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DATA ON THE FEMALE REPRODUCTION OF *CHALCIDES MERTENSI* KLAUSEWITZ, 1954 (REPTILIA: SCINCIDAE) FROM TUNISIA

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Abstract. *Chalcides mertensi* is an Algero-Tunisian endemic three-toed skink. It inhabits perennial meadows in north-western Tunisia. The main characteristics of its reproduction traits were studied in six populations, by counting the total number of embryos in pregnant females in relation to their snout-vent lengths (SVLs) and measuring the lengths of newborn individuals. The species is viviparous, with SVLs of gravid females ranging between 103.2 and 220.3 mm, making this species one of the largest among its congeners. The maximal number of embryos per gravid female was 28, ranging between zero and 15 in each of the right and left oviducts. Selection apparently favoured larger females to maximize the number of embryos since a correlation between the number of embryos and female SVL was found. Births occurred during the first week of July, one month earlier than dates given for three-toed *Chalcides* species in Europe. The species produced one clutch each year.

INTRODUCTION

The genus *Chalcides* is the most diverse one among North African Scincidae, with many endemics in the region (Pasteur 1981; Mateo et al. 1995; Bons and Geniez 1996; Schleich et al. 1996; Carranza et al. 2008). Within the genus, the "grass swimming clade" (Carranza et al. 2008) contains seven species with marked body elongation and limb reduction (Caputo et al. 1995; Greer et al. 1998) which occupy mostly grassy habitats (Doumergue 1901; Chirio and Blanc 1997; Rugiero 1997; Escoriza et al. 2018).

Within this clade, tridactyl skinks are represented by five species (Caputo 1993) with two Moroccan endemics: *Chalcides minutus* and *C. pseudostriatus*. *C. striatus* is restricted to the Iberian Peninsula, southern France and northern Italy. *C. chalcides* occupies peninsular and insular Italy, Tunisia and Libya where its presence is questionable (Bauer et al. 2017). In Tunisia, a high genetic diversity of the species was noted compared to Italian populations (Giovannotti et al. 2007; Stöck et al. 2016). Finally, *C. mertensi* is an Algero-Tunisian endemic. The species may be abundant in some areas; its distribution is discontinuous, as noted for Algeria where its presence was linked to water (Chirio and Blanc 1997). However, in Tunisia, the species was not found far from water in many of its habitats, but it lives in humid areas.

Studies on reproduction of three-toed skinks started since the 19th century, with Studiati's work on the reproduction of *Chalcides chalcides* (Studiati 1855; Blackburn et al. 2015). He was followed by Giacomini (1891) who described the histological structures of the species placenta (Blackburn et al. 2017). Then, a few other authors treated the same subject (Mingazzini 1892; ten Cate-Hoedemaker 1933).

More recently, intensive studies on the viviparity of *Chalcides chalcides* were conducted during the 1990s (Angelini and Ghiara 1991; Blackburn 1993; Caputo 1993; Blackburn and Callard 1997; Rugiero 1997; Blackburn et al. 1998; Caputo et al. 2000). Studies on the reproduction of other three-toed skinks concerned *Chalcides striatus* (Pérez-Quintero 1988; Caputo et al. 2000), *Chalcides guentheri* (Goldberg 2012) and *Chalcides mauritanicus* (Bogaerts 2020). Information on the reproduction of *C. pseudostriatus* was given by Caputo (1993) and on *C. minutus* by Caputo et al. (2000).

This study is restricted to populations of *Chalcides mertensi* inhabiting the north-western parts of Tunisia (Figure 1) without dealing with more southern populations (Giovannotti et al. 2013). Among tridactyl *Chalcides* species, *Chalcides mertensi* is the last one for which there is no data on its reproduction. Therefore, the main objective of this paper is to present basic information on the reproduction of this species and to compare it with other three-toed congeners.

METHODS

Study sites

The studied populations of *Chalcides mertensi* belong to six localities: Ain Soltane (36°31'17", 8°21'05",

Figure 1. Adult specimen of *Chalcides mertensi* in its natural habitat, environs of Ain Draham, June 2020.

676 m (altitude)), El Feidja (36°31'12", 8°18'51", 1121 m), Fernana (36°40'04", 8°40'55", 285 m), Tabarka (36°56'04", 8°46'43", 2 m), Nefza (36°58'02", 9°01'59", 51 m) and Sejnane (37°04'16", 9°12'11", 119 m; Figure 2).

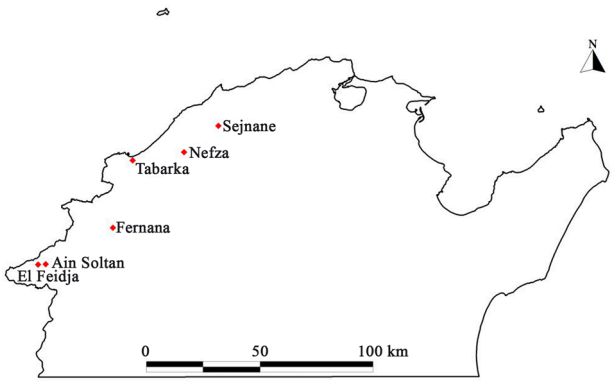

Figure 2. Geographical distribution of six studied populations of *Chalcides mertensi* in north-western Tunisia.

Ain Soltane and El Feidja are very close, and they will be regarded as one site. In El Feidja, animals live in open firebreak trenches and open meadows. The species is not frequently found in the field. Its habitats are mainly covered with annual herbaceous plants, *Asphodelus ramosus* and *Agrimonia eupatoria*. The habitat occupied by the species in Ain Soltane is hilly with many landslides. It is entirely covered with annual and perennial Poaceae, but the landscape is dominated by two spiny plants: *Cynara cardunculus* and *Galactites tomentosa*. *C. cardunculus* constitutes a safe refuge for animals when disturbed. This population was particularly abundant.

In Tabarka, animals inhabit perennial meadows. The permanent vegetation cover is made up of perennial Poaceae (*Cynodon dactylon* and *Bolboschoenus glaucus*) associated with other species, particularly *Asphodelus ramosus* and *Drimia maritima*, mainly in areas under intense grazing pressure and in the boundary separating plots of land. Shrub vegetation includes rushes (*Juncus* sp.) and *Dittrichia viscosa*. In these meadows, the density of animals is often high. In recent years, certain plots started to be cultivated, and the burning of herbaceous vegetation took place at the end of summer, with unfavourable consequences for animals living there. In Nefza, the habitat of *Chalcides mertensi* consists of a sloping olive grove on the edge of a dam. As the soil is not ploughed, it is mostly covered with *Asphodelus ramosus*, *Drimia maritima* and *Dittrichia viscosa*. The clay soil cracks in the hot season, and animals take refuge inside these fissures. Finally, in the region of Sejnane, the habitat of the species consists of a permanent meadow adjoining a *Eucalyptus* plantation. A continuous vegetation cover is formed by Poaceae mixed with *Asphodelus ramosus*. The meadow is much extended and is floodable in winter.

Sampling

Female skinks were collected from their natural habitats (permanent meadows) during their activity period, between April 2002 and June 2006, from different areas in north-western Tunisia. Skinks were fixed in alcohol and dissected later. A pregnant female was taken in July 2003 from Tabarka. It was maintained in captivity until parturition (for one day). Once hatchlings were born (n = 9), their date of birth was noted, measured (snout-vent length (SVL) and tail length) and released with their mother in the place from which it was captured.

Analyses

MapInfo 5 was used to represent the distribution of the six localities from which females were studied. IBM

SPSS Statistics (version 20) was used to analyse the collected data. Mean values are presented with their standard errors.

For each female, SVL was measured by a calliper (to the nearest 0.1 mm). Sexually active females have enlarged ova which migrate to the oviducts after fecundation (Schleich et al. 1996). Their colour turns into yellow. Inactive females have small white oocytes still in the ovary. After dissection, the number of embryos in each oviduct was counted.

As female SVL seems to vary between populations, a Mann Whitney U test was done between the SVL of females from Tabarka and Ain Soltane (13 and 40 females, respectively). The same test was conducted to compare the total number of embryos between females from Tabarka and Ain Soltane.

To check the asymmetry in embryo numbers between oviducts, a Mann Whitney test was conducted to compare the number of embryos in the left and right oviducts after testing for normality.

Finally, linear regression was used to analyze the relationship between female SVL and the total number of embryos. The Spearman correlation coefficient was also calculated. Alpha was set at 0.05.

RESULTS

Data collected from the pregnant females are summarized in Table 1. The smallest gravid female had a SVL of 103.2 mm (Nefza), while the largest one had a body length of 220.3 mm (Ain Soltane). The total number of embryos per gravid female varied between 3 and 28. It varied between zero and 15 and 1 and 14 in the right and left oviduct, respectively. Statistics of the data concerning all gravid females are presented in Table 2.

A significant difference in SVL was found between females from Tabarka and Ain Soltane (U = 152.0, $p = 0.0263$). However, no significant differences were found in the number of embryos between both populations (U = 166.0, $p = 0.0515$). There was also no significant difference in the number of embryos between oviducts (U = 1854, $p = 0.973$).

There was a significant correlation between SVL and the total number of embryos (Figure 3).

The Spearman correlation coefficient was 0.846 and was significant ($p < 0.0001$).

All females with a SVL larger than the smallest gravid one were not pregnant. Indeed, five females from Ain Soltane having a body length larger than 107 mm were not gravid (one of which caught in June). Their SVL varied between 107.5 and 132 mm, accounting for only 11.1% of the total sexually mature females. It is to be noted that the smallest gravid female in this population had a SVL of 116.4 mm and that all females with a SVL higher than 140 mm were gravid (Figure 4).

Hatchlings were born on the 5th of July 2003. Data concerning their body measurements are presented in Table 3. Body length varied between 42.9 and 47.9 mm, while total length varied from 85.2 to 93.4 mm.

DISCUSSION

Although information on the reproduction of other *Chalcides* species is available in the literature (Caputo 1993; Rugiero 1997; Caputo et al. 2000), this paper provides the first data on the reproduction of *Chalcides mertensi*. Data presented by some other authors (Orsini and Cheylan 1981) are confusing because they concern a complex of three-toed *Chalcides* species.

Table 2. Summary statistics of the data on *Chalcides mertensi* reproductive output. R.O., right oviduct; L.O., left oviduct.

	n	Minimum	Maximum	Mean	SE
R.O.	61	0	15	5.89	0.468
L.O.	61	01	14	5.61	0.356
Total	61	03	28	11.44	0.784

Table 3. Summary statistics on newborn *Chalcides mertensi* (n = 9).

	SVL (mm)	Tail length (mm)	Total length (mm)
Mean	45.52	43.97	89.49
SE	0.60	0.61	0.95
Min	42.90	42.30	85.20
Max	47.90	48.30	93.40

Table 1. Summary data collected from females of *Chalcides mertensi* from north-western Tunisia.

	Ain Soltane	El Feidja	Fernana	Nefza	Sejnane	Tabarka	Total
n	40	1	4	1	2	13	61
SVL*	155.053 ± 4.72	183.70	168.35 ± 12.96	103.20	144.60 ± 29.90	171.97 ± 5.48	158.80 ± 3.72
R.O.**	5.43 ± 0.612	8.0	8.75 ± 1.03	3.0	6.0 ± 3.0	6.46 ± 0.91	5.89 ± 0.47
L.O.***	5.30 ± 0.483	7.0	7.50 ± 0.87	2.0	4.00 ± 1.0	6.38 ± 0.52	5.61 ± 0.36
Total	10.65 ± 1.04	15.0	16.25 ± 1.65	5.0	10.0 ± 4.0	12.85 ± 1.38	11.44 ± 0.78

* mean ± SE; ** right oviduct: mean ± SE, *** left oviduct: mean ± SE.

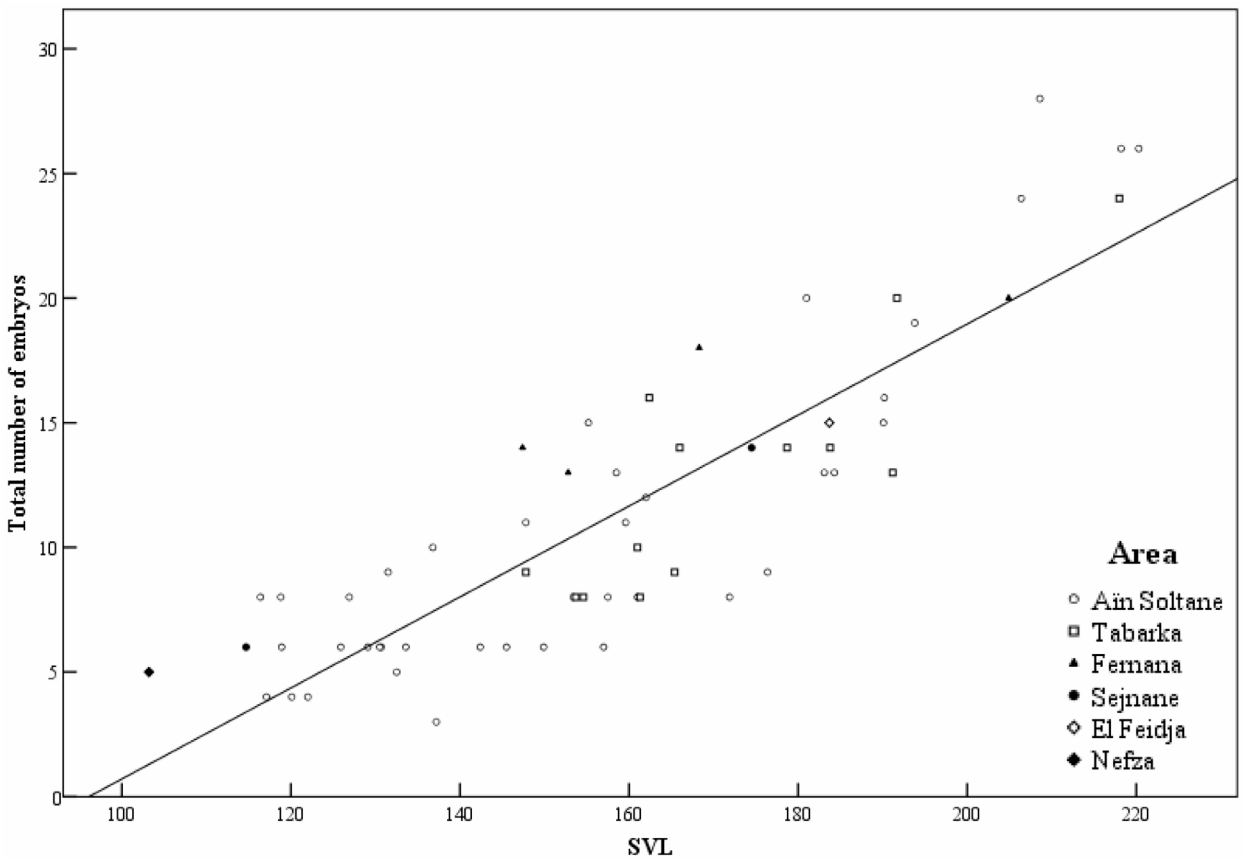

Figure 3. Linear regression between body length and litter size (number of embryos) in six Tunisian populations of *Chalcides mertensi*.

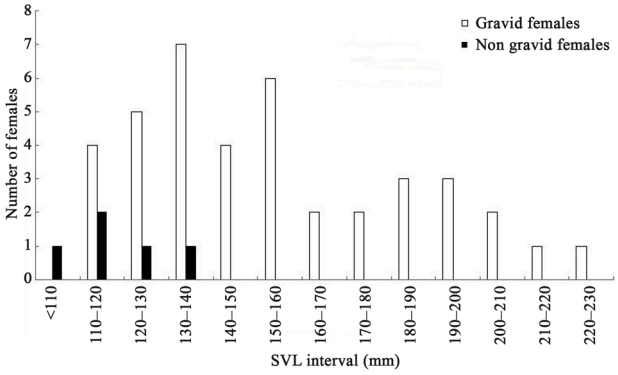

Figure 4. Number of gravid and non-gravid females with a SVL greater than the smallest pregnant female in Ain Soltane.

Table 4. Litter size of the "grass-swimming clade of *Chalcides*" *sensu* Carranza et al. (2008).

Species	Litter size (range)	(*n*)	Author
C. chalcides	7.8 (3–19)	60	Caputo et al. 2000
=	5.9 (4–9)	15	Rugiero 1997
C. striatus	10	1	Caputo et al. 2000
=	4.23 (1–8)	26	Pérez-Quintero 1988
C. pseudostriatus	12.67 (6–22)	3	Caputo 1993
C. minutus	4.0 (2–7)	6	Caputo et al. 2000
C. guentheri	5.8 (3–8)	5	Goldberg 2012
C. mauritanicus	2.5 (2–3)	2	Bogaerts 2020
C. mertensi	11.44 (3–28)	61	This study

In this study, despite that the smallest pregnant female had a SVL of 103.2 mm (Nefza), we found some larger females not pregnant. This implies that mature females do not reproduce annually (Figure 4). On the other hand, all females caught after the 10th of July were not sexually active (empty oviducts), indicating that births took place earlier. Only one female was captured on the 15th of March and was not sexually active (oocytes still attached to the ovary, empty oviducts).

The fact that the largest gravid female had a body length of 220.3 mm makes *Chalcides mertensi* the largest three-toed *Chalcides* species (Pérez-Quintero 1988; Caputo 1993; Caputo et al. 1995). Maximum body length known for tridactyl *Chalcides* species is 202 mm for *C. pseudostriatus* (Caputo 1993) and 204 mm for a Moroccan *Chalcides* species (Busack 1986). Both of these records are shorter than the largest *C. mertensi* found in this study (Table 2). Thus, it is not possible to compare the weight of the study species with *C. pseudostriatus* (more than 150 g; Mateo et al. 1995) since the weight of *C. mertensi* was not registered.

It should be noted that the largest individual did not contain the maximal number of embryos (26). While the one having the highest number of embryos (28)

had a body length of 208.6 mm. Both of them were from Ain Soltane. The mean number of embryos of the "grass-swimming" clade of *Chalcides sensu* Carranza et al. (2008) is presented in Table 4.

This table shows that the mean litter size in *C. mertensi* is smaller than that noted for *C. pseudostriatus* (n = 3). This result may be a consequence of sample size. In the studied sample, litter size was 16.25 in Fernana (n = 4) or 12.85 in Tabarka (n = 13). In *C. mertensi*, clutch size was significantly correlated with female body size. This finding was also noted for *C. chalcides* (Caputo 1993; Caputo et al. 2000).

The species starts its annual activity by the first week of March, and stays active until the end of September. The earliest activity period in the year for a tridactyl *Chalcides* species was given by Pérez-Quintero (1988) for *C. striatus* in Spain (February) and by Doumergue (1901) in Algeria for the same month. In north-western Tunisia, active *Chalcides* species during February have never been found, even on hot days. As noted by Rugiero (1997) for its congener *C. chalcides* or by Pérez-Quintero (1988) for *C. striatus*, active animals become rare since mid-July till the end of their activity period. No copulation was seen in the field, and newborn individuals were found active by the end of the first week of July. In the studied samples, the difference in body length between the smallest and largest newborn individuals was 5 mm, while difference in total length was 8.2 mm. Such differences were also recorded for newborn *C. mauritanicus* (Bogaerts 2020). As individuals were not sexed, it is not possible to ascertain if these length differences are related to sexual dimorphism or not. As adult females are larger than adult males, they may be larger at birth. It may also be related to different nutritional intake from the mother during pregnancy, but this hypothesis still needs to be proved.

As noted for related species (Ghiara et al. 1987; Pérez-Quintero 1988; Angelini and Ghiara 1991; Blackburn 1992, 1993; Blackburn and Callard 1997; Blackburn et al. 1998; Caputo et al. 2000; Corso et al. 2000; Galán 2003; Sánchez-Hernández et al. 2013), *C. mertensi* is viviparous and produces only one clutch each year. Copulation in this species occurs probably in late March, and births occur up to the first week of July. This cycle is the same as that of *C. chalcides*, even if parturition is noted later in Italy (late July–August; Angelini and Ghiara 1991; Rugiero 1997). It is important to note that in Tunisia parturition occurs in the same period for *C. chalcides* (pers. obs.). The noted difference with Italian populations may be caused by environmental factors (higher temperatures in North Africa compared to Europe). The same dates of birth are also given for *Chalcides bedriagai* in Spain (Galán 2003) or *C. viridanus* in the Canary Islands (Sánchez-Hernández et al. 2013). It seems that, out of Tunisia, both in *C. chalcides* and other pentadactyl species, copulation occurs later than in *C. mertensi*.

C. mertensi females (Table 1) were larger than males (mean SVL of males 115.41 ± 3.39 mm, n = 65; data not shown; see Caputo (1993) for a smaller sample size). This is also the case of *C. chalcides* (Caputo 1993; Caputo et al. 2000), *C. striatus* (Pérez-Quintero 1988) and *C. mauritanicus* (Martín et al. 2015). Larger female bodies are selected to allow the development of more embryos (Vitt and Blackburn 1991; Perry 1996). Indeed, in this study larger females had the highest number of embryos (Figure 1).

Further studies are needed to investigate embryo growth during pregnancy, placental structures (such as those described for *Chalcides chalcides* (Angelini and Ghiara 1991; Blackburn and Callard 1997)), and quantify the embryo intake of nutrients during pregnancy.

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