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Abstract. Sexual dimorphism in the size and shape of the Sardinian cave salamander (*Atylodes genei*) was analysed using morphometric measurements. Males and females are roughly equal in body size (mean snout-vent length SVL: 53.8 and 53.4 mm, respectively) but differed in body shape. Relative to their SVL, males had comparatively larger heads, longer limbs and tails than females, which agrees with patterns of sexual dimorphism in other closely related species. This suggests the existence of phylogenetic conservatism in sexual differences in body shape. The lack of dimorphism in body size could be an ancestral trait in *Atylodes genei*.

Key words: Plethodontidae, body size, body shape, *Speleomantes*

Introduction

Sexual dimorphism – the expression of different phenotypes in the two sexes – has intrigued evolutionary biologists since Darwin (1871). The main driver for the evolution of sex-specific morphological structures is usually considered to be sexual selection (Rowe & Arnvist 2013). This kind of selection acts either through competition between males to gain access to females or through mate choice, whereby females evaluate the evolutionary fitness of their potential mates according to morphological traits specifically developed for this purpose (Andersson 1994).

However, aside from sexual selection, three further factors may lead to the evolution of sexual dimorphism: (i) fecundity selection, which favours larger bodies in females providing more space for eggs; (ii) competition between sexes, which should lead to sexual dimorphism in body size or food consumption structures (Selander 1972); and (iii) mating performance through intrasexual competition, which favours morphological structures maximizing the number of successful mating attempts (Gvoždík & Van Damme 2003).

Although sexual differences in body size have previously been investigated in many species of salamanders (De Lisle & Rowe 2013), dimorphism in body shape has received much less attention (Malmgren & Thollesson 1999, Kalezić et al. 2000, Romano et al. 2009, Hasumi 2010). Many species of salamanders – as well as frogs – seem to follow the predicted patterns of sexual size dimorphism in ectotherms whose females are larger than males (De Lisle & Rowe 2013). The opposite pattern is rare and may be associated with the existence of contests between males for females (Shine 1979); nevertheless, a large variety of types of behaviour related to inter- and intrasexual interaction exists in salamanders, including scramble competition, mate guarding, courtship, fighting for resources and biting (Levgenius & Parzefall 1992, Mathis et al. 1995, Kawamichi & Ueda 1998).

The plethodontid salamander *Atylodes genei* lives in caves and rocky-outcrops in Mediterranean forests and is geographically restricted to the area of Sulcis-Iglesiente in south-west Sardinia (Lanza 1999). To date, the only study examining sexual dimorphism

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in this species reported sexual differences in total and body lengths, and provided information about differences in body shapes between males and females based on ratios but lacked any statistical analysis (Lanza et al. 1995). In this study, we used both body size and shape to describe patterns of sexual dimorphism in *Atylodes genei*, which will facilitate comparisons with other available data for this species (Lanza et al. 1995, 2007) and other European lungless salamanders (Salvidio & Bruce 2006).

Material and Methods

Field sampling of *Atylodes genei* was carried out in south-west Sardinia on 23-26 April 2009. A total of 97 individuals were collected, of which, despite the large sampling effort, the sex ratio was severely biased towards males, probably due to differences in activity patterns between the sexes. Salamanders were caught by hand and anaesthetised using tricaine methanesulphonate (MS-222), one of the anaesthetics most commonly used in the field sampling of amphibians (e.g. Schumacher 1996). Sexual determination was based on the detection of the mental gland, a typical male structure; individuals larger than the smallest male and lacking this trait were considered as females. Twelve linear morphometric traits were measured with a digital calliper to a resolution of one millimetre: 1: snout-vent length (SVL), from the tip of the snout to the rear margin of the cloaca; 2: head length, from the tip of the snout to the gular fold; 3: maximum head width; 4: head width between the eye orbits; 5: head width at the gular fold; 6: maximum head height; 7: interorbital distance, equivalent to the minimum distance between the eyes; 8: forelimb length from the point of insertion to the tip of the longest finger; 9: hindlimb length from the point of insertion to the tip of the longest toe; 10: axilla-to-groin length; 11: tail length; and 12: trunk length, calculated as SVL minus head length. All measurements were taken by the same person (F. Amat). Sexual size dimorphism was estimated using an index (Lovich & Gibbons 1992) defined as the quotient between the sizes of the largest and smallest sex −1. By convention this index is given as a positive value when females are the largest sex and as a negative value when males are largest.

Statistical analyses were performed using the logarithmic value (log10) of the morphometric variables. Sexual size and shape dimorphism were investigated using a two-way ANOVA with sex and geographical area as factors. In order to maximise the sample size, populations were pooled into three groups
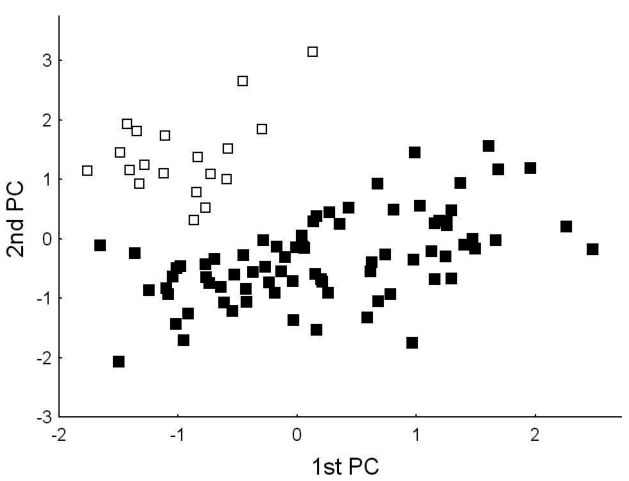

Fig. 1. Plot of the examined individuals of *Atylodes genei* on the two first PC: open squares – females and black squares – males.

Table 1. Means, standard errors of the twelve linear morphometric traits by sexes of sampled individuals of *Atylodes genei*.

	Mean ± SE	
	Males	Females
SVL	53.8 ± 4.2	53.4 ± 2.8
Head length	14.0 ± 1.4	12.7 ± 0.9
Trunk length	39.7 ± 3.2	40.6 ± 2.6
Maximum head width	10.6 ± 1.5	8.4 ± 0.4
Head with at eye orbits	7.9 ± 1.1	7.3 ± 0.8
Head with at gular fold	7.3 ± 0.9	6.3 ± 0.4
Head depth	5.3 ± 0.7	3.7 ± 0.3
Interorbital distance	5.4 ± 0.6	4.8 ± 0.3
Forelimb length	14.4 ± 1.4	13.4 ± 1.1
Axila-groin length	26.6 ± 2.4	27.0 ± 1.5
Hindlimb length	16.6 ± 1.5	14.8 ± 0.9
Tail length	45.8 ± 4.9	34.9 ± 2.2

based on geographical proximity (Rivera et al. 2015) as follows: Domusnovas (cave and surroundings), 42 salamanders, of which 11 were females and 31 males; Nuxis (two caves), 24 salamanders, of which 8 were females and 16 males; and Fluminimaggiore (three caves), 31 salamanders, of which all were males. In total 78 males and 19 females from these three localities were analysed. Pearson correlations were performed to assess whether the SVL was positively associated with the other 11 morphometric variables (results not shown). In order to evaluate sexual differences in body shape whilst controlling for the effect of body size on the other variables, a two-way MANCOVA test was conducted with SVL as a covariate. An examination of the multivariate

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Table 2. Coefficients of linear morphometric variables of the sampled specimens of *Atylodes genei* in the two first factors, corresponding eigenvalues and percentage of explained variance.

Variable	1st PC	2nd PC
Trunk length	0.680	0.650
Head length	0.840	–0.156
Maximum head width	0.854	–0.198
Head with at orbits	0.672	0.041
Head with at gular fold	0.818	–0.078
Head height	0.857	–0.351
Interorbital distance	0.792	–0.029
Forelimb length	0.718	0.073
Axila-groin length	0.677	0.657
Hindlimb length	0.866	–0.064
Tail length	0.855	–0.260
Eigenvalues	7.170	0.925
% explained variance	0.608	0.084

patterns of correlation between the 11 morphometric variables was performed using a principal component analysis (PCA) to visualise sexual differences in shape between sexes (Romano et al. 2009). All analyses were performed using Statistica 4.5.

Results

Sexual size dimorphism was extremely small (males 53.8 ± 4.2 and females 53.4 ± 2.8 mm). Females matured at larger sizes than males (minimum SVL, 49.9 and 44.9 mm in females and males, respectively), but males had larger maximum SVL than females (63.1 and 60.6 mm, respectively). Thus, sexual and geographical differences had statistically a non-significant influence on body size (ANOVA: geographical area, $F_{1,62} = 1.290$, $P = 0.259$; sex, $F_{1,62} = 2.843$, $P = 0.096$; interaction, $F_{1,62} = 0.405$, $P = 0.526$). However, the analysed populations had significant sexual dimorphism in body shape – including mean head width, trunk length and tail length (Table 1, $P < 0.001$) – combined with geographical variation; however, there was no interaction between these two factors (MANCOVA: geographical area, $F_{1,51} = 0.689$, $P = 0.038$; sex, $F_{1,51} = 0.076$, $P < 0.001$; interaction, $F_{1,51} = 0.743$, $P = 0.127$). The PCA had a high total variance accounted for by the first two factors (72.4 %). The first PC had the typical structure of a size vector (Table 2), in which all the variables are positively and strongly weighted. Thus, the first PC only described individual variation in the overall body size, irrespective of sex. The second PC was positively influenced by the weights of trunk length and axilla-groin length, while the other variables – above all, maximum head width, height and length, and tail length – were both strongly and negatively weighted. Plotting the data of these two PCs revealed a pattern of sexual-shape dimorphism (Fig. 1). Thus, males were characterised by relatively bigger heads in all three dimensions (length, width and height), longer tails and longer hindlimbs. Females, though, had opposing patterns in the relative proportions of their heads and tails.

Discussion

The commonest pattern of sexual size dimorphism in ectothermic organisms is a bias towards larger females (Fairbairn 1997). This is a general rule in amphibians such as salamanders (De Lisle & Rowe 2013, Han & Fu 2013, Amat & Meiri 2017). Nevertheless, our study recorded roughly equal body size between sexes in *Atylodes genei* in agreement with previous studies (mean SVL: males 52.5 mm and females 52.0 mm, Lanza et al. 1995). Male combat has been proposed as one of the main selective factors enhancing sexual size dimorphism biased towards males, especially in frogs (Shine 1979, Han & Fu 2013). Despite the inherent difficulties in observing the behaviour of European plethodontid salamanders, there is no evidence that male *Atylodes* or *Speleomantes* species fight (Zanetti & Salvidio 2006, Sguanci et al. 2010). On the other hand, females of these salamander species have been reported to show aggressive behaviour towards conspecifics or predators when guarding their clutches (Stefani & Serra 1966, Mutz 1998, Oneto et al. 2014). In the case of two continental *Speleomantes* (Salvidio & Bruce 2006), females are larger than males; however, the opposite pattern was observed in the Sardinian *S. sarrabusensis* (Tessa et al. 2008). Remarkably, the most comprehensive and descriptive analysis of morphological differentiation in European lungless salamanders (Lanza et al. 1995) reports widespread sexual size dimorphism biased towards females in all these species with the exception of *Atylodes genei*.

Sexual differences in body size in amphibians are due to faster maturity in one sex rather than to differences in growth rates (Zhang & Lu 2013), although one experimental study carried out with caecilians did show the importance of both factors (Kupfer et al. 2004). There are no available data for the timing of sexual maturity in *Atylodes genei* that could validate this pattern; nevertheless, faster male maturation has been reported in one European lungless salamander

(*Speleomantes ambrosii*: 3.5 and 5 years for males and females, respectively; Salvidio 1993). By contrast, populations of the Sardinian *S. sarrabusensis* showed faster sexual maturation occurring at an identical minimum age in both sexes (two years, Tessa et al. 2008), with males being larger than females. It has been suggested that insularity could affect life history and body size in ectotherms (Novosolov et al. 2013) through selective pressure given that predation and competition for resources acts in differently on islands than in the mainland. Therefore, insular conditions could have modified the patterns of sexual maturity and, in turn, sexual size dimorphism in Sardinian lungless salamanders in relation to continental species. Alternatively, the basal position of *Atylodes genei* in the phylogeny of the European lungless salamanders (Van der Meijden et al. 2009) could be evidence of an ancestral condition of little or no sexual size dimorphism.

Based on our results, *Atylodes genei* exhibits a clear pattern of sexual shape dimorphism characterised by larger heads, limbs and tail in males, albeit with some geographical variation. Previous research (Lanza et al. 1995) found the same pattern of sexual dimorphism in limb proportions but opposite patterns in relative head lengths, as well as sexual differences in head width and tail length that varied between the studied populations. Sexual differences in body shape could have implications for this species' ecology. For example, cave salamanders use their tails as a fifth limb to move up and down vertical surfaces (Lanza 1991) and so if they have proportionally longer tails than females, males may be obliged to avoid steeper slopes. Nevertheless, none of the few available studies of the spatial ecology of cave salamanders have ever found sexual segregation in their use of vertical habitats (Ficetola et al. 2013). Although larger hind limbs in males may increase their climbing ability and compensate for the negative effect of longer tails, it is likely that the magnitude of the difference we found is not large enough to have any real effect. Another significant sexually dimorphic trait found in our study was head width, which was proportionally larger in males, a pattern shared with most plethodontid salamanders including the closely related *Speleomantes* species (Salvidio & Bruce 2006). Two different hypotheses have been proposed to explain this pattern. Firstly, contests between males to control mating territories may promote the development of proportionally bigger heads with powerful jaw muscles as, for example, in the plethodontid salamanders of the genus *Aneides* (Davis 2002); nonetheless, this behaviour has never been observed in the European cave salamanders (Zanetti & Salvidio 2006). Secondly, sexual differences in head size could be explained by a segregation of trophic niches reducing intraspecific competition since prey size might be constrained by mouth size. However, previous studies on Sardinian cave salamanders have found no support for this hypothesis and only report an ontogenetic effect on diet structure (Lunghi et al. 2017, Salvidio et al. 2017).

Our results suggest that a change in the patterns of sexual size dimorphism in *Atylodes genei* compared to other European plethodontid salamanders has taken place; by contrast, shape dimorphism has remained mostly unchanged. Future research on sexual dimorphism, age at maturation and fecundity in other lungless salamanders could help clarify whether these differences are adaptations to insularity or, alternatively, the result of the conservation of an ancestral condition.

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