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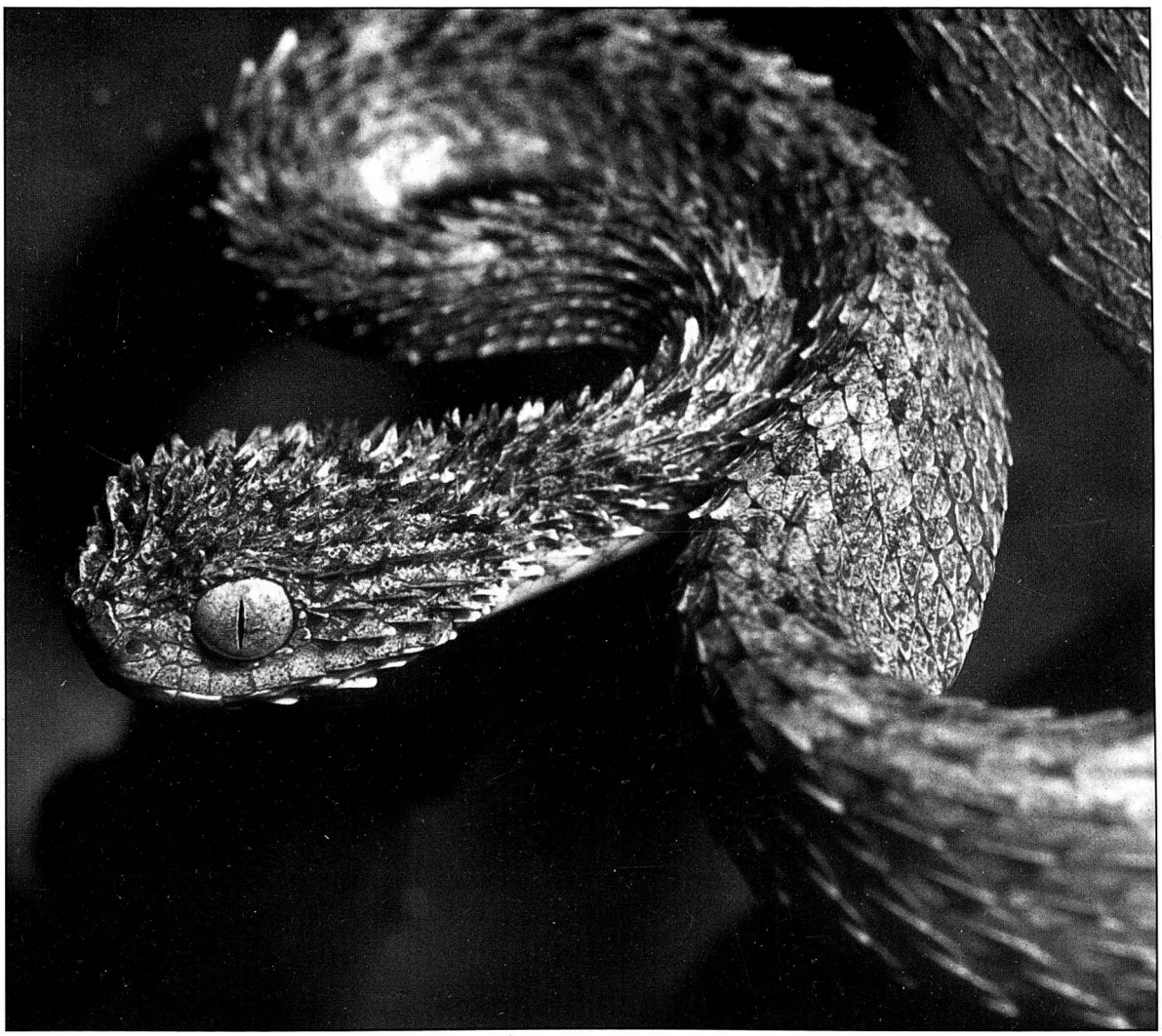

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THE USE OF DOSED AND HERBAGE *N*-ALKANES AS MARKERS FOR THE DETERMINATION OF INTAKE, DIGESTIBILITY, MEAN RETENTION TIME AND DIET SELECTION IN GALAPAGOS TORTOISES (*GEOCHELONE NIGRA*)

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Eleven captive Galapagos tortoises (*Geochelone nigra*) were used as study subjects to estimate digesta kinetics, diet composition, intake and apparent digestibility (aD) using *n*-alkanes. The results were compared to observed intakes and digestibility estimated through total faecal collection. Acid-insoluble ash (AIA) and acid-detergent lignin (ADL) were compared to the alkane method for the estimation of aD. Mean retention times (MRT) were estimated in four adult tortoises (ca 40-60 years old) and four juvenile tortoises (4-5 years old) fed a pulse dose of Co-EDTA, Cr-mordanted fibre (particle size <2 mm) and *n*-alkane hexatriacontane (C_{36}). Average MRT for the liquid phase marker Co was nine days in both adult and juvenile tortoises. For the particle phase markers Cr and C_{36}, MRT was 12 days in adult tortoises and eight and nine days, respectively, in juveniles. Digestibility, diet intake and diet composition were estimated in nine Galapagos tortoises fed for 32 days on a standardized diet containing the synthetic *n*-alkanes octacosane (C_{28}), dotriacontane (C_{32}) and C_{36}. In four juvenile tortoises kept individually, total faecal collection was performed and the faecal recovery rates of *n*-alkanes were estimated for pentacosane (C_{25}), heptacosane (C_{27}), C_{28}, nonacosane (C_{29}), hentriacontane (C_{31}), C_{32}, tritriacontane (C_{33}) and C_{36}. Intakes calculated with the alkane-pair C_{31} and C_{32} overestimated intake by a factor 1.5. After correction for the relative recoveries of alkanes there was no significant difference between estimated and observed intakes. Observed aD of organic matter (OM) was 67.5%. Estimated aD of OM with the internal marker C_{36} alkane, ADL and AIA were 48.5%, 38.9% and 18.3%, respectively. Estimates of diet composition using alkanes in individual animals accurately reflected directly-observed composition. Observed selection of a certain feedstuff was recognized with the alkane method. This is the first report of the use of *n*-alkanes as digestive markers in reptiles and it confirms that *n*-alkanes may be used for determining diet intake and the passage through the gut of the particulate digesta phase, and for estimating diet composition. The possibility of estimating different aspects of digestive strategies with the same marker type is a major asset of the alkane technique.

Key words: tortoise, reptile, *n*-alkane, *Geochelone*, digestibility markers, digestion, intake, diet composition

INTRODUCTION

In domesticated animals, digesta markers are used routinely to calculate faecal output and to estimate kinetics within the digestive tract. It may be because no reptile has been domesticated that a pulse or continuous dose marker system has not been studied in depth in any reptile species. The large number of marker systems (see Hatch & Afik (1999) for a summary of passage time markers) used in nutrition studies in reptiles, sometimes without thorough validation, is a disadvantage for the comparison of results obtained in different studies. A concentration on fewer marker systems would therefore be an important achievement. A marker system that could be of special interest under these circumstances uses the *n*-alkanes, which have been used to study different aspects of digestive strategies, such as digestibility, diet intake, food selection, and digesta kinetics in mammals and birds (Dove & Mayes, 1996; Hameleers *et al.*, 1996). These markers, which are found in the epicuticular waxes of plants as mixtures of different carbon-chain lengths, have received considerable attention in the last 15 years. Comprehensive reviews on the alkane method in domestic ruminants have been published (Dove & Mayes, 1991; Dove & Mayes, 1996). A major advantage of the *n*-alkane technique is that it allows the estimation of digestibility and intake with the same marker system and therefore considerably reduces labo-

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ratory work. Whereas digestibility is estimated by the use of an odd-chain *n*-alkane, naturally present in plants, as internal marker, the method for estimating intake is based on the combined use of a natural odd-chain *n*-alkane and a dosed even-chain *n*-alkane. The low, but measurable, concentrations of the even-chain alkane normally present in the diet is taken account of in the intake calculation. In theory, the reliability of intake estimates is not affected by the digestibility of the ration, or the marker recoveries in the faeces, if the recoveries are sufficiently similar between the natural and dosed alkanes (Dove & Mayes, 1991). In domestic ruminants the alkane combinations C_{31}/C_{32} and C_{32}/C_{33} have been the most reliable for this procedure. The *n*-alkanes have also been used as markers to estimate dietary proportions of different plant species or plant components (Dove, 1992; Malossini *et al.*, 1994; Salt *et al.*, 1994; Dove & Moore, 1995). As different plant species tend to have differing mixtures of odd-chain alkanes (chain lengths in the range 21 to 35 carbon atoms) diet composition can be estimated from the patterns of alkanes in the faeces and in the dietary components. Similarly, the dietary proportions of different component feedstuffs can be estimated by having them labelled with separate synthetic *n*-alkanes (usually even-chain) (Hatt *et al.*, in press).

A further application of *n*-alkanes as markers for digesta kinetic studies has been demonstrated in recent studies in ruminants (Mayes *et al.*, 1997; Hatt *et al.*, 1998).

Most research has been conducted with sheep and cattle (Dove & Mayes, 1996), but the alkane method has also been applied to non-domestic ruminants and non-ruminant species, such as the giraffe (*Giraffa camelopardalis*) (Hatt *et al.*, 1998; Clauss, 1998), pigs and horses (Mayes *et al.*, 1995; Ordakowski *et al.*, 2001), mountain hares (*Lepus timidus*) (Hulbert, 1993) and rabbits (Letso, 1996). Gudmundsson & Halldorsdottír (1995) successfully used synthetic alkanes incorporated into diets to estimate dietary intake and digestibility in farmed fish. In birds, *n*-alkanes have been evaluated in chickens (Hameleers *et al.*, 1996) and pigeons (*Columba livia*) (Hatt *et al.*, in press).

The present experiments were conducted to evaluate the use of *n*-alkanes as markers in a reptilian species, just as they have been applied to study digestive strategies in mammalian and in avian species. The aim was to validate the alkane method in tortoises in two experimental trials. In Trial 1 we compared estimates of digesta mean retention times (MRT) obtained using *n*-alkanes as markers with those derived from the use of the respective liquid-phase and particulate-phase markers, Co-EDTA and Cr-mordanted fibre. The use of alkanes for estimating dietary intake, digestibility and composition was examined in Trial 2. Digestibilities determined using *n*-alkanes were compared with measurements obtained from the internal markers acid-detergent lignin (ADL) and acid-insoluble ash (AIA), and from total faecal collection. Estimates of the dietary proportions of different feed components acquired from dietary and faecal *n*-alkane patterns were compared to directly observed diet composition.

MATERIAL AND METHODS

ANIMALS

Eleven Galapagos tortoises (*Geochelone nigra*) of various body masses and ages from Zurich Zoo (Switzerland), were used in this study. All juvenile animals (between 4 and 8 years) were hatched at Zurich Zoo. Adults were wild-caught animals and their ages could only be guessed, based on the time they had been kept at the zoo. The animals shared the enclosure with five adult Aldabra tortoises (*Geochelone gigantea*).

The heated indoor enclosure measured 65 m^2 and the flooring was concrete except for a 3 m^2 sand pit for egg deposition. The outside enclosure measured 265 m^2 and the floor consisted of grass, gravel and concrete. There was a heated shelter (20-25°C) in the outside enclosure into which all animals could retreat if it was cold outside. Animals were kept in their usual enclosure throughout the study, except that during Trial 2 juvenile tortoises (nos. 11, 15, 28, 29) were separated in individual pens of 1.5 m^2 each for individual feeding and total faecal collection. Juveniles were kept indoors and adults outdoors during Trial 1 (August/September). Throughout Trial 2 (February/March) all animals were housed indoors.

The ambient temperature (aT) and relative humidity (rH) were determined daily during both trials. Juvenile animals nos. 11, 15, 28 and 29 were weighed before and after every trial. For logistical reasons all other animals were only weighed before and two months after the entire study period.

DIETS

During Trial 1 the tortoises were fed their usual diet. Adult animals, which were kept outdoors, had access to fresh and dried rye-grass. Browse (*Ficus* and *Salix*) was offered on a daily basis, and a mix of vegetables (fennel, celery, parsley, carrots), fruit (apples, pears, banana), cottage cheese and a vitamin/mineral supplement was offered twice daily. The diet of juvenile tortoises contained a higher proportion of herbs (basil, parsley, sage) than the diet of adults, and it lacked fruit. Grass was offered only twice a week. The diet of juveniles was cut in small pieces (approximately 1 cm), whilst the diet of adults was chopped into pieces several centimetres. in length. All animals had access to water *ad libitum*.

In Trial 2 all animals received the same diet, which consisted of a total daily amount of 5470 g on a fresh matter (FM) basis for the entire group, and was composed of 75% dried grass, 14% tortoise pellets, 8% apples and 3% lettuce on a dry matter (DM) basis. The DM content of the entire diet was 51%. Pellets were commercial tortoise pellets (Dorswal, Roswal Products,

Zurich, CH) into which *n*-alkanes at a concentration of approximately 1700 ppm per kg DM for each *n*-alkane were mixed before pelleting. The alkanes used were octacosane (C_{28}), dotriacontane (C_{32}) and hexatricontane (C_{36}) (Fluka Chemie GmbH, Buchs, CH). The reason for including three *n*-alkanes was to allow estimation of the faecal recovery factors relative to their carbon-chain lengths.

Digesta Kinetics (Trial 1)

The four adult tortoises and four of the juveniles (nos. 11, 15, 28 and 29) were fed a pulse dose of indigestible markers with their morning food. Juveniles were fed the marker mixed with chopped cabbage (*Brassica oleracea*). Adults were individually offered markers in kiwi fruit and tomato halves.

The preparation of the sodium salt of the monovalent Co-EDTA anion was made according to Udén *et al.* (1980). The salts (15.5 g) were redissolved in 200 ml distilled water and mixed with the tortoise pellets described above. The final Co-concentration was analysed to be approximately 1700 ppm on a DM basis and animals were dosed with 0.8 mg Co per kg body mass (BM). For the identification of the particulate phase, two markers – Cr-mordanted cell wall of dried grass and tortoise pellets labelled with C_{36} alkane – were used. The preparation of Cr-mordanted fibre (1% Cr on a DM basis) with a particle size of <2 mm was performed according to Udén *et al.* (1980). Each animal was dosed with 20 mg of Cr.

For the preparation of alkane-labelled pellets, C_{36} was dissolved in *n*-hexane (C_6) at 40°C and subsequently mixed with pellets to achieve a concentration of 2300 ppm on a DM basis. Hexane was left to evaporate overnight. Animals were dosed with 46 mg C_{36} per kg BM.

In an earlier investigation with the same juvenile animals dosed with carmine red (66 mg kg^{-1} BM) it was observed that the marker was excreted in faeces for up to 18 days (Liesegang *et al.*, 2000). It was expected that in adults the marker would be excreted over a longer period than in juveniles, so faecal samples were collected daily for 25 d after dosing. As faeces and renal excrements were voided separately, faecal collection was possible without contamination. After collection the samples were frozen at -20°C for at least 48 h and subsequently freeze-dried to constant mass. Before laboratory analysis, the samples were ground to a particle size of approximately 1 mm with a coffee grinder. The analysis for *n*-alkanes was carried out by an adaptation of the method of Mayes *et al.* (1986a). Duplicate dried, ground faecal and food samples of 0.5 g were weighed into 100 x 20 mm borosilicate glass culture tubes fitted with screw caps. All tubes and caps were rinsed prior to use with 2 ml of petroleum spirit to eliminate possible hydrocarbon contamination. A solution of tetratriacontane (C_{34}) (0.8 mg/g) in *n*-hexane was added to the sample by weight (0.2 g) as an internal standard, followed by 7 ml of ethanolic KOH (1M). The tubes were then capped and heated for 16 h at 90°C in a dry-block heater. After cooling to 50 - 60°C, the alkanes were extracted by adding 7 ml of *n*-hexane and 2 ml of doubly distilled water. The supernatant (non-aqueous) layer was separated into new glass culture tubes. The extraction was repeated and subsequently the *n*-hexane was evaporated to approximately 4 ml on a dry block (at 50°C). The extracts were then applied to a column containing silica gel (Kieselgel 60, 70-230 mesh) with a bed volume of 5 ml. Hydrocarbons were eluted into 23 x 54 mm scintillation vials by addition of 2 x 4 ml of *n*-hexane to the column, and subsequently the samples were evaporated to dryness. The purified hydrocarbon extracts were redissolved with 0.5 ml of *n*-hexane and transferred to an autosampler vial for analysis by gas chromatography (for technical details see Appendix A).

For the Co and Cr analysis, 0.35 g of freeze-dried, ground sample was mixed with 5 ml of 72% sulphuric acid and left on a shaker at room temperature overnight. Subsequently, 40 ml of distilled water was added and after vigorous shaking, 20 ml of supernatant was passed through a paper filter. Co and Cr concentrations were measured in the filtrate by atomic absorption spectrometry (for technical details see Appendix B).

The MRT in hours of each marker in the entire gastrointestinal tract was calculated according to Thielemans *et al.* (1978). The following equation was applied:

$$MRT = (\Sigma t \times C \times \delta t) \times (\Sigma C \times \delta t)^{-1}$$

where C is the marker concentration in the faecal sample (mg kg^{-1} DM) at time t (h after administration) and δt is the interval (h) represented by the respective sample.

Diet Intake, Digestibility and Diet Composition (Trial 2)

The experimental period of Trial 2 lasted 32 days, i.e. 25 days of adaptation to the diet to reach a steady state in marker excretion and 7 days of faecal collection. In adults and juveniles (Nos. 3, 5 and 6) individual faecal samples were collected on a daily basis. Total faecal collection was performed for animals nos 11, 15, 28 and 29.

Food was offered at 08.00 h, 10.00 h and 13.00 h. Juvenile tortoises nos. 11, 15, 28 and 29, which were fed and housed individually, were offered the following amounts of food on a FM basis: 45 g, 22 g, 30 g, and 29 g, respectively. There were no refusals during the entire trial. Juveniles defecated once or twice a day. Adults defecated more frequently, up to five times a day. After collection the samples were frozen at -20°C for at least 48 h and subsequently freeze-dried to constant mass. On days where two faecal samples were collected from individual animals, samples were pooled in equal amounts. Samples of diets and pellets were analysed for composition and internal marker concentrations. The procedure for analysis of *n*-alkanes was identical to that used in

TABLE 1. Analysis of diets fed to Galapagos tortoises (*Geochelone nigra*). Values are expressed per unit fresh matter (FM) or dry matter (DM).

		Diet Trial 1 (Juveniles)	Diet Trial 2 (Entire Group)
Dry matter	% FM	16.8	51.0
Crude protein	% DM	20.1	11.3
Crude fibre	% DM	14.9	20.5
Organic matter	% DM	89.9	95.7
Acid-insoluble ash	% DM	-	2.6
Acid-detergent lignin	% DM	-	5.0

Trial 1. In addition, samples were analysed for DM according to Padmore (1990), and crude protein (CP), crude fibre (CF) and organic matter (OM) were determined using standard procedures for Weender analysis. Furthermore, ADL was analysed according to the systems of Van Soest (1994), and AIA was estimated gravimetrically as the filtrate residue after ashing in a muffle furnace at 550°C for 16 h and hydrolysis in 12% hydrochloric acid (4 N).

Intakes (I, kg DM d^{-1}) were estimated from the faecal ratios of ingested external and internal *n*-alkanes, the administration rate of the external alkane, and internal alkane concentration in the consumed diet based on the method described by Mayes *et al.* (1986*b*):

$$I = \text{Faeces}/(1 - \text{Digestibility})$$
$$= (D_{32}/F_{32})/(1 - [1 - H_{31}/F_{31}])$$
$$= (D_{32} \times F_{31})/(F_{32} \times H_{31})$$

where F_{31}, F_{32} and H_{31} represent the respective *n*-alkane concentrations (mg per kg DM) in faeces (F) and test diet (H). D_{32} represents the dose (mg) of *n*-alkane ingested per day, which was known from direct observation. The even-chain *n*-alkane C_{32} was used as external (dosed) alkane and hentriacontane (C_{31}) was the internal alkane.

Apparent digestibility coefficients (aD) of organic matter (OM) (% DM) were calculated on the basis of total faecal collection and by using C_{36} *n*-alkane, AIA and ADL as internal markers.

For the estimation of aD of OM (aD_{OM}) with the total faecal collection method, the following standard equation was used:

$$aD_{OM} = 100 \times (I_{OM} - E_{OM})/I_{OM}$$

where I_{OM} represents the amount of OM ingested (g DM) and E_{OM} is the amount of excreted faecal OM (g DM).

For the estimation of aD_{OM} with the markers AIA, ADL, and C_{36}, the following standard equation was used:

$$aD_{OM} = 100\text{-}[(M_d/M_f) \times (N_f/N_d) \times 100]$$

where M_d and M_f are respective percentages of marker in diet and faeces and N_d and N_f represent percentages of nutrient (OM) in diet and faeces, respectively.

The estimation of diet composition with the alkane method was performed with the least-squares optimization procedure described by Newman *et al.* (1995), using the MathCAD 2.52 software (MathSoft Inc.). The method is based on an approximation and uses an iterative algorithm similar to those adopted by Salt *et al.* (1994) and Dove & Moore (1995).

Results are presented throughout as means ± standard error of the mean (SEM) and *n* is the number of individuals or samples. Repeated measures analysis of variance (ANOVA) and the Scheffé *F*-test were used to

TABLE 2. Body masses (BM, kg), age (y) and mean retention times (MRT, h) within the age class (adults and juveniles: Nos. 11,15,28 and 29) of cobalt (Co), chromium (Cr) and n-alkane (C_{36}) in juvenile (J) and adult (A), male (m) and female (f) Galapagos tortoises (*Geochelone nigra*). [a,b] Different superscripts within the same line indicate significant differences ($P<0.05$) by Scheffé *F*-test.

Animal No.	Age (y)	BM (kg) at Trial 1	BM (kg) at Trial 2	Average MRT (h) Co (liquid phase)	Cr (particle phase)	C_{36} (particle phase)
1A f	~60	99.5	98.0	217	270	280
10A m	~40	135.0	-	222	281	267
20A m	~40	139.5	-	189	279	306
30A m	~40	207.0	210.0	260	317	308
Mean±SE				222±30[a]	287±21[b]	290±20[b]
3J f	8	-	36.1	-	-	-
5J m	7	-	28.1	-	-	-
6J f	7	-	37.8	-	-	-
11J m	5	12.3	14.0	217	185	215
15J f	5	6.1	6.8	159	183	206
28J f	4	8.1	8.9	251	195	208
29J f	4	8.1	9.2	227	213	258
Mean±SE				214±39[a]	198±23[a]	222±25[a]

test the difference in MRT between the three markers within the same age group, and difference in intakes and digestibilities between markers and direct observation. For the statistical analysis of the MRT between the age groups an unpaired *t*-test was used. A probability $P<0.05$ was accepted as level of significance.

RESULTS

Environment and Animals

Analyses of diets fed to juveniles in Trial 1 and to all the animals in Trial 2 are summarized in Table 1. Ambient temperatures were relatively stable throughout the study. In the outdoor enclosure aT was 21.3±2.6°C in the afternoon (17.00 h) and rH was 73.5±15.4%. In the inside enclosure aT was 23.2±1.0°C and rH was 88.5±9.7%. All animals were active and appeared healthy throughout the study. No adverse effect of marker feeding was observed.

Body Mass and Mean Retention Times

Values of body mass of individual animals and average MRT within the age class are given in Table 2. Markers were ingested voluntarily by all animals within ten minutes of being offered, with the exception of animal no. 20 which ingested the Cr-mordanted fibre only 24 h later, when this marker was offered in a second attempt. This time difference was taken into account in the MRT calculation.

The calculated MRT are represented in Table 2. Differences in MRT of the liquid phase marker Co-EDTA between adults and juveniles were not significant (*t*-test, $P=0.74$). The MRT was approximately nine days. Adult tortoises had significantly longer MRT (12 days) for the particle phase markers Cr and C_{36}, compared to the juveniles, where the MRT were eight and nine days, respectively (Cr: $P=0.001$; C_{36}: $P=0.005$). Adult tortoises showed a selective retention factor of 1.3. The selective retention of each of the particulate markers was significant (ANOVA Co-EDTA: Cr – at $P<0.05$ Scheffé-*F*-value 14.3; Co-EDTA: C_{36} – at $P<0.05$ Scheffé-*F*-value 15.9). Juvenile tortoises in contrast showed no selective retention (Co-EDTA: Cr – at $P<0.05$ Scheffé-*F*-value 0.46; Co-EDTA: C_{36} – at $P<0.05$ Scheffé-*F*-value 0.14).

Typical excretion patterns of the adult and juvenile tortoises in this study are exemplified by the animals nos. 10 and 15 respectively (Fig. 1). In juveniles a steep increase or indeed a pulse excretion (for Co) in marker excretion was observed, whereas in adults excretion increased gradually. In adults and juveniles decline of the liquid phase marker Co was more gradual than that of the particle phase markers Cr and C_{36}, which were excreted as a pulse. In both age groups Co reached a maximum of excretion earlier than Cr and C_{36}.

Diet Intake and Digestibility

Although it is common practice to force-feed reptiles during digestibility trials, we let the animals feed voluntarily and accepted the probability that some food would not be eaten. However, this certainly influenced the rate of defecation by some animals within a group, as some individuals ate more food than others. Furthermore, two animals (nos. 10 and 20) refused to eat the diet on a regular basis and had to be excluded from the study.

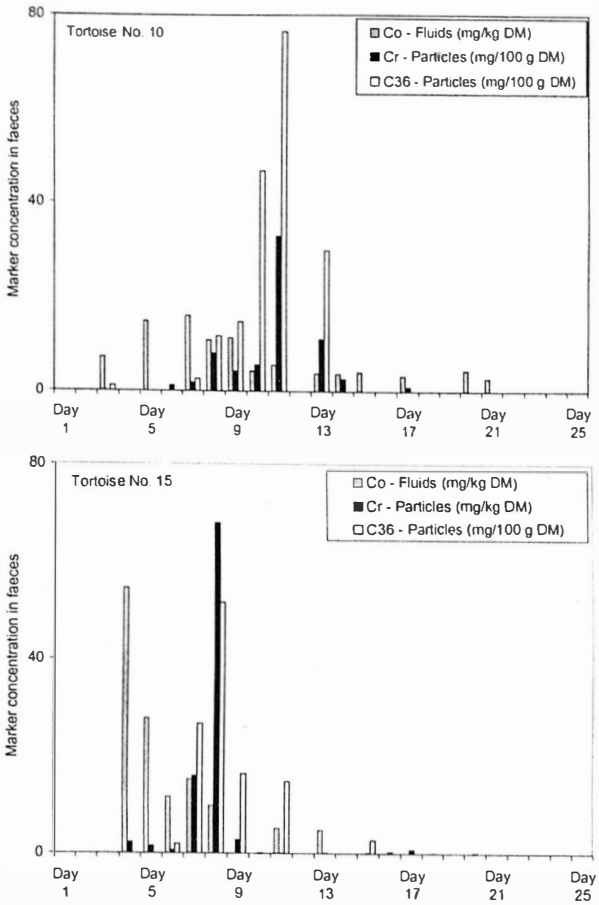

FIG. 1. Faecal concentrations of digesta markers from a single oral dose given to Galapagos tortoises (*Geochelone nigra*) of ~40 y (top) and of 5 y (bottom)

Faecal output of individuals was very variable. On an average daily basis, tortoise no. 11 had an output of 10.2 g DM (number of defaecations, n=6), no. 15 produced 3.3 g DM ($n=4$), no. 28 produced 3.4 g DM ($n=5$) and no. 29 produced 8.2 g DM ($n=5$).

Analyses of feedstuffs offered in Trial 2 are presented in Tables 1 and 3. The preparation of pellets with alkanes produced distinct concentrations of C_{28}, C_{32} and C_{36}. In the four juvenile tortoises kept individually it was possible to calculate *n*-alkane recovery rates based on the average daily intake over the 25 preceding days and faecal samples collected for seven days thereafter (Table 3). Using the *n*-alkane-pair C_{31} and C_{32}, the unadjusted average daily intake was estimated to be 25.0±3.58 g DM. The actual daily mean intake was 16.7±5.14 g DM, so the alkane method overestimated intake significantly (ANOVA at $P<0.05$ Scheffé-*F*-value 15.5), by a factor of 1.5. After taking account of differences in recovery of C_{31} and C_{32} alkanes (C_{31}/C_{32} = 1.36), the estimate was lowered to 18.4±2.64 g DM, which did not differ significantly from the actual daily mean intake (ANOVA at $P<0.05$ Scheffé-*F*-value 0.50).

TABLE 3. Concentration of *n*-alkanes (mg kg^{-1} DM) in feeds offered during Trial 2 and faecal recovery rates calculated for the Galapagos tortoises (*Geochelone nigra*) nos. 11, 15, 28 and 29. * Synthetic alkanes incorporated into tortoise pellet.

	C-chain length of *n*-alkanes											
	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{36}
Diet Mix	0.0	25.0	0.0	76.1	339.3	221.4	0.0	140.5	342.0	25.4	-	225.3
Pellet	0.0	0.0	0.0	0.0	1805.6*	0.0	0.0	0.0	1723.7*	0.0	-	1613.2*
Apple	0.0	19.2	0.0	204.2	19.5	701.0	0.0	0.0	0.0	0.0	-	0.0
Hay	0.0	24.1	0.0	42.5	0.0	135.5	0.0	191.5	0.0	42.5	-	0.0
Lettuce	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	0.0
Recovery rates	-	0.67	-	0.48	0.86*	0.71	-	0.91	0.67*	1.07	-	0.70*

Digestibility of organic matter, determined from total faecal collection, was 67.5±12.77%. Estimates of OM digestibility derived from alkane C_{36} (48.5±13.35%), AIA (18.3±15.83%) and ADL (38.9±13.67%) were smaller than the observed digestibility, but the alkane method yielded results closest to those obtained by total faecal collection. A significant difference resulted only between total faecal collection and AIA (ANOVA at $P<0.05$ Scheffé-F-value 7.8578).

DIET COMPOSITION

In Trial 2 diet composition was calculated on the basis of the average faecal *n*-alkane concentrations over seven days and was compared with the observed average composition of the consumed diet during the entire study period. The results (Fig. 2) have been grouped for the adults, juveniles that were fed individually (nos. 11, 15, 28, 29) and remaining juveniles (nos. 3, 5, 6) that were kept together with the adults. Lettuce was not included in the diet composition calculation as its *n*-alkane levels were below detection level (Table 3). The diet composition was thus compared with the actual diet composition having omitted the lettuce (pellet 15%, hay 77% and apple 8% on a DM basis). The correspondence between actual and estimated diet composition was very good, particularly for adult tortoises (Fig. 2).

DISCUSSION

To our knowledge this is the first study to investigate the use of *n*-alkanes as digestive markers in reptiles. In Trial 1 we evaluated the C_{36} *n*-alkane as a marker for measuring the rate of passage of the particulate phase of

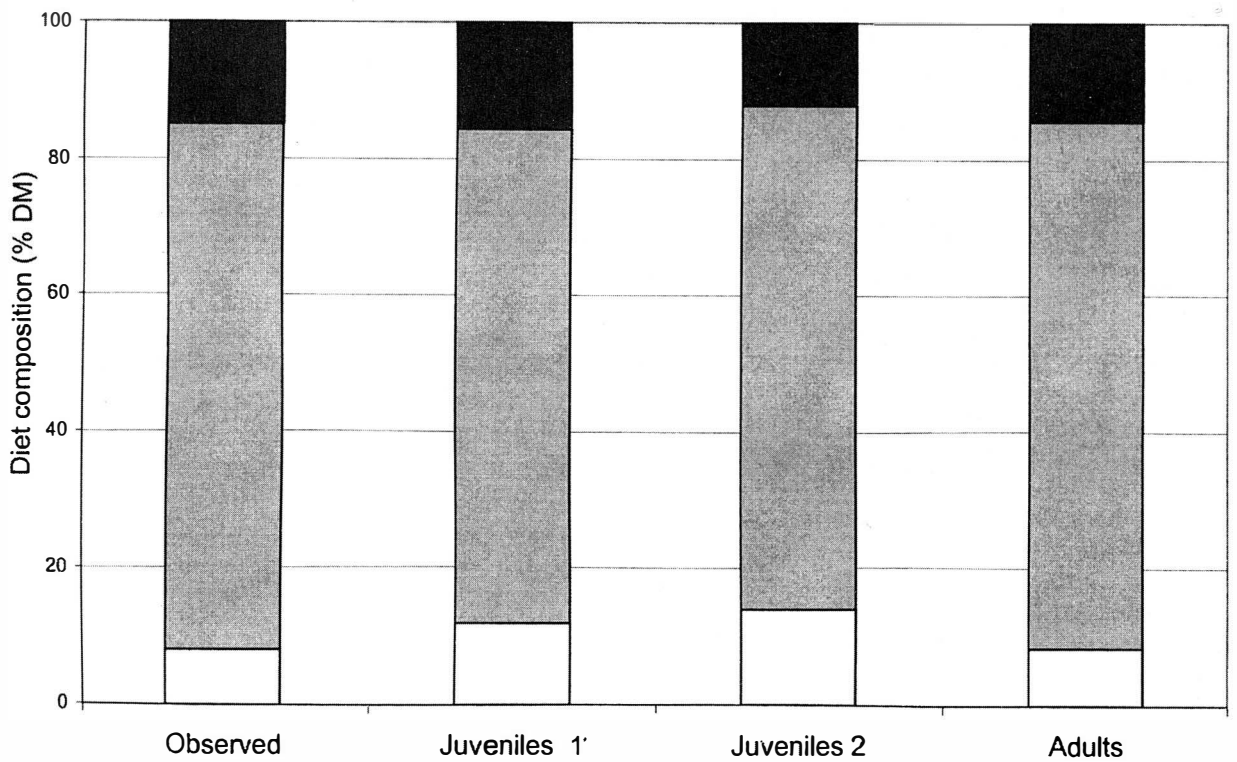

FIG 2. Directly observed diet composition (% of dietary DM) in Galapagos tortoises (*Geochelone nigra*) in Trial 2 compared to mean estimates obtained using *n*-alkanes as faecal markers. Juveniles 1 (n=4) were fed individually. Juveniles 2 (n=3) and Adults (n=2) were fed as a group. Black bars, pellet; shaded bars, hay; open bars, apple.

digesta and compared it with the kinetic marker Cr-mordanted fibre (particle size <2 mm). In our study it appears that C_{36} had a MRT which was not significantly longer than that of the mordanted Cr. The average MRT of the digesta particulate phase in juvenile and adult tortoises was approximately 10 days and this was significantly longer than that of the liquid phase marker, Co-EDTA. This observation agrees with previous results that compared passage-rates of different digesta phases in reptiles (Barboza, 1995; Hatch & Afik, 1999).

Based on the current knowledge of digesta kinetics, two observations in Trial 1 deserve further discussion. Firstly, although the average body mass of adults differed from the juveniles' by a factor of 17 there was no significant difference in the MRT of the liquid phase. This would suggest that body mass does not have an influence on MRT. Secondly, MRT of particulate and liquid phases were significantly different in adults but not in juveniles. This suggests that juveniles did not show any selective food retention of particles <2 mm, whereas in adults there was significant selective retention.

As for the first observation, it is reasonable to assume that length of gastrointestinal tract increases with body-size. Bjorndal (1979) compared the length of the gastrointestinal tract of two green turtles weighing 50 kg and 82 kg, and the larger animal had a longer gastrointestinal tract. Accordingly, we would expect that adult tortoises should have longer MRT of the liquid phase than juveniles. This was not the case in our study. Indeed, there is other evidence that body size may not have a major influence on MRT in tortoises. In the study of Meienberger *et al.* (1993), where the body masses of tortoises differed by a factor of 12 (0.25 – 3.1 kg), no differences in transit times were noted in relation to body mass. Bjorndal & Bolten (1992) compared 12 g hatchlings with 3000 g adults of *Pseudemys nelsoni*, a herbivorous freshwater turtle, and found MRT of 56 h and 81 h respectively; i.e. body mass differed by a factor of 250, but MRT differed only by a factor of 1.4. If the equation of Illius and Gordon (1992) for the estimation of MRT in mammalian hindgut fermenters is applied in the latter case, one might have expected MRT to differ by a factor of at least 4.1.

In our study, a possible explanation for the adults having the same MRT of the liquid phase as the juveniles could be that MRT in the adults was relatively low owing to the higher amount of fibre in their diet, as a result of the inclusion of larger amounts of browse and dried and fresh grass.

Our observation that juvenile tortoises, in contrast to adults, did not appear to have a selective retention of particles may have been due to different particle sizes of diets. Juveniles' food was cut to sizes of approximately 1 cm, whereas adults' vegetables and fruits where chopped to pieces of several centimetres. As reduction of food particle size in reptiles is limited by poor mastication (Throckmorton, 1976; Norman & Weishampel, 1985) it is reasonable to assume that adults ingested larger food particles than juveniles. Large particles probably do experience some degree of selective retention compared to the fluid phase and travel as boli through the digestive tract of tortoises. The particulate markers used in this study may have been associated with boli and as a result underwent selective retention. In juvenile tortoises, which had a more homogenous diet, this separation of particles and fluids did not occur at a significant level, as shown in Fig. 1. This could explain the steeper increase of marker excretion, or even pulsed excretion (for Co), observed in juveniles, compared to adult tortoises, where selective retention resulted in a more gradual increase and decrease of marker excretion. To test this hypothesis, gut content at different sites would have to be examined, which was not feasible in this study.

We encourage further testing of our observation that body mass appeared not to have a significant influence on MRT of the liquid phase, by comparing MRT of the same diet offered in different particle sizes to tortoises of various sizes.

Concerning the use of *n*-alkanes as a marker system for the particulate phase in digesta kinetic studies, this class of marker can be recommended for further studies in reptiles. Compared to Cr-mordanted fibre at a particle size <2 mm, no significant difference was observed with C_{36}-labelled pellets. An advantage of the alkane method over Cr-mordanted fibre is that different chain lengths of this marker may be combined to study the MRT of particles with different sizes.

One aim of Trial 2 was to estimate *n*-alkane recovery rates. Whereas in domestic ruminants and birds faecal recovery increases with chain length (Dove & Mayes, 1996; Hameleers *et al.*, 1996; Hatt *et al.*, in press), no such pattern could be recognized in the Galapagos tortoises. Our results are in general agreement with observations made in mammalian hindgut fermenters, such as the horse and the pig, where recoveries did not vary significantly with chain length (Mayes *et al.*, 1995; Ordakowski *et al.*, 2001). In our study, however, the recoveries were more erratic than in pigs and horses. An explanation for this may be the MRT and the distribution of alkanes between the solid and liquid phases of digesta. Synthetic alkanes have higher, but still low, proportions in liquid phase than natural alkanes (Mayes *et al.*, 1988). The long MRT of tortoises compared to mammals may have increased differences in recoveries due to different rates of passage. Emulsifier systems in the gut and processes of lipid absorption from the gut may also be important. Further studies on the recovery of alkanes in tortoises and other reptilian species will be needed to clarify this point.

In the present study C_{36} had a recovery rate of 70%. As a result apparent OM digestibility in the four juvenile tortoises (nos. 11, 15, 28 and 29) was underestimated without correction for the recovery rate. Nevertheless the *n*-alkane produced a more accurate digestibility co-

efficient than the internal markers, AIA and ADL. In situations where total faecal collection is not possible the alkane method may be applicable especially for comparative purposes. Further work is necessary to evaluate the variability in faecal recovery of C_{36} alkane under a range of conditions; if such variability were low, reliable estimates of digestibility could be obtained using a recovery correction factor.

A possible reason for ADL not producing satisfactory results is that lignin content in our diet was too low. Van Soest (1994) states that lignin is a better marker in diets with high ADL content, especially above 5% on a DM basis. In our diet the content was 5% (Table 1). The internal marker AIA in the present study had the lowest recovery, as based on the degree to which it caused an underestimation of digestibility. In our case it appears reasonable to assume that AIA was absorbed and excreted in the urine, as this has been described in ruminants (Kotb & Luckey, 1972; Owens & Hanson, 1992). Besides absorption, which causes an underestimation of digestibility, a further disadvantage of the AIA method is the possibility of contamination of faeces, resulting in an overestimation of digestibility. Minerals in the diet that are insoluble in acid may arise from two sources: biogenic mineral fractions in the forage, and contamination from soil and dust (Van Soest, 1994). The latter has already been of concern in Aldabra tortoises studied in the field (Hamilton & Coe, 1982) but contamination may also result through the uptake of sand (geophagy) or stones (lithophagy) which has been described in several species of tortoises and terrapins (Gans & Gans, 1978; Zwart, 2000).

The above mentioned problems with ADL and AIA show that a major disadvantage of these markers, in contrast to the *n*-alkane C_{36}, is that they are not discrete chemical entities; the ADL and AIA in faeces may not be the same material as that determined in the diet.

For the intake estimations the comparatively high recovery of C_{31} in relation to C_{32} resulted in an overestimation of digestibility relative to faecal output, hence an overestimation of intake. This difference in recoveries of synthetic and natural alkanes had to be included for accurate intake estimates, but the results show the utility of the alkane method.

In the present study the intake of dose of C_{32} was known from direct observation. However, in future studies without direct observation it will be important to develop a technique for dosing tortoises reliably. Based on the findings of this study, applying the dosed marker three times daily for 25 days will result in a marker excretion that allows intake calculations.

Estimation of diet composition produced satisfactory results in all animals (Fig. 2). Apples were less precisely estimated than hay and pellets, probably because of their respective alkane concentrations. Whereas hay and pellets could easily be identified by their individual *n*-alkane profiles (C_{31} in hay; C_{28} and C_{32} in pellets), the alkane profile of apples was less characteristic. Nevertheless, individual selection is suspected inasmuch as the estimations suggest that juveniles selected apples more strongly than adults in the mixed-age group. This conclusion is supported by observations during the experiment and goes along with the conclusion of Bjorndal & Bolten (1992) that young tortoises improve the nutritional value of their diet by more effective selection, compared to adult tortoises. The alkane method proved to be valuable for estimating diet composition and it might be of special interest in field trials with respect to seasonal changes in diet and effects on digestion.

In conclusion, this study has clearly demonstrated that alkanes have good potential as dietary markers in herbivorous tortoises. Alkanes were successfully used as markers for the particle digesta phase and for diet composition estimates. Further studies are encouraged to improve recovery of alkanes, and as a result, estimation of diet intake and apparent digestibility coefficients. The possibility of estimating different aspects of digestive strategies with the same marker type is a major asset of this technique and might allow considerable progress towards understanding digestive strategies in tortoises.

ACKNOWLEDGMENTS

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APPENDIX A

Gas chromatograph type: Hewlett Packard 6890 GC Series.
Mode (packed or capillary column): Capillary.
Autosampler type: Hewlett Packard 6890 GC Series.
Injection system: Hewlett Packard 6890 GC Series Injector system.
Injection volume: 1.0 ml.
Detector: FID.
Column: Supelco SPB 1™ Fused Silica Capillary Column 15 m x 0.53 mm ID, film thickness 1.5 mm.
Column temperatures - Programmed: 1 min at 230°C; 7°C/min to 280°C; 10 min at 300°C.
Injector temperature: 300°C.
Detector temperature: 320°C.
Carrier gas: H_2, 4.8 ml/min.
Make-up gas: Nitrogen, 15 ml/min.
Time for analysis: Temperature programmed - 25 min.
Time between run: 5 min.
Replicate injections: none.
Data collection system: Hewlett Packard Chemstation 4.01 linked to Pentium 5/133 Hewlett Packard Vectra Series 4.
Other information: Standard mixture (C_{24} - C_{36}) injected after every 15 sample vials. Response factors calculated after all samples have been run on chromatograph.

APPENDIX B

Atomic absorption spectrometer type: Perkin Elmer Model 3300.
Light: Cobalt WL 240.7 nm Chrom WL 357.9 nm.
Slit Width: 0.2 nm.
Flame gas: air acetylene (C_2H_2).
Gas flows: Oxidant 8.0 l/min Fuel 1.6 l/min.
Calibration: linear plot with 3 standard solutions (0.5 mg/l, 1 mg/l, and 2 mg/l).
Read time: 0.5 s Sample replicates: 3.
Read delay: 0.5 s Standard replicates: 3.
Software: AA Lab Reporter.

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A NEW *ATHERIS* SPECIES (SERPENTES: VIPERIDAE), FROM TAÏ NATIONAL PARK, IVORY COAST

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We describe a new species of the genus *Atheris* from Taï National Park, a large rainforest area in south-western Ivory Coast. *Atheris* sp. nov. shows close affinities to *A. squamigera*, but is distinguished from it by a combination of scale characteristics, as well as morphometric differences in head proportions.

Key words: Serpentes, Viperidae, *Atheris* sp. nov., Taï National Park, Ivory Coast;

INTRODUCTION

The genus *Atheris* has undergone several taxonomic revisions (Broadley, 1996, 1998). However, due to the huge variability within the genus (Jacobi, 2001), the taxonomic status of several taxa still remains to be settled. Very recently two new species of *Atheris* have been described: *A. acuminata* from Uganda (Broadley, 1998) and *A. broadleyi* from Cameroon (Lawson, 1999). The resurrection of *A. subocularis* from the synonymy of *A. squamigera* has currently been published (Lawson, Noonan & Ustach, 2001). The latter species is especially renowned for its variability (Spawls & Branch, 1995). However, previous papers may have inadvertently increased its known variation in many characters by combining distinct species (Lawson, 1999).

According to Hughes (pers. comm.), without a thorough re-examination of existing museum specimens, it cannot be decided whether *A. squamigera* is a geographically variable species or a species complex. However, there is good evidence of more than one species being involved. *Atheris hirsuta* sp. nov. from Taï National Park (TNP), Ivory Coast, is one of these species that shows close affinities to *A. squamigera,* but differs sufficiently to be recognized at the species level.

MATERIAL AND METHODS

For comparison, we investigated museum specimens of *A. squamigera* from the entire Central and West African range of the species (Fig. 1). Museum specimens under investigation originated from, and are deposited in, the following collections: Staatliches Museum für Naturkunde Stuttgart (SMNS), Zoologisches Forschungsinstitut und Museum Alexander Koenig Bonn (ZFMK), Zoologisches Museum der Humboldt Universität zu Berlin (ZMB), Zoologisches Institut und Zoologisches Museum der Universität Hamburg (ZMH), Zoological Museum, University of Copenhagen (ZMUC), and Zoologische Staatssammlung München (ZSM).

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The geographical co-ordinates were obtained from Geographic Names Processing System - Phase IV, and a portable GPS (Garmin 12XL). The map was drawn with the mapping program Versamap version 2.07.

In order to ensure comparability, we investigated the same characters used in recent treatments of the genus (Broadley, 1998; Lawson & Ustach, 2000): suprarostrals (SRO), internasals (INS), interorbitals (IOS), maximum transverse head scales (MTHS), circumorbital scales (COS), interoculabials (IOL), interocunasals (ION), supralabials (SL), infralabials (IL), pairs of sublinguals (PSL), dorsal scale rows at mid-body (MSR), ventrals and subcaudals. For discussion of the diagnostic significance of these characters, see Broadley (1998). In addition we investigated morphometric proportions of the head, as well as structure and shape of scales. Scale terminology is according to Broadley (1998).

Head width (HW) was always taken as the maximum head width possible. Head length (HL) was measured from snout to the quadratum. Interorbital distance (IOD) was measured between the circumorbital scale rows. Snout-eye distance (SED) was measured from the anterior corner of the eye to the snout tip. Scale and head measures were taken to the nearest 0.1 mm using a dial calliper. When scale counts differed between the sides of the head, we gave both values. Snout-vent length (SVL) was taken to the nearest mm using a metre rule. Results are summarized in Tables 1, 2 and 3. Scale microstructure was examined by means of a scanning electron microscope (SEM).

SPECIES ACCOUNT

ATHERIS HIRSUTA SP. NOV.

Holotype. SMNS 11333, male, about 6 km West of the "Station de Recherche en Ecologie Tropicale" (SRET 5°50'N 7°19'W), TaïNational Park, Ivory Coast, 20 September 2000, 07.00 hrs, R. Ernst leg.

Diagnosis. Slender tree viper with rather short head, IOD/SED 2.3; heavily carinated scales, especially on head and neck, giving the snake a bristly appearance; keels run in long curves towards a sharp tip; six suprarostrals; eight to nine infralabials; three pairs of sublinguals; elongate dorsal scales; 16 scale rows around mid-body.

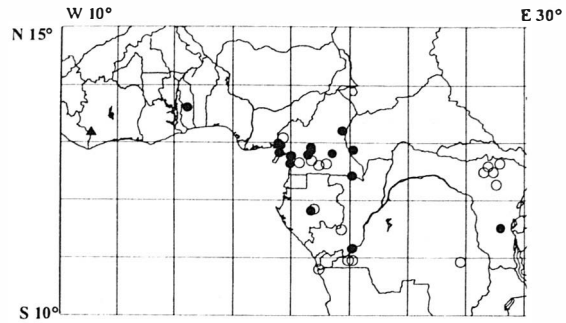

FIG. 1. West and Central African distribution of *A. squamigera* (circles) and type-locality of *A. hirsuta* sp. nov. (triangle). Closed symbols represent localities of checked specimens, open symbols represent unchecked literature records; sources: Blanc & Frétey, 2000; Broadley, 1998; Lawson & Ustach, 2000.

Description. Slender tree viper, 480 mm total length, tail 95 mm; very short and sturdy head, IOD/SED 2.3; rostral three times as wide as deep, surmounted by six suprarostrals; viewed from the front the three median suprarostrals in the lower row increase in size from left to right; the upper row consists of three scales, the lateral ones larger than the median (Fig. 2c); five internasals strongly carinated; nasals undivided; a single scale row separates nasals from circumorbital scales; head scales heavily carinated and lanceolate; nine interorbitals; 14 scales between posterior supralabials; eye large, 3.1 mm in diameter; eye diameter 2.5 times the eye-border to lip distance; no interoculabial; 14 and 15 circumorbitals, four in contact with supralabials; nine and 10 supralabials; mental twice as wide as long; eight and nine infralabials, first pair in contact posterior to mental; three pairs of sublinguals; three preventrals; two to three rows of keeled gular scales; dorsal scales heavily keeled, especially on head and anterior third of body; keels run curved to a sharp tip; lateral scales without serration; no reticulate microdermatoglyphic pattern of cell boundary lines; size of the scale keels decreases from head to tail; keels at mid-body shorter and with rather blunt tips; 16 scale rows around mid-body; 14 scale rows in the neck region; 14 scale rows anterior to vent; 160 ventrals, slightly keeled; 58 subcaudals.

Coloration. Dorsal coloration in life is bronze; several scale tips and keels being dark-brown, forming an irregular pattern of broken crossbars; ventrally cream-white to yellowish; iris yellow (Fig. 3). In preservative (3 % formaldehyde transferred to 70 % ethanol after two months), colour changed to reddish-brown dorsally; ventral scales turned uniform clear reddish brown, being more intense in posterior third of body and tail, fading anteriorly.

Etymology. The name refers to the hirsute appearance of the snake (lat. hirsutus = hairy, hirsute).

TABLE 1. Head-measurements and indices of specimens examined. Data for *Atheris squamigera* are based on all material examined (n=23). Damaged specimens were excluded from the analysis. Measurements in mm. For abbreviations see the material and methods section.

	HL	HW	IOD	SED	HL/HW	IOD/SED	HW/IOD
Atheris hirsuta sp. nov.	14.3	10.7	6.9	3.0	1.3	2.3	1.6
Atheris squamigera							
Mean	20.2	15.0	8.1	4.8	1.4	1.7	1.8
Mode	16.4	11.1	8.2	5.3	1.1	1.6	1.4
SD	4.1	3.3	1.4	1.0	0.1	0.1	0.2
Min	12.5	8.5	5.3	3.3	1.1	1.4	1.4
Max	28.9	22.1	11.8	7.2	1.7	1.9	2.2

TABLE 2. Head scalation of specimens examined. Data for *Atheris squamigera* are based on the entire material examined (n=44). Damaged specimens were excluded from the analysis. For abbreviations see the material and methods section.

	SRO	INS	IOS	MTHS	COS left	COS right	IOL	ION	SL left	SL right	IL left	IL right	PSL
Atheris hirsuta sp. nov.	6.0	5.0	9.0	14.0	15.0	14.0	0.0	1.0	10.0	9.0	9.0	8.0	3.0
Atheris squamigera													
Mean	4	4	8	14	14	14	0	1	10	10	11	11	5
Mode	3	4	8	15	14	14	0	1	10	10	11	11	5
SD	1.5	0.6	0.7	1.1	1.4	1.3	0.3	0.4	1.0	0.9	1.0	1.2	0.6
Min	3	3	7	12	11	11	0	1	8	8	8	8	4
Max	8	6	10	17	17	17	1	2	12	12	12	13	7

TABLE 3. Body scalation and SVL in mm of specimens examined. Data for *Atheris squamigera* are based on all material examined (n=44). Damaged specimens were excluded from the analysis. [a] = + anal. For abbreviations see the material and methods section.

	MSR	Ventrals[a]	Sub-caudals	SVL
Atheris hirsuta sp. nov.	16.0	160	58.0	480
Atheris squamigera				
Mean	19	154	52	428
Mode	20	158	55	450.0
SD	1.6	7.7	5.7	93.3
Min	16	140	31	220
Max	22	171	65	605

Habitat. The TNP (6°10'-5°10'N, 7°20'-6°50'W) is the largest protected rain forest area in West Africa. Annual precipitation reaches 2200 mm in the south-west and 1700 in the north-east of the park. Precipitation is highest from April-May to June-July and from September to October-November. A first dry period lasts from December to February. A second dry period normally occurs in August. Daily mean temperature varies between 20°C and 30°C; diurnal fluctuations in temperature are up to 10°C. Mean annual temperature is about 25°C. Humidity fluctuates from 85% during the day to 90-100% during the night. This area is situated within the equatorial climate zone, which is influenced by the southern passat (Riezebos, Vooren & Guillaumet, 1994). Floristically it belongs to the Guinea-Congo Region (Guillaumet, 1967). The holotype was found after heavy rain, on a dirt road between the small town of Taï and the SRET. This area is characterized by secondary rain forest.

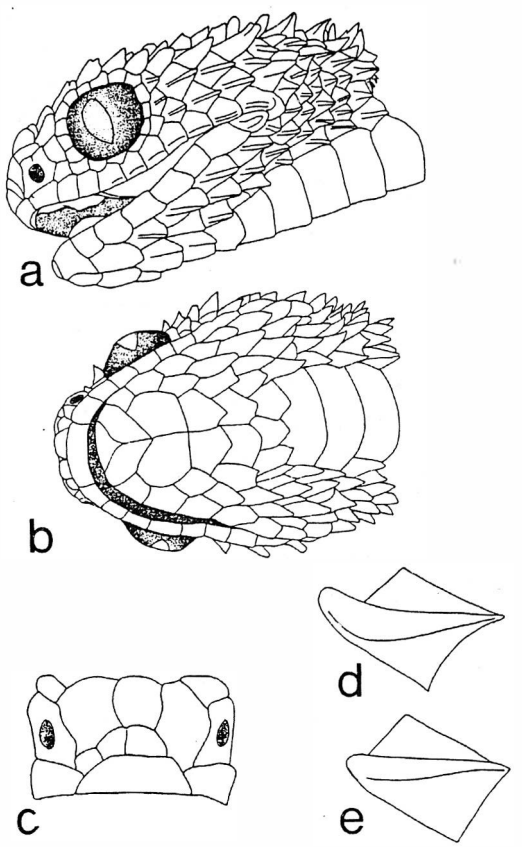

FIG. 2. Lateral (a) and ventral (b) aspect of *Atheris hirsuta* sp. nov. (holotype, SMNS 11333) from Taï-National Park, Ivory Coast; scalation of the snout (c); dorsal scales from *Atheris hirsuta* (d) and *A. squamigera* (e) (ZSM 375 / 1909).

Behavioural remark. Compared to the sympatric *A. chlorechis*, *A. hirsuta* seemed to be more aggressive. When disturbed, it immediately coiled the anterior third of the body in an S-shape, ready to strike.

Distribution. So far only known from the type locality (Fig. 1).

DIFFERENTIAL DIAGNOSIS AND DISCUSSION

Atheris hirsuta sp. nov. resembles *A. squamigera* in appearance but can be distinguished from the latter by several morphometric, and morphological characters. Its most intriguing difference is the elongated dorsal scalation, especially on the head and anterior body, which could not be observed to such an extent in any specimens of *A. squamigera*. Further distinguishing characters were: the number and arrangement of suprarostrals (SRO) (six in *A. hirsuta;* three or up to eight in *A. squamigera,* but usually odd numbers occur); the number of pairs of sublinguals (PSL) (three pairs in *A. hirsuta*; four to seven in *A. squamigera*); and the shape of the head and neck scales. Scales in *A. squamigera* are never as strongly bent as in *A. hirsuta* (Fig. 2 d, e), which in this respect appears similar to *A. hispida* and *A. acuminata*. *Atheris hirsuta* has a much shorter snout and larger eye than *A. squamigera*, giving the head a more blunt and stocky appearance. This is expressed by the IOD/SED index, which is 2.3 in *A.*

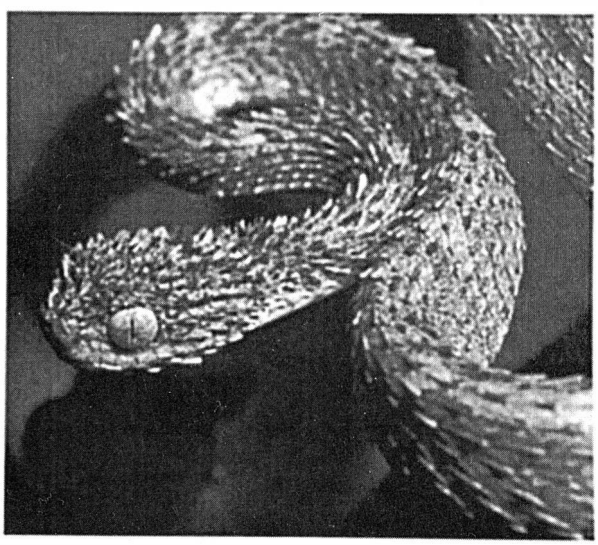

FIG. 3. *Atheris hirsuta* sp. nov. (holotype, SMNS.11333) from Taï National Park, Ivory Coast.

hirsuta, compared with a maximum of 1.9 (mean 1.7) in *A. squamigera*. The number of mid-dorsal scale rows (MSR) in the holotype of *A. hirsuta* is comparatively low – only 16, which equals the lowest counts found in *A. squamigera* (SMNS 4213; ZMB 20481).

Atheris hirsuta differed from the sympatric *A. chlorechis* by the number of suprarostrals (7-8 in *A. chlorechis*, arranged in a lower row of four and an upper row of three to four scales) and the maximum transverse head scale count (MTHS) between the posterior supralabials (SL) (25-27 in *A. chlorechis*; 14 in *A. hirsuta*). *Atheris chlorechis* has higher infralabial counts (IL) (10-11; 8-9 in *A. hirsuta*) and higher numbers of dorsal scale rows at mid-body (25-37). Scales of *A. chlorechis* are not as strongly keeled and the keel only stretches across two thirds of the entire scale, leaving a smooth, slightly depressed area uncovered. Our SEM-photos of *A. hirsuta* revealed no serration of keels on lateral scales, known to occur in some *A. chlorechis* (Groombridge, 1980, cited by Broadley, 1996, 1998).

Atheris hirsuta differs from the East African species *A. nitschei*, *A. rungweensis*, *A. desaixi*, *A. katangensis* and *A. ceratophora* by having fewer transverse head scales, fewer interocunasals (ION) and higher numbers of dorsal scale rows at mid-body. Dorsal scalation in these species is never as elongate as in *A. hirsuta*. The latter lacks serrated keels on lateral scales, present in the species mentioned above. The lack of elongate supraoculars that form horn-like projections above the eyes furthermore distinguishes *A. hirsuta* from *A. ceratophora*. Keels in *A. rungweensis* and *A. desaixi* end before the scale tip, whereas they reach the tip in the other members of this group, as well as in *A. hirsuta*. *A. rungweensis* and *A. desaixi* have more interoculabials (IOL), as well as higher numbers of infralabials. The number of pairs of sublinguals is higher in *A. desaixi*. *Atheris broadleyi* from Cameroon is distinguished from *A. hirsuta* by having more interoculabials, a higher number of scale rows at mid-body and fewer interorbitals (IOS), and by its consistent dorsal colour pattern, also used to distinguish it from the closely related *A. squamigera* (Lawson, 1999). Two distinctive East African species, *A. hispida* and *A. acuminata*, also posses acuminate or lanceolate dorsal scales, but differ from *A. hirsuta* by having fewer transverse head scales and a lower number of suprarostrals, as well as fewer pairs of sublinguals. In addition, *A. hispida* has a higher number of interocunasals. *Atheris acuminata* has a lower number of circumorbital scales, as well as lower numbers of supralabials. For a summary of differences between *A. hirsuta* and other *Atheris* species, see Table 4.

Several authors pointed out that the high variability within the genus *Atheris*, and especially *A. squamigera*, may at least partially be due to incorrectly combining distinct species within a single taxon (Lawson, 1999; Hughes pers.comm.). For example, *A. hispida* had long been taken as a synonym of *A. squamigera* (Laurent, 1955). The same is true for *A. broadleyi*, which had been referred to as a colour morph of *A. squamigera* (Perret & Mertens, 1957; Broadley, 1998), until its description by Lawson (1999). The resurrection of the species status of *A. subocularis* has recently been published (Lawson et al. 2001). We herein adopted the recognition of *A. anisolepis* as junior synonym of *A. squamigera* by Lawson & Ustach (2000). However, these authors do not consider the difference in suprarostral scale counts (seven to eight in *A. anisolepis*, three to seven in *A. squamigera*), mentioned in Broadley (1998). We found this character to be consistent in four museum specimens (SMNS 8361; ZMB 28987; ZSM 275/1996; ZSM 375/1909;) and an additional specimen (ZMUC R68269) previously recognized as *A. anisolepis* (Broadley, 1998). Referring to data presented by Broadley (1998), Hughes (pers. comm.) has pointed out the great range in ventral counts (males: 133-169, variation: 36 scales; females: 141-175, variation: 34 scales) in *A. squamigera*. In his opinion, a variation of more than 30 in ventral scale counts within one sex is suggestive of more than one species being involved.

TABLE 4. Comparison of characteristics among *Atheris hirsuta* sp. nov. and other *Atheris* species. Differences to *Atheris hirsuta* are marked bold. SLS = serrated keels on lateral scales; ESO = elongate supraoculars; * = no data; + = present; - = absent. For further abbreviations see the material and methods section. Data referring to *Atheris squamigera* represent mean values of specimens examined. Data referring to species other than *Atheris hirsuta* and *Atheris squamigera* adapted from: Broadley, 1998 and Lawson, 1999.

	SRO	INS	IOS	MTHS	COS	IOL	ION	SL	IL	MSR	PSL	SLS	ESO
hirsuta	6	5	9	14	14-15	0	1	9-10	8-9	16	3	-	-
squamigera	**4**	**4**	**8**	14	14	0	1	10	**11**	**19**	**5**	-	-
nitschei	3-7	4-5	6-12	**18-20**	10-17	0-2	**2-5**	8-13	9-15	**23-34**	3-6	+	-
rungweensis	3-7	5-6	9-13	**24-26**	15-18	1-2	**3-4**	9-12	**11-13**	**23-33**	2-3	+	-
desaixi	6-7	5	8-11	**22**	14-17	1-2	**2-3**	**10-12**	**11-14**	**21-31**	**4-6**	+	-
ceratophora	5-9	4-5	7-11	**19-20**	13-19	0-1	**2-4**	7-11	8-12	**19-27**	1-3	+	+
katangensis	3-6	5-6	9-11	**20-22**	14-17	0-1	**2-3**	9-12	**11**	**23-31**	3	+	-
chlorechis	**7-8**	5	8-14	**25-27**	15-20	0-2	**3-4**	9-12	**10-11**	**25-37**	**1-2**	+	-
broadleyi	3-7	3-5	**3-8**	*	12-16	0	**3**	9-12	9-12	**17-23**	*	-	-
hispida	**3**	4-6	6-10	**12**	9-15	0	**2**	9-10	8-10	15-19	**1-2**	-	-
acuminata	**2**	3	**5**	**10**	11-12	0	1	**6**	7-8	**14**	**1**	-	-

Characteristic threat displays involving stridulation, that have been reported for *A. desaixi* (Ashe, 1968) and erroneously for *A. nitschei* (Goetz, 1975), were not observed when handling A. *hirsuta*. The dorsal scales of *A. hirsuta* showed no micro-ornamentation, as has been illustrated by Price (1982, Fig. 6a) and Groombridge (1980, Figure 184, cited in: Broadley, 1996) for *A. squamigera*. In our opinion, it remains questionable whether this is really a feature of diagnostic value. Among the material examined in SEM-analyses, we found *A. squamigera* showing such ornamentation as well as specimens showing no ornamentation at all.

Atheris hirsuta clearly differs from the frequently observed colour patterns in *A. squamigera*. The latter are most often apple green to turquoise blue with yellow crossbands, occasionally uniform spectrum yellow (Lawson, 1993) or violet (Lawson, 1999), or a mottled combination of these colours, and sometimes light brown (this paper).

Another strong argument for the distinctiveness of *A. hirsuta* from *A. squamigera* is their allopatric distribution. The reports of the latter species from Senegal (Lawson, 1999) and the Ivory Coast (Spawls & Branch, 1995; McDiarmid, Campbell & Touré, 1999) are not substantiated by voucher specimens and are most probably repetitions of earlier misidentifications in the literature. The westernmost verified *A. squamigera* record is from "Togo" (Werner, 1897; Sternfeld, 1910), based on ZMB 13777. There were no precise locality data for Buettner's specimens, but they probably came from the forested Togo hills, which now lie on the border between Togo and Ghana, since former western Togo now constitutes the Volta Region of Ghana, accounting for the inclusion of Ghana within the range of this species by Leeson (1950) and Hughes & Barry (1969). Werner (1897) provided data for four uncatalogued specimens of *A. squamigera* with low counts for midbody scale rows (17-19), and a head which he assigned to the East African species *A. ceratophora*. However, Sternfeld (1910) only listed two *A. squamigera* from Togo, collected by Buettner, with the Berlin Museum number 13777, but claimed that the species was new for Togo. This "Togo" population is separated from populations in south eastern Nigeria east to Kenya by the "Dahomey Gap", an area well known to form a zoogeographic barrier for forest species (e.g. Schiøtz, 1967). This area is occupied by "forest-savanna mosaic", with only small patches of forest remaining, and has existed in its present form for some 10 000 years. The present situation may be the cumulative effect of successive Pleistocene dry periods, due to climatic oscillations (Moreau, 1963, 1969; Jahns *et al.*, 1999).

Although our description of *A. hirsuta* is based on a single specimen, we believe the differences to be sufficient to justify its description at the species level, thereby making the name accessible for further studies of the snake fauna of the Ivory Coast. Rödel & Mahsberg (2000) recorded 39 snake species from TNP. From their experience in other regions (Rödel, Grabow, Böckheler & Mahsberg, 1995; Rödel, Kouadio & Mahsberg, 1999) they suggest that approximately only two thirds of the snake fauna of TNP has been found so far. With this description the known species score of TNP rises to 40.

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APPENDIX

COMPARATIVE MATERIAL EXAMINED:

Atheris squamigera

BURUNDI: ZSM 275/1996, E:VIII.93-VI.96, Burundi, Musigati;

CAMEROON: ZMH R06316 old no. 1760, Cameroon; ZMH R06321 old no. 1861, Cameroon; ZMH R06311 old no. 4973, Jaundebezirk (Yaoundé) an der Kribistrasse, S Yaoundé, Cameroon; ZMH R06323 old no. 4493, Esosung, Bakossi Mountains, Bezirk Johann-Albrechts-Höhe (=Kumba) 1060 m a.s.l., Cameroon, Africa; ZMH R06313 old no. 4949, Kuti, Bamum Country, 1040 m asl, Cameroon; ZMH R06328 old no. 5131, Central Africa Molundu; ZSM 375/1909a, Dibongo bei Abdea Kamerun; ZSM 375/1909b, Dibongo bei Abdea Kamerun; ZSM 96/1978, Esosung, Bakossi Mountains, Kamerun; ZMB 20342, Douala, Yossplatte; ZMB 20481, Douala, Yossplatte; ZMB 15887-88, Joh.Albrechtshöhe; ZMB 21727, Longji, Kamerun; ZMB 28987, Longji, Kamerun; ZMB 24302, Njong, Neu-Kamerun; ZMB 30690, Ajoshöhe am Nyong; ZMB 30719, Ajoshöhe am Nyong; SMNS 4213, Acra, Kamerun; SMNS 4264, Malimba, W.Afrika; ZFMK15619, Kamerun & Fernando Pó, 1951/54 1963, Kumba; 1951/54 1963, Moka /Bioko; ZFMK5452-56, Kamerun+Fernando Pó, 1951/54 1963, Moka /Bioko; ZFMK 15618, Kamerun & Fernando Po, 1951/54 1963, Nyasoso/Kupe;

CONGO: ZMUC R68269 (800), Ménengué, 4°16'S-11°47'E, Congo; ZMUC R68270 (RJD 12),Tchissanga, 4°32'S-11°46'E, Congo; ZSM 130-137/1999 V.1995, Brazzaville umgeb.;

DEMOCRATIC REPUBLIC OF CONGO (ZAIRE): ZMH R06317 old 124, Belgisch Kongo, Democratic Republic of Congo; SMNS 8361, Zaire, Afrika, Prov.Kivu Station Irangi; ZMB 37709, Leopoldville; ZMB 37807, Leopoldville; ZMH R06326 old 184, Leopoldville: Kinshasa;

GABON: ZMH R06320 old 1098,Gabon;

TOGO: ZMB 13777,Togo.

Gazetteer (only localities of West and Central African distributional range of *A. squamigera* included):
CAMEROON: 34 km N of Lolodorf, 3°14'N 10°44'E; Njong, 3°17'N 9°54'E; Bitye, 3°1'N 12°22'E; Metet, 3°26'N 11°45E; Dibongo bei Abdea 3°47'N 10°6'E; Jaundebezirk (Yaoundé) an der Kribistrasse, S Yaoundé, 3°52'N 11°31'E; Longji, 3°5'N 9°58'E; Boumir Camp, Dja Forest Reserve, 3°9'N 13°0'E; Ajoshöhe am Nyong, 4°0'N 13°34'E; Fernando Pó, Kumba, 4°43'N 9°11'E; Southwest Province, 5°25'N 9°20'E; Esosung, Bakossi Mountains, Bezirk Johann-Albrechts-Höhe (=Kumba) 1060 m a.s.l. Africa, 5°59'N 14°26'E; Molundu, 2°2'N 15°13'E; Douala, Yossplatte, 4°3'N 9°42'E;
CONGO: Leketi, 2°34'S 14°17'E; Ménengué, 4°16'S 11°47'E; Tchissanga, 4°32,S 11°46'E; Lukolela, 5°23'S 24°32'E; Prov.Kivu Station Irangi, 2°30'N 28°0'E; Brazzaville umgeb., 4°16'S 15°17'E;
DEMOCRATIC REPUBLIC OF CONGO (ZAIRE): Banana, 6°1'S 12°24'E; Leopoldville: Kinshasa, 4°18'S 15°18'E; Leopoldville,4°19'S 15°13'E; Avakubi, Kinshasa,1°20'N 27°34'E; Niapu, Kinshasa, 2°25'N 26°28'E; Medje, Kinshasa, 2°25'N 27°18'E; Akenge, Kinshasa, 2°56'N 26°50'E; Rungu, Kinshasa, 3°11'N 27°52'E; Mbanza-Ngungu, Kinshasa, 5°15'S 14°52'E; Kinsuka, Kinshasa, 5°15'S 15°13'E;
GABON: Makandé, 0°47'S 11°58'E;
IVORY COAST: Taï NP, 5°50'N 7°19'W;
TOGO: Togo, no precise locality available.

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THERMAL AND REPRODUCTIVE ECOLOGY OF THE SNAKE *PSAMMOPHIS PHILLIPSI* FROM THE RAINFOREST REGION OF SOUTHERN NIGERIA

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Aspects of the thermal and reproductive ecology were studied in the colubrid snake *Psammophis phillipsi* from south-eastern Nigeria. The annual reproductive cycle was well synchronized and seasonal: females were gravid in the middle part of the dry season, and oviposition occurred in the second half of the dry season (February-March), whereas egg hatching occurred by the onset of the rainy season (mid April to early June). The mean number of eggs was 9.2±0.9 (n = 23), and was related to maternal size. Gravid and non-gravid specimens did not differ significantly in terms of mean SVL, mean T_b, or T_s. Mean T_b tended to increase regularly from 0600 to 1200 hrs, attained the higher values in the middle of the day (1200-1500 hrs), and tended to decrease after 1800 hrs. Gravid individuals differed from non-gravid individuals in that their variances of T_b were notably lower both in the early morning and in the middle of the day, but higher at other times of day. Multiple regressions showed that the interaction of T_a and T_s had a significant effect on T_b, whereas T_b was not influenced by the number of eggs produced in gravid animals.

Key words: body temperatures, Colubridae, Psammophiinae, reproductive biology, tropical Africa

INTRODUCTION

The notion that thermoregulation is a central factor of reptilian biology is at the forefront of modern herpetology (e.g. see Avery, 1979; Huey & Slatkin, 1976; Peterson *et al.*, 1993) and has stimulated very sophisticated approaches and analyses (e.g. see Hertz, 1992; Hertz *et al.*, 1993, 1999; Christian & Weavers, 1996; Webb & Shine, 1998; but see Alexander & Currin, 1999; Currin & Alexander, 1999; Hertz *et al.*, 1999, for diverging opinions on the theoretical framework). In recent years, however, whether thermoregulation is indeed important for most reptiles has been called into question (Shine & Madsen, 1996). After studying the thermal ecology of Australian pythons, Shine & Madsen (1996) concluded that thermoregulation is of very limited relevance to the biology of these tropical reptiles. They also suggested that as thermal challenges to reptilian life should be trivial in the tropical environment, it is likely that thermoregulatory biology is of only minor relevance for most reptiles. Indeed, the majority of reptile species occur in the tropics and not in temperate regions where the majority of previous studies on reptilian thermoregulation is concentrated. Based on these contrasting views, it is obvious that, if we are to place the thermoregulatory biology of reptiles in its proper perspective, we need more information on the thermal ecology (and its links with the reproductive biology) of a wide variety of tropical reptiles, especially snakes, which have been neglected until now in this respect (but see Luiselli & Akani, 2002*a*). Although tropical environments may not present the same problems associated with low temperatures as temperate regions, there may be problems of excessively high temperature, whose avoidance is a form of thermoregulation. Thus, studies of thermoregulation in temperate climates may not be of great relevance to species in the tropics, but it would be wrong to suggest that thermoregulation is unimportant in the tropics (Luiselli & Akani, 2002*a*).

The scope of the present paper is, therefore, to convey detailed field data on the body and environmental temperatures – and on their links with the reproductive biology – of a diurnal, fast-moving, mid-sized Afrotropical snake (*Psammophis phillipsi* Hallowell, 1844: Colubridae, Psammophiini), which has been totally unstudied in the past with regard to these aspects of biology. This species was selected as study model because of (1) its ecological characteristics (diurnality and fast-moving) which are entirely different from those exhibited by the pythons studied by Shine & Madsen (1996) or by the semi-aquatic species studied by Luiselli & Akani (2002*a*), and (2) its abundance in our study area (southern Nigeria) (e.g. see Akani, Barieenee, Capizzi & Luiselli, 1999), thus allowing easy accumulation of field data even within short time-spans of research.

MATERIALS AND METHODS

The field study was carried out at a site in south-eastern Nigeria (vicinity of Eket, Akwa-Ibom State, 04°50' N, 07°59' E), characterized by a moist rainforest patch

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growing along the banks of the River Quo-Ibo (= Kwa-Ibo), partially inundated during the wet season, and surrounded by areas of subsistence cultivation (pineapple, banana, plantain, cassava, yam, etc). The study area –which is located within the Guinea-Congolian rainforest block (White, 1983) and has a typical tropical climate (wet season: April-September; dry season: October-March) – was already well explored because it was previously used for other ecological studies on both snakes (Luiselli *et al.*, 1998) and other reptiles (Luiselli *et al.*, 1999).

Field data on the thermal ecology of the snakes were collected, both on sunny and on rainy days, during the dry seasons of 2000 (March) and 2001 (January-February-March), and during the wet season of 2000 (April to June) and 2001 (April and May), whereas field data on the reproduction of snakes were collected in the period September 1996 to July 2001.

Snakes were searched for along standardized routes within the various microhabitats frequented by them at the study area (see Luiselli & Akani, 1999, 2002*b*). Each snake eventually captured was measured for snout-vent length (SVL, to the nearest 0.1 cm), and individually marked by ventral scale clipping for future identification. For females, eventual pregnancy status and numbers of eggs were determined by abdominal palpation (precision: ±1 egg). In addition, specimens found already dead during our surveys (e.g. snakes killed by farmers, squashed by cars, etc.) were dissected to determine their eventual numbers of eggs. Because determination of sex is often problematic in *P. phillipsi* even in adults (if not using invasive techniques) and may lead to misidentifications (Akani *et al.*, unpublished), we subdivided our specimens into two categories: gravid (GR) and non-gravid (NGR). GR were all females obviously carrying eggs, and NGR were all adults which were obviously not pregnant (i.e. males plus non-gravid females). To discriminate between juveniles and adults, we followed Butler (1993), who found that the smallest mature *phillipsi* female was 650 mm SVL, and that females were significantly smaller than males. Thus, we assumed that all specimens shorter than 650 mm were immatures, and these were excluded from the analyses.

Body (cloacal) temperature of each captured snake was measured within 60 seconds from capture, by a rapid-recording Schultheis thermometer (range: 0/50°C, manufactured by Miller & Weber Inc., New York). At each capture spot, air temperature and substratum temperature were also measured. Air temperature was measured at approximately 100 cm above the ground, and substratum temperature was measured at soil level at the site of the snake. Both air and substratum temperatures were always taken in the shade, as all specimens used for body temperature recording were captured in total or partial shade. In addition, the hour of the day (Lagos standard time), substratum type, and the activity type exhibited by the snakes at the time of capture were recorded. To avoid statistical problems caused by pseudo-replication of the data (cf. Hurlbert, 1984; Licht *et al.*, 1966; Mathur & Silver, 1980), we took body temperature only once from each snake, i.e. the recaptured individuals were never used again for body temperature recording. This procedure was applied in consideration of the abundance of *P. phillipsi* at the study area (where it is the most common snake species, cf. Luiselli *et al.*, 1998; Luiselli & Akani, 1999), thus making the above procedure feasible.

All statistical tests were two-tailed, with alpha set at 5%. In the text, the means are followed by ±1SD. Body temperatures are indicated by T_b, air temperatures by T_a, and substratum temperatures by T_s. All tests were done using STATISTICA (version 5.0 for Windows) computer package (Statsoft, 1996). When the effects of the interaction of reproductive status (gravid versus non-gravid) and hour of the day on T_b were analysed, it was done after having verified that the interaction effect explained more of the T_b than a simple additive model (one-way ANOVA) based on the two factors taken separately.

RESULTS

Reproductive Cycle

Heavily gravid specimens of *P. phillipsi* were found in the field only during the dry season: 17 specimens were collected in November, 14 in December, 11 in January, and 9 in February. No gravid specimens were seen in any other month, but newborn specimens (i.e. specimens with highly visible umbilical scars; SVL: 23.7±2.1 cm, n=18) were collected at the end of April (n=2), March (n=11) and early June (n=5). This suggests that the annual reproductive cycle of *P. phillipsi* is well synchronized and strongly seasonal in southern Nigeria: females are gravid in the middle part of the dry season, and likely to oviposit in the second half (February-March), whereas egg hatching probably occurs by the

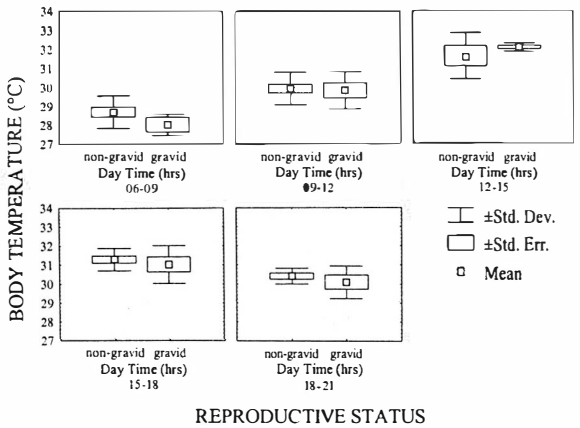

FIG. 1. Hourly variations of means (and dispersion measures) of body temperatures of gravid and non-gravid *Psammophis phillipsi,* throughout five time intervals. Numbers of specimens: 06-09 hrs – gravid n=5, non-gravid n=8; 09-12 hrs – gravid n=6, non-gravid n=11; 12-15 hrs – gravid n=5, non-gravid, n=6; 15-18 hrs – gravid n=6, non-gravid n=9; 18-21 hrs – gravid n=5, non-gravid n=9. For statistical details see the text.

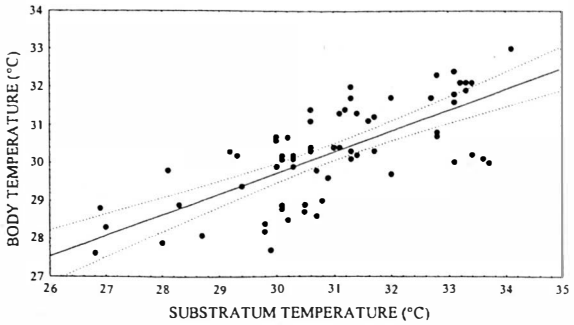

FIG. 2. Relationships between body temperature and substratum temperature in *Psammophis phillipsi*. For statistical details, see the text.

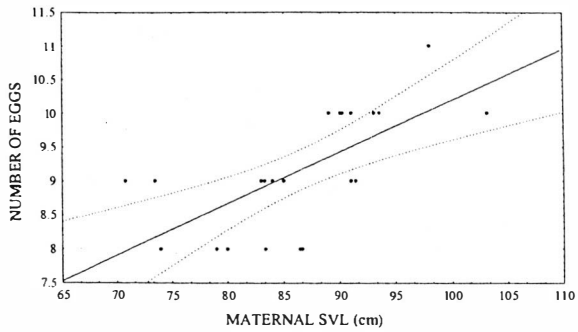

FIG. 3. Relationships between body temperature and number of eggs in gravid female *Psammophis phillipsi*. For statistical details, see the text.

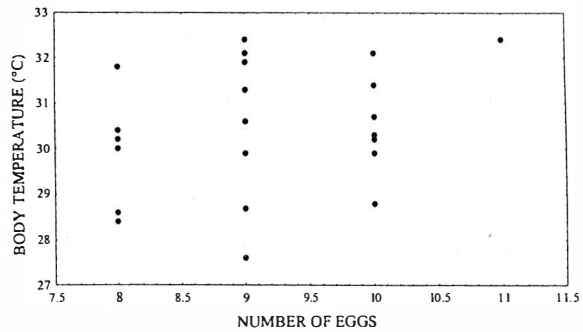

FIG. 4. Relationships between maternal SVL and number of eggs in gravid female *Psammophis phillipsi*. For statistical details, see the text.

onset of the rainy season (i.e. mid April to early June). The mean number of eggs was 9.2±0.9 (n=23).

THERMAL ECOLOGY AND ITS LINKS TO REPRODUCTIVE BIOLOGY

Gravid snakes averaged 86.6±7.83 cm SVL (n=23), compared to 88.7±14.62 cm SVL in non-gravid specimens (n=44). The two samples did not differ significantly in terms of mean SVL (one-way ANOVA: $F_{1,65}$= 0.41, P=0.523); mean T_b (non-gravid: 30.2±1.28° C, n=44, gravid: 30.4± 1.37° C, n=23; one-way ANOVA: $F_{1,65}$=0.53, P=0.468), and mean T_s (non-gravid: 30.9±1.69° C, n=44, gravid: 31.1±1.77° C, n=23; one-way ANOVA: $F_{1,65}$=0.28, P>0.65).

The hourly variations in T_b (means and dispersion measures) for both gravid (total examined n=27) and non-gravid specimens (n=40) are presented in Fig. 1. Data are grouped into five time intervals, each three-hours long. In general, it appears that mean T_b tended to increase regularly from 0600 to 1200 hrs, attained the higher values in the middle of the day (1200-1500 hrs), and tended to decrease after 1800 hrs. This daily pattern was evident in both gravid and non-gravid specimens (Fig. 1).

After pooling gravid and non-gravid specimens, the hourly variations in T_b were significant ($F_{4,62}$=27.3, P<0.001), and a Levene test for heterogeneity of variances indicated no significant effect of the time of day on the variances ($F_{4,62}$=0.67, P=0.617). The results of the analyses were not changed by log-transforming the T_b data. The interaction of reproductive status (gravid versus non-gravid) and hour of the day had a very significant effect on T_b (MS effect = 8.198, $F_{9,57}$=12.00, P<0.001). Post-hoc comparisons (Tukey HSD test) showed that the variance of T_b of gravid and non-gravid specimens did not differ significantly at time intervals 0900-1200, 1500-1800 and 1800-2100 hrs, but the gravid specimens had significantly lower variances than non-gravid specimens at time intervals 0600-0900 hrs and 1200-1500 hrs. Also in this case, the results of the analyses were not changed by log-transforming the T_b data.

T_s had significant effect on T_b (T_s: r=0.720, P<0.001; equation: T_b = 13.230 + 0.550 T_s, see Fig. 2). Concerning gravid specimens, T_b was not influenced by the number of eggs produced (r=0.276, ANOVA: $F_{1,21}$=1.727, P=0.203; equation: T_b=26.532+0.425 number of eggs, see Fig. 3), which was in turn positively related to maternal SVL (r=0.672, P< 0.01; equation: number of eggs = 2.578 + 0.076 SVL, see Fig. 4).

DISCUSSION

REPRODUCTIVE CYCLE

Butler (1993) described the annual reproductive cycle of *P. phillipsi* captured from several localities north of the equator as a monoestrous dry season cycle (*sensu* Saint Girons, 1982; Saint Girons & Pfeffer, 1971), and observed that testicular recrudescence begins at the end of the rainy season, vitellogenesis by the end of the rainy season, oviposition by mid-dry season, and egg hatching at the onset of the rainy season. In addition, Senter (2001) confirmed the occurrence of dry season oviposition in *P. phillipsi* from Liberia. Our data agree completely with the observations by these authors, and indicate that the annual cycle of reproduction is nearly perfectly synchronized in all *P. phillipsi* populations from the west African rainforest block (Senter, 2001, and part of the data given in Butler, 1993) to the Nigeria-Cameroon rainforest block (present study, data in Butler & Reid, 1990, and part of the data given in Butler, 1993).

In addition, a remarkable similarity between the present study and that of Butler (1993) was found in other aspects of the reproductive biology, including the mean offspring SVL, which was very similar in the two

studied samples (between sample comparison: t=1.92, df=26, P=0.661). On the other hand, the number of eggs produced by females in our study was less than that of females studied by Butler (1993) (between sample comparison: t=3.61, df=30, P=0.0011), but our data are based on abdominal palpation, whereas those of Butler (1993) are based on dissection, which may have partially biased our estimates (i.e. lowering our egg counts). Moreover, Butler (1993) did not find any significant relationship between maternal size and the number of eggs produced, whereas our study did. In this case, it is likely that Butler's study was based on too small a number of gravid specimens (n=9) to find any significant correlation between these variables, and so the apparent differences between the two studies may be explained by sample sizes differences rather than actual ecological divergence.

THERMAL ECOLOGY AND ITS LINKS TO REPRODUCTIVE BIOLOGY

Our detailed data on field T_b, and its relationships with external temperatures suggest that thermoregulation is not very important for the fast moving, highly active *P. phillipsi*. In fact, T_b's were very significantly correlated to T_s, which suggests a degree of thermo-conformity in this species (although far from ideal poikilothermy), in which the main requirement is to lose heat (due to the high external temperatures) rather than to accumulate heat. To lose heat, these snakes rest in the shade, and when basking, they always avoid resting fully exposed to the sun (Akani *et al.*, unpubl. obs.). Thus, the fact that snakes select shady spots is due to their avoid ance of thermally stressful exposure to full solar radiation, and is certainly a form of behavioural thermoregulation. In addition, the fact that gravid specimens maintained the same T_b as non-gravid specimens at nearly all periods of the day is further evidence of the fact that during pregnancy, elevation of T_b by thermoregulation is not necessary (e.g. see Avery, 1979). However, gravid specimens tended to maintain higher T_b than non-gravid specimens during the middle of the day, which is quite difficult to interpret at present. In this regard, it is necessary to stress that many previous studies (e.g. Hertz, 1992; Hertz *et al.*, 1993, 1999) have suggested that gravid females differ from non-gravid animals not so much in the mean temperature selected, but in the degree of precision. This pattern, with variances notably lower for gravid animals, is also emerging in our study, but only in the middle of the day (see Fig. 1). Indeed, according to Fig. 1, it is the gravid females that have the more variable mean T_b (over the first three periods), which is contrary to the idea that gravid females keep their T_b within a narrower range. A possible interpretation of the significant interaction between time period and reproductive status is that T_b was higher in gravid than in non-gravid females at some times of day, but lower at other times. Thus, the lower variance of gravid females' T_b at some times of the day may be nothing to do with thermoregulation, but perhaps a quirk of relatively small sample sizes. Mean T_b of *P. phillipsi* was quite constant throughout the day (but lower at night), and was also relatively similar to that exhibited by several other colubrids from temperate regions (e.g., Dmi'el & Borut, 1972; Mushinsky *et al.*, 1980). However, it was lower than the preferred T_b of other diurnally active, fast moving colubrids (Fitch, 1963; Hirth & King, 1969; Vitt, 1974).

Although based on a relatively small sample size, our study also showed that the numbers of eggs carried by a female does not have any influence on the female T_b, which supports the idea that thermoregulation plays a relatively minor role in some aspects of the life-history of this tropical colubrid. However, it is noteworthy that the reproductive females continue feeding even when heavily gravid (Akani *et al.*, 2003), and thus it would be of great interest to further investigate the interrelationships between feeding rates, reproductive condition and thermal ecology in this Afrotropical species. We suggest that *Psammophis phillipsi* is a potential model species for the study of these issues in the Afrotropics. Whereas almost nothing is known of the links between energy intake, thermal ecology and reproductive biology in snakes from this geographic region, *Psammophis phillipsi* is abundant and widespread, is relatively easy to study, and displays some life history characters of remarkable interest (e.g. dietary shift during pregnancy, feeding extended over the gestation period, etc.; see Akani *et al.*, 2003). Comparisons of *Psammophis phillipsi* with species from temperate regions – which are often regarded as model organisms for herpetological study (e.g. Bonnet *et al.*, 2001) – would be of interest.

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USE OF FLUORESCENT PIGMENTS AND IMPLANTABLE TRANSMITTERS TO TRACK A FOSSORIAL TOAD (*PELOBATES FUSCUS*)

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Compared to other vertebrate groups, movement patterns and microhabitat use in amphibians has been little studied. The two goals of this study were (1) to compare two different methods of tracking amphibians (implantable transmitters and fluorescent pigments); and (2) to characterize movement patterns and habitat use in the nocturnal, fossorial spadefoot toad (*Pelobates fuscus*). A fluorescent pigment method was useful for microhabitat studies, as trails could be detected in all kinds of terrestrial habitats, even under wet conditions. Using this method it was possible to trace complete nocturnal movement patterns (maximum distance moved: 73 m). Implantable transmitters were particularly appropriate for fossorial species, such as *Pelobates fuscus*. Diel home range and microhabitat preferences were more precisely defined using a combination of telemetry and pigments. In addition, the vertical component of habitat use could be assessed. The spadefoot toad was more likely to use areas of bare soil or short vegetation and seemed to avoid shrub-covered areas. Mean distance moved between two successive burrows was higher in females (22.9 m) than in males (12.9 m).

Key words: amphibian movement, fluorescent pigments, microhabitats, *Pelobates fuscus*, radiotelemetry

INTRODUCTION

Patterns of animal movement can provide useful information on migration, dispersal, homing activity, activity area, and site selection for reproduction. Given world-wide declines in amphibian populations (Barinaga, 1990; Halliday, 1998; Pechmann *et al.*, 1991; Wake, 1991), herpetologists have taken a special interest in amphibian movements and habitat use (Demaynadier & Hunter, 1998; Dodd & Cade, 1998; Gibbs, 1998; Sjogren-Gulve, 1998). Management practices may enhance amphibian dispersal (Seabrook & Dettmann, 1996), enabling new populations to establish. Alternatively, management can be used to restrict dispersal and thereby minimize mortality by road-kill.

Small body size technically limits the study of movement by small vertebrates. With amphibians, capture-mark-recapture studies (e.g. Clarke, 1974; Haapanen, 1974; Denton & Beebee, 1992) are labour-intensive outside the breeding season and often provide limited data on individual, day-to-day movements. Radio-isotope tracing has been used occasionally (e.g. Ashton, 1994; Barbour *et al.*, 1969), and a tracking device (a sewing machine bobbin in a holder tied around the waist) was used with large, non-burrowing individuals for short study periods (e.g. Dole, 1965; Sinsch, 1987; Sinsch, 1990). Although the development of small and lightweight radio-tracking systems seems promising (Nuland & Claus, 1981), attaching the transmitter is problematic (van Gelder, Aarts & Staal, 1986*a*; Golay, 1996; Tramontano, 1997). External transmitters are especially difficult to use with burrowing species, so implantable ones may be more practical (Madison, 1997; Olders, van Gelder & Krammer, 1985).

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Telemetry studies of amphibians are growing in number. External transmitters with various modes of attachment have been used on anurans such as *Bufo americanus* (Tester, 1963 quoted in van Nuland & Claus, 1981), *B. marinus* (Seabrook & Dettmann, 1996), *B. bufo* (van Gelder *et al.*, 1986*a*; van Nuland & Claus, 1981), *B. viridis* (Baumgart, unpublished data), *B. calamita* (Golay, 1996), *Rana temporaria* (Fiorito *et al.*, 1994; Tramontano, 1997), *Rana muscosa* (Matthews & Pope, 1999) and *Buergeria buergeri* (Fukuyama, Kusano & Nakane, 1988). Oldham & Swan (1992) forced adult *Rana temporaria* and *Bufo bufo* to swallow transmitters, as did Pearson & Bradford (1976) with *Bufo spinulosus*. Implantable transmitters were used in anurans, such as *Bufo bufo* (van Gelder *et al.*, 1986*b*; Olders *et al.*, 1985), *B. americanus* (Werner, 1991), *B. spinulosus* (Sinsch, 1991), *B. calamita* (Sinsch, 1992), *B. canorus* (Martin, unpublished data), *Rana clamitans* (Lamoureux & Madison, 1999) and *Hoplobatrachus occipitalis* (Spieler & Linsenmair, 1998), and in urodeles such as *Cryptobranchus alleganiensis* (Blais, 1996 quoted in Madison, 1997; Stouffer *et al.*, 1983), *Ambystoma maculatum* (Madison, 1997), *A. tigrinum* (Madison, 1998; Madison & Farrand, 1998), *A. gracile* (Stringer, 1997), *Salamandra lanzai* (Riberon, Miaud & Guyétant, 1997) and *Triturus cristatus, T. marmoratus* and their hybrids (Jehle & Arntzen, 2000). Even larval *Dicamptodon tenebrosus* have been radio-tracked (Colberg *et al.*, 1997).

Even so, telemetry studies have their limitations. For example, the precision of tracking depends on the number of location points, and the presence of an observer may influence the behaviour of the studied animal. One approach that may circumvent many difficulties is the use of fluorescent pigments that rub off onto the ground when the animal moves. These have been used for indirect visual tracking of small, nocturnal

rodents (Duplantier *et al.*,, 1984; Frantz, 1972; Lemen & Freeman, 1985), lizards (Fellers & Drost, 1989; Dodd, 1992), hatchling turtles (Butler & Graham, 1993) and tortoises (Blankenship, Bryan & Jacobsen, 1990). Recently, this method has been adapted for, and tested on, amphibians (Lodé, 1996; Eggert, Peyret & Guyétant, 1999).

We used fluorescent pigment for indirect visual tracking and telemetric studies with implantable transmitters on the spadefoot toad (*Pelobates fuscus*) to gain information on habitat use by this secretive, fossorial and nocturnal toad. The present paper describes these two tracking procedures, and also reviews their advantages and limitations for obtaining movement information critical for population management and conservation.

MATERIALS AND METHODS

Five adult toads (two males aged 2 and 3 years and three females aged 4, 7 and >2 years) were caught between 6 and 25 May, 1998 while leaving two natural breeding ponds in north-eastern France (see Eggert & Guyétant, 1999 for site description and age estimation method). The transmitter implantations were done in the laboratory under general anaesthesia with 2-phenoxyethanol, an anaesthetic used for fish (Deacon, White & Hecht, 1997). Approximately 0.1-0.2 ml phenoxyethanol was mixed in 100 ml water and the toads were immersed until muscular relaxation was observed (30-40 minutes). After making a small incision mid-laterally in the left flank of each toad, the transmitter (BD-2GH, Holohil Systems, Ontario, Canada) was placed in the body cavity. Sutures of 6-0 polypropylene thread were made before washing the toad in running tap water. The animals recovered after 10 to 30 mins and, after observation for two days to verify full recovery, were released in the field at their exact places of capture. The implant volume (16 x 9 x 8 mm) represented about 10% of the normal abdominal volume of eggs, and its mass (2 g) was 9 to 11% of the body mass.

Animals were located in their burrows every day until 18 July (i.e. 52 to 71 days) using classical local triangulation methods and recorded to a precision of 10 cm, using a compass and a measuring tape. Searches for active, radio-tracked and non-radio-tracked toads were made over 34 nights. Every captured toad was tagged with a Passive Integrated Transponder (PIT tag) for individual identification.

Orange and yellow fluorescent pigments (Radiant Color, Ltd) were used with four of the radio-tracked toads and with 37 other, non-radio-tracked adults found active at night in the same area, so that the availability of habitat types could be considered the same for all toads. The dye was diluted in paraffin oil and applied in the field with a brush to the undersides of the four legs of the toads. We did not dig up any toads. As *P. fuscus* burrows daily, the colour pigments were removed by burrowing. During the following night we used a 6 W UV lamp to locate the pigment that had rubbed off onto the ground or vegetation. We marked the path with a blue, fluorescent spray, visible under normal daylight. Movement patterns were then reduced to a series of points, assessed with the same procedure as for radio-tracking data, and filmed using a video camera for further microhabitat analysis. To test habitat-type preferences, we estimated habitat availability in the central region of the toads' range by measuring the length of each vegetation structure encountered along randomly chosen lines. For this, four 50 m-long lines forming the branches of two randomly situated crosses were used. Then, differences between availability and use by toads were tested with *G*-tests for goodness-of-fit. A Mann-Whitney *U*-test was performed when data normality assumptions were not met. Sandy, open areas were made up of natural dune and small, newly-created areas cleared of vegetation.

Active toads were rarely found near a burrow, so almost all observed fluorescent trails were incomplete parts of the respective nocturnal excursions. Therefore, fluorescent trails were used to estimate diel activity areas of the radio-tracked toads in two ways: (1) with the two burrows used before and after nocturnal movement (located during the day with transmitters), or (2) with only the fluorescent trail.

Minimum convex polygons and 99% probability Jennrich-Turner bivariate normal ellipses were calculated using the Ranges V computer program (Kenward, 1990).

RESULTS

All five toads consumed food within 24 hr of anaesthesia, and recovered normal locomotory activity within this period. Although transmitters were all the same model, signal range varied from 25 to 60 m, depending on the unit. As toads never moved far in two successive days (maximum distance observed: 88.7 m), and all toads preferred to dig in previous burrow sites, field work did not suffer from this low signal range. Toads' burrows could rarely be recognized because toads take care to cover the entrance (e.g. Kuzmin, 1999) or because entrances were hidden *de facto* by surrounding vegetation. Classical local triangulation methods allowed the location of toads' burrows in, at worse, a half square metre area. Passive Integrated Transponders allowed confirmation of the exact location of the toads' burrows by scanning the half square metre with the PIT tag reader.

During the period of the study, each toad used five to eight burrows and moved from one site to another between five and 15 times. Thus, the same hole could be used again several days after desertion (up to 11 days). One toad burrowed in the same place on 37 consecutive days. We observed that not all individuals emerged every night. A five-day period of inactivity was often observed in both sexes. The shortest distance between two successive burrows was 0.2 m and the longest was 88.7 m. The mean distance moved between two successive burrows was higher in females (22.9 m, SD=13.0

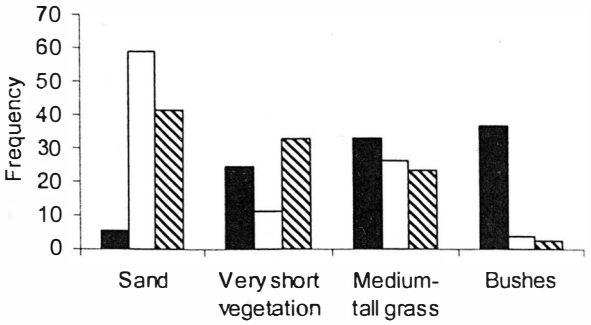

FIG. 1. Proportions of terrestrial habitat in four categories available in the field (black bars) and proportions of distance travelled by toads in each category (open bar: radio-tracked toads; striped bar: non-radio-tracked toads).

m, n=23) than in males (12.9 m, SD=19.6 m, n=12) (Mann-Whitney U-test, U=62, P<0.008).

Relocating the same toad on subsequent nights would have been almost impossible without radio-tracking, mainly because of the very cryptic coloration pattern. As a result, home-range would have been difficult to estimate.

Orange pigment was the most visible colour under ultraviolet light. The nocturnal trail was detectable along its entire course for the most part, although greater care was needed to find the fluorescent grains after a trail exceeded 15 to 25 m. The maximum distance recorded was 73 m, in a smooth, sandy area, but crossing vegetation (grass, moor) reduced the maximum trail length. No toads moved exclusively in vegetation during a night.

Detection of the trail was possible in all kinds of terrestrial habitat, even when wet. Pigments persisted on the ground and the vegetation for several days, even after light showers – this could be distracting when individuals moved around the same area several times. However, the blue fluorescent dye used to display the trails enabled us to distinguish individual trails, and it was possible to display all the trails in one area until the first heavy rain.

For radio-tracked and non-radio-tracked toads a significant difference between habitat availability and habitat use was observed (Fig. 1; G-tests for goodness-of-fit, respectively G=1833, df=3, P<0.0001 and G=2231, P<0.0001). Distributions of habitat use between the two categories of toads were distinct (χ^2=39, df=3, P<0.01) because of the different use of sandy areas and areas with very short vegetation. The greater use of sandy areas by radio-tracked toads was mainly due to two individuals that, when first crossing a small, newly-created sandy area, kept within this area more precisely. No other toads ever crossed this test area. Overall, sandy places were clearly attractive, whereas shrub-covered areas were avoided by toads both with and without transmitters.

The vertical components of movements were obvious, particularly in grass – in dense, tall grass movements occurred throughout the vegetation (about 5 to 10 cm high), whereas in sparsely vegetated areas the toads moved on the ground.

Because of daily homing behaviour, it was more relevant to calculate diel home range than total activity area (Mullican, 1988). As there were no significant differences in diel home range between all radio-tracked individuals (Mann-Whitney test P>0.05), we pooled data from these toads to compare estimates of mean diel activity area obtained with pigment alone and with pigment and burrow information (Table 1).

Estimates of diel home-range were larger when radio-tracking data were included (P=0.06, Table 1).

DISCUSSION

PIGMENTS

Fluorescent pigments gave information similar to continuous radio-tracking methods (but without a time component) and gave better spatial accuracy, and so were better for estimating the diel home-range (Mullican, 1988). The pigment method is inexpensive and easy to set up. It could be used on many individuals as long as their trails could be distinguished from one another. This method can be used to study dispersal (Gibbs, 1998; Seabrook & Dettmann, 1996) as, for example, at the post-metamorphic stage (Demaynadier & Hunter, 1999; Sinsch, 1997). Edge effects, corridor use and foraging strategy might be inferred with increased precision. A possible drawback is that stress from handling might affect the behaviour of studied individuals, as reported for lizards (Dodd, 1992). However, *Pelobates fuscus* are placid and were not obviously disturbed by short handling periods of 10 to 30 seconds. Regarding physical stress on animals and impact on locomotion or burrowing behaviour, both will be lower with fluorescent pigments than with Dole's (1965) trailing device. Moreover, pigments could be used with very small individuals.

TABLE 1. Estimated daily activity area determined by fluorescent pigments for 14 diel trails of radio-tracked toads, with and without the location of the starting and ending burrows. The burrows were located using telemetry. Statistical comparisons used the paired t-test.

	Pigments + telemetry (m²)			Pigments only (m²)			
Method	Mean	Range	SD	Mean	Range	SD	P
Minimum convex polygon	23.5	2.2-98	28.4	16.4	2.1-80	20.4	0.06
Ellipse	148.2	11.4-572.9	183.1	105.24	12.2-379	112.4	0.11

The vertical component of habitat use is seldom considered in amphibian microhabitat studies. Fluorescent pigments provided useful data on this aspect, and as we diluted the powder in paraffin oil, the persistence of tracks was better under dew or in the wind than pigments used alone (pers. obs.). Moreover, unlike other methods (Mullican, 1988), the technique worked equally well on bare ground and in thick vegetation, except that plant cover reduced the detectable trail length. Fluorescent dye is especially appropriate for most species of amphibian because of their relatively short daily movements. *Pelobates fuscus* is a 4-7 cm long walking species with thin skin. Thus, we applied only small amounts of coloured paraffin oil. With other species that are larger, or that rely more on jumping, it should be possible to improve the maximum length of trail detection by using more paraffin oil and dye.

Implantable Transmitters

Implantable transmitters appear more suitable than external ones, especially in fossorial species, as they do not injure the animals when they burrow into the ground. As observed in other studies (Madison, 1997; Madison & Farrand, 1998; Olders *et al.*, 1985) the implants seemed not to influence behaviour. Individuals with implanted transmitters were easily located, allowing observation of temporal and spatial activity patterns without artefacts resulting from troublesome harnesses (Golay, 1996). Similar implantation procedures have proven effective (Colberg *et al.*, 1997; Madison, 1997; Older *et al.*, 1985; Sinsch, 1988, 1991; Spieler & Linsenmair, 1998; Werner, 1991). The main limitation of telemetry with implantable transmitters, besides transmitter size and battery duration, is the quite low signal range. This restriction could became problematic with very mobile species, for example during migration (van Gelder *et al.*,1986*a*; Sinsch, 1990). Signal range will probably increase with the coming improvement of small batteries.

Combined Use of Transmitters and Pigments

The combined use of implantable transmitters and fluorescent pigments in amphibian movement studies allows additional observations that cannot be obtained with either method alone. Without telemetry it would be difficult to relocate cryptic animals such as spadefoot toads, and thus to reapply fluorescent dyes on successive days. In the case of daily homing behaviour or movements in patchy environments, radio-tracking was informative for estimating home range or activity area only with intensive relocation efforts. Usually, this disturbs the animal, changing its movement patterns, and reduces the reliability of the results (Mullican, 1988). This did not happen with the pigment method because of the absence of an observer during animal movements. Pigments allowed us to get more data points and more information on use of microhabitats and burrowing sites, with quality-control.

As a general rule, cryptic toads were much more easily discovered in open areas than in tall grass or shrub-covered areas. Consequently, the occurrence in vegetation of toads without transmitters is more likely to have been underestimated than the occurrence in vegetation of radio-tracked toads.

Spadefoot Toad Habitat Use and Conservation Implications

Toads strongly preferred areas of bare ground or low vegetation. Shrub-covered places were avoided. We even observed that a toad that passed directly beneath a copse of willows without leaves during its migration to a breeding pond in April, stopped five meters in front of the same, leafy bushes after breeding in early June. In the end, the toad retreated about 50 m and went round this 400 m^2 copse. We could therefore assume that visual signals play a major role in such behaviour.

It is well known that spadefoot distribution is restricted locally to specific areas with a friable soil texture, because of the fossorial behaviour of the species (e.g. Nöllert, 1990; Kuzmin, 1999). River banks with alluvial sand deposits should be the most suitable spadefoot toad habitat according to Meissner's (1970) soil choice experiments. Our observations suggest that dense copse can inhibit adult spadefoot toads' dispersal. This was in agreement with Kauri's (1946) observations concerning the species' range expansion following deforestation by farmers. Nevertheless, spadefoot toads can be found in open forest (Kuzmin, 1999). As dense, shrubby vegetation represents the natural succession of vegetation in our studied area, population management measures in progress focus greatly on controlling vegetational succession.

Diel home range and microhabitat use can be better defined with the combined use of telemetry and pigments. These two methods can give relevant information about movement patterns that are required for habitat restoration and population management.

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SHORT NOTES

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INCREASED USE OF PONDS BY BREEDING NATTERJACK TOADS, *BUFO CALAMITA*, FOLLOWING MANAGEMENT

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Key words: toad, habitat management, amphibian conservation

Natterjack toads, *Bufo calamita*, breed at fewer than 50 sites in the UK (Banks, Beebee & Cooke, 1994). The species was originally more widespread, but since the beginning of the 20th Century they have disappeared from more than 70% of their former localities, most notably from heathland in south-east England (Banks *et al.*, 1994). Much of the decline is a consequence of habitat loss, or pond acidification resulting from atmospheric pollution (Beebee *et al.*, 1990).

The natterjack toad colonies around the Solway coast represent one of the few remaining strongholds for this species in the UK. Despite their location at the north-western limit of the European range, in the early 1990s they accounted for up to 23% of the total British population (Banks *et al.*, 1994). One of the largest Solway populations breeds in pools on saltmarsh and adjoining agricultural land at Caerlaverock, south-west Scotland. On the Wildfowl and Wetlands Trust (WWT) reserve at Eastpark Farm, Caerlaverock, an extensive network of ponds has been surveyed annually for evidence of natterjack toad breeding attempts since 1991. After two years of poor breeding success in 1994 and 1995, WWT initiated an intensive management programme involving clearance of aquatic vegetation, excavation and redefinition of existing ponds. Although this approach has been adopted at several other sites (Banks & Beebee, 1987; Fleming, Mearns & Race, 1996; Beebee, Denton & Buckley, 1996), as far as we are aware, the WWT programme is the first to be conducted on such a large scale, with a total of 17 ponds excavated or cleared since 1995.

This paper documents changes in pond usage at WWT Caerlaverock over a nine-year period, and in particular, highlights the effectiveness of pond management as a tool for natterjack toad conservation. This is the first published account of natterjack toad breeding at the WWT reserve at Caerlaverock, and the first study to determine the average time lag between excavation and colonization or re-colonization for such a large sample of managed ponds.

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The WWT reserve at Caerlaverock (54°58'N 3°27'W) is adjacent to a National Nature Reserve managed by Scottish Natural Heritage (SNH), where natterjacks were studied previously in a small number of ponds (e.g. Banks *et al.*, 1994). At the WWT reserve, natterjacks breed in shallow pools (<1m deep) about 10m a.s.l. These pools were created in 1970-71 when soil was excavated from field margins to form banks which screened access to two bird observation towers (see Fig. 1). During the winter months, the toads hibernate in the soft, sandy soil forming the banks. Many of the pools are subject to occasional inundation by seawater on high spring tides during the winter, and tend to dry out by the end of most, but not all summers. Both processes are presumed to reduce the number of aquatic predators of natterjack tadpoles during the spring. Each of 24 distinct pools was assigned a unique code in 1991, and a further three were distinguished when the numbering system was updated in 1995.

WWT initiated a management programme in 1995, part-funded by SNH, involving excavation and redefinition of part or all of the existing ponds using heavy machinery. The purpose was primarily to clear all emergent vegetation and encroaching rushes (*Juncus* spp.) down to the bare soil, and leave a vegetation-free base of comparable depth to the original pond. This work was carried out in the autumn, after the end of the natterjack breeding season, and began with the ponds that were most overgrown, in some cases with very little open water remaining. In spring 1997, one additional pond (B2) was constructed (primarily for waterfowl) at a distance of c. 100m from the nearest bank (Fig. 1). In recent years, electric fences were also placed around all monitored ponds during the summer to exclude any cattle or sheep grazing in the adjacent pasture. The electric fences were removed after toadlet emergence had ceased, allowing access for cattle to crop the sward and trample the vegetation, slowing the rate at which ponds were overwhelmed by marginal vegetation and creating suitable habitat around the margin for foraging adult natterjacks the following spring.

All monitored pools (see Fig. 1) were visited on a minimum of 4 occasions, and generally more frequently, during May-August, 1991-1992 and 1994-1999, to check for evidence of breeding natterjacks (spawn strings, tadpoles or toadlets). Natterjack tadpoles were distinguished from common toad *Bufo bufo* and common frog *Rana temporaria* tadpoles on the basis of size, colour and the presence of a white throat patch. In most years, additional evening visits were made in May and June to record numbers of spawning adults. All fieldwork was carried out under licence from SNH.

Counts of the number of adult natterjack toads present during the evening were very variable within and between years. We do not know how much of this variation was due to differences in timing of our visits in relation to weather, so we do not regard the counts as reliable indicators of population size (see also Fleming *et al.*, 1996). However, the highest number counted on

FIG. 1. Location of study site and map of pond network (ponds coded A-Z).

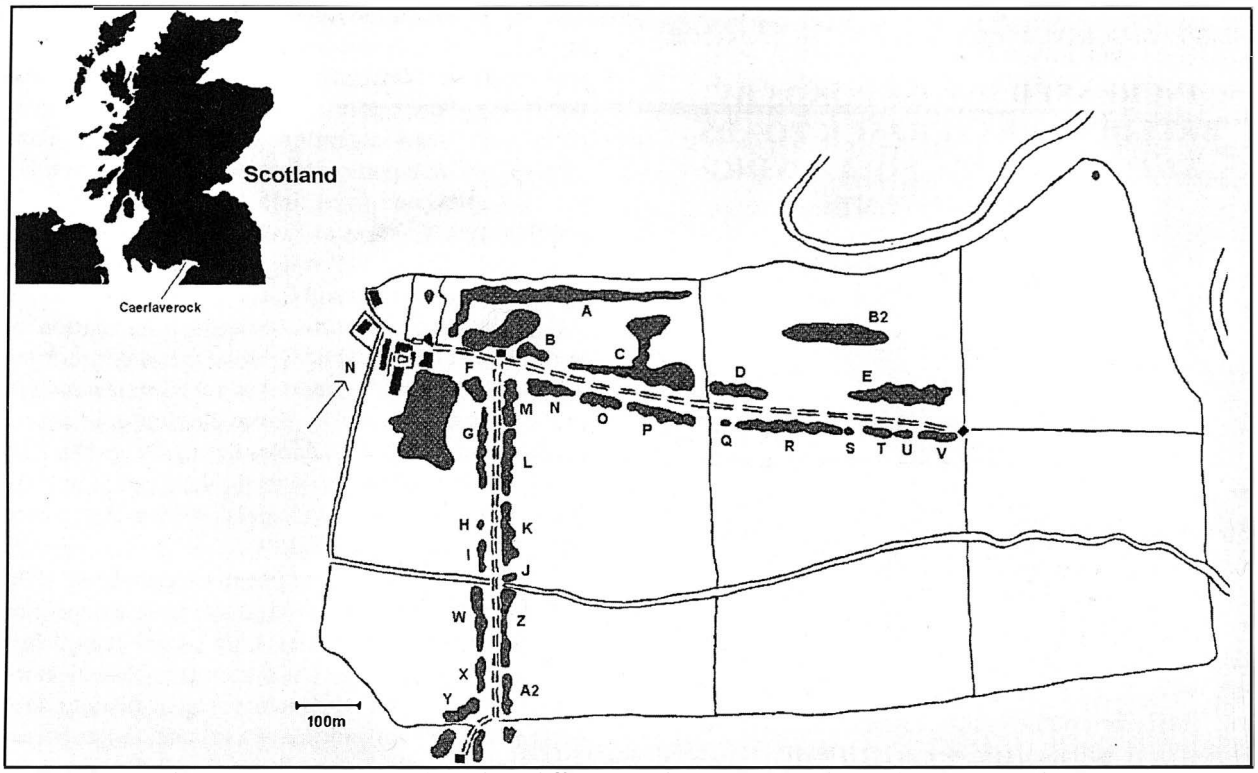

TABLE 1. Breeding attempts by natterjack toads in different ponds at WWT Caerlaverock, 1991-92 and 1994-99. The arrows indicate that vegetation clearance and re-shaping of at least part of a pond occurred in the autumn of the previous year. Note that pond B2 (see Fig. 1) was excavated as part of a separate project in spring 1997. N/d: No data; N/p: No pond.

Pond	Year							
	1991	1992	1994	1995	1996	1997	1998	1999
A					⇒	*	*	
B								
C					*	⇒	*	*
D	*			*	*	* ⇒	*	*
E	*	*	*	*	*	*	*	*
F	*	*			*			
G	*		*		*		*	*
H								⇒
I					*		*	⇒ *
J								
K						⇒	*	*
L	N/d	N/d	N/d		⇒ *	* ⇒	*	*
M	*		*		⇒ *	*	*	*
N	*	*	*	*	⇒ *	*	*	*
O					⇒ *	*	*	*
P				*	*	*		
Q			N/d					
R			*	*	*		*	
S			N/d			*	*	
T	*					⇒ *	*	*
U						⇒ *	*	*
V						⇒ *	*	*
W					*	*		⇒ *
X						⇒ *	*	*
Y					⇒ *	*	*	*
Z	*	*	*	*	*	*	*	*
A2	*	*		*	*		*	
B2	N/p	N/p	N/p	N/p	N/p	⇒ *	*	*

the WWT reserve on a single night was 390 adults, on 1 May 1995. The actual population size was obviously in excess of this, as not all males are likely to call or females to be present on a single night (Beebee, 1979).

Changes in the use of pools by spawning natterjacks in relation to maintenance is indicated in Table 1. In eight cases where pools had not been used by natterjacks for the previous two or more years, spawning took place in the spring following management work (ponds K, O, T-V, X and Y) or in the next spring (pond A). In no case where a pond was used previously by natterjack toads (ponds C, D, I, L-N and W) did pond maintenance in the autumn result in abandonment of that pond for breeding the next year. Of the pools cleared in 1995, five out of six were used in the four seasons up until 1999. Of those cleared in 1996, all were used by natterjacks for breeding in every season up to and including 1999. Furthermore, pond B2, which was excavated in early 1997 (although not specifically for natterjacks), held tadpoles in 1997, 1998 and 1999.

Considering the original 11 pools that were left unmanaged until at least 1998 (coded B, E-G, J, P-S, Z and A2; see Table 1), the number showing positive signs of natterjack breeding changed little throughout the study period (Fig. 2). In contrast, use of the 12 pools (coded A, C, D, K, M-O, T-V, X, Y) that were actively managed in 1995-1998 increased from four to 11 ponds during the period 1991-1999.

By 1997-99, natterjack tadpoles were recorded in a total of 23 different ponds on the WWT reserve at Caerlaverock. In 1999, in addition to these regularly-monitored sites, natterjacks used six other pools on the reserve or adjacent NNR, and although no systematic searches were made, it is likely that spawning occurred in other pools on the upper saltmarsh. There are only an estimated 250-300 individual pools used regularly by natterjacks in the UK and of the 47 extant colonies in 1989, only 12 held 100s to 1000s of adults (Banks *et al.*, 1994). With 29 or more breeding ponds in current use and a breeding population of at least 400 adults, the Caerlaverock area is clearly of considerable national importance in terms of natterjack conservation. The size of the local population presumably reflects a high density of suitable sites for breeding, including both saltmarsh pools subject to occasional tidal inundation, and ephemeral ponds on adjacent farmland which generally dry up during the late summer. Either set of conditions serves to eliminate or reduce the numbers of potential predators of tadpoles in the spring, which can significantly reduce tadpole survival, especially in deeper, more permanent ponds (Banks & Beebee, 1988; Beebee, Fleming & Race, 1993).

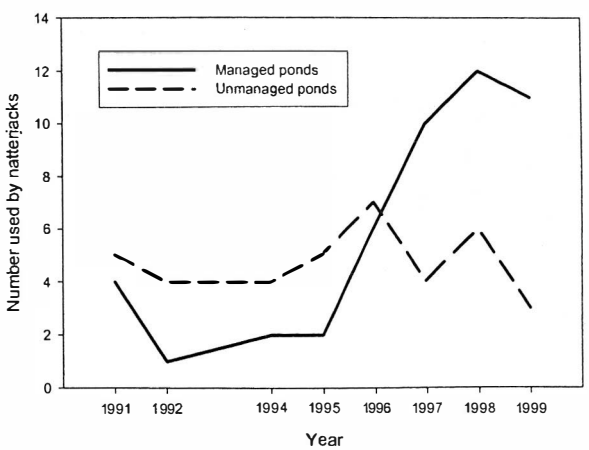

FIG. 2. Changes in number of unmanaged ponds (n=11) and ponds managed in 1995-1997 (n=12) with evidence of breeding natterjacks, 1991-1992 and 1994-1999.

Excavation and clearance of emergent vegetation and encroaching rushes from ponds at WWT Caerlaverock had an immediate effect on their attractiveness to natterjacks, with a significant increase in the median number of ponds used from 6.5 during 1991-1995 to 15.5 during 1996-1999 (Table 1). However, it is worth noting that in the long-term, one potential disadvantage to natterjacks of increasing the availability of ponds by effective management may be a concomitant increase in numbers of common frogs and toads. Growth rate and survival of natterjacks are reduced and emerging toadlets are smaller in high-density single- and mixed-species ponds (Griffiths, 1991; Tejedo & Reques, 1994; Bardsley & Beebee, 1998). Moreover, given a choice, female natterjacks will avoid breeding in ponds with high tadpole densities (Banks & Beebee, 1987). Although there is no information on population trends, common frogs and toads are both reasonably abundant at the WWT reserve, and for example were found in 76% of pools used by natterjacks in 1998 (WWT, unpublished data). Therefore, the possibility that inter-specific competition might become a problem in the future for natterjacks cannot yet be excluded.

In almost every case, managed ponds at Caerlaverock were colonized by natterjacks in the spring immediately following clearance of encroaching vegetation and redefinition of ponds in the autumn. These ponds were the most overgrown and in need of re-instatement. The ability to colonize new sites is a characteristic of natterjacks and facilitates the rapid exploitation of ephemeral ponds in unpredictable habitats (Beebee, 1979). In addition, modification of existing breeding pools at Caerlaverock never resulted in desertion. That natterjacks continued to use these pools for breeding despite substantial alterations further underlines their high degree of flexibility as regards site selection. Most importantly from a conservation perspective, it suggests that a similar management programme, if adopted elsewhere, would be unlikely to have a detrimental effect on spawning rates. This is especially noteworthy, as simply from the rate at which they have declined in recent decades, natterjack toads might otherwise be regarded as a highly sensitive species. However, our results indicate that increasing the number and presumably also the variability of pools is an extremely effective strategy for natterjack conservation. This is likely to be particularly useful at sites with low botanical interest where potential reduction in plant diversity is not an issue.

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FIRST RECORD OF THE LEOPARD GECKO *EUBLEPHARIS ANGRAMAINYU* (REPTILIA: SAURIA: EUBLEPHARIDAE) FROM ANATOLIA

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Key words: gecko, geographical distribution, new record

The leopard gecko *Eublepharis angramainyu* Anderson & Leviton, 1966 occurs in the western foothills of the Zagros Mountains and the Mesopotamian plain in Iran, Iraq and north-eastern Syria, with a vertical distribution of 300-1000 m (Anderson & Leviton, 1966; Nader & Jawdat, 1976; Leviton *et al.*, 1992; Martens & Kock, 1991; Anderson, 1999). It is a nocturnal lizard inhabiting stony hills and ruins, and can be seen most often in the middle of the night. It feeds among stones, on crickets, scorpions, solpugids, large spiders and beetles, as well as small geckos of the same and different species. In Iran, egg-laying occurs from the end of May to the beginning of June (Szczerbak & Golubev, 1996; Anderson, 1999). *Eublepharis angramainyu* has not previously been reported from south-east Anatolia (Basoglu & Baran, 1977; Baran & Atatür 1998; Sindaco *et al.*, 2000). We present here the first record of this genus and species from Anatolia.

An adult female was collected from Kara Dagh-Arsanli, approx. 8 km south-east of Birecik, Vilayet Sanliurfa (Fig.1), Turkey, 36° 59' N, 38° 02' E, 9 June, 2001, Leg. B. Göçmen, M. Tosunoglu & D. Ayaz. The specimen is now in Ege University, ZDEU 31/2001.

The terminology used in describing the specimen conforms to Szczerbak & Golubev (1996) and Anderson (1999). The specimen (Fig. 2) is an adult female with a partly regenerated tail. Snout-vent length (SVL) 148 mm, partly regenerated tail length 66 mm, supranasal scales separated by a single, almost hexagonal internasal scale. The width of the internasal scale is more than its length, six small additional nasal scales surround the nostril; 11 supra- and 11 infralabial scales; the ear is large, with its length (5 mm) 2.5 times its width (2 mm); pentagonal mental is shorter than wide and followed by four rows of enlarged scales (postmentalia); chin shields (the first row of postmentalia) in contact with first infralabials; dorsal tubercles on the flanks almost touching each other; ventral scales hexagonal and non-imbricate, with 26 hexagonal ventral scales across midbody; 13 feebly marked (preanal) pores arranged between the anal cleft and the ventral scales in the form of an inverted "V"; 24 smooth subdigital lamellae on both hind feet; three transverse rows of ventral scales in each caudal whorl. The background colour of the body is ochreous with lilac-brown spots; on the head these spots are roughly arranged in longitudinal rows with wider interspaces, bordering the pale continuous stripe from the neck to the tail base; on both sides this strip is bordered by pale transverse bands as well as by dark interrupted stripes which can merge in a longitudinal direction; three wide, dark transverse bands on shoulder, midbody and lumbar region. Limbs covered with randomly distributed lilac-brown dots and spots; tail covered with numerous dark spots, which can be oriented transversely. Lower surfaces of the body whitish, except regenerated part of the tail, which is covered irregularly with a few small lilac-brown spots.

FIG. 1. Map showing the new locality (open circle) and the previously known most westerly locality (solid circle) for *Eublepharis angramainyu*.

The specimen was found at an elevation of 400 m, on ground enriched by clay-limestone. The vegetation was sparse and composed of various grasses. It was night time, around 23.00 hr; air temperature was 25°C.

In pholidosis and coloration, the Sanliurfa specimen almost agrees with the descriptions of *Eublepharis angramainyu* given by Anderson & Leviton (1966), Leviton *et al.* (1992), Grismer (1988), Szczerbak & Golubev (1995), and Anderson (1999), except for the higher supra- and sublabial counts (11 instead of 10) and higher ventral scale count (26 instead of 24) when we take into consideration Szczerbak & Golubev's (1995) data. A similar tendency towards higher numbers of ventral and labial scales in the gecko *Asaccus elisae* from the same location (Birecik, Vilayet Sanliurfa), compared with Syrian and Iraqi specimens, was reported by Tok *et al.* (1997). Such geographic differences within a

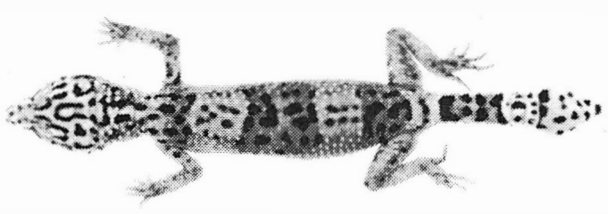

FIG. 2. Dorsal aspect of *Eublepharis angramainyu* (ZDEU 31/2001-female) from Kara Dagh (Arsanli-Birecik, Vilayet Sanliurfa), Turkey.

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species may be correlated with local conditions. Taking into account also the statement by Anderson (1999), that the ventral scale count varies between 27 and 38, we are of the opinion that our specimen indeed belongs to the species *Eublepharis angramainyu.*

A single voucher specimen of *E. angramainyu* (SMF 74240) from the Khabur river region (Khatüniyah, Al Hasakah) in north-eastern Syria, near the Syria-Iraq border (Fig. 1) (Martens & Kock, 1991; Rösler, 1995: *sic* = W Syria) formerly marked the westernmost edge of the species' known range (Martens & Kock, 1991; Anderson, 1999). Thus, our record of *E. angramainyu* from the western part of SE Anatolia extends its known distribution some 290 km air distance to the north-west.

Anatolia is located at an important transitional zoogeographical region between Asia and Europe. In its eastern parts, there are no natural boundaries with the neighbouring countries and, therefore, little endemism. Syroeremic herpetofaunal elements such as *Cyrtopodion scaber, Stenodactylus grandiceps, Asaccus elisae, Acanthodactylus boskianus, Coluber ventromaculatus, Eirenis coronella* and *Spalerosophis diadema* (Baran & Atatür, 1998; Franzen, 1999) are known from sites close to the Syrian border. All of these species are adapted to the semi-arid conditions of the northern reaches of the Mesopotamian plain of the Turkish-Syrian border region. However, *E. angramainyu* seems to be an Iranoeremic herpetofaunal element from its known distribution (Disi & Böhme, 1996; Anderson, 1999). A penetration of Iranoeremic forms into western SE Anatolia and Syria, as exemplified by *E. angramainyu*, shows the zoogeographical importance of SE Anatolia and Syria, connecting Afrotropical and Palaearctic elements of the herpetofauna in the eastern Mediterranean region (Disi & Böhme, 1996).

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DIET, GASTROLITH ACQUISITION AND INITIATION OF FEEDING AMONG HATCHLING MORELET'S CROCODILES IN BELIZE

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Key words: *Crocodylus moreletii*, crocodile, hatchling, stomach flushing

Morelet's crocodile (*Crocodylus moreletii*) inhabits freshwater wetlands throughout much of the Atlantic lowlands of Mexico, Guatemala and Belize (Groombridge, 1987). Nesting occurs during June-July, and hatchlings emerge in August-September after an incubation period of approximately 75 days (Platt, 1996). The ecology of hatchling Morelet's crocodiles has not been investigated, and indeed few field studies have been conducted on hatchling crocodilians of any species (Neill, 1971; Valentine, Walther, McCartney & Ivy, 1972; Delany, 1990; Platt, Rainwater & Thorbjarnarson, 2002). Such studies are difficult undertakings owing to the small body size, cryptic habits and largely nocturnal activity of hatchling crocodilians. In particular, little is known concerning the diet of hatchling *C. moreletii*. Alvarez del Toro (1974) stated in a largely anecdotal account that hatchlings consume only terrestrial and aquatic insects, and Schmidt (1924) found primarily aquatic insect remains in the stomachs of seven juveniles ranging from 29 to 74 cm in length. Furthermore, the acquisition of gastroliths by neonate crocodilians is poorly documented (Fitch-Snyder & Lance, 1993), and our literature search revealed that virtually nothing is known concerning the initiation of feeding by wild hatchlings. Dietary studies are fundamental to understanding the ecology of an organism (Rosenberg & Cooper, 1990), and among crocodilians, diet is known to affect growth, behaviour and reproduction (Lang, 1987).

Here we characterize the diet, and report on the acquisition of gastroliths and initiation of feeding by hatchling *C. moreletii*. Our study was conducted during 1994 at Gold Button Lagoon (GBL) in northern Belize (Platt, 1996). GBL (17° 55' N; 88° 45' E) is a 142 ha man-made lagoon located on the 10 526 ha privately owned Gold Button Ranch, approximately 32 km south of Orange Walk Town in Orange Walk District. An estimated 175 non-hatchling crocodiles inhabit GBL (Platt, 1996), one of the highest densities (1.2 crocodiles/ha) found anywhere in Belize (Platt & Thorbjarnarson, 2000). GBL is characterized by dense stands of *Typha* and *Eleocharis* along the shoreline, and floating mats of *Nymphaea* in shallow water. Water levels fluctuate depending on the amount of local rainfall, and are highest late in the wet season (September to November).

Crocodile nests were monitored during incubation from June through September as part of a larger study on nesting ecology (Platt, 1996). Each nest was initially inspected weekly to determine losses from predation and flooding, but daily inspections (usually before 1000 hours) were conducted as the estimated hatching date approached. Once hatching occurred, we returned after dusk to capture hatchlings by hand with the aid of a headlight. Hatchlings were generally found in dense aquatic vegetation adjacent to the nest site. The following morphometric data were collected from each hatchling: total length (TL, tip of snout to tip of tail measured along the ventral surface), snout-vent length (SVL, tip of snout to anterior margin of cloacal vent measured along the ventral surface), and head length (HL, measured dorsally from the tip of snout to median posterior edge of the cranial roof). Each hatchling was permanently marked for future identification by notching the dorsal edge of a unique series of three caudal scutes (Jennings, David & Portier, 1991), and then released at the capture site, usually within an hour.

Hatchlings were recaptured on subsequent nights and stomach contents obtained by stomach flushing (Taylor, Webb & Magnusson, 1978). A flexible plastic tube (5.5 mm exterior diameter), lubricated with vegetable oil was eased down the oesophagus and into the stomach. Water (c. 4 cm^3) was slowly poured into the tube until the abdomen became visibly distended. Gently palpating the abdomen caused a mixture of water and stomach contents to surge into the tube. The hatchling was then inverted, and this mixture directed across a fine mesh screen. The process was repeated (usually three to four times) until only water free of stomach contents was obtained. No hatchling was stomach flushed more than once in a seven-day period. Although we did not evaluate the effectiveness of this technique, Fitzgerald (1989) sacrificed juvenile caimans (*Caiman crocodilus*) after stomach flushing, and concluded most food items were recovered. Stomach contents were sorted and prey items identified to the lowest possible taxonomic category. Non-food items such as stones, seeds and vegetable matter were also recorded. Occasionally unmarked hatchlings were found among pods of marked hatchlings during recapture efforts. Because pods typically consist

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TABLE 1. Frequencies and percentages of occurrence of prey taxa and non-food items recovered from hatchling Morelet's crocodiles (n = 71) at Gold Button Lagoon, Belize (August to October 1994). N= number of hatchlings containing a particular item. Percentages do not sum to 100 because multiple items were often recovered from a single hatchling.

Stomach contents	N	%
Prey		
Insects	60	84.5
Arachnids	21	29.5
Gastropods	2	2.8
Fish	12	16.9
Non-food items		
Vegetation	4	5.6
Stones	8	11.2
Seeds	2	2.8

of neonates from a single nest (Platt, 1996), we assumed unmarked hatchlings had simply eluded our initial capture efforts and age was assigned based on that of other pod members.

One hundred and six *C. moreletii* hatchlings from six pods were captured and marked at GBL from 17 August to 1 October 1994. Mean (±1SD) hatchling morphometrics were: TL=26.9±1.3 cm (range = 23.9 to 29.5 cm); SVL=13.0±0.5 cm (range = 11.8 to 14.1 cm); HL= 4.0±0.2 cm (range = 3.6 to 4.5 cm); HL/SVL ratio=0.30±0.009 (range = 0.27 to 0.32). Seventy-two hatchlings ranging from 4 to 24 days old were recaptured and stomach flushed 86 times. Stomach contents were recovered from 71 hatchlings; 15 had empty stomachs and were excluded from our analysis. Results are presented in Table 1. To our knowledge, no mortality occurred as a result of capture or stomach flushing and numerous recaptures have since been made (Platt, Rainwater & McMurry, unpubl. data).

Our results indicate that hatchling *C. moreletii* feed primarily on invertebrates. Insects were the most frequently recovered prey, followed by arachnids. Insects, arachnids or both were recovered from 69 (97.1%) hatchlings. Most remains consisted of highly macerated bits of chitin and fleshy material not identifiable to a particular taxon. However, we were able to identify representatives of three insect orders: Coleoptera, Orthoptera and Odonata. Others have photographed hatchling *C. moreletii* capturing adult Odonata (Richard & Carol Foster, pers. comm.). Likewise, the few available studies suggest that insects are important prey for hatchlings of other species as well (*Caiman crocodilus*: Staton and Dixon, 1975; Thorbjarnarson, 1993; *Caiman yacare*: Cintra, 1989; *Alligator mississippiensis*: Delany, 1990).

Aquatic gastropods appear to be relatively unimportant prey for hatchling *C. moreletii*. Only two opercula (length = 5 mm) were found among the stomachs we examined, most likely from *Pomacea flagellata*, a freshwater ampullarid snail abundant at GBL. The opercula length indicates these were small juvenile snails.

Fish were the only vertebrates recovered from hatchling *C. moreletii*, but comprised a minor component of the diet. The occurrence of fish was inflated by the recovery of *Petenia splendida* scales from six hatchlings in a single pod. Based on scale size, the fish was estimated to be an adult 15 to 20 cm long and undoubtedly consumed as carrion, perhaps supplied by the attending female. However, while maternal provisioning of hatchling crocodilians has long been suggested (McIlhenny, 1935), it remains to be convincingly documented (Lang, 1987). With the exception of *Astyanix fasciatus* fins recovered from a single hatchling, other fish remains consisted of unidentifiable bits of bone and flesh.

Despite observations to the contrary (McIlhenny, 1935; Neill, 1971; Delany, 1990), Fischer, Mazzotti, Congdon & Gatten (1991) contend that hatchling American alligators (*A. mississippiensis*) are unable to capture small prey effectively owing to a long snout in relation to body size (Mean HL/SVL ratio=0.31; SE=±0.001; n=288), and instead rely on metabolism of residual yolk as an energy source. However, there was no significant difference between the HL/SVL ratios of *C. moreletii* and *A. mississippiensis* hatchlings (ANOVA; $F_{1,392}$=0.62; P>0.05), and the prevalence of prey remains among stomach contents indicates that *C. moreletii* neonates are adept predators. Therefore, if *A. mississippiensis* hatchlings fail to capture small prey it is unlikely to be due to morphological constraints imposed by the HL/SVL ratio, and the conclusions of Fischer *et al.* (1991) should be reassessed. Allsteadt & Lang (1995) concluded that the importance of residual yolk has been overstated; it probably serves as an immediate post-hatching energy source, diminishing in importance once feeding is initiated, and feeding may even stimulate its absorption.

Non-food items recovered from hatchling *C. moreletii* include fragments of vegetation, small stones (range = 1 to 5), and hard seeds (range = 1 to 6). Vegetation is likely consumed incidental to prey capture and has no nutritional value (Coulson & Hernandez, 1983). Stones and other hard objects are deliberately consumed and serve as gastroliths (Davenport, Grove, Cannon, Ellis & Stables, 1990; Fitch-Snyder & Lance, 1993). We found small stones and hard seeds in *C. moreletii* hatchlings ranging from 4 to 19 days old, which to our knowledge is the earliest record of gastrolith acquisition for any crocodilian. Fitch-Snyder & Lance (1993) reported stones in two six-month old *A. mississippiensis* and observed a 14-dayold hatchling unsuccessfully attempting to consume stones. Davenport *et al.* (1990) speculated that stones found in captive *Crocodylus porosus* were present when the animals were initially obtained as wild hatchlings. Our study and others indicate that gastrolith acquisition may occur soon after hatching.

Although not essential for digestion, gastroliths are thought to facilitate the breakdown of ingested prey in a manner similar to grit in the avian gizzard, and may be important for hatchling and juvenile crocodilians that consume chitin-rich diets (Sokol, 1971; Platt, Brantley & Hastings, 1990; Fitch-Snyder & Lance, 1993). Davenport *et al.* (1990) found that gastroliths rapidly disperse throughout the stomach contents after food ingestion, and probably enhance digestion by squeezing fluids from punctured arthropods. However, Taylor (1993, 1994) states that gastroliths are unimportant in digestion and serve primarily as ballast for buoyancy control in a variety of aquatic organisms, but concludes their function in crocodilians remains unresolved and warrants further investigation.

Fifteen 4-day old hatchlings from a single pod were the youngest individuals we stomach-flushed; 12 (80%) contained no prey, although a small stone was recovered from one. The remaining 71 hatchlings were $\geq$ 7-days old and prey were recovered from 68 (95.7%), suggesting that neonate *C. moreletii* initiate feeding four to seven days post-hatching. Our findings are consistent with other observations on hatchling crocodilians under both wild and captive conditions. According to McIlhenny (1935), wild *A. mississippiensis* hatchlings begin feeding "shortly after leaving the nest", and Joanen & McNease (1976, 1977) noted that feeding in captivity was initiated three to four days post-hatching. Captive *Crocodylus niloticus* hatchlings "accept food soon after emergence" (Pooley, 1962), although Cott (1961) stated that wild hatchlings usually eat nothing until about two weeks old. Platt *et al.* (2002) reported that *C. acutus* neonates in northern Belize begin feeding within one week of hatching. Initiation of feeding is probably dependent on a variety of factors including air and water temperature, and even geographic origin of the hatchlings (Lang, 1981; Webb, Whitehead & Manolis, 1987).

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REASSESSMENT OF COMPARATIVE GENETIC DISTANCE IN REPTILES FROM THE MITOCHONDRIAL CYTOCHROME *B* GENE

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Key words: base composition, Cytochrome *b*, genetic distance, reptiles

Mitochondrial cytochrome *b* is one of the most widely sequenced genes in vertebrates. As such it is commonly used as a genetic "yardstick", especially for comparing congeneric species for which it is typically phylogenetically informative and not severely saturated (Moritz, Dowling & Brown, 1987). Recently, Johns & Avise (1998) presented data culled from Genbank to make comparisons of genetic distances between congeneric species and confamilial genera across the major vertebrate classes. They also presented percentage base compositions across all sites, and for the third positions only. However, for those interested in reptiles this study was limited by the data available at the time – of the 16 genera examined, seven were snakes and three iguanids, thus possibly causing distortion towards averages from these groups. Also, some researchers unfortunately fail to follow the standard convention of submitting the sense strand for protein coding sequences, and instead submit the reverse compliment to Genbank. In at least one case these were included in the previous analysis in the reverse direction relative to the other sequences (Johns, pers. comm.). This affects the base composition estimates for all positions. Here I include data for reptile groups that were not previously available, increasing the number of families examined from eight to 13, and the number of genera from 16 to 35. I also calculate the correct base compositions as compared to those previously published (Johns & Avise, 1998).

TABLE 1. Base composition in third position sites of cytochrome *b* sequences.*Chi squared tests indicate significant heterogeneity of base frequencies across taxa within the Iguanidae.

Taxon	Percentage base composition in third position sites			
	A	C	G	T
Teiidae	41.6	34.5	3.2	20.7
Xantusiidae	39.1	34.7	3.9	22.3
Scincidae	37.7	38.7	4.3	19.3
Gekkonidae	36.1	44.1	5.3	14.6
Chelonidae	44.6	42.2	1.8	11.4
Emydidae	43.2	39.7	3.4	13.6
Testudinidae	41.0	38.8	2.4	17.8
Colubridae	35.5	39.1	3.3	22.1
Elaphidae	37.8	34.3	3.8	24.1
Viperidae	31.8	44.3	4.9	18.9
Anguidae	40.6	35.2	4.0	20.2
Iguanidae*	40.0	39.1	3.5	17.5
Lacertidae	31.2	38.4	3.3	27.1
Mean	38.5	38.7	3.6	19.2
Johns & Avise (1998)	29.7	28.1	18.0	24.2
All positions average	28.2	28.8	13.7	29.2
Johns & Avise (1998)	27.7	27.6	16.1	28.6

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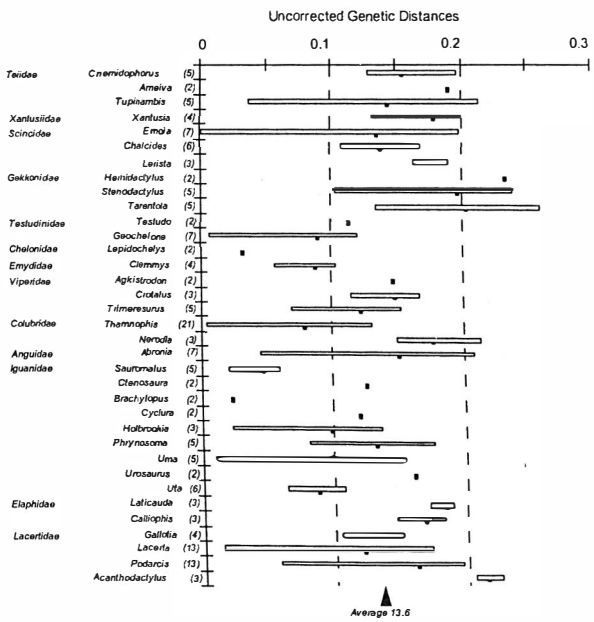
All sequences were downloaded from Genbank and aligned by eye. The alignment is available on request from the author. There were no indel insertions or deletions. All analyses were carried out in PAUP* 4.0 (Swofford, 2000). Average overall, and third position only, base compositions were calculated for each family, and uncorrected genetic distances were estimated for all pairwise comparisons within genera (Table 1, Fig. 1).

Base composition in the third positions is now very different from that previously reported, and much more similar to that obtained for other vertebrate groups. In particular, the very low proportion of guanosines (3.4-5.5% in other vertebrates) is similar in the reptiles (3.6%; not the 18% reported by Johns & Avise, 1998). Overall base compositions are also significantly different. Assessment of the correct base composition is very important, as it is often used as an indication that mito-

FIG. 1. Uncorrected genetic distances in cytochrome *b* gene sequences between reptile species within 35 genera. Numbers of species assayed are given in parentheses. Mean genetic divergence estimates and ranges are shown. Only a monophyletic subset of sequences from *Lacerta* was included.



chondrial DNA has been amplified for phylogenetic studies, as opposed to nuclear-integrated copies (e.g. Macey *et al.*, 1999). There is significant variation in base composition between genera within the Iguanidae, something which will be problematic for researchers using cytochrome *b* to assess phylogenetic relationships within this family (e.g. Wiens & Hollingsworth, 2000). The mean cytochrome *b* genetic distance for congeneric reptiles is higher than the previous estimate (13.6%, compared to 12%).

This reassessment, like the study of Johns & Avise (1998), highlights the important role Genbank can play in large scale analyses, but it also exposes some problems with the present system. Despite the fact that Johns & Avise (1998) rejected two sequences as probable pseudogenes, three of the published and analysed sequences (from *Crotalus* and *Agkistrodon,* Cullings *et al.*, 1997) require single base pair insertions to align them with those of other reptiles. A similar error has been reported for the analysed *Gallotia* sequences, where one of the published sequences has an erroneous single base pair insertion (Carranza *et al.*, 1999). These errors will affect any analyses in which they are not detected. Another problem is that some assessment of taxonomy is required. For example, the analysed genus *Lacerta* is known to be paraphyletic (Arnold, 1989; Harris, Arnold & Thomas, 1998). Further, there is little agreement on the generic status of some lizards – sequences from the lizard endemic to Madeira have been deposited in Genbank simultaneously under *Teira dugesii, Lacerta dugesii* and *Podarcis dugesii.* Any attempt to use Genbank to assess congeneric levels of variation will run into difficulties in this situation unless researchers are aware of these problems.

While cytochrome *b* is still the most obvious genetic yardstick for most vertebrate groups, the inclusion of new data has altered both the previously reported base compositions (Johns & Avise, 1998), and average congeneric divergences. Clearly, as more data are submitted to Genbank both can be quantified across a wider range of taxa, allowing more accurate calibration.

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ERRATUM

Hernández, M., Maca-Meyer, N., Rando, J. C., Valido, A. & Nogales, M. (2001). Addition of a new giant lizard from La Gomera Island to the phylogeny of the endemic genus *Gallotia* (Canarian archipelago). *Herpetological Journal* **11**, 171-173:

Page 171, left column, line 23: Rando *et al.* (1997) recognised four species and not five.

Page 171, left column, lines 35-36: *Gallotia simonyi* not *Gallotia simonyi machadoi*.

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(revised July 2002)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: *Full Papers* (no length limit); *Reviews* and *Mini-reviews* (generally solicited by a member of the editorial board); *Short Notes*; controversies, under *Forum* (details available from the Editor); and *Book Reviews*. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the *Herpetological Journal* is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

2. *Two* copies of all submissions, and illustrations, should be sent to the Scientific Editor, together with a computer diskette (IBM formatted) containing the text and, if possible, figures. Alternatively, submission by e-mail is possible - please contact the Scientific Editor for information. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigor, and the degree of general interest in the subject matter. The Editor's decision will be final.

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer, and double-spaced with wide margins all round. The journal is typeset direct from the author's electronic text, so all manuscripts should be prepared using a word processor (preferably on a PC-compatible microcomputer). If figures are prepared using computer graphics, they should be supplied separately and NOT embedded in the text of the word processor file. Preferred formats are MS Word for Windows (text) and MS Excel, Bitmap, TIFF, Windows Metafiles (.wmf, .emf) or JPEG files (graphics).

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of five words or less, and the name and address of the corresponding author with (if available) an email address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted. *Short Notes* (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. A *sans serif* font (e.g. Universe or Helvetica) is preferred.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. TABLE 1; they should be typed double spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. FIG. 1. Colour photographs can only be included at cost to the author (quotes can be obtained from the Managing Editor). If an illustration has more than one part, each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. Illustrations produced using other types of computer printer are not usually of suitable quality. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated —"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the first author's surname followed by *et al.* should be used (Smith *et al.*, 1972). In the list of references the full title of the journal should be given. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal:

   Bellairs, A. d'A. (1957). *Reptiles*. London: Hutchinson.

   Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* **2**, 206–210.

   Dunson, W. A. (1969*a*). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

   Dunson, W. A. (1969*b*). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American Journal of Physiology* **216**, 995–1002.

9. Final acceptance of a paper will depend upon the production by the author of a typescript, illustrations and computer file(s) ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

10. Proofs should be returned to the Managing Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

11. Twenty-five offprints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.

12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

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