Where to live in Lisbon: urban habitat used by the introduced Italian wall lizard (*Podarcis siculus*)

Ricardo Ribeiro[*], Paulo Sá-Sousa

ICAAM – Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Pólo da Mitra, Ap. 94, 7002-554 Évora, Portugal

*Correspondence: E-mail: ricardoavribeiro@gmail.com

Exotic animal invasions constitute a major threat to biodiversity worldwide. Our assessment determined the core range occupied by the Italian wall lizard (*Podarcis siculus*) in the urban area of Parque das Nações (Lisbon, Portugal), where it was accidentally introduced two decades ago. Despite the apparent current scenario of non-expansion, the alien species interferes with the local distribution pattern of the native *P. virescens*, with both lizard species shown to use the available microhabitats differently. The native *P. virescens* population displays an overall heterogeneous distribution in the urban matrix. On the other hand, the occupancy of the exotic species clusters within the original area of introduction (garden in front of the Lisbon Oceanarium), as well as in adjacent gardens. There, *P. siculus* replaced the native *P. virescens* as the only lizard species present. Appa-rently, there is no known reason there that prevents the alien lizard from colonizing more available geographic area and expanding.

Invasive species currently represent one of the major problems in nature conservation (Schulte, 2012). The damage caused by invasive reptiles mainly comes from disruptions and changes in native food webs and ecosystem functions, with impacts ranging from predation on sensitive species, intoxication of predators, competition and hybridization with native species, vectorization of new parasites, as well as direct and indirect impacts on humans (Kraus, 2009; Fujisaki *et al.*, 2010). Among the European reptile species, the Italian wall lizard (*Podarcis siculus*, Sauria: Lacertidae) is considered as both an opportunistic species and a successful exotic colonizer, characterized by wide eco-logical tolerance and plasticity, allied with high propagation capacity (Nevo *et al.*, 1972; Capula & Ceccarelli, 2003; Crnobr-nja-Isailovic *et al.*, 2009; Silva-Rocha *et al.*, 2014). *Podarcis siculus* is distributed conti-nuously throughout the Italian Peninsula and East Adriatic coast, as well as for big islands - Sardinia (where recent studies suggested that it may be allochtonous instead of native, Senczuk *et al.*, 2017) and Sicily - and a multitude of adjacent islets (Corti & Lo Cascio, 2002). Unlike the reported pet trade introductions in the USA (Kolbe *et al.*, 2013), it might have been introduced by ship trade into Corsica, Balearics and several port areas around the Mediterranean Basin e.g. SW France,

Greece, Turkey, Tunisia or Lybia (CARRETERO & SILVA-ROCHA, 2015).

Towards Lisbon, some *P. siculus* individuals might have been transported along with rough materials and ornamental plants (structures in which they would most likely be sheltered in or basking on), from their place of origin, during the Expo98 event (GONZÁLEZ DE LA VEGA *et al.*, 2001; SILVA-ROCHA *et al.*, 2012). This appears to be a recurrent issue, as similar cases were reported by RIVERA *et al.* (2011) and VALDEÓN *et al.* (2010), after the observation of several *P. siculus* individuals in olive trees, imported from Calabria (Italy) to Catalonia (Spain).

In urban environments, which are typically characterized by spatially and temporally heterogeneous thermal mosaics, ecological interactions with potential competitors and predators may also influence patterns of occurrence and abundance of individuals of a given species (KOLBE *et al.*, 2016). In those environments, it has been observed that sympatric lacertids (namely *Podarcis muralis* and *P. siculus*) are spatially organized according to each species specific ecological needs, and therefore not limited to interspecific interactions (CAPULA *et al.*, 1993). Lizard species often tend to be associated with specific microhabitat patches, with minimized interspecific competition through their spatial niche distribution (SMITH & BALLINGER, 2001; RUGIERO & LUISELLI, 2007; VITT & CALDWELL, 2014). However, there may be cases where the microhabitat occupied by a certain species is restricted by the presence of another (ARNOLD, 1987). That could be the case with autochthonous *Podarcis virescens* (GENIEZ *et al.*, 2014; DIAS *et al.*, 2016), which might be affected by this *P. siculus* introduction, although negative impacts are still unknown (such as expansion, hybridization or extinction of native species). Both species under study coexist in sympatry with other lacertids in their native distributions, evidencing ecological segregation (CAPULA, 1993; CAPULA *et al.*, 1993; CARRETERO, 2008; VALDEÓN *et al.*, 2010; GRANO *et al.*, 2011; CARRETERO & SILVA-ROCHA, 2015; CARRETERO & SALVADOR, 2016).

However, the introduction of the Italian wall lizard in other areas has been shown to be detrimental to autochthonous lacertids, especially in micro-insular environments, with evidence of competitive spatial coexistence or exclusion (NEVO *et al.*, 1972; CAPULA, 2002; CAPULA & CECCARELLI, 2003; PODNAR *et al.*, 2005; VALDEÓN *et al.*, 2010; MATEO *et al.*, 2011; CARRETERO & SILVA-ROCHA, 2015).

Based on previous personal reports from Paulo Sá-Sousa and bibliography from LOUREIRO *et al.* (2008), this study's main objectives consisted in determining the current range area occupied by the introduced *P. siculus* and assessing the degree of spatial segregation exerted by the exotic population upon the autochthonous species. This required the knowledge of the current spatial distribution of both species in Lisbon, as well as each species microhabitat preferences, according to the available space.

MATERIALS AND METHODS

The study area covered the location where *P. siculus* was first introduced and consequently reproduced (GONZÁLEZ DE LA VEGA *et al.*, 2001), within the so-called *Parque das Nações*, a leisure and residential area of Lisbon (38°45'43.7"N 9°05'39.0"W,

Figure 1: Total prospected area limited in red; *Podarcis siculus* introduction spot highlighted by a black square.

Fig. 1). For lizard sampling one large polygon was delimited to address the possible expansion and establishment of new populations towards adjacent spots. The sampling area was subsequently divided in 16 plots or sectors with approximate dimensions, to facilitate the visual encounter of lizards. Although these plots consisted of urban areas, there are contrasting differences between them, ranging from those that show predominance of artificial elements, to those that hold some garden spots with lawn or a more rustic appearance, usually consisting of an eclectic selection of plant species from various regions of the world (Pedrosa, 2013).

Due to statistical and data independence reasons, the sectors were sampled following a random order. Each sector was surveyed by walking at a slow, steady pace, during one sunny day (when there

were favourable weather conditions for lizard thermoregulation). However, some sectors took longer to cover in cases of high occurrence of observations. All lizard observations (each corresponding to one individual) consisted of visual encounters (Lambert, 1984; Eekhout, 2010), where the following data was recorded: GPS point of location, species (*P. siculus* or *P. virescens*), behaviour activity, perch type, height and slope, distance to vegetation cover, refuge type and distance to refuge, along with the substrate texture, number of trees, shrubs, non-woody plants and dead branches/trunks, found within a four meters radius surrounding each observation point (the dissertation from which this article was adapted covered a wider range of variables, including sex and size class, which were considered negligible for the study's purpose, primarily focused on
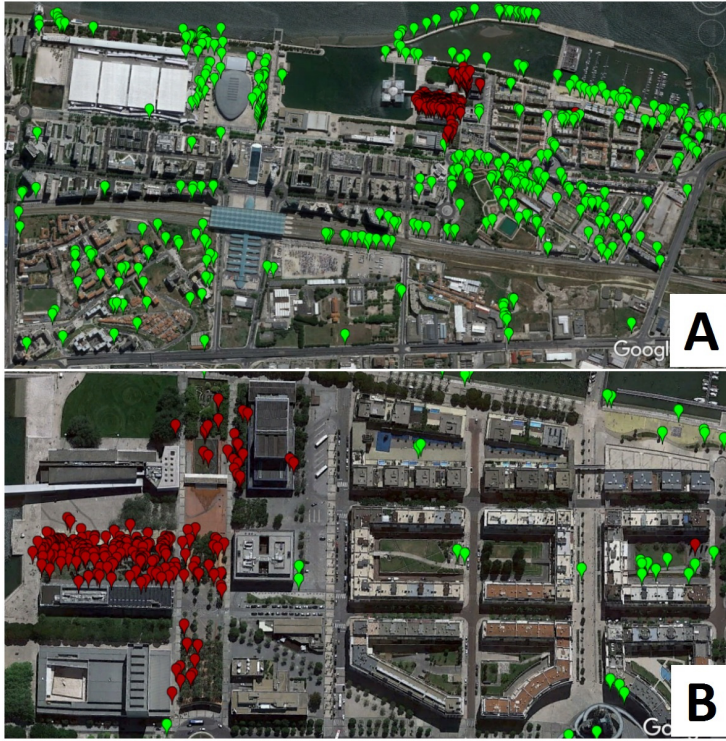
Figure 2: Spatial distribution of both species (A - total area; B - focus in the presence of *Podarcis siculus*). Red symbols – introduced *Podarcis siculus*; Green symbols - autochthonous *Podarcis virescens*.

habitat use and microspatial distribution, and therefore discarded from this paper). The first round of sampling covered all (16) sectors, providing a general acquaintance of both the study area and its lizard occupancy. Based on this previous data assessment, the second and third rounds covered only 8 of the 16 sectors, excluding those where *P. siculus* was clearly absent in the first round. Thus, the first round took place between April 25th, 2016 and July 3rd, 2016; the second began on September 8th, 2016 and ended on September 21st, 2016; the third lasted from September 24th, 2016 to October 11th, 2016.

Further statistical analyses were performed using the SigmaPlot® 13.0, Statistica 13® and Microsoft Excel 2016 softwares, tested for a confidence level of 95% (α =

0.05) (STRODE & BROKAW, 2015). The chi-squared test was used for comparison between species, regarding the data collected on the constituent elements of the micro-habitats used by both species, where the formulated null hypothesis was that "there are no differences between the use given by the invasive and autochthonous species to the elements present in the microhabitat". For this test's application we used raw data, that is, simple counts (observations), instead of percentages or proportions (STRODE & BROKAW, 2015), presented in table, which included the observed and expected values for several categories, within each considered parameter (DYTHAM, 2011). Since multiple tests were performed simultaneously, further statistical corrections were made using the Holm-Bonferroni method,

Table 1: Sampled sector maximums for both species in each sector. The three sectors with occurrence of both species highlighted in grey.

Sector	Max. *P. virescens*	Max. *P. siculus*
1	26	1
2	9	26
3	22	96
4	41	0
5	13	0
6	7	0
7	4	0
8	8	0
9	27	0
10	24	0
11	2	0
12	10	0
13/14	33	0
15	9	0
16	16	0
Sub-total	**251**	**123**

narrowing the possibility of generating a statistically significant result (type I error) (HOLM, 1979). To enlighten these intricate multiple results, a Multiple Correspondence Analysis test was performed comprehending all parameters, to visualize the pattern of relationships between the microhabitat features and their usage by both species (ABDI & VALENTIN, 2007).

RESULTS

Lizard occupancy

A total of 699 observations were registered, 476 (68%) belonging to the native *P. virescens* and 223 (32%) to the introduced *P. siculus*, present in only three sectors (Table 1).

The native *P. virescens* population shows an overall heterogeneous distribution in the urban matrix. On the other hand, the occupancy of the invasive species only clusters within the original area of introduction (garden in front of the Lisbon Oceanarium), as well as in adjacent gardens (Fig. 2a). A single individual of *P. siculus* was also o-bserved in a previously unknown location (a garden spot placed some hundreds of meters away from the main area), where *P. virescens* often occurs (Fig. 2b).

Degree of spatial segregation between Podarcis siculus *and* Podarcis virescens

The Chi-squared tests of independence support the main differences found in the spatial variables tested between *P. siculus* and *P. virescens*, as explained in the following sections concerning habitat, through the analysis of the graphics and tables ($P < 0.001$ for nearly all values, Table 2), regarding the behaviour shown by the lizards during observation, the elements present in the microhabitat and its use by both species. The only exception was the parameter "Distance to Refuge", which was similar for both species ($P = 0.223$, Table 2). The Holm-Bonferroni correction method confirmed the obtained results, whereas the previously mentioned parameter remained as the only to not reject the null hypothesis.

The Multiple Correspondence Analysis test plots for both species (Fig. 6) showed that, despite overlapping for the most part, *P. siculus* only occupies part of the spatial niche of *P. virescens* at the multivariate level, which could provide some answers regarding the possible event of future expansion and its impacts on *P. virescens*.

Table 2: Results of the chi-Squared statistical tests, used for comparison between species. Significant *P*-values highlighted in bold. Adjusted Holm-Bonferroni *P*-values on the right.

Tested Parameters	$\chi 2$	df	*P*-value	Holm-Bonferroni P-values
Behaviour	19.401	3	***P*< 0.001**	*P* = 0.01
Perch type	81.089	3	***P*< 0.001**	*P* = 0.005
Perch height	51.731	3	***P*< 0.001**	*P* = 0.03
Perch slope	22.236	2	***P*< 0.001**	*P* = 0.01
Refuge type	98.363	6	***P*< 0.001**	*P* = 0.01
Distance to refuge	4.379	3	*P* = 0.223	*P* = 0.01
Number of Trees	34.2	1	***P*< 0.001**	*P* = 0.02
Number of Shrubs	43.719	2	***P*< 0.001**	*P* = 0.01
Number of non-woody plants	12.945	2	***P*= 0.002**	*P* = 0.05
Number of dead branches/trunks	33.585	3	***P*< 0.001**	*P* = 0.004
Substrate texture	62.558	2	***P*< 0.001**	*P* = 0.01
Distance to vegetation cover	21.584	2	***P*< 0.001**	*P* = 0.01

Among the variables which contributed the most for the relative inertia values, were the Refuge and Perch Type Rock, as well as Vertical Perch Slope, and Artificial Perch Type and Substrate Texture.

Habitat use

Both species seem to occur in larger numbers (84% of the *P. siculus* total and 97% of *P. virescens*) in areas with few or no trees (0 to 2) (Fig. 3a), with the autochthonous species being less dependent on the existence of arboreal and non-woody plants cover on site (Fig. 3c - the congeners showed a marked preference for areas without non-woody plants, 73% of *P. siculus* and 80% *P. virescens*). Similarly, 58% of *P. siculus* and 56% of *P. virescens* individuals appeared in spots without shrubs (Fig. 3b)

but were also present in areas with 1 to 5 (23% and 39%) shrubs. Both species used areas with a moderate presence of dead wooden matter (up to 30 branches/trunks/etc.), counting for 71% of *P. siculus* and 56% of *P. virescens* observations (Fig. 3d). Substrate texture (Fig. 3e) enhances *P. siculus* as having occupation for earthy open spaces (79%), while *P. virescens* appears to be a more generalist species, though often found in earthy substrate (50%), dividing the remaining half of observations almost equally between artificial and mixed grounds.

Micro-habitat features

The most used perch for both lacertids (Fig. 4a) appears to be the ground (more evident for *P. siculus*, 81%, than for *P. virescens*, 53%), whereas *P. virescens* also choos-
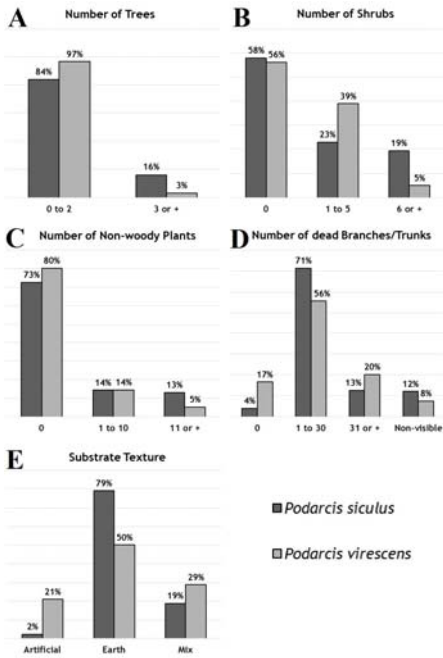
Figure 3: Results concerning micro-habitat elements: number of Trees (A), Shrubs (B), Non-woody Plants (C), dead Branches/Trunks (D) and Substrate Texture (E).
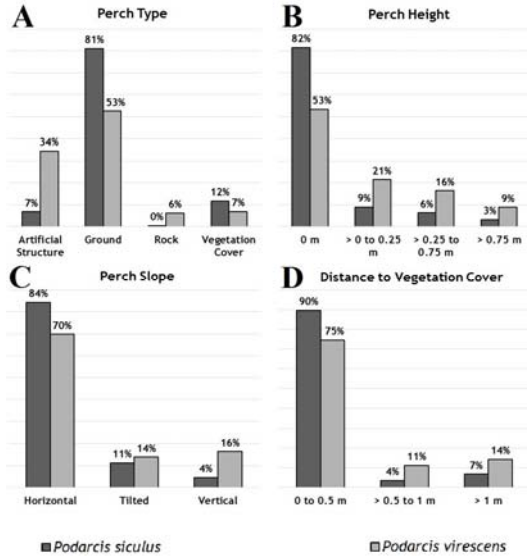

Figure 4: Results concerning each species use of the available micro-habitat: Perch Type (A), Height (B), Slope (C) and Distance to Vegetation Cover (D).

es perches located in artificial stru-ctures (34%). The rocky perches were the least chosen, by both species. Concerning to the perch height, soil level was the most observed (Fig. 4b), with *P. siculus* showing ground dwelling attributes (82%), similar to slightly more than half of the *P. virescens* individuals. Of the latter, 21% are still distributed at a reduced height (from 0 to 0.25 m), with only 9% of the individuals found at heights above 0.75 m. The slope assumed by each lizard at perch site (Fig. 4c) was mostly horizontal for both species (84% of *P. siculus* and 70% of *P. virescens*). The "Vertical" posture was the second most observed in *P. virescens* and the least observed in *P. siculus*. High dependency

(90% for *P. siculus* and 75% for *P. virescens)* of vegetation cover (herbaceous, shrubs, trees – Fig. 4d) near to the perch (up to 0.5 m) was detected.

The behaviour activity of lizards (Fig. 5a) shows that both species were often seen basking (57% for *P. siculus* and 64% for *P. virescens*), but also active (18 to 20%), suggesting a similar daily tasks pattern. When disturbed, the lizards often seek the available vegetation cover as refuge (Fig. 5b), which was evident (50%) for *P. siculus*, while the native lizard chose between ve-getation cover and artificial structures (26% and 32%, respectively), the latter one which the exotic species apparently tends to avoid. Approximately 22%
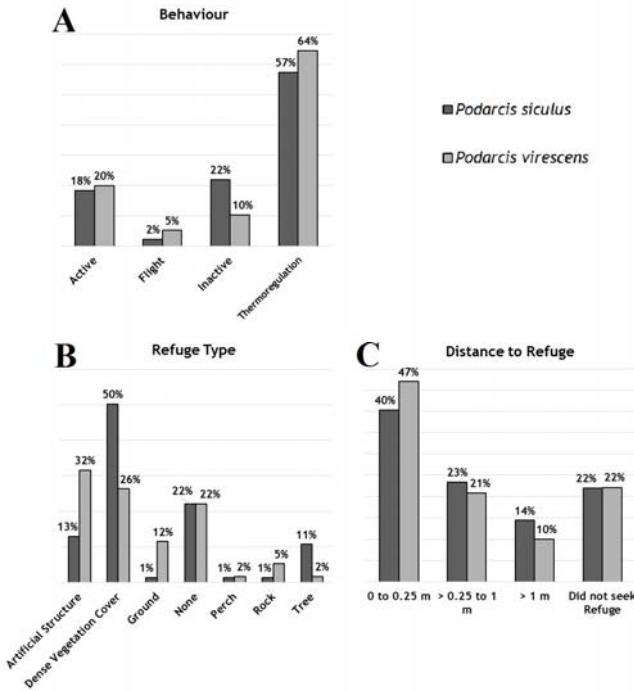
Figure 5: Results concerning each species use of the available micro-habitat: Behaviour (A), Refuge Type (B) and Distance to Refuge (C).

of the observations for both species did not seek refuge, remaining in the perch without mo-ving, or escaping without seeking shelter in the nearby elements. About 40% of *P. siculus* and 47% of *P. virescens* prefer to carry out their daily tasks near (up to 0.25 m) to a possible refuge (Fig. 5c), with the number of observations decreasing as the distance to refuge increases.

DISCUSSION

Although there were found some significant differences in the use of several categories of habitat tested between both lizard species, that is not the major evidence. Indeed, all observations in Parque das Nações (Lisbon) point to a scenario of absence of expansion by the alien species *P. siculus*, as previously suggested by LOUREIRO *et al.* (2008) and Sá-Sousa's personal ob-

servations. There, this species individuals show the behaviour and ecological patterns typical of their own species (CORTI & LO CASCIO, 2002). However, the obtained data suggests that local spatial segregation was most probably due to competitive exclusion, as *P. siculus* replaced *P. virescens* as the only lizard species present at the original introduction site. In fact, *P. siculus* has a larger body size than *P. virescens*, which is probably an advantage in competitive encounters since, according to DOWNES & BAUWENS (2002), larger species tend to be dominant over smaller ones. This appears to have favoured the exotic wall lizard to the detriment of the native species, since in Lisbon the species are shown to be sympatric but never syntopic.

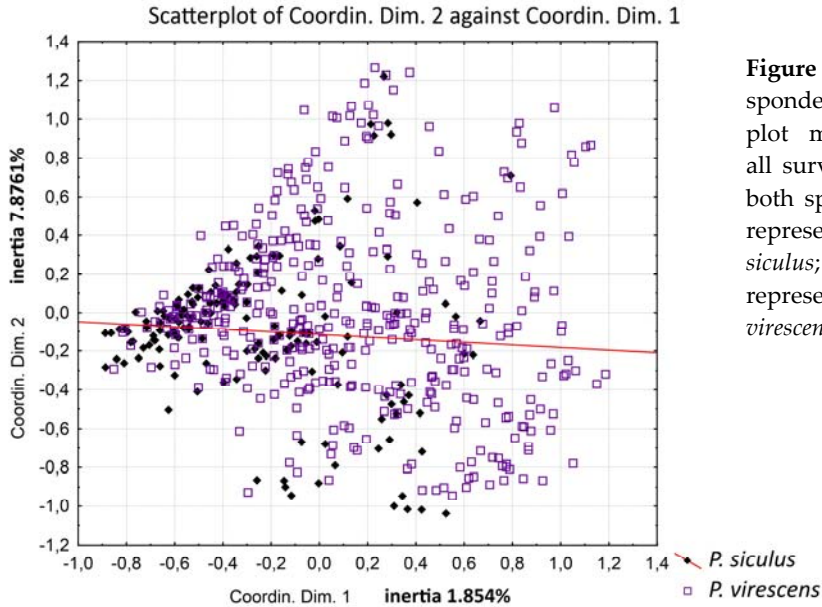Sympatric species, when in direct competition, often spatially separate them-

Figure 6: Multiple Correspondence Analysis Scatterplot map, comprehending all surveyed parameters for both species. Black symbols represent introduced *P. siculus*; purple, open symbols represent autochthonous *P. virescens*.

selves due to differences in the structural niche (e.g. diameter and height of the chosen perch) and, in some cases, in the microclimatic environment (eg. open vs. shaded areas) or by displacement of individuals through both competition and anti-predatorial/survival behaviour (DOWNES & BAUWENS, 2002; ŽAGAR *et al.*, 2015). Syntopic lizard populations were shown to alter their predation risk by using specific habitats based on the availability of refu-ges that provide protection against shared predators, which act as competition mediators, indirectly inducing asymmetrical competition and shifts in segregation patterns (SMITH & BALLINGER, 2001; ŽAGAR *et al.*, 2015). The results in Lisbon suggest that both species use the available microhabitats differently.

The exotic species *P. siculus* preferentially uses microhabitats having a mostly earthy substrate texture, avoiding fully artificial substrates. It shows, to some extent, dependence on the existence of some type of vegetation cover (herbaceous, shrubs, trees) relatively close to the perch, being mainly found at soil level. Despite its observable proximity to vegetation cover, the microhabitats used by *P. siculus* tend to be poor in tree and shrub cover, with most of the individuals observed also showing preference for areas with little to none dead wooden matter on the ground. It was also observed, in this species, tendency to avoid artificial structures as possible shelter, preferring the available vegetation cover for this purpose - and thus perhaps limiting its distribution in this predominantly urban area.

It is tricky to explain the present lack of expansion of *P. siculus* in Lisbon, especially since this species often spreads into urban areas in native Italy, where it is sympatric with *P. muralis*: *P. siculus* prefers the

driest places, while *P. muralis* the moisten ones (CORTI & LO CASCIO, 2002). Perhaps it is still a question of a short time lapse (two decades) after the presumed date of introduction (not yet the sufficient time and effort required to efficiently use the newly occupied habitat - STAMPS, 1995), the possibility of the exotic species larger body size being a disadvantage at this particular introduction site (making this lizard more prone to predation or less capable of dealing with the introduction site's conditions), and/or lack of a reproductive effective high enough to promote recruitment of young lizards towards new adjacent spots (more individuals = more potential dispersers - VIGNOLI *et al.*, 2012).

This diminished reproductive effective might also be due to high predation pressure, as several individuals of *Tarentola mauritanica* were observed in the *P. siculus* place of occurrence and nearby sites, a species which may consume individuals of the latter and other species of lacertids, both immature and adult (PELLITTERI-ROSA *et al.*, 2015). This population growth control may be carried out by *P. siculus* adult individuals as well, in cases of cannibalism (such as those previously described by OUBOTER, 1981, BURKE & MERCURIO, 2002, CATTANEO, 2005, CAPULA & ALOISE, 2011 and GRANO *et al.*, 2011), or by cats, dogs or seagulls present in the area (BURKE & NER, 2005). Other lacertid found inside the study area limits was *Psammodromus algirus*, which could spark interactions with yet unknown consequences in case of future contact with the exotic population.

Although the introduction area in Parque das Nações is surrounded by artificial structures that apparently provide little or no shelter to these *P. siculus* individuals, they might attempt to expand. If landscape work is carried out in the adjacent areas and gardens, it may possibly promote the dispersal of *P. siculus*, by altering their previously unfavourable conditions and resources, since dispersal behaviour seems to be in some aspects influenced by external factors, such as intraspecific competition and habitat quality (VIGNOLI *et al.*, 2012). This brings us to the single individual found off the core area of introduction, which may occur most likely due to the translocation of plants or trees between gardens of Parque das Nações, an action witnessed during the sampling rounds. Most of these movements are usually carried out in the winter, due to the reduced plant activity, which facilitates the success of the transplant, but also favours exotic reptile species introductions, often hibernating inside available cavities in trees or plants, so that these movements do not cause the individual to escape from the refuge (VALDEÓN *et al.*, 2010). Despite the contribution of such activities and regular maintenance work to introductions, they can also rake the soil and diminish the available vegetation cover, destroy lizard eggs buried in the ground or sand, reduce the availability of perches for thermoregulation and disturb the existing microclimate at the place of introduction – yet again providing a plausible argument for the absence of expansion. This clustering could be due to high predation pressure, short time span since introduction, competition with autochthonous species, lack of available favourable resources and/or microhabitat degradation – or synergy of all the previous factors. If expansion is

to occur, data suggests that it possibly would not exclude the autochthonous species entirely, since *P. siculus* seems to not occupy all the available habitats in the Parque das Nações area, with a reduced usage of the more artificial and/or rocky ones.

Nevertheless, the present study demonstrates how there is no homogeneity of introduction/invasion cases, as naturalized populations can vary enormously in their ecological dominance, from those that remain in small numbers in a single locality, to those that spread like wildfire on a large scale and become numerically dominant (Kraus, 2009). Similarly, for the other known introduced populations of *P. siculus* in the Iberian Peninsula, there is no evidence of high expansion in Almeria, while it seems to be decreasing in Cantabria or even being extirpated from La Rioja and Catalonia (Carretero & Silva-Rocha, 2015). Since the eradication of alien reptile populations is virtually impossible once established (Kraus, 2009), we suggest the monitoring and containment of the exotic population, as well as planning maintenance works considering the presence of the species, as prevention measures to avoid future territorial expansion and consequent impacts on native fauna.

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