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FILLING THE GAPS: ADDITIONAL NOTES ON THE REPRODUCTION OF THE KÜHNE'S GRASS LIZARD (*TAKYDROMUS KUEHNEI* VAN DENBURGH, 1909; SQUAMATA: LACERTIDAE) FROM SOUTHWESTERN TAIWAN

GERRUT NORVAL[1], JEAN-JAY MAO[2], AND STEPHEN R. GOLDBERG[3]

[1]Applied Behavioural Ecology & Ecosystem Research Unit, Department of Environmental Sciences, UNISA, Private Bag X6, Florida, 1710, Republic of South Africa, e-mail: a_sagrei@hotmail.com
[2]Department of Forestry & Natural Resources, National Ilan University. No. 1, Sec. 1, Shen-Lung Rd., Ilan, 260, Taiwan, Republic of China
[3]Department of Biology, Whittier College, Whittier, California 90608, USA

***Abstract*.—The Kühne's Grass Lizard (*Takydromus kuehnei*) is a poorly studied indigenous species in Taiwan. In this report we incorporate additional information concerning reproduction with our previous observations to provide a more comprehensive description of reproduction in *T. kuehnei* from southwestern Taiwan. We collected 48 *T. kuehnei* (18 male, 19 female) from a secondary forest in Santzepu, Sheishan District, Chiayi County, as part of a herpetofauna survey in the area. We also obtained five clutches of eggs from these lizards. The smallest reproductively active female was 49 mm snout-vent length (SVL). Oviposition took place from April to July. Clutch sizes ranged from one to two eggs with an average of 1.8. A histological examination of five museum specimens confirmed that females may produce multiple clutches in the same year. We obtained 11 eggs that had an average length, width, and mass of 10.6 mm, 6.1 mm, and 1.8 g, respectively. The average relative clutch mass was 16.7%. Five eggs successfully hatched after an average incubation period of 32.6 days. The hatchlings had an average SVL of 23.8 mm, total length of 51.4 mm, and mass of 0.3 g.**

Key Words.—clutch size; multiple clutches; relative clutch mass; size at maturity

INTRODUCTION

Reporting in detail on the reproductive biology of a species not only contributes to the understanding of the biology of the species in question, but can also contribute to the development of more comprehensive life-history models and elucidate phylogenetic relationships (Tinkle 1969). Even studies of limited data can be helpful in providing a basis for comprehensive studies, and/or highlight aspects that warrant in-depth empirical studies. Lacertid lizards in the genus *Takydromus* (= speed runner) are slender, long-tailed lizards from Southeast Asia, China, Taiwan, Ryukyu Islands, Japan, and extreme southeastern Russia (Pianka and Vitt 2003; Arnold et al. 2007). All members of this genus are oviparous (Arnold et al. 2007). Clutch sizes of some *Takydromus* species consist of 1–2 eggs, while others may have 4–9 eggs (Takenaka 1989; Arnold 1997; Arnold et al. 2007).

The Kühne's Grass Lizard (*Takydromus kuehnei*), formerly classified as *Tachydromus kuehnei* and *Platyplacopus kuehnei* (Arnold et al. 2007), is a diurnal species that lives in forest margins, at altitudes below 1000 m, in Taiwan, southeastern parts of China (Van Denburgh 1909; Pope 1935), and Vietnam (Ziegler and Bischoff 1999; Ziegler 2002; Bobrov 2003). Ziegler and Bischoff (1999) described the reproductive state of some *T. kuehnei* female specimens from China, Taiwan, and Vietnam, as well as a hatchling from a clutch of eggs that were found on the ground, under some leaf litter, near a brook in Vietnam (Ziegler 2002). Previously, we reported on one *T. kuehnei* clutch (Norval and Mao 2006), and also gave a brief description of the oviductal eggs of a dissected *T. kuehnei* (Norval and Mao 2008), both from lizards collected from southwestern Taiwan. Yet, the reproductive biology of *T. kuehnei* in Taiwan remains poorly studied, and aspects such as the duration of the reproductive season and the minimum sizes at which the sexes become sexually mature are undescribed. Here we report additional information concerning reproduction, which we incorporate with our previous observations to provide a more comprehensive description of reproduction in *T. kuehnei* from southwestern Taiwan.

MATERIALS AND METHODS

***Field collection*.**—From June 2005 to July 2008, we collected *T. kuehnei* with short-fence funnel-trap units that were set in a small fragment of a secondary forest in Santzepu, Sheishan District, Chiayi County (23º25'N, 120º29'E; elevation = ca. 90 m; datum: WGS84) as part

FIGURE 1. One of the short-fence funnel-trap units used in this study. Funnel-traps were covered with a cotton fabric (not shown here) to protect the trapped animals from the elements.

of a herpetofauna survey in the area. Forest canopy cover was about 80% and was created by the crowns of Betelnut Palms (*Areca catechu*) and *Macaranga tanarius*. In addition to the saplings of *A. catechu*, the undergrowth of the secondary forest consisted of *Alocasia odora*, *Alpinia zerumbet*, *Ardisia squamulosa*, *Bidens pilosa*, *Cinnamon camphora*, *Clerodendrum paniculatum*, *Dendroclalmus latiflorus,* and *Ipomoea cairica*.

Each short-fence funnel-trap unit (Fig. 1) consisted of three corrugated polypropylene plastic sheets (each 100 cm in length, 30 cm in height, and 0.5 cm in thickness). The plastic sheets were taped together with duct-tape and held upright and in place by hairpin-shaped wires that were inserted, through holes created by the corrugations of the fence material, into the soil. Soil was used to fill any gaps that could allow animals access beneath the fence. We placed a 30 × 30 cm PVC celuka board, with a 15 cm diameter hole, over which a modified shrimp funnel trap was attached, at each end of the fence.

For all collected lizards, we measured snout-vent length (SVL) and tail length (TL) with a transparent plastic ruler to the nearest mm, scored the tail as complete or broken, and weighed lizards to the nearest 0.1 g with a digital scale. If lizards had suffered tail-loss in the past, the regenerated portion of the tail was also measured with a transparent plastic ruler to the nearest mm. To avoid injury to the lizards, we made no attempts to determine the sex of the lizards, other than visually examining the tail bases for the presence of enlarged hemipenal pouches.

Captive husbandry, eggs and hatchlings.—We temporarily (< 5 days) housed collected lizards in small glass cages (L × W × H = 25 × 25 × 27 cm), after which they were released in the same area they had been collected. We covered the floor of every cage with a thin layer (ca. 10 mm deep) of clean river sand overlain by a layer (ca. 20 mm deep) of sphagnum moss (*Sphagnum* sp.). Each cage also contained two small Petri dishes for food and water. We provided food, which consisted of mealworms (*Tenebrio molitor*) and clean fresh water *ad libitum*. The cages were placed on a shelf in a room where they were exposed to direct sunlight early in the morning. We did not take any other measures to control light and temperature regimens. We checked the cages daily for eggs, by carefully searching through the sphagnum moss.

While in captivity, five females laid eggs, and for each clutch of eggs, we recorded the clutch size and measured the length and width of each egg with a dial caliper to the nearest 0.01 mm. If the eggs were not attached to each other, we weighed them individually to the nearest 0.1 g with a digital scale. When eggs were attached to each other, no attempts were made to separate them, so their combined weight was measured to the nearest 0.1 g with a digital scale. We weighed the female again to the nearest 0.1 g with a digital scale to obtain the maternal post-oviposition body mass, and then calculated the relative clutch mass by using the formula (Shine 1980):

$$\text{relative clutch mass} = (\text{the total clutch weight} / \text{post-oviposition body mass}) \times 100$$

However, on two occasions, more than one female was housed in the same cage, so it was not possible to determine conclusively which female had laid the eggs. Therefore, no maternal data could be obtained.

After the dimensions and weights of the eggs were measured, we placed the eggs on top of a clean river-sand substrate, with a depth of ca. 50 mm, in a plastic small-animal cage. We covered eggs and substrate with sphagnum moss, and we created a moist environment within the cage by mist-spraying the interior of the cage with water every 2–3 days. As we have done before (Norval and Mao 2006), we incubated the eggs at room temperature (25–32 ºC) by placing the cage on the floor in a room where it was exposed to sunlight during the morning.

To avoid injury to the hatchlings, we made no attempts to determine their sex. They were measured by placing them next to a small ruler under a white sheet on an Epson Professional 1650 scanner, set to scan at 400 dpi and 100%. All hatchling measurements were then recorded from these scanned images (Mao et al. 2009). We weighed the hatchlings with an electronic scale to the nearest 0.1 g, and all were released a few days after hatching in the area where the adult females were collected.

Histology of museum specimens.—We also examined histologically five *T. kuehnei* females from the same area to determine their reproductive states. We deposited these specimens in the herpetology collection of the

TABLE 1. Summary of the ranges of morphological data of *Takydromus kuehnei* in this study. The means and standard deviations are given in parentheses. Hatchlings were from eggs laid by females in captivity.

	n	SVL (mm)	Tail-length (mm)	Mass (g)
Males	18	49–58 (53.4 ± 2.9)	19–183 (142 ± 46.9)	1.7–3.4 (2.5 ± 0.5)
Females (all)	19	49–59 (53.4 ± 2.6)	95–160 (135.2 ± 18.8)	1.3–2.9 (2.3 ± 0.4)
Females (gravid)	5	49–59 (54.8 ± 3.8)	115–160 (137.4 ± 21.3)	1.8–2.9 (2.4 ± 0.5)
Lizards < 49 mm SVL	11	33–48 (42.2 ± 4.8)	41–143 (111.4 ± 32.7)	0.6–1.9 (1.3 ± 0.4)
Hatchlings	5	23–24 (23.8 ± 0.5)	45–55 (51.4 ± 3.9)	0.3–0.3 (0.3 ± 0.0)
Dissected specimens	5	33–59 (47 ± 10.7)	41–160 (114.4 ± 51.3)	0.6–2.9 (1.7 ± 1.1)

Natural History Museum of Los Angeles County, Los Angeles, California, USA. The specimens consisted of an intact *T. kuehnei*, which was recorded as a prey item of a *Sibynophis chinensis chinensis* (Norval and Mao 2008), and four *T. kuehnei* that were accidentally collected in 2007 and 2008 in pitfall traps set in Santzepu, Sheishan District, Chiayi County, southwestern Taiwan, as part of a study to examine the impact of the exotic lizard, *Anolis sagrei*, on arthropod diversity in the area (Huang et al. 2008). We removed the left gonad of the specimens, dehydrated them in an ascending series of ethanol, embedded them in paraffin, sectioned them at 5μ, and mounted them on glass slides, which was stained with Harris' hematoxylin followed by eosin counterstain. We examined the left ovaries of all the specimens visually or microscopically, and assigned them to one of four stages of the ovarian cycle: (1) inactive - no yolk deposition; (2) yolk deposition in one or more ovarian follicles; (3) oviductal eggs present and yolk deposition in one or more ovarian follicles; or (4) oviductal eggs present, and no yolk deposition in ovarian follicles.

Statistical analysis.—We used a one-way analysis of variance ($\alpha = 0.05$) to compare the SVL of mature males and females and also to compare the mean dimensions of the eggs from the various clutches.

RESULTS

Field collection.—During the study period we collected 48 *T. kuehnei*. We could positively determine the sex of 18 of the collected lizards as males by their enlarged tail bases. Males had a mean SVL of 53.4 mm (Table 1). Because the smallest of these individuals (n = 2) had a SVL of 49 mm, any lizard with a SVL of ≥ 49 mm, and without an enlarged tail base, was regarded as a female. Using these criteria, we classified 19 of the collected lizards as females. Females also had a mean SVL of 53.4 mm (Table 1). Mean SVL length did not differ ($F_{1, 35} = 0.0007$, $P = 0.98$) between mature males and females. Five of the females laid eggs while in captivity, and the smallest reproductively active female had a SVL of 49 mm (Table 1).

Captive husbandry, eggs and hatchlings.—We recorded oviposition from April to July (Table 2). The size of five clutches ranged from 1–2 eggs (1.8 ± 0.4). The relative clutch mass ranged from 14.3–19.1% (n = 3; mean ± SD = 16.7 ± 2.4; Table 2). We obtained 11 eggs, all of which were white and oval shaped. Average length (± SD) of eggs was 10.6 ± 0.9 mm (range = 9.3–11.85 mm), average width 6.1 ± 0.6 mm (5.34–7.21 mm), and average mass 1.8 ± 0.4 g (0.2–0.3 g). Mean lengths ($F_{5,5} = 15.25$, $P = 0.005$) and widths ($F_{5,5} = 6.53$,

TABLE 2. The snout-vent length (SVL in mm), post-oviposition body mass (POBM in g), clutch size (CS), and relative clutch mass (RCM) of the reproducing *Takydromus kuehnei* females. Also presented are mean ± SD of egg length (MEL), egg width (MEW), and egg mass (MEM).

Date	SVL	POBM	CS	MEL	MEW	MEM	RCM
25 May 2007	57	2.8	2	9.5 ± 0.1	6.0 ± 0.6	0.2 ± 0.0	14.29
25 May 2007	49	2.4	2	9.8 ± 0.3	5.8 ± 0.1	0.2 ± 0.0	16.67
25 April 2008	--	--	2	10.8 ± 0.5	6.0 ± 0.1	0.3 ± 0.0	--
25 April 2008	--	--	1	11.42	5.34	0.2	--
15 June 2008	--	--	2	11.8 ± 0.1	7.0 ± 0.1	0.3 ± 0.0	--

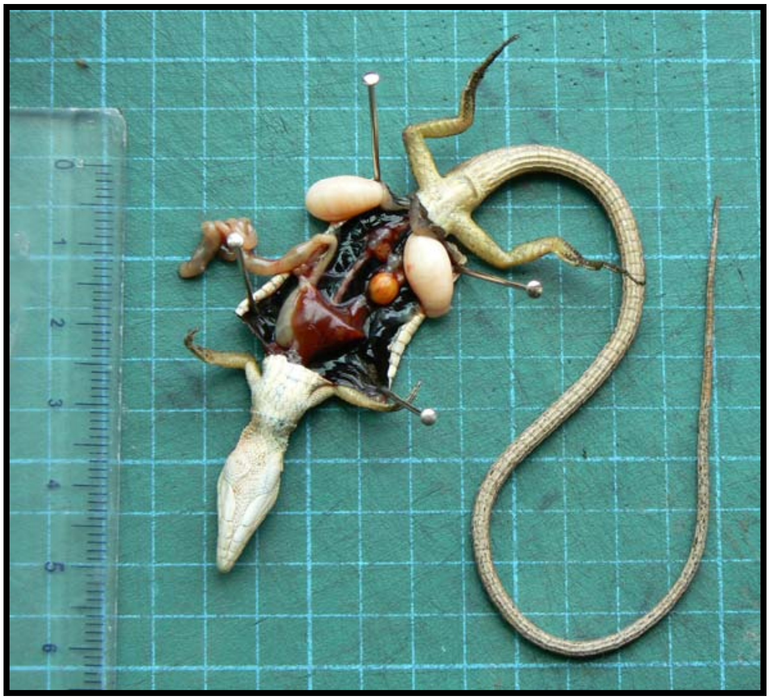

FIGURE 2. A ventral view of a female *Takydromus kuehnei* (LACM 180390). Two shelled oviductal eggs and the enlarged ovarian follicles are evident.

P = 0.03) of the eggs differed significantly among clutches. Only five eggs successfully hatched after an incubation period that ranged from 28 to 37 days (mean 32.6 ± 4.3). Average hatchling SVL was 23.8 ± 0.5 mm (range = 23–24 mm) and average total length was 51.4 ± 3.9 mm (45–55), and all had a body mass of 0.3 g.

Histology of museum specimens.—Of the five specimens that we examined histologically, one female (55 mm SVL, LACM 172594, collected 11 May 2008) had two oviductal eggs, but no yolk deposition in any of the ovarian follicles. Another female (59 mm SVL, LACM 180390, collected 16 May 2007) had two oviductal eggs (Fig. 2), and was undergoing yolk deposition in the ovarian follicles (Fig. 3). A third female (48 mm SVL, LACM 180389, collected 25 May 2007) had no oviductal eggs or yolk deposition in any of the ovarian follicles, despite being collected during the breeding season. The remaining two females (33 mm SVL, LACM 180387, collected 25 May 2007; and 40 mm SVL, LACM 180368, collected 25 May 2007) were immature.

DISCUSSION

Even though some references have been made pertaining to reproduction of *T. kuehnei* (Table 3), the information is incomplete and does not allow detailed comparisons. Our study thus provides a foundation for future research on the species' reproduction. The minimum sizes at which *T. kuehnei* become sexually mature are not known. The snout-vent lengths of *T. kuehnei* females described elsewhere (Table 3) exceeded the SVL of the smallest reproducing female from this study. Because a female (48 mm SVL) from our study was non-reproductive when collected in late May, it seems that *T. kuehnei* females become sexually mature at ca. 49 mm SVL. Interestingly, the smallest male with an enlarged tail base also had a SVL of 49 mm. In *T. sauteri*, the only species we are aware of in which the minimum size of sexual maturity of males has been described (Huang 2006), the sizes at which the sexes become sexually mature are very similar. This aspect of not only *T. kuehnei*, but also the other members of this genus, requires further empirical study.

Although clutch sizes of up to four eggs for *T. kuehnei* have been reported, our observations again support the reported clutch sizes of one to two eggs (Table 3). We also found that although the eggs from each clutch were quite similar in size, the sizes of the eggs among clutches varied quite substantially. This was also noted by Ziegler (2002) for *T. kuehnei* from Vietnam, and is thus not out of the ordinary. A study involving *T. septentrionalis* found that these lizards are income breeders, and that reduced energy at low and high body temperatures result in fewer and smaller eggs per season, but had no effect on the clutch sizes (Luo et al. 2010). In another study (Ji et al. 2007), *T. septentrionalis* produced

FIGURE 3. A histological view of the right ovaries of LACM 180390 (magnification 40x). Note the corpus luteum (single arrow) from the oviductal egg, and the yolk deposition that is in progress for the next clutch (double arrow). This is evidence that more than one clutch is produced in the same reproductive season.

different numbers and sizes of eggs at different times of the year, illustrating that there are trade-offs between reproduction and growth, that an optimal fraction of accessible resources is allocated to current reproduction, and that seasonal shifts in reproductive output and egg sizes are determined by natural selection.

Although we found that *T. kuehnei* females may produce multiple clutches in the same year, it was not possible to determine the exact number of clutches that a female can produce. According to Ziegler (2002), there are claims that *T. kuehnei* can produce up to four clutches per year, but the author gave no reference, so the claim can not be verified. If the claim is based on *T. kuehnei* in captivity, the observation should be interpreted with caution. For example, Lin et al. (2004) recorded four clutches of eggs in less than two months from a *T. stejnegeri* in captivity, while Cheng and Lin (1987) recorded only two to three clutches per year under natural conditions. Similarly, Telford (1969) found some differences between the number of clutches produced by *T. tachydromoides* in captivity and those under natural conditions and used this as an example of the danger of using captive lizards to describe ecological parameters of wild populations.

The *T. kuehnei* hatchlings from our study were all similar in size, and all resembled adults in coloration. Although the hatchling of *T. kuehnei* from Vietnam, described by Ziegler (2002), is slightly larger (Table 3) than the ones described herein, the difference is small and could simply be due to the methodology used to measure hatchlings. Still, it should be noted that in studies involving *T. septentrionalis* from different localities, there was geographic variation in the mean sizes of the maternal lizards and their eggs, and that some populations produced larger eggs and larger offspring (Du et al. 2005, 2006, 2010). It is thus also possible that the differences observed between the *T. kuehnei* hatchlings from Taiwan and Vietnam are due to spatial inter-population variation. However, additional data would be required to verify this.

In oviparous reptiles, it is common that the incubation period increases as temperatures decrease within an acceptable range within which successful development can take place (Birchard 2004). Studies done on *T. stejnegeri* (Chen et al. 2010) and *T. septentrionalis* (Du and Ji 2006) found that an increase in incubation temperature decreased the incubation period. The variation we observed in the incubation period was thus most likely due to temperature differences at which the eggs were incubated.

The exact duration of the reproductive cycle of *T. kuehnei* is not known. Ziegler (2002) stated that it is during the dry season in Vietnam, and that a female collected in the beginning of September 1997 exhibited signs of recent oviposition, suggesting oviposition at least until August. Interestingly, Huang (2006) found that *T. sauteri* females become reproductively active as early as February, and females with oviductal eggs are first apparent in April, while reproduction ceased in August. Similarly, it was found that in *T. stejnegeri* females, reproduction commenced in March and ceased in August (Cheng and Lin 1987), while *T. viridipunctatus* females are reproductive from May to August (Lue and Lin 2008), and *T. hsuehshanensis* and *T. luyeanus* females are reproductive from April to August (Huang 1998; Lue and Lin 2008). Based on our findings, we conclude that the reproductive season of *T. kuehnei* females in Taiwan is most likely similar to that of *T. hsuehshanensis*, *T. luyeanus*, *T. sauteri*, *T. stejnegeri*, and *T. viridipunctatus*, occurring during the spring and summer period.

Information on the reproductive patterns of reptiles and amphibians is critical for species conservation efforts (Gibbons, 1994), so there is a need for an understanding of aspects such as the period of sperm production, timing of yolk deposition, and number and sizes of clutches produced. A detailed empirical study is thus suggested to determine the exact period of the reproductive cycle of *T. kuehnei* in Taiwan. As *T. kuehnei* is a fairly rare species (Van Denburgh 1912;

TABLE 3. Summary of reported information on reproduction for *Takydromus kuehnei* (measurements in mm and mass in g). Values are reported as ranges, and where the information is available, the mean and standard deviation are given in parentheses. The sample size, n, refers to the number of clutches described, SVL = snout-vent length, and POBM = post-oviposition body mass (g). An asterisk (*) indicates values from dissected specimens.

	Shang and Lin (2001)	Lue et al. (2002)	Norval and Mao (2006)	This study	Ziegler et al. (1998); Ziegler (2002)	Ziegler and Bischoff (1999)	Ziegler and Bischoff (1999)	Ziegler and Bischoff (1999)
Location	Taiwan	Taiwan	Taiwan (SW)	Taiwan (SW)	Vietnam	China	Taiwan	Vietnam
Time of oviposition	--	Summer	July	April - June	July - Aug.	--	--	--
n (clutches)	--	--	1	7	3	3	1	1
Maternal SVL	--	--	54	49–59 (55.0 ± 4.3)	55–61	--	59	52
Maternal POBM	--	--	2.1	1.8–2.9 (2.5 ± 0.5)	--	--	--	--
Clutch size	1–2	1	2	1–2* (1.9 ± 0.4)	2–3*	3–4*	3*	3*
Egg length	--	--	10.7–10.9 (10.8 ± 0.1)	9.3–11.9 (10.6 ± 1.0)	7.5–14.5	--	6	10–11
Egg width	--	--	5.6–6.4 (6.0 ± 0.6)	5.3–7.2 (6.1 ± 0.6)	6–7.5	--	5–6	5–6
Egg mass	--	--	(0.2)	0.2–0.3 (0.24 ± 0.1)	--	--	--	--
Incubation period	--	--	35	28–37 (32.6 ± 4.3)	--	--	--	--
Hatchling SVL	--	--	24 (24 ± 0.0)	23–24 (23.8 ± 0.5)	26	--	--	--
Hatchling mass	--	--	0.3 (0.3 ± 0.0)	0.3 (0.3 ± 0.0)	--	--	--	--

Ziegler et al. 1998), we suggest that existing museum specimens be used where possible.

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GERRUT NORVAL (pictured above with a Leopard Tortoise, *Stigmochelys pardalis*, in South Africa) received a National Certificate (N. Cert.) in Production Management from Technikon Witwatersrand (1997). He also received a N. Cert: Nature Conservation (2003) and a National Diploma: Nature Conservation (2010) from the University of South Africa (UNISA). Since 2000, Gerrut has been actively involved in field research on squamates in Taiwan, and has been an Associate Researcher of the Applied Behavioural Ecology and Ecosystem Research Unit (ABEERU), Department of Environmental Sciences, UNISA, since 2004. He has broad research interests in the ecology of lizards and snakes, reptiles as invasive species, and reptile parasitology. Currently Gerrut is undertaking his M.Sc. in Nature Conservation through UNISA. His main research is on the exotic invasive population of the Brown Anole (*Anolis sagrei*) in Taiwan. (Photographed by Andries W. Norval)

JEAN-JAY MAO (pictured above collecting *Anolis sagrei* with a B.B. gun, for research purposes) is an Assistant Professor in the Department of Forestry and Natural Resources, National Ilan University, Taiwan. He received his M.S. from the Department of Biological Science at the National Sun Yat-Sen University, Kaohsiung, Taiwan, and his doctoral degree in Natural Science (Dr. *rer*. *nat*.) from Biogeographie, Trier Universität, Germany. He is interested in ecological studies, conservation, and the management of squamates. His current research focus is on the spatial ecology of wetland water snakes and other related organisms, as well as how wetland hydrological regulation affects population characteristics of semi-aquatic snakes. As for conservation, he is interested in the management of venomous snakes that accidentally enter homes in Taiwan, and the feasibility of venom collection from these snakes. (Photographed by Lan-Wei Yeh)

STEPHEN ROBERT GOLDBERG (left) is a Biology Professor at Whittier College, Whittier, California, USA. He received a B.A. degree from Boston University in 1962 and studied at the University of Arizona, Tucson under the well-known herpetologist, Charles H. Lowe, where he received a M.S. in 1965 and Ph.D. in 1970. He has been teaching Biology at Whittier College, Whittier, California, from 1970 to present. He had a very productive career in research and authored or co-authored over 600 publications in scientific journals. His areas of expertise are reproductive histology and parasitology. He was a Research Associate in the Herpetology Section of the Natural History Museum of Los Angeles County starting in 1976 and donated thousands of amphibians and reptiles to their collection. He had an extremely productive collaboration in parasitology with Charles R. Bursey (Pennsylvania State University, Shenango), which led to over 400 publications on parasitology of amphibians and reptiles. (Photographer unknown)