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Sex determination and optimal development in the Moorish gecko, *Tarentola mauritanica*

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Abstract

Under temperature sex determination (TSD), sex is determined by temperature during embryonic development. Depending on ecological and physiological traits and plasticity, TSD species may face demographic collapse due to climate change. In this context, asymmetry in bilateral organisms can be used as a proxy for developmental instability and, therefore, deviations from optimal incubation conditions. Using *Tarentola mauritanica* gecko as a model, this study aimed first to confirm TSD, its pattern and pivotal temperature, and second to assess the local adaptation of TSD and variation of asymmetry patterns across four populations under different thermal regimes. Eggs were incubated at different temperatures, and hatchlings were sexed and measured. The number of lamellae was counted in adults and hatchlings. Results were compatible with a TSD pattern with males generated at low and females at high incubation temperatures. Estimated pivotal temperature coincided with the temperature producing lower embryonic mortality, evidencing selection towards balanced sex ratios. The temperature of oviposition was conservatively selected by gravid females. Asymmetry patterns found were likely related to nest temperature fluctuations. Overall, the rigidity of TSD may compromise reproductive success, and demographic stability in this species in case thermal nest choice becomes constrained by climate change.

KEYWORDS
asymmetry, development, developmental stability, geckos, *Tarentola mauritanica*, temperature-dependent sex determination

1 | INTRODUCTION

Sex determination is a crucial biological process implemented through highly diverse mechanisms, roughly dictated genetishcally, environmentally or both. Under environmental sex determination (ESD), sex is established in response to a nongenetic factor experienced within a discrete period after fertilization (Bull, 1983). Within ESD, temperature sex determination (TSD) is a special case where the sex of progeny is determined by the temperature experienced during their embryonic development (Bull, 1980; Janzen & Paukstis, 1991; Valenzuela & Lance, 2004). Among reptiles, all crocodilians and sphenodontians have exclusively TSD, whereas squamates and turtles can exhibit either TSD or genetic sex determination (GSD) (Janzen & Phillips, 2006; Sabath et al., 2016) with a few species combining both (e.g. *Pogona vitticeps* in Quinn et al., 2007). Three different patterns of TSD can be found in vertebrates: lower temperatures result in males and higher in females (MF pattern); lower temperatures result

in females and higher in males (FM pattern); and a third pattern where intermediate temperatures result in males and the extremes in females (FMF pattern) (Bull, 1983; Pezaro et al., 2017; Shine, 1999). The temperature that produces an even sex ratio is known as the pivotal temperature, around which there is a transitional range of temperatures (TRT), where mixed offspring sex ratios take place in variable proportions (Bull, 1980; Hulin et al., 2009).

While TSD may be adaptive (e.g. Pen et al., 2010; Rhen & Lang, 1995; Spencer & Janzen, 2014; Warner & Shine, 2008), it also carries substantial environmental risks, namely, if thermal availability for offspring development becomes limited. It is now widely recognized that recent shifts in climate regimes are affecting the phenology and survival of many different animal and plant species (Cunze et al., 2013; Thomas et al., 2004; Thuiller et al., 2008). Certainly, TSD species have survived various climatic extremes throughout recent paleoclimatic history (e.g. glaciations), which suggests acclimation or adaptation. However, increasing evidence indicates that contemporary global warming is taking place at such a fast pace that it might overcome biological responses and shift sex ratios, provoking demographic collapse (e.g. feminization in sea turtles in Hawkes et al. (2007), Jensen et al. (2018), Santidrián Tomillo et al. (2015) and Tanner et al. (2019); masculinization in the tuatara in Mitchell and Janzen (2010) and Mitchell et al. (2010)). If these findings are to be generalized, it will depend on species' ecological and physiological traits and their evolutionary potential (Mitchell & Janzen, 2010). How could then TSD species mitigate the effects of contemporary climate change? It seems that a wider transitional range of temperatures (TRT) provides more resilience towards increasing temperatures (Hulin et al., 2009). Species that are not able to find appropriate thermal environments for generating balanced sex ratios in their progeny would be forced to shift their distribution paralleling environmental changes or perish (see Boyle et al., 2016). In between, nesting behaviour can be adjusted spatially and temporally. These biological responses may result either from rapid evolutionary changes in organisms with shorter generation times (Mainwaring et al., 2017) or from individual phenotypic plasticity in long-living species (Fox et al., 2019). As such, reptiles with TSD may balance sex ratios in thermally variable environments by nesting earlier in the season (Hawkes et al., 2007; Telemeco et al., 2009) or by selecting nesting sites with different shade covers. This indicates a behaviorally plastic mechanism compensating for the change in temperatures during embryonic development (Pezaro et al., 2016; Refsnider & Janzen, 2010, 2012; Telemeco et al., 2009, 2013).

Beyond a biased sex ratio, suboptimal incubation temperatures may carry other costs. Environmental perturbations (e.g. temperature, pollution and subnutrition) during embryonic development may lead to developmental instability, translated into morphological deviations from symmetry in paired structures of bilateral organisms (Arnold & Peterson, 2002; Ji & Qiu, 2002; Löwenborg et al., 2011; Qualls & Andrews, 1999; Zhdanova & Zakharov, 2006). Thus, asymmetry should be lower under optimal developmental conditions and, therefore, becomes an indicator of developmental instability (Palmer, 1994). Different asymmetry patterns could be used to infer putative differences in thermal optimality between sexes and across populations. Fluctuating asymmetry (FA, lateral variation normally distributed around zero) must be disentangled from other types of asymmetry commonly found in organisms, namely, directional asymmetry (DA, laterally biased variation with a mean different than zero) and antisymmetry (AS, when right and left sides are equally common and more frequent than zero). These patterns, due to their probabilistic nature, are a property of the population rather than the individual (Palmer, 1994).

The Moorish gecko, *Tarentola mauritanica* (Linné, 1758), native to the western Mediterranean Basin, is recognized as a species complex with one of its six genetic lineages (European/North African clade) occupying an extensive geographic distribution and a wide array of environmental conditions (Harris, Batista, Carretero et al., 2004a; Harris, Batista, Lymberakis et al., 2004b; Rato et al., 2012, 2015, 2016), being therefore an ideal model to study patterns of adaptation and phenotypic plasticity. Besides the undetectability of sex chromosomes (De Smet, 1981; Odierna et al., 1994), studies of incubation suggest this species has a TSD MF pattern (Hielen, 1992; Nettmann & Rykena, 1985), although sample sizes, incomplete result exposition and pooling with other *Tarentola* species make this inference preliminary. In this context, Hielen (1992) tentatively suggested a pivotal temperature of 26.8°C based on experiments performed on geckos from Menorca, Balearic Islands, now ascribed to the European clade, according to genetic evidence (Rato et al., 2012, 2016). Nevertheless, the study from Hielen (1992) was inconclusive with respect to the detection of TSD in *Tarentola* species, since at temperatures higher than 30°C, males were also obtained. Later on, Köhler (2005) compiled a series of improved studies on the same *Tarentola* species used in Hielen (1992) (*T. angustimentalis*, *T. bottgeri* and *T. delalandii*), and the results are that, between 23 and 26°C, the authors obtained 100% males and between 27 and 30°C, 100% females.

Beyond this composed evidence, experimental studies on the adaptation/plasticity of TSD components such as oviposition temperature choice, sex ratio and pivotal temperature in response to local conditions, are still lacking.

Therefore, this study aims to (1) confirm the existence and describe the TSD pattern in this species; (2) evaluate the existence of adaptation/plasticity in key behavioural and physiological TSD components, namely oviposition temperature choice and pivotal temperature, to local thermal environments; and (3) to evaluate the existence of developmental instability inferred from different patterns of scalation asymmetry at the population level. To this end, clutches obtained from breeding couples collected in four wild populations with different climate regimes were experimentally incubated at different temperatures, and the resulting progeny was examined for sex and morphology (size and scalation). Moreover, the scalation pattern of hatchlings incubated at different temperatures and their mothers is compared in order to make inferences about heritability, optimal conditions and individual fitness. As such, we expect to shed light on the ecological and evolutionary dynamics of TSD in *T. mauritanica* and its vulnerability to climate change.

2 | MATERIALS AND METHODS

2.1 | Sampling

Adult individuals of *T. mauritanica* of both sexes (identified by visual inspection of the tail base, through the presence of hemipenal bulges and femoral pores in males) from four Iberian populations (Ayamonte, Portimão, Évora, Torres Vedras, Figure 1) were collected during spring 2019. All captures were performed with the necessary permits requested to national authorities of both Portugal (27,497/2019/DRNCN/DGEFF) and Spain (Consejería de Agricultura, Ganadería y Pesca y Desarrollo Sostenible de la Junta de Andalucía [SGIB/AF], code: 64oxu821PFIRM-AbNASMFdjKTCmO/uw.). In order to avoid phylogenetically related differences, all sampled populations belong to the European clade, previously identified by molecular markers in Rato et al. (2016). Animals were caught using a noose (following García-Muñoz & Sillero, 2010) or by hand, sometimes using a laser pointer to attract them (Cole, 2004). The identification of potential nests was based on what is described in the literature for this species, on the researchers' experience in the field, and on the detection of empty gecko shells and animal couples on site. In nature, these geckos lay their eggs under rocks, tree branches and pieces of wood or bury them in the soil (González de la Vega, 1988). Two potential nests per population were identified in the field, each with one iButton datalogger (i-button DS1922L, precision 0.001°C, accuracy 0.001%) to obtain overall monitoring of temperature in the potential nests throughout the reproductive season (April to August). They were retrieved in 2020, and local nest thermal data were downloaded.
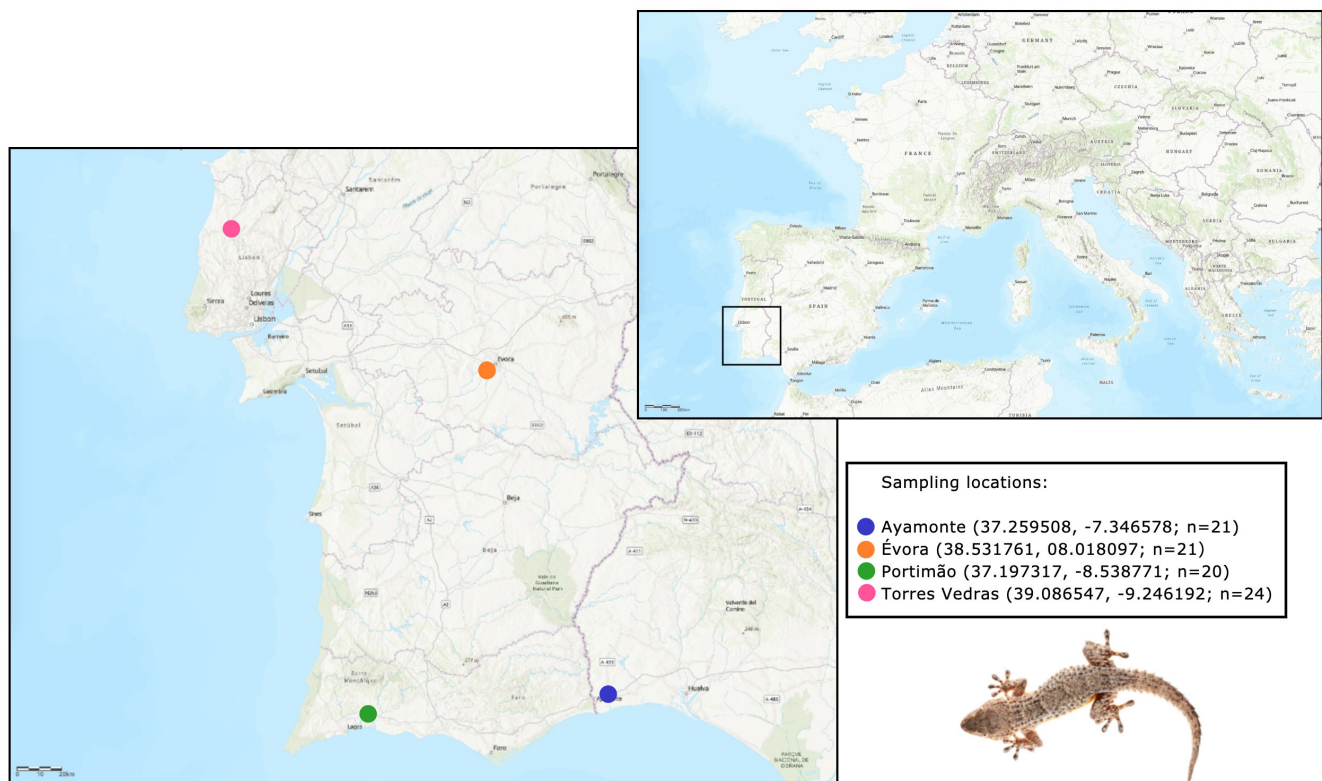

FIGURE 1 Map with the sampling locations of the populations included in this study. *N* indicates the number of couples collected [Colour figure can be viewed at wileyonlinelibrary.com]

2.2 | Husbandry

Once in the laboratory, animals were kept in captivity under the protocol approved by the Committee of Animal Experimentation of the University of Porto (Portugal) under the Directive 2010/63/EU of the European Parliament (27497/2019/DRNCN/DGEFF). Individuals from the same population were kept in couples, and since *Tarentola* are characterized by having stable couples in the wild (Vasconcelos et al., 2017), when possible, the couples found in the field were kept in the laboratory. All animals were maintained under controlled temperature conditions, at 18–20°C room temperature and routinely heated with 150W infrared light bulbs, in 50×30×25 cm terraria with a water dish and securely stacked rocks serving as refugia places. Snout-vent length (SVL) with an electronic calliper to the nearest 0.01 mm and weight with a digital scale to the nearest 0.0001 g were measured. In order to optimize reproduction, individuals were fed every day with live animals, for enrichment, namely house crickets (*Achetus domesticus*) and mealworm larvae (*Tenebrio molitor*) powdered with vitamin and calcium supplements. Although the clutch size per female is 1–2 eggs, female Moorish geckos can lay multiple clutches (up to seven in captivity) throughout the reproductive season (Martínez-Rica, 1974). Females were examined weekly for signs of advanced egg development. Identified gravid females were individually exposed to a thermal gradient (22–45°C), in a 100×30×40 cm acrylic terrarium covered with tissue paper, with a 150W infrared bulb fixed 15 cm above the substrate and maintaining a natural photoperiod (Figure S1, following Carretero, 2008 and Rato & Carretero, 2015). As in Rato and Carretero (2015), to provide continuous refuge for the female geckos along the gradient while letting them select their preferred temperatures, a 0.05×1 m aluminium plate was placed at the bottom of the terraria, separated longitudinally by a thin wooden laminate to provide robustness to the structure.

The selected temperature for oviposition was recorded with an infrared thermometer (Fluke® 68, precision 0.1°C, accuracy according to the manufacturer ±1%) pointed to the eggs at 1 cm distance. Once the experiments were through, the animals were released back into the wild, at their respective sampling locations.

2.3 | Egg incubation and TSD data collection

Immediately after oviposition, each egg was placed over vermiculite in one of six egg incubators (RCOM Juragon Pro), previously set to constant temperatures ranging from 25 to 30°C (following Hielen, 1992) and 90% humidity. The eggs were distributed to have a more or less even number per population at each incubation temperature. In order to avoid pseudo-replication at the clutch level and to disentangle between maternal and temperature effects on phenotypic sex, when possible, eggs from the same clutch were incubated at distinct temperatures, unless they were attached to each other and impossible to separate without compromising their integrity. Incubators were checked daily in search of hatchlings. After hatching, hatchling SVL was measured to the nearest 0.01 mm using a digital calliper and weight was taken with a precision scale (precision 0.0001 g; CPA model 224S, Sartorius Bohemia). Sex identification of the juveniles was performed by inspection of the gonadal conducts with the help of an electronic magnifier, after being euthanized following the "cooling then freezing" protocol from Shine et al. (2015), which consisted of cooling the animal in the fridge for 3 h for their brain activity to decline followed by placing them in the freezer to euthanize them. This was the planned end of the experiment, and no animals were euthanized before. Hatching success and hatchling sex ratio were recorded.

2.4 | Asymmetry data collection

Photos of the lamellae (toe structures made of keratinaceous filaments, setae, that split into spatulae at the ends, that enable van der Waals interaction in geckos and substrates; Heim et al., 2010) below the fifth finger (LFF) were taken of the arms on both sides of the body of hatchlings, mothers and putative fathers (since paternity was not genetically tested and sperm retention from copulation in the field cannot be discarded; Siegel et al., 2014), in order to disentangle between incubation temperature and heritability effects (Figure S2). LFF is not only important for the taxonomy of *Tarentola* (Bshaena & Joger, 2013; Joger, 1984) but, since subtle differences likely do not have a direct effect on geckos' locomotion, it can serve as a proxy of suboptimal developmental conditions, as observed in many other studies on reptiles (Laia et al., 2015; Lazić et al., 2013; Seligmann, 2006; Seligmann, 2011). Furthermore, the number of lamellae (~15–20) is large enough to adjust to normal and minimize threshold effects and reliably assess developmental instability (Soule, 1967). For each individual, LFF was recorded twice (always by Marques, V.) from the digital photos available, allowing several days of rest between the first and the second counting and randomizing the order of examined specimens to assess measurement error, ensuring the independence of trait counts. Assessment of symmetry departure was represented by the frequency distribution of right trait measure minus left trait measure (R - L).

TABLE 1 Post hoc Tukey test results comparing LogSVL of each sex of all populations. Highlighted in bold are the differences between the sexes within each population

	AY-F	AY-M	EV-F	EV-M	PT-F	PT-M	TV-F
AY-M	1.000	–	–	–	–	–	–
EV-F	0.991	1.000	–	–	–	–	–
EV-M	**0.005**	**0.018**	0.053	–	–	–	–
PT-F	1.000	1.000	0.986	**0.005**	–	–	–
PT-M	**<0.001**	**<0.001**	<0.001	0.942	**<0.001**	–	–
TV-F	0.996	1.000	1.000	**0.027**	0.993	**<0.001**	–
TV-M	**<0.001**	**0.002**	**0.007**	1.000	**<0.001**	0.994	**0.002**

FIGURE 2 Means plot of (a) LogSVL categorized by population and sex (LM1, $p = .017$) and (b) LogBRI categorized by sex (LM2, $p < .0001$). Vertical bars denote 95% confidence intervals [Colour figure can be viewed at wileyonlinelibrary.com]
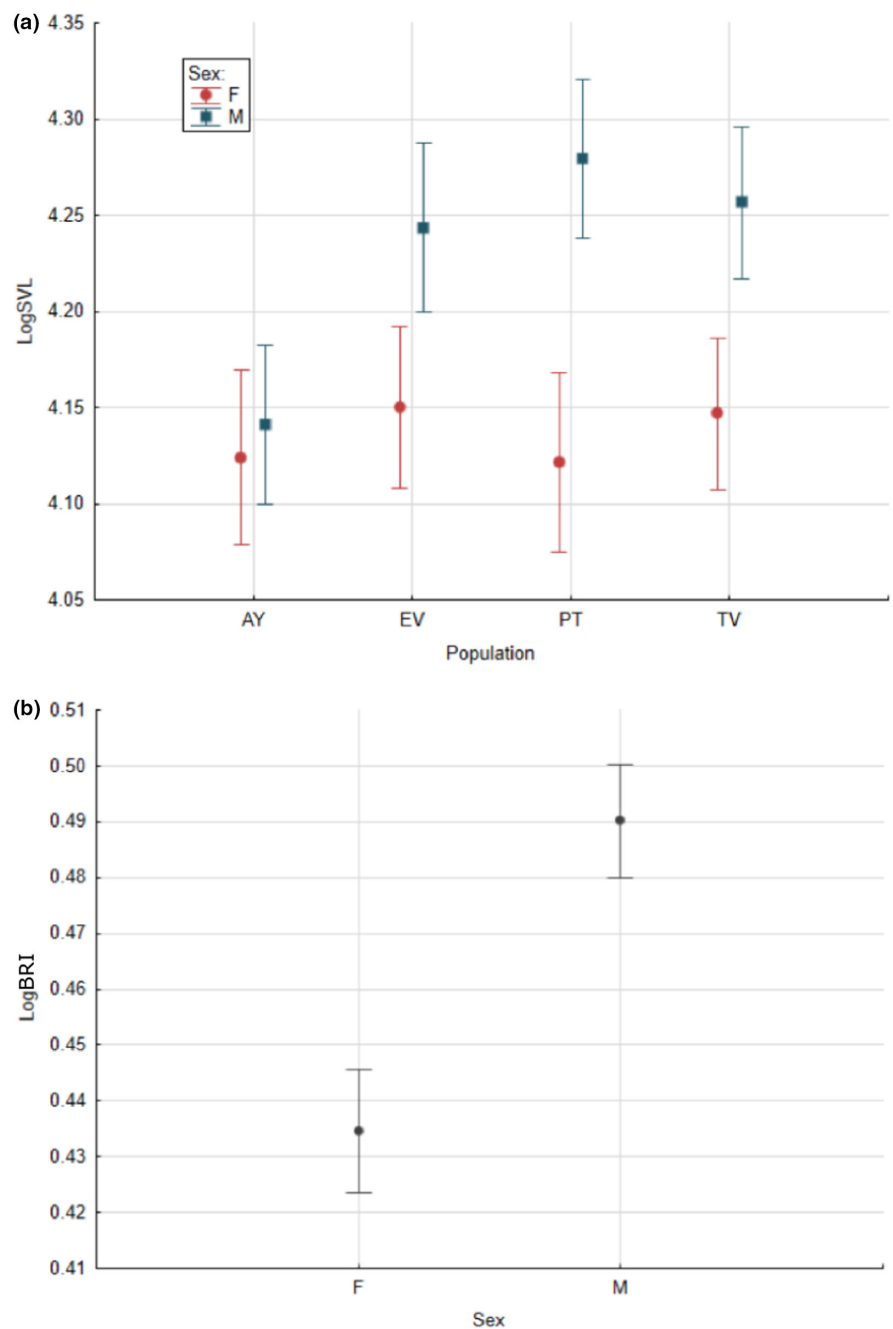
2.5 | Statistical analyses

Viability was determined from the hatching success at different incubation temperatures. Shapiro–Wilk tests indicated no deviation of the data (adult SVL, hatchling SVL, hatchling weight, oviposition temperature, adult asymmetry, hatchling asymmetry) from a normal distribution. The variable adult weight deviated from normality, so both adult SVL and adult weight were log-transformed. The adult and hatchling body ratio index (BRI) was calculated (logWeight/logSVL for adults and weight/SVL for hatchlings). Viability and total incubation time were also not normally distributed and due to the low sample sizes, nonparametric tests were applied. Hourly thermal data from the dataloggers were reduced to the maximum, minimum and mean daily temperatures, and daily thermal variance was obtained.

Once assumptions of normality and homoscedasticity were met, data analysis proceeded. Weight and SVL were tested for correlation both in adults and hatchlings. Two generalized linear models (GLM) were performed: GLM1, a logistic regression model to analyse hatchling sex ratio as the outcome of different incubation temperatures and to determine the pivotal temperature; GLM2, building with log link function and with the variables oviposition date, population, weight and BRI, in order to know which variables best explained oviposition temperature chosen by females. The model building approach followed in Zuur et al. (2009) was adopted: the models with the lowest Akaike's Information Criterion (AIC) score, lower variable complexity, ΔAIC <2 (Burnham & Anderson, 2004) and the most explanatory significant results would be selected. A total of seven general linear models (LM) were performed in the following order: LM1 and LM2 to evaluate the morphometric differences and infer the effects of population, sex and their interaction on adult SVL and BRI, respectively; LM3 to determine whether hatchling weight was affected by mother weight and/or incubation temperature; LM4 to check whether incubation temperature had any effect on hatchling BRI; LM5 to check for possible heritability of the asymmetry trait (effects of mother asymmetry on hatchling asymmetry); LM6 to determine the effects of population and/or sex and their interaction on adult asymmetry; LM7 to infer the effects of incubation temperature on hatchling asymmetry. For LM6 and LM7, the (R - L) asymmetry values were transformed to a discrete variable with the values "symmetrical" and "asymmetrical." Post hoc Tukey tests were performed on significant factors to detect specific differences. Two Kruskal–Wallis tests were performed with incubation temperature as independent and viability and total incubation time as dependent variables, since these were not normally distributed. All analyses and plotting were conducted using the software Statistica v.13 (Statsoft, Inc, 2018).

3 | RESULTS

3.1 | Sexual dimorphism

Adult weight and SVL were strongly correlated (df = 156, r = .91, p < .001). Adult SVL differed significantly both between sex and across populations, with p < .0001 and p = .003, respectively, and their interaction was also significant (p = .017, LM1, Table S1). A post hoc Tukey test confirmed a significant difference between the sexes (males > females) in Portimão (p < .001) and Torres Vedras (p = .002), while in Évora (p = .053) and Ayamonte (p = 1.000) this difference was not significant; in Ayamonte, males were very similar in size to females and significantly smaller than the males of all other populations (Table 1, Figure 2a). Adult BRI was significantly affected by sex (p < .0001) but not by population (p = .11, LM2, Table S2). Males were more robust than females (Figure 2b).

3.2 | Viability, sex ratio and pivotal temperature

Throughout the reproductive season, of the 86 females captured, 71 survived throughout the reproductive season, and a total of 60 eggs were incubated; due to the incubator malfunctioning, eggs could only be incubated at 25, 28 and 30°C (11 eggs at 25°C, 28 at 28°C and 21 at 30°C). While trying first to achieve an even number of eggs per population at each incubation temperature, unfortunately, the discrepancy across populations (Figure 4) explains the different sample sizes at each temperature. Out of those 60 eggs, only 28 were viable and hatched. The highest viability was recorded at 28°C, with 67.9% of the incubated eggs successfully hatching; 18.2% of eggs incubated at 25°C were viable, followed by 30°C, with 33.3% of incubated eggs hatching (Figure S3A). The Kruskal–Wallis test confirmed statistically significant differences in viability across incubation temperatures (df = 2, H = 9.97 and p = .007). The total incubation days decreased significantly with incubation temperature (Kruskal–Wallis test: df = 2, H = 18.28 and p = .0001 showing significance (Figure S3B). Similar to the adults, the hatchling weight was significantly correlated with SVL, with df = 21, r = .79 and p < .0001. Hatchling weight was not affected by incubation temperature (p = .26), but instead by mother weight, with p = .01 (LM3, Table S3 and Figure S3C). Incubation temperature did not have an effect on hatchling BRI (LM4). Sex ratio

varied significantly with temperature (GLM1, df = 1, χ^2 = 11.11, p = .0008) with offspring being female-biased (100% females) at 30°C, male-biased (100% males) at 25°C and 94.4% females at 28°C. Unfortunately, due to the high mortality rate, the sample size for the extreme temperatures was low, with only two eggs at 25°C. Sex identification for the remaining unhatched eggs at 25°C was not possible, since embryonic development was interrupted before the phenotypic sex was settled. The putative pivotal temperature was located at 27.78°C (Figure 3).

3.3 | Oviposition temperature choice

None of the tested models showed statistical significance in describing the oviposition temperature chosen by females (GLM2). The model with oviposition date as the only variable presented an AIC = 134.06, followed by a model with only the female weight, with an AIC = 134.89 (Table 2). The model containing only the population variable presented an AIC of 135.99. A box plot of the different oviposition temperatures of each population illustrates subtle differences that could simply belong to one large population of data (Figure 4). Females from Ayamonte chose the highest temperatures to lay their eggs (mean = 31.44 ± 2.33°C), while females from Portimão chose the lowest (mean = 27.60 ± 3.30°C). The temperatures chosen by females from Torres Vedras fall somewhat in the middle, with the least deviation (mean = 30.05 ± 2.12°C). Évora shows the broadest range of temperatures of all populations (mean = 29.46 ± 4.18°C).

3.4 | Developmental instability

LM5 model discarded the heritability of the LFF asymmetry trait (p = .70, Figure S3D). LM6 model indicated that adult asymmetry was significantly affected by population, with p = .006, and discarded a significant effect of sex (p = .75, Table S4). A post hoc Tukey test indicated a statistical difference between Portimão and the remaining populations (Table 3). All populations presented zero-centered normal distributions meeting the criteria for FA, with Portimão being clearly more symmetrical and Évora slightly skewed towards asymmetrical values (Figure 5a). As for the asymmetry in hatchlings, although the ones incubated at 28°C seem slightly more symmetrical than the others, there were no significant effects of incubation temperature (p = .78) and there was a lack of data for the extreme temperatures of 25 and 30°C (model LM7, Figure 5b).

3.5 | Local thermal conditions

Average daily nest temperatures and daily nest thermal variance data obtained from the dataloggers are presented in Figure 6a,b, respectively. Unfortunately, the dataloggers placed in Évora could not be retrieved due to habitat disturbance. In Ayamonte, the mean nest temperatures rose significantly over the months of March through June, reaching the highest values in July and August, at times 32°C, dropping quite abruptly in September. This locality experienced extremely high thermal variance, with values sometimes above 35°C. In Portimão, the average
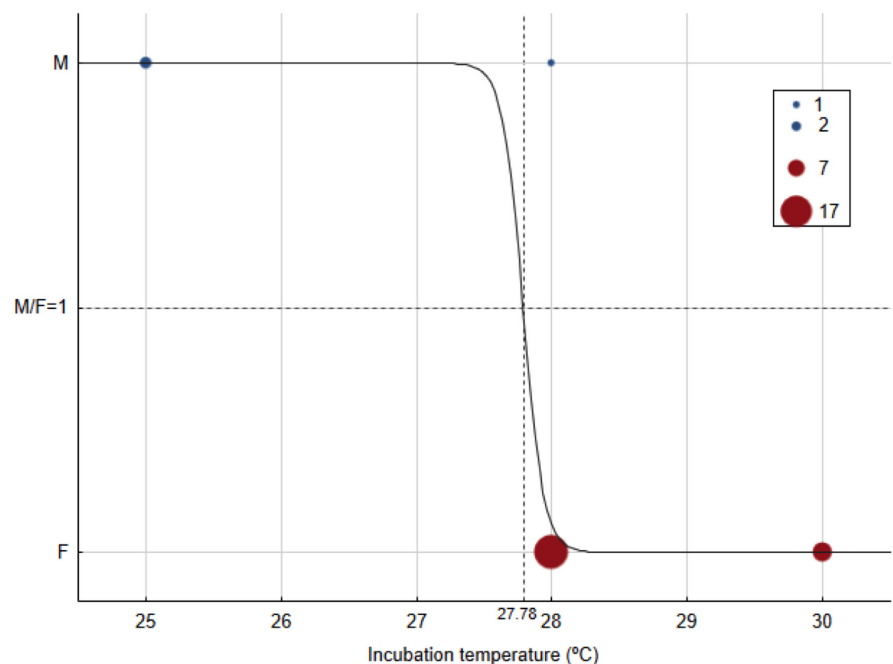

FIGURE 3 Frequency of each sex at different incubation temperatures. Logistic regression fitting of male yield (GLM1). Pivotal temperature = 27.78°C, p = .0008 [Colour figure can be viewed at wileyonlinelibrary.com]

Variable 1	Variable 2	Df	AIC	ΔAIC (AICi-AIC1)	L. Ratio χ2	p
Oviposition date		1	134.060	0	1.712	.191
Weight		1	134.886	0.826	0.886	.347
BRI		1	134.923	0.864	0.849	.357
Weight	Oviposition date	2	135.313	1.253	2.459	.292
BRI	Oviposition date	2	135.404	1.344	2.368	.306
Oviposition date	Population	4	135.692	1.632	6.080	.193
Population		3	135.997	1.947	3.776	.287

TABLE 2 GLM2 model building results for oviposition temperature. Normal distribution; log function
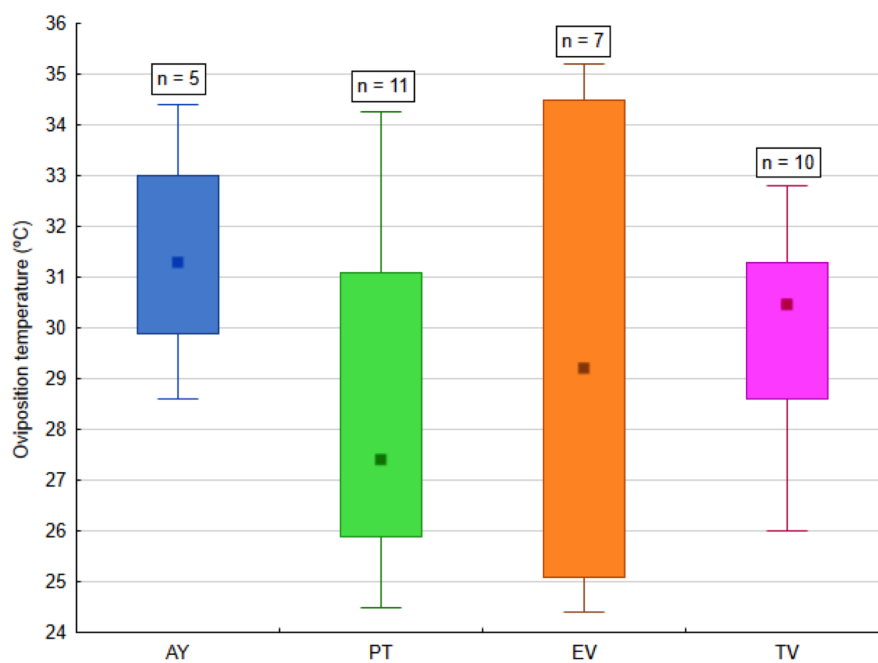

FIGURE 4 Female oviposition temperature choice categorized by population. *N* represents the number of trials [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Post hoc Tukey test results comparing levels of asymmetry among populations

Population	AY	PT	EV
PT	**0.020**	–	–
EV	0.995	**0.009**	–
TV	0.999	**0.023**	0.980

*Note:* Bold significance values indicates <0.01 and <0.05

temperatures of potential nests reached their peak in July and August but stayed around 24–26°C, never even going above 29°C and, again, dropping abruptly at the beginning of September. In Torres Vedras, the months of June and July experienced the highest average daily temperature, mostly in between 22 and 24°C, oscillating in a more gradual way in the preceding and following months. Contrary to Ayamonte, the Portimão and the potential nests at Torres Vedras underwent clearly more stable temperatures, with the thermal variance staying at around 5–10°C.

4 | DISCUSSION

Results of this experimental study suggest TSD as the most likely mechanism for sex determination in *Tarentola mauritanica*, as first proposed by Hielen (1992) and Nettmann and Rykena (1985) and later reviewed in Köhler (2005). The sex ratio obtained at different temperatures allied with the lack of sexual chromosomes in *T. mauritanica* (De Smet, 1981; Odierna et al., 1994) is also good evidence that in fact, this gecko species might have TSD. Although the recent paper from Gamble et al. (2015) using RAD-seq was able to uncover sex chromosomes in previously classified TSD species, they did not analyse any *Tarentola*

FIGURE 5 Asymmetry patterns in (a) adults of both sexes, categorized by population, and (b) hatchlings categorized by incubation temperature
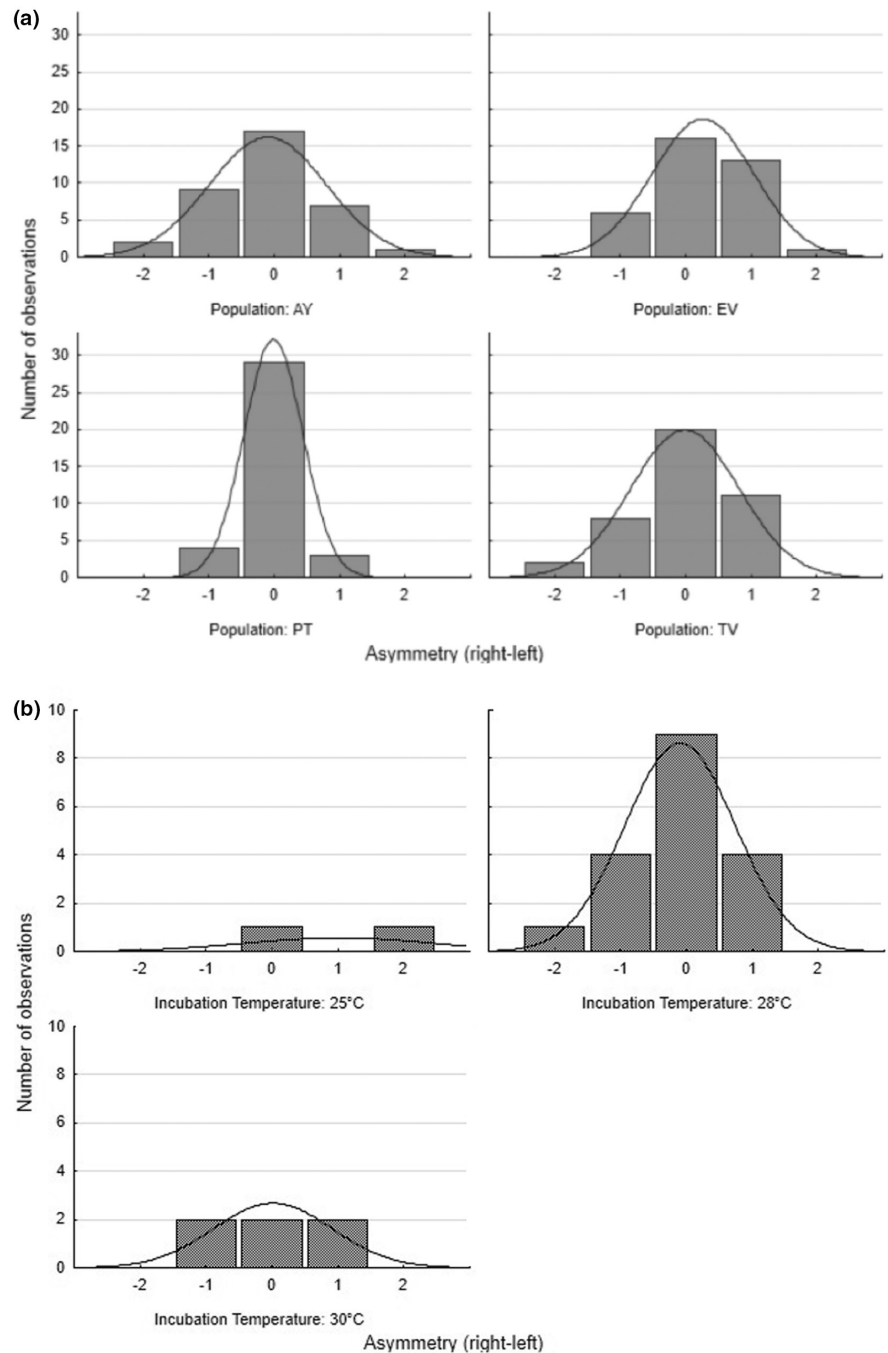
specimens. Hence, for now, the only available evidence for a lack of sex chromosomes in *T. mauritanica* derives from the traditional cytogenetic methods mentioned above.

However, examining the results obtained here, a sex-reversal hypothesis cannot be discarded as well. Sex reversal resulting from environmental temperature is known to be widespread across reptiles (Testudines, Lacertidae, Agamidae, Scincidae, Gekkonidae) (reviewed in Holleley et al., 2016). More recently, water restriction was also observed to override the heterogametic sex determination in two viviparous snakes (Dupoué et al., 2019). Hence, sex determination systems are now interpreted within a

continuum of environmental and genetic effects, obscuring the lines between GSD and TSD patterns, which were once considered as strictly dichotomous.

Furthermore, the results indicate that biological responses to thermal heterogeneity are mainly based on phenotypic plasticity rather than on adaptation, which has repercussions under the current disturbance in climate regimes across the species range.

The results on hatchling viability suggest that extreme incubation temperatures (25 and 30°C) will result in increased mortality. In fact, Hielen (1992) recorded no hatchlings at constant temperatures below 25°C, evidencing
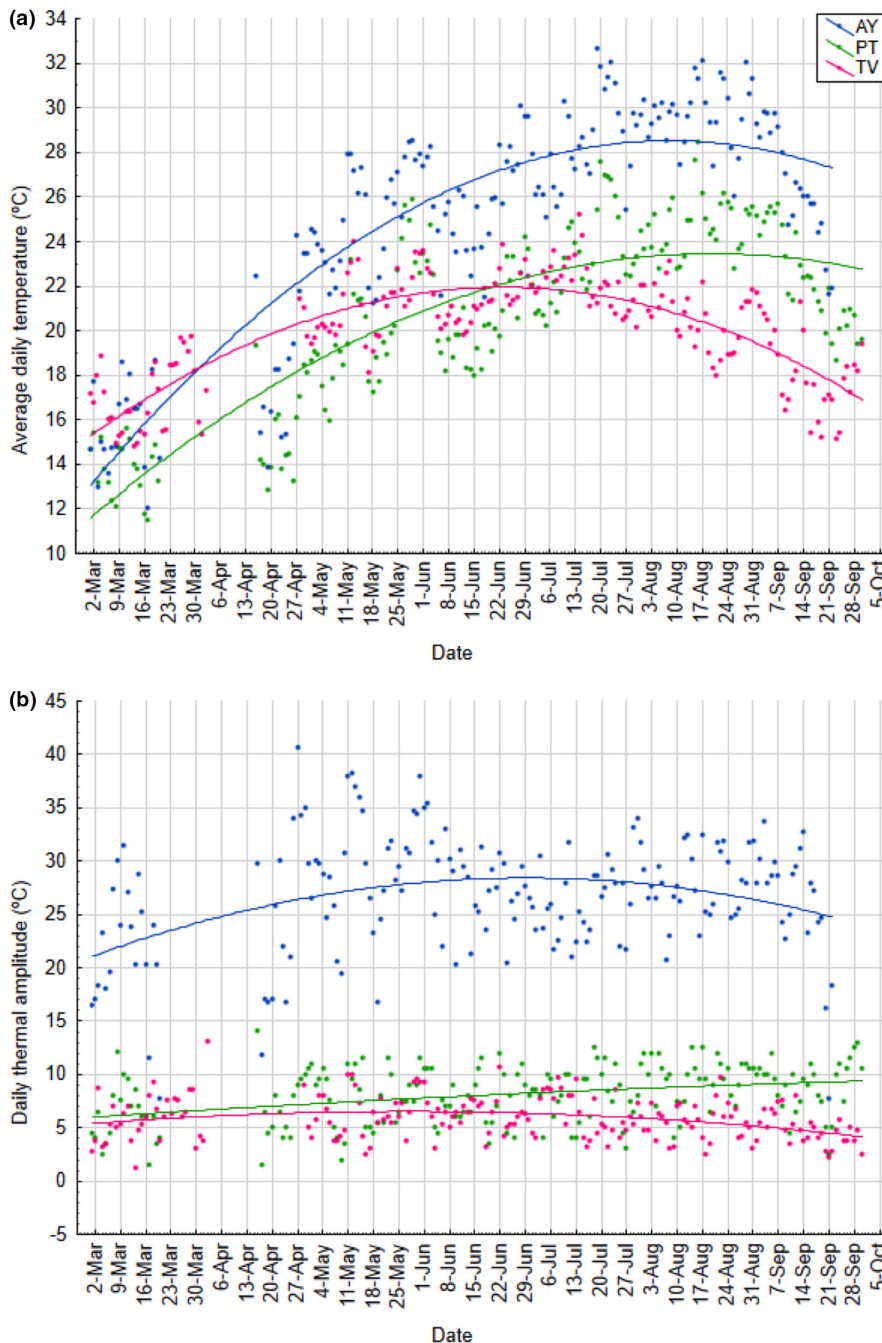
FIGURE 6 Average daily nest temperatures (a) and daily nest thermal amplitudes (b) recorded in each population during the reproductive season. Blue—Ayamonte; green—Portimão; pink—Torres Vedras [Colour figure can be viewed at wileyonlinelibrary. com]

that this temperature may represent a conservative threshold to viable embryonic development shared with other *Tarentola* species. A study performed on different reptile species showed that embryos incubated at colder temperatures allocate additional metabolic effort to maintenance costs because these temperatures require maintenance functions and do not allow embryonic growth or differentiation (Du et al., 2009), which might increase embryo mortality.

So far, there is only one study documenting the existence of a sex ratio embryo mortality bias in reptiles (Burger & Zappalorti, 1988), while there is another one recording sex-biased mortality in larval amphibians in

the wild (Lambert et al., 2021). This lack of studies/ evidence could indicate that this is a rather rare mechanism in herps, but it cannot be discarded as a possible explanation for the obtained results, only because there are few studies demonstrating this phenomenon. On the contrary, this seems to be a common mechanism well described in mammals, birds and some fishes (Firman, 2020; Kato et al., 2017; Morán et al., 2016). In all these examples, sex bias is correlated with a decrease in fecundity resulting from the sex-biased mortality of embryos or eggs.

The obtained bias on hatchling sex ratio at higher and lower incubation temperatures goes in line with the

hypothesis of an MF TSD pattern in *T. mauritanica* advanced previously from incubation data in Hielen ([1992]) and Nettmann and Rykena ([1985]). However, our experimental results support a putative pivotal temperature at around 27.78°C (Figure 3), differing 0.98°C from the 26.8°C suggested by Hielen ([1992]). It is important to note that an ampler range of samples for incubation temperatures could significantly change this value. Furthermore, the abrupt TRT can be explained by the very low male to female ratio at 28°C, which may also be slightly warping the pivotal temperature, pulling it towards higher values. Definitely, further data need to be collected in the future with more fine-tuned incubation tests, especially at intermediate temperatures, in order to infer more specific conclusions about how the sex ratio changes across distinct incubation temperatures and the exact pivotal temperature. Nevertheless, considering that an incubation temperature of 28°C also renders the lowest mortality, it is reasonable to assume that pivotal temperature results from selection favouring a balanced sex ratio.

Although egg size does not have an effect on sex ratio, it carries repercussions on hatchling size and survival. The finding that mother weight is positively related to hatchling weight may be linked to this species' life-history trait of two-egg clutches. *T. mauritanica* females never lay more than two eggs, so they invest their extra resources in producing larger juveniles, which will, naturally, have a higher survival rate after hatching (Sinervo, [1990]). Because of multiple two-egg clutches per reproductive season (Martínez-Rica, [1974]), this species falls somewhat in between K-selection (more energy is put into each individual offspring, increasing individual fitness, but decreasing fecundity) and r-selection (the production of many offspring, each with a relatively low probability of surviving to adulthood (Pianka, [1970])). This relationship between mother and hatchling weight was also recorded in the leopard gecko, *Eublepharis macularius*, by Tousignant and Crews ([1995]).

The lack of correspondence between oviposition temperature and climate regime of populations clearly indicates the absence of adaptation to local conditions, suggesting instead phylogenetic conservativeness, as seen in other lizard species (i.e. Dial & Grismer, [1992]). As such, *T. mauritanica* should rely on phenotypic plasticity to cope with variations in the thermal environment associated with nesting. If the inability for adjusting nest-site choice is confirmed, an increase in mortality when thermal incubation limits are exceeded or simply trends to extremely unbalanced sex ratios as expected from the rapid climate shifts may have severe demographic consequences for, at least, the populations analysed here. It is worth noting that, although lab

conditions were designed to emulate the natural environment as closely as possible, captivity may have increased background noise, causing some females to lay eggs induced by stress and not by temperature preference, which increases variation in the results.

There is an overall low sample size, which is even more pronounced when analysing each individual population. In the case of Ayamonte, for example, there is a lack of representative data across the reproductive season, because females stopped reproducing early (June 24). In fact, according to González de la Vega ([1988]), the reproductive season of *T. mauritanica* from Huelva takes place between March and July, which seems sooner than in the other populations. Further collections of animals during March are needed to obtain the full oviposition temperature data spectrum. In Torres Vedras, apart from reproduction seemingly starting later, the temperature for oviposition across the season is somewhat stable. Nevertheless, if all eggs were incubated at the mean female-selected oviposition temperature, all embryos would develop as females (Figure 4). This suggests that male production may be the result of other environmental constraints (e.g. climate, variation in substrate temperatures), rather than of a specific choice of the mothers. Still, one should also consider the phenology of animals from different populations: when and where mating takes place and females lay their eggs. This interplay between female behaviour and environment might result in geographic variations in sex ratio in hatchlings but not necessarily at the adult stage. The adult sex ratio of wild populations of *T. mauritanica* is around 1:1 (Martínez-Rica, [1974]), while the social system in *Tarentola* species is based on male territoriality (Pereira et al., [2019]) and stable couples (Vasconcelos et al., [2017]). In these conditions, deviations from balance should be negatively selected due to the lower survival of the juveniles of the most frequent sex.

Even though the annual temperature data from Évora were unavailable due to the loss of the dataloggers, considering its innermost location, it is expected to display the highest thermal variance. Although nest temperatures differ from air temperatures, data from meteorological stations indicate that this is, indeed, the locality with both the highest maximum and the lowest minimum daily temperatures across the reproductive season (data from Weatherspark, https://weatherspark.com/). Assuming the data can be combined, essentially: the southernmost localities were warmer, and the innermost locality displays the highest thermal amplitude. Since scalation in reptiles seems fixed at birth (Maderson, [1965]), FA informs on the levels of developmental instability due to incubation conditions. As such, for this trait, adult geckos from all populations displayed FA and inter-sexual differences were minimal. The southern coastal population

(Portimão) was the most symmetric, which matches the more stable nest conditions. However, the northernmost locality showed equally stable conditions while not being as symmetrical. The reason for this might be lower mean temperatures, differing the most from the optimal conditions, considering both hatchling viability and constant oviposition temperature selected by the females. It would be expected that the southern innermost location (Ayamonte) and eventually the northern innermost location (Évora) would be the least symmetrical compared. However, this is not the case, suggesting that females from both northern (Torres Vedras) and inland (Ayamonte and Évora) populations may face constraints when finding thermally suitable nesting sites. It is possible that factors other than temperature like female temperature before oviposition (Shine et al., 2007) and local environmental disturbance (Eeva et al., 2000; Hardersen, 2000) are also affecting development.

On the other hand, asymmetry levels in hatchlings incubated in the lab did not show evidence of heritability but instead revealed the effects of temperature on developmental instability. Despite the small sample size, it is remarkable that hatchlings incubated at 28°C were more symmetrical than those incubated at extreme temperatures. Interestingly enough, this was the temperature with the lowest associated mortality and approached the putative pivotal temperature. Combining these findings, it seems that 28°C is the optimal temperature both for embryo development and for producing a sex-balanced progeny. However, in the thermal gradients, females seem to select higher temperatures for oviposition. Although this could be suboptimal in terms of hatchling viability, other factors such as operational sex ratio or phenological constraints may be involved. Indeed, colder incubation temperatures result in longer incubation (Du et al., 2009), and hatchlings born later in the season might not be strong enough to withstand winter brumation (Sinervo, 1990). In other TSD studies, selected incubation temperatures were cooler in southern/warmer populations (e.g. *Chrysemys picta* in Schwarzkopf & Brooks, 1987 and Refsnider et al., 2014, *Chelydra serpentina* in Ewert et al., 2005). The choice of temperature was essentially dependent on the degree of exposure of the nest (i.e. with vegetation). Hence, although our results of female oviposition temperature choice suggest conservativeness, there are definitely other behavioural traits that need to be explored.

5 | CONCLUSION

The results provide quantitative evidence compatible with TSD in *Tarentola mauritanica* and, specifically,

suggest an MF pattern. However, temperature sex-reversal or sex-biased mortality are also plausible explanations for the obtained results. Indeed, a larger sample size, wider incubation temperatures and detection of sex chromosomes based on RAD-seq are foreseen in this species to make conclusions. The results also indicate that the different thermal components of its reproductive ecology (pivotal temperature, developmental optimum, incubation time) are highly integrated and mostly conservative across part of the geographic range of the species, with no evidence of local adaptation in female thermal nest choice, a key TSD trait. Being thermally labile, scalation asymmetry provided a reliable indicator of developmental instability, suggesting less suitable conditions for nesting in the northern and inland populations. While a larger sample size and more populations may allow a wider generalization, this is one of the few studies in TSD geckos. If thermal rigidity was widespread in TSD Gekkota, it may compromise reproductive success and demographic stability if nesting becomes constrained by climate change. This opens promising research avenues on the functional analysis of the effects on environmental shifts on TSD species and, hence, on the repercussions of global warming and its minimizing measures.

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