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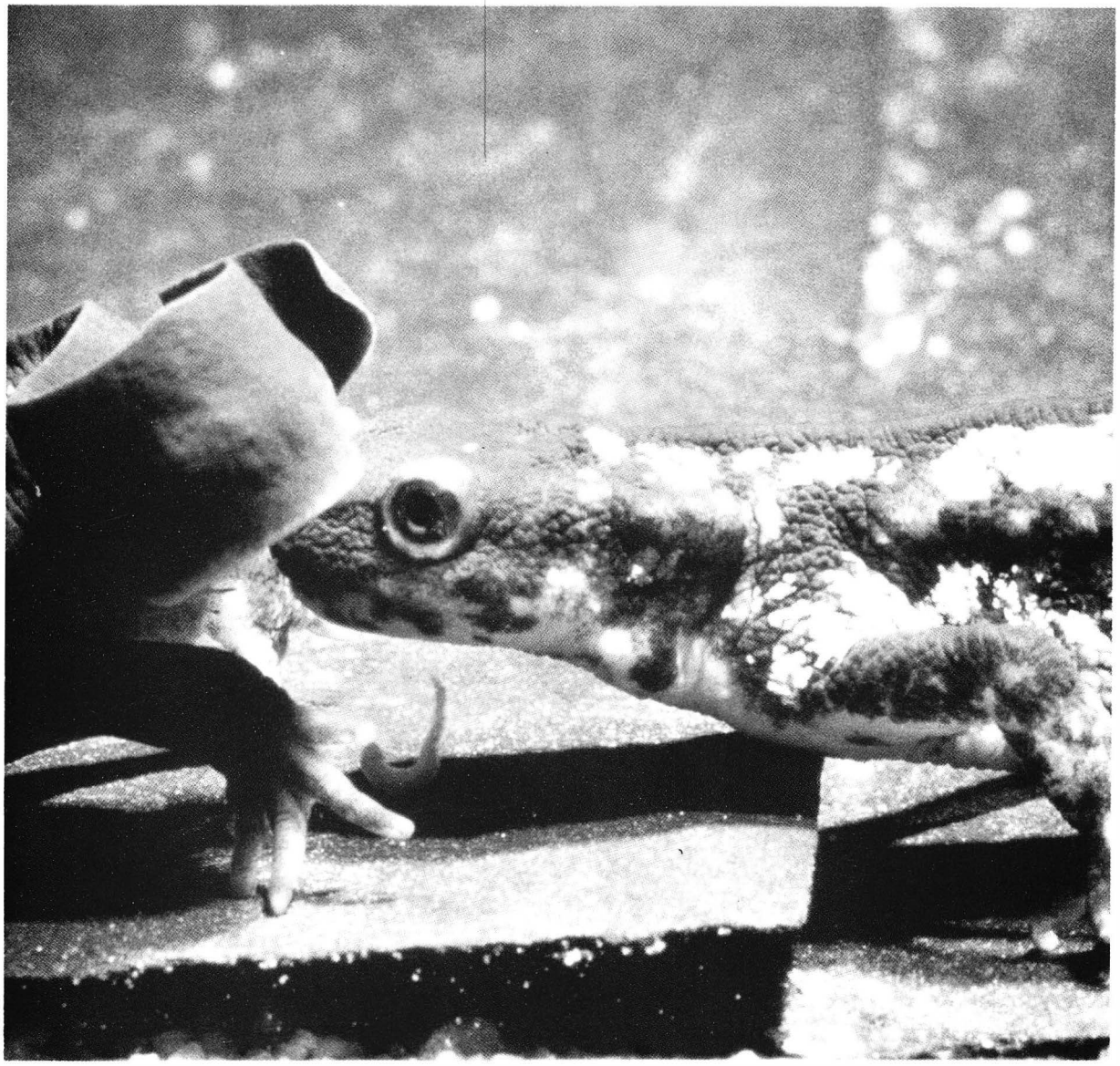

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HERPETOLOGICAL JOURNAL, Vol. 5, pp. 293-297 (1995)

MATING CALLS OF THREE SPECIES OF ANURANS FROM BORNEO

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Mating calls of three anuran species from Borneo, *Rana glandulosa*, *R. signata* and *Polypedates leucomystax* were analysed. The audiospectrograms obtained were compared with previously published descriptions of the calls of these species in different geographic areas. The comparisons revealed a high level of heterogeneity of the calls within the range of *R. signata* and *P. leucomystax*. The possible taxonomic implications of these findings are also discussed. The first description of the mating call of *R. glandulosa* is presented.

INTRODUCTION

The anuran fauna of SE Asia, and particularly that of Borneo, holds one of the highest levels of diversity found in the world (Frost, 1985; Groombridge, 1992). In spite of this phenomenon, knowledge about the biology of most species is extremely limited, especially if compared with the available information about the amphibian fauna from other neotropical areas. Recent publications have contributed significantly to the advance of the knowledge of anuran taxonomy of SE Asia (e.g., Inger, 1989, Inger & Stuebing, 1991). However, it is difficult to determine the actual number of species of amphibians present in many areas, including Borneo (Inger & Stuebing, 1989). Most published taxonomic published studies are based on morphological characters, while only a few take into account aspects of natural history and ecology of the species (e.g. Alcala & Brown, 1956; Matsui, 1979); and even fewer consider genetic aspects for taxonomic comparisons (e.g. Matsui *et al.*, 1986; Kuramoto & Yong, 1992). The importance of advertisement calls as mechanisms for pre-mating isolation have been emphasized for a number of species of the temperate zone (Littlejohn, 1981, 1988; Gerhardt, 1988, 1991). The modern techniques of sound analysis provide an efficient quantification of the temporal and spectral components of call parameters, which allows quantitative comparisons at different levels (Duellman & Pyles, 1983; Márquez *et al.*, 1993). As for the Asian anuran fauna, some studies have also addressed the importance of the advertisement calls as a relevant characteristic to define the taxonomic status of different taxa (Alcala *et al.*, 1986, Brzoska *et al.*, 1986; Matsui *et al.*, 1986).

The present study contributes to the knowledge of three anuran species from Borneo, describes their mating calls and provides information about calling behaviour. Whenever possible, the results of the analysis of the recordings are compared with previously published descriptions of the same species, in an effort to determine the homogeneity of the calls within the ranges of distribution of the species. These comparisons may suggest lines for future taxonomic research.

MATERIAL AND METHODS

Specimens were recorded in Borneo in July and August 1991. Recordings were obtained in Gunung Mulu National Park (4° N 115°E) and in Bako National Park (2°N 110°E), in the region of Sarawak, Borneo, Malaysia. Recording equipment included a portable tape recorder Sony WM D3 and a directional microphone ATR 55 Telemike. Recordings were edited and analysed with an Apple Macintosh-based digital sound analysis system. Sounds were digitised at a sampling rate of 44.1 KHz and with 16 - bit resolution, with Sound Tools hardware (Digidesign 1360 Willow Rd, Menlo Park, CA 94025 U.S.A.). Numerical parameters of the spectral and temporal components, as well as audiospectrograms and oscillograms, were obtained with Signalyze software (Infosignal Inc. CP 73, CH-1015 Lausanne, Switzerland). Spectral components were based on fast Fourier transform (FFT) with window width of 1024 points. Seven call characteristics were measured: call duration, number of pulses or pulse groups per call (*sensu* Schneider & Sinsch, 1992), pulse rate (pulses per second), fundamental frequency, dominant frequency, other emphasized frequencies, and calls per minute.

Recorded specimens were identified visually *in situ*, based on their external morphology (Inger, 1966). Photographs were taken in order to confirm subsequently the initial identification. The photographic material was deposited in the photographic archive of the Museo Nacional de Ciencias Naturales de Madrid (Archive numbers: 7960-7966). Recordings were obtained at a temperature range of 22-26°C in Bako, and 20-24°C in Gunung Mulu. Recorded specimens were not collected: in agreement with the laws of the national

TABLE 1. Summary of numerical parameters of the advertisement calls. For each value, the mean, standard deviation (in parentheses) and range are shown. * in *R. signata* the values shown represent no. of pulse groups and pulse groups/sec.

	Rana glandulosa	*Rana signata*	*Polypedates leucomystax*
Number of individuals	1	1	3
Number of calls/individual	19	3	1·3·5
Duration (ms)	1502.2 (176.3) 1278 - 1802.8	620.7 (5.5) 614.3 - 624.4	143.5 (19.3) 130.5 - 165.7
Number of pulses	10.1 (1.1) 9 - 12	9 (0)* 9 - 9	13.4 (1.2) 11.3 - 15
Pulses/second	6.7 (0.2) 6.3 - 7	14.5 (0.1)* 14.4 - 14.7	94.0 (11.9) 84.3 - 107.3
Calls/minute	20.0 (1.2) 18.2 - 21.6	1.3 (0.8) 0.8 - 1.9	3.7 (0.6) 3.3 - 4.2
Fundamental frequency (Hz)	824.7 (19.5) 787.5 - 868.2	1130.8 (20.2) 1110.6 - 1151	2623.7 (273.3) 2398.9 - 2927.9
Dominant frequency (Hz)	2439.0 (63.7) 2281.7 - 2564.4	2402.9 (20.2) 2382.7 - 2423.1	2623.7 (273.3) 2398.9 - 2927.9
Other emphasized frequency (Hz)	1649.4 (38.7) 1575.0 - 1736.5		
	3286.0 (136.6) 2907.7 - 3392.3		
	5080.0 (337.1) 4866.3 - 6138.5		

parks where the recordings were obtained, they were freed in their capture sites immediately after recording and identification.

RESULTS AND DISCUSSION

Table 1 shows the summary of the numerical parameters measured from the recordings.

Rana (Pulchrana) glandulosa Boulenger, 1882.

The known geographic range of this species includes Thailand, peninsular Malaysia, and Sumatra and Borneo in the Sunda Archipelago (Inger, 1966). Although Inger & Stuebing (1989) reported that this frog occurs in flooded areas near the coast, our specimens were recorded in Gunung Mulu (Deer cave area, 4°N 114° 7'E) in a primary forest located more than 80 km away from the nearest coast. Males were widely dispersed. They were isolated, calling either from the ground or perched in low vegetation about 20-80 cm above ground. No choruses were found. The call of this species (Fig. 1A) consists of a loud and extremely long note (mean duration, 1502.2 ms) with 9-12 pulses. Its mean dominant frequency is 2440 Hz, and three additional harmonics (1650 Hz, 3286 Hz and 5080 Hz) are other emphasized frequencies. The first harmonic, or fundamental frequency (825 Hz), is not emphasized. The call is repeated at a fast rate (about 20 calls per minute). To the best of our knowledge no previous publication of the call of this species is available for comparison.

Rana (Pulchrana) signata (Günther, 1872).

This species is known to occur in Thailand, Peninsular Malaysia, Sumatra, Borneo and Philippines (Frost, 1985) and is usually associated with medium-sized streams (Inger, 1966). Our recordings were obtained in a primary forest in the vicinity of Melinau Camp, in Gunung Mulu National Park (4° 3'N 114° 9'E). Males called in isolation with several metres between individuals and no choruses were observed. Most calling males were found on the ground along a stream bank. Only a few individuals were seen calling while perched up to 150 cm on the riparian vegetation.

The call has a mean duration of 620.7 ms. It is composed of a sequence of nine, evenly spaced, pulse groups repeated at regular intervals, with decreasing intensity and duration (average pulse group duration 35 ms, mean number of pulses per pulse group 7.7)

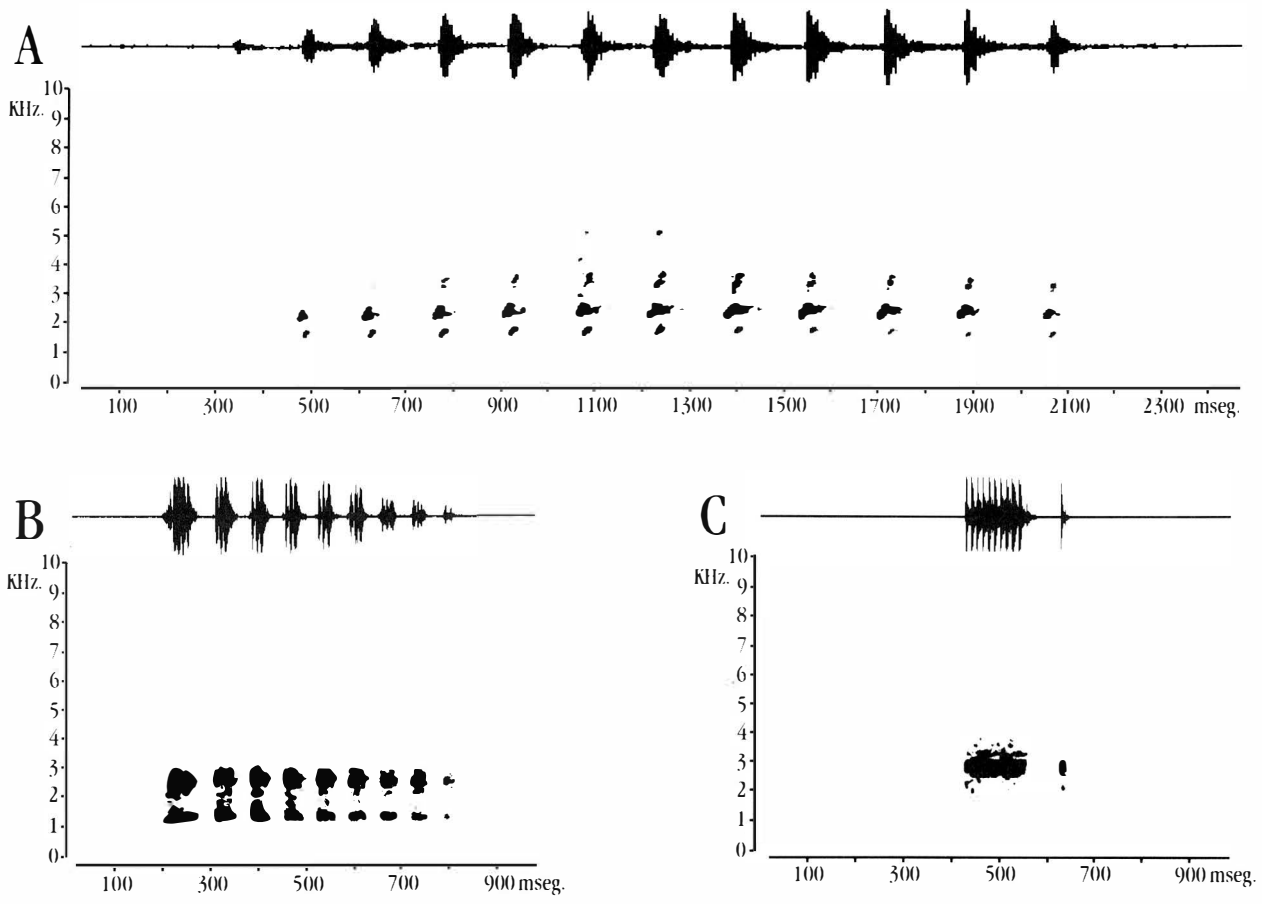

FIG 1. Audiospectrograms and oscillograms of a characteristic advertisement call of each species. A, *Rana glandulosa*; B, *Rana signata*; C, *Polypedates leucomystax*.

(Fig 1B). The mean dominant frequency is 2402.9 Hz while the average fundamental frequency is 1130.8 Hz. Alcala *et al.* (1986) described the mating calls of individuals from Malinao, in the vicinity of Puerto Princes (Palawan Island, Philippines. 10°N 119°E). These individuals were identified by Inger (1954), as belonging to the subspecies *R. s. moellendorffi* Boettger. The calls of the Philippine specimens do not coincide with our recordings either in temporal or in spectral features. Alcala *et al.* (1986) described a complex call with distinct note types: some initial tuned notes (tuned, *sensu* Heyer *et al.*, 1992) that do not resemble any of the sounds obtained in our recordings, and some final notes with a complex spectral structure that are somewhat more similar to the notes described by us, but have different emphasized frequencies (dominant frequency 1350 Hz, and power in the second harmonic at 2800 Hz). The differences in the mating calls found between our recordings for the Sarawak individuals and the results of Alcala *et al.*(1986) from Malinao, are substantial enough to suggest that further studies on the differentiation of these populations may prove fruitful.

Polypedates leucomystax (Gravenhorst, 1829).

This taxon is considered to be a generalist species (Inger, 1966), having a vast distribution, ranging from Nepal, through SE Asia and continental China, up to Taiwan (Inger, 1966; Dubois 1976). The species has been also introduced in the Ryukyu archipelago (Matsui *et al.*, 1986). The individuals recorded were found calling near human quarters in the Bako National Park (Sarawak, 1° 7'N 110° 4'E). Males aggregated and formed choruses perched on the vegetation around ponds and flooded areas at variable heights (from a few centimetres to several meters above ground). The mating call is composed of a single pulsed note (10-16 pulses), relatively short (mean duration 143.5 ms), and with a mean dominant frequency of 2623.7 Hz. This note may be followed by an isolated supernumerary pulse (duration 4.3 ms) which has a similar dominant frequency (Fig. 1C). Other call types obtained in our recordings were shorter (40.55 ms duration), lower in intensity, composed of only three to five pulses, and with a dominant frequency slightly lower that that of the mating call (2380 Hz). These calls were not included in Fig. 1., since their intensity was comparatively

very low and the recording level was not adequate for a complete description. Heyer (1971) described the calls of *Polypedates leucomystax* from Sakaerat, Thailand. He described two note-types: the longer note (230-380 ms) showed a dominant frequency between 2500 and 2600 Hz, resembling our recordings from Borneo. However, other call characteristics differ. Thus, the spectral structure of the recordings from Thailand (300-2600 Hz) and the temporal structure of such calls (4-5 pulses) are clearly different from our recordings. On the other hand, the shorter note of the specimens from Thailand was described as being 120-250 ms in duration, and with 2-4 pulses. Such notes do not occur in our recordings, although perhaps the pulse structure resembles our supernumerary pulses. Nevertheless, the differences between the calls from Thailand and ours are substantial.

Matsui (1982) described the mating calls of *Polypedates leucomystax* from Ranau (Sabah, Malaysia). His description of the primary mating call is similar to our results, both in temporal features (duration 130 ms, 12.8 pulses/call) and spectral structure (dominant frequency 2488 Hz). The recordings of Matsui (1982) seem to show some emphasized harmonics that do not appear in our analyses; but differences in recording equipment or in recording or analysis techniques may account for this lack of agreement. Matsui (1982) also described a type of vocalization that he termed "after call", composed of tonal (i.e. well tuned) notes of variable duration. We did not record any of those call types. However, there is substantial agreement between the description of the main call in Matsui (1982) and our recordings. On the other hand, Kuramoto (1986) described the calls of *Polypedates leucomystax* from three sites in Taiwan. The calls of the Taiwanese individuals are extremely brief pulses with complex spectral structure (500-3300 Hz), emitted in groups of one to five pulses forming calls that lasted from 30 ms (one pulse) up to 581 ms (five pulses emitted separately). This call type does not resemble our recordings, but shows some similarities with the recordings described for specimens from Thailand (Heyer, 1971). Brzoska *et al.* (1986) described in detail the calls of *P. leucomystax leucomystax* Boie (subspecific status after Inger, 1966) in Negros, Philippines (9°N 123°E). They described three call types, although only one of them was considered to be an advertisement call. This call is slightly longer than those obtained by us (average duration 192 ms), and is composed of a similar number of pulses to the calls shown in our recordings (average 16.2 pulses/call). The fundamental frequency of some of the Philippine calls (Figs 3a, and 3b, in Brzoska *et al.* 1986) also appear to be similar to our recordings. Brzoska *et al.* (1986) also described some shorter notes which appear to coincide with our lower-intensity notes. Therefore, the similarity in advertisement call is further supported by the partial concordance in acoustic repertoire. In summary, the advertisement calls of *P. leucomystax* from Borneo and the Philippines appear to be similar; while those of Taiwan and Thailand, though not identical, show a characteristic pulsed structure which could suggest a more recent common origin. This call pattern is also shared by the rest of the racophorid frogs from Taiwan (Kuramoto, 1986). Our results are in agreement with the conclusions of Matsui *et al.* (1986) who consider that the Taiwanese specimens of *Polypedates* are *P. megacephalus*, a distinct species from the Bornean population. Our bibliographical research on the published audiospectrograms of the mating calls of the sister species in the *Polypedates leucomystax* complex (*sensu* Inger, 1966), (Matsui, 1979) shows a high degree of variability in acoustical characteristics. This result supports the conclusions of Inger (1966), who found a high variability in the group, based on morphological characters.

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We are grateful to R. Inger, who kindly confirmed the identification of the species based on photographs. I. De la Riva, M. García-París and M. Tejedo contributed to the bibliographical research. B. Sanchíz provided useful comments to an earlier version of the manuscript. M. J. Littlejohn and H. Schneider critically read the manuscript and provided suggestions that improved it substantially. Sound analyses were performed in the facilities of the Estación Bio-Geológica del Ventorrillo (C.S.I.C.). This study was partially funded by projects CYCIT PB92-0091 and CYCIT PB91-0115-CO2.

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CRYSTALLINS IN LENSES OF GEKKONID LIZARDS (REPTILIA, GEKKONIDAE)

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The ocular lenses of the Gekkonidae differ in their crystallin compositions. The lenses of all species investigated contain variable amounts of α-, β-, γ-, and τ-crystallins, which are commonly found in lenses of various vertebrates. Additionally, in lenses of the distantly related genera *Phelsuma* and *Lepidodactylus* another crystallin has been found which is a 38 kDa monomer and comprises about 20% of the total amount of crystallins. All other gekkonid species investigated, both nocturnal and diurnal, do not possess this crystallin. This distribution is highly surprising. It neither supports a possible correlation between similarity of crystallin composition and similarity of habits, nor a possible correlation between crystallin composition and phylogenetic relationship. Closer examination of the biochemical properties of the 38 kDa 'gecko' crystallin and comparison with other taxon-specific crystallins, especially the ε-crystallin of some other sauropsids, leads to the conclusion that this 38 kDa crystallin may be a so far undescribed novel type of crystallin.

INTRODUCTION

Vertebrate eye lenses consist mainly of numerous lens fiber cells which contain high concentrations of water-soluble proteins called crystallins. Despite the similar functions, i.e., light transmission and focus, of the lenses, they differ in shape, protein content and especially in their crystallin composition. These heterogenous crystallins can be divided into two main groups (reviews: Wistow & Piatigorsky, 1988; de Jong, Hendriks, Mulders & Bloemendal, 1989; Wistow, 1993). The first group comprises the α-, β- and γ-crystallins which are present in almost all vertebrates. The second group of crystallins is taxon-specific and frequently involves crystallins which are related or identical to enzymes of metabolic pathways (Wistow & Piatigorsky, 1987, 1988). Some of these taxon-specific enzyme crystallins have preserved their enzymatic activity, e.g. the ε-crystallin of some birds and crocodiles, which is identical with lactate dehydrogenase B4, and the τ-crystallin of several fishes, reptiles and birds, which is related to α-enolase (Wistow, Mulders & de Jong, 1987; Wistow *et al.,* 1988). Other enzyme crystallins do not show enzymatic activity, e.g. the η-crystallin of elephant shrews, which is identical with aldehyde dehydrogenase (Wistow & Kim, 1991).

Both the correlation between the distribution of taxon-specific crystallins and the phylogenetic relationships between these taxa, and the correlation between the distribution and the similarities in habits are still unclear. On the one hand, the scattered occurrence of the ε-crystallin does not correlate to phylogenetic relationships between the avian orders. On the other hand, this crystallin is also expressed in crocodiles (Stapel *et al.*,1985) which are supposed to be closely related to birds. Among birds, the ε-crystallin is found in diurnal species which occupy habitats with high ambient light intensities (Wistow *et al.,* 1987). So far, the ε-crystallin has not been found in nocturnal birds. In contrast, the expression of the δ-crystallin seems to be more closely connected with phylogenetic relationships (Wistow, Anderson & Piatigorsky, 1990). Despite the very similar feeding habits of chimney swift (*Chaetura pelagica*) and barn swallow (*Hirundo rustica*) only the latter possesses the δ-crystallin. The crystallin composition in the swift resembles that in Anna's hummingbird (*Calypte anna*). Swifts and hummingbirds are closely related but have very different habits.

To clarify the role of either relationship or similarity of habits, it seemed logical to choose a group of animals which combines close phylogenetic relationships with markedly different habits. Among the Reptilia, these conditions are met by the Gekkonidae. This family comprises both nocturnal and diurnal genera. According to the transmutation theory of Walls (1934, 1942), the nocturnal geckos are supposed to descend from primarily diurnal lizard ancestors whose visual cells had been cones with coloured oil droplets. Thus, the rods of 'secondarily' nocturnal geckos, which generally lack oil droplets, had transmuted from cones. Furthermore, Walls (1934, 1942), suggested that some of the gekkonid species which are now diurnal have reverted from nocturnal gecko ancestors. Their visual cells have undergone a second transmutation from rods back to cones. Most of these cones lack oil droplets, but as a substitute for these droplets the lenses of the 'tertiarily' diurnal geckos are yellow, in contrast to the colourless lenses of nocturnal species. Perhaps these decisive changes in habits resulted in both morphological modifications of the visual cells and biochemical modifications of the lenses.

TABLE 1. Occurrence of 38 kDa crystallin and of yellow colour in lenses of gekkonid species with different activity periods. C-D: crepusculo-diurnal, D: diurnal, D-N: diurno-nocturnal, N: nocturnal.

Species	Activity	Yellow lens	38 kDa-crystallin
Ailuronyx seychellensis	D-N	-	-
Gekko gecko	N	-	-
Hemidactylus frenatus	N	-	-
Lepidodactylus lugubris	D-N	-	+
Lygodactylus picturatus	D	+	-
Oedura castelnaui	N	-	-
Pachydactylus geitje	N	-	-
Pachydactylus maculatus	N	-	-
Paroedura pictus	N	-	-
Phelsuma andamanensis	D	+	+
Phelsuma barbouri	D	+	+
Phelsuma dubia	D	+	+
Phelsuma guentheri	D-N	+	+
Phelsuma madagascariensis	D	+	+
Phelsuma standingi	D	+	+
Sphaerodactylus cinereus	C-D	-	-
Stenodactylus sthenodactylus	N	-	-
Uroplatus henkeli	N	-	-
Uroplatus phantasticus	N	-	-

MATERIALS AND METHODS

ANIMALS

The lenses were obtained from nineteen species of the Gekkonidae (Table 1). *Sphaerodacylus cinereus* belongs to the subfamily Sphaerodactylinae, *Oedura castelnaui* belongs to the subfamily Diplodactylinae, whereas all other species belong to the subfamily Gekkoninae. Only adult animals were used.

PREPARATION OF LENS EXTRACTS

The animals were anaesthetized by chilling to 4°C and then humanely killed by decapitation. The eyes were enucleated and either used immediately or kept frozen at -80°C. The lenses were removed, weighed and carefully homogenized in minimum volumes of various buffer solutions depending on the different experiments. Insoluble fractions were removed by centrifugation at 4°C for 15 min at 15 000 x g. Protein concentrations of the different lens extracts were determined by a modified method of Neuhoff, Zimmer & Mesecke (1979) using bovine serum albumine as standard.

ENZYME ASSAY

Lactate dehydrogenase (LDH) activities of freshly prepared water-soluble proteins were determined in a standard assay mixture containing 100 m*M* bis-Tris-propane buffer pH 7.0, 0.15 m*M* NADH, 1 m*M* pyruvate and an appropriate amount of extract in a total volume of 1 ml. The reactions were started by the addition of pyruvate and monitored spectrophotometrically by following the decrease in absorbance at 340 nm as a function of time. One unit of enzyme activity is defined as the amount of enzyme that caused the oxidation of 1 µMol NADH/min (equivalent to the generation of 1 µMol lactate/min) at pH 7.0 and 25°C. A molar absorption coefficient of 6.2 10^6 cm^2/Mol for NADH was used for calculation.

GEL ELECTROPHORESIS

Samples for sodiumdodecyl sulphate (SDS) electrophoresis gels were prepared in two different ways. For the preparation of detergent-soluble proteins, lenses were homogenized in a solution containing 50 m*M*

Tris-HCl buffer pH 8.8, 3.75% SDS, 20 m*M* dithioerythritol and 10 m*M* dithiothreit. These samples were incubated at 30°C for 16 hours. After centrifugation (10 000 x g, 15 min) the protein concentrations of the supernatants were determined. Aliquots with 5, 10 or 20 mg protein brought to 30% mercaptoethanol and 6% glycerol with bromphenol blue, were separated on 6-20% gradient polyacrylamide gels containing 0.1% SDS and 3.2 *M* urea.

For the preparation of water-soluble proteins only, lens extracts were prepared in 125 m*M* Tris-HCl buffer pH 6.8. After determination of the protein concentration, the samples were denatured in a solution containing 5% SDS, 2% mercaptoethanol and 10% glycerol with bromphenol blue and boiled for 3 - 5 min. Aliquots with either 10 or 20 mg protein were run on 14% polyacrylamide gels containing 0.1% SDS. Protein bands were stained with Coomassie Brilliant Blue R250.

The molecular weights of crystallin subunits were calculated from the mobilities of the following standard marker proteins: phosphorylase b 92.5 kDa, bovine serum albumin 69 kDa, ovalbumin 45 kDa, carbonic anhydrase 29 kDa, soybean trypsin inhibitor 22 kDa, myoglobin from horse skeletal muscle 17.8 kDa, cytochrome c 12.5 kDa, lung trypsin inhibitor 6.5 kDa.

WESTERN BLOTTING

Water-soluble lens proteins of some gekkonid species were separated by sodiumdodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (14% polyacrylamide), and then electrophoretically transferred to an Immobilon PVDF membrane. Blots were saturated in a solution of 10 m*M* TRIS, 150 m*M* NaCl (TBS) pH 7.2, 1% Tween 20 for at least 1 hr at room temperature and then incubated with anti-duck ε-crystallin antiserum for 4 hr at room temperature. The antiserum was kindly provided by Prof. W. W. de Jong, Nijmegen. After washing with TBS, 0.05% Tween 20 the blots were incubated with peroxidase-conjugated anti-rabbit IgG antiserum for two hours at room temperature. After washing, 4-chloro-naphthol was used for colour development.

GEL FILTRATION CHROMATOGRAPHY

Extracts of water-soluble lens proteins in elution buffer were subjected to fast protein liquid chromatography (FPLC). Samples of 200 ml containing between 1.8 and 2.5 mg protein were applied to a column of Sephacryl 100. The proteins were eluted with 100 m*M* Tris buffer, pH 7.0, at a flow rate of 60 ml/h at room temperature. Protein was monitored by the absorbance at 280 nm. Native molecular weights were estimated by using chromatography of the following standard proteins on the same column: aldolase 160 kDa, bovine serum albumin 67 kDa, ovalbumin 45 kDa, chymotrypsinogen 25 kDa and cytochrome c 12.4 kDa.

Peak fractions of samples were pooled and concentrated 30- to 90-fold over Centricon 10 filters. Aliquots with about 10 mg protein were supplied to a 14% polyacrylamide gel.

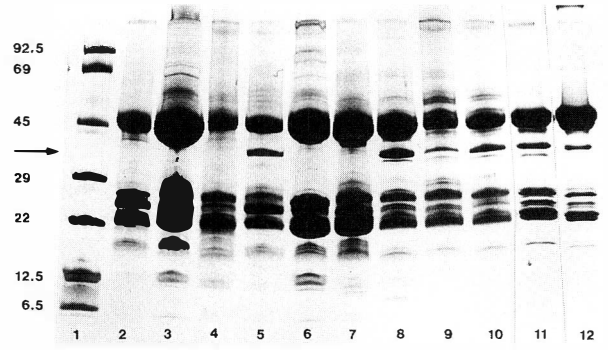

FIG. 1. Protein composition of crude lens extracts. SDS-PAGE (6-20% polyacrylamide) of detergent-soluble proteins (20 µg per lane). Lanes 2 to 7: nocturnal species, lanes 8-12: diurnal species. 2: *H. frenatus*, 3: *G. gecko*, 4: *P. maculatus*, 5: *L. lugubris*, 6: *P. pictus*, 7: *A. seychellensis*, 8: *P. dubia*, 9: *P. barbouri*, 10: P. *andamanensis*, 11: *P. standingi*, 12: *P. madagascariensis*. Lane 1: marker proteins. The molecular weights are given in kDa (left). The arrow indicates the 38 kDa crystallin.

RESULTS

PROTEIN COMPOSITION OF CRUDE LENS EXTRACTS

The isolated lenses of most diurnal gekkonid species are yellow with the exception of the lens of *Sphaerodactylus cinereus*, whereas the lenses of nocturnal geckos are colourless (Table 1). The lenses are generally of a soft consistency. With about 25-30% (e.g. *S. sthenodactylus*: 25%; *L. lugubris*: 27%; *O. castelnaui*: 29%) protein content in relation to lens wet weight; the gecko lenses belong to the more watery lenses, comparable to those of birds (e.g., eider duck *Somateria mollissima*: 26%).

The gel electrophoretic separations of detergent-soluble lens proteins of 19 different gekkonid species reveal remarkable differences in their compositions: A polypeptide of about 38 kDa occurs in considerable amounts in the diurnal genus *Phelsuma* and in the diurno-nocturnal species *L. lugubris* (Fig. 1). The 38 kDa polypeptide is found neither in other nocturnal nor in other diurnal species (Table 1). Thus, it does not correlate with the yellow colour of lenses from diurnal geckos.

In all species investigated, apart from *Lygodactylus*, protein patterns are similar in the ranges of 69 to 45 kDa and 29 to 12.5 kDa. The major protein δ-crystallin shows up as a relatively broad band with a molecular weight of about 50 kDa (Fig. 1). If the gel is not overloaded, the broad δ-bands of all species except *L. lugubris* are resolved into two bands, whereas the δ-crystallin of the latter always migrates as a single band (Fig. 2). The lens protein composition of *L. picturatus* characteristically has a relatively broad band in the range of about 15 kDa and only a very small one in the range of 20-22 kDa (Figs. 2, 3A). The latter is due to a reduced expression of α-crystallin.

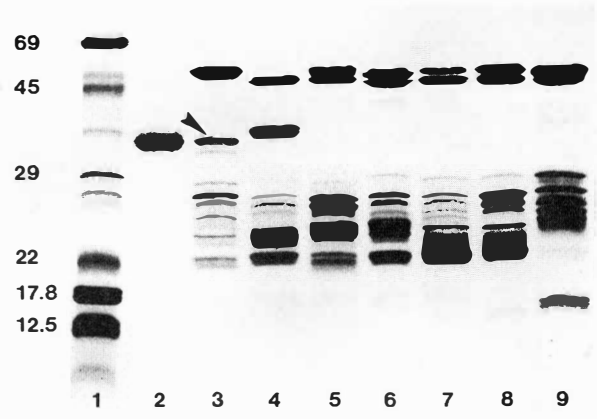

FIG. 2. Protein composition of crude lens extracts of sauropsid species and comparison with LDH. SDS-PAGE (14% polyacrylamide) of water-soluble proteins (10 µg per lane). Lane 1: marker proteins, molecular weights in kDa, lane 2: LDH from pig heart muscle, lane 3: eider duck *S. mollissima*, lane 4: *L. lugubris*, lane 5: *O. castelnaui*, lane 6: *S. sthenodactylus*, lane 7: *U. henkeli*, lane 8: *U. phantasticus*, lane 9: *L. picturatus*. The arrowhead indicates the avian ε-crystallin.

The 38 kDa protein comprises about 20 to 22% of the total amount of water-soluble lens proteins and is, therefore, regarded as one of the major crystallins (Fig. 2, 3A). Concerning the protein pattern of detergent-soluble lens proteins, the amount of this crystallin seems to be smaller (10-12%) (Fig. 1). This difference is due to the use of detergents during the extraction procedure, as these samples contain not only the cytosolic proteins but also the membrane proteins of the numerous lens fibres.

The 38 kDa crystallin seems not to be restricted to the Gekkonidae, because it also occurs in the diurnal lizard *Egernia cunninghami* (Scincidae) (unpublished observation). There are no appreciable differences in the mobility of the denatured 38 kDa crystallins between the genera *Phelsuma*, *Lepidodactylus* and *Egernia*.

Regarding the molecular weight of this special crystallin and its scattered occurrence in the Gekkonidae, it resembles on the one hand the ε-crystallin, identical with LDH, of some avian and reptilian genera, and on the other hand the ρ-crystallin of the amphibian genus *Rana*. Thus, a comparison of the lens crystallin compositions in some of those vertebrates seems useful. The lens protein patterns of *Rana temporaria* and *P. dubia* or *L. lugubris* are quite different (Röll, 1990). The ρ-crystallin has a higher mobility on the gel than the 38 kDa crystallin, pointing to an apparent molecular weight of 36 kDa. The protein pattern of the eider duck *S. mollissima*, is similar to that of *L. lugubris* (Fig. 2). Both the special gekkonid lens protein, the ε-crystallin of *S. mollissima* and the enzyme LDH have, in their denatured form, molecular weights of about 38 kDa (gekkonid crystallin: 38.4 ± 0.9 kDa, n=15; ε-crystallin: 38.1 ± 0.5 kDa, n=3; LDH: 38 ± 0.7 kDa, n=9). So it seems possible that this gekkonid and the avian ε-crystallin are identical or at least related.

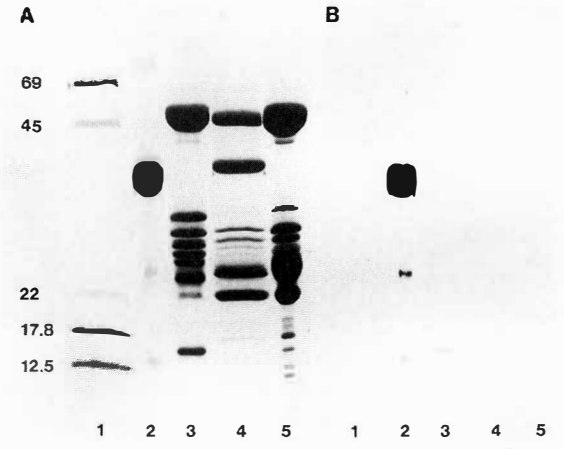

FIG. 3. SDS-PAGE (14% polyacrylamide) (A) and immunoblot (B) of gekkonid water-soluble lens proteins. A Crystallin composition of three gekkonid species and comparison with LDH (20 µg each lane). Lane 1: marker proteins, molecular weights in kDa, lane 2: LDH, lane 3: *L. picturatus*, lane 4: *L. lugubris*, lane 5: *S. sthenodactylus*. B Blotted proteins of the same extracts in the same sequence as in A. Proteins are stained with anti-duck ε-crystallin antiserum.

LDH-ACTIVITY IN GEKKONID LENSES

The ε-crystallin shows high LDH-activity (Wistow *et al.*, 1987; Chiou, Lee & Chang, 1990). Thus, it was examined whether gekkonid lens extracts also develop enzymatic activity. Three species were chosen for the LDH-activity assay: the diurno-nocturnal *L. lugubris* possessing the 38 kDa crystallin in comparison to the diurnal *L. picturatus* and the nocturnal *S. sthenodactylus*, both without this crystallin. The LDH-activities of these different samples are very similar (Table 2). The activity of the sample with the 38 kDa crystallin, presumed to be related to LDH, is not higher than the activities of the other samples. Thus, these activities must be due solely to the cytosolic LDH in the lens cells.

IMMUNOBLOTTING WITH ANTI-DUCK ε-CRYSTALLIN ANTISERUM

Water-soluble proteins of gekkonid lenses and pig heart muscle LDH, electrophoretically separated and blotted onto a PVDF-membrane, were incubated with an antiserum against the duck ε-crystallin (Fig. 3A, B).

TABLE 2. LDH-activity in crude lens extracts of three gekkonid species.

Species	mU/mg lens wet weight	mU/mg total protein
L. lugubris	28.4	104
L. picturatus	17.7	112
S. sthenodactylus	15.1	59

This antiserum does not react with any protein band of the gekkonid lens extracts subjected to the immunoblotting procedure (Fig. 3B, lanes 3-5), but it clearly shows a positive reaction with both the purified pig LDH (Fig. 3B, lane 2) and the ε-crystallin of the lens extract of the eider duck (not shown). Consequently, the 38 kDa and the ε-crystallin are not immunologically related.

GEL FILTRATION CHROMATOGRAPHY

The elution profiles of lens extracts from *L. lugubris* and *S. sthenodactylus* show five peaks each (Fig. 4A). Four peaks of the *L. lugubris* profile correspond in their elution volumes to four peaks of the *S. sthenodactylus* profile. The gel electrophoretic analysis of the protein patterns of these peak fractions reveals that the peaks at nearly identical elution volumes are composed of nearly identical crystallin subunits (Fig. 4B). The first peaks of the two profiles (S1, L1) contain α-crystallin, which is composed of two types of subunits (21 and 23 kDa). The major protein of S2 and L2 is the δ-crystallin, which has subunits of about 50 kDa. S2 and L2 additionally contain a small amount of α-crystallin subunits of the preceding peak fractions. Peak fractions S3 - only a shoulder of the broad peak S4 - and L3 are composed of crystallin subunits of 50 kDa which resemble the τ-crystallin of birds and reptiles. Peak fractions S5 and L5 reveal monomeric γ-crystallins of 22 kDa.

The peak fractions S4 and L4 differ in both their elution volumes and their protein compositions. S4 contains four subunits of 25-30 kDa, probably of β-crystallin, and additionally a 50 kDa protein. In contrast, L4 contains mainly the monomeric 38 kDa crystallin. The additional small bands of low molecular weights (27-30 kDa) could either be parts of ß-crystallin subunits or fragments of the main protein in this fraction. In lenses of *L. lugubris* the 38 kDa crystallin seems to partly replace the ß-crystallin subunits (Fig. 4B). Aliquots of the peak fractions L4 do not show enzymatic activity in the LDH activity assay.

DISCUSSION

The gel electrophoretic separations of gekkonid lens crystallins reveal a considerable difference in compositions. It concerns a major 38 kDa crystallin occurring in the lenses of all species investigated of the genus *Phelsuma* and in the lens of *L. lugubris*, where it comprises 20-22% of the total amount of crystallin (Fig. 2, Table 1). This distribution is highly surprising. It supports neither a possible correlation between similar crystallin compositions and similar or nearly identical habits nor a conceivable correlation between similar crystallin compositions and the degree of relationship.

Concerning the first possibility, the virtually complete lack of the 38 kDa crystallin in *L. picturatus* is remarkable. Both the genus *Phelsuma* and the genus *Lygodactylus* share numerous common characteristics: generally, they are strictly arboreal and insectivorous, active during the day ('tertiarily' diurnal), and constantly exposed to bright sunlight. Additionally, both genera possess yellow lenses. Because of these nearly identical habits it could be assumed that similar requirements on the function of the lenses result in very similar lens protein compositions.

P. guentheri is the only extant member of the genus *Phelsuma* which is not exclusively diurnal. The morphology of the eye of *P. guentheri* is virtually identical to that of fully diurnal *Phelsuma* species. *P. guentheri* is mainly active during late afternoon up to the evening (5 pm - 8 pm); in the course of night its activity decreases and ceases at about 2 am (Langebaek, 1979). Because of the very close relationships an identical crystallin pattern is not surprising.

The sphaerodactyline *S. cinereus* does not possess the 38 kDa crystallin though being mainly diurnal (Table 1). However, this is not unexpected because the members of this genus generally live in the herbaceous layer, where they have secretive if diurnal habits. Thus, it is seldom exposed to bright sunlight.

If the crystallin composition could be viewed as a measure of the degree of relationship, the 38 kDa crystallin is expected to occur in the lenses of the genera *Phelsuma*, *Ailuronyx* and *Lygodactylus*. These are closely related and are included in the monophyletic Afro-Madagascan group (Joger, 1985). But both *Lygodactylus* and *Ailuronyx* lack the 38 kDa crystallin (Figs. 1, 2). The lens protein pattern of *A. seychellensis* resembles that of the nocturnal species. Indeed, this gecko is mainly, but not exclusively, active at night.

The unexpected presence of the 38 kDa crystallin in *L. lugubris* can be explained neither by similar habits nor by a close phylogenetic relationship to *Phelsuma*. The 38 kDa crystallins of *Lepidodactylus* and *Phelsuma* show identical mobilities on SDS-gels. Though this does not prove their identity, there is so far no evidence to the contrary.

Several vertebrate enzyme-crystallins with molecular weights of their subunits in the range of 35-38 kDa have been described. These are the ζ-crystallin of guinea pig, degu rock cavy, camel and llama, the λ-crystallin of rabbits and hares, the μ-crystallin of some marsupials and the ρ-crystallin of the genus *Rana*.

The ζ-crystallin is a homotetramer (Huang, Russell, Stone & Zigler 1987), while the λ-crystallin is a dimer or a tetramer (Mulders *et al.*, 1988). The ρ-crystallin of the genus *Rana* is an oligomer (Tomarev, Zinovieva, Dolgilevich, Luchin, Krayev, Skryabin & Gause 1984) or, more likely, a monomer (Bindels *et al.*, 1983; Fujii *et al.*, 1990). In any case, the ρ-crystallin of *Rana temporaria* has a higher mobility on my gels than the 38 kDa crystallin of geckos, pointing to an apparent molecular weight of 36 kDa. As the μ-crystallin of marsupials, the quaternary structure of which seems to be unknown, was not even observed in all marsupial species investigated (Wistow & Kim, 1991), it is highly unlikely that this crystallin should occur in gekkonid lenses.

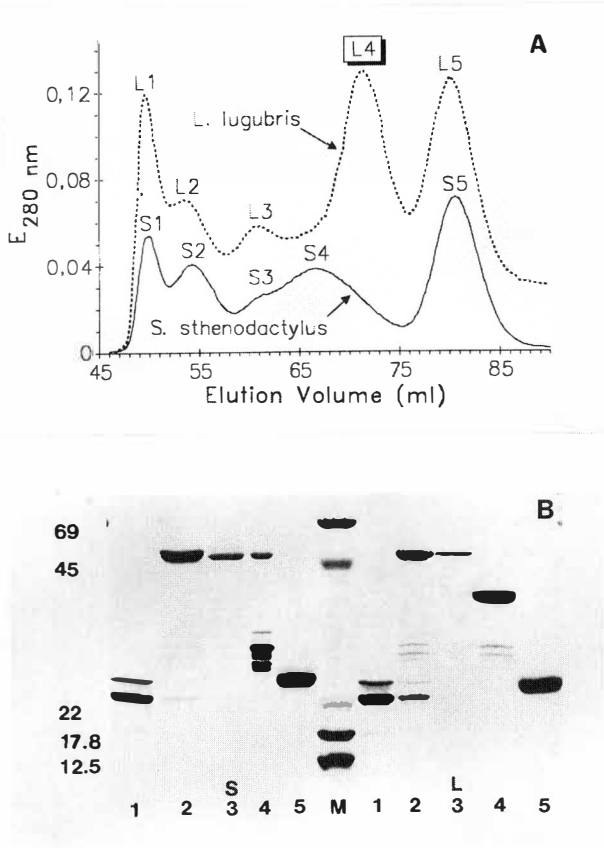

FIG. 4. A. Elution profiles of water-soluble lens proteins of *L. lugubris* and *S. sthenodactylus*. B. Analysis of peak fractions by SDS-PAGE (14% polyacrylamide). Numbering of peak fractions as in A. L4 contains the 38 kDa crystallin. Molecular weights of marker proteins (M) are given in kDa.

The monomeric 38 kDa crystallin of some geckos seems rather to be more similar to the ε-crystallin. The scattered presence of the latter in sauropsid taxa, birds and crocodiles, supports an evolutionary relationship between the ε and the 38 kDa crystallin. The ε-crystallin is identical with LDH-B4. Chiou, Chang & Lin (1988) found a molecular weight of 150 kDa and suggested a tetrameric structure. The isoenzymes of vertebrate LDH are tetrameric proteins with native molecular weights of 140 kDa; only LDH of invertebrates are found to be tetrameric or dimeric (Urich, 1990). In any case, the ε-crystallin possesses high enzymatic activity. The τ-crystallin of reptiles and fishes is closely related to the glycolytic enzyme α-enolase. The normal enzyme, however, is a dimeric protein (Chin, Brewer & Wold 1981), whereas τ-crystallin, isolated from turtle and lamprey, is predominantly monomeric (Stapel & de Jong, 1983; Wistow & Piatigorsky, 1987). Despite being apparently a monomer, the τ-crystallin exhibits reduced enolase activity (Wistow *et al.*, 1988). Thus, lens extracts of *L. lugubris* possessing the 38 kDa crystallin were tested for LDH activity in comparison with extracts of *L. picturatus* and *S. sthenodactylus*. The enzymatic activities of these samples reach only low values in the range of 60 to 110 mU per mg of total protein (Table 2). These activities are due to the LDH activities of the normal lens metabolism, particularly of anaerobic glycolysis. Hockwin & Orloff (1981) found a LDH-activity of 7.5 mU per mg protein of whole bovine lenses. In contrast to this low enzymatic activity, the isolated duck ε-crystallin possesses an extremely high activity of 100 U per mg (Wistow *et al.*, 1987). Even higher values of LDH-activity of isolated duck and swan ε-crystallin, 510/590 and 550 U per mg protein, respectively, have been reported by Chiou, Chang & Lai (1989) and Chiou *et al.* (1990). The isolated 38 kDa crystallin shows no enzymatic activity at all. This is, besides the different quarternary structure, an additional difference between the 38 kDa crystallin and the ε-crystallin.

The lack of enzymatic activity alone does not necessarily imply the lack of structural similarity of subunits. The 38 kDa crystallin might have lost its enzymatic activity because of only small modifications in the protein structure concerning the residues required for LDH activity. In this case the immunological relationship between the 38 kDa crystallin and the ε-crystallin should be retained. As the anti-duck ε-crystallin antiserum does not react with the 38 kDa crystallin (Fig. 3B), the dissimilarity of the gekkonid lens protein and the ε-crystallin is clearly revealed.

On account of the differences between the 38 kDa crystallin and ε-crystallin, it is supposed that the gekkonid crystallin may be a new, undescribed crystallin but only the unravelling of its amino acid sequence can be a real proof for this assumption.

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HABITAT ASSOCIATION OF THE TORTOISES *GEOCHELONE PARDALIS* AND *KINIXYS SPEKII* IN THE SENGWA WILDLIFE RESEARCH AREA, ZIMBABWE

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There have been few studies of the mechanism of niche separation in sympatric tortoises. This paper examines the habitat association of *Geochelone pardalis* and *Kinixys spekii* at the Sengwa Wildlife Research Area, using data on 460 tortoises marked from 1982 to 1992. Tortoises were found in most of the vegetation types present. Habitat niche breadth was slightly greater in *G. pardalis* (B=0.48) than in *K. spekii* (B=0.36). There was considerable niche overlap between the two species (O=0.76), the only major difference being the greater use of riverine grassland by *G. pardalis*. Home range areas of individuals recaptured in several years were significantly larger in *G. pardalis* (mean 26 ha) than in *K. spekii* (mean 3.1 ha). The pattern of refuge use differed between the two species; *K. spekii* used burrows, and *G. pardalis* used thickets and felled trees.

INTRODUCTION

Much of the species diversity of tortoises (Testudinidae) reflects geographical replacement of one species by another. For example, the four species in North America are allopatric (Lamb, Avise & Gibbons, 1989). In areas where sympatric tortoises occur, the means of coexistence have not been investigated in detail. Wright, Steer & Hailey (1988) found that *Testudo hermanni* and *T. graeca* were separated by habitat utilization in north-eastern Greece, and the same may apply to *T. hermanni* and *T. marginata* in western and southern Greece (Willemsen, 1991).

Southern Africa has the most diverse tortoise fauna of any region of the world (Swingland & Klemens, 1989); the mechanisms of niche separation in this region are clearly of interest. Three species occur in Zimbabwe; the leopard tortoise *Geochelone pardalis*, found over most of the country, and the hingeback tortoises *Kinixys belliana* and *K. spekii*. The latter has only recently been confirmed as a full species (Broadley, 1993). *Kinixys spekii* occurs over most of Zimbabwe, while *K. belliana* is restricted to the Eastern Highlands, where populations of the two species may be found close together (Broadley, personal communication).

Geochelone pardalis and *Kinixys spekii* are thus broadly sympatric. These two tortoises have been studied in the Sengwa Wildlife Research Area, Gokwe District, the largest area in Africa devoted solely to ecological research (Cumming, 1983). The present paper describes the habitat association of the two species, using data collected over a ten year period. Additional information is presented on their long-term movements and use of refuges.

METHODS

Tortoise sightings were incidentally recorded during routine work in the Sengwa Wildlife Research Area (18° 6' S, 28° 12' E) from 1982 to 1992, mostly by game scouts. Tortoises were brought into the Sengwa Wildlife Research Institute office, marked with numbered metal tags glued on to the centre of the 3rd vertebral scute (Gaymer, 1973) with epoxy adhesive, and immediately returned to the exact capture location. Only the first capture of each individual was used in the analysis of habitat utilization. Locations were recorded to the nearest 100 m using a grid reference system. The vegetation type at each capture location was found from the 1:50 000 scale vegetation map of the Sengwa area (Cumming, 1970), a simplified version of which is given by Cumming (1975). Tortoise capture locations were plotted by computer to the same scale, together with a 1 km grid to facilitate comparison with the vegetation map.

The scale over which vegetation types varied was measured to compare with the 100 m resolution of tortoise locations. Four rectangles, totalling 108 square km, were selected (on the basis of excluding the Sengwa and Lutope rivers). The number of areas of different vegetation identified within these rectangles totalled 66 (including all patches more than half inside the rectangle boundaries). The mean size of an area of vegetation was therefore about 1.6 square km, which would correspond to 160 tortoise grid locations. It was concluded that the grid system had sufficient resolution to identify the vegetation types used by tortoises. The scale over which vegetation types varied was smaller around the Sengwa and Lutope rivers, but this was felt to be unimportant for two reasons. First, several

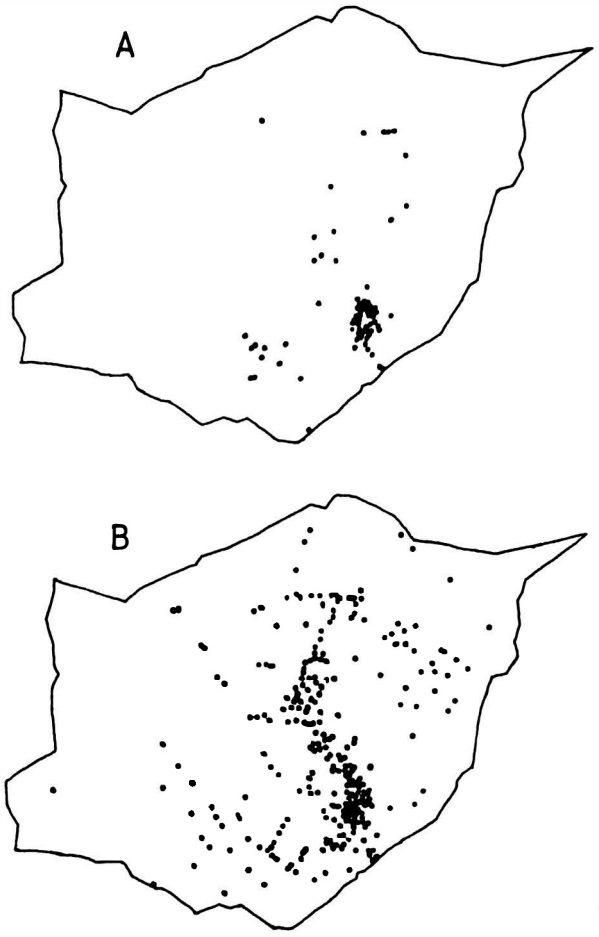

FIG. 1. Maps of the capture locations of *Kinixys spekii* (A) and *Geochelone pardalis* (B) in the Sengwa Wildlife Research Area, 1982-1992.

of the riverine types were combined in analysis. Second, recording of tortoise locations was probably more accurate around the rivers, which formed obvious landmarks.

Niche breadth (B) was calculated as:

$$B = \frac{1/\Sigma p_i^2}{n}$$

where p_i is the proportion of the i^{th} category used from n possible categories (Pianka, 1973). Niche overlap was calculated using Pianka's (1973) symmetric measure:

$$O = \frac{\Sigma p_{ij} p_{ik}}{\sqrt{\Sigma p_{ij}^2 \Sigma p_{ik}^2}}$$

where p_{ij} and p_{ik} are the proportions of the ith category for species j and k.

Home ranges were calculated for tortoises captured five or more times in three or more different years. Capture locations were plotted and the home range area was measured as a minimum convex polygon. Information on the refuges used by thread-trailed tortoises is also presented here. Eight *K. spekii* and nine *G. pardalis* were trailed for up to ten days in January and February 1992. They were relocated each morning. When the tortoise was clearly in a refuge, the characteristics of the refuge were noted.

RESULTS

Records of both species were clustered in the area around the Institute office and staff compound (Fig. 1). The extent of this clustering differed significantly between the two species. The two 1 km squares of the office area included 59.8% of the 107 records of *K. spekii*, but only 19.3% of the 353 records of *G. pardalis* ($\chi^2 = 66.0$, 1 df, $P<0.001$). Possibly game scouts were less likely to see the small *K. spekii* when away from

TABLE 1. Vegetation types used by *G. pardalis* and *K. spekii*.

	All observations		Except office area	
Type	*G. p.*	*K. s.*	*G. p.*	*K. s.*
a	25.2	32.7	15.8	11.6
b	18.7	17.8	21.4	32.6
c	2.8	6.5	3.5	16.3
d	2.3	0.9	2.8	2.3
e	2.0	0	2.5	0
f	2.5	0.9	3.2	2.3
g	15.9	1.9	19.6	4.6
h	2.8	0	3.5	0
i	3.4	6.5	3.2	13.9
j	0.3	1.9	0.4	4.6
k	5.9	3.7	6.7	4.6
l	3.1	0	3.9	0
m	7.4	0.9	9.1	2.3
n	3.1	1.9	3.5	2.3
o	2.0	15.0	0.3	0
p	2.5	9.3	0.7	2.3
N	353	107	285	43
B	0.447	0.345	0.478	0.358
O	0.841		0.761	

Data are percentages of N observations for each species, with niche breadth (B) and overlap (O). Vegetation types are: a, *Brachystegia-Julbernardia* mixed woodland (Miombo); b, *Colophospermum mopane* woodland (Mopane); c, *C. mopane-Combretum-Erythroxylum* woodland and bushland; d, *C. mopane-Acacia nigrescens-Ximenia* woodland and bushland; e, *Baikiaea-Baphia-Combretum* wooded bushland thicket; f, *Commiphora-Combretum* wooded bushland thicket; g, *Acacia tortilis-Grewia* riparian communities; h, *Combretum-Terminalia* low woodland and bushed grassland; i, *Julbernardia-Vellozia* wooded and bushed grassland; j, *Brachystegia boehmii-Combretum* wooded and bushed grassland; k, *C.mopane-Combretum-Tristachya* wooded and bushed grassland; l, *Acacia albida-Hyparrhenia* wooded grassland; m, River terrace (flood plain) grassland; n, Grassland on alluvial or saline soil, and drainage line grassland (vlei); o, Grassland with *C. mopane* scrub; p, Dense riverine woodland. Types a to o correspond to those in Fig. 2 of Cumming (1975); n includes two of Cumming's types. Type p is an addition; it occurs mostly along the Kove river. (See Appendix 1 for full vegetation map coder).

TABLE 2. Tests of association between the two tortoise species and vegetation type. Values are the percentage of the total records of each species which occur in that vegetation type. Also shown is a χ^2 test of association between species and vegetation (against all other vegetation types pooled): NS = $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.

	All observations				Except office area			
Vegetation type	*G. p.*	*K. s.*	χ^2	*P*	*G. p.*	*K. s.*	χ^2	*P*
Miombo woodland (a)	25.2	32.7	2.34	NS	18.7	11.6	0.50	NS
Miombo total (a,i,j)	28.9	41.1	5.66	*	19.3	30.2	2.72	NS
Mopane woodland (b)	18.7	17.8	0.05	NS	14.4	32.6	2.64	NS
Mopane total (b,c,d,o)	31.7	43.9	5.40	*	34.7	55.8	7.08	**
Open riverine (g,m)	23.2	2.8	22.74	***	28.8	7.0	9.24	**

the office, because they were then concentrating on large game and/or poachers. The occurrence of the tortoises in the different vegetation types has therefore been analysed both including and excluding the two 1 km squares around the office.

The two analyses are broadly similar (Table 1). The major effect of excluding the data from around the office was to decrease the proportion of records from miombo woodland, which was the major vegetation type in that area. Niche breadth increased slightly for both species, and the overlap was slightly lower, when the office area was excluded. Niche breadth was slightly higher in *G. pardalis* than in *K. spekii*: about 0.45 and 0.35, respectively, in both analyses. There was considerable overlap between the two species: $O = 0.76$ for the restricted data set and $O = 0.84$ for the full data.

The number of sightings was low in several vegetation types. A test of the significance of the differences in habitat association thus depends on the appropriate grouping of vegetation types. The basis of the grouping used is three *a priori* hypotheses suggested by observations of tortoises in 1992 and 1993 (rather than derived from the data being analysed):

(1). *Kinixys spekii* occurs more freqently in miombo woodland than does *G. pardalis*. In fact there was no significant association between tortoise species and miombo woodland (Table 2). There was an association, in the expected direction, when mixed miombo/grassland vegetation types (i, j) were included, but this was significant only for the total data.

(2). *Geochelone pardalis* occurs more frequently in mopane woodland than does *K. spekii*. There was no significant association between tortoise species and mopane woodland (Table 2). There was an association when mopane scrub and mixed mopane/grassland vegetation types (c,d,o) were included. This association was significant for both the total data and the data excluding the office area, but was in the opposite direction to that predicted.

(3). *Geochelone pardalis* occurs more frequently in open riverine vegetation than does *K. spekii*. There was a significant association between tortoise species and open riverine habitats (*Acacia tortilis* open woodland and riparian grassland), for both the full data and the data excluding the office area. The association was in the expected direction.

Four individuals of both species were captured on five or more occasions in three or more years. *Geochelone pardalis* were captured more frequently, but over shorter periods (Table 3); both species were captured on average in four different years. Home ranges of *G. pardalis* (mean: 26 ha) were about ten times larger than those of *K. spekii* (mean: 3.1 ha). The difference between the two species was significant: analysis of variance of log-transformed data, $F_{1,6} = 16.7$, $P<0.01$.

Two thirds of the refuges of *K. spekii* were the burrows of mammals (Table 4), principally those of the springhare (*Pedetes capensis*) and the antbear (*Orycteropus afer*). The pangolin (*Manis temmincki*) burrows were constructed by springhares or antbears, but had been occupied by a radiotracked pangolin. *Geochelone pardalis* was not found in burrows, though on two occasions the thread trail entered and left antbear holes. This species used mostly shrubs, thickets and felled trees. The association between species and use of burrows vs other refuges was highly significant ($\chi^2 = 46.3$, $P<0.001$).

TABLE 3. Movements of recaptured tortoises. Values are means (with range), or ±SD. Duration is the number of years from the first to the last capture, inclusive.

	G. pardalis	*K. spekii*
Number of tortoises	4	4
Number of captures	11.2 (6-21)	6.2 (5-8)
Number of years	4.0 (3-6)	4.0 (3-6)
Duration (years)	4.2 (3-6)	6.0 (4-7)
Home range (ha)	26±14	3.1±1.1

TABLE 4. Refuge use by trailed tortoises. Data are the percentages of *N* refuges. * Shrubs were: *Brachystegia boehmii, Colophospermum mopane, Combretum* spp., *Diplorhynchus condylocarpon, Erythroxylum zambesiacum, Friesoldielsia obovata, Grewia monticola, Julbernardia globiflora, Lannea stuhlmanni, Pseudolachnostylis maprouneifolia, Catunaregum spinosa.*

	G. pardalis	*K. spekii*
Springhare burrow	0	30
Antbear burrow	0	20
Pangolin burrow	0	16
Shrub/thicket*	46	12
Felled tree (dead)	37	18
Felled tree (live)	15	4
Grass	2	0
N	46	50

Apart from one *G. pardalis* in a grass pallet, all the refuges occupied by the tortoises provided both shelter and protection. Nevertheless, there was no indication that the tortoises selected thorny shrubs. Only one (*Catunaregum spinosa*) of the eleven taxa identified had thorns, in an environment where thorny shrubs were rather common.

DISCUSSION

Sightings of tortoises were made incidental to other work, due to the their low population density; the sighting frequency of tortoises at Sengwa was under one per observer per day (both species combined), even for an experienced observer searching specifically for them. As a result, there was no attempt to sample all habitats equally, or to avoid bias from greater ease of sighting in open habitat types. It is therefore not possible to calculate habitat preferences: that is, the number of sightings in relation to the area of each vegetation type. Nevertheless, these problems will not affect a comparison of the habitat associations of the two species of tortoise. Tortoises were found in most vegetation types, which therefore appear to have been sampled adequately.

Both species of tortoise used almost all of the vegetation types present at Sengwa. *Geochelone pardalis* has previously been noted as a habitat generalist (Greig & Burdett, 1976; Rall, 1985; Scoones, 1986). There was considerable habitat overlap between *G. pardalis* and *K. spekii*, which suggests that either (1) separation is along some other dimension, such as food, or (2) there is no competition because populations are limited by some other factor, such as predation. The diets of the two species do in fact differ widely. *Geochelone pardalis* is completely herbivorous, taking grasses as well as herbs (Milton, 1992, Rall & Fairall, 1993, and personal observations at Sengwa). *Kinixys spekii* is omnivorous, taking herbs, invertebrates and fungi (Hailey, Coulson & Loveridge, in preparation), as do other species of *Kinixys* (Blackwell, 1968).

The pattern of habitat association did not confirm two of the three hypotheses tested. The expectation that *K. spekii* was associated with miombo woodland was perhaps due to the concentration of observations around the Institute office, where this vegetation predominated. The second hypothesis, association of *G. pardalis* with mopane, was also rejected; indeed, *K. spekii* was more associated with mopane when mixed vegetation types were included. This is perhaps due to the wide variety of habitats which include mopane trees. While in our experience only *G. pardalis* are found in large areas of pure mopane woodland, both species use small patches of mopane, and mixed mopane habitats. The third pattern was confirmed; an association between *G. pardalis* and open riverine habitats, which occur along the Sengwa and Lutope rivers. This does not seem to be an association with water as such, as *K. spekii* was associated with the dense riverine woodland along the Kove river (vegetation type p).

Home range areas determined by recaptures were similar to those found by thread-trailing over a ten day period: 1.9 and 26 ha in *K. spekii* and *G. pardalis*, respectively. Bertram (1979*a*) also found a home range of 1.9 ha in a female *K spekii* radiotracked for two years. (That paper refers to the animal as *K. belliana*, but this would now be *K. spekii*; Broadley, personal communication). This female spent most time in a core area of only 0.24 ha, and it seems likely that the true home range area of *K. spekii* is indeed only a few hectares. The true home range area of *G. pardalis*, however, is likely to be greater than that measured by short-term studies or those based on few capture locations. Rall (1985) found an average home range area of 12 ha in three *G. pardalis* radiotracked over two weeks, but other studies have found substantially larger home ranges. Four home ranges estimated from Figure 3 of Van Zyl (1966) average 107 ha, and Bertram (1979*b*) gives a home range area of 160 ha for a radiotracked adult female.

Geochelone pardalis and *K. spekii* used significantly different refuges, probably related to the inability of the larger species to enter mammal burrows. Both African tortoises used refuges providing both protection and shelter, in contrast to the Mediterranean *Testudo hermanni* which often utilizes flimsy pallets (Hailey, 1989). The burrows of springhare in particular are deep and long (Butynski & Mattingly, 1979) and provide excellent protection for the small *K. spekii*, which is vulnerable to predation by several animals at Sengwa, including the ground hornbill and spotted hyaena.

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APPENDIX 1

The lettered vegetation types used here correspond to the following numerical codes for the full Sengwa vegetation map (Cumming, 1970).

a-2; b-4; c-7; d-6; e-3, 18; f-17; g-5, 10, 14, 19; h-8, 9; i-12; j-11; k-13; l-15, 16; m-22; n-23, 24, 25, 26; o-20, 21; p-1.

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NOTES ON THE LIFE-HISTORY AND REPRODUCTIVE BEHAVIOUR OF *CYNOPS ENSICAUDA POPEI* (AMPHIBIA: SALAMANDRIDAE)

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Field observations of the sword-tailed newt, *Cynops ensicauda popei* (Inger 1947), have yielded preliminary data on life-history traits and reproductive behaviour. In April 1993 the animals were abundant in the breeding ponds and on land. Compared to European species of *Triturus*, this species has an extended breeding season. Few of the observed courtship encounters progressed to the spermatophore transfer phase. Competitive encounters were frequent. The sexual behaviour of *Cynops ensicauda popei* is characterised by a short duration of display behaviour and a small repertoire of courtship behaviour patterns. The reproductive behaviour is compared to that of related salamandrids.

INTRODUCTION

Although Japanese newts of the genus *Cynops* are common within their range, their life history has not been the subject of detailed study. Aspects of the reproductive biology of the Japanese fire-bellied newt, *Cynops pyrrhogaster*, have been studied by Tsutsui (1931), Kawamura & Sawada (1959), Sawada (1963*a,b*) and Arnold (1972). Much less is known of *Cynops ensicauda*, a closely related species (Hayashi & Matsui, 1988), consisting of two subspecies *C. e. ensicauda* and *C. e. popei*, both occurring at tropical latitudes on the southern islands of Japan (Stejneger, 1907; Inger, 1947; Thorn, 1968).

Observations of the sexual behaviour under experimental conditions suggest that this species may have a long breeding period, during which the animals can mate several times. In encounters with several males present there may be strong competition, with males scrambling for females and, in the process, reducing one another's mating success by interrupting on-going courtship sequences in various different manners (Sparreboom, 1994). These observations suggest that the patterns of courtship behaviour and reproductive strategy of this species may differ from the better-known species of the sister genus *Triturus*.

To obtain complementary data on the reproductive behaviour of the species and to gather data on its life-history, the newts were studied in their natural habitat.

METHODS

The present observations were made on *C. e. popei* on Zamami-jima, from 3 to 14 April 1993. This island is located some 40 km W of Okinawa-jima. From among the many water bodies where the newts occurred, six different habitats were selected for regular inspection: (1) a 90 m stretch of roadside ditch 1 m wide with concrete walls, functioning as a drain and containing water of 5-10 cm depth, covered with debris and algae; (2) a hole in a marshy meadow, measuring 200 x 100 cm and some 40 cm deep, dug out mechanically and serving as a cattle drinking place; (3) a puddle in the same meadow, measuring 400 x 250 cm and 20 cm deep, overgrown with reeds, grasses and algae; (4) a series of cattle waterholes with submerged vegetation and cow dung, varying from 100 to 300 cm in diameter, in the marshes 2 km west of Zamami port; (5) two puddles filled with water spilt over from three large water containers on top of the hill about 1 km west of Zamami port (see below) and (6) a recently dug cattle drinking place in the meadow bordering the dam, 200 x 200 cm and some 40 cm deep, about 2 km north of Zamami port.

Sexes could be distinguished by the tail, which is longer in the female, and also by the shape and size of the cloaca, which is larger in the male and swollen in breeding individuals (Inger, 1947). Males with swollen cloacae were regarded as sexually mature adults. Sexual maturity of females was more difficult to determine since they did not all have distended abdomens. Females found in water were considered adult. Terrestrial animals of similar size were also considered adult. In animals ranging from 90 to 95 mm total length, sex could already be distinguished on the basis of relative tail-length. Females of that size were found exclusively on land and considered sub-adult.

Estimates of the sex-ratio were made by intensive dip-netting in the various water bodies of site 4 and by capturing specimens found walking in the open in different habitats, in particular the forest edge. For the study of reproductive behaviour, the sites with better visibility were monitored twice daily. In the evenings the animals could be watched under torch-light. A total of 29 hrs were spent on observation of courtship behaviour, of which 12 hrs by day and 17 hrs in the evening between 19 and 22 hrs.

The best place for observations of sexual behaviour was the tank-spill pond (site 5). This waterbody measured 100 x 200 cm and was 10 to 25 cm deep. There

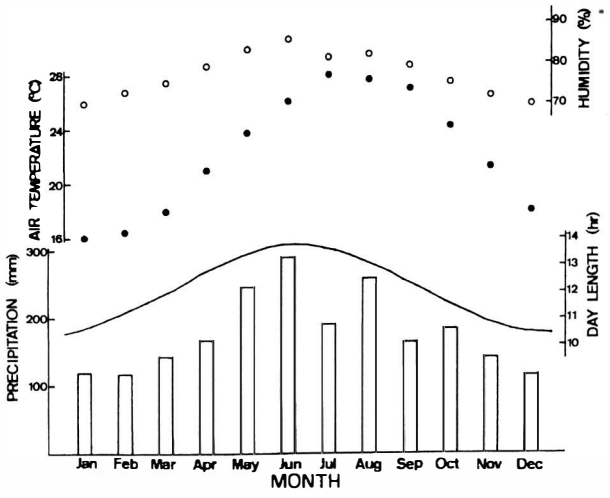

FIG. 1. Climatological data for Naha, Okinawa-jima (ca 40 km E of Zamami-jima), taken from JMAOB (1989). Open bars represent monthly precipitation; solid line day length; closed circles monthly average air temperature; open circles monthly average humidity. All values represent averages from 1951 to 1980.

were some reeds, algae and submerged grasses on one side; the floor consisted of a light coloured sandy clay and the water was mostly clear, allowing observation at very close range, close enough to recognise the newts individually by the pattern of spots on the dorsum. Newts were also wandering on land around the pond and could be seen entering and leaving the water. Other good observation sites were open spots in drainage channels, under bridges where there was less growth of weeds and algae and where the newts were also active during the day.

Fig. 1 summarises the annual climatological data for Naha, Okinawa-jima, some 40 km E of Zamami-jima, which can reasonably be expected to be similar to those for Zamami-jima. Precipitation is relatively high all year round, humidity hardly falls below 70%. During the study period there were mostly overcast skies, some rain and temperatures from 15 to 23° C.

The rains brought out the newts in great numbers. Breeding reportedly takes place from March to June or July, with the peak in April (Tago in Inger, 1947; Thorn, 1968).

LIFE-HISTORY

ADULTS

Adult newts were found in great abundance in the water bodies. They were even more numerous on land, where they were found at night roaming about. Some were seen eating. They were also active during daytime, particularly during and after rainfall. The size of adult females ranged from about 110 to about 140 mm, of males 93 to about 120 mm total length, which is broadly in agreement with existing data (Inger, 1947).

Colour patterns were extremely varied. Some animals were uniformly dark-brown to black on the dorsum. Other individuals were spotted with brown, yellow or greenish-white patches, which were scattered irregularly over the body, occasionally tending to form broad longitudinal bands over body and tail. Some animals also showed two, more or less distinct, reddish-brown dorso-lateral stripes. The ventral colour varied from a pale yellow to shades of orange and bright red, with irregular black spots or stripes. In some animals the belly showed no black markings at all, whereas in others the black parts obliterated most of the bright belly colour, leaving only a narrow coloured stripe on throat and vent. Most animals showed irregular black spots on an orange coloured vent. No sexual dimorphism was evident in the colour patterns. Two females were found which showed abnormal colouration, with mottled orange and greenish-white patches covering the entire back. Animals in the water normally had a smooth skin surface, whereas the majority of animals found on land had a more granular skin. But this was not consistent, both skin states being found amongst aquatic and terrestrial newts.

EGGS

Eggs were found mainly inside submerged and decaying grass-stalks, or between the stalks and leaves of grasses at the waters edge and between thin plant roots sticking out from the bottom and edges of ponds. Most eggs were well-hidden, and the more exposed ones were actively being preyed upon by the adult newts. This was observed on at least four occasions. Females laid their eggs singly and could be observed ovipositing both by day and at night. The females did not wrap the leaves of grasses and plants around the eggs in the well-known *Triturus* fashion, but simply pressed the leaves or twigs against the egg with their hind-feet. Eggs may also be deposited between dead leaves (Ikehara, Yonashiro, Miyagi & Toyama, 1984), or occasionally on land near the water.

LARVAE

Larvae were found in all waterbodies described above. They were most numerous in the cattle drinking holes containing vegetation (site 4). They could be spotted on the bottom of the observation ponds, contrasting with the sand-coloured floor. They were also found amongst vegetation pulled up during dip-netting. Larvae of all sizes were found in the same pond, ranging from 13 mm with remains of yolk still present, up to 47 mm, nearing metamorphosis.

JUVENILES

Juvenile newts, measuring from 50 to 89 mm total length, were found mainly during and after rain. They were found in and along the gutters, bordering a gravel road leading out of the marshes, to the west of Zamami port. These marshes probably form the main centre of dispersal of the newly metamorphosed animals. Table 1 shows the body size distribution. This finding suggests that most of the juveniles that were captured had recently completed metamorphosis. No juveniles were

TABLE 1. Body size distribution of juvenile *Cynops ensicauda popei* ($n = 75$), collected on Zamami-jima in April 1993.

Class of total length	Number	Ratio to total number
50-54 mm	10	(13 %)
55-59 mm	18	(24 %)
60-64 mm	19	(25 %)
65-69 mm	17	(23 %)
70-74 mm	5	(7 %)
75-79 mm	2	(3 %)
80-84 mm	2	(3 %)
85-89 mm	2	(3 %)

found in the water. The smallest animal found in the water was a male of 93 mm total length, with a swollen cloaca.

ADULT SEX-RATIO

The sex-ratio of newts captured in the ponds was skewed toward an excess of males, with 85% males and 15% females ($n = 222$; $\chi^2 = 109.6$; $P<0.001$), whereas the ratio of animals caught on land was almost even, with 51% males and 49% females ($n = 838$; $\chi^2 = 0.31$; $P> 0.05$). Interpreting these estimates is problematic due to possible differential 'catchability' of the sexes. The sex-ratio found in the aquatic habitat, where the animals breed, may be closer to the operational sex-ratio than that found on land.

FOOD

Newts were observed foraging both on land and in water. They were seen hunting very small animals, which could not be identified, in the clay on the bottom of the ponds. They were seen snapping at water striders (Gerridae) and could be seen eating their own species' eggs. On one occasion an adult newt was seen devouring a small, dead fish. In one of the gutter pits, an adult was observed with part of the half-decayed body of a juvenile sticking out of his mouth. Freshly captured newts, kept in a small plastic box, egested tiny snail-shells of the genus *Paludinella*, a taxon living at the water edge. On land, the newts were seen swallowing big earthworms and trying to devour snails, the shell sticking out of their mouths.

PREDATION AND MORTALITY

Adult newts appear to have few natural enemies. Cattle egrets (*Bubulcus ibis*) and white herons (*Egretta garzetta*) which were present in the marshes might incidentally prey on *Cynops*, but the newt's toxic skin secretion probably makes the animal unpalatable to many potential predators, including the birds. Even crows, which can normally be seen eating corpses on the street, did not touch the dead newts which could be found in great quantity on and along the roads. The natricine snake *Amphiesma pryeri* has been reported as preying occasionally on *Cynops* (Takara, 1962; Tanaka, 1986) and its larvae (Imaizumi, 1953). There is also an observation on record that the bullfrog, *Rana catesbeiana*, a species introduced in Okinawa-jima, preys on newts (Otani, 1987).

Many newts were found trapped in roadside gutters. These gutters are made of polished cement with 30 cm deep vertical sides, making it impossible for most animals, once fallen in, to escape. After every period of rain, the gutters were full of newts, many of which would die within a day if exposed to the sun. (Over a distance of 280 m, a gutter along a new road from the marshes eastward contained 347 recently dead adults and juveniles).

OTHER AMPHIBIANS

Rana limnocharis and *Microhyla ornata* and its larvae were found in the breeding ponds. Freshwater crabs and shrimps were found in several newt breeding localities, particularly in the sewage drains in the village and in the marshes.

SEXUAL BEHAVIOUR

BEHAVIOUR PATTERNS

The following patterns of sexual behaviour were distinguished in staged encounters of this species (Sparreboom, 1994; *in prep.*) and were also observed in the natural habitat. The terminology follows Halliday (1977) in as far as the behaviour patterns are similar to *Triturus*.

- Pursuit of the female (PURSUIT);
- Tail-fanning display (DISPLAY);
- Creeping ahead of the female (CREEP);
- SPERMATOPHORE DEPOSITION;
- SPERMATOPHORE PICK-UP;
- COMPETITION by one or more other males during fanning display;
- SEXUAL INTERFERENCE during the creeping stage.

Pursuit of the female is part of the orientation of the male towards the female: the male may swim or walk after the female with slow movements, nudge and sniff at her. He may also dash at her and try frantically to move in front of her if the female moves away from the male or ignores him.

The display consists of tail-fanning, a rapid vibration of the distal part of the tail, which is folded against the male's body on the side facing the female. Tail-fanning is performed in short bouts, alternated with pauses during which the tail is held stationary. During this display the male is more or less positioned perpendicular toward the female's longitudinal axis. Fanning may be performed after a direct approach if the female remains stationary, or after a period of pursuit. Pursuit and display may be rapidly alternating and cannot easily be separated as different stages.

Creeping is the term for a type of behaviour of the male, usually performed after some tail-fanning bouts. He turns away from the female, one or two air bubbles escape from his mouth ('guffing'), and he slowly

creeps ahead of the female, while she is following him and nudging his undulating tail.

Spermatophore deposition follows after the male has crept forward for a while and received tail-touches from the female. He pauses and raises his tail slightly, extruding a spermatophore in front of the female. When the female continues to follow the creeping male, her cloaca moves over the spermatophore. If all goes well, the sperm-cap sticks to her cloaca. The male continues creeping ahead without turning back to the female (Sparreboom, 1994).

Competition is here used to denote an attempt by two or more males to court the female simultaneously. A common form of competition consists of several males swimming after a female, trying to take up a position in front of her and attempting to fan at her. A male may also squeeze himself between a female and a fanning male and thus interrupt the courtship.

The term 'sexual interference' refers to a male's attempt to take over a female engaged in an on-going courtship encounter with another male during the creeping stage (see Arnold, 1976): the rival male puts himself between the female and the tail of the creeping male, occasionally inducing him into a fruitless spermatophore deposition by touching his tail. Subsequently the intruder displays to the female himself, or may move to creep straight away and deposit his own spermatophore (Sparreboom, *in prep.*).

Observed Patterns of Sexual Behaviour

During daytime four to ten animals were visible in the observation pond, whereas at dusk, from 10 to 18 animals could be counted. Usually the males outnumbered the females. There were four to six females in the pond; the observed number differed due to poor visibility of females during oviposition and a come and go of animals. In the course of the evening more animals would become visible or appear from outside the pond. Usually at least two or three females were laying eggs among the grasses in one corner. The others were immobile or foraging. They were only occasionally approached by males, who were usually more or less spaced out over the bottom of the pond. Occasionally the same males could be found at the same spot during several consecutive evenings, but they were not seen defending the site in any way against intruders. Their activity consisted of sitting still, foraging and slowly swimming short distances. Occasionally they would approach, sniff, nudge and briefly fan at a female.

Although females regularly attracted some attention from the males if they moved out of the reeds to the floor of the pond, there was a noticeable increase in male activity within a circle of about 40 cm, if a new and probably unmated female entered the pond. Such a female could be recognised by a silvery hue on her body, due to many tiny air bubbles on the dry skin. This appearance could last for a full day, after which her skin would become indistinguishable from that of the others. She would enter the pond and walk forward slowly, stopping from time to time, her buccal movements suggesting that she was perceiving odours from the water.

One or more males would approach the female, sniff at her and start fanning display. During this display the male would touch the female's body from time to time with his neck or snout or he would actually squeeze his snout between her and objects in the pond, when she moved away. If a displaying male was not disturbed by other approaching males, he would continue fanning, occasionally for three minutes. As a reaction to little movements of the female, the male would adjust his fanning position by moving back and forth a few steps, and in this way directing the water current more precisely to the female's snout. The female would either swim away or stay completely still for a short while and then turn her head towards the male. On this signal the male would turn round and start creeping ahead of her. If she followed him and touched his tail several times, he would deposit a spermatophore. If the female kept following the male's tail, she would eventually be led over the spermatophore which then might become attached to her cloaca. In the two observed sequences which resulted in sperm pick-up, one female was inseminated one hour after her entering the pond, another within ten minutes after arrival.

Quantification of Behaviour Patterns

A total of 100 courtship encounters were observed, 65 of which were in the tank-spill pond, the main ob-

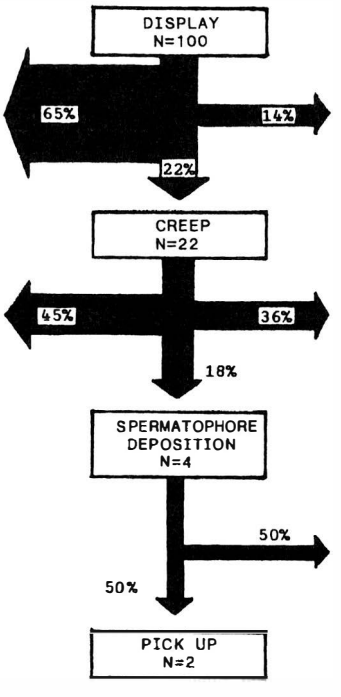

FIG. 2. Transitions between stages of courtship for sexual encounters between male and female *Cynops ensicauda popei*. Arrows that exit to the left indicate interruption of the courtship by the female moving away, arrows exiting to the right indicate that the sequence was broken off by the male, due to competition by one or several intruders.

servation site. 28% of the encounters were observed in daytime, 72% in the evening in darkness. Of the 22 courtships that progressed to the creep stage, 9 (41%) were observed by day and 13 (59%) in the evening.

Fig. 2 depicts the transitions from display behaviour onward. All courtship encounters were observed until the end of the encounter. Out of 100 courtship encounters only four progressed to the deposition of one or two spermatophores (6 in total). Of these four instances, one creep was interrupted by sexual interference without prior pick-up of the spermatophore cap, one other was also interrupted, but sperm pick-up could not be verified. In the two remaining sequences the first spermatophore was picked up successfully, but it did not progress to further spermatophore pick-ups due to interference (so the success rate was: 33% of 6 spermatophore depositions, 9% of courtships that progressed to the creep stage and 2% of the total number of observed courtship sequences).

Competitive Encounters

As there were invariably more sexually active males than females in the pond at the same time, a new female usually attracted the attention of more than one male at a time. A female might be followed persistently by two or more males and males might compete for a position to fan at the female.

Sexual interference, the form of competition taking place during the creeping stage, was observed particularly when a courting male had just turned round to creep: at this moment, neighbouring males who had not paid attention to the courting couple until that time, would suddenly approach the couple with rapid and agitated movements and attempt to move between the tail of the creeping male and the snout of the female. Alternatively, they could simply touch the female's tail, possibly depending on the position from where they started their assault on the on-going courtship sequence. Occasionally, such an approach was of a more determined nature, with the interfering male nudging forcefully or gently biting and thereby displacing the female as she was following the first creeping male. Usually, the female would react negatively to such behaviour and swim away, leaving the males behind, frantically swimming over and under and around one another, but losing the female from sight.

No instance was recorded where an interfering male succeeded in taking over the courtship and inseminating the female himself. The most successful cases of interference were those where creep and spermatophore transfer were interrupted and the first courting male was prevented from completing his courtship.

Quantification of Competitive Behaviour

Among the 100 observed courtship encounters, 25 instances of some form of competition were recorded: On 14 occasions this was an interruption in the display phase, during fanning. On eight occasions sexual interference took place during creep and on three occasions after a spermatophore deposition when the couple was creeping on (see Fig. 2). In 18 of the 25 competitive encounters two males were involved, in five encounters three males and in two encounters five different males were present at the same time.

DISCUSSION

Behaviour patterns and reproductive strategies may vary according to the progression of the breeding season, the availability of mates (operational sex-ratio) and other factors such as density of individuals at the breeding site (Verrell, 1989; Verrell & McCabe, 1988). The present observations are limited to a short period of what appears to be a peak in a prolonged breeding season and may therefore not give a fully representative picture of the mating system. However, they suggest a considerable flexibility of this species: the animals easily alternate between water and land and do not appear to be very selective in their choice of breeding habitat. Courtship takes place in deep (about 100 cm) as well as very shallow water (about 2 cm). Eggs can be laid in water and also on land. Not all adult animals simultaneously take part in reproduction. The simultaneous presence of adults in and out of breeding condition, of eggs and larvae in all stages of development, and of recently metamorphosed juveniles points to an extended reproductive season (potentially from October to June or July: Ota, *pers. observ.* in 1987 and 1990). With winter temperatures generally not falling much below 15 °C (see Fig. 1), there is no necessity for a winter break or hibernation. Precipitation and air humidity are generally high all year round (Fig. 1) and create the damp conditions under which the animals could in principle remain active a major part of the year. On the nearby island of Akajima many adult newts were found in slowly flowing and shallow waters of 28 °C. This suggests that the animals can tolerate relatively high water temperatures, so the species is able to exploit not only a variety of habitats but also a great part of the year for breeding activity. This and the scarcity of natural enemies may be an explanation for this species' abundance.

Related salamandrids such as species of *Triturus* have mating strategies that seem to be more adapted to dealing with specific ecological constraints and have more clearly synchronised periods of sexual activity (Verrell & McCabe, 1988; Verrell, 1989). Compared with such 'specialists', *Cynops ensicauda* is a 'generalist'.

The behavioural interactions observed in this study are qualitatively similar to descriptions of courtship and sexual interference in the laboratory (Sparreboom, 1994 and *in prep.*). This concurs with results obtained in similar but more extensive work on *Triturus vulgaris* (Verrell, 1984; Verrell & McCabe, 1988). The successful transfer of a spermatophore was rarely observed in the field (in 2 out of 100 encounters). This also parallels field observations of other species

(Giacoma & Crusco, 1987; Massey, 1988; Verrell & McCabe, 1988; Hedlund, 1990; Zuiderwijk, 1990; Pavignano, Sacchetto & Giacoma, 1993; Faria, in press). It may not be coincidental that the successful spermatophore pick-ups were observed on occasions where fresh and possibly unmated females entered the pond. Once inseminated, females may be unresponsive for some time. This could however not be verified at the observation site. What emerges from this study and the others cited is that successful insemination may be a relatively rare event in nature.

The observations on sexual interference suggest that a creeping male produces a substance (possible a courtship pheromone) that not only attracts the following female but also alerts other males. This may be worth testing experimentally.

Clearly the data reported in this paper are fragmentary and of a preliminary nature. Given the relatively good observability of this species as compared with others, a more extensive study of several populations and in different months of the breeding season may well be rewarding.

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SHORT NOTES

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FOOD HABITS OF MEDITERRANEAN POPULATIONS OF THE SMOOTH SNAKE (*CORONELLA AUSTRIACA*)

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Some European snakes (e.g. *Coronella austriaca*, *Natrix natrix* and *Vipera berus*) have large distribution ranges and occur from regions with cold climates at northern latitudes (e.g. Scandinavian peninsula) to regions with Mediterranean climates at the southern latitudes (e.g. Italian peninsula). These broadly distributed species are normally found in very different habitats in the various parts of their geographic ranges and thus may use different food resources.

The smooth snake (*Coronella austriaca*), a small (< 80 cm long) live-bearing colubrid, occurs from the Scandinavian peninsula (latitude about 64°N) to the Mediterranean regions of southern Europe (Greece, southern Italy) and is found from mountainous areas over 2000 m elevation to sea level (Bruno, 1984). Despite its very broad distribution range, the ecology of the smooth snake is poorly understood because of its highly secretive behaviour. Some data on the dietary habits of *Coronella austriaca* are available from southern Britain, Sweden and western France (Duguy, 1961; Andrén & Nilson, 1976; Spellerberg & Phelps, 1977; Goddard, 1981, 1984), as well as from mountain areas of the Italian peninsula (Darsa, 1972), but no data from Mediterranean habitats is available.

In this paper we present (1) data on the food habits of the smooth snake in Mediterranean environments of central Italy, and (2) address some remarks on trophic and ecological relationships of snakes in such Mediterranean habitats. All data given here were collected between spring 1987 and summer 1994 in a Mediterranean region of central Italy (Tolfa Mountains, province of Rome, about 150-400 m above sea level), during the course of long-term ecological research on the snakes of this area, especially *Vipera aspis, Coronella girondica* and *Elaphe longissima* (e.g. see Luiselli & Agrimi, 1991; Luiselli & Rugiero, 1993; Agrimi & Luiselli, 1994).

Coronella austriaca is the rarest snake species of the Tolfa Mountains (Bruno, 1977; Capula *et al.*, unpublished), where it occurs with scattered populations only at the bushy edges of wooded zones (*Quercus cerris*, *Q. pubescens* and *Fagus silvatica* forests), especially in the vicinities of spiny shrubs (e.g. *Rubus*). The rarity and the highly secretive habits of this species in the study area placed a constraint on the amount of data that could be collected, thus explaining the relatively small sample sizes.

When encountered in the field, the smooth snakes were captured by hand, sexed, measured for body length, weighed and processed in order to obtain food items, by collection and analysis of both stomach contents and faecal pellets. These were collected by gentle palpation of the snake abdomen until regurgitation or defecation occurred (see Monney, 1990; Luiselli & Agrimi, 1991). This procedure does not harm the handled specimens. The food items were identified to the lowest taxon possible. The ingested biomass (calculated only from prey contained in stomachs) was calculated by the methods of Braña *et al.* (1988), based on fresh weight if the prey was in optimal condition, or the mean weight of the species if it was not. After identification and measurement of food items, we forced the snakes to reingest the disgorged prey. When the snake did not reingest the food, the item was placed in 75% ethanol and preserved in the private collections of the authors. After laboratory examination, all remains from faecal pellets were preserved in 75% ethanol for further analysis. The data were analysed with an SAS computer package (version 6.0), all statistical tests being two-tailed and with alpha level = 0.05.

The handling of 86 different smooth snake individuals during 1987-1994 yielded a total of 44 prey items from the stomachs and faeces of 43 individuals (25 males and 18 females) (Table 1). Unfortunately, as our sample was composed only of adult snakes, we cannot give any consideration to eventual ontogenetic shift in this species' diet. However, an ontogenetic dietary shift in *Coronella austriaca* has been suggested by Goddard (1981) who predicted an innate preference for lizard prey in juveniles. A similar dietary pattern was proved to occur in other Mediterranean snakes, for instance *Vipera aspis francisciredi* (Luiselli & Agrimi, 1991).

The diet consisted almost exclusively of small vertebrates, but a single invertebrate item was found. This was a carabid beetle (genus *Pterostichus*) possibly ingested secondarily by the snake. Indeed, the stomach also contained the remains of a lizard (*Podarcis muralis*) that is an insectivorous generalist, frequently eating small terrestrial beetles (Capula, Luiselli & Rugiero, 1993). The other prey items were reptiles and small mammals. Lacertid lizards were the principal prey types in terms of both frequency of occurrence in stomachs (over 81% of the total diet) and ingested biomass (about 55% of the total) (Table 2). The wall lizard (*Podarcis muralis*) was preyed on by smooth snakes significantly more frequently than the other lacertid lizards (χ^2 test, $P < 0.05$). This may have been related to the much higher frequency of occurrence of this taxon in the relatively wet habitats frequented by *Coronella austriaca*. Other reptiles (a juvenile slow worm, *Anguis fragilis*, and a newborn viper, *Vipera*

TABLE 1. Summary of the diet data obtained from *Coronella austriaca* of the Tolfa Mountains (Rome, Italy). Data come from analysis of both faecal pellets (31 specimens) and stomach contents (12 specimens) of living snakes.

Prey type	*N* in faeces	*N* in stomach contents	*N* total	%
INSECTA				
Coleoptera (Carabidae)	1	-	1	2.27
REPTILIA				
Podarcis muralis	13	6	19	43.18
Podarcis sicula	1	2	3	6.81
Podarcis sp.	5	-	5	9.08
Lacerta viridis juv.	4	-	4	9.08
Lacertidae sp.	6	-	6	13.63
Anguis fragilis	-	1	1	2.27
Vipera aspis	-	1	1	2.27
MAMMALIA				
Muridae (*Apodemus*)	2	2	4	9.08
Total	32	12	44	100

aspis) were also found, but their relevance in terms of biomass percentage was small (Table 2). Small mammals (Muridae) were rarely preyed on by *Coronella austriaca*, but their biomass contribution to the total diet was relevant. Mediterranean smooth snakes preyed on both nesting and subadult mice. In southern Britain smooth snakes were found to prey upon adult pygmy shrews (*Sorex minutus*), with two shrews being found in the stomach of the same individual (T. Gent, personal communication). Mean ingested biomass per snake was 7.16 ± 4.05 g, that means about 17.50% of the average snake body mass ($\bar{x}$ = 40.91 ± 10.94 g). The biggest prey item found was a murid (*Apodemus*), weighing 16.3 g. The mean prey mass/predator mass ratio was 0.175 ± 0.114. This ratio ranged between 0.058 and 0.416. We did not find any correlation between prey mass and predator total length ($P > 0.9$), or between prey mass and predator mass (in g) ($P > 0.9$). Moreover, the correlation between prey mass (in g) and snake "weight status" (sensu Forsman & Ås, 1987) was not statistically significant ($P > 0.2$).

The populations of *Coronella austriaca* studied here are, to our knowledge, the southernmost populations of this taxon analysed for dietary data. Comparisons with populations living at more northern latitudes are therefore interesting to study the geographic variation in the diet composition of this widely distributed species.

Despite several hundred kilometres and a great deal of habitat difference separating our smooth snake populations from those studied in Britain, Sweden and France, the dietary habits of the Mediterranean *Coronella*

TABLE 2. Relative biomass (B in g and as percentage of the total biomass, % B) of the prey items found in the stomach contents of *Coronella austriaca* from the Tolfa Mountains (Rome, Italy).

Prey (*N*)		B	%B
Podarcis muralis	(*N* = 6)	37.0	43.02
Podarcis sicula	(*N* = 2)	10.3	11.97
Anguis fragilis	(*N* = 1)	3.6	4.18
Vipera aspis	(*N* = 1)	5.8	6.74
Apodemus sp.	(*N* = 2)	29.3	34.07
Total biomass:		86.0	100.00

austriaca populations appear to be very similar to those of other populations of this species studied previously (Rollinat, 1934; Duguy, 1961; Appleby, 1971; Darsa, 1972; Goddard, 1984). In our study area the principal prey of smooth snakes was the commonest lizard species (*Podarcis muralis*) and the commonest rodent species (*Apodemus sylvaticus*) available in the field. Thus the prey ratio taken by smooth snakes in our study areas was 9 lizards : 2 mice : 1 snake, and the ratio of abundance of different prey types was estimated, by mark-and-recapture studies, to be 12 lizards : 2 mice : 1 snake. Thus, these findings indicate some opportunism in the choice of prey by the Mediterranean populations of the smooth snake. The same was also observed in smooth snakes from central France (eating principally *Podarcis muralis*, Rollinat, 1934; Duguy, 1961) and in those from southern Britain (eating principally *Lacerta vivipara*, Appleby, 1971). With regard to British *Coronella austriaca* populations, Goddard (1984) reports that small mammals and lizards are taken in relation to their abundance and that, contrary to popular opinion, *Lacerta agilis* is not a primary prey for this snake. In our studied populations, moreover, there was also a single case of ophiophagy. Ophiophagy has been documented in this species (e.g. Darsa, 1972), but is possibly not a frequent occurrence in the field (Monney, Luiselli & Capula, *in prep.*). Lizard eggs were not observed in the guts of the examined *Coronella austriaca*, though this prey type was eaten by French specimens of the species (Saint Girons, 1955).

The stomach contents were always small. This probably results from the reduced swallowing ability of *Coronella austriaca* because of the small size of its mouth. Considering that the small size of the mouth would represent a strong constraint on the feeding performance of *Coronella austriaca*, it is reasonable to hypothesize that the remarkable homogeneity in the diet composition of smooth snake populations inhabiting very different geographic areas and experiencing very different environmental conditions, depends not only on genetically induced food preferences but also on the need for prey organisms to be either small-sized or, possibly, elongated. Goddard's (1981, 1984) view was that the genetic preference for lizards may be altered

in life to allow feeding on small mammals, depending on local resource availability.

In the territory of the Tolfa Mountains, *Coronella austriaca* is usually sympatric with both strictly terrestrial (*Vipera aspis*, *Coluber viridiflavus*) and semi-arboreal snakes (*Elaphe longissima*, Bruno, 1977). On the other hand, it is parapatric with the congeneric *Coronella girondica* and with *Elaphe quatuorlineata*, both species usually selecting more dry and sunny spots. Moreover, *Coronella austriaca* populations are especially concentrated in places where the density of the other snakes, primarily *Coluber viridiflavus*, is very low (Filippi *et al.*, unpublished). Though our data on this issue are very preliminary, we suggest that this depended on both direct predation pressure by these sympatric snakes (e.g. *Coluber viridiflavus*, that may prey on snakes, including the venomous species, Duron & Acolat, 1956) and strong interspecific competition for food. In fact, the dietary habits of young *Vipera aspis*, adults and young *Coronella girondica* and *Coronella austriaca*, young *Coluber viridiflavus* and *Elaphe longissima,* are extremely similar to each other in this Mediterranean region (e.g. compare this study with data given in Luiselli & Agrimi, 1991; Luiselli & Rugiero, 1993; Agrimi & Luiselli, 1994), and interspecific competition may be very strong amongst small snakes essentially feeding on *Podarcis muralis* and other lacertid lizards. In this regard it is noteworthy that the average biomass per ha of snakes (considering together all the species of this area) was always greater than that of lizards in 12 different study areas within the territory of the Tolfa Mountains (Filippi *et al.*, unpublished).

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HERPETOLOGICAL JOURNAL, Vol. 5, pp. 319-320 (1995)

CLOACAL SCRATCHING AS POST SEXUAL DISPLAY IN THE PALMATE NEWT (*TRITURUS HELVETICUS*)

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The courtship behaviour of European newts (*Triturus*) has been the subject of a wide literature. Following the first classification of sexual display events by Salthe (1967; see also Joly & Delsol, 1977), the elementary acts of the sequence of sexual behaviour of the smooth newt, *Triturus vulgaris,* have been precisely established by Halliday (1974, 1975). Halliday's nomenclature has provided the basis for the description of sexual behaviour in other species, these studies including the description of new acts (Halliday, 1977; Rafinski & Czaja, 1984; Pecio & Rafinski, 1985; Giacoma & Sparreboom, 1986; Faria, 1993). The courtship of *Triturus helveticus* has been described in this way by Halliday (1977) and then more precisely by Wambreuse & Bels (1984). According to these authors, the sexual sequence may end in the same way as in *T. vulgaris*. The last spermatophore transfer may be followed either by surface breathing by one or the two sexes (Halliday & Sweatman, 1976), or the male may move away from the female after performing residual ineffective acts (Halliday, 1976). The aim of the present note is to describe in *T. helveticus* a new sequence of male behaviour, which may follow spermatophore transfer, and consists of female cloacal scratching.

Newts were collected during February in the Dombes region (North-East from Lyon, South-Eastern France). Males and females were kept separately in 40 x 40 x 10 cm tanks, the bottom of which was covered with sand. Aquatic plants provided shelter. The newts were fed with *Chironomus* larvae. Observations were conducted during teaching courses in March and April, in circular 60 cm diameter dishes, the bottoms of which were covered with a fine layer of sand. Sequences were monitored according to Halliday (1975). Approximately thirty experiments involved single pairs and twenty experiments involved assemblages of two males and one female. Several sequences were monitored by a videotape recorder for a more precise description.

In about half of the successful encounters (45%), the end of the courtship was followed by a sequence of scratching of the female cloacal region by the male. This behaviour always consisted of a continuous sniffing of the female's cloaca, associated with scratching-like movements by one or both forelegs (Fig. 1). This scratching was oriented towards the anterior and posterior parts of the cloacal region. The frequency of scratching movements was between two and three movements per second. A scratching sequence was composed of 7 to 10 bouts and each of them consisted of 4 to 11 scratching movements (mean duration of a bout: 3.43 ± 0.59 s, n = 16; shortest duration: 0.58 s; longest duration: 9.33 s).

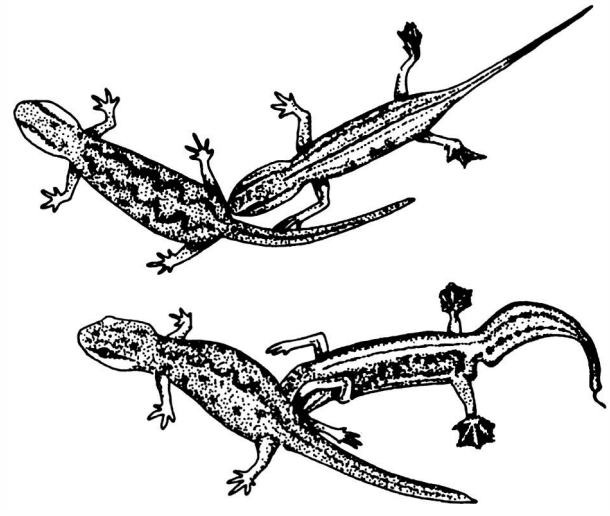

FIG. 1. Cloacal scratching behaviour in *Triturus helveticus*. Top: the male (on the right) sniffs the cloaca of the female and scratches the lower part of her tail with his left forelimb. Bottom: scratching of the female's cloacal region with the two forelimbs of the male lifted over its head.

In all cases, this behaviour did not incite the female to move away. But neither did the females always remain motionless. When a female moved while the male performed scratching, the male persistently followed the female, trying to keep close contact between his snout and her cloaca. The duration of an entire sequence was very variable, from 30 s to several minutes. Scratching ended when the male finally moved away from the female.

In most cases, scratching followed male and female post-mating breathing. It has been observed exclusively with females which had successfully taken at least one spermatophore. In the two-male experiments, the sniffing of the cloaca of a postmating female only elicited the scratching behaviour in the male from which the spermatophore(s) originated. Cloacal sniffing by the other male was always followed by his turning away. The behaviour of the scratching male may be assumed to be caused by the recognition of his own odour from the spermatophore. But it is also possible that such a behaviour can only be performed after the male displayed a complete sexual sequence including at least one spermatophore deposition. The function of such a behaviour may be to stimulate sperm transfer from the spermatophore to the female spermathaeca.

Post-copulatory display has been described in *Taricha granulosa* by Propper (1991). In this species, it consists of an amplexus which may last from 4 h to 4

days. Such a post-copulatory amplexus is assumed to prevent insemination by another male in inducing inhibition of female sexual receptivity. In *Triturus helveticus*, cloacal scratching may be supposed to play the same role.

The fact that this behaviour has not been described previously might suggest a local specificity of the South-eastern French populations. The goal of this paper also is to promote new observations in other populations, and also in the closely related species *T. vulgaris, T. boscai, T. italicus and T. montandoni.*

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SIZE STRUCTURE AND SEX RATIO OF DWARF CAIMAN IN THE SERRA AMOLAR, PANTANAL, BRAZIL

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Paleosuchus palpebrosus is one of five caiman species that occur in Brazil. With a maximum length of about 1. 6 m (Medem, 1981), it is among the smallest crocodilians in the world. *P. palpebrosus* has a highly ossified skin and, although some illegal hides have been confiscated in Amazonas (Rebelo & Magnusson, 1983), the species is of negligible commercial value. On the other hand, it is possible that some populations might be threatened by habitat modification.

This dwarf caiman apparently has strict habitat requirements in central Amazonia, where it is largely restricted to flooded forests along the margins of large rivers and lakes (Magnusson, 1985). Nevertheless, the species can be encountered from Amazonia southward through the drainages of the São Francisco, Paraná and Paraguay rivers, excluding central areas of the Pantanal (Ross & Magnusson, 1989). Despite this extensive geographic distribution, there are no published studies of *P. palpebrosus* populations in the wild. This general lack of ecological information is one factor that could eventually affect the species conservation (Thorbjanarson, 1992).

In this study we evaluated densities, size structure and sex ratios in two streams of the Serra Amolar, Pantanal. We also analysed stomach contents of dwarf caiman in one of the streams. The study area, Acurizal Ranch, is located to the north of Corumbá, next to the Bolivian border. The elevation is about 600 m above mean sea level. The two clear-water streams studied are the Fundão and the Cafezal. They arise in the nearby mountains and flow to the Paraguay River. They have bottoms of sand and rock. Water depth is 30-120 cm; width varies between 3 and 10 m.

In June 1993 and August 1994 we walked 4 km stretches of the two study streams on two consecutive nights. Dwarf caiman were captured, measured and marked. In 1993, 19 dwarf caiman were captured in Cafezal stream and 19 in Fundão stream. In 1994, 11 dwarf caiman were found in Cafezal stream and 13 in Fundão stream. Stomach contents were obtained from five juveniles (< 40 cm snout-vent length - SVL) and one adult (57.5 cm SVL) by the wash-method of Taylor *et al.* (1977).

In 1993 in Fundão, the observed density was 6.5 caiman/km and in Cafezal it was 8 caiman/km. In 1994, the observed density was 2 caiman/km in Fundão and 2.5 caiman/km in Cafezal. Caiman less than 20 cm were not included in density calculations.

The size structure in Fundão was dominated by smaller individuals, and the largest caiman we captured there was 65.6 cm SVL (Fig. 1A). In Cafezal, animals were often larger (Fig. 1B) and we found a dead caiman with SVL of 92 cm; this was larger than any individual measured by Medem (1981).

The male:female sex ratio of caimans captured in Fundão stream was 1:7.5 and 1:2 in Cafezal stream. The stomachs of five juveniles were empty except for small rocks. The one adult contained remains of crabs.

At least in 1993, observed densities of *P. palpebrosus* in the two streams of Serra Amolar were higher than those observed for *P. trigonatus* in Amazonia (Magnusson & Lima, 1991). However, in 1994, when the streams were very dry, we saw fewer caiman. Possibly, the caiman had moved to other areas within the forest, as has been observed for *P. trigonatus* (Magnusson & Lima, 1991). In addition, our recapture rate was quite low (5%), and this suggests that individuals may move into and out of the streams or that

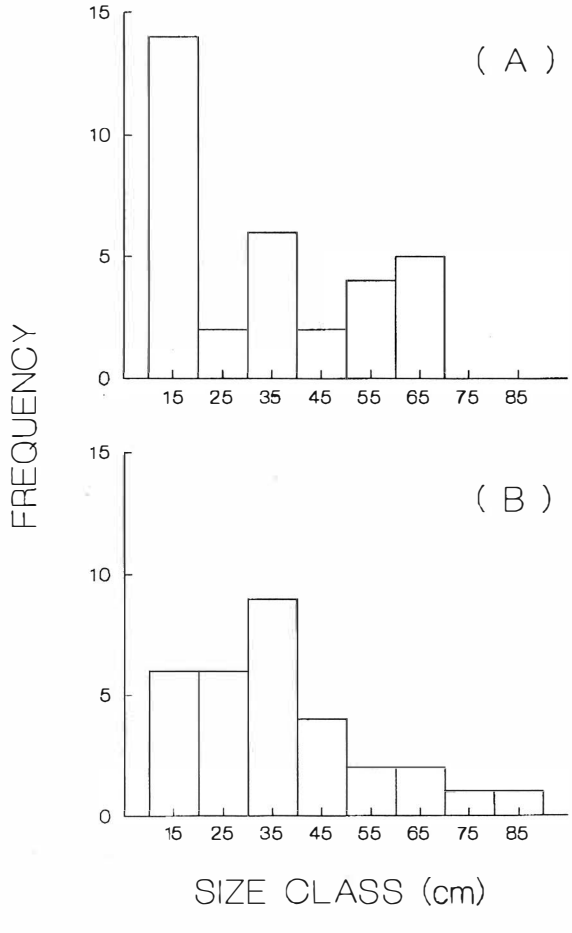

FIG. 1. Size structure (snout-vent length) of *P. palpebrosus* in (A) stream Fundão and (B) stream Cafezal, Serra Amolar, Pantanal. Size classes are in 10 cm intervals centered on the values shown.

many animals are not resident in the 4 km stretches. Furthermore, the abundance of hatchlings in both streams during both years indicates that reproduction is occurring annually. Nothing is known about the sex ratio of wild *P. palpebrosus*, but our observed female-biased sex ratio suggests that the region's relatively cool temperature might produce a female-biased hatch, as has been observed for *P. trigonatus* in Amazonian rainforest (Magnusson *et al.*, 1987).

Caiman populations and their habitat are now reasonably well protected within our study area. However, we also examined two apparently similar streams in Serra Urucum, near Corumbá. Both of these streams had been impacted by mining pollution, and we found no dwarf caiman in them

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THE ORIGIN OF OVIGEROUS LOGGERHEAD TURTLES (*CARETTA CARETTA*) RECORDED IN NORTHERN EUROPE

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Although loggerhead sea turtles (*Caretta caretta*) do not nest in northern Europe, they are occasionally recorded in this area (Brongersma, 1972). Likely possibilities for the area of origin for these specimens are the western Atlantic and the eastern Mediterranean, since major loggerhead rookeries exist in both of these regions (Dodd, 1988). Brongersma (1972) suggested that the juvenile loggerhead turtles found in northern Europe are most probably carried from the western Atlantic on the Gulf Stream and North Atlantic Current. However, since mature loggerhead turtles are capable of strong swimming, their area of origin is less readily resolved. In this study, we use the known difference in the size of mature females from western Atlantic and eastern Mediterranean rookeries to provide evidence for the probable area of origin for ovigerous loggerhead turtles reported in northern Europe.

Two loggerhead turtles were reported by Brongersma (1972) that contained large well developed eggs, indicating that they were mature females. The first was found alive on 27 December 1894 on the coast of the Netherlands and contained 1150 eggs, the largest of which were 35 mm in diameter. The second was reported alive from Scotland on 13 December 1923 and contained 1020 eggs, the largest of which were 28.6 mm in diameter. There are various methods for measuring the carapace length of turtles, with a general dichotomy between measurements made with a flexible tape-measure over the curve of the carapace (curve length or CL) and measurements of the straight line carapace length made with callipers (straight length or SL). The ovigerous loggerhead turtle reported from the Netherlands had a carapace length of 96.5 cm, although whether this referred to CL or SL was not detailed, while the specimen reported from Scotland had a CL of 104.1 cm. We compared the size of these two specimens with the size of nesting loggerhead turtles on two islands in Greece, Cephalonia (Hays & Speakman, 1991, 1992) and Zakynthos (Margaritoulis, 1982), and from Turkey (Erk'akan, 1993) (Fig. 1). The mean CL of nesting loggerhead turtles on Cephalonia was 82.8 cm (n = 57, SD = 3.7) and hence the individual probabilities of specimens of 96.5 cm and 104.1 cm coming from this population are both < 0.001 (Z = 3.7 and 5.8 respectively). Similarly, the mean CL of nesting loggerhead turtles on Zakynthos was 80.4 cm (n = 27, SD = 6.2) and hence the individual probabilities of specimens of 96.5 cm and 104.1 cm coming from this population are also both < 0.001 (Z = 2.6 and 3.8 respectively). Erk'akan (1993) reported a mean carapace length of 73.1 cm (n = 49, SD = 5.3) for nesting loggerhead turtles from Turkey, although whether this referred to CL or SL was not stated. We assumed that this measurement refers to SL and, using the relationship between CL and SL observed elsewhere in the Mediterranean (Hays, 1992: CL = 1.03(SL + 1.9)), we estimated the mean CL for this population to be 77.3 cm (and by proportional scaling SD = 5.6). Hence, as with the Greek populations, we calculated that the individual probabilities of loggerhead turtles of 96.5 cm and 104.1 cm coming from this Turkish population are both < 0.001 (Z = 3.4 and 4.8 respectively). In contrast, the length of the two ovigerous specimens recorded in northern Europe lies well within the size range for nesting loggerhead turtles in Florida and Georgia, USA (Fig. 1), suggesting, therefore, that these two specimens originated from the western Atlantic rather than from the Mediterranean.

In our calculations we have assumed that the measurement of the ovigerous loggerhead turtle recorded in the Netherlands refers to CL. In terms of our conclusions regarding the origin of this turtle, this is a conservative assumption, since if the measurement was for SL rather than CL, then the probability that this turtle was of Mediterranean origin would be even less than that calculated.

It has been suggested that some juvenile loggerhead turtles from the western Atlantic may be carried by current systems into the Mediterranean where they may then mature and breed (Groombridge, 1988). In sup-

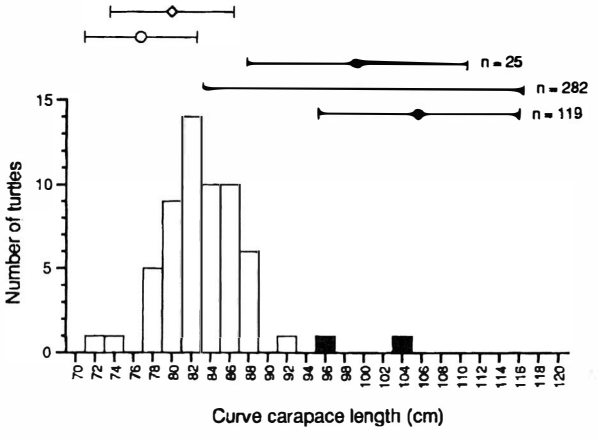

FIG. 1. The size of two ovigerous loggerhead turtles recorded in northern Europe (solid bars) (from Brongersma, 1972); the CL of nesting loggerhead turtles on Cephalonia, Greece (open bars) (from Hays & Speakman 1991, 1992); the mean CL (and range) for nesting loggerhead turtles in Florida and Georgia, USA, (●)(from three studies reviewed by Dodd 1988, the number of turtles measured in each study is indicated); the mean CL (and SD) for nesting loggerhead turtles from Zakynthos, Greece (diamond) (from Margaritoulis, 1982); the mean CL (and SD) for nesting loggerhead turtles from Turkey (open circle) (calculated from Erk'akan, 1993).

port of this suggestion, recent genetic evidence suggests first that some of the juvenile loggerhead turtles found in the Mediterranean are of western Atlantic origin (Laurent *et al.*, 1993) and that interchange between rookeries in Florida and Greece occurs at a rate of about one individual per generation (Bowen *et al.*, 1993). The evidence presented in the current study suggests that, in addition to juveniles, mature female loggerhead turtles may also traverse the Atlantic and hence this may be an additional mechanism by which trans-Atlantic gene flow may be mediated in this species.

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Accepted: 6.4.95

BOOK REVIEW

Revision of the Neotropical Snake Genus Chironius Fitzinger (Serpentes, Colubridae). J. R. Dixon, J. A. Wiest Jr., and J. M. Cei. (1993). 279 pp. Monografie XIII. Museo Regionale di Scienze Naturali, Torino, Italy. (cloth).

Chironius is a very easily recognised genus of New World snakes; it has an even number, twelve or ten, rows of scales at mid-body. Of the nearly 3000 museum specimens examined, less than one half of one per cent were misplaced to genus. On the other hand it is clear that the genus was in need of revision; nearly one half of the specimens were either misidentified or unidentified! That the species were not easily resolved is suggested by the key; there are several alternative routes by which one may arrive at identification of some of the species. The authors recommend that a specimen for identification be tested against every couplet of the key.

South America has an old and diverse colubroid snake fauna, the xenodontines. *Chironius* is a member of a more recent colubrine invasion; its affinities lie in North America and the Old World. The maxillary teeth show a slight graded increase in size from front to rear but are not otherwise differentiated; a Duvernoy's gland is however retained. With thirteen species, one of them described here and, several of them sympatric, it has achieved a modest radiation. They range from Honduras to northern Argentina. They are active diurnal snakes of terrestrial or arboreal habits. Some reach lengths in excess of two metres. The extensive data on stomach contents indicate that they prey almost wholly on frogs. There are some good colour photographs. Judging by the numbers of specimens in museums it seems that *Chironius vincenti*, confined to the island of St Vincent, is the only one which may be endangered; few specimens have ever been collected, and most of those more than a hundred years ago.

The characters employed and the method of recording them are described in detail and the scores for the taxonomic units recognised are very fully tabulated. Within four of the species the authors recognise subspecies. They depend wholly or largely on colour pattern; it is not clear that two or more characters covary across a boundary zone.

Fifteen characters are selected for the phylogenetic analysis. The states of each character are ordered and a primitive state is recognised. With a total of more than forty character states we have sufficient for the analysis of thirteen species. The species have been ranked according to the number of derived character states. This appears to provide the basis for a "pencil and paper" analysis. As the numerical characters were coded on a scale of never more than six steps a parsimony procedure could have been used. We have a plausible dendrogram on which character transformations are marked. We cannot however judge whether there are alternative analyses which merit consideration. A final portion of the work outlines a history of the genus in relation to climatic change.

Garth Underwood
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ANNOUNCEMENTS

The followng application was published on 30 June 1995 in Vol. 52, Part 2 of the *Bulletin of Zoological Nomenclature*. Comment or advice on the application is invited for publication in the *Bulletin* and should be sent to the Executive Secretary (I.C.Z.N.), c/o The Natural History Museum, Cromwell Road, London, SW7 5BD, UK.

Case 2850

***Phyllophis carinata* Günther, 1864 (currently *Elaphe carinata*; Reptilia, Serpentes): proposed conservation of the specific name**

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Abstract. The purpose of this application is to conserve the specific name of *Phyllophis carinata* Günther, 1864, which has had long usage in an extensive literature, for a snake occurring in SE China, northern Vietnam, Taiwan and southern Japan (Ryukyus). For a very short time (March-October 1891) the species was considered to be congeneric with the Central and South American snake *Coluber carinatus* Linnaeus, 1758, rendering Günther's name a junior secondary homonym. *Coluber phyllophis* Boulenger, 1891 was thus established as a replacement for *C. carinatus* (Günther, 1864) and the latter is thus formally permanently invalid. However the name *phyllophis* has rarely been used and has not appeared at all since 1929.

THIRD WORLD CONGRESS OF HERPETOLOGY, PRAGUE

2-10 AUGUST 1997

The Third World Congress of Herpetology will be held in Prague, Czech Republic, 2-10 August 1997. It will cover all fields of herpetology, including palaeoherpetology. The scientific programme will be divided into two parts by one day of professional excursions that will include areas that served for the past 50 years as military exercise fields closed to the public, with populations of amphibians and reptiles developed within this period of time in natural conditions not affected by anthropogenic activities. Other events will include an exhibition in the National Museum, 'Evolution of European Herpetofauna', and an exhibition of living European amphibians and reptiles prepared by the Terrarium Society of Prague. Social programme will include a beer party in a medieval Prague brewery, steamboat cruise on the River Vltava, and a concert of old Czech music. A wide choice of day-trips into various parts of the country for accompanying persons will be available. Post-congress tours, prepared by CEDOK travel agency, will include herp-watching in Central Asia, palaeoherps of Central Europe, and a historical tour through the former Czechoslovakia and Austria.

Futher information is available from: Dr Zbynek Rocek, Congress Director, Department of Palaeontology, Academy of Sciences, Rozvojova 135, 165 00 Prague 6 - Suchdol, Czech Republic.

Tel: ++422 24311421; Fax: ++422 24311578; E-mail: rocek@gli.cas.cz.

The British Herpetological Society,
c/o The Zoological Society of London,
Regent's Park,
London, NW1 4RY, UK

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THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS

(revised January 1992)

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.

2. *Three* copies of all submissions, and illustrations, should be sent to the Editor. All papers will be subject to peer review by at least two referees

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 (at least near-letter quality, avoid worn ribbons), and double-spaced with wide margins all round. Typesetting is greatly assisted if accepted manuscripts can be supplied on microcomputer diskettes. Authors are therefore strongly encouraged to produce manuscripts using a wordprocessor (preferably on a PC-compatible microcomputer).

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 5 words or less. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. Short notes (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. 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.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. Table l; they should be typed double spaced on separate sheets with a title/short explanatory paragraph underneath.

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. 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. 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 complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. 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 J. Physiol.* **216**, 995–1002.

9. Final acceptance of a paper will depend upon the production by the author of a typescript and illustrations 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 Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

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