

Sexual size dimorphism in a Tunisian painted frog *Discoglossus pictus auritus* population

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Abstract

Sexual size dimorphism in a Tunisian painted frog *Discoglossus pictus auritus* population. Sexual size dimorphism is a hot topic in evolutionary ecology as it is influenced by a plethora of selective pressures. In this study, we investigated this issue in a painted frog *Discoglossus pictus auritus* population from south Tunisia, while controlling for age effects. Our results showed no significant sexual dimorphism in body size, classifying the painted frog among the few anuran species where the females are not larger than the males. However, foot webbing length showed a significant male-biased dimorphism, a pattern that may reflect selective pressure favoring swimming in males. Enlarged webbings could possibly improve a male's mating success through greater aptitudes to win competition against rivals for egg fertilization. Therefore, our findings suggest a male-biased dimorphism in a locomotion-related character in an anuran species, a trait possibly under sexual selection.

Key words: *Discoglossus pictus auritus*, Sexual size dimorphism, Tunisia, Morphometric sexing

Resumen

Dimorfismo sexual en el tamaño en una población de sapillo pintojo, *Discoglossus pictus auritus*, en Túnez. El dimorfismo sexual en el tamaño es un tema candente en ecología evolutiva, ya que está influenciado por una gran cantidad de presiones selectivas. En este estudio, investigamos este tema en una población de sapillo pintojo mediterráneo, *Discoglossus pictus auritus*, del sur de Túnez, controlando los efectos de la edad. Nuestros resultados no mostraron un dimorfismo sexual significativo en el tamaño corporal y nos permitieron clasificar al sapillo pintojo mediterráneo como una de las pocas especies de anuros en las que las hembras no son más grandes que los machos. Sin embargo, encontramos que la longitud de las membranas interdigitales mostraba un dimorfismo significativo, ya que era superior en los machos, lo que puede reflejar una presión selectiva que favorece su capacidad de natación. Es posible que las membranas agrandadas permitan mejorar el éxito de apareamiento de los machos al darles mayores aptitudes para ganar la competencia contra rivales por la fertilización de los huevos. Por lo tanto, nuestro estudio permite concluir la existencia de dimorfismo sexual en un carácter relacionado con la locomoción en una especie de anuro, lo que indicaría la posibilidad de que este rasgo esté influenciado por la selección sexual.

Palabras clave: *Discoglossus pictus auritus*, Dimorfismo en el tamaño sexual, Túnez, Sexado morfométrico

Introduction

Understanding the factors and processes shaping animal morphology has long been a major goal in ecology and evolutionary biology (Wainwright and Reilly 1994). In this context, valuable insights can be gained through the investigation of sexual size dimorphism (hereafter SSD) regarding either the entire body or certain body parts (Andersson 1994). SSD may reflect divergent selective pressures operating on each sex, traditionally thought to be shaped by interacting effects of sexual selection that may increase mating chances. Also, SSD may be affected by natural selection, influencing traits that enhance growth, survival, and fecundity (Littleford-Colquhoun et al 2019).

In Anurans, there is a tendency towards female-biased SSD, as females are larger than males in most species (90% of anurans reviewed by Shine 1979). This pattern is often interpreted in terms of fecundity selection favoring larger females, simultaneously increasing fecundity (Shine 1979, but see Pincheira-Donoso and Hunt 2017). On the other hand, some body parts, such as heads, nuptial pads, and limbs, tend to be larger in males, the hypothesis being they evolve under sexual selection pressures where such traits are an advantage for males regarding access to females and mating success (Pincheira-Donoso et al 2021). However, it is increasingly recognized that ecological niche segregation between sexes may also result in significant SSD, but clear predictions regarding which sex would be larger than the other are lacking (Pincheira-Donoso et al 2021).

Given that amphibians have indeterminate growth, it is crucial to interpret data concerning size of the individuals concerned in relation to age (Halliday and Verrell 1986). In this context, it has been argued that most SSD variation among anuran species can be explained by differences between sexes in age structure. In other words, because males reach sexual maturity relatively early, females, who are older, appear larger (Monnet and Cherry 2002, Kupfer 2007, Zhang and Lu 2013). Intraspecific-level studies have highlighted similar conclusions, suggesting that longevity and age at first reproduction are the main determinants of SSD (review in Kupfer 2007; e.g. Liao and Lu 2010, Cadeddu et al 2012). These findings underscore the necessity of explicitly considering individual age as a possible confounding factor when conducting morphometric comparisons between sexes in order to increase the reliability of results and conclusions about SSD in anurans.

Here we studied SSD in painted frogs *Discoglossus pictus auritus* occurring in oases along the Gulf of Gabès, in south-eastern Tunisia. This semi-terrestrial anuran inhabits places where water is available, although it lives primarily out of water. It is active by night and hides during daytime in shelters (Dhib et al 2024). Using a set of morphometric measurements recorded for 111 painted frogs, whose ages were determined by skeletochronology, we studied the sexual dimorphism in total body size and the size of various body parts. We also aimed to identify powerful morphometric discriminators between sexes and derive a reliable morphometry-based method that would be useful for rapid sex identification in the field.

Methods

Study site and data collection

We sampled painted frogs from in five oases in south-eastern Tunisia (table 1). In all five oases the vegetation has three distinct layers: palm trees, fruit trees, and herbaceous plants (Kassah 1996, Selmi and Boulmier 2003). Within these oases, private fields are irrigated on a rotational basis using groundwater pumped from wells and distributed through networks of cemented canals, pipes, and ditches. In these irrigation networks, water loss is common, especially from old and poorly maintained canals, which create permanent shallow pools used by the painted frog.

Fieldwork was spread over four years, between 2019 and 2022, and was always carried out in spring (from late February to early June). Painted frogs were searched for visually and captured by hand along old, cemented canals and around nearby shallow pools. Captured frogs were kept in wet cloth bags while in the field, and then taken to the laboratory for handling. Frogs were weighed using a digital scale to the nearest 0.1 g, and the following measurements (in mm) were taken using a digital caliper to the nearest 0.01 mm: snout-vent length (SVL), trunk length (TRL), head length (HL), head width (HW), eye diameter (EYED), distance between the tip of the snout and the front edge of the eye (SEYD), nostril-eye distance (NEYD), inter-orbital distance (IORD), distance between the nostrils (NOSD), and a series of hind-limb measurements: femur length (FEML), tibia length (TIBL), foot length (FOOL), length of the metatarsal tubercle (MTUL), lengths of the fingers 1 to 5 (FINL1, FINL2, FINL3, FINL4, and FINL5), and webbing length (WEBL). For bilateral traits, the right and left sides were measured, and the mean values were calculated for the statistical analyses. The frogs were euthanized and used as study models in practical activities of Animal Biology and Physiology for undergraduate Biology students.

Sex was determined by observation of the gonads, and a finger was preserved in 70% ethanol for subsequent age determination by means of skeletochronology (Comas et al 2016, Zhao et al 2019). After several trials to estimate the time needed for decalcification, the samples were decalcified in 3% nitric acid for 20 minutes. Decalcified samples were conserved in a phosphate-buffered saline solution with sucrose and kept at 4°C for at least 48 h. The samples were then sectioned with a freezing microtome (CM1850 Leica) to a thickness of 12 µm. The cross-sections were stained with Harris hematoxylin for 20 minutes; excess stain was rinsed by washing the slides in tap water for 5 minutes. Stained sections were then dehydrated in an alcohol chain, fixed with DPX (mounting medium for histology), and mounted on slides. The cross-sections were examined for the presence of lines of arrested growth (LAG) using a light microscope (Leica DM1000LED) at magnifications from 20 to 125x. We took several photographs with a camera (Leica ICC50W) at the University of Granada (Spain), selecting sections in which the size of

the medullar cavity was at its minimum and that of the periosteal bone at its maximum. The number of LAGs detected in the periosteal bone was independently counted three times by two authors (MC and JD), but on different occasions, and always blinded in terms of specimen identification (Comas et al 2016). If the three readings did not match, we carefully examined all sections of the individual concerned, and made additional age calculations. Sections with the highest number of coincidences (at least three) were used for the final age estimate. This approach has proven to provide highly repeatable age estimates (Comas et al 2016).

Data analyses

We used a *t*-test to check the significance of differences between sampled females and males regarding age and morphometric traits. We then conducted ANOVAs to assess the relevance of age class (3 classes: < 3 years, 3 years, > 3 years) and sex (two classes) as predictors of each of the morphometric traits studied. Age was categorized into 3 classes given that 3 years was the mode and the frequency of individuals with extreme values of age was low (80% of individuals were 2-3 years old), and also because we could not assume linear relationships of age (as a quantitative variable) with the morphometric traits investigated. In these ANOVA models, we also considered an age*sex interaction term to account for possible age-related sex effects. Subsequently, the only morphometric trait that showed a significant difference between sexes (namely WEBL; see Results) was used for investigating allometry. WEBL was regressed against SVL in males and females separately, and the estimated slopes (β) were tested for deviation from 1 by means of a *t*-test. Significant deviation from 1 would indicate allometry, either positive ($\beta > 1$) or negative ($\beta < 1$), otherwise, there would be isometry ($\beta = 1$). We used ordinary least-square regression rather than standardized major axis regression, as has recently been recommended (see McCambridge et al 2019). All analyses were conducted on \log_{10} -transformed data.

As we were also interested in identifying a simple and reliable method useful for sex identification in the field, we conducted a Discriminant Function Analysis (DFA) on the WEBL-to-SVL ratio. The validity of the obtained function was verified using a cross-validation (jackknife) procedure (Andrew et al 2015). For each individual, we calculated a discriminator score and a posterior probability of being a male. The cut-off point to assign an individual as female or male was determined as the midpoint between the mean scores for females and males (van Franeker and ter Braak 1993). All statistical analyses were conducted using SAS software (SAS Institute 2008).

Results

The study included a 111 painted frogs (46 females and 65 males; table 1). Individual ages ranged from 1 to 5 in females and 1 to 6 in males

Table 1. List of study sites and sample size of painted frogs: F, females, M, males, T, total.

Tabla 1. Lista de las localidades de estudio y el tamaño de muestra de los sapillos pintojos mediterráneos: F, hembras; M, machos; T, total.

Site	Coordinates	Sample size		
		F	M	T
Chenini	33° 52' N-10° 04' E	6	3	9
Ghannouche	33° 56' N-10° 02' E	1	4	5
Kettana	33° 45' N-10° 12' E	20	10	30
Laawinet	33° 59' N-10° 00' E	10	21	31
Mareth	33° 38' N-10° 18' E	5	15	20
Matmata nouvelle	33° 40' N-10° 00' E	4	12	16
Total		46	65	111

(mean \pm SD = 2.98 \pm 0.93 and 2.75 \pm 0.97, respectively; $t_{109} = 1.220$, $P = 0.224$). Based on raw data, WEBL was the only morphometric trait that differed between sexes, being larger in males than in females (table 2). This was confirmed by the results of ANOVAs (fig. 1). ANOVAs also showed significant relationships of all morphometric traits with age, but no significant interaction age*sex (table 3).

WEBL showed significant positive relationships with SVL in both males and females, separately (females: $R^2 = 38\%$, $\beta \pm SE = 1.195 \pm 0.231$, $F_{1,44} = 26.660$, $P < 0.001$; males: $R^2 = 91\%$, $\beta \pm SE = 2.955 \pm 0.118$, $F_{1,63} = 627.820$, $P < 0.001$; fig. 2). The estimated regression slope did not differ from 1 in females ($F_{1,44} = 0.710$, $P = 0.404$), but it was significantly larger than 1 in males ($F_{1,63} = 274.850$, $P < 0.001$; fig. 2), suggesting isometry in females but positive allometry in males.

Using the WEBL-to-SVL ratio, we obtained a discriminant function allowing 95% (106/111) of correct sex identification: $DF = -9.657 + 141.775 \times \text{WEBL/SVL}$, with positive scores indicating males and negative scores indicating females (fig. 3). All sampled females were classified correctly, but 5 males (8%) were miss-classified as they had negative scores (fig. 3).

Discussion

Little information is available on the painted frog at the southern limit of its geographic range. Here we report morphometric data on one of its southernmost populations (Ben Hassine and Nouira 2012, Nouira et al 2022). Our results highlight the absence of sexual dimorphism in body size, this being consistent with the results reported for populations from Malta, Sicily, northern Tunisia, Algeria, and Morocco (Sammot and Schembri 1991, Capula and Corti 1993, Amor et al 2010). However, Oromi et al (2016) reported a male-biased sexual size dimorphism in painted frogs from northwest Algeria and an introduced population

Table 2. Descriptive statistics of morphometric traits recorded in female and male painted frogs. All traits are in mm, except for body weight (g). (See Methods for the trait abbreviations):

Tabla 2. Estadística descriptiva de los rasgos morfométricos registrados en hembras y machos de sapillo pintojo mediterráneo. Todos los rasgos se expresan en mm, excepto el peso corporal (g). (Véase el apartado Methods para consultar las abreviaciones de los rasgos).

Trait	Females (n = 46)				Males (n = 65)			
	Min	Max	Mean	SD	Min	Max	Mean	SD
W	2.90	22.80	11.58	4.26	1.00	23.50	11.87	4.85
SVL	30.87	64.41	48.75	6.82	22.41	61.18	47.79	7.99
TRL	20.64	46.41	34.16	5.22	15.13	45.55	33.25	5.86
HL	9.75	18.82	14.59	2.13	6.76	17.62	14.54	2.42
HW	11.65	24.07	17.57	2.50	8.42	22.47	17.77	3.05
EYED	3.12	6.25	4.86	0.71	2.50	6.04	4.91	0.75
SEYD	4.16	8.18	6.20	0.91	2.63	8.15	5.95	1.10
NEYD	2.55	4.90	3.79	0.58	1.41	5.48	3.63	0.62
IORD	2.37	4.26	3.41	0.45	1.68	4.88	3.39	0.60
NOSD	2.58	4.60	3.66	0.40	1.97	4.66	3.61	0.54
FEML	11.91	27.25	19.89	2.68	8.54	25.07	19.96	3.46
TIBL	14.38	30.39	21.73	2.95	9.78	26.45	21.41	3.45
FOOL	21.62	44.67	32.76	4.37	15.85	40.62	33.02	5.09
MTUL	0.95	3.01	1.60	0.40	0.62	2.95	1.63	0.39
FINL1	2.67	5.21	4.15	0.66	1.52	6.04	4.46	0.91
FINL2	5.58	11.18	8.43	1.24	4.05	11.09	8.93	1.61
FINL3	9.33	19.20	14.08	1.96	6.73	18.11	14.37	2.37
FINL4	13.49	27.00	19.94	2.72	8.68	24.89	19.89	3.29
FINL5	8.72	17.13	12.63	1.85	5.61	17.19	13.00	2.40
WEBL	0.89	2.79	1.97	0.52	0.27	7.44	4.99	1.58

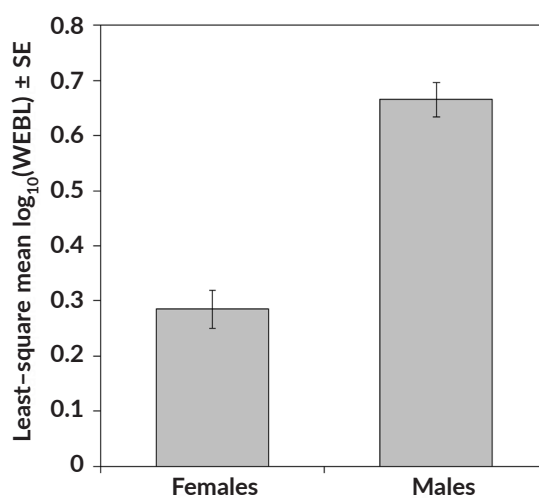


Fig. 1. Least-square means of webbing length (WEBL, \log_{10} -transformed) in female and males painted frogs, as estimated from an ANOVA model accounting for the effects of age.

Fig. 1. Medias de mínimos cuadrados de la longitud de las membranas interdigitales (WEBL, con transformación logarítmica en base 10) en hembras y machos de sapillo pintojo mediterráneo, estimadas a partir de un modelo ANOVA controlando los efectos de la edad.

Table 3. Results of ANOVAs on investigated morphometric traits (\log_{10} -transformed) as functions of age (3 classes: < 3 years, 3 years, > 3 years), sex (2 classes), and their interaction. (See Methods for the trait abbreviations): T, typy III sum of squares; Ms, mean squares.

Tabla 3. Resultados de las pruebas ANOVA de los rasgos morfométricos estudiados (con transformación logarítmica en base 10) como una función de la edad (3 clases: < 3 años, 3 años, > 3 años), el sexo (2 clases) y la interacción de ambos. (Véase el apartado Methods para consultar las abreviaciones de los rasgos): T, tipo III suma de cuadrados; Ms, cuadrados medios.

Trait	R ² (%)	Source	df	T	Ms	F-ratio	P
W	14	Age	2	0.622	0.311	5.480	0.006
		Sex	1	0.000	0.000	0.000	0.959
		Age*sex	2	0.064	0.032	0.560	0.573
SVL	16	Age	2	0.081	0.041	7.130	0.001
		Sex	1	0.002	0.002	0.390	0.534
		Age*sex	2	0.007	0.003	0.580	0.561
TRL	14	Age	2	0.085	0.042	6.620	0.002
		Sex	1	0.005	0.005	0.710	0.402
		Age*sex	2	0.009	0.004	0.690	0.503
HL	15	Age	2	0.078	0.039	6.830	0.002
		Sex	1	0.000	0.000	0.000	0.952
		Age*sex	2	0.004	0.002	0.360	0.697
HW	16	Age	2	0.070	0.035	5.930	0.004
		Sex	1	0.000	0.000	0.000	0.991
		Age*sex	2	0.013	0.006	1.090	0.339
EYED	16	Age	2	0.051	0.026	5.300	0.006
		Sex	1	0.000	0.000	0.040	0.851
		Age*sex	2	0.024	0.012	2.430	0.093
SEYD	14	Age	2	0.055	0.028	4.390	0.015
		Sex	1	0.013	0.013	2.000	0.161
		Age*sex	2	0.024	0.012	1.920	0.151
NEYD	12	Age	2	0.053	0.027	4.570	0.013
		Sex	1	0.008	0.008	1.330	0.251
		Age*sex	2	0.003	0.002	0.290	0.747
IORD	11	Age	2	0.043	0.022	4.060	0.020
		Sex	1	0.000	0.000	0.040	0.839
		Age*sex	2	0.003	0.002	0.290	0.751
NOSD	16	Age	2	0.048	0.024	7.030	0.001
		Sex	1	0.001	0.001	0.200	0.657
		Age*sex	2	0.009	0.004	1.300	0.276
FEML	13	Age	2	0.055	0.028	4.530	0.013
		Sex	1	0.000	0.000	0.040	0.852
		Age*sex	2	0.018	0.009	1.440	0.242
TIBL	15	Age	2	0.059	0.030	5.670	0.005
		Sex	1	0.003	0.003	0.500	0.483
		Age*sex	2	0.019	0.010	1.830	0.166
FOOL	17	Age	2	0.068	0.034	7.100	0.001
		Sex	1	0.000	0.000	0.000	0.994
		Age*sex	2	0.009	0.004	0.910	0.406
MTUL	15	Age	2	0.121	0.060	5.710	0.004
		Sex	1	0.005	0.005	0.450	0.502
		Age*sex	2	0.002	0.001	0.100	0.901
FINL1	13	Age	2	0.062	0.031	3.430	0.036
		Sex	1	0.014	0.014	1.550	0.215
		Age*sex	2	0.013	0.006	0.700	0.500
FINL2	16	Age	2	0.072	0.036	5.230	0.007
		Sex	1	0.010	0.010	1.470	0.227
		Age*sex	2	0.014	0.007	1.020	0.363
FINL3	15	Age	2	0.059	0.030	5.190	0.007
		Sex	1	0.000	0.000	0.080	0.779
		Age*sex	2	0.013	0.007	1.150	0.320
FINL4	14	Age	2	0.060	0.030	5.100	0.008
		Sex	1	0.001	0.001	0.090	0.760
		Age*sex	2	0.009	0.005	0.780	0.461
FINL5	12	Age	2	0.059	0.030	4.320	0.016
		Sex	1	0.002	0.002	0.240	0.627
		Age*sex	2	0.013	0.007	0.950	0.389
WEBL	46	Age	2	0.256	0.128	2.690	0.073
		Sex	1	3.236	3.236	67.980	< 0.001
		Age*sex	2	0.196	0.098	2.060	0.133

in Catalonia (northeast Spain). Still, all these works, including our study, are in agreement to consider the painted frog among the few species of anurans where females are not larger than males. It would also be interesting to verify whether this morphological characteristic is common to all *Discoglossus* species.

A key result of our study is the significant SSD in foot webbings, these being more than twice as large in males than females. Indeed, webbing length (expressed relative to total body size) has proven to

be a powerful discriminator between sexes, and we suggest that it can be confidently used for rapid sex identification in the field. According to the discriminant function obtained, individuals with positive scores were males, whereas 90% of individuals with negative scores were females. Furthermore, investigations of allometric relationships showed that greater body size was associated with greater enlargement in foot webbings in males, while this trait grew proportionally to the entire body in females.

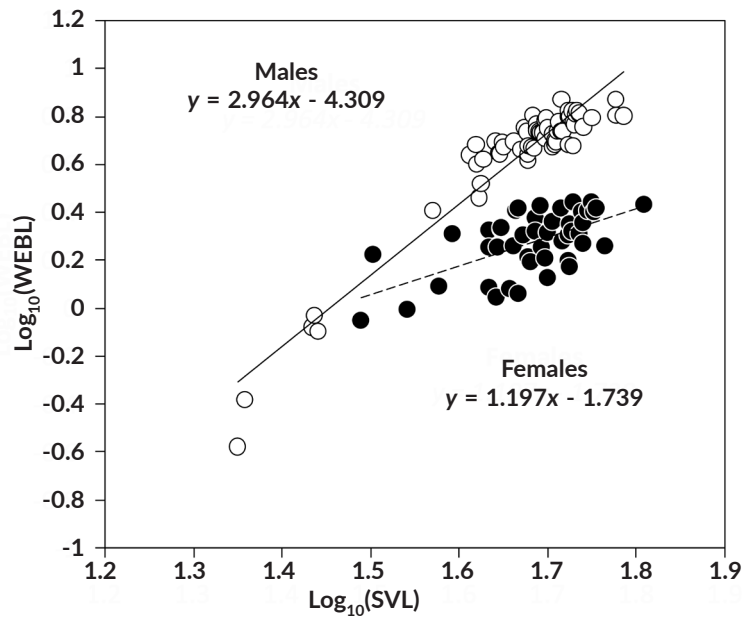


Fig. 2. Relationships of webbing length (WEBL) with snout-vent length (SVL) in females (black symbols and solid line) and males (white symbols and dashed line) painted frogs, using \log_{10} -transformed data.

Fig. 2. Relación de la longitud de las membranas interdigitales (WEBL) con la longitud hocico-cloaca (SVL) de las hembras (símbolos negros y línea continua) y los machos (símbolos blancos y línea discontinua) de sapillo pintojo mediterráneo, usando datos con transformación logarítmica en base 10.

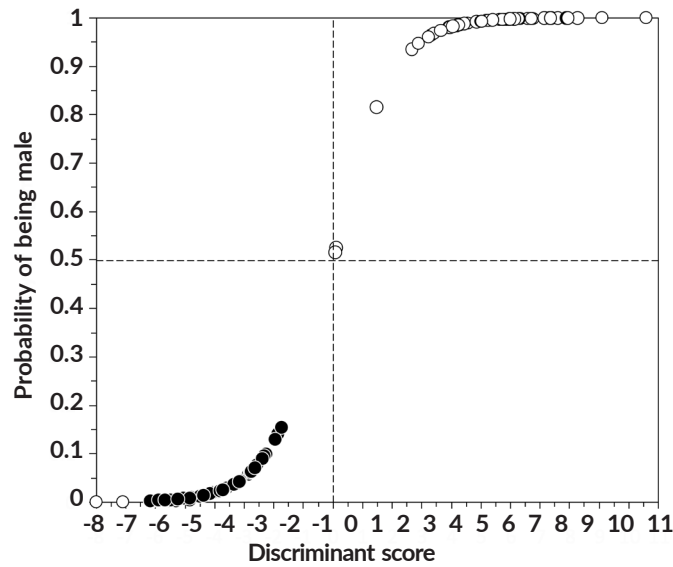


Fig. 3. Plot of the probability of being categorized as a male as a function of the discriminant score, derived from a Discriminant Function Analysis of webbing length (WEBL) to snout-vent length (SVL) ratio (WEBL-to-SVL ratio), for female (black symbols) and male (white symbols) painted frogs. The horizontal dashed line represents a 50% probability of being male, and the vertical line represents the cut-off discriminant score.

Fig. 3. Gráfico en el que se muestra la probabilidad de clasificar un ejemplar como macho en función del valor discriminante calculado a partir de un análisis de función discriminante de la razón de la longitud de las membranas interdigitales (WEBL) con respecto a la longitud hocico-cloaca (SVL) (WEBL-to-SVL ratio) en las hembras (símbolos negros) y los machos (símbolos blancos) de sapillo pintojo mediterráneo. La línea horizontal representa el 50% de probabilidad de que un ejemplar sea macho y la vertical, el valor de corte de la función discriminante.

Because foot webbings are mainly involved in swimming, the male-biased dimorphism we report here may suggest selection pressure favoring males' swimming aptitudes (see Zamora-Camacho 2023). Because staying in water is associated with mating and fertilization of eggs in adult painted frogs (Doumergue 1901), it is reasonable to suspect that the larger male' feet webbings is linked to their aquatic mating behavior. In effect, in this species, mating takes place in the water, with males calling to attract females and competing for mating (Doumergue 1901, Salvador 1996, García-Paris 2000). This behavior suggests possible advantages for those males with enlarged webbings and high swimming abilities. For example, enlarged webbings would increase a male's swimming speed, improving its chances to win scramble competition against rivals and first reach a female who has just entered the mating pond. Enlarged feet webbings may also increase the ability of a male to swim while dragging the female away from rival males, thereby avoiding their participation in the fertilization of the eggs. It should, however, be noted that an exaggerated increase in foot webbings may alter the ability of males to move on land, causing possible survival costs. In this scenario, the size of males' foot webbings would be largely shaped by the opposing demands of jumping performance, affecting survival, and swimming ability, and influencing breeding success. These supposed effects, however, still require empirical validation. Another possible advantage of enlarged foot webbings in males could be related to the extrusion of the female's ova and/or their mixing with the sperm during copulation. Further observations on mating behavior are needed to verify whether this behavior exists in the painted frog, and whether it increases the rate of fertilization of eggs.

In conclusion, our study highlights significant male-biased dimorphism in the painted frog concerning the size of foot webbings, a swimming-related morphological trait. We thus report a new example of male-biased SSD in locomotion-related characters in anurans (see Petrović et al 2017). We hypothesize that this trait is under sexual selection pressure, but more relevant information regarding mating behavior and swimming/jumping performance of this species is needed.

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Author contributions

S Selmi designed the study; **J Dhib** conducted the fieldwork and morphometric measurements; **M Comas** and **J Dhib** carried out the lab work on skeletochronology, while **G Moreno-Rueda** provided the funds for this lab work; **S Selmi** performed the statistical analyses; **J Dhib** wrote the main body of the manuscript with input from the other authors on specific aspects.

Conflict of interest

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