*	*(present study)*
DB11144	Morocco	35.3333	-5.6028	*n/a*	—	*(present study)*
DB11145	Morocco	33.3645	-4.6912	*n/a*	*n/a*	*(present study)*
DB11146	Morocco	35.7706	-5.6052	*n/a*	*n/a*	*(present study)*
DB11147	Morocco	35.2004	-5.2662	*n/a*	—	*(present study)*
DB11148	Morocco	35.3703	-5.5541	*n/a*	*n/a*	*(present study)*
DB11151	Morocco	35.0333	-4.9833	*n/a*	*n/a*	*(present study)*
DB11152a	Morocco	35.0333	-4.9833	*n/a*	*n/a*	*(present study)*
DB11152b	Morocco	35.0333	-4.9833	*n/a*	*n/a*	*(present study)*
DB11157	Morocco	35.1405	-5.1345	*n/a*	*n/a*	*(present study)*
DB11251	Morocco	33.8667	-3.0333	*n/a*	*n/a*	*(present study)*
DB11287	Morocco	32.2165	-5.5497	—	*n/a*	*(present study)*
DB11661	Morocco	31.2057	-7.8577	*n/a*	*n/a*	*(present study)*
DB11681	Morocco	31.2057	-7.8577	*n/a*	*n/a*	*(present study)*
DB11684	Morocco	32.1699	-5.9301	*n/a*	*n/a*	*(present study)*
DB11705	Morocco	32.1581	-5.3684	*n/a*	—	*(present study)*
DB11911	Morocco	32.2025	-6.0010	*n/a*	*n/a*	*(present study)*
DB11927	Morocco	32.2025	-6.0010	*n/a*	*n/a*	*(present study)*
DB11928	Morocco	32.2025	-6.0010	*n/a*	*n/a*	*(present study)*
DB13251	Morocco	31.2057	-7.8577	*n/a*	*n/a*	*(present study)*
DB13254	Morocco	31.2057	-7.8577	*n/a*	*n/a*	*(present study)*
DB13544	Morocco	35.1820	-2.4295	*n/a*	*n/a*	*(present study)*
DB14461	Morocco	33.9370	-3.0538	*n/a*	*n/a*	*(present study)*
DB14463	Morocco	33.9370	-3.0538	*n/a*	*n/a*	*(present study)*
DB14466	Morocco	33.9370	-3.0538	*n/a*	*n/a*	*(present study)*
DB14695	Morocco	31.2035	-7.8617	*n/a*	*n/a*	*(present study)*
DB14718	Morocco	33.4054	-5.1033	*n/a*	*n/a*	*(present study)*
DB14719	Morocco	33.4036	-5.1016	*n/a*	*n/a*	*(present study)*
DB14772	Morocco	33.4036	-5.1016	*n/a*	*n/a*	*(present study)*
DB14777	Morocco	31.2906	-7.3816	*n/a*	*n/a*	*(present study)*
DB14779	Morocco	31.2906	-7.3816	*n/a*	*n/a*	*(present study)*
DB14781	Morocco	31.2906	-7.3816	*n/a*	*n/a*	*(present study)*
DB15507	Morocco	33.6466	-4.9796	*n/a*	*n/a*	*(present study)*
DB15508	Morocco	33.6466	-4.9796	*n/a*	*n/a*	*(present study)*
DB15509	Morocco	33.6466	-4.9796	*n/a*	*n/a*	*(present study)*
DB15510	Morocco	33.6466	-4.9796	*n/a*	*n/a*	*(present study)*
DB15550	Morocco	35.0596	-5.1938	*n/a*	*n/a*	*(present study)*
DB15551	Morocco	35.0643	-5.0999	*n/a*	*n/a*	*(present study)*

DB15554	Morocco	35.2668	-4.8424	*n/a*	*n/a*	*(present study)*
DB15555	Morocco	35.2668	-4.8424	*n/a*	*n/a*	*(present study)*
DB15561	Morocco	35.5667	-5.5587	*n/a*	*n/a*	*(present study)*
DB15571	Morocco	35.6632	-5.6232	*n/a*	*n/a*	*(present study)*
DB20152	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20153	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20155	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20156	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20166	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20167	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20169	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB20170	Morocco	31.1896	-7.8536	*n/a*	*n/a*	*(present study)*
DB22373	Morocco	32.9797	-5.0661	*n/a*	*n/a*	*(present study)*
DB23771	Morocco	32.0360	-5.4658	*n/a*	*n/a*	*(present study)*
DB23814	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23827	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23828	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23856	Morocco	32.0360	-5.4658	*n/a*	*n/a*	*(present study)*
DB23857	Morocco	33.1124	-5.0277	*n/a*	*n/a*	*(present study)*
DB23870	Morocco	33.1419	-5.0515	*n/a*	*n/a*	*(present study)*
DB23873	Morocco	32.0360	-5.4658	*n/a*	*n/a*	*(present study)*
DB23877	Morocco	32.0360	-5.4658	*n/a*	*n/a*	*(present study)*
DB23880	Morocco	32.0360	-5.4658	*n/a*	*n/a*	*(present study)*
DB23891	Morocco	32.0360	-5.4658	*n/a*	—	*(present study)*
DB23895	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23897	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23910	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23920	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB23930	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB24101	Morocco	30.7439	-7.6096	*n/a*	—	*(present study)*
DB24152	Morocco	31.0353	-7.7086	*n/a*	*n/a*	*(present study)*
DB24153	Morocco	31.0353	-7.7086	*n/a*	*n/a*	*(present study)*
DB24155	Morocco	31.0353	-7.7086	*n/a*	*n/a*	*(present study)*
DB24157	Morocco	31.0353	-7.7086	*n/a*	*n/a*	*(present study)*
DB25213	Morocco	33.0209	-5.0727	*n/a*	*n/a*	*(present study)*
DB25248	Morocco	33.0209	-5.0727	*n/a*	*n/a*	*(present study)*
DB25301	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25302	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25303	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25304	Morocco	32.0353	-5.4675	*n/a*		*(present study)*
DB25305	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25306	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25307	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25308	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25309	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25310	Morocco	32.0353	-5.4675	—	*n/a*	*(present study)*
DB25311	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25312	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25313	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25314	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25315	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25316	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25317	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25318	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25319	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25320	Morocco	32.0353	-5.4675	*n/a*	—	*(present study)*
DB25321	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25322	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*

DB25323	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB25324	Morocco	32.0353	-5.4675	*n/a*	—	*(present study)*
DB25325	Morocco	32.0353	-5.4675	*n/a*	*n/a*	*(present study)*
DB26262	Morocco	34.1295	-4.0315	*n/a*	*n/a*	*(present study)*
DB26714	Morocco	34.1038	-4.0729	*n/a*	*n/a*	*(present study)*
JH51	Morocco	31.6000	-6.5100	*n/a*	*n/a*	*(present study)*
JH52	Morocco	31.6000	-6.5100	*n/a*	*n/a*	*(present study)*
JH65	Morocco	31.5199	-6.2763	*n/a*	*n/a*	*(present study)*
JH66	Morocco	31.5199	-6.2763	*n/a*	*n/a*	*(present study)*
JH70	Morocco	31.5559	-7.0002	*n/a*	*n/a*	*(present study)*
JH80	Morocco	30.9810	-8.7544	*n/a*	*n/a*	*(present study)*
JH81	Morocco	30.9810	-8.7544	*n/a*	—	*(present study)*
JH82	Morocco	30.9810	-8.7544	*n/a*	—	*(present study)*
JH122	Morocco	31.2059	-7.8662	*n/a*	—	*(present study)*
DQ081172	Morocco	31.2000	-7.8500	—	DQ081172.1	Pinho *et al.*, 2006
DQ081173	Morocco	33.5000	-4.5000	—	DQ081173.1	Pinho *et al.*, 2006
DQ081174	Morocco	35.0500	-5.2000	—	DQ081174.1	Pinho *et al.*, 2006
DQ081175	Spain	36.7200	-6.0900	—	DQ081175.1	Pinho *et al.*, 2006
DQ081176	Spain	37.2600	-6.9400	—	DQ081176.1	Pinho *et al.*, 2006
DQ081177	Tunisia	36.1800	9.7000	—	DQ081177.1	Pinho *et al.*, 2006
DQ081178	Tunisia	36.7700	8.6800	—	DQ081178.1	Pinho *et al.*, 2006
DQ081179	Morocco	30.7300	-7.6000	—	DQ081179.1	Pinho *et al.*, 2006
DQ081180	Morocco	30.7300	-7.6000	—	DQ081180.1	Pinho *et al.*, 2006
EF081075	Spain	—	—	—	EF081075.1	Pinho *et al.*, 2007
EF081076	Spain	—	—	—	EF081076.1	Pinho *et al.*, 2007
EF081077	Spain	—	—	—	EF081077.1	Pinho *et al.*, 2007
EF081078	Spain	—	—	—	EF081078.1	Pinho *et al.*, 2007
EF081079	Spain	—	—	—	EF081079.1	Pinho *et al.*, 2007
EF081080	Morocco	—	—	—	EF081080.1	Pinho *et al.*, 2007
EF081081	Morocco	—	—	—	EF081081.1	Pinho *et al.*, 2007
EF081082	Morocco	34.2200	-4.0200	—	EF081082.1	Pinho *et al.*, 2007
EF081083	Morocco	—	—	—	EF081083.1	Pinho *et al.*, 2007
EF081084	Morocco	—	—	—	EF081084.1	Pinho *et al.*, 2007
EF081085	Morocco	—	—	—	EF081085.1	Pinho *et al.*, 2007
EF081086	Morocco	—	—	—	EF081086.1	Pinho *et al.*, 2007
EF081087	Morocco	—	—	—	EF081087.1	Pinho *et al.*, 2007
EF081088	Morocco	—	—	—	EF081088.1	Pinho *et al.*, 2007
EF081089	Morocco	—	—	—	EF081089.1	Pinho *et al.*, 2007
EF081090	Morocco	—	—	—	EF081090.1	Pinho *et al.*, 2007
EF081091	Morocco	—	—	—	EF081091.1	Pinho *et al.*, 2007
EF081092	Morocco	—	—	—	EF081092.1	Pinho *et al.*, 2007
EF081093	Morocco	—	—	—	EF081093.1	Pinho *et al.*, 2007
EF081094	Morocco	33.4200	-5.2200	—	EF081094.1	Pinho *et al.*, 2007
EF081095	Morocco	—	—	—	EF081095.1	Pinho *et al.*, 2007
EF081096	Morocco	—	—	—	EF081096.1	Pinho *et al.*, 2007
EF081097	Morocco	—	—	—	EF081097.1	Pinho *et al.*, 2007
EF081098	Morocco	—	—	—	EF081098.1	Pinho *et al.*, 2007
EF081099	Morocco	33.5200	-5.0800	—	EF081099.1	Pinho *et al.*, 2007
EF081100	Morocco	—	—	—	EF081100.1	Pinho *et al.*, 2007
EF081101	Morocco	—	—	—	EF081101.1	Pinho *et al.*, 2007
EF081102	Morocco	—	—	—	EF081102.1	Pinho *et al.*, 2007
EF081103	Morocco	—	—	—	EF081103.1	Pinho *et al.*, 2007
EF081104	Morocco	—	—	—	EF081104.1	Pinho *et al.*, 2007
EF081105	Morocco	—	—	—	EF081105.1	Pinho *et al.*, 2007
EF081106	Morocco	—	—	—	EF081106.1	Pinho *et al.*, 2007
EF081107	Morocco	—	—	—	EF081107.1	Pinho *et al.*, 2007
EF081108	Morocco	—	—	—	EF081108.1	Pinho *et al.*, 2007
EF081109	Morocco	—	—	—	EF081109.1	Pinho *et al.*, 2007

EF081110	Morocco	34.9700	-4.9200	—	EF081110.1	Pinho *et al.*, 2007
EF081111	Morocco	34.9700	-4.9200	—	EF081111.1	Pinho *et al.*, 2007
EF081112	Morocco	35.3200	-5.4800	—	EF081112.1	Pinho *et al.*, 2007
EF081113	Morocco	35.3200	-5.4800	—	EF081113.1	Pinho *et al.*, 2007
EF081114	Morocco	35.0000	-5.3800	—	EF081114.1	Pinho *et al.*, 2007
EF081115	Morocco	35.8700	-5.4000	—	EF081115.1	Pinho *et al.*, 2007
EU269585	Morocco	30.7300	-7.6000	—	EU269585.1	Pinho *et al.*, 2008
EU269587	Tunisia	36.7700	8.6800	—	EU269587.1	Pinho *et al.*, 2008
GQ856084	Algeria	36.3700	-5.0500	—	GQ856084.1	Lima *et al.*, 2009
GQ856085	Algeria	36.3700	-5.0500	—	GQ856085.1	Lima *et al.*, 2009
GQ856086	Algeria	35.8700	-1.9300	—	GQ856086.1	Lima *et al.*, 2009
GQ856087	Algeria	35.8700	-1.9300	—	GQ856087.1	Lima *et al.*, 2009
GQ856088	Algeria	36.3700	-4.2700	—	GQ856088.1	Lima *et al.*, 2009
GQ856089	Algeria	36.3700	-4.2700	—	GQ856089.1	Lima *et al.*, 2009
GQ856090	Algeria	36.4700	-3.9800	—	GQ856090.1	Lima *et al.*, 2009
GQ856091	Algeria	36.4700	-3.9800	—	GQ856091.1	Lima *et al.*, 2009
GQ856092	Algeria	35.2800	-1.2500	—	GQ856092.1	Lima *et al.*, 2009
GQ856093	Algeria	35.2800	-1.2500	—	GQ856093.1	Lima *et al.*, 2009
GQ856094	Algeria	34.8300	-1.2800	—	GQ856094.1	Lima *et al.*, 2009
GQ856095	Algeria	34.8300	-1.2800	—	GQ856095.1	Lima *et al.*, 2009
GQ856096	Algeria	34.5500	2.7800	—	GQ856096.1	Lima *et al.*, 2009
GQ856097	Algeria	34.5500	2.7800	—	GQ856097.1	Lima *et al.*, 2009
GQ856098	Algeria	33.7200	1.1700	—	GQ856098.1	Lima *et al.*, 2009
GQ856099	Algeria	33.7200	1.1700	—	GQ856099.1	Lima *et al.*, 2009
GQ856100	Algeria	35.3500	6.6200	—	GQ856100.1	Lima *et al.*, 2009
GQ856101	Algeria	35.3500	6.6200	—	GQ856101.1	Lima *et al.*, 2009
GQ856102	Algeria	35.6700	6.0700	—	GQ856102.1	Lima *et al.*, 2009
GQ856103	Algeria	35.6700	6.0700	—	GQ856103.1	Lima *et al.*, 2009
GQ856104	Algeria	36.8700	7.6200	—	GQ856104.1	Lima *et al.*, 2009
GQ856105	Algeria	36.8300	8.4000	—	GQ856105.1	Lima *et al.*, 2009
GQ856106	Algeria	36.7500	4.4200	—	GQ856106.1	Lima *et al.*, 2009
GQ856108	Tunisia	36.7500	4.4200	—	GQ856108.1	Lima *et al.*, 2009
HQ898012	Tunisia	37.1000	8.9800	—	HQ898012.1	Kaliontzopoulou *et al.*, 2011
HQ898013	Tunisia	36.8100	8.7200	—	HQ898013.1	Kaliontzopoulou *et al.*, 2011
HQ898014	Spain	37.6900	-4.4800	—	HQ898014.1	Kaliontzopoulou *et al.*, 2011
HQ898015	Spain	37.6900	-4.4800	—	HQ898015.1	Kaliontzopoulou *et al.*, 2011
HQ898016	Spain	37.8900	-4.7800	—	HQ898016.1	Kaliontzopoulou *et al.*, 2011
HQ898017	Spain	37.1800	-3.6000	—	HQ898017.1	Kaliontzopoulou *et al.*, 2011
HQ898018	Spain	37.1833	-3.6000	—	HQ898018.1	Kaliontzopoulou *et al.*, 2011
HQ898019	Spain	36.9730	-5.6631	—	HQ898019.1	Kaliontzopoulou *et al.*, 2011
HQ898020	Spain	38.3000	-5.2700	—	HQ898020.1	Kaliontzopoulou *et al.*, 2011
HQ898021	Spain	36.4900	-4.7800	—	HQ898021.1	Kaliontzopoulou *et al.*, 2011
HQ898022	Spain	36.5200	-5.6200	—	HQ898022.1	Kaliontzopoulou *et al.*, 2011
HQ898023	Spain	36.9500	-4.5400	—	HQ898023.1	Kaliontzopoulou *et al.*, 2011
HQ898026	Spain	35.8900	-5.3200	—	HQ898026.1	Kaliontzopoulou *et al.*, 2011
HQ898028	Morocco	31.1400	-7.9100	—	HQ898028.1	Kaliontzopoulou *et al.*, 2011
HQ907939	Spain	37.4645	-3.9247	—	HQ907939.1	Kaliontzopoulou *et al.*, 2011
HQ907940	Spain	37.4645	-3.9247	—	HQ907940.1	Kaliontzopoulou *et al.*, 2011
HQ907941	Spain	37.7800	-3.7900	—	HQ907941.1	Kaliontzopoulou *et al.*, 2011
HQ907942	Spain	37.7786	-3.7851	—	HQ907942.1	Kaliontzopoulou *et al.*, 2011
KY576649	Algeria	34.5520	2.7960	—	KY576649.1	Beddek *et al.*, 2018
KY576650	Algeria	36.5670	2.4460	—	KY576650.1	Beddek *et al.*, 2018
KY576651	Tunisia	37.0640	8.9920	—	KY576651.1	Beddek *et al.*, 2018
KY576652	Tunisia	37.0640	8.9920	—	KY576652.1	Beddek *et al.*, 2018
KY576653	Algeria	35.3410	1.2380	—	KY576653.1	Beddek *et al.*, 2018
KY576654	Algeria	36.7900	4.5150	—	KY576654.1	Beddek *et al.*, 2018
KY576655	Algeria	36.6930	4.6080	—	KY576655.1	Beddek *et al.*, 2018
KY576656	Algeria	36.7450	5.6080	—	KY576656.1	Beddek *et al.*, 2018

KY576657	Algeria	36.7450	5.6080	—	KY576657.1	Beddek *et al.*, 2018
KY576658	Algeria	36.8750	6.4330	—	KY576658.1	Beddek *et al.*, 2018
KY576659	Algeria	35.5990	6.0370	—	KY576659.1	Beddek *et al.*, 2018
KY576660	Algeria	36.6930	4.6080	—	KY576660.1	Beddek *et al.*, 2018
KY576661	Algeria	36.6930	4.6080	—	KY576661.1	Beddek *et al.*, 2018
KY576662	Algeria	36.4470	4.1230	—	KY576662.1	Beddek *et al.*, 2018
KY576663	Algeria	35.6870	-0.9060	—	KY576663.1	Beddek *et al.*, 2018
KY576664	Algeria	36.4580	4.1100	—	KY576664.1	Beddek *et al.*, 2018
KY576665	Algeria	34.5690	2.7810	—	KY576665.1	Beddek *et al.*, 2018
KY576666	Algeria	35.8650	1.9680	—	KY576666.1	Beddek *et al.*, 2018
KY576667	Tunisia	37.0640	8.9920	—	KY576667.1	Beddek *et al.*, 2018
KY576668	Algeria	35.6220	-0.8890	—	KY576668.1	Beddek *et al.*, 2018
KY576669	Algeria	36.7490	3.0280	—	KY576669.1	Beddek *et al.*, 2018
KY576670	Algeria	35.8720	1.9470	—	KY576670.1	Beddek *et al.*, 2018
KY576671	Algeria	36.5670	2.4460	—	KY576671.1	Beddek *et al.*, 2018

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Supp. 2. Sample codes, country, sampling coordinates, GenBank accession codes, and author for *Psammodromus algirus* sequences used in this study. Samples in bold were sequenced specifically for this study.

Sample Code	Country	Sampling Coordinates		GenBank Accession Codes		Author(s)
				MC1R	ND4	
DB90	Morocco	32.6620	-5.4994	n/a	—	*(present study)*
DB143	Morocco	32.1964	-5.6429	n/a	n/a	*(present study)*
DB441	Morocco	31.5892	-6.9369	n/a	—	*(present study)*
DB929	Morocco	34.0249	-6.7171	—	n/a	*(present study)*
DB943	Morocco	33.4191	-5.17841	n/a	n/a	*(present study)*
DB951	Morocco	34.2073	-5.6895	n/a	n/a	*(present study)*
DB1624	Morocco	32.2714	-5.1170	n/a	n/a	*(present study)*
DB2357	Morocco	33.5427	-5.3169	n/a	n/a	*(present study)*
DB2389	Morocco	32.5691	-3.7043	n/a	n/a	*(present study)*
DB3767	Morocco	35.0626	-5.1951	n/a	n/a	*(present study)*
DB3770	Morocco	34.1305	-4.0292	n/a	n/a	*(present study)*
DB3771	Morocco	34.8782	-4.6109	n/a	n/a	*(present study)*
DB3774	Morocco	33.4054	-5.1033	n/a	n/a	*(present study)*
DB3985	Morocco	33.4054	-5.1033	n/a	n/a	*(present study)*
DB4068	Morocco	34.8333	-2.4167	n/a	n/a	*(present study)*
DB4235	Morocco	34.1305	-4.0292	n/a	—	*(present study)*
DB9364	Morocco	30.8081	-7.5837	n/a	n/a	*(present study)*
DB11922	Morocco	—	—	n/a	n/a	*(present study)*
DB14454	Morocco	33.8653	-3.0324	n/a	n/a	*(present study)*
DB14455	Morocco	33.8653	-3.0324	n/a	n/a	*(present study)*
DB14457	Morocco	33.8653	-3.0324	n/a	n/a	*(present study)*
DB15506	Morocco	33.5954	-4.9684	n/a	n/a	*(present study)*
DB15549	Morocco	34.9520	-5.0585	n/a	n/a	*(present study)*
DB15559	Morocco	35.3796	-4.9947	n/a	n/a	*(present study)*
DB23812	Morocco	30.7439	-7.6096	n/a	n/a	*(present study)*
DB24246	Morocco	30.8022	-7.7674	n/a	n/a	*(present study)*
DB25246	Morocco	31.9159	-6.6609	n/a	n/a	*(present study)*
DB25444	Morocco	33.2618	-5.0244	n/a	n/a	*(present study)*
JH1	Morocco	35.4298	-6.0591	n/a	n/a	*(present study)*
JH5	Morocco	33.0059	-5.0752	n/a	n/a	*(present study)*
DQ150368	Morocco	35.5720	-5.3540	—	DQ150368.1	Busack & Lawson, 2006
DQ150369	Morocco	33.2900	-5.3370	—	DQ150369.1	Busack & Lawson, 2006
DQ150370	Morocco	31.1950	-7.8550	—	DQ150370.1	Busack & Lawson, 2006
DQ150371	Morocco	35.7810	-5.9260	—	DQ150371.1	Busack & Lawson, 2006
DQ150372	Spain	—	—	—	DQ150372.1	Busack & Lawson, 2006
DQ150373	Spain	—	—	—	DQ150373.1	Busack & Lawson, 2006
DQ150374	Spain	—	—	—	DQ150374.1	Busack & Lawson, 2006
DQ150375	Spain	—	—	—	DQ150375.1	Busack & Lawson, 2006
DQ150376	Morocco	35.8270	-5.5640	—	DQ150376.1	Busack & Lawson, 2006
DQ150377	Spain	—	—	—	DQ150377.1	Busack & Lawson, 2006
DQ150378	Spain	—	—	—	DQ150378.1	Busack & Lawson, 2006
DQ150379	Spain	—	—	—	DQ150379.1	Busack & Lawson, 2006
DQ150380	Morocco	35.7596	-5.8341	—	DQ150380.1	Busack & Lawson, 2006
FJ587970	Spain	—	—	—	FJ587970.1	Fitze *et al.*, 2011
FJ587971	Spain	—	—	—	FJ587971.1	Fitze *et al.*, 2011
FJ587972	Spain	—	—	—	FJ587972.1	Fitze *et al.*, 2011
FJ587973	Spain	—	—	—	FJ587973.1	Fitze *et al.*, 2011
FJ587974	Spain	—	—	—	FJ587974.1	Fitze *et al.*, 2011
FJ587975	Spain	—	—	—	FJ587975.1	Fitze *et al.*, 2011
FJ587976	Spain	—	—	—	FJ587976.1	Fitze *et al.*, 2011
FJ587977	Spain	—	—	—	FJ587977.1	Fitze *et al.*, 2011
FJ587978	Spain	—	—	—	FJ587978.1	Fitze *et al.*, 2011
FJ587979	Spain	—	—	—	FJ587979.1	Fitze *et al.*, 2011
FJ587981	Spain	—	—	—	FJ587981.1	Fitze *et al.*, 2011
FJ587982	Spain	—	—	—	FJ587982.1	Fitze *et al.*, 2011
FJ587983	Spain	—	—	—	FJ587983.1	Fitze *et al.*, 2011
FJ587984	Spain	—	—	—	FJ587984.1	Fitze *et al.*, 2011
FJ587985	Spain	—	—	—	FJ587985.1	Fitze *et al.*, 2011
KC621630	Spain	—	—	—	KC621630.1	Noonan *et al.*, 2013
KX080785	Morocco	33.4351	-5.2246	—	KX080785.1	Mendes *et al.*, 2016
KX081068	Morocco	31.7240	-6.9700	—	KX081068.1	Mendes *et al.*, 2016
KX081069	Morocco	33.4330	-5.2290	—	KX081069.1	Mendes *et al.*, 2016
MF684952	Spain	—	—	MF684952.1	—	Mendes *et al.*, 2017
MF684953	Spain	—	—	MF684953.1	—	Mendes *et al.*, 2017
MF684954	Spain	—	—	MF684954.1	—	Mendes *et al.*, 2017
MF684955	Morocco	34.2104	-3.9996	MF684955.1	—	Mendes *et al.*, 2017

MF684956	Spain	—	—	MF684956.1	—	Mendes *et al.*, 2017
MF684957	Spain	—	—	MF684957.1	—	Mendes *et al.*, 2017
MF684958	Morocco	30.7439	-7.6096	MF684958.1	—	Mendes *et al.*, 2017
MF684959	Spain	—	—	MF684959.1	—	Mendes *et al.*, 2017
MF684976	Spain	—	—	—	MF684976.1	Mendes *et al.*, 2017
MF684977	Tunisia	—	—	—	MF684977.1	Mendes *et al.*, 2017
MF684978	Spain	—	—	—	MF684978.1	Mendes *et al.*, 2017
MF684979	Morocco	34.2100	-4.0010	—	MF684979.1	Mendes *et al.*, 2017
MF684980	Spain	—	—	—	MF684980.1	Mendes *et al.*, 2017
MF684981	Spain	—	—	—	MF684981.1	Mendes *et al.*, 2017
MF684982	Morocco	30.7439	-7.6096	—	MF684982.1	Mendes *et al.*, 2017
MN030204	Morocco	34.8119	-2.4027	—	MN030204.1	Garcia-Porta *et al.*, 2019
MN030222	Morocco	35.7596	-5.8341	—	MN030222.1	Garcia-Porta *et al.*, 2019
319 other unpublished *P. algirus* sequences from Spain given upon request						Díaz *et al.*

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