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Rediscovery of the golden-striped salamander *Chioglossa lusitanica* of Sintra, Portugal

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The southern distribution limit of the Iberian endemic and threatened golden-striped salamander (*Chioglossa lusitanica*) is located about 170 km NE of Lisbon, Portugal. In 1943 Anthero Seabra reportedly introduced a few specimens in the Sintra mountains, about 20 km NW of Lisbon, but the exact introduction site is not known. The existence of a reproducing population in Sintra became a recurrent topic among herpetologists and, despite the efforts of several individuals and teams, was not confirmed until now. After a fortuitous finding of one individual, we report here the results of a monitoring program involving photoidentification of adults and juveniles conducted during the autumn and winter of 2015/16 and 2016/17. We found a reproducing population living along a 107 m stretch of a single stream. Phenology and larval sizes were similar to those of other populations. Notable aspects of this population are its small size (estimated at 339 ± 35 individuals) and confinement to a very small area, the low proportion of individuals that were recorded moving along the stream and the very short distances travelled by those individuals, and the large size of several adults, including the longest individual recorded so far.

Key words: amphibia, assisted migration, caudata, historical introduction, isolate

INTRODUCTION

The golden-striped salamander, *Chioglossa lusitanica* Barbosa du Bocage, 1864, is endemic to North-western Iberian Peninsula, typically living near small brooks with fast-flowing, well-oxygenated water and dense surrounding vegetation, in mountains where annual precipitation exceeds 1000 mm (Arntzen, 1981). Adults are mainly nocturnal, sheltering in caves, or under rocks or leaf litter, during the day. In Portugal, reproduction may occur between September and May depending on climatic factors, mainly precipitation (Arntzen, 1981; Sequeira, Ferrand and Crespo, 2003).

Golden-striped salamanders have a slender body and an exceedingly long tail that corresponds up to two thirds of its total length (Arntzen, 1994). Other unusual traits that distinguish it from other European caudates are a protractile tongue, extreme lung reduction and tail autotomy (Bocage, 1864; Arntzen, 1981). Due to their unique morphology, ecology and endemicity, *C. lusitanica* is a species of high conservation interest, yet threatened by habitat destruction and agrochemical pollution of streams. It is listed as vulnerable in both the IUCN Red List of Threatened Animals (Arntzen et al., 2009) and the Red Data Book of Vertebrates of Portugal (Cabral et al., 2005).

The geographical distribution of *C. lusitanica* has been studied by several researchers (Arntzen & Teixeira, 2006) and is currently well documented, at least in Portugal (Loureiro et al., 2008). The species occurs from Asturias and Galiza in Spain to north-western and central Portugal (Fig. 1.2) with the Alvéolos Mountains, just north of the Tejo river, as the southern-most location (Loureiro et al., 2008). However, there are three historical indications of population isolates in the south (Fig. 1.2): i) the single record south of the river Tejo, in Elvas, for which it is now clear that arose from misreading a faded label (Crespo, 2008); ii) "La Serrota" in Ávila, Central Spain (Pérez-Arcas, 1874); iii) the Sintra mountains (Seabra, 1943). In 1943, the zoologist Anthero Seabra captured "a few specimens" (the exact number is unknown) of golden-striped salamanders in the Buçaco mountains, central Portugal and introduced them in the Sintra mountains (Seabra, 1943). These mountains are about 170 km SW of the recorded species distribution limit and 20 km NW of Lisbon (Fig. 1.1). Seabra (1943) mentions that environmental conditions at Sintra were similar to those of the northern mountains where the species was common, and would therefore probably be adequate for the establishment of a reproducing population.

Even though many a herpetologist has tried to find *C. lusitanica* in Sintra mountains since then, it has remained elusive (Almaça, 1959; Malkmus, 1979; Teixeira et al., 1998; Loureiro et al., 2008). Around 1990, Gaston-Denis Guex found one single individual in Sintra while looking for *Salamandra salamandra* (Arntzen, 1999). Unfortunately, the exact location of that individual was not recorded. In 2015, Nuno Reis and João Martins

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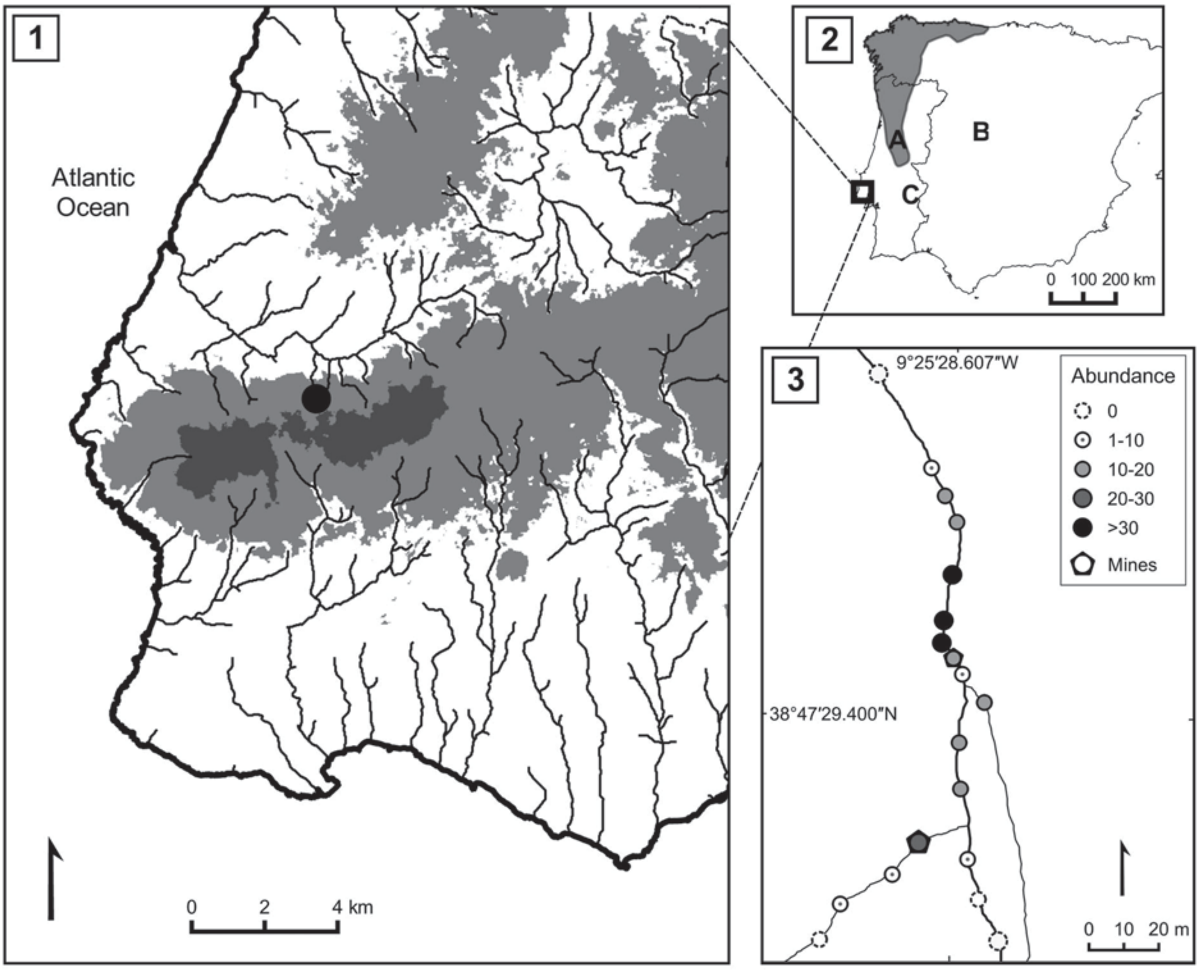

Figure 1. (1) Sintra mountains hydrographic network and topography (light grey – above 150 m; dark grey – above 350 m a.s.l.); **(2)** Current distribution of *C. lusitanica* on the Iberian Peninsula (grey); A –Buçaco; B – La Serrota; C- Elvas; **(3)** Local distribution and abundance (number of individuals first found in each stream section) of *C. lusitanica* along the streams.

photographed one adult golden-striped salamander that they found in a stream near Sintra. We report here on the distribution, population size, phenology, movements and body size distribution of this *C. lusitanica* population.

METHODS

Study area

The field survey was conducted in Sintra mountains from November 2015 to April 2016 (Year 1) and from October 2016 to April 2017 (Year 2) in the two streams that flow along the valley where the first individual was spotted (valley coordinates: 38˚47'44 N, 9˚25'25 W to 38˚47'19 N, 9˚25'30 W - Fig. 1.2). Altitude of the surveyed area varies between 82 m and 281 m a.s.l. The climate is Mediterranean with Atlantic influences, with a hot dry summer and a mild rainy winter. The average annual precipitation is 727 mm and average annual temperature is 15.3˚ C (data from Colares meteorological station, 2.3. km NW of the surveyed area; SNIRH, 2018). Both streams flow along large granite boulders, forming small caves and inner pools; one of the streams is fed by waters flowing out of two abandoned mine galleries. Non-native invasive trees, such as *Pittosporum undulatum* and *Acacia* sp., are the dominant vegetation along the banks. In addition, *Eucalyptus globulus* and the native *Quercus suber*, *Castanea sativa* and *Pinus pinea* are present in low density. The sub-arboreal stratum is composed by mosses, vines, shrubs, ferns and leaf litter.

A total of 37 visits (23 on the first year and 14 on the second) were conducted at dusk and during the night. The time between visits was 6.32 ± 5.36 days in the first year and 11.57 ± 7.96 days in the second. About 1 km of stream plus a 3-meter band bordering each margin were prospected; additional prospecting for eggs or developing embryos was conducted on sheltered locations, such as under large boulders and crevices, or in the mines. Water temperature and pH were measured in all visits and ranged from 11˚ C to 15˚ C and from 6.2 to 6.8 respectively.

Distribution and population structure

Sixty survey points were defined along the two streams according to landscape features, and each captured individual was assigned to the nearest point. If no individuals were found after searching for 30 minutes and 3 sampling days, it was assumed that none was present in that stream section. This procedure was repeated on the beginning of the second year at all survey points, regardless of its status on the first year. The distribution map depicted in Figure 1.3 was produced using QGIS software, version 2.14 Essen.

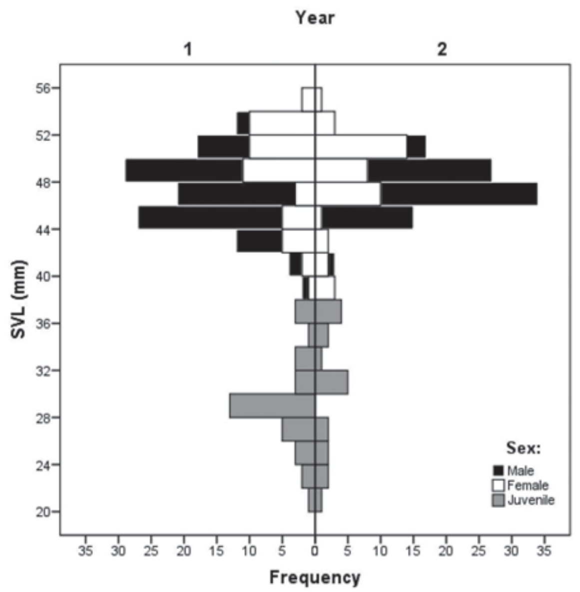

Figure 2. Histogram of body size classes (SVL, mm) on Years 1 and 2

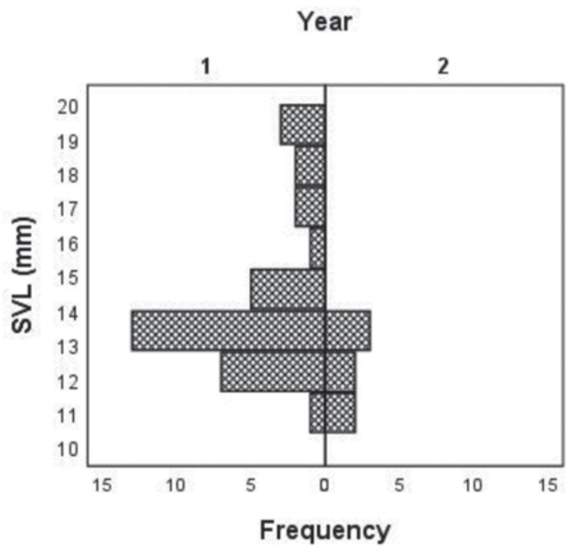

Figure 3. Histogram of body size classes (SVL, mm) for larvae on Years 1 and 2

Snout-vent length (SVL) of all captured individuals was measured from snout to the insertion of the hind limbs, using Fiji (Schindelin et al., 2012) software tools over dorsal photographs, with animals photographed in a natural, curved position. Several pictures of each individual were measured; the measurement error was 0.47 ± 0.37 mm for adults (1% of their average length). Individuals with SVL ≥ 38.0 mm were considered adults (Arntzen, 1981). Adults and juveniles were weighed with a digital scale to the nearest 0.01 g. Sex identification was based on the presence (males) or absence (females) of cloacal swelling and any unusual observations (e.g. body injuries) were registered.

The sex-ratio was calculated by dividing the number of males by the number of females. Body condition was estimated using the scaled mass index proposed by Peig & Green (2009). This index standardises the mass of the salamanders to an average SVL (46.9 mm for males and 48.4 mm for females, in our sample) using the scaling relation between log mass and log SVL.

Photo-identification was based on the unique dorsal pattern. Growth rate was calculated from the difference between final SVL and initial SVL of a recaptured individual divided by the number of days between captures. We grouped the data as: "autumn" - October and November; "winter" - December and January; "spring" - February to April (this final interval included three months as there were few captures in April). We then tested for differences in physical condition among seasons in each sex with ANOVA, after checking for homocedasticity. Net displacement distance was assumed as the distance between the first and final points of capture for each individual, and were calculated within each year and between years. Results are expressed as mean ± SD; α was set to 5%. Statistical analyses were conducted with the software SPSS (version 24).

Population size estimate

To estimate population size, we used the open-population POPAN model incorporated into the software programme MARK (White & Burnham, 1999). As sampling was not constant thought time, capture histories were built by aggregating 30 successively captured adults within each year, resulting in 10 groups (six in the first year and four in the second). A goodness-of-fit (GOF) test was run to test the capture-mark-recapture assumptions of the model using the program RELEASE GOF (Burnham et al., 1987), also included in MARK. Based on the result of TEST 2 + TEST 3 of RELEASE, a *post hoc* variance inflation factor ($\hat{c}$) was estimated by [(TEST 2+TEST 3)/df = $\chi 2$/df]. Since $\hat{c}$ =2.88, the most parsimonious model was identified using the Quasi-Akaike Information Criterion (QAIC) (Burnham & Anderson, 2002).

A POPAN model can estimate four parameters: the survival rate (φ), the probability of capture (*p*), the probability of an individual to enter the population studied (*pent*) and the size of the population (*N*). Given the short migration distances and the limited scope for emigration or immigration (see Results), we considered *pent* = 0 and modeled φ and *p* as constant (.) or time varying (t). The following functions were used: sinus or logit link function for φ and *p* and log-link and identity link function for *N*.

RESULTS

Distribution and population structure

A total of 308 different golden-striped salamanders (225 adults, 40 juveniles and 43 larvae) were found along a continuous section of 107 m in one of the streams and its tributaries, which represents 11% of the total length of the prospected streams. Within this section there are two main nuclei separated by 50 m (Fig. 1.3), both near the mines. No other individuals were found in any of the adjacent streams.

Table 1. POPAN models for the *C. lusitanica* population in the Sintra Mountains. Model-choice criteria: corrected Quasi-Akaike Information Criterion (QAICc), and difference of QAICc values from the best fitting model (ΔQAICc). w_i - model weight. *K* - number of estimated parameters. For model descriptions, see Methods.

Model	QAICc	ΔQAICc	w_i	*K*
φ(t) *p*(.) (logit link for φ, sinus link for *p*, identity link for *N*)	216.516	0.000	0.870	3
φ(t) *p*(t) (logit link for φ and *p*, log-link for *N*)	221.654	5.138	0.067	13
φ(.) *p*(t) (logit link for φ and p, identity link for *N*)	221.920	5.404	0.058	12
φ(.) *p*(.) (logit link for φ and *p*, identity link for *N*)	226.916	10.400	0.005	3

Males were smaller than females (t_{228} = 3.55, P < 0.001; Fig. 2). This was also seen in their median SVL (47.0 mm for males and 49.6 mm for females). With an SVL of 55.8 mm, the largest female captured was the largest *C. lusitanica* individual ever recorded, 1.8 mm longer than the previous maximum, found at Galicia, about 350 km N of Sintra (Brizzi et al., 1999). Despite its large size, this individual grew 3.53% of its initial SVL in 33 days. The smallest juvenile was recorded in year 1 with SVL of 20.2 mm (Fig. 2). Larval SVL varied from newly hatched (9.9 mm) to pre-metamorphic (19.2 mm), with a bimodal distribution in year 1 (Fig. 3). There were low frequency injuries such as tail autotomy (24 individuals). Other anomalies were also rare: unusual short toes (16 individuals), skin deformities (3 individuals) and bloat-like disease (1 individual).

Matings were only seen in November (both years) and gravid females were seen from late October to early January. In the first year, most of the adults were found in March (22.98%), while in the second year most adults were found in October (38.23%) and March (14.13%). The sex-ratio in the first year was 1.60 and in the second year was 1.39. Males were more frequent during autumn and winter - sex ratios of 2.00 in November and of 3.09 in December of the first year; of 2.29 in October of the second year. Females were more frequent only in late spring, with sex ratios of 0.50 and 0.67 in April of the first and second years, respectively. The body condition of males was significantly higher in spring than in the other seasons (ANOVA, $F_{(2, 131)}$ = 8.71, P < 0.001; Tukey HSD test, P < 0.001 for Autumn vs Spring; P = 0.02 for Winter vs Spring). This pattern was not found in females (ANOVA, $F_{(2, 83)}$ = 1.02, P = 0.36).

Newly hatched larvae (near their egg capsules) were seen in January (year 1) and in November (year 2). Egg capsules and developing embryos (N=41) were found underneath rock crevices in one of the mines and in the stream adjacent to it. At the stream, larvae were more frequent in February of year 1 (48.6% of the larvae of that year) and in November of year 2 (50%).

Movements and population size estimate

Considering the adults, recaptures represented 36.8% of captures by the end of the first sampling year and 46.2% by the end of the second; only 31.1% of the adults captured in the second year had been captured in the first. At the final visit, 10 out of 17 adults were new individuals. As for juveniles, recaptures constituted 14.71% the captures at the end of the first year and 11.11% at the end of the second. No juveniles were recaptured between years.

Most recaptures occurred on the same stream section – only 8% of the salamanders moved between stream sections in the first year (three juveniles, six males and one female), and 4.7% (five males) in the second. Considering the animals captured in both years, displacements were more frequent - 36.36% of the recaptures (seven males and five females) were found in different stream sections. Most movements were upstream (eight out of ten in the first year, four out of five in the second year and seven out of 12 between years). The maximum net distance moved was 48.2 meters downstream in the first year, by a male (average 15.38 ± 12.99 m), 26.3 meters upstream in the second, also by a male (average 23.73 ± 20.16 m) and 69 meters downstream between years, again by a male (average 16.86 ± 7.27 m).

The GOF test results showed that the data did not follow all assumptions (TEST 2: χ^2_{11} = 13.28, P = 0.27; TEST 3: χ^2_{11} = 50.07, P < 0.001; TEST 2 + TEST 3: χ^2_{22} = 63.37, P < 0.001). However, if $\hat{c}$ values are ≤ 3, the lack of fit is acceptable (Lebreton et al., 1992). The most parsimonious model considered a constant capture probability (of 0.1) and a survival probability dependent of time (Table 1). In this model, survival between visits was estimated to vary from 0.82 to 1. This population was followed for only two years and therefore it is not possible to estimate annual survival. Population size was estimated to consist of 339 ± 35 individuals, which corresponds to 3.2 salamanders per linear meter of the occupied stream section.

DISCUSSION

Finding a reproducing population of an iconic West-Iberian endemic salamander so close to Lisbon and in an area regularly surveyed by several herpetologists (including *R. Rebelo* and *E. Crespo*, both senior authors of this paper) over several decades is remarkable. The simplest explanation for its presence is the introduction reported by A. Seabra in 1943, although Seabra does not refer to the exact location of the introduction. However, the fact that the Sintra Mountains are an interglacial refuge for other Iberian north-western species, such as *Lacerta schreiberi* Bedriaga, 1878, a lacertid that maintains an isolated population in Sintra, in habitats similar to those of *C. lusitanica* (Brito et al., 1996), points at the possible presence of a relic population of this salamander (Alexandrino et al., 2007). In fact, climate and orography-based distribution models predicted the suitability of the north-facing slopes of Sintra mountain for *C. lusitanica* (Arntzen & Teixeira, 2006), corroborating the opinion of Seabra (1943). Genetic analyses will most likely contribute to identifying the origin of this isolated population.

The Sintra salamanders live near abandoned mines and subterranean streams, microhabitats that maintain high ambient humidity and constitute optimal shelter and reproduction sites for the species (Sequeira, Ferrand & Crespo, 2003) and that have been regularly identified as summer refugia for local populations (Arntzen, 1981; Arntzen, 1994; Sequeira et al., 2001; Arntzen, 2015). The population size structure shows a continuous distribution across juveniles and adults, indicating successful reproduction over the years. The bimodal larval size distribution of the first year's sampling is consistent with the sizes of the first and second-year larval cohorts of the northern populations (Arntzen, 1981; Lima, Arntzen & Ferrand, 2000). Our very small sample of metamorphs and small juveniles does not allow for statistical comparisons with other populations; nevertheless, the sizes of the pre-metamorph and of the smallest juveniles at Sintra were similar to that reported by Arntzen (1981) for two populations near Porto. The very large body sizes of some adults may result from long lifespans, high growth rates (possibly influenced by the relatively higher autumn and winter temperatures at Sintra, when compared to the condition in the NW of the Iberian Peninsula), or both.

Chioglossa lusitanica can be either remarkably faithful to a favourable site (e.g., mines) or to migrate rapidly over long distances (Arntzen, 1994). Migrants can constitute a relatively high proportion of a population (up to 49%; Arntzen, 1994), but this was not found at Sintra, where less than 10% of the individuals were found to move between stream sections within the same year. Both the average and maximum distances moved at Sintra are much shorter than at the two northern populations studied by Arntzen (1994), where displacements over 350 metres were registered in just one night; they are however, similar to the values recorded by Sequeira et al. (2001), also near Porto, but in a more intensively human-modified habitat (abandoned farm with dry-stone walls). The higher sedentarism of the Sintra salamanders may be due to the inhospitable habitat surrounding the stream, considering both microclimate and vegetation cover (Vences, 1993).

Reproduction at Sintra occurred from November through January, coinciding with the known reproductive season for this species in its main distribution range (Vences, 1990; Sequeira, Ferrand & Crespo, 2003). The physical condition of males was significantly higher at the end of the activity season, suggesting that they were accumulating reserves for aestivation (Arntzen, 1981). For females this trend was not noticed, as the variability in their condition was much higher, most likely due to the egg development and laying processes. The first observation of eggs and larvae occurred much later in the first than in the second year, which may have been due to the very strong, torrential rains of October 2015 (150 mm, twice the normal precipitation for that month; SNIRH, 2018). The torrential rains may have inhibited egg laying and/or driven the larvae to sheltered, inaccessible pools. However, the strong stream flow did not displace all the larvae downstream, as a fair number of larvae from the second year cohort was seen later at the stream.

Low human disturbance and absence of water or soil contamination have probably contributed for the continued existence of the Sintra population. Arntzen (2015), showed that larval survival is a crucial factor for the maintenance of *C. lusitanica* populations. We could not estimate larval survival up to metamorphosis, but our data on larval size distribution and metamorph sizes, although very limited, do not support the hypothesis of a poor habitat for larval development. On the other hand, the terrestrial habitat at the occupied site is not favourable for the salamanders and may hamper population increase and expansion, as a eucalyptus cover was already shown to be associated with less food resources for *C. lusitanica* (Vences, 1993), and eucalyptus plantations were related to a severe local decline (Arntzen, 2015). Our estimate of population size revealed a small effective, especially when compared with populations from the main distribution range that reach abundances of 11-12 salamanders per meter of stream (in some locations up to 16-17 salamanders per meter of stream) (Arntzen, 1981; Teixeira et al., 1998). In other locations, the size of the breeding population was found to consist of 1/5 to 1/6 of the total population (Arntzen, 2015) and a similar pattern may explain the low probability of capture, the relatively high number of new individuals captured up to the final visit and the violation of the TEST 3 of RELEASE, which is affected by heterogeneity in capture rates (White & Cooch, 2012). Continued sampling may ultimately reveal a larger population.

CONCLUSION

It is startling how amphibian populations continue to be rediscovered, even near dense populated areas like Lisbon. This population may have remained undetected for more than 70 years (and much more, if it is a remnant from glacial eras). The rediscovery of species and populations of amphibians previously thought to be extinct or nonexistent is not uncommon, as the case of the iconic Mallorcan midwife toad, *Alytes muletensis*, which was considered extinct until its discovery in 1980 (Mayol & Alcover, 1981), or the more extreme rediscovery of *Latonia nigriventer* Mendelssohn & Steinitz, 1943 in Israel (Biton et al., 2013). Although the origin of this population remains uncertain, *C. lusitanica* is a threatened and protected species. If future studies reveal that the Sintra population results from a 70-year old introduction, an interesting conservation dilemma will result, adding to the current discussion on whether the conservation of introduced species in their non-native range is justifiable (e.g., Gibson & Young, 2017), even in the cases where they reveal an invasive character (Marchetti & Engstrom, 2015). Furthermore, the longterm survival of this single population of a species restricted to favourable microhabitats and with so many exquisite ecological requirements bring some hope for future reintroductions, or even assisted migration to sites previously unoccupied, but deemed climatically favourable for other amphibian species.

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