Reproduction in Kotschy's gecko *Mediodactylus kotschyi* (Squamata: Gekkonidae) from the Greek Islands and Israel

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KOTSCHY'S gecko *Mediodactylus kotschyi* (Steindachner, 1870) is widely distributed in the Old World and has a discontinuous distribution that includes Israel, Jordan, Iran, Lebanon, Syria, Asia Minor, Greece, Agean Islands, Cyprus, Albania, Turkey, Bulgaria, Hungary and the Ukraine, (Uetz & Hosek 2011). There are anecdotal accounts of *M. kotschyi* reproduction in Werner (1930), Werner (1966), Loveridge (1972), Beutler & Gruber (1979), Valakos & Vlachopanos (1989), Szczerbak & Golubev (1996), Baran & Atatür (1998), Corti & Lo Cascio (2002), Szczerbak (2003), Beshkov & Nanev (2006), Valakos et al. (2008), Baier et al. (2009) and Stojanov et al. (2011). The purpose of this paper is to add information to the reproductive biology of *M. kotschyi* by reporting on a histological analysis of gonadal material from the Greek Islands and Israel. Information on the reproductive cycle such as timing of spermiogenesis, number of egg clutches produced and period of vitellogenesis provides essential life history data for formulating conservation policies for lizard species.

MATERIALS AND METHODS

A sample of 29 *M. kotschyi* from Greece (Greek Islands) (10 males, mean SVL = 37.3 mm ± 4.5 SD, range = 30-44 mm; 14 females, mean SVL = 40.2 mm ± 3.0 SD, range = 35-44 mm; 5 subadults, mean SVL = 22.0 mm ± 1.4 SD, range = 21-24 mm) was borrowed from the Peabody Museum of Natural History (YPM), Yale University, New Haven, Connecticut, USA. A further 25 *M. kotschyi* from Israel (8 males, mean SVL = 35.8 mm ± 5.1 SD, range = 28-42 mm; 16 females, mean SVL = 37.5 mm ± 5.2 SD, range = 29-45 mm; 1 subadult, SVL = 25 mm) were borrowed from the Tel-Aviv University, Zoological Museum (TAUM), Tel Aviv, Israel for a reproductive study. Lizards from the Greek Islands were collected in 1959, 1961, 1991 and 1999, and from Israel in 1950, 1952, 1953, 1955, 1958, 1959, 1962, 1964, 1965, 1972, 1975, 1984, 1988, 1989, 2002, 2005. *Mediodactylus kotschyi* examined are listed in the appendix.

For histological examination, the left testis was removed from males and the left ovary was removed from females. Enlarged follicles (> 3 mm length) or oviductal eggs were counted. Tissues were embedded in paraffin and cut into sections of 5 µm. Slides were stained with Harris haematoxylin followed by eosin counterstain (Presnell & Schreibman, 1997). Slides of testes were examined to determine the stage of the spermatogenic cycle. Slides of ovaries were examined for the presence of yolk deposition or corpora lutea. Histology slides were deposited at TAUM or YPM. An unpaired t-test was used to compare *M. kotschyi* male and female mean body sizes (SVL) using Instat (V. 3.0b, Graphpad Software, San Diego, CA).

RESULTS

Mean body sizes (SVL) were not significantly different between males from the Greek Islands and Israel (unpaired t-test, $t = 0.69$, $df = 16$, $P = 0.50$). Monthly stages in the testicular cycle in the Greek Islands and Israel are shown in Table 1. Two stages were present in the testicular cycle; (1) Recrudescence: characterised by a proliferation of germ cells for the next period of spermiogenesis (sperm formation), primary and secondary spermatocytes predominate; (2) Spermiogenesis, whereby lumina of the seminiferous tubules are lined by clusters of spermatozoa and/or rows of metamorphosing spermatids. Epididymides were not sectioned but they were enlarged and whitish in colour, consistent with them containing sperm. Testes undergoing recrudescence were noted in March in the Greek Islands and February and November in Israel. The epididymides from these

Month	n	Recrudescent	Spermiogenesis
		Greek Islands	
March	2	2	0
May	6	0	6
July	2	0	2
		Israel	
February	2	1	1
March	2	0	2
April	1	0	1
May	1	0	1
November	2	2	0

Table 1. Monthly stages in the testicular cycles of *Mediodactylus kotschyi* from Israel (n = 8) and the Greek Islands (n = 10).

three males were not enlarged. Males undergoing spermiogenesis occurred in May and July in the Greek Islands and February to May in Israel (Table 1). The smallest reproductively active male (spermiogensis in progess) from the Greek Islands (YPM 5795) measured 30 mm and was collected in May; from Israel (TAUM 2965) the smallest measured 31 mm and was collected in April.

Mean body sizes (SVL) were not significantly different between females from the Greek Islands and Israel (unpaired t-test, t = 1.7, df = 28). Monthly stages in the ovarian cycle in the Greek Islands and Israel are in Table 2. Three stages were present in the ovarian cycle of *M. kotschyi*; (1) Quiescent: no yolk deposition was noted; (2) Early yolk deposition: basophilic yolk granules present in the ooplasm; (3) Enlarged ovarian follicles > 3 mm; (4) Oviductal eggs were present. The single clutch from Israel (TAUM 4135) consisted of two eggs. The seven clutches from the Greek Islands (YPM 5798, 5809, 5811, 5812, 5828, 5829, 15154) had a mean of: 1.86 ± 0.38 SD, range: 1-2. The smallest reproductively active female from the Greek Islands (two follicles > 3 mm) measured 35 mm and was collected in May (YPM 15154) whereas those from Israel both measured 45 mm SVL (TAUM 4135); two follicles > 4 mm from January and (TAUM 6081) two oviductal eggs from May. This large minimum body size for reproduction for *M. kotschyi* females from Israel likely reflects my small female sample size. There was evidence from the Greek Islands (YPM 5809, 5798, 5812) and Israel (TAUM 4135) that female *M. kotschyi* produce multiple clutches during a single year. This was indicated by the presence of oviductal eggs and concomitant yolk deposition in the same females.

Five subadults from the Greek Islands collected in March (mean SVL = 22.0 mm ± 1.4 SD) were presumably born late the previous fall. One subadult from Israel (SVL = 25 mm) collected in September was presumably born earlier in the same year.

DISCUSSION

Beutler & Gruber (1979) reported that subadults of *M. kotschyi* (20 to 25 mm SVL) became active in March in the Greek Islands and reached adult sizes (30 to 34 mm SVL) in May. *Mediodactylus kotschyi* has an extensive geographic range that encompasses a variety of climates ranging from

Month	n	Quiescent	Early Yolk Deposition	Enlarged Follicles > 3 mm	Oviductal Eggs
			Greek Islands		
March	4	4	0	0	0
May	7	1	1	1	4*
June	2	0	0	2	0
July	1	1	0	0	0
			Israel		
January	3	2	0	1	0
February	1	1	0	0	0
March	3	3	0	0	0
May	2	1	0	0	1**
June	2	2	0	0	0
October	2	2	0	0	0
December	3	3	0	0	0

Table 2. Monthly stages in the ovarian cycles of *Mediodactylus kotschyi* from Israel (n = 16)/Greek Islands (n = 14); *three of four females, **one female, with oviductal eggs and concomitant yolk deposition for a subsequent clutch.

Location	Clutch Size	Number Clutches	Reproductive Period	Source
Bulgaria	2	?	summer	Beshkov & Nanev (2006)
Bulgaria	1-2	?	summer	Stojanov et al. (2011)
Crimea	1-2	1	May to July	Szecerbak & Golubev (1996)
Cyprus	2	?	March to June	Baier et al. (2009)
Greece	mean = 2.25	?	?	Valakos & Vlachopanos (1989)
Greece	1-2	?	Year-round	Valakos et al. (2008)
Greek Islands	1	?	May	Werner (1930)
Greek Islands	2	?	June, July	Loveridge (1972)
Greek Islands	?	?	May to July	Beutler & Gruber (1979)
Israel	1-2	?	May to September	Werner (1966)
Italy	?	1	?	Corti & Lo Casio (2002)
Turkey	2-6	?	?	Baran & Atatür (1998)

Table 3. Previous reports on reproduction of *Mediodactylus kotschyi*.

tropical, temperate to arid. One would thus suspect some geographic variation in the reproductive cycle. Previous reports on reproduction are mainly anecdotal and are listed in Table 3. Herein I have provided information that multiple clutches are produced both in the Greek Islands and Israel. Whether multiple clutches are produced by other populations of *M. kotschyi* will require additional investigation. There is a report of year-round reproduction in Greece by Valakos et al. (2008) which requires verification, as it contrasts with other reports of spring-summer reproduction listed in Table 3. My finding of males with testes undergoing recrudescence (renewal) prior to spring in both the Greek Islands and Israel suggests *M. kotyschyi* exhibits a seasonal reproductive cycle, typical of temperate zone geckos (see for example, Goldberg, 2006), as opposed to tropical lizards which exhibit continuous breeding (Fitch, 1982). The clutch size range of 1-2 is identical to that reported for other geckos (Vitt, 1986). The larger clutches (2-6) reported for *M. kotschyi* from Turkey by Baran & Atatür (1998) should be verified. In view of its extensive geographic range, subsequent analyses of the different populations of *M. kotschyi* are needed before variations in reproductive cycles of these geckos can be ascertained.

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APPENDIX

Mediodactylus kotschyi examined from Israel (TAUM) (by District) and the Greek Islands (YPM) with longitudes and latitudes.

Israel: Central (32°08'N, 34°92'E): 481, Haifa (32°48'N, 34°59'E): 479, 1321, 2021, 2024, 2965, 9921,13776, 13790, 14879; Jerusalem (31°75'N, 35°00°E): 12823, 15232; Northern (32°70'N, 35°30'N): 477, 3050, 5040, 6081, 12810, 12812, 13624; Southern (31°07'N.35°12'E): 721-723, 4135; West Bank (31°42'N, 35°12'E): 10841; Unknown: 5813.

Greek Islands: Andros Island (37°52'N, 24°46'E): 5813, 5814, 5815, 5816, 5817, 5818, 5819, 5820, 5821, 5822 Astypalaia Island (36°32'N, 26°22'E): 5812, 5830; Crete (35°15'N, 25°00E): 15154, 15155, 15156; Milos Island (36°44'N, 24°25'E): 5761, 5762, 5763; Naxos Island (37°04'N, 25°22'E): 5828; Paros Island (37°02'N, 25°11'E): 5808, 5809, 5810, 5811, 5829; Siros Island (37°25'N, 24°57'E): 5794, 5795, 5796, 5797, 5798.