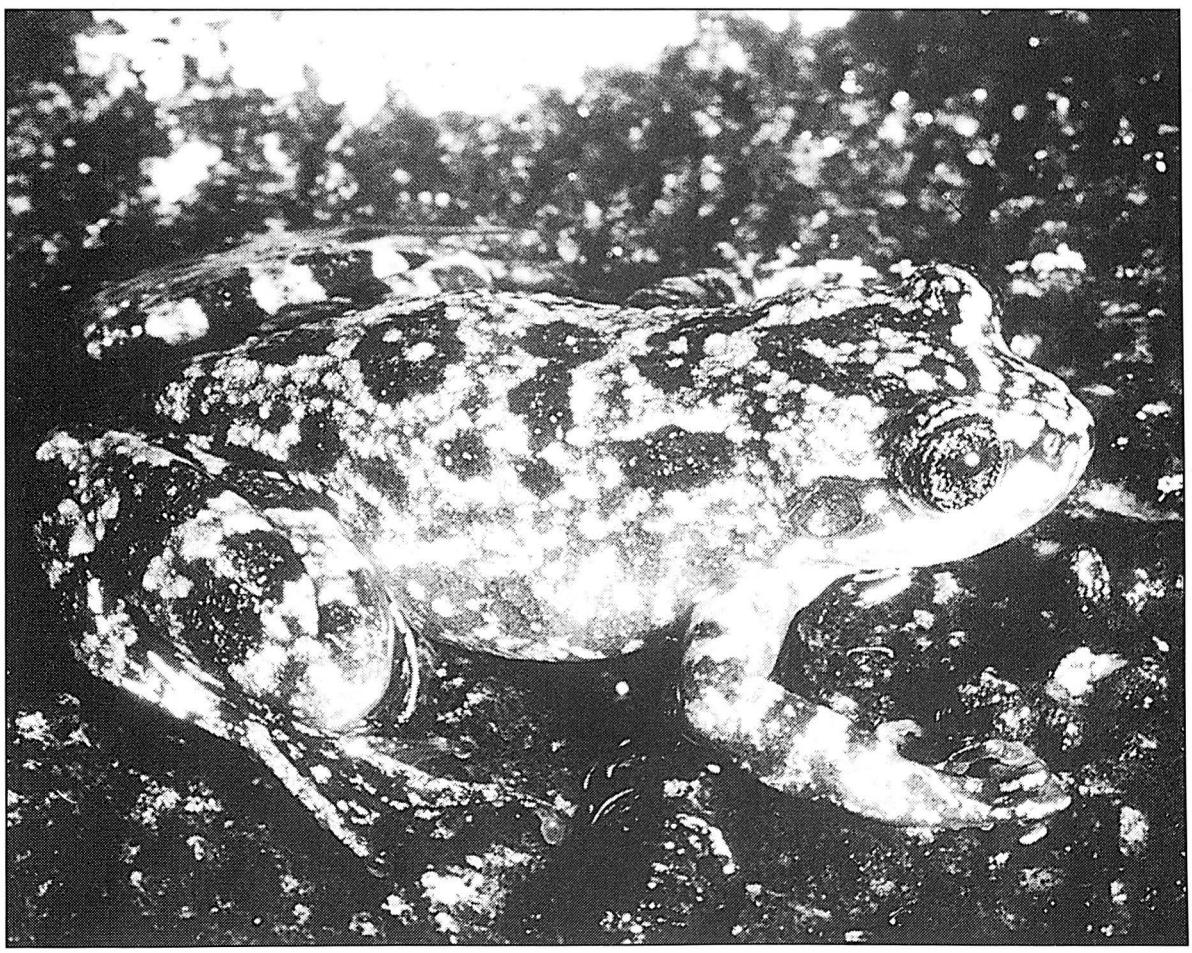
Volume 9, Number 3

July 1999
ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

The Herpetological Journal is published quarterly by the British Herpetological Society and is issued free to members. Articles are listed in *Current Awareness in Biological Sciences*, *Current Contents*, *Science Citation Index* and *Zoological Record*.

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FRONT COVER: *Mantidactylus madecassus* (see Vences & Glaw, pp. 101-110)

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 89-94 (1999)

HOW MANY WAYS CAN A SNAKE GROWL? THE MORPHOLOGY OF SOUND PRODUCTION IN *PTYAS MUCOSUS* AND ITS POTENTIAL MIMICRY OF *OPHIOPHAGUS*

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As part of its defensive display the Indian rat snake, *Ptyas mucosus*, mediolaterally compresses the anterior portion of its body and expands its throat region ventrally. During this postural display *P. mucosus* produces a deep, rumbling defensive growl. Acoustic analysis of this growl revealed it to be a long moderately loud sound dominated by lower frequencies. Binding the neck and anterior portion of the body with surgical tape prevented the postural display; while the defensive sounds produced by bound specimens had the same duration and amplitude, their frequency increased significantly. The tracheal membrane of *P. mucosus* is unusually wide and expands away from the tracheal rings into the body cavity. We hypothesize that during the defensive postural display the expansive tracheal membrane is collapsed forming isolated pockets which have a resonance effect on the exhalent airstream. *Ptyas mucosus* may be an acoustic Batesian mimic of the king cobra, *Ophiophagus hannah*.

Key words: sound production, acoustics, Batesian mimicry, colubrids, defensive behaviour

INTRODUCTION

Snakes exhibit a wide variety of defensive behaviours (for reviews see Carpenter & Ferguson, 1977; Greene, 1988) including postural displays, expulsion of a variety of fluids, and production of a variety of defensive sounds. While some of these defensive behaviours have been extensively studied, for most the underlying mechanisms and behavioural significance remain poorly known (see Greene, 1997). Similarities in defensive displays have led to several claims of Batesian mimicry in snakes. Some of the proposed examples of Batesian mimicry are generally accepted (e.g. *Echis* and *Dasypeltis*, see Gans, 1961), while others (e.g. the coral snake complex) remain contentious (see Pough, 1988; Roze, 1996).

The Dhaman or Indian ratsnake (*Ptyas mucosus*) is a large (up to 3.5 m) colubrid that is found throughout most of southern Asia and western Indonesia. *Ptyas mucosus* is often described as a nervous snake which is both quick to strike and prone to defensive displays, including the production of a distinctive low frequency sound (e.g. Wall, 1921; Minton, 1966; De Rooij, 1917). There is substantial overlap between the geographic distribution and ecological preferences of *P. mucosus* and the king cobra, *Ophiophagus hannah* (see Smith, 1943). There are several accounts in the literature describing *P. mucosus* as resembling or mimicking that of *O. hannah*, particularly in regards to their defensive behaviours and the defensive sounds they produce (e.g. Flower, 1899; Soderberg, 1973; Whitaker, 1978; Murthy, 1986; Greene, 1997). As part of its defensive display *O. hannah* passes an exhalent airstream over a series of tracheal diverticula which act as resonating chambers, acoustically modifying the hiss into a low frequency growl (Young, 1991). While similar tracheal diverticula are found in other snakes, including *P. korros*, they are lacking in *P. mucosus* (Young, 1992).

The reported similarities between the sounds of *P. mucosus* and *O. hannah*, coupled with the absence of resonating diverticula in *P. mucosus*, raise several questions regarding the acoustic biology of this species. Is the sound produced by *P. mucosus* really acoustically similar to the growl of *O. hannah*, and how is the sound produced without resonating diverticula? The current study examines the morphological basis of sound production in *P. mucosus* in an effort to document the acoustic properties of this defensive sound, the mechanics of sound production, and the extent of mimicry between *P. mucosus* and *O. hannah*.

MATERIALS AND METHODS

ACOUSTICS

Eight specimens of *Ptyas mucosus* (mean SVL = 136.4 cm, SD = 14.8) were caught in the wild in the vicinity of the Madras Crocodile Bank Trust, India. Individual specimens were placed in a large quiet room and their defensive behaviour elicited by the presence of the investigator. The defensive sounds were recorded using a ND 757B (ElectroVoice) microphone (frequency response 50-22 000 Hz), positioned approximately 20 cm from the head of the snake, and a FOSTEX X-18 recorder (frequency response 20-12 000 Hz). Amplitudes of the hisses were determined using a 840029 Digital Sound Meter (SPER Scientific), positioned approximately 30 cm away from the snake's head. At least five defensive sounds were recorded from each specimen. Acoustic analyses were performed by inputting the recordings into an Instrunet Analog/Digital converter (GW Instruments) coupled to a Power Macintosh 6500 (Apple Computers) which supported the

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SoundScope software (GW Instruments), and WLFDAP 1.0 digital filter (Zola Technologies).

Once a series of defensive hisses had been recorded, the animal was restrained and porous surgical tape (Leukopor) was wrapped around the snake's body, beginning just caudal to the head and extending for approximately one-quarter of the snake's length. The surgical tape was applied tightly enough to prevent vertical expansion of the neck region, but was not so tight as to interfere with ventilatory airflow. Once the tape was in place, the animal was released and a second series of defensive sounds was recorded as described above. Following this second series of defensive sounds, the animals were again restrained, the surgical tape was removed, and small plugs of sterile cotton were packed into the external nares and sealed in place with surgical tape. The animals were again released and their defensive sounds invoked and recorded. After this third series of recordings the narial plugs were removed and the snakes returned to the wild.

MORPHOLOGY

The upper respiratory tract was examined in three adult specimens of *P. mucosus* from the private collection of the senior author. In one specimen the trachea and larynx were dissected. In the second specimen segments of the trachea were removed, dehydrated in an ethanol series, cleared in Hemo-De (Fisher), embedded in paraffin, and 10 μm sections were cut in both the parasagittal and transverse planes. The head of the third specimen was bisected sagittally, decalcified in Cal-Ex (Fisher), dehydrated in an ethanol series, cleared in Hemo-De (Fisher), embedded in paraffin, and 12 μm sections were cut in the parasagittal plane. Slides were stained with a modified form of Van Gieson's stain (Young *et al.*, 1995); Verhoff's elastin stain (Luna, 1968) was also used to test for elastin in the sections through the trachea.

RESULTS

DEFENSIVE BEHAVIOUR

Adult specimens of *Ptyas mucosus* are alert diurnal snakes which are quick to respond to provocation. When provoked *P. mucosus* adopts a defensive posture in which approximately the anterior fifth of the body is held horizontally several centimetres off the ground. The neck and anterior portion of the body expands ventrally and narrows in width, this expansion is most pronounced immediately behind the head (Fig. 1). These postural behaviours are normally accompanied by multiple open-mouth strikes. In between these defensive strikes, while the neck region is still expanded ventrally, *P. mucosus* produces a low raspy hiss which is best described as a growl. This defensive sound is produced during exhalation while the mouth is closed – little sound is produced during inhalation .

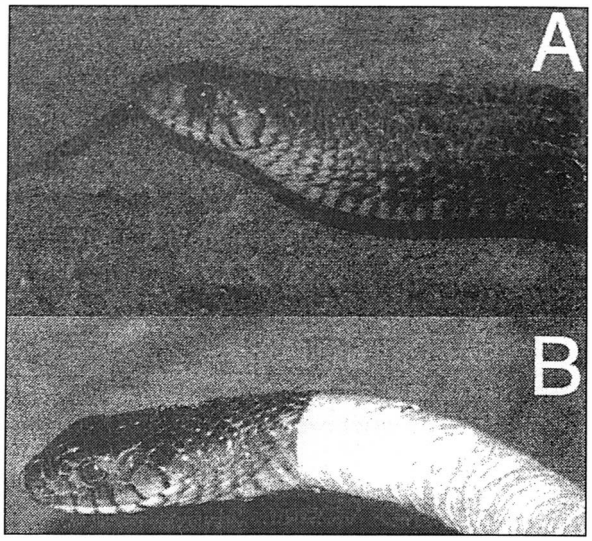

FIG. 1. Defensive posture of a 120 cm SVL specimen of *Ptyas mucosus*. Unbound state showing the ventral expansion of the throat region during growling (top); same specimen following binding of the anterior portion of the body with surgical tape (bottom).

MORPHOLOGY OF THE UPPER RESPIRATORY TRACT

The trachea of *P. mucosus* is located near the ventral surface of the body, displaced slightly to the left-hand side (Fig. 2A,C). The tracheal membrane, which connects the opposing tips of the cartilaginous rings, is expansive and extends to the right-hand side of the animal and then dorsally (Fig. 2B,C). Although this tracheal membrane is quite wide, it neither supports diverticula nor is it compressed into distinct pleats. The tracheal rings of *P. mucosus* show a gradation of cartilage types: the distal tips of the tracheal rings are hyaline cartilage; however, the majority of the tracheal ring is composed of elastic cartilage, as evidenced by differential reaction with Verhoff's elastin stain (Fig. 2D). The tracheal membrane supports a simple cuboidal epithelium and does not show any specializations for gas exchange. Between the basement membrane of the epithelium and the surrounding connective tissue, there is a distinct layer of longitudinally arranged smooth muscle fibres (Fig. 2E). The larynx of *P. mucosus* does not include any septa, partitions or diverticula.

The choana and nasopharyngeal duct of the specimens examined were large, owing to the large size of adults of this species; however, other than a constriction at the cranial end of the nasopharyngeal duct, they show no anatomical specializations. The cavum nasi proprium is also large, particularly the choanal tube. The concha is distinct, as is the expansive dorsolateral choanal zone. The choanal tube opens into the dorsomedial portion of the nasal vestibulum, which lacks any distinctive anatomical specializations. The external nares do not support an occluding flap or sphinctered valve.

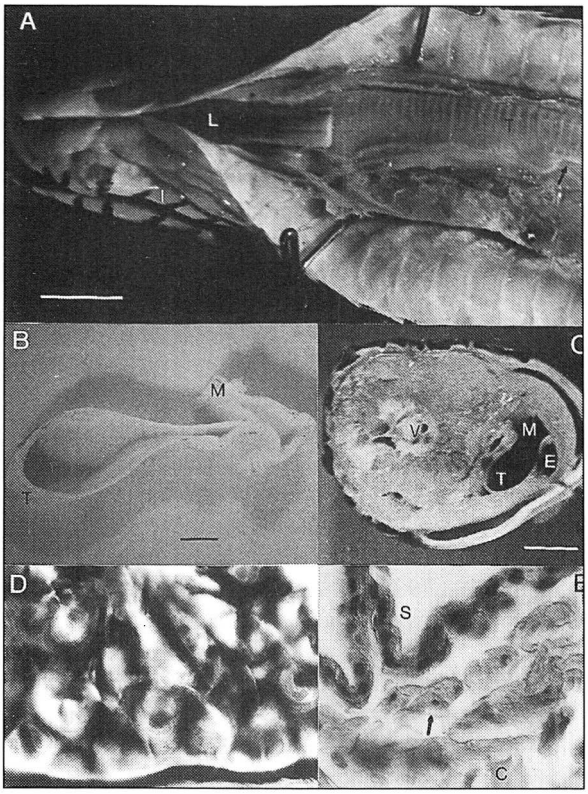

FIG. 2. Morphology of the trachea of *Ptyas mucosus*. A - ventral view of the throat region following removal of the oesophagus and caudal portion of the tongue: note the lateral displacement of the larynx and the dorsolateral position of the tracheal membrane (*arrow*) (scale bar = 1cm); B - transverse section of the excised trachea showing the relative expansion of the tracheal membrane (scale bar = 1mm); C - cranial view of a transverse section through the throat region showing the position of the trachea and the expansion of the tracheal membrane (scale bar = 5mm); D - transverse section through the tracheal cartilage stained with Verhoff's elastin stain: note the dark basement membrane and the black elastic fibers among the chondrocytes (scale bar =0.1mm); E - transverse section through the tracheal membrane stained with a modified Van Gieson's stain: note the presence of smooth muscle fibres (*arrow*) between the lumenal epithelium and the surrounding connective tissue (scale bar = 0.1mm). Abbreviations: C - loose irregular connective tissue; E - oesophagus; I - infralabial scale; L - tongue; M - tracheal membrane; S - lumenal epithelium of the tracheal membrane; T - tracheal rings; V - vertebra.

ACOUSTICS OF THE DEFENSIVE HISS

When initially approached all eight specimens produced a deep growling sound. This growl had a mean duration of 1.98 sec (SD = 0.53, range = 1.10-2.90, n = 8) and a mean amplitude of 53.9 dB SPL (SD = 2.67, range = 50.2-57.0, n = 8). Acoustic analyses (Fig. 3) revealed the growls to have a mean dominant frequency of 1019 Hz (SD = 250, range = 722-1619, n = 8), a mean maximum frequency of 6370 Hz (SD = 180, range = 6103-6803, n = 8), and a mean minimum frequency of 414 Hz (SD = 62, range = 262-546, n = 8). The growling sound showed neither temporal patterning, regular amplitude modulation, frequency modulation, nor distinct harmonics (Fig. 3).

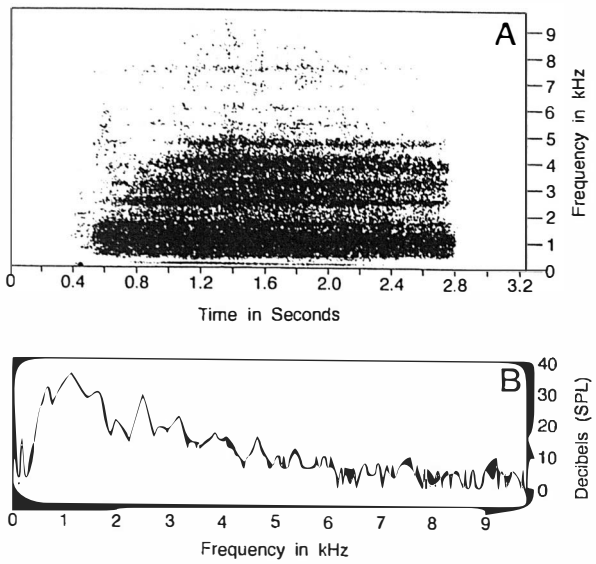

FIG. 3. Acoustic analysis of the "growl" of *Ptyas mucosus*. A - sonogram of a 2.3 sec growl: note the absence of frequency or amplitude modulation; B - power spectral analysis (FFT of 2048 points) of the same growl: note the dominance of low frequency sounds and the absence of any prominent harmonics.

Wrapping the neck and anterior portion of the body in surgical tape prevented the snake from ventrally expanding its throat region (Fig. 1). However, the specimens still produced defensive sounds. Qualitatively, these sounds were quite distinct from the growl of the unbound snake in lacking the low frequency rumbling quality of the growl, and may be more properly referred to as a hiss. The hisses recorded had a mean duration of 1.78 sec (SD = 0.5, range 1.17-3.11, n

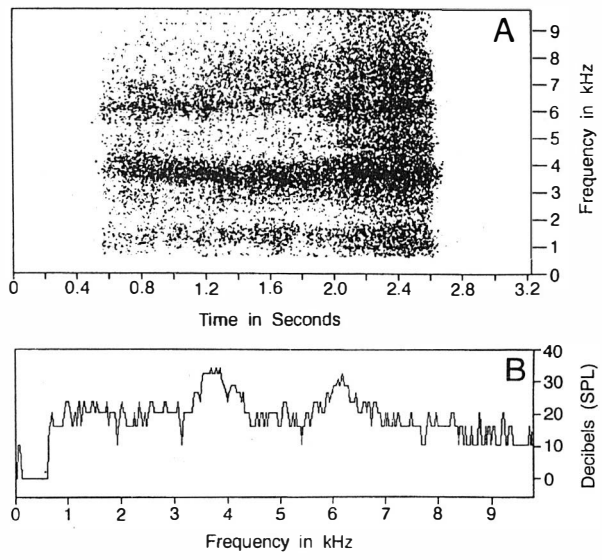

FIG. 4. Acoustic analysis of the hiss of *Ptyas mucosus*; these sounds were recorded after binding surgical tape around the anterior portion of the body of the same specimen analysed for Fig. 3. A - sonogram of a 2.0 sec hiss; note the absence of frequency or amplitude modulation and the shift to higher frequency when compared to the growl (Fig. 3); B - power spectral analysis (FFT or 2048 points) of the same hiss: note the shift in dominant frequency following the binding of the throat region.

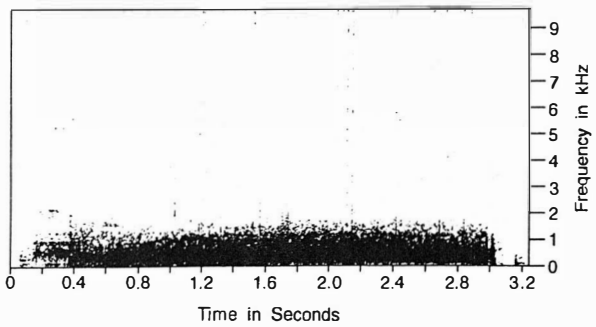

FIG. 5. Sonogram of the growl produced by a nine foot king cobra (*Ophiophagus hannah*) recorded and analysed in the same fashion as described for *P. mucosus*: note the lower frequency of the growling sound of *O. hannah* compared to *P. mucosus*.

= 8) and a mean amplitude of 53.9 dB SPL (SD = 2.67, range 50.2-57.0, $n = 8$). Sonogram and spectral analyses (Fig. 4) show that the hiss has a dominant frequency of 2,322 Hz (SD = 1037, range 897-3675, $n = 8$) a maximum frequency of 6500 Hz (SD = 371, range = 5775-7568, $n = 8$) and a minimum frequency of 566 Hz (SD = 169, range = 306-875, $n = 8$). The hiss of *P. mucosus* is an acoustically simple sound lacking modulation, harmonics, or temporal patterning (Fig. 4). Plugging the external nares eliminated defensive sound production and appeared to impede the overall activity pattern of the snake.

Statistical analysis revealed no significant regression between body size and the quantified features of the defensive sounds. Student's *t*-test revealed significant differences between the growls and the hisses in both the dominant ($t = 5.36$, df = 17, $P = 0.0001$) and minimum ($t = 3.47$, df = 17, $P = 0.0029$) frequencies. The duration, amplitude, and maximum frequency of the hiss of *P. mucosus* were not significantly different (using Student's *t*-test with a $P = 0.05$ level) from the corresponding features of the growl.

DISCUSSION

Any claim of mimicry or close resemblance between *Ptyas mucosus* and *Ophiophagus hannah* must be placed in a very restricted context. While these are both large, thick-bodied species, there are several distinctive features including the prominent occipital shields of *O. hannah*. While the general body features of *P. mucosus* are distinct from those of *O. hannah*, it is the defensive repertoire that is frequently cited as being similar (e.g. Flower, 1899; Soderberg, 1973; Whitaker, 1978; Murthy, 1986; Greene, 1997). A brief inventory of defensive behaviour makes the distinction between these two species clear: (1) body posture–*P. mucosus* holds the anterior portion of its body horizontally; *O. hannah* holds the anterior portion of its body vertically: (2) throat expansion–*P. mucosus* expands the throat region ventrally causing a mediolateral compression; *O. hannah* expands the throat region laterally into a small hood causing dorsoventral compression: (3) strike–*P. mucosus* is quick to strike (even while the harasser is well out of range, most strikes are performed with the mouth open, and multiple strikes are common); *O. hannah* is more inclined to maintain a postural stance, (most strikes are performed with the mouth closed, and multiple strikes are not as common); and (4) sound production–both species produce a low rumbling growl.

The growl produced by *P. mucosus* is acoustically similar to that of *O. hannah* (compare Figs. 3 and 5). The growl produced by *O. hannah* is of a lower frequency range (Young, 1991), but the frequency ranges of the two sounds overlap extensively. The growl of *O. hannah* is produced by a series of connective tissue diverticula which extend off the tracheal membrane and are continuous with the lumen of the trachea; airflow through the trachea produces resonance within these tracheal diverticula which results in the growling sound (Young, 1991,1992). *Ptyas mucosus*, although lacking these tracheal diverticula (Fig. 2), is capable of producing a similar growling sound which differs acoustically from most exhalatory sounds made by snakes (Young, 1991, 1997; Young, Sheft & Yost, 1995; Young & Lalor, 1998).

The nasal passageway of *P. mucosus* has a large diameter, presumably reflecting the large head and body size of this species. Other ophidians with large heads (e.g. *Bitis arietans*) produce higher frequency exhalatory sounds (Young *pers. obs.*), as do even larger monitor lizards (Young, *et al.*, 1998). Although the larynges of some ophidian species have anatomical specializations associated with sound production (Young *et al.*, 1995), no such features were observed in the larynx of *P. mucosus* (Young, 1998). When standing close to a growling *O. hannah*, it is easy to localize the growling sound as emanating from the throat, not the head; similarly, the growl of *P. mucosus* appears to emanate from the throat region. For these reasons, we concentrated our manipulations on the throat region of *P. mucosus*.

The amplitude and duration of the hisses produced by *P. mucosus* when the anterior portion of its body was wrapped with surgical tape were not significantly different from the amplitude and duration of the growls produced by the snake in its natural state. This similarity of amplitude and duration indicates that the surgical tape did not interfere with the exhalatory airflow. The significant differences in the acoustic properties of the hiss and the growl (Figs. 3 and 4), coupled with no significant differences in the amplitude and duration of these sounds, support our contention that the nasal passageway and larynx do not play a key role in producing the growl (since they were unaffected by the surgical tape) and further indicate that the growl is dependent on changes in the state of the trachea associated with the ventral expansion of the throat.

The structure and position of the trachea are such that at rest, both the tracheal lumen and the space de-

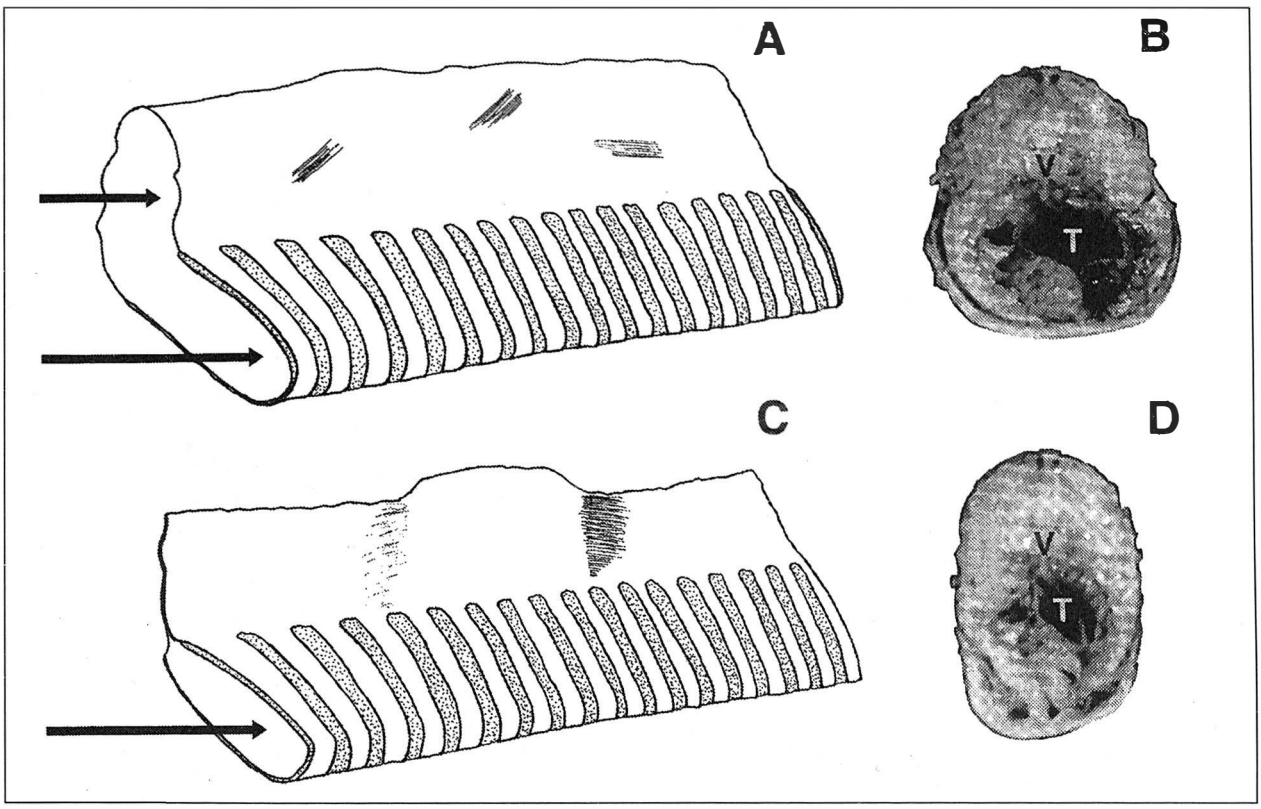

FIG. 6. Illustration of the hypothesized morphological basis of growling in *Ptyas mucosus*. A - illustration of a patent trachea and tracheal membrane allowing for complete airflow; B - transverse section near the middle of the throat showing the patent trachea and membrane; C - illustration of the trachea upon compression of the throat region showing distortion of the tracheal rings, collapse of the tracheal membrane, and localized expanded regions in the tracheal membrane; D - same transverse section used in B but with pressure applied to the lateral surfaces to produce compression and ventral expansion, note the alteration in the size and shape of the tracheal lumen. Abbreviations: T - tracheal lumen, V - vertebra.

fined by the expanded tracheal membrane should be patent (Fig. 6). Mediolateral compression of the throat region results in two changes in the trachea: the tracheal cartilages flex slightly (which brings the opposing tips of the trachea together), and the expanded tracheal membrane is collapsed down by the body wall (Fig. 6). We hypothesize that this collapse of the tracheal membrane is incomplete, particularly in the neck region, and that isolated portions of the tracheal membrane remain patent. Localized expansions of the tracheal membrane may be promoted by contraction of the longitudinal smooth muscle in the tracheal membrane. These isolated patent segments of the tracheal membrane would then function like tracheal diverticula, producing the resonant growling sound (Fig. 6). When the snake calmed down and the mediolateral compression stopped, the elastin in the cartilage would enable the trachea to return to its original state, the spreading of the opposing tips of the tracheal rings serving to expand the tracheal membrane.

There is considerable morphological variation in the structure of the tracheal membrane in ophidians, including narrow membranes, expanded membranes, diverticulae, and tracheal lungs (see Young, 1992; Wallach, 1998). The acoustic role of many of these morphological features has been explored (Young, 1997), and the tracheal membrane may also play a role in some postural displays by allowing the snake to expand its throat region (Noble, 1921; Young, 1992). If our hypothesis for sound production in *P. mucosus* is correct, it would represent the first example of a dynamic shape change in the trachea associated with sound production, and a strong example of a functional interrelationship between postural display and sound production (see Kinney, Young & Abishahin, 1998).

At the very least, our analyses document the acoustic similarities between the growls produced by *P. mucosus* and *O. hannah*. Since these sounds are only produced during defensive displays, these animals have considerable overlap in geographic range and habitat preference, and *O. hannah* has a highly toxic venom, this defensive growl would appear to be an example of acoustic Batesian mimicry.

ACKNOWLEDGEMENTS

This work was made possible by a research grant to BAY from the Academic Research Committee of Lafayette College. Part of this work was conducted at the Madras Crocodile Bank Trust; the authors are indebted to Romulus Whitaker, Indraneil Das, Gerard Martin, and the staff of the MCBT for their hospitality and assistance.

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Accepted: 7.10.98

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 95-99 (1999)

CHANGES IN BODY FLUID OSMOLALITY, ION CONCENTRATION AND NITROGEN BALANCE IN *PELOBATES SYRIACUS* DURING ONTOGENESIS

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The larvae of *Pelobates syriacus* have a long developmental period to metamorphosis. This is atypical of xeric- inhabiting anurans and suggests that the populations of this species in Israel are relicts from the time when this region enjoyed a milder climate. In contrast, metamorphic climax is rapid. In the course of a few days the external appearance changes from that of tadpole to toadlet. During the same short period, together with other internal reorganizations, the kidney completes its maturation from pronephros to mesonephros. The production of urine, a fluid which is almost identical to plasma in the tadpoles, ceases, and when it is resumed in post-metamorphic toadlets, it is dilute and adult-type with nitrogen as urea replacing ammonia. There is a simultaneous increase in plasma osmolality. In *P. syriacus* a long period of development followed by the emergence of a relatively large metamorph may be an adaptation to a hostile environment. Coupled with the rapid metamorphic climax, these life history features may increase the opportunities to disperse and burrow into soil that remains moist, at the beginning of a long hot and dry summer.

Key words: *Pelobates*, ontogenesis, osmolality, ion-balance, nitrogen excretion

INTRODUCTION

Pelobates syriacus (Boettger, 1889) is a rare and endangered amphibian in Israel. Its tadpoles are much larger than those of most anurans. Individual tadpoles may attain a gross weight of more than 25g. Development from hatching to metamorphosis is slow, taking 4-5 months. Maximum size is achieved as the first limb-buds appear and is maintained until all four legs have erupted. Weight decreases over a variable period of 1 to 2 months and the total body water content: weight of dry matter ratio falls from about 10:1 to about 5:1. Urine, which accounts for 15% or more of the gross weight in the less mature tadpoles, is reduced to a barely measurable volume as tail-length shortens.

Unlike the extended developmental period, the metamorphic climax is rapid. About a week after feeding ceases, the already shortened tail is resorbed, the skin develops the markings typical of an adult and the toadlet seeks dry land – all within a further 3-4 days. The external morphological changes are accompanied by maturation of the kidney to a functional mesonephros (Warburg & Gealekman, in prep.), an increase in plasma osmolality and a change from ammonotelism to ureotelism (Degani & Nevo, 1986). Many changes occur within a very short period; however, external features are not an accurate enough indication of the stage of internal reorganization.

P. syriacus females require fairly deep (at least 0.5 m) water in which to lay their eggs (personal observations, MRW). In years when the first heavy winter rains are late, slow development poses a serious problem so that an occasional breeding season may occur in which no tadpoles metamorphose successfully before the ponds begin to dry out. The large size and slow development of the tadpoles provide a rare opportunity to collect both blood and urine from them at several different stages and to compare the composition of those fluids with those of both newly metamorphosed toadlets and adults.

The purpose of this study was to follow the changes taking place in the osmolality and ion content of plasma and urine as well as in mode of nitrogen excretion throughout the metamorphic cycle of this rare toad. We wished to compare the pattern of stage-related changes in the plasma composition with those previously determined in the similarly large tadpoles of *Rana catesbeiana* (Just *et al*., 1977) and to determine the composition of tadpole urine.

METHODS

A limited number of larval *Pelobates syriacus* were collected from breeding ponds in Upper Galilee in the springs of the years 1990-1993 by special permit from the Nature Conservation Board, as this toad is on the Red List of species for Israel. They were maintained in the laboratory for up to three months in aged tap-water, which was changed every 1-2 days, and were fed on washed lettuce leaves. Toads which had metamorphosed in previous years were kept, individually, in moist soil and fed mealworms. Tadpoles were selected for assay at various stages (according to Zabroda & Ilyenko, 1981) from the appearance of the first leg bud (Stage 11), through attainment of maximum size (Stage 14), to reduction of the tail until considerably shorter than the hind legs (Stage 17). Four toadlets within two weeks post-metamorphosis (Stage 19), three second

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year juveniles and five adults 3-8 years old were also included.

The animals that were used to determine water-content were measured (snout-vent and snout-tail-tip), lightly blotted and weighed. Bladder urine was obtained with a fine catheter through the cloaca. In toads and toadlets, gentle pressure was applied to the abdomen to expel the bladder contents but in tadpoles, several millilitres of a clear fluid were frequently voided when the external opening of the cloaca was very lightly touched with the tip of the catheter. This fluid proved to be identical in osmolality and electrolyte composition to that obtained when the catheter was inserted. As a precaution against possible faecal contamination, samples were centrifuged for 5 min at 2000 rpm but no sediment was visible. All animals were killed rapidly to avoid water influx across the skin by immersion in 0.02M MS222 (Sandoz). This is a higher concentration than that employed for anaesthesia. The carcasses were blotted and re-weighed before being dried to constant weight at 95°C.

Animals used to assay major osmolytes in the body fluids were treated similarly, but after killing and blotting, a ventral incision was made and blood from the heart and/or fluid from the body cavity was collected. The liver of some specimens in the later stages of development was removed for the assay of the ornithine urea cycle enzymes, carbamyl phosphate synthetase (CPS) and arginase.

Osmolality was determined immediately after sample collection (Wescor 5500 Vapour Pressure Osmometer) and the remainder of the sample frozen at -20°C for later analyses. Urea and ammonia concentrations were determined colorimetrically (Sigma bulletin No. 640). Sodium and potassium were measured by flame photometry (Corning 480), and chloride on a Radiometer CMT10 chloride titrator. Ornithine urea cycle enzymes were assayed by the method of Brown & Cohen (1959). Results are expressed as mean values ± SD. Mean values were compared by Student's t-test.

RESULTS

Between stages 11 and 17, mean plasma osmolality increased from about 185 to about 215 mOsm/kg (Fig. 1) (t=2.853, df=13, P<0.01) correlating with a decrease in percentage body water content from 93.5 to 80.2 (Fig. 2; t=5.882, df=13, P<0.001). This was found to have decreased further, to 75.2%, in a single 8-year old adult. While this reflects the concentrating effect of water loss, the constant levels of potassium and chloride over the same period (Fig. 1) indicate some regulation of these ions. The electrolyte composition of body fluid and urine was almost identical in each individual tadpole throughout this period of development during which weight (3.7-25.4 g; Fig 2) and total length (5.0-16.5 cm) both fluctuated, peaking around stage 14.

At stage 11, urine accounted for about 6% of total body weight. This increased as the hind-legs developed and by stage 14 had reached about 15%. It decreased to about 3% (stage 16) as the fore-legs erupted, and very little urine remained at stage 17. Within two weeks post-metamorphosis, the urine volume of a single specimen was within the normal adult range (Fig. 3).

The osmolality of the toads' plasma was significantly higher than that of the tadpoles' body fluid

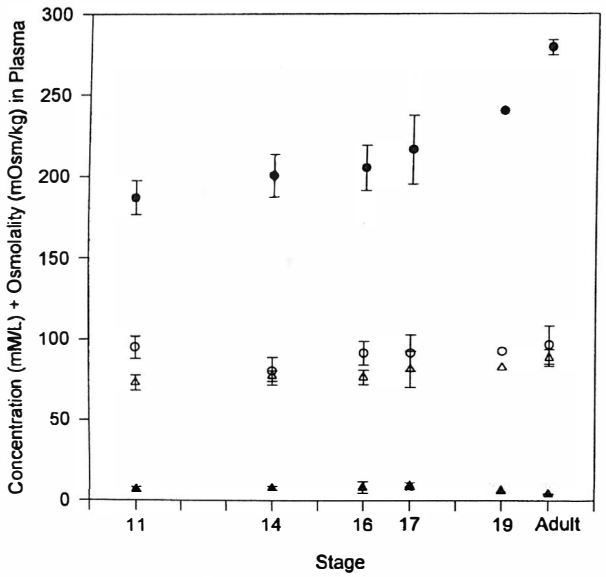

FIG 1. Total osmolality and concentrations of major electrolytes in plasma of *P. syriacus* tadpoles, stages 11-17 (mean±SD). Filled circles: osmolality; open circles: sodium; filled triangles: potassium; open triangles: chloride. Stage 11, n=5; stage 14, n=3; stage 16, n=5; stage 17, n=10; stage 19, n=1; adult, n=4.

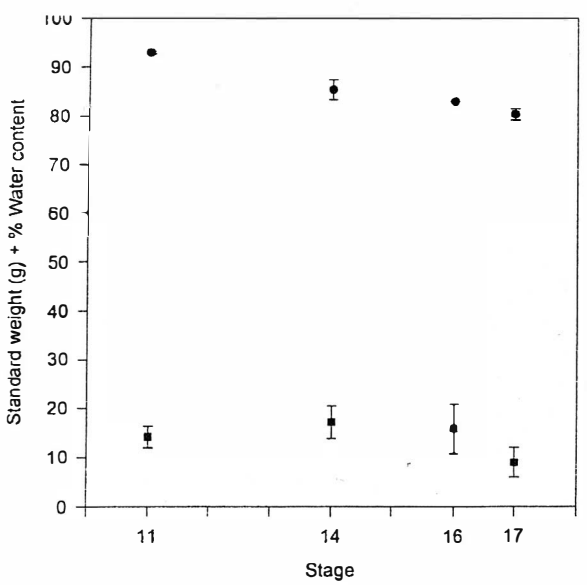

FIG 2. Percent water content and standard weight of *P. syriacus* tadpoles, stages 11-17 (mean±SD). Circles: water content; squares: standard weight. Sample sizes (stages 11-17) as in Fig. 1.

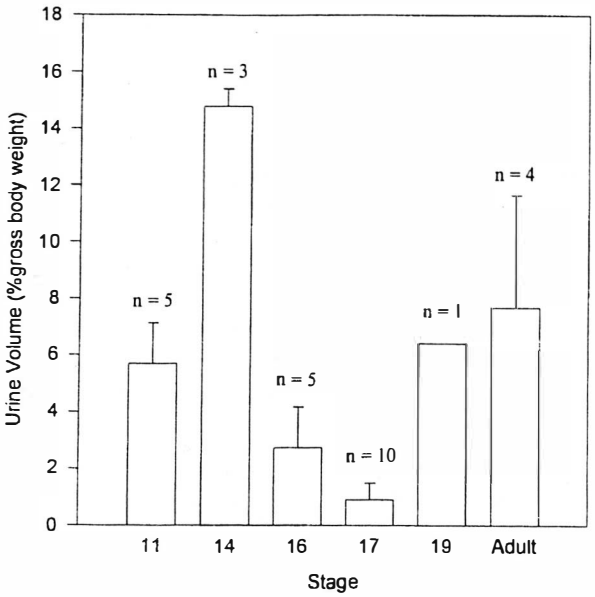

FIG 3. Urine volume expressed as percent total body weight, which itself reaches maximum at stage 14. The stages (Zabroda & Ilyenko, 1981) are not of equal duration; 11-16 together take several weeks, while 17 is completed in a few days. *n* = number of animals.

(t=8.282, df=36, P<0.001). The concentrations of sodium and chloride were increased (sodium: t=1.829, df=33, P<0.05; chloride t=5.090, df=37, P<0.001), and the presence of urea contributed to the increased total osmolality (Table 1). Apart from an increased concentration of urea, there was no significant difference in the composition of the blood of a single 8-year old animal and that of three newly metamorphosed individuals (Table 2). The urine was dilute, with the summed sodium and chloride concentrations about 17% of that in the plasma. Weights varied from 4.1 g in a 1-year old toad (6.2 g in a newly metamorphosed toadlet) to 46.0 g at 3 years; water content decreased from 81.6 to 75.2%. The data were, necessarily, collected from a small number of animals (according to permit issued by the Nature Conservation Board).

Ammonia ($Amm_T = NH_3 + NH_4^+$) was found at concentrations between 5 and 15 mM in the plasma of all stage 11 tadpoles, but not in stages 12-14. Much higher concentrations (~50 mM) were present in a few stage 16 and several stage 17 animals, although none was present in others at the same apparent level of development. The urine profile was similar but with slightly lower values to that of plasma.

TABLE 2. Plasma osmolality and standard (bladder-empty) weight in *Pelobates syriacus* toadlets and toads.

Stage	Age	*n*	Osmolality (m Osm/kg)	Mass (g)
Postmetamorph	5 days	1	228	6.1
Postmetamorph	1 week	2	252	6.2
Postmetamorph	2 weeks	1	240	6.6
Juvenile	1 year	3	293±12.0	4.3±0.55
Adults	2 years	2	279	25.4
Adults	3-4 years	2	280	12.3
Adults	8 years	1	264	46.0

Low levels of arginase activity (sp. act. ~15 µmoles urea mg^{-1} protein-h^{-1}) were detected in the liver of some tadpoles just before the onset of metamorphic climax (stage 17). This remained unchanged at stage 19, but in juveniles and adults the specific activity of this enzyme was more than twice as high. The earliest appearance of any CPS activity (*ca.* 10 µmoles^{-3} citrulline mg^{-1} protein h^{-1}) was in a toadlet 1 week after metamorphosis. This was within the range of adult values (10-20 µmoles^{-3} citrulline mg^{-1} protein h^{-1}).

DISCUSSION

During development from stages 11-17, tadpole plasma osmotic pressure gradually increased from 185 to 215 mOsm/kg. The difference was significant but cannot be attributed to an increased sodium concentration (Fig. 1) as was found in *R. catesbieana* (Just *et al.*, 1977). The total plasma osmolality and concentrations of major osmolytes in toads ranging in age from newly metamorphosed to 8 years, are in the range found previously in this species (Degani *et al.*, 1983; Shpun *et al.*, 1992; 1993), but the osmolality of the urine is higher largely because of an unexplained elevated chloride concentration. No previously published data could be found which gave changes in these fluids at metamorphic climax, but in *R. catesbieana* plasma osmolality rose from 160 to 259 mOsm/l, and in *Scaphiopus hammondi* from 190-290 mOsm/l (Funkhouser, 1977). The increase in *P. syriacus* is of a similar magnitude.

The urine (but not plasma) of larval *Caudiverbera caudiverbera*, an anuran which is fully aquatic throughout its life-history, was collected at various stages of development (Zamorano *et al.*, 1988), but only the ex-

TABLE 1. Plasma and urine composition (Mean ± SD) of *Pelobates syriacus* tadpoles (stages 11-17), toadlets and toads.

	Tadpoles				Toadlets and Toads			
	n	Plasma	*n*	Urine	*n*	Plasma	*n*	Urine
Osmolality (mOsm/kg)	26	206±23.1	25	203±26.1	12	270±19.8	7	89±29.8
Sodium (mM/l)	27	88±10.8	20	88±10.3	8	96±11.1	3	12±1.7
Potassium (mM/l)	27	9.0±0.77	20	3.3±0.50	8	4.6±1.04	3	0.8±0.33
Chloride (mM/l)	27	76±9.1	24	78±11.6	12	94±12.4	7	22±6.6
Ammonia (mM/l)	22	17±20.8	24	10±17.1	-	-	-	-
Urea (mM/l)		-		-	12	32±25.3	6	26±12.8

cretion rates of ammonia and urea were published. *C. caudiverbera* larvae gradually become ureotelic before the completion of metamorphosis. In *P. syriacus* the changeover is abrupt. Little or no urine appears as metamorphic climax approaches, and the fluid produced by post-metamorphic toadlets is the first to contain urea. This coincides with the first appearance of CPS activity in the liver and these two observations, together, suggest that the ornithine urea cycle becomes functional, in this species, only at metamorphosis.

Renal function also undergoes a rapid change during metamorphosis. The amphibian larval kidney, a pronephros, persists in anurans until metamorphosis (Fox, 1963; Michael & Yacob, 1974). Its primary function is to excrete water in order to balance the influx across the water-permeable integument. During the tadpoles' growth the mesonephros develops and gradually takes over. In *P. syriacus* tadpoles, urine is so similar in composition to that of the plasma that it must be presumed that plasma electrolytes are replaced adequately from diet as the bathing fluid is almost salt-free (Na^+ 5mM, Cl^- 15mM). The pronephros degenerates and is replaced by a mesonephros which on completion of metamorphosis has assumed exclusive function (Pons *et al.*, 1982). Adult-type urine is produced within the first week of emergence on to dry land.

The long period of larval development of *P. syriacus*, although found in other members of this genus living in more temperate climes, is atypical of xeric inhabiting anurans. Rapid growth and a short larval period to ensure metamorphosis before ephemeral ponds dry out is much more usual (Warburg, 1988). The long developmental period, coupled with the females' "reluctance" to deposit eggs in shallow water, is habitat restrictive, in that it confines breeding to a limited number of ponds; consequently, it places the species at considerable risk from the cumulative effects of those human activities which involve changes in land usage. The sympatric anuran, *Bufo viridis*, is not subject to the same constraints. It breeds successfully even in temporary pools in the Negev desert (Dimentman & Margalit, 1981) and develops more rapidly than *P. syriacus*. Despite the same depredations, *B. viridis* remains fairly widely distributed from Galilee to the Negev.

P. syriacus, in Israel, is probably a relict population from the time when this region enjoyed a milder climate, at the end of the last glaciation period (about 10,000 years ago; Butzer, 1958). It owes its limited survival, in great part, to its longevity (15 years old from tadpole in our lab). The relationship between maximal larval size and metamorph size is therefore worthy of further exploration in this species. It has been suggested that larger metamorphs have greater locomotory capabilities or greater stamina (Newman & Dunham, 1994) either of which confers an advantage in the first, vital search for a suitable habitat. Once it leaves its pond, the toadlet must quickly find a patch of moist soil in which to burrow. The rapidity of the final stages leading to metamorphosis may be an adaptation to climatic conditions but the trigger is not known.

In the laboratory, under controlled conditions of temperature and food availability, animals successfully metamorphose about two weeks before the summer solstice. This regularity suggests that while growth rate and development of tadpoles are strongly influenced by abiotic factors – particularly the size of ephemeral breeding ponds which are dependent on winter rainfall – the time of actual metamorphosis is possibly regulated by an internal clock. In the present climatic conditions of this region, this timing is not optimal for a successful search for a microhabitat of damp soil and may represent behaviour inherited from earlier times.

ACKNOWLEDGEMENTS

We thank Mrs Mira Rosenberg for her skilled technical assistance. We also wish to thank the Nature Conservation Board for a special permit to collect and experiment on a limited number of *P. syriacus* tadpoles and toadlets.

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Accepted: 18.1.99

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 101-110 (1999)

VARIATION IN *MANTIDACTYLUS MADECASSUS* MILLOT & GUIBÉ, 1950, A LITTLE KNOWN MALAGASY FROG, WITH RESURRECTION OF *MANTIDACTYLUS PAULIANI* GUIBÉ, 1974

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Based on morphological differences, specimens currently attributed to the Malagasy montane frog species *Mantidactylus madecassus* can be divided into two distinct groups, which correspond to the geographically separated populations from the mountain massifs of Ankaratra and Andringitra. The Ankaratra populations differ from the Andringitra populations by the lack of distinctly bilobed subarticular tubercles on the fingers; more extended webbing between the toes; and a less contrasting dorsal colour pattern. Furthermore, they are distinguished morphometrically. The name *Mantidactylus pauliani* Guibé, 1974 is available for the Ankaratra specimens and is hereby resurrected. A lectotype of *M. madecassus* is designated. The two species share a lack of vomerine teeth and both possess a very short snout, and should be considered as closely related allopatric sister taxa. So far as is known, they occur between 1500 and 2500 m altitude (mainly above 2000 m), in brooks and their tributaries in areas of ericoid vegetation or of rock formations with rupicolous plant communities. A short review of Malagasy montane amphibian species is provided, confirming that montane habitat in Madagascar harbours an important diversity of species specialized to high-altitudes.

Key words: Anura, Ranidae, Mantellinae, *Brygoomantis*, montane herpetofauna, Madagascar

INTRODUCTION

Madagascar contains a rich diversity of habitat types, mainly due to the variety of climates. The eastern rainforest belt is separated from the western arid regions by a high plateau on which special montane ecosystems are found. The three highest massifs are Tsaratanana in the north (2876 m), and Ankaratra (2642 m) and Andringitra (2658 m) in central Madagascar. The Malagasy montane herpetofauna is known from extensive collections, harboured mainly in the Museum National d'Histoire Naturelle (MNHN), Paris, but basic information on the biology and ecology of most species is still lacking.

Recently, Raxworthy & Nussbaum (1996*a*) reviewed the montane amphibian and reptile communities of the Malagasy massifs of Andringitra, Ankaratra, and Tsaratanana, based on their own surveys. They found that a relatively large number of species are restricted to the montane heathland, and rejected the hypothesis that this habitat is artificial and faunistically depauperate. In another publication (Raxworthy & Nussbaum, 1996*b*), the same authors emphasized the similarities in the montane herpetofauna between the Ankaratra and Andringitra massifs.

One frog species so far known only from high altitudes of these two massifs is *Mantidactylus madecassus* (see Millot & Guibé, 1950; Blommers-Schlösser & Blanc, 1991), which was not collected in the survey of Raxworthy & Nussbaum (1996*b*), and thus not included in the list of montane amphibians from Madagascar published by Raxworthy & Nussbaum (1996*a*).

M. madecassus is a representative of the most speciose and heterogeneous anuran genus in Madagascar. According to the most recent descriptions (Vences *et al.*, 1997; Glaw & Vences, 1997; Andreone *et al.*, 1998), the endemic genus *Mantidactylus* currently contains 63 described species classified into 12 subgenera (Dubois, 1992; Glaw & Vences, 1994). *M. madecassus* is included in the subgenus *Brygoomantis* Dubois, 1992 (formerly *Mantidactylus ulcerosus* group) which currently consists of seven valid species (Blommers-Schlösser & Blanc, 1991, Glaw & Vences, 1994). *Brygoomantis* are distinguished from representatives of other subgenera of *Mantidactylus* by a derived karyotype (chromosome number $2n = 24$, see Blommers-Schlösser & Blanc, 1991; not known in *M. madecassus*) and a combination of femoral gland structure (glands including a prominent rounded structure with external median depression, rudimentary glands present in females), sexual dimorphism in tympanum size (males having a larger tympanum than females), slightly distensible single subgular vocal sac in males, slightly enlarged finger and toe discs, semiaquatic and partly diurnal habits, tadpoles with generalized mouthparts and distinct spiral-shaped intestine visible through ventral skin, and advertisement call structure (series of pulsed calls with low intensity).

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In the course of our ongoing revisions of the frog genera of Madagascar, we examined the type material of *Mantidactylus madecassus* and of its junior synonym *M. pauliani*. In the present paper, we give a detailed redescription of both taxa and revalidate *M. pauliani* based on several morphological and morphometric differences. We also provide an updated list of montane amphibians of Madagascar, and a comparison between the herpetofaunas of Andringitra and Ankaratra.

MATERIALS AND METHODS

The following morphological measurements were taken with a calliper to the nearest 0.1 millimeter: SVL (snout-vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus). All available specimens of the taxon *pauliani* were measured, whereas in *M. madecassus* specimens measured were selected according to their state of fixation, in order to get a sample in a comparable state to the *pauliani* specimens. All measurements were taken by the same person (MV). Institutional abbreviations are as listed in Leviton *et al.* (1985). Webbing formula follows Savage & Heyer (1967) as modified by Myers & Duellman (1982) and Savage & Heyer (1997). To facilitate comparisons with other species of *Mantidactylus,* we also give the formula used by Blommers-Schlösser (1979) and most subsequent authors who have published accounts on Madagascan anurans.

Femoral glands were examined and photographed under a stereo-microscope. Our description refers to the macroscopic appearance of the gland on the ventral femur. In a few specimens, we also carefully removed the skin of the ventral femur and turned it upside down; by this procedure, the gland structures, which remain completely attached to the skin, could be examined in more detail and could easily be distinguished from simple granular thigh patches as present in many frogs.

Morphometric data were processed statistically with the software package SPSS for Windows, version 6.1.2. Samples were compared for representative ratios by non-parametric Mann-Whitney *U*-tests. Data were transformed logarithmically ($\log_{10}$) to render relationships between them linear. A Principal Component Analysis (PCA) was carried out using the $\log_{10}$-transformed data (three factors extracted).

RESULTS

VARIATION IN *M. MADECASSUS*

According to Blommers-Schlösser & Blanc (1991), *M. madecassus* is known from seven localities: Ankaratra, Nosiarivo, Ivangomena, Andohariana, Ambalamarovandana, and Anjavidilava. The first two localities are located in the Ankaratra massif and refer to the type series of the taxon *Mantidactylus pauliani* Guibé, 1974 (which was synonymized with *M. madecassus* by Blommers-Schlösser & Blanc, 1991), and to several ZMA specimens previously also referred to *M. pauliani* (see Blommers-Schlösser, 1979). All other localities are located in the Andringitra massif according to the map in Blommers-Schlösser & Blanc (1991), which did not include information on voucher specimens. We found MNHN vouchers for all mentioned sites except Ambalamarovandana. Several other localities are corroborated by MNHN specimens and by one ZFMK specimen (Fig. 1); all these sites are located close to each other within the Andringitra massif.

Detailed examination and direct comparison of all available specimens demonstrated that they can be classified into two distinct groups, corresponding to the Andringitra and Ankaratra samples, respectively. Differences are (a) subarticular tubercles on fingers (very prominent, mostly bilobed in Andringitra specimens, but single and indistinct in those from Ankaratra; Fig. 2); (b) webbing (more extended in Ankaratra, generally reaching the disc of the fifth toe); and (d) colouration (darker and more uniform in Ankaratra).

Additionally, distinct morphometric differences between both samples were found. Measurements of all available Ankaratra specimens and of a representative

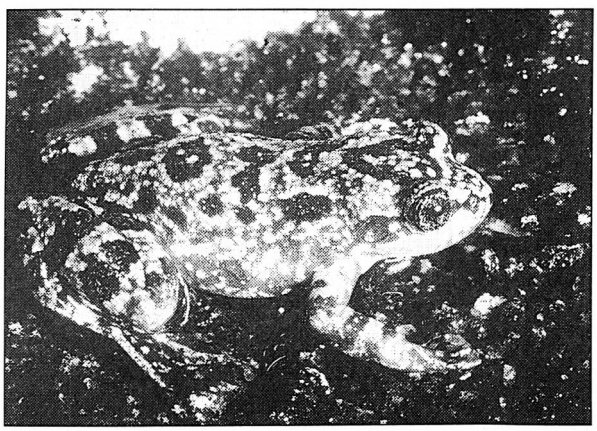

FIG. 1. *Mantidactylus madecassus* (ZFMK 57416 from Cuvette Boby, Andringitra) in life.

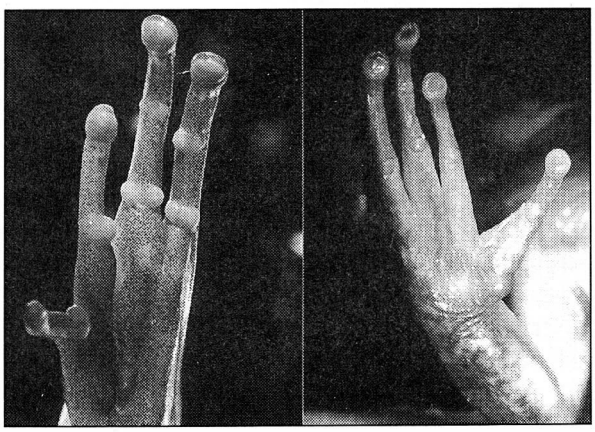

FIG. 2. Ventral side of hands of type specimens of *M. madecassus* (MNHN 1953.246; left) and *M. pauliani* (MNHN 1972.1508, right). Not to scale. Note difference in shape of subarticular tubercles.

TABLE 1. Measurements (in mm) of adult specimens of *Mantidactylus madecassus* and *M. pauliani*. See Materials and Methods section for abbreviations of characters. LT = lectotype, PLT = paralectotype, HT = holotype, PT = paratype.

	Status	Sex	SVL	HW	HL	TD	ED	END	NSD	NND	FORL	FOTL	HAL	HIL	FL
M. madecassus															
MNHN 1953.246	LT	F?	27.5	9.6	9.7	1.9	3.1	1.8	2.2	3.0	16.6	22.4	7.9	47.0	15.3
MNHN 1989.3591	PLT	M?	25.4	8.0	8.6	1.6	2.2	1.7	1.8	3.0	12.7	21.3	6.2	36.1	12.0
MNHN 1989.3592	PLT	F?	31.0	9.6	10.2	2.3	3.0	1.8	2.0	3.0	16.7	21.0	7.6	43.1	14.5
MNHN 1989.3594	PLT	F?	29.2	10.6	10.3	2.9	3.2	2.0	2.2	3.3	16.3	17.2	7.7	43.7	14.7
MNHN 1972.1182	-	F	32.0	10.8	11.1	2.3	3.5	1.9	2.6	3.2	17.2	24.8	8.5	51.0	18.3
MNHN 1972.1185	-	F	32.4	10.9	11.0	2.2	3.3	2.1	2.7	3.1	17.1	25.2	8.7	50.5	17.7
MNHN 1972.1190	-	M?	27.0	9.2	9.9	1.7	3.1	2.0	2.2	2.8	15.3	21.1	7.0	42.4	14.2
MNHN 1972.1192	-	F	33.7	10.9	11.2	2.6	3.5	2.0	2.5	3.4	18.0	25.1	8.4	49.6	16.7
MNHN 1972.1198	-	M	27.0	9.4	9.9	2.0	3.3	1.7	2.4	3.0	15.5	20.1	7.8	45.4	15.0
MNHN 1972.1199	-	M	29.8	9.9	10.7	2.9	3.4	2.0	2.1	3.3	17.6	23.7	8.8	48.6	16.8
MNHN 1972.1204	-	F	29.3	9.5	10.0	2.0	3.1	1.8	2.2	3.3	16.2	25.3	7.7	44.7	13.1
MNHN 1972.1206	-	F	31.4	10.5	10.2	2.1	3.5	2.1	2.3	3.4	19.0	21.4	8.5	51.4	17.2
M. pauliani															
MNHN 1972.1508	HT	M	31.0	11.0	11.0	2.5	3.1	1.8	2.3	2.6	18.0	25.7	8.9	50.4	17.2
MNHN 1972.1509	PT	F?	29.7	11.2	11.7	2.8	3.1	1.9	2.1	3.1	17.4	24.4	9.0	47.5	17.0
MNHN 1972.1510	PT	F	33.7	11.5	10.8	2.2	4.0	1.6	2.2	3.1	18.7	24.2	8.9	48.7	17.0
MNHN 1972.1511	PT	F	31.1	10.7	10.8	2.0	3.3	1.5	2.1	2.7	17.6	23.8	8.3	47.1	16.0
MNHN 1972.1512	PT	F?	27.7	10.0	10.5	2.2	3.4	1.5	2.1	3.0	17.5	24.2	8.7	42.1	17.0
MNHN 1972.1513	PT	F?	25.8	9.6	9.7	2.1	3.4	1.7	1.9	2.7	16.6	23.1	8.4	44.9	15.8
MNHN 1972.1514	PT	M	29.5	11.0	11.3	2.7	3.3	1.6	2.1	3.0	17.7	24.5	9.0	47.4	16.7
MNHN 1972.1515	PT	F	32.9	10.6	11.1	2.2	3.4	1.6	2.0	3.1	18.2	23.8	9.6	49.1	16.7
MNHN 1972.1516	PT	M?	31.6	11.5	11.9	3.1	3.5	1.7	2.3	3.0	18.9	25.5	8.9	50.1	17.3
ZMA 6803 (1184)		F	31.6	10.1	10.4	2.0	3.4	1.4	2.3	3.0	16.5	22.1	8.1	45.5	15.6
ZMA 6803 (1185)		F?	27.5	9.2	9.3	2.3	3.4	1.4	1.7	3.1	15.7	21.8	7.8	42.8	15.2
ZMA 6803 (1186)		F?	26.9	8.8	9.2	1.6	3.2	1.4	1.8	2.7	16.0	21.4	8.6	44.8	14.7
ZMA 6803 (1187)		F?	24.4	8.6	9.1	1.6	3.1	1.3	1.7	2.8	15.0	20.2	7.4	41.6	14.0
ZMA 6803 (1188)		M?	24.8	8.8	9.1	1.8	3.2	1.5	1.8	2.7	15.4	20.5	8.0	41.1	14.0

TABLE 2. Principal component loadings (PCL) of the first three principal components from a principal component analysis of data in Table 1 (log-transformed), given separately for analyses of male and female data. The five most influential PCLs for each principal component are marked by a superscript ranking.

	PCA of males			PCA of females		
Variable	PCL1	PCL2	PCL3	PCL1	PCL2	PCL3
SVL	0.107	0.170[5]	-0.077	0.094	0.093	0.122
HL	0.115[2]	0.037	-0.040	0.102[4-5]	0.024	0.268
HW	0.110[4-5]	0.033	-0.076	0.107[1]	-0.016	0.123
END	0.005	0.406[1]	-0.059	0.060	0.419[1]	-0.278[5]
NND	0.015	0.293[3]	0.547[1]	0.062	0.363[2]	0.292[4]
NSD	0.071	0.301[2]	-0.316	0.083	0.238[5]	-0.623[2]
ED	0.077	-0.010	0.461[2]	0.085	-0.288[3]	-0.106
TD	0.105	0.062	0.262[3]	0.086	0.113	0.811[1]
FORL	0.117[1]	-0.050	0.011	0.102[4-5]	-0.149	0.033
HAL	0.099	-0.214[4]	0.097	0.092	-0.263[4]	0.032
HIL	0.114[3]	-0.001	-0.108[5]	0.102	-0.029	-0.458[3]
FOL	0.108	-0.118	-0.065	0.106[2-3]	-0.087	-0.100
FOTL	0.110[4-5]	-0.115	-0.170[4]	0.106[2-3]	-0.099	-0.114

sample of Andringitra specimens are given in Table 1. We performed a statistical comparison of both samples using Mann-Whitney *U*-tests on the ratios in Table 3; males and females were tested separately. Males and females from Ankaratra had relatively longer hands ($P<0.05$ in females; $P<0.1$ in males), shorter eye-nostril distances (relative to head length; $P<0.05$ in males; $P<0.001$ in females), shorter nostril-snout tip distances (relative to head length; significant only in females; $P<0.005$), and shorter nostril-nostril distances (relative to head width; $P<0.05$ in males and females). In both samples, relative tympanum width was larger in males than in females (ratios TD/ED and TD/SVL), but these differences were not statistically significant.

A multivariate PCA resulted in a clear separation of the two samples both in separate and combined analyses of males and females. After removal of the first factor (equivalent to the size or growth effect), the second and third factors (equivalent to the most important shape factors) were plotted in Fig. 3. Both samples were mainly separated along the second principal component. The most influential loadings of the second principal component (Table 2) corresponded largely to those variables previously identified as significantly different between the samples: END, NSD, NND, HAL and SVL in males; END, NND, ED, HAL and NSD in females.

Table 3 summarizes the most important morphological and morphometric differences between the Ankaratra and Andringitra samples. A discriminant analysis using the data in Table 1 predicted correctly group membership of all specimens with $P<0.0001$.

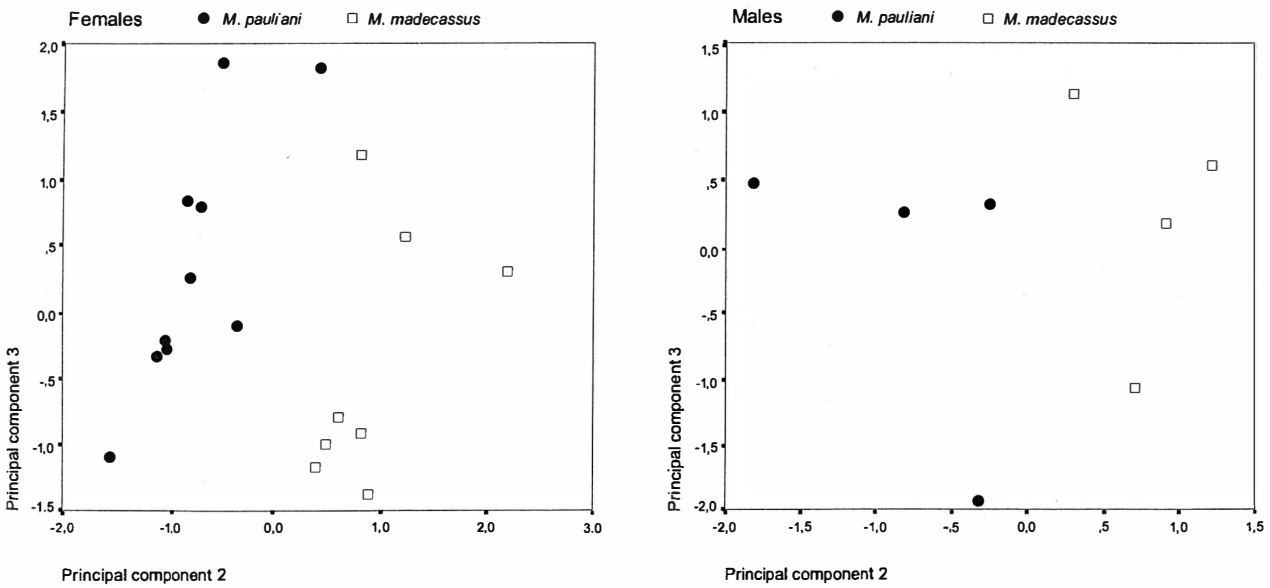

FIG. 3. Scatterplots of second and third principal components of a PCA of log-transformed data in Table 1, analyzed separately for males and females.

TABLE 3. Differential characters between *Mantidactylus madecassus* and *M. pauliani*. Variable ratios are given as mean ± SD (minimum and maximum values in parentheses). Data were compared using Mann-Whitney *U*-Tests. Significant differences between data pairs are marked with asterisks: * $P<0.05$, ** $P<0.005$, *** $P<0.001$.

Species	*M. madecassus* (Andringitra)	*M. pauliani* (Ankaratra)
SVL (males)	27.0-29.8 mm	24.8-31.6 mm
SVL (females)	25.4-33.7 mm	24.4-33.7 mm
HAL/SVL (males)	0.27 ± 0.02 (0.26-0.30)	0.30 ± 0.02 (0.28-0.32)
HAL/SVL (females)	0.26 ± 0.02 (0.24-0.29) *	0.29 ± 0.02 (0.26-0.33) *
FOTL/HIL (males)	0.48 ± 0.02 (0.45-0.50) *	0.51 ± 0.01 (0.50-0.52) *
FOTL/HIL (females)	0.49 ± 0.01 (0.47-0.50)	0.50 ± 0.03 (0.48-0.57)
END/HL (males)	0.19 ± 0.01 (0.18-0.20) *	0.15 ± 0.01 (0.14-0.17) *
END/HL (females)	0.18 ± 0.01 (0.17-0.21) ***	0.15 ± 0.01 (0.13-0.18) ***
NSD/HL (males)	0.21 ± 0.01 (0.20-0.22)	0.20 ± 0.01 (0.19-0.21)
NSD/HL (females)	0.23 ± 0.02 (0.20-0.25) **	0.19 ± 0.01 (0.18-0.22) **
NND/HL (males)	0.31 ± 0.02 (0.28-0.33) *	0.26 ± 0.03 (0.24-0.30) *
NND/HL (females)	0.31 ± 0.02 (0.28-0.35) *	0.29 ± 0.02 (0.25-0.33) *
HL/SVL (males)	0.36 ± 0.01 (0.34-0.37)	0.37 ± 0.01 (0.36-0.38)
HL/SVL (females)	0.34 ± 0.01 (0.32-0.37)	0.35 ± 0.02 (0.32-0.39)
HW/SVL (males)	0.34 ± 0.02 (0.34-0.36)	0.36 ± 0.01 (0.36-0.37)
HW/SVL (females)	0.33 ± 0.01 (0.31-0.35)	0.35 ± 0.02 (0.32-0.38)
TD/ED (males)	0.74 ± 0.17 (0.55-0.91)	0.77 ± 0.14 (0.56-0.89)
TD/ED (females)	0.67 ± 0.07 (0.60-0.77)	0.63 ± 0.11 (0.50-0.90)
Colour in preservative	mostly beige with dark markings.	mostly uniformly dark with indistinct darker markings
Subarticular finger tubercles	distinct, bilobed.	indistinct, not bilobed.
Foot webbing	ends just below disc on toe 5.	reaches disc on toe 5.

Within the genus *Mantidactylus* (and also other Malagasy anurans), there is no other example of such important morphological differences (especially in the shape of the subarticular tubercles) between populations included in a single species. Generally, even slight morphological differences between populations are paralleled by bioacoustic (see data in Glaw & Vences, 1994) or, as in the genus *Mantella*, by genetic differentiation (Vences *et al.*, 1998). Sometimes, bioacoustically well differentiated *Mantidactylus* species are even virtually indistinguishable by morphology (Glaw & Vences, 1994).

We consider the differences found between the Andringitra and Ankaratra populations attributed to *Mantidactylus madecassus* as sufficient support for a distinction at the specific level. Because the name *Mantidactylus pauliani* is available for the Ankaratra populations, we resurrect this name from the synonymy of *M. madecassus*.

REDESCRIPTION OF *M. MADECASSUS* AND *M. PAULIANI*

MANTIDACTYLUS MADECASSUS MILLOT & GUIBÉ, 1950

Diagnosis. A species of the genus *Mantidactylus* as indicated by the lack of nuptial pads in males and the presence of femoral glands (verified in ZFMK 57416). A member of the subgenus *Brygoomantis* as indicated by (a) sexual dimorphism in tympanum size (males having a relatively slightly larger tympanum than females); (b) only slightly enlarged finger and toe discs; (c) well developed webbing between toes; and (d) presence of femoral glands including a rounded structure with external median depression, and of rudimentary femoral glands in females (verified in ZFMK 57416).

M. madecassus is distinguished from all other species of *Mantidactylus* by the presence of distinct, bilobed subarticular tubercles on the fingers (never bilobed in other *Mantidactylus*); further from all other *Mantidactylus* except *M. pauliani* by the unique head shape, with a broadly rounded, very short snout; from other species of the subgenus *Brygoomantis* except *M. pauliani* by the lack of vomerine teeth; and, additionally, from *M. betsileanus, M. biporus* and *M. alutus* by the great extent of the webbing between the toes. For further distinction from *Mantidactylus pauliani*, see below.

Name bearing type. Lectotype (hereby designated) MNHN 1953.246 (Fig. 4). Collected by J. Millot at Cirque Boby, Andringitra massif. Specimen in mediocre state of preservation. Colour patterns well preserved, dorsally beige with irregular large dark brown markings, ventrally uniformly light. Fingertips slightly enlarged. Fingers with very distinct bilobed

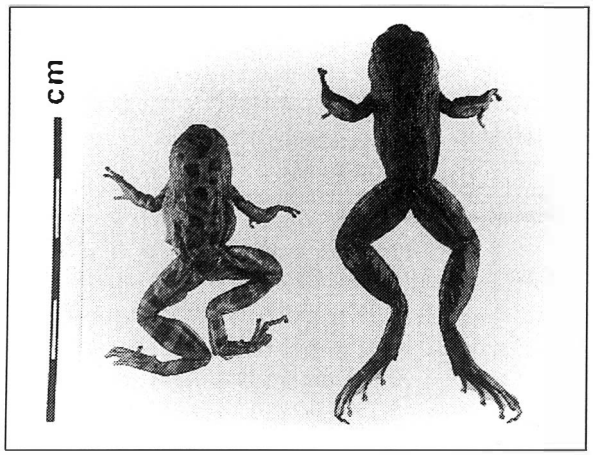

FIG. 4. Lectotype of *M. madecassus* (MNHN 1953.246, left) and holotype of *M. pauliani* (MNHN 1972.1508) in dorsal view.

subarticular tubercles. No femoral glands visible (possibly due to poor fixation). Webbing formula of foot: I 1^+-2^- II $1-2$ III 1^+-2^+ IV 2^+-1 V. Webbing formula according to the notation of Blommers-Schlösser (1979): 1(0.25), 2i(0.75), 2e(0), 3i(1), 3e(0.25), 4i(1.5), 4e(1.25), 5(0). Toe 5 of same length as toe 3. Foot longer than tibia. Tibiotarsal articulation reaches the centre of the eye.

Other types. Eight paralectotypes (MNHN 1989.3590-3597; originally all subsumed under MNHN 1953.246) in a mediocre state of preservation, partly in worse state than the lectotype, all with the same collection dates and locality as the lectotype. The original catalogue entry reads 10 specimens under MNHN 1953.246, but only eight specimens (beside the lectotype, which retained the original number) were given new numbers in 1989. The missing specimen is probably BMNH 1950.1.2.80, which is labeled as cotype and was received in 1950 from J. Guibé. This specimen, in a better state of preservation than the MNHN specimens, must therefore also be regarded as paralectotype.

All paralectotypes agree morphologically with the lectotype. The following data refer to variation in three specimens (MNHN 1989.3591-3592, 1989.3594). Webbing formula I $(1-1^+)-(1^+-2)$ II $(1-1^+)-(2-2^+)$ III $1^+-(2-3)$ IV $(2-3^-)-(1-1^+)$ V. Webbing formula according to the notation of Blommers-Schlösser (1979): 1(0-0.25), 2i(0.5-1), 2e(0-0.5), 3i(1-1.5), 3e(0.25), 4i(1-2), 4e(1-1.75), 5(0-0.5). Toe 5 of same length as toe 3. Foot longer than tibia. Tibiotarsal articulation reaches posterior eye margin in MNHN 1989.3591 and 3594, tympanum in MNHN 1972.3592.

The prominent bilobed subarticular tubercles on the fingers are visible in all specimens. Femoral glands are not clearly recognizable in any paralectotype, probably due to their poor state of preservation.

Additional material examined. ZFMK 57416 (Cuvette Boby, Andringitra, collected by F. Glaw and M. Vences on 18 January 1994) and 26 specimens from the MNHN, all collected by C. P. Blanc and co-workers during their 1970/71 expedition to the Andringitra massif: MNHN 1972.1181 (Cuvette Boby, 28.11.1970), 1182-3 (Marositry), 1184-5 (Andohabatomanara, Varavarana, ruisseau, 18.11.1970), 1186 (Plateau Andohariana, riv. Riambavy, ca. 2030 m altitude, 6.12.1970), 1189 (Cuvette Boby, 25.11.1970), 1190 (Ibory face ouest, 17.12.1970), 1191 (Cuvette Boby), 1192 (Ibory face ouest, 25.11.1970), 1193-5 (Cuvette Boby), 1196-7 (Ibory face nord, ruisseau, 16.12.1970), 1198-9 (Antsifotra, 4.12.1970), 1200-1 (plateau Andohariana, riv. Riambavy, 2.12.1970), 1202-3 (Anjavidilava, 9.1.1971), 1204-5 (Ibory face sud, 16.12.1970), 1206 (Cirque Boby, 26.11.1970). MNHN 1972.1187 (Ibory, 16.12.1970) and 1188 (Ivangomena) were not examined; in the MNHN catalogue, they are provided with the comment "echangé Duellman 1977" and almost certainly they correspond to the specimens KU 173060-61 (L. Trueb, *pers. comm.*).

All examined specimens correspond well with the type series. Femoral glands (a small, rudimentary structure with median depression and additional irregular structures towards the anal region) are recognizable in ZFMK 57416 (probably a female), but are not evident in MNHN specimens, probably due to poor fixation and preservation.

Distribution. Locality coordinates and altitudes are given according to Paulian *et al.* (1971) and Goodman (1996*a,b*). The species is only known from the Andringitra massif and was found at 9-10 localities between ca. 1500 and 2500 m altitude (most localities higher than 2000 m): (1) Cirque Boby, 2520 m (see Blommers-Schlösser & Blanc 1991: 254), probably 22°11'S/46°53'E; (2) Cuvette Boby, ca. 2470 m, 22°11'S/46°53'E; (3) Andohariana plateau, ca. 2030 m, 22°09'S/46°54'E; (4) Anjavidilava, 1800-2100 m, 22°09'S/46°57'E; (5) Marositry, ca. 2000 m, 22°10'S/46°56'E; (6) Varavarana 1500-1850 m, 22°08'S/46°57'E; (7) Antsifotra, ca. 2000 m, 22°10'S/46°56'E (altitudes and coordinates referring to Antsifotra River); (8) Ivangomena, 2100-2500 m, 22°09'S/46°53'E; (9) Ibory (north, west and south slope), probably referring to Pic Bory, up to 2630 m, 22°12'S/46°55'E; possibly also (10) Ambalamarovandana, altitude ca. 1530 m, 22°08'S/46°57'E (locality in Blommers-Schlösser & Blanc 1991, possibly referring to a personal observation of C. P. Blanc).

Natural history. MNHN 1989.3593 (SVL 31.3 mm) contained 10 very large (diameter 3.8 mm) uniformly yellowish oocytes. MNHN 1972.1206 (SVL 31.4 mm) contained 28 yellowish to light brown oocytes of 2-2.3 mm diameter. We found two specimens in a stagnant tributary of a brook, surrounded by ericoid vegetation (Glaw & Vences, 1994). Specimens were sitting in or near water, and readily dived when disturbed.

MANTIDACTYLUS PAULIANI GUIBÉ, 1974

Diagnosis. A species of the genus *Mantidactylus* as indicated by the lack of nuptial pads in males and the presence of femoral glands. A member of the subgenus

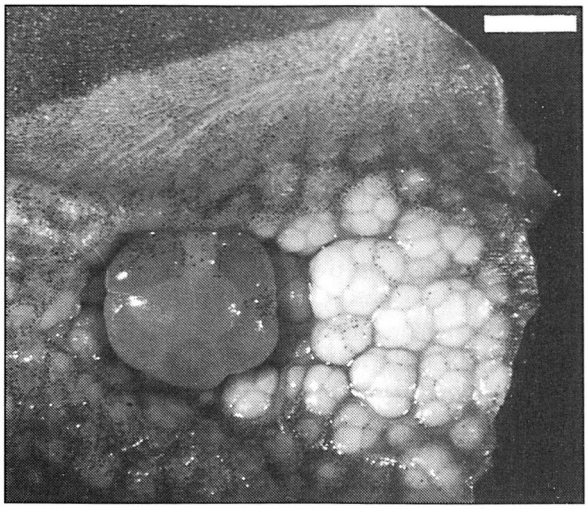

FIG. 5. Femoral gland of *Mantidactylus pauliani* (MNHN 1972.1514, male) in internal view (on the underside of ventral femur skin after dissection). Scale = 1 mm.

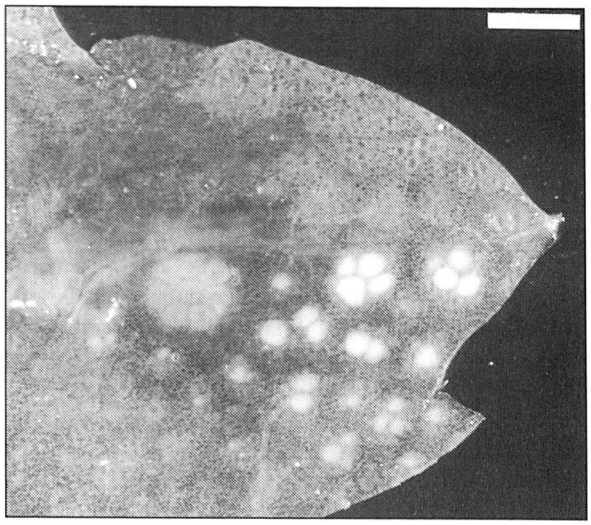

FIG. 6. Femoral gland of *Mantidactylus pauliani*, MNHN 1972.1511, female, in internal view (on the underside of ventral femur skin after dissection). Scale = 1 mm.

Brygoomantis as indicated by (a) sexual dimorphism in tympanum size (males having a relatively slightly larger tympanum than females); (b) only slightly enlarged finger and toe discs; (c) well developed toe webbing; and (d) presence of femoral glands including a prominent rounded structure with external median depression, and of rudimentary femoral glands in females.

M. pauliani is distinguished from all other *Mantidactylus* except *M. madecassus* by the unique head shape, with a broadly rounded, very short snout; from other species of the subgenus *Brygoomantis* except *M. madecassus* by the lack of vomerine teeth; and, additionally, from *M. betsileanus, M. biporus* and *M. alutus* by the great extent of the foot webbing. *M. pauliani* is distinguished from *M. madecassus* by the single and indistinct subarticular tubercles on the fingers, by the great extent of the webbing between the toes (webbing extending onto disc of fifth toe), and by a less distinct dorsal pattern.

Name bearing type. Holotype MNHN 1972.1508 (Fig. 4). Collected by C. P. Blanc at Nosiarivo, Ankaratra massif. Adult male in excellent state of preservation. Dorsal colour uniformly dark brown, with a few light elements on femur resulting in slight crossband pattern. Ventrally uniformly cream with a slight fading from ventral to dorsal colour along the flanks. Finger tips barely enlarged. Greyish outer metatarsal tubercle present, rather large and distinct, slim (2.2 x 0.9 mm).

Webbing formula I $1-1^{+}$ II $1-2$ III $1^{+}-2$ IV $1^{+}-0^{+}$ V. Webbing formula according to the notation of Blommers-Schlösser (1979): 1(0), 2i(0.5), 2e(0), 3i(1), 3e(0.25), 4i(1), 4e(0.25), 5(0). Toe 5 of same length as toe 3. Foot longer than tibia. Tibiotarsal articulation reaches the tympanum.

Femoral glands present, consisting of one larger, rounded externally prominent structure with a median depression, and irregular, less prominent groups of granules proximally. A rosette-like structure of several of these small granule groups is recognizable.

Other types. Eight paratypes. Four males (MNHN 1972.1509, 1972.1512, 1972.1514, 1972.1516), three females (MNHN 1972.1510, 1972.1511, 1972.1515), and one possibly subadult male (MNHN 1972.1513). MNHN 1972.1509-1513 with the same collecting dates and locality as the holotype; MNHN 1972.1514-1516 collected by C. P. Blanc at Betay forest, Ankaratra massif. All in excellent state of preservation. Dorsal colour rather uniform, but lighter with large dark brown markings in a few specimens (e. g. MNHN 1972.1509).

The following data refer to variation in three paratype specimens (MNHN1972.1510, 1972.1514-1515). Webbing formula I $1-1^{+}$ II $1-2$ III $1^{+}-2$ IV $(1^{+}-2)-0^{+}$ V. Webbing formula according to the notation of Blommers-Schlösser (1979): 1(0), 2i(0.5), 2e(0), 3i(1), 3e(0.25-0.5), 4i(1), 4e(0.25-1), 5(0). Toe 5 of same length as toe 3. Foot longer than tibia. Tibiotarsal articulation reaches the posterior eye margin in MNHN 1972.1514, the tympanum in the other two specimens.

Femoral glands are visible in all specimens, also in females (although clearly smaller). The gland section with external median depression is small in the males as compared with other *Brygoomantis*. The smaller, proximal groups of granules are also present in females, but rosette-like structures are less distinct in most females. It is not easy to distinguish externally between male and female glands. Females are therefore only tentatively sexed except MNHN 1972.1511 which was dissected (see below).

After dissection of one male (MNHN 1972.1514), it could be recognized that the gland section externally showing the median depression consists of two median granules surrounded circularly by seven slightly smaller granules, and the proximal smaller irregular structures are groups of several much smaller granules (Fig. 5). In a female (MNHN 1972.1511), the rudimentary median depression section was a central granule surrounded by seven granules of similar size, and the proximal irregular groups of granules were smaller and more widely spaced (Fig. 6).

Additional material examined. ZMA 6803, five specimens individually labeled with the field numbers 1184-1188, collected by R. Blommers-Schlösser on 21.3.1973 at Nosiarivo, Ankaratra massif, 2200 m altitude.

Distribution. Known from two collecting sites, located close to each other in the Ankaratra massif at altitudes above 2000 m: (1) Nosiarivo, 2200 m (see Blommers-Schlösser 1979); (2) Betay forest (according to Viette 1991: Ambohimirandrana, 2100/2200 m).

Natural history. One female (MNHN 1972.1511) contained 16 mature uniformly yellowish oocytes of 3.5 mm diameter. According to Blommers-Schlösser (1979), specimens were found under boulders in rapids.

DISCUSSION

Relationships of *Mantidactylus madecassus* and *M. pauliani*

Mantidactylus madecassus and *M. pauliani* can be considered as strictly montane amphibian species, occurring between 1500 and 2500 m altitude, mainly above 2000 m. Habitats, so far as is known, are brooks and their tributaries in areas of ericoid vegetation or of rock formations with rupicolous plant communities (compare locality lists with site descriptions in Paulian *et al.*, 1971; Blommers-Schlösser, 1979; Glaw & Vences, 1994; Goodman 1996*a*).

The distinguishing features between *M. madecassus* and *M. pauliani* have already been shown by Guibé (1978) in his drawings of the hands and feet of both species (figs. 14-15 versus 72-73; *M. madecassus* having bilobed to paired subarticular tubercles on fingers, and its webbing not reaching the disc of the fifth toe). Guibé (1978) also mentioned the special subarticular tubercles of *M. madecassus* as "élargis transversalement, ceux du doigt 3 parfois doubles". However, neither in the original description of *Mantidactylus pauliani* (Guibé, 1974) nor in his monograph (Guibe, 1978) did he mention the obvious external resemblance of *pauliani* and *madecassus*. Their similarity was seemingly first noted by Blommers-Schlösser & Blanc (1991). These authors, however, did not recognize the differences between the taxa (characterizing the subarticular tubercles as "peu saillants", probably based on examination of *pauliani* specimens only) and synonymized them without any discussion.

The head shape shared by *Mantidactylus madecassus* and *M. pauliani* is unique among Malagasy frogs and may be regarded as a derived state. The rounded, short snout strongly resembles that of European newts, genus *Triturus*, in their aquatic phase, and may be a morphological adaptation to largely aquatic habits.

Attribution of the two species to the subgenus *Brygoomantis* is mainly based on femoral gland morphology, because the other main synapomorphies of the subgenus (reduced chromosome number and spiral-shaped intestine of tadpole) are unknown. However, the similarity of the structure of the femoral glands to certain species of *Brygoomantis*, such as *Mantidactylus curtus,* is striking (*pers. obs.*), and the subgeneric attribution of *M. madecassus* and *M. pauliani* is therefore rather certain.

Relationships of the Montane Amphibian Fauna of Ankaratra and Andringitra Massifs

A recent analysis of montane herpetofaunas in Madagascar (Raxworthy & Nussbaum, 1996*a*) compared the high mountain communities endemic to altitudes higher than 1500 m of the three highest massifs in Madagascar: Andringitra, Ankaratra and Tsaratanana. As these authors found no additional species of amphibians and reptiles during the final 3-5 survey days at each site, they considered the species lists compiled during their surveys as "nearly complete". Their reptile data actually appear to be relatively complete, in comparison with available literature. According to Raxworthy & Nussbaum's (1996*a*) list, however, they only recorded the amphibians *Anodonthyla montana, Plethodontohyla tuberata, Boophis microtympanum, Mantidactylus aerumnalis, M. alutus*, and *M. domerguei* as high mountain species of the Ankaratra and Andringitra massifs. At least half of the mentioned species also occur lower than 1500 m altitude and therefore cannot be viewed as high mountain endemics in the sense defined by Raxworthy & Nussbaum (1996*a*). *M. domerguei* is known between 900-1800 m altitude (Glaw & Vences, 1994), and *M. alutus* occurs at least down to 1200 m altitude. *M. aerumnalis* sensu Andreone & Gavetti (1994) is only known from the type locality Andrangoloaka (1389 m according to Viette, 1991) and An'Ala (ca. 850 m, *pers. obs.*) and therefore is clearly not a montane species. The records of *M. aerumnalis* of Raxworthy & Nussbaum (1996*a*) may actually refer to *M. brevipalmatus* (which in the past was considered as a synonym of *M. aerumnalis*; see Blommers-Schlösser & Blanc, 1991), but even this species is possibly not a high mountain endemic. According to Blommers-Schlösser & Blanc (1991) it is known from Mandraka, which lies at about 1200 m altitude. Equally, *Boophis microtympanum* is possibly not a high mountain endemic, but the taxonomy of populations from the lower altitudes of Andringitra needs further study (see Glaw & Vences, 1994). Although *Plethodontohyla tuberata* has been recorded from Angavokely (Blommers-Schlösser & Blanc, 1991) which is at 1400 m (Viette, 1991), this species and *Anodonthyla montana* can be considered as high mountain endemics, at least in a less strict sense, according to current knowledge.

Species which are more likely to be true high mountain endemics were apparently not found in the surveys of Raxworthy & Nussbaum (1996*a,b*), and thus not included in their accounts. *Boophis williamsi* is only known from 2200 m altitude (Guibé 1974, Blommers-Schlösser, 1979), whereas *B. laurenti* occurs 1500-2650 m above sea level (Guibé, 1974). The few

exactly known localities for *Scaphiophryne madagascariensis* are between 1530 m (Ambalamarovandana) and 2030 m (Andohariana), and *Scaphiophryne pustulosa* occurs at similar altitudes. Finally, *Mantidactylus madecassus* and *M. pauliani* appear to be confined to areas above 1500 m and can therefore be regarded as high mountain endemics.

An updated list of the montane amphibians (defined by a general restriction to altitudes of ca. 1500 m or higher) occurring on the three Malagasy massifs of Andringitra, Ankaratra, and Tsaratanana (and partly at other localities of high altitude; see Glaw & Vences 1994) is therefore as follows (data from Raxworthy & Nussbaum, 1996*a*; Andreone *et al.*, 1998; and as discussed above): *Boophis laurenti* (Andringitra), *B. williamsi* (Ankaratra), *B. ankaratra* (Andringitra, Ankaratra), *Mantidactylus madecassus* (Andringitra), *M. pauliani* (Ankaratra), *Anodonthyla montana* (Andringitra), *Plethodontohyla tuberata* (Ankaratra), *P. guentherpetersi* (Tsaratanana), *P.* sp. A (Tsaratanana), *P.* sp. B (Tsaratanana), *P.* sp. C (Tsaratanana), *Platypelis tsaratananaensis* (Tsaratanana), *P.* sp. A (Tsaratanana), *Stumpffia* sp. A (Tsaratanana), *Scaphiophryne madagascariensis* (Andringitra), *S. pustulosa* (Ankaratra), and *S.* sp. A (Tsaratanana). Additional species which may be montane endemics but which need taxonomic revision are *Boophis microtympanum* (Andringitra, Ankaratra), *Mantidactylus brevipalmatus* (Andringitra, Ankaratra), and *Mantidactylus elegans* (Andringitra, Tsaratanana). This list contains up to 17, possibly 20 species, whereas Raxworthy & Nussbaum (1996*a*) listed 14 montane amphibian species. The general conclusion drawn by these authors – that the montane heathlands of Madagascar harbour a relatively diverse endemic amphibian fauna – is therefore strongly confirmed.

Three species pairs of montane amphibians may be examples of vicariant speciation between the Andringitra and Ankaratra massifs. *Mantidactylus madecassus* (Andringitra) and *M. pauliani* (Ankaratra) appear to be sister species due to the probably synapomorphic head shape and lack of vomerine teeth. *Scaphiophryne madagascariensis* (Andringitra) and *S. pustulosa* (Ankaratra) share a greenish colouration with sharply bordered brown markings which are not known in other *Scaphiophryne* (see illustrations in Glaw & Vences, 1994). *Boophis laurenti* (Andringitra) and *B. williamsi* (Ankaratra) share a large relative foot length and probably large blackish tadpoles (data in Blommers Schlösser & Blanc, 1991; Glaw & Vences, 1994). An additional example is found in reptiles: the geckos *Lygodactylus intermedius* (Andringitra, 1700-2600 m, see Pasteur, 1995) and *L. mirabilis* (Ankaratra, 2300-2640 m). Andringitra and Ankaratra populations of all other amphibian and reptile species which can be considered as mountain endemics (*Boophis ankaratra, Phelsuma barbouri, Calumma hilleniusi, Furcifer campani, Amphiglossus* sp., *Mabuya boettgeri, Mabuya madagascariensis*) appear to be conspecific on both massifs (data from Glaw & Vences, 1994; Raxworthy & Nussbaum, 1996*a*). Other species such as *Plethodontohyla tuberata* and *Anodonthyla montana* are known only from one of the two massifs and have no closely related sister species in the other massif. These species are therefore not helpful in indicating any vicariant speciation events between Ankaratra and Andringitra. The same is true for *Mantidactylus elegans* which occurs in Andringitra up to 2500 m, but is not known from Ankaratra.

The climatic history of the Quaternary in Madagascar included dynamic shifts between drier and more humid periods (Battistini, 1996). According to the data summarized in Burney (1996), ericoid vegetation of Madagascar's highest mountain ranges may have flourished at times during the Pleistocene down to elevations of ca. 1000 m. This continuous montane habitat was probably populated by the ancestor of the *M. madecassus/pauliani* clade, as well as by the ancestors of the other allopatric sister species. Due to a period of warmer climate, it is possible that such high mountain habitats and their associated amphibian fauna became restricted to the high altitude refuges of Ankaratra and Andringitra. In this scenario, the subsequent temperature decreases were not intense enough to allow the montane habitats of both massifs to get into long-term contact at lower altitudes again; hence, montane endemics of both massifs, such as the *Mantidactylus madecassus* and *M. pauliani* ancestor, remained isolated and several of them evolved into separate species.

ACKNOWLEDGEMENTS

We are grateful to Annemarie Ohler and Alain Dubois (MNHN, Paris), Berthus van Tuijl (ZMA, Amsterdam) and Barry Clarke (BMNH) for making material accessible. Linda Trueb (KU, Kansas) provided information on specimens held in her care. The fieldwork of FG was financially supported by "Deutscher Akademischer Austauschdienst" (DAAD). The work of MV was supported by a grant from the "Graduiertenförderung" of the Nordrhein-Westfalen state. The research in Madagascar was made possible by cooperation between the University of Antananarivo and the "Zoologisches Forschungsinstitut und Museum A. Koenig", Bonn. We are indebted to the Malagasy authorities for research permits, and the permits to export voucher specimens.

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Accepted: 2.2.99

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 111-117 (1999)

MALE RESPONSE TO LOW FREQUENCY OF FEMALE REPRODUCTION IN THE VIVIPAROUS LIZARD *LIOLAEMUS* (TROPIDURIDAE)

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Female *Liolaemus elongatus* and *Liolaemus pictus* have biennial and triennial reproductive cycles respectively, with a low availability of reproductive females during the breeding season. Previous results have shown slight interspecific differences in sexual dimorphism between *L. pictus* and *L. elongatus*, coinciding with differential accessibility to females. Present results show major interspecific differences in the timing of spermatogenesis. Male *L. pictus* begin to reproduce during the first year of adulthood and remain active during a long breeding season. In contrast, male *L. elongatus* delay reproduction for one year and reproduce during a narrower period, in synchrony with the female reproductive cycle. Male *L. elongatus* showed an increasing testicular size during spermatogenesis, and a reduction in size when the first spermatozoa appear. *Liolaemus pictus* had spermatozoa in seminiferous tubules for the entire sampling period, from spring to autumn. The existence of severe thermal constraints for vitellogenesis and pregnancy set the following chain of causal events: environmental conditions - female cycle - male cycle and male dimorphic traits. This sequence of events shows how environmental cues can constrain the female cycle, and female availability, and thereby also affect the male cycle and the development of male dimorphic traits.

Key words: Sexual dimorphism, *Liolaemus*, male reproductive cycles

INTRODUCTION

The factors determining sexually dimorphic traits are complex and can involve natural or sexual selection (Mouton & van Wyk, 1993), and/or non-adaptive circumstances (Huang, 1996*a*). In the context of sexual selection (Harvey & Bradbury, 1991), sexual dimorphism may result from inter-sexual selection (e.g. female choice) or intra-sexual selection (e.g., male-male competition). Sexual dimorphism occurs frequently in lizards and is commonly reflected in differences in body size (Censky, 1995; Huang, 1996*a*), body proportions (Mouton & van Wyk, 1993), colour pattern and presence of pre-anal glands (Cei, 1986; 1993). It has been widely studied in many reptile groups (Mouton & van Wyk, 1993; Censky, 1995; Huang, 1996*a*), including Liolaeminae (Cei, 1986; 1993; Lobo & Laurent, 1995; Ibargüengoytía & Cussac, 1996; 1998; Vega, 1997).

Liolaemus pictus lives in temperate habitats up to moderate altitudes (520 to 1600 m), in leaf-mould and under logs and it is the most common lizard in the Andean-Patagonian forest of Nahuel Huapi National Park (Christie, 1984). *Liolaemus elongatus* lives on rock promontories in the steppe and in the transition rainforest-steppe where it may be seen in the understory as well as the rocks along the shores of lakes (Ibargüengoytía, Cussac & Ubeda, 1997). The species are sympatric in the transition rainforest-steppe (Ibargüengoytía *et al.*, 1997) of Northern Patagonia.

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The sexual dimorphism found in *L. pictus* and *L. elongatus* corresponds to pre-anal glands present only in males (Cei, 1986) and to differences in body shape, particularly a bigger head and a larger vent width in males (Ibargüengoytía & Cussac, 1996; 1998). Sexual differences observed in *L. pictus* were due to an allometric increase in male head length relative to juveniles. In *L. elongatus* head width grows in a negative allometric way in juveniles and adult females, whereas in adult males head width is isometric. The vent width of *L. elongatus* grows in a positive allometric manner in juveniles and adult males, whereas in adult females growth is isometric. Differences between adult male and female body size, or between male and female maximum juvenile size, were not found in these species (Ibargüengoytía & Cussac, 1996; 1998).

Dimorphic head size can be a consequence of (a) resource partitioning between the sexes; (b) differential energy allocation for reproduction; or (c) a response to selective pressures due to the social structure (Mouton & van Wyk, 1993). Notwithstanding the need for further studies on the diet to test the first hypothesis, three phenomena make it worth considering the last two hypotheses:

(1) *Liolaemus pictus* and *L. elongatus* are viviparous, suggesting that females are the sex with higher investment in offspring (Bull & Shine, 1979; Krebs & Davies, 1993).

(2) Both species have a low frequency of female reproduction with biennial to triennial (*L. pictus*) and annual to biennial (*L. elongatus*) female reproductive cycles so, even though the relation between male and female captures is near 1:1, the calculated proportion of reproductive females ranges between 0.5 and 0.33 in *L.*

pictus, and between 1 and 0.5 in *L. elongatus.* Reproductive females are therefore a limited resource (Ibargüengoytía & Cussac, 1996; 1998).

(3) In both species, the exponent of the allometric relationship of male head size is greater in adults than in juveniles (Ibargüengoytía & Cussac, 1996; 1998).

Bias in sex ratio and differential parental effort may affect the intensity of sexual selection, since male ability to acquire a mate becomes important (Krebs & Davies, 1993), increasing male-male competition (Dearing & Schall, 1994).

The male component of sexual dimorphism has its functional parallel in the male reproductive cycle. *Liolaemus pictus* shows high testicular diameters from the end of spring to early autumn, suggesting a prolonged reproductive activity period (Ibargüengoytía & Cussac, 1996). In contrast, *L. elongatus* shows the largest testicular size in mid-spring, a minimum in summer, and a clear gonadal recrudescence, reaching its peak value in autumn, suggesting a prolonged spermatogenetic period of one year (Ibargüengoytía & Cussac, 1998). Testis mass and volume correlate with spermatogenic activity in some lizards, such as *Eumeces elegans* (Huang, 1996*b*), and three species of the genus *Uma* (Mayhew & Wright, 1970). However, the relationship between testicular size and male reproductive cycle is complex in species such as *Liolaemus gravenhorsti* (Leyton, Morales & Bustos Obregón, 1977), *L. alticolor, L. bitaeniatus, L. darwini, L. scapularis* (Ramírez-Pinilla, 1992), *L. aymararum, Tropidurus peruvianus* (Leyton, Veloso & Bustos Obregón, 1982*)*, *Platysaurus capensis* and *P. minor* (van Wyk & Mouton, 1996).

The existence of severe thermal constraints for vitellogenesis and pregnancy, and their effects on the female cycle (Ibargüengoytía & Cussac, 1996, 1998), suggest the existence of female influence on male cycle and male dimorphic traits. Differences between *L. pictus* and *L. elongatus* sexual dimorphism and male cycle (testicular size-based) seem to be related to distinct female reproductive cycles and different accessibility of females. In the present work we consider the male cycle as the functional counterpart of the male component of sexual dimorphism. We investigated major inter-specific differences in the reproductive biology of male *L. elongatus* and *L. pictus,* mainly based on spermatogenic traits, and explore the possible existence of differences in male life history. Particularly, we test the hypothesis of dependence between testicular size and spermatogenic processes, through a comparative study, in order to make use of this tool for studying the consequences of low frequency of female reproduction on male reproductive traits.

MATERIALS AND METHODS

SAMPLING PROTOCOL

The sample studied included four groups of specimens of both sexes: (A) *L. elongatus, n*=39, collected from October to March (1981 to 1984) and *L. pictus, n*=186, collected from November to April (1982 to 1984) at Nahuel Huapi and Lanín National Parks; (B) *L. elongatus, n*=35, collected from November to March (1993 to 1995) and *L. pictus, n*=30, collected from October 1993 to April 1994 at San Carlos de Bariloche. Localities of samples A and B are situated between 39° and 41.5° S, and between 71.6° and 70.5° W, at altitudes of 500 to 1800 m high. Specimens are deposited in the Centro Regional Universitario Bariloche of the Universidad Nacional del Comahue. The third sample, included *L. elongatus, n*=130, collected from December to January in Neuquén (1963 to 1973) and Mendoza (1961 to 1994). These localities are situated at 32° to 41.1° S and 66.5° to 72° W, 1200-4000 m altitude, and specimens are deposited in the Instituto de Biología Animal of the Universidad Nacional de Cuyo. The fourth group, D, corresponded to a capture-recapture sample of *L. pictus, n*=8, and *L. elongatus, n*=16, caught along the shore of the Moreno Lake near San Carlos de Bariloche (41.2° S, 71.5° W, 760 m altitude) from September 1996 to April 1997 (see Ibargüengoytía *et al.,* 1997). Sample D was the only group originating from a single locality.

The morphological study was based on the four groups of specimens, but the histological study considered only 13 specimens of *L. elongatus* and five specimens of *L. pictus* from groups A and B. Adulthood criteria followed Ibargüengoytía & Cussac (1996, 1998). Therefore, maximum juvenile size (53.74 mm) in *L. elongatus* was determined by taking into account (a) the size of the smallest female with ovulated oocytes, or uterus with medium size folds spread all over the organ surface, and (b) the smallest male showing testicular growth during the breeding season. In *L. pictus* the maximum juvenile size was considered less than the size of the smallest vitellogenic female (49 mm).

AUTOPSY PROCEDURES AND HISTOLOGY TREATMENT

Lizards were killed by intraperitoneal administration of sodic thiopental, fixed in Bouin's solution for 24 hr, and preserved in 70 % ethanol. Male gonads were removed and dehydrated in ethanol series and embedded in paraffin. Sections of 4 to 7 μm were stained with Masson tricromic or Hematoxylin and Eosin (Martoja & Martoja Pierson, 1970).

DATA RECORDED

In the case of samples A, B and C, capture dates (DATE) were considered as days of a single standard year. The following data were recorded: capture date (DATE), testicular size as antero-posterior diameter (TS), snout-vent length (SVL), maximum body perimeter (BP, only for sample D), and body weight (BW, only for sample D). Following Mayhew & Wright (1970), spermatogenesis stages (SS) were determined by the most advanced cell type present at the luminal margin of the seminiferous tubule: (1) for spermatogonia, (2) for spermatocytes, (3) for spermatids, and (4)

for spermatozoa. Cell type recognition was based on Pudney (1995). Light microscopy examination of left and right testis of two individuals of each species did not show differences in the SS, so gonads were considered equally in the subsequent analysis. The seminiferous tubule diameter (TD) and epithelium height (EH) was recorded for each testis from 16 slides. The TD and EH averages from each individual were used in the analysis. Female reproductive cycles, inferred from individuals of groups A, B and C, were taken from Ibargüengoytía & Cussac (1996, 1998).

STATISTICAL ANALYSIS

Data were studied using regression, correlation, Student's *t*-test, cluster (CA, centroid and euclidean distance as measures) and discriminant (Wilks' Lambda as method) analysis. Normality and variance homogeneity assumptions were tested comparing predicted and observed frequencies by means of the Kolmogorov-Smirnov test, and by analysis of residuals or Levene's test, respectively (Sokal & Rohlf, 1969; Norusis, 1986). Kruskal-Wallis, Kolmogorov-Smirnov and Mann-Whitney tests were used as non-parametric tests.

RESULTS

TESTICULAR SIZE AND SPERMATOGENESIS

Liolaemus elongatus. Testicular diameter (TS) and snout-vent length (SVL) were related (Fig. 1, upper left panel). A subset of males (n=12) analysed for SVL, TS,

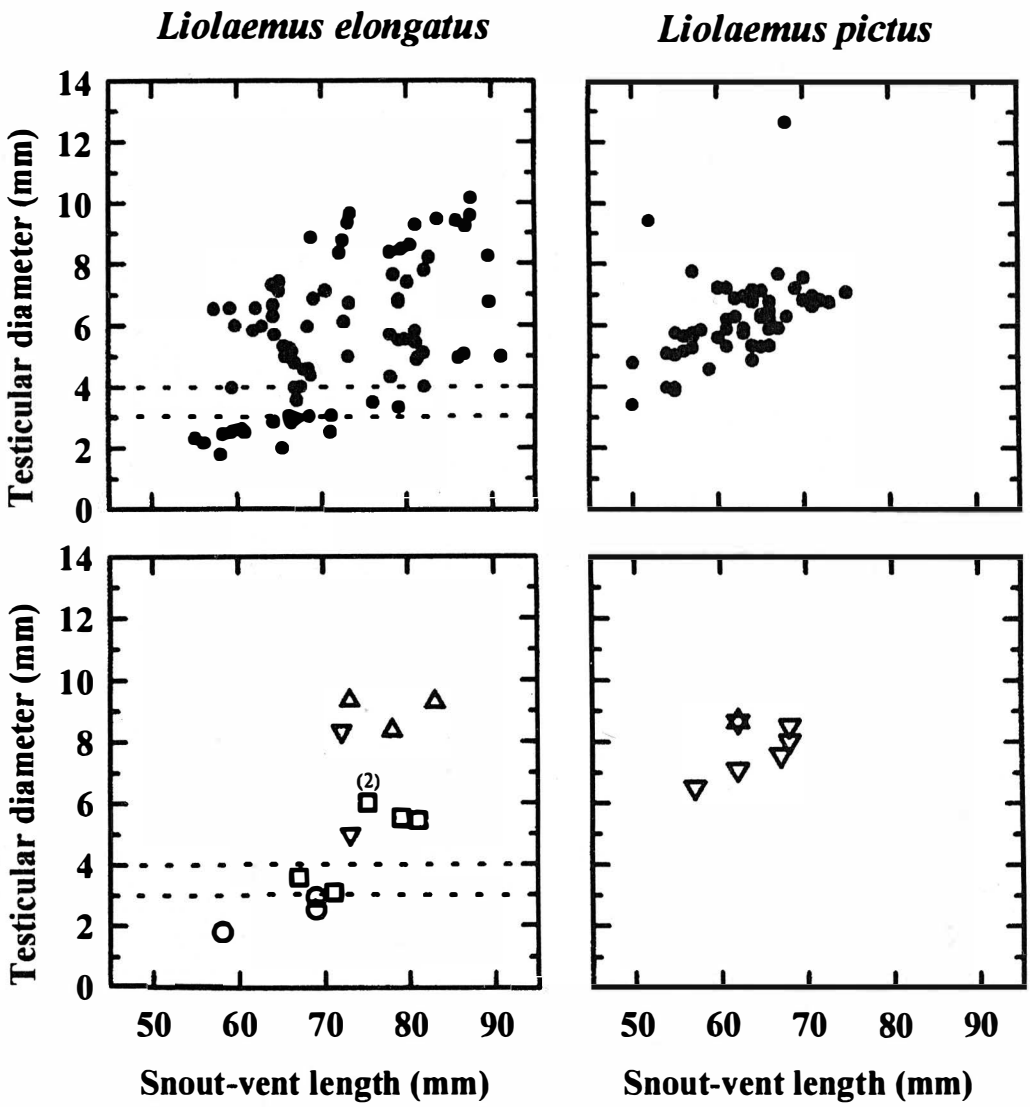

FIG. 1. Testicular diameter (TS) versus snout-vent length (SVL) of *L. elongatus* (left) and *L. pictus* (right). Lower panels show the spermatogenic stages (SS) resulting from the histological study (circles SS 1, squares SS 2, triangles SS 3, inverted triangles SS 4; numbers in parenthesis indicate coincident values). TS of *L. elongatus* was related to SVL (Regression, F=28.33, df=82, P<0.0001, upper left panel). The TS showed differences among SS (Kruskal-Wallis, χ^2=33.85, df=3, P<0.0001, lower left panel). Dashed lines (left panels) indicate males with testes greater or smaller than 4 mm TS, which are significantly discriminated (Discriminant, WL=0.330, n=14, P<0.0004), and the cut-off point between males with and without spermatozoa in the testes (3 mm). Considering all the data, the SVL shows differences between male groups with TS smaller and greater than 3 mm (Mann-Whitney, Z=4.4018, n=83, P<0.0001, upper left panel). TS of *L. pictus* was related to SVL (Regression, F=13.44, df=52, P<0.0001, upper right panel). All the specimens, except one, have advanced SS (lower right panel), so the relationship between TS and SS could not be statistically analysed.

and SS (2 left and 12 right testes were considered) could be clustered into two groups (CA) and significantly discriminated on the basis of TS (Fig. 1, lower left panel), grouping males with testes greater (n= 9) or smaller (n= 5) than 4 mm TS. Particularly, histological observation showed that spermatocytes, spermatids, and spermatozoa are absent in testes smaller than or equal to 3 mm TS (i.e. stage 1), and no stage 1 male has testes greater than 3 mm TS. Considering all the data, the SVL showed significant differences between male groups with TS smaller and greater than 3 mm (Fig. 1, upper left panel).

The TS showed a significant relationship between SVL and DATE (1 in Table1). However, analysis of variance among SS, taking into account SVL and DATE as covariates, was not possible due to lack of variance homogeneity. Spermatogenic stage 4 was found only at the end of spring and at the end of summer. The TS showed significant differences among SS (Fig. 1, lower left panel and Fig. 2): in particular, TS corresponding to spermatogenic stage 1 was significantly smaller than all others, and TS of spermatogenic stage 3 was significantly larger than those of spermatogenic stage 2 and 4.

A significant relationship was found between TD and EH (2 in Table 1). The TS showed a significant relationship with TD (3 in Table 1), and EH (4 in Table 1).

Liolaemus pictus. TS was related to SVL (Fig. 1, upper right panel). All the specimens, except one, have advanced SS (4) so, the relationship between TS and SS could not be statistically analysed (Fig. 1, lower right panel).

A significant relationship was found between TD and EH (5 in Table 1). The TS did not show a significant relationship either with EH (6 in Table 1), or with TD (7 in Table 1).

BODY SIZE

Liolaemus elongatus. Male SVL in samples A, B and C ranged from 55.06 mm to 90.9 mm (n=84). Male SVL from capture-recapture sample (D) ranged from 57.3 mm to 77.1 mm (n=15). Female SVL from samples A, B and C ranged from 53.7 mm to 85.9 mm (n= 88). Female SVL from sample D ranged from 63.7 to 76.8 mm (n=8). Male and female SVL distributions (all individuals) did not show significant differences (Fig. 3). In sample D, males and females show significantly different BP (8 in Table 1) but not different BW (9 in Table 1).

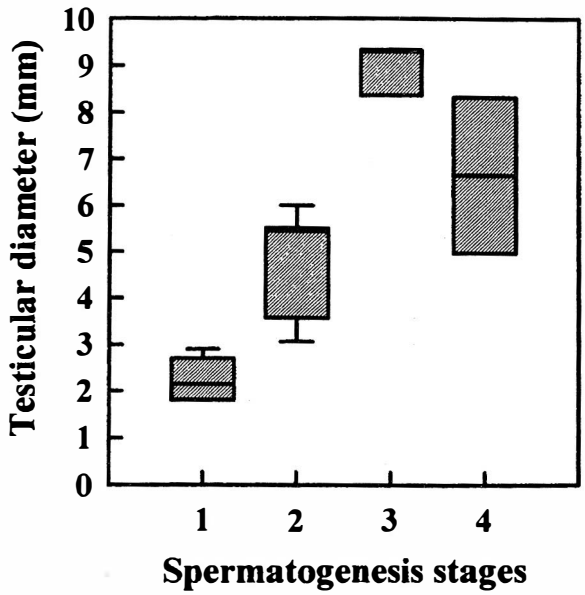

FIG 2. Testicular diameter versus spermatogenesis stages for male *L. elongatus*. Box plots indicate median, quartiles, and 10th and 90th percentiles (no data outside). The TS corresponding to spermatogenic stage 1 was significantly smaller than all others (Mann-Whitney, $Z_{1,2}$=4.1033, n=28, P< 0.0001, $Z_{1,3}$=3.5341, n= 17, P<0.0004, $Z_{1,4}$=3.4427, n=16, P < 0.0006), and TS of spermatogenic stage 3 was significantly larger than those of spermatogenic stage 2 (Mann-Whitney, Z=4.2797, n=29, P<0.0001) and 4 (Mann-Whitney, Z=3.5523, n=17, P<0.0004).

Liolaemus pictus. Male SVL ranged from 50 mm to 75 mm (samples A and B, n=55), while SVL of males from the capture-recapture sample (D) ranged from 53.5 mm to 61.8 mm (n=5). Female SVL from samples

TABLE 1. Summary of statistical analyses.TS, testicular size; SVL, snout-vent length; DATE, date of capture; TD, seminiferous tubule diameter; EH, epithelium height; BP, maximum body perimeter; BW, body weight.

	Test	Variables	Statistic	df or n	P
1	Multiple Regression	TS vs. SVL and DATE	F=37.57	df=44	P<0.001
2	Correlation	TD vs. EH	r=0.95	n=14	P<0.0001
3	Correlation	TS vs. TD	r=0.91	n=14	P<0.0001
4	Correlation	TS vs. EH	r=0.84	n=14	P<0.0001
5	Correlation	TD vs. EH	r=0.94	n=7	P<0.002
6	Correlation	TS vs. EH	r=0.08	n=7	P>0.86
7	Correlation	TS vs. TD	r=0.09	n=7	P>0.84
8	Student's t	BP, between sexes	t=4.70	df=19	P<0.0001
9	Student's t	BW, between sexes	t=0.01	df=11	P>0.90
10	Student's t	BP, between sexes	t=0.58	df=6	P>0.58
11	Student's t	BW, between sexes	t=-0.40	df=7	P>0.70

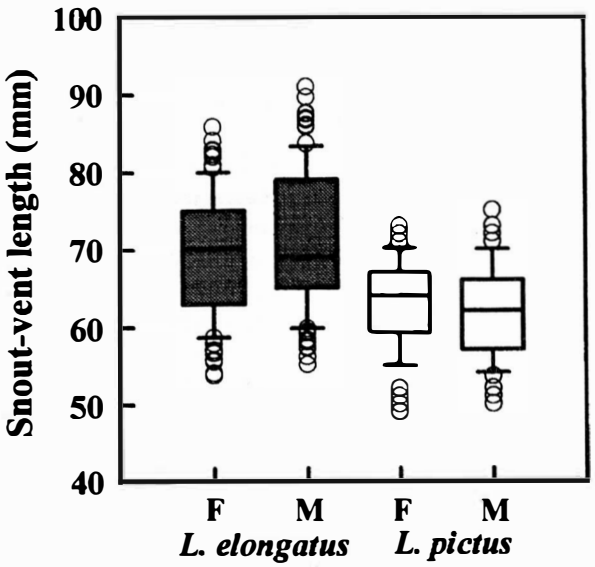

FIG. 3. Female (F) and male (M) snout-vent length of adult *L. elongatus* (n=194, samples A, B, C and D) and *L. pictus* (n=143, samples A, B and D). Box plots indicate median, quartiles and data outside 10th and 90th percentiles. No significant difference between sexes could be found (Kolmogorov-Smirnov, *L. elongatus*, Z=1.042, n=194, P>0.22, *L. pictus*, Z=0.819, n=143, P>0.51).

A and B ranged from 49 mm to 73 mm (n=79), while SVL of females from the capture-recapture sample (D), ranged from 55.8 mm to 68.9 (n=4). Male and female SVL were not significantly different (Fig. 3). In sample D, males and females did not show significantly different BP (10 in Table 1) nor different BW (11 in Table 1).

ADULT GROWTH

Liolaemus elongatus. Capture-recapture data from sample D (Fig. 4) show individual growth of two females and four males and particularly, a notable growth of two males during summer.

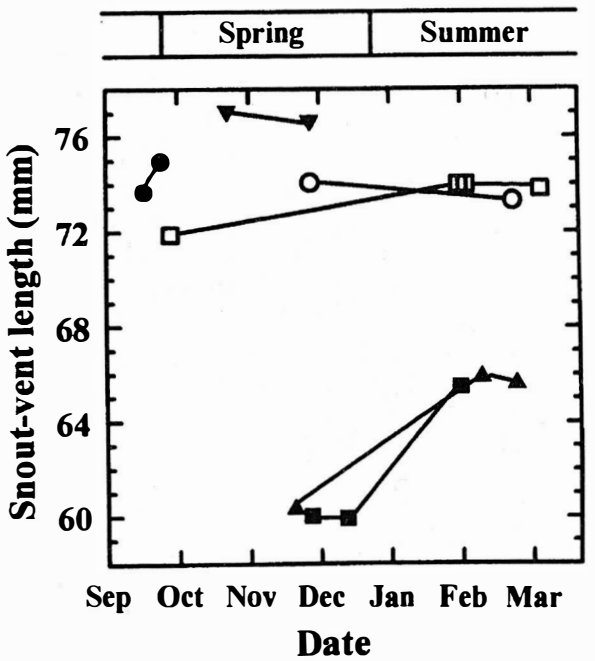

FIG. 4. Snout-vent length versus DATE recapture data of female (hollow symbols) and male (solid symbols) *L. elongatus*. Lines link records of the same animal.

Liolaemus pictus. Only one male lizard was recaptured (sample D), so no inter-sexual comparison was possible.

DISCUSSION

Reptile reproduction is considered to be cyclical in temperate zones. Pudney (1995) considered two patterns of spermatogenesis. In the typical "postnuptial" pattern the mating occurs in spring/early summer, followed by a period of testicular regression. Spermatogenic recrudescence is initiated in the late summer/autumn. For some species spermatogenesis is completed before winter, with spermatozoa stored in the epididymides (*sensu* Pudney, 1995) for the next breeding season. In the "prenuptial" pattern, spermatogenesis undergoes arrest in the winter (or proceeds at a very slow rate) to resume again in the spring, and is completed just before mating (Pudney, 1995). Most oviparous *Liolaemus* species can be ascribed to one of these patterns (Ramírez-Pinilla, 1991; Cruz & Ramírez-Pinilla, 1996; Vega, 1997). Viviparous *Liolaemus* species have "prenuptial" - for example *L. gravenhorsti* (Leyton *et al.*, 1977) - or "postnuptial" patterns - for example, *L. aymararum* - but also different reproductive patterns not clearly ascribed to "prenuptial" or "postnuptial" profiles such as those of *L. multiformis, L. jamesi* and *L. alticolor* (Leyton *et al.*, 1982). *Liolaemus gravenhorsti* has a yearly, continuous spermatogenesis, with a period of maximal activity in summer-autumn and minimal activity in spring-summer. Female *L. gravenhorsti* do not have a sperm reservoir and oocytes are ovulated in spring (Leyton *et al.*, 1977). *Liolaemus aymararum* has spermatozoa in the epididymides in mid- and late autumn and pregnancy in late spring. In *Liolaemus multiformis* and *L. jamesi* the availability of gametes is synchronized in males and females in autumn. Pregnancy takes place during winter and spring (Leyton *et al.*, 1982).

Male *L. elongatus* and *L. pictus* show two major inter-specific differences in their reproductive biology. First, we found a small group of non-reproductive adult male *L. elongatus* alongside reproductive lizards. Non-reproductive adult males lack germinal cells other than spermatogonia and have smaller testes and shorter SVL. The capability of TS to discriminate a group of small males, with testes poorly developed reinforces the relationship between TS and SS. This seems to be the case for *Platysaurus* (van Wyk & Mouton, 1996) in which young adult males, while entering the mature group may exhibit a delay in reproduction relative to larger individuals. It is tempting to consider stage 1 males as juveniles, but the presence of similar-sized males with large testes still supports the 53.74 mm SVL (Ibargüengoytía & Cussac, 1998) as maximum juvenile size. The non-reproductive character of small adult males would probably allow the coexistence of males with a wide range of body sizes, as can be seen in the

SVL distribution of sample D (see Results), instead of the spatial displacement of newly mature males by larger adult ones, as happens in *Anolis limifrons* (Andrews & Stamps, 1994). This delayed reproduction, probably for one year, reduces the reproductive cost over the entire life cycle by preventing male-male encounters (and indirectly, exposure to predators), and by allowing smaller males to allocate energy to growth. Such a strategy augments, in *Ameiba plei* (Censky, 1995), the possibilities of future mating and, ultimately, increases the reproductive success. Large *A. plei* males win intrasexual encounters, guard females and are the only males observed to mate (see also Sugg, Fitzgerald & Snell, 1995). We cannot ignore the fact that, curiously, this phenomenon was not observed in *L. pictus*, though the latter has lower availability of reproductive females (Ibargüengoytía & Cussac, 1996). Other factors, such as environmentally dependent differences in predation risk, probably change the final consequences of male-male encounters.

Secondly, the observed relationships between TS and SS also show two species-specific characteristics. As in *L. gravenhorsti* (Leyton *et al.*, 1977), male *L. elongatus* show an increasing TS during spermatogenesis and a decrease when the first spermatozoa occur. However, the positive relationship among TS, TD and EH in *L. elongatus* differs from *L. gravenhorsti,* where the tubules maintain a wide diameter even when testicular weight decreases. It is important to keep in mind, when considering similarities between these species, that the female reproductive cycle of *L. elongatus* closely resembles *L. gravenhorsti* (Leyton *et al.,* 1977; Leyton, Miranda & Bustos Obregón, 1980; Ibargüengoytía & Cussac, 1998).

Liolaemus elongatus had spermatozoa in the seminiferous tubules at the end of spring and at the end of summer. In a rather different way, *L. pictus* had spermatozoa in seminiferous tubules throughout the sampling period, from spring to autumn, and displayed no significant relationship between TS and EH and TD, suggesting a different male cycle. The absence of a relationship between testicular and tubular size would be related to interstitial hydration (Leyton *et al.*, 1977). Here, it seems that the same strategy is used in two different situations. Male neotropical lizards, such as *Tropidurus peruvianus,* encounter reproductive females throughout the year (Leyton *et al.*, 1982). Male *L. pictus* face, in a temperate environment, a low availability of reproductive females, due to the biennial to triennial female reproductive cycle (Ibargüengoytía & Cussac, 1996) and probably, a low and unpredictable encounter rate during the spring-autumn breeding season. For both species, males have a permanent supply of spermatozoa.

The absence of dependence between female reproductive condition and adult female body length in both species (Ibargüengoytía & Cussac, 1996; 1998), and female growth rates that are lower than males in *L. elongatus,* agree with the idea of female preferential investment in present reproduction (Smith, 1992; Sugg *et al.*, 1995). However, in *L. elongatus* and *L. pictus* (present results and Ibargüengoytía & Cussac, 1996; 1998), adult male and female mean body length showed neither intersexual differences nor differences in size at the time of sexual maturity. Different growth rates but similar adult size suggest a cause that remains to be tested - that of greater predation on larger males.

Past (Ibargüengoytía & Cussac, 1996; 1998) and present results point out slight interspecific differences in sexual dimorphism and major differences in male cycle between *L. pictus* and *L. elongatus,* coinciding with a differential accessibility to females. Male *L. pictus* begins to reproduce during its first year of adulthood and remains active during a long breeding season. In contrast, male *L. elongatus* delay reproduction for one year and reproduce during a narrower period, in synchrony with the female reproductive cycle.

The male cycle can be considered as the functional counterpart of the male component of sexual dimorphism. The causal relationships between female and male reproductive cycles are mutual but the existence of severe thermal constraints for vitellogenesis and pregnancy (Ibargüengoytía & Cussac, 1996, 1998) set the following chain of causal events: environmental conditions - female cycle - male cycle and male dimorphic traits. This sequence of events, compared for two species of *Liolaemus* in Patagonia, indicates how environmental cues can constrain female cycle and female availability, and in consequence affect male cycle and the development of male dimorphic traits.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Mariana Lozada for the critical review of the manuscript and to Margrid Leben for the English revision. We also acknowledge the constructive comments of the three anonymous reviewers. This work was partially supported by Universidad Nacional del Comahue.

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Accepted: 12.2.99

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 119-123 (1999)

EGG DEPOSITION STRATEGIES OF THE SMOOTH NEWT (*TRITURUS VULGARIS*) IN AN UNPREDICTABLE ENVIRONMENT

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Studies carried out in England on the reproduction of smooth newts have revealed that under relatively constant, favourable conditions, they spend a period of up to six months in the water, with oviposition lasting about 4-6 weeks. The reproductive strategies of two Romanian smooth newt populations inhabiting unpredictable, highly variable and hostile environments were analysed for comparison. Females in both populations had an average body weight at the beginning of the reproductive period of 1.09 and 0.96 g respectively, one third of the average body weight of adult females in England. The average snout-vent lengths were 34.9 and 33.8 mm, about 25% shorter than those from English populations. The average numbers of eggs deposited by female newts from the two populations were 74 and 51 respectively, compared to an average of 300 eggs in England. The average age of females from one of the populations studied was 4.2 years and age at first reproduction was estimated at three years, similar to another English population studied. At the end of the oviposition period females still contained yolked oocytes, suggesting that clutch size cannot be correctly estimated by counting the initial numbers of yolked ovarian oocytes. Their reproductive effort was reduced since, due to environmental hostility, body size was significantly diminished and this leads to a smaller clutch size.

Key words: *Triturus*, newts, reproductive strategies, variable environments

INTRODUCTION

Female newts of the genus *Triturus* deposit eggs individually over several weeks. Four to ten days after insemination (Diaz-Paniagua, 1989; Pecio, 1992), the females start depositing the eggs fertilized with the sperm stored in a special organ, the spermatheca (Sever, 1994). The oviposition period lasts from several days up to three months (Verrell, Halliday & Griffiths, 1986), during which time the female feeds actively and may be inseminated by several different males (Pecio, 1992; Gabor & Halliday, 1997).

Large differences between the number of eggs in the ovaries and the number of deposited eggs, and between the fertility estimates, have been reported by authors studying populations in different countries (Hagström, 1980; Baker, 1992). Estimates vary between a minimum of 25-80 eggs deposited (Verrell, 1986), up to 637 eggs (Baker, 1992), while oocyte counts in the ovaries can reach an average of 1000, ranging between 581-1573 (Hagström, 1980).

Studies carried out in England on the reproduction of smooth newts (*Triturus vulgaris*) have revealed that under relatively constant, favourable conditions, they spend a period of up to six months in water, with oviposition lasting on average 4-6 weeks (Verrell & Halliday, 1985; Verrell, Halliday & Griffiths, 1986; Baker, 1992). These results contrast with observations made in other European countries (Accordi, Massarek & Nobili, 1990; Fasola & Canova, 1992; Pecio, 1992; Kalezic, Cvetkovic, Djorovic & Dzukic, 1996) indicating that newts tend to spend shorter periods in water. I investigated the reproductive strategies of two Romanian smooth newt populations (*T. v. vulgaris*) inhabiting unpredictable, highly variable environments.

I wanted to test whether (1) the parameters describing the rate of oviposition are similar to the ones reported by Baker (1992) for an English smooth newt population; (2) the predicted correlation between body size and clutch size is valid for the studied populations; (3) ovarian oocyte numbers are reliable estimates of clutch size; and (4) clutch size and oviposition period are correlated.

MATERIALS AND METHODS

The two Romanian smooth newt populations studied, are exposed to different degrees of environmental stress. The first population studied inhabits a pond several hundred square metres in area, with a maximum water depth of less than 1 m. The pond is located in a forest consisting mainly of oak and lime trees (Baneasa forest, north of Bucharest). The pond is subjected to prolonged drought and lasts in spring for only four to six weeks. Other amphibian species reproducing in the pond are *Triturus cristatus, Rana dalmatina, Pelobates fuscus*, and *Hyla arborea.* Due to the brief hydroperiod, there are years when amphibians cannot reproduce, while in other years only *Rana dalmatina* - an early breeder - achieves metamorphosis. Only in rainy years are newt and spadefoot toad (*Pelobates fuscus*) larvae able to reach metamorphosis. The second population studied inhabits a temporarily flooded area on an island in the lower Danube floodplain (latitude 44°47'52", longitude 27°49'05"), frequently subjected to flooding

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TABLE 1. The main parameters (mean, standard deviation, and range) describing egg deposition in two smooth newt populations from Romania, compared with the population studied by Baker (1992) in England.

Locality	Number of eggs deposited	Oviposition period (days)	Female initial weight (g)	Female SVL (mm)	Average rate of oviposition (eggs/day)	Maximum no. eggs deposited per day
Banesa Forest	Mean=51.2	Mean=11.9	Mean=0.96	Mean=33.8	Mean=5.51	24
n=22	SD=30.8	SD=8.9	SD=0.1	SD=2.72		
	Range 15-105	Range 2-33	Range 0.7-1.4	Range 30.2-38.4	Range 1.7-17	
Danube	Mean=74.6	Mean=12.3	Mean=1.09	Mean=34.9	Mean=5.85	37
floodplain	SD=61.4	SD=6.4	SD=0.24	SD=2.34		
n=13	Range 13-195	Range 1-21	Range 0.76-1.64	Range 28-39.8	Range 1.6-10.6	
England	Mean=300	Mean=36.9	Mean=2.7	Mean=45.9	Mean=8.7	54
(Baker, 1992)	SD=189	SD=19.7	SD=0.8	SD=3.81		
n=10	Range 88-637	Range 11-74	Range 1.6-4.2	Range 40-52	Range 3.9-17.3	

(see Cogalniceanu, Cristofor & Vadineanu, 1997 for a detailed description of the site).

The Baneasa forest population was visited in 1996, four days after a warm spring rain had melted most of the remaining snow. Also present were large numbers of actively breeding *Pelobates fuscus*, *Triturus cristatus,* and freshly laid *Rana dalmatina* spawn. The floodplain population was visited in 1995, shortly after severe spring floods had covered large parts of the island and had filled the pond. In both populations, smooth newt pairs in courtship were frequently observed.

Twenty-two females from Baneasa forest and 17 from the floodplain population were captured and kept individually in aquaria. Identical experimental conditions were provided during both years. Newts show a high individual variability in their response to the stress induced by captivity. Captivity-induced stress is easily detectable because animals fail to feed and emaciate rapidly. Care was taken to avoid unnecessary stress that can induce the loss of reproductive condition.

The body weight of females was measured at the beginning and end of the experiment on an electronic Gibertini balance with a precision of 0.01 g. Snout-vent length (SVL) was measured with dial callipers at the beginning of the breeding season with a precision of 0.1 mm. Captive females were fed every other day with *Tubifex* sp. Plastic strips were provided for egg deposition, and the eggs were collected and counted daily according to Arntzen & Hedlund (1990). Males were added to each aquarium for at least two days/week to ensure that females were inseminated (Pecio, 1992).

Four females from the Danube floodplain population were sacrificed immediately after capture, before oviposition started (control group). Six of the remaining thirteen females oviposited for less than two weeks on average. After a week without any further eggs being laid they were also sacrificed (group 1). The remaining seven females oviposited for almost a month and were sacrificed after a week without deposition. Females were first anaesthetized in MS-222 (Sandoz), and then sacrificed. The ovaries were dissected, stored in Gilson's solution (Montori, 1989) and the remaining oocytes counted. Yolked oocytes with a diameter larger than one mm were counted as mature. The hind limb was removed and stored in alcohol. Age was assessed by skeletochronology following Miaud (1991).

RESULTS

Females in the two populations had, at the beginning of the reproductive period, body weights averaging 1.09 and 0.96 g respectively, representing about one third of the average body weight of adult females in England. Their average SVL's were 34.9 and 33.8 mm respectively, about 75% of the SVL of females in England. Both initial body weight and SVL of females did not differ significantly between the two populations (ANOVA: F=3.70, P>0.05, df=2,33). The average numbers of eggs deposited were low, 74 and 51 respectively, but differences were not significant (ANOVA: F=3.46, P=0.07, df=2,33). The oviposition rates and the maximum number of eggs deposited in a day were also lower (Table 1; Fig. 1). Oviposition rates did not differ significantly between the two populations (ANOVA: F=0.34, P>0.5, df=2,33).

Females from the Danube floodplain population decreased in body weight on average by 0.31±0.05 g

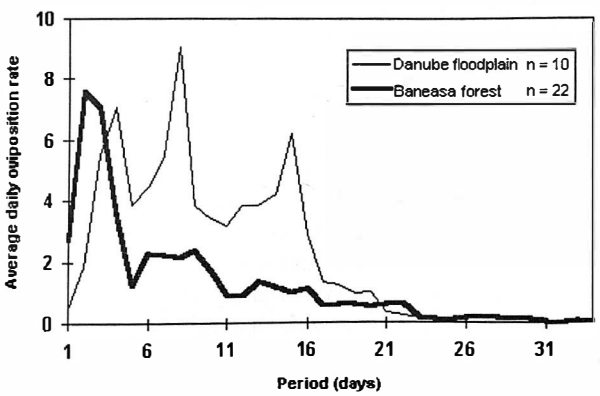

FIG. 1. Mean daily egg deposition rate of female smooth newts.

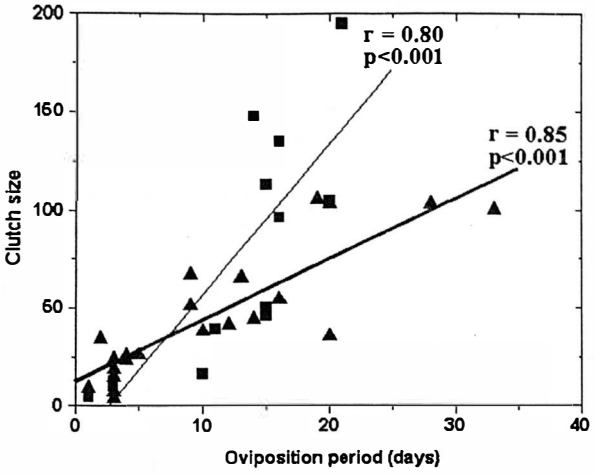

FIG. 2. Relationships between oviposition period and clutch size. Bold line, triangles, Banesa Forest (n=22); thin line, squares, Danube floodplain (n=13).

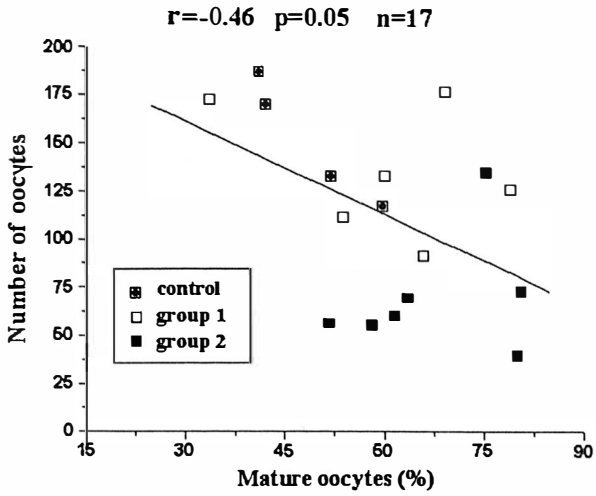

FIG. 3. Relationship between the total no. of ovarian oocytes present at the end of the experiment and the percentage of mature oocytes in the three groups from the Danube floodplain population. See text for details.

(ranging between 0.09 and 0.75, n=13) during the oviposition period, corresponding to an average body weight loss of 26.8% (ranging between 11.2 and 45.3%). Both weight loss and percentage weight loss were positively correlated with clutch size (r=0.63, P=0.02, n=13 and r=0.69, P=0.009, n=13 respectively).

Clutch size and oviposition period were significantly related in both Romanian populations studied, but the slopes of the regression lines differed significantly (t=2.59, df=33, P<0.01; Fig. 2).

The number of remaining ovarian oocytes was negatively correlated with clutch size in the Danube floodplain population (r=-0.79, P=0.001, n=13), suggesting that the number of ovarian oocytes before reproduction should only be used to estimate potential clutch size. They do not represent actual clutch size, since not all oocytes are deposited.

The females from the Danube floodplain population sacrificed at the beginning of reproduction (control group), had on average 151 oocytes in their ovaries. The females that oviposited for only a few days and were sacrificed ten days later (group 1) had on average 135 oocytes left, while the females which oviposited for almost a month (group 2) had on average only 70 oocytes left. The differences between the means of the three groups were significant (ANOVA: F=10.54, P=0.0016, df=2,16). A Tukey-Kramer multiple comparison test showed that the differences between the means were not significant for the control group and group one, but were highly significant for group 1 and group 2 (P<0.01), and for the control group and group 2 (P<0.01).

After summing the number of eggs deposited and the number of oocytes left in the ovaries, similar values were obtained for all three groups (mean±SE; control group 151.5±16.2; group 1 157.1±14.9; group 2 189.2±12.8). There was no significant variation in oocyte numbers between the groups (ANOVA: F=2.06, P=0.16, df=2,16).

When comparing the percentage of mature oocytes and the number of oocytes (mature and immature) present after deposition in the ovaries, it appears that the females in the control group and in group 1 had higher number of oocytes, but lower percentages of mature oocytes than the females in group 2. In females belonging to group 2 the percentage of mature ovarian oocytes increased to almost 80%, suggesting that vitellogenesis continued during the oviposition period (Fig. 3).

The mean age of 13 females from the Danube floodplain, estimated by skeletochronology, was 4.23±0.34 years (Cogalniceanu & Miaud, in prep.). The minimum age was three years (n=5), suggesting that this is the age of the first reproduction.

TABLE 2. Correlations between the main parameters describing oviposition in smooth newt females.* P< 0.05; ** P < 0.01; *** P < 0.001; NS - not significant.

Locality	Initial body weight and clutch size	Initial body weight and deposition rate	Clutch size and oviposition period
Baneasa forest	r = 0.07NS	r = - 0.17NS	r = 0.85***
Danube floodplain	r = 0.42NS	r = 0.40NS	r = 0.80***
England (Baker, 1992)	r = 0.61*	r = 0.89**	-

DISCUSSION

Severe environmental conditions affecting the two Romanian populations studied probably influenced their body size. Feeding in water lasts for only short periods while feeding opportunities on land are dis-

rupted by long unfavourable intervals (floods or drought respectively). The population studied by Baker (1992) inhabited a permanent pond, with a humid, more favourable surrounding habitat, allowing for prolonged feeding during both the aquatic and terrestrial phases. The weight lost during the aquatic phase is usually regained during the terrestrial phase (Verrell & Halliday, 1985; Fasola & Canova, 1992). Unfavourable conditions, mainly drought, can reduce this gain and impose smaller body dimensions. The two populations studied from Romania are both subjected to strong environmental pressures, either from drought or from high floods, with temperatures fluctuating between up to 40°C in the summer and as low as -20°C in the winter during most years. The Baneasa forest population inhabits an area where, due to the lowering of the underground water level during the last 20 years, the pond dries very rapidly, usually before metamorphosis of the larvae. Prolonged periods of inundation in the lower Danube floodplain were shown to cause a decrease in the body condition and fitness of green frog populations (Cogalniceanu, 1997). Inundation was also shown to cause increased mortality in two species of toads, especially in areas deprived of refuges from floods (Bosman, van Gelder & Strijbosch, 1997), and to increase the risk of predation from fish (Aronsson & Stenson, 1995). These factors might act in favour of an early breeding strategy, with all the potential advantages that it has (Nilsson & Svenson, 1996).

Although the data were collected during consecutive years, the parameters describing egg deposition do not differ significantly between the two populations. The only difference between the two populations studied appears to be that of larger clutches laid over shorter periods of time by females from the Danube population, compared to the Baneasa population (Fig. 2). This might be explained by the fact that females from the Danube population have a more diverse food supply (thus stimulating oviposition) but are also prone to stronger predation. Overall, despite the fact that environmental pressures are different, reproductive strategies appear similar in the two populations.

The body weights of the females in the populations studied, around one gramme, are extremely low. In Italy larger females were also reported with an average female body weight of 1.9 g (Fasola & Canova, 1992). Verrell & Halliday (1985) indicate an average female body mass of 2.6 g in another population from southern England.

These results compare favourably with the study by Kalezic *et al.* (1996) who found a similar number of oocytes in the ovaries, an average of 152 (ranging between 99 and 206), but no correlation between oocyte number and female size.

The hypothesis that actual clutch size can be measured by counting the ovarian oocyte number before reproduction, confirmed by Baker (1992), is not supported by this study, since not all oocytes are deposited. Similar results were obtained by Hagström (1980), who reported that after reproduction females still contained yolked oocytes. The small clutch size might also be caused by lack of further insemination. Pecio (1992) reported that females inseminated with only one spermatophore laid on average 52 eggs, while females further inseminated laid 137 eggs/season.

The average age of females from the floodplain population and the British population studied by Verrell & Francillon (1986) are similar (Mann-Whitney test, $z=1.44$, $P>0.05$). The range of age classes in smooth newt females is nevertheless different, three to six years in the Romanian floodplain population and two to five years in the British population. Similar results are reported by Kalezic *et al.* (1996), who estimate the time of attainment of sexual maturity for females at 3.2 years. Thus, reproduction at a younger age is not the cause for the large differences in body size.

The present results indicate great variability in the reproductive effort of populations that inhabit unpredictable and highly variable environments. In unfavourable conditions, *Triturus alpestris* females can stop depositing eggs or can reproduce only every other year (Vilter & Vilter, 1963). There seems to be a trade-off between clutch size and resource allocation. A smaller clutch size is preferred since resources also have to be allocated to somatic maintenance because of irregular feeding opportunities (Rosenheim, 1996). I suggest that female newts have a higher reproductive potential than previously estimated. When females terminate oviposition this is not a consequence of the depletion of mature oocytes but it is probably triggered by environmental factors.

ACKNOWLEDGEMENTS

Special thanks are due to Sorana Topa and Stan Evelina for their help during the experiment, and to Dorel Rusti, Pim Arntzen, John Baker, Richard Griffiths and an anonymous referee for helpful comments on earlier drafts. Claude Miaud was of great help in the age assessment.

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Accepted: 16.2.99

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 125-132 (1999)

A LATITUDINAL CLINE OF DARK PLASTRAL PIGMENTATION IN THE TORTOISE *TESTUDO HERMANNI* IN GREECE

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The area of dark pigmentation on the plastron of the tortoise *Testudo hermanni* shows a latitudinal cline over about 400 km in Greece, with populations in the south being darker. The carapace did not show the clinal trend, and pigmentation was not significantly related to longitude or altitude. We examined several possible explanations for the cline, including an effect of incubation temperature, random genetic variation, and adaptation to several environmental variables. The most likely explanation is selection for thermoregulation, with decreased dark pigmentation in the north reducing heat loss to the substrate by infra red radiation during activity. This hypothesis was supported by data on body (T_b) and substrate (T_s) temperatures in populations from northern, central and southern Greece. T_b was generally above T_s, showing that heat would generally be lost rather than gained through the plastron, and the mean difference T_b-T_s was greatest in the north: +6.6 °C, compared to +2.4 °C in the south. Mean T_b was lowest in the south (26.9°C, compared to 29.3°C in the north) and the slope of T_b on T_s was about 1 (compared to 0.5 in the north). Thermoregulation in southern Greece is similar to that of tropical tortoises, with avoidance of overheating being the major problem, rather than elevation of T_b for activity.

Key words: cline, pigmentation, *Testudo*, thermoregulation, tortoise

INTRODUCTION

Many species of reptiles show marked variation in colour pattern within and among populations, particularly in the level of dark pigmentation (Crisp, Cook & Hereward, 1979). Genetic differences in pigmentation may be the result of selection for camouflage (Gibbons & Lillywhite, 1981), reptiles on darker soils often being darker coloured (Lewis, 1949; Lawrence & Wilhoft, 1958). Dark pigmentation also has consequences for thermoregulation, melanistic individuals reaching higher body temperatures than normally pigmented individuals (Gibson & Falls, 1979). Dark pigmentation may also be involved in protecting the tissues from damaging ultraviolet (UV) radiation (Porter & Norris, 1969; Cloudsley-Thompson, Constantinou & Butt, 1985). Pigmentation may, however, be directly affected by incubation temperature in reptiles (Ewert, 1979; Murray, Deeming & Ferguson, 1990), so that differences among populations are not necessarily genetic.

There is considerable variation in the extent of dark pigmentation on the plastron of the Mediterranean tortoise *Testudo hermanni*, particularly between the western (*T. h. hermanni*) and eastern (*T. h. boettgeri*) subspecies (Guyot & Devaux, 1997). Differences of coloration are of taxonomic significance in some chelonians (Fritz, 1992). The plastron of tortoises is rarely exposed, and is therefore a good candidate for a selectively neutral characteristic of value in differentiating between the subspecies of *T. hermanni*. Nevertheless, plastral pigmentation varies substantially between populations of *T. h. boettgeri* in Greece; animals from the south have notably dark plastrons, similar to those from western Europe. This paper describes the variation in dark plastral pigmentation of *T. hermanni* in Greece, in relation to geographic and environmental variables. Several hypotheses to explain this variation are then evaluated, in particular that this is due to thermoregulation; data on body and substrate temperatures are used to assess the direction and relative rate of heat exchange through the plastron in different populations.

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METHODS

Pigmentation of *T. hermanni* was studied at 16 sites in Greece (Fig. 1), which are described by Willemsen & Hailey (1989). Tortoises were marked in the field with unique codes by filing the marginal scutes (Stubbs *et al.*, 1984); each pattern considered here is from a different individual. Straight carapace length was measured with a specially-constructed flat-bed calliper or 'tortometer'. Sex was determined on the basis of external characteristics: *T. hermanni* can be sexed from a straight carapace length of 10 cm (Stubbs *et al.*, 1984), and only animals larger than 10 cm are considered here. The pattern of pigmentation was recorded by photographing the carapace and/or plastron as colour slides.

Slides were projected on to squared paper and the area of dark pigmentation was traced out. The area was measured on one side of the plastron and on the costal scutes of one side of the carapace. Dark pigmentation occurs in characteristic areas of the plastron and carapace (typical patterns of dark plastral pigmentation in *T. hermanni* are shown in Fig. 3 of Guyot & Devaux,

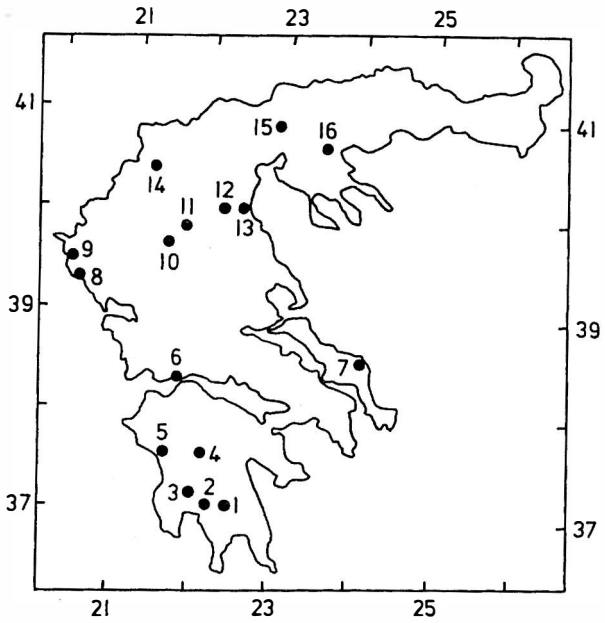

FIG. 1. Map of Greece showing the locations of the sites, and latitude (°N) and longitude (°E). Sites are: 1, Sparta; 2, Kalamata; 3, Arfai; 4, Langadia; 5, Olympia; 6, Antirrion; 7, Kymi; 8, Parga; 9, Igoumenitsa; 10, Meteora; 11, Deskati; 12, Agios Dimitrios; 13, Litochoron; 14, Kastoria; 15, Kilkis; and 16, Mikra Volvi.

1997), and the pattern is approximately bilaterally symmetrical in each individual. The area of pigmentation was then calculated as a percentage of the total area of the half-plastron or costal scutes, respectively. The arcsine transformation (Sokal & Rohlf, 1981) was used for multivariate analysis of percentage data (but not for calculating means and SDs).

The primary environmental data for each site comprised its latitude, longitude and altitude. The environmental temperature at each site was summarised as the effective temperature (ET; Stuckenberg, 1969), calculated as $ET=(8\bar{T}+14T_r)/(T_r+8)$, where $\bar{T}$ is the mean annual temperature and T_r is the annual range of temperature. Mean temperature in each month was calculated as (mean daily maximum + mean daily minimum)/2 (Meteorological Office, 1996, page viii); $\bar{T}$ is the mean temperature averaged across all months, and T_r is the difference between the means of the warmest and coldest months. A multiple regression showed that ET in Greece and the Balkans south of 41°N was significantly related to latitude ($P<0.001$) and altitude ($P<0.001$), but not to longitude ($P=0.066$) (Willemsen & Hailey, 1999). A multiple regression of the significant variables explained most ($r^2=88.3\%$) of the variation of ET from latitude and altitude, and was used to predict ET at the tortoise sites.

Substrate temperatures (T_s) were measured, simultaneously with body temperatures (T_b) of active tortoises, at three sites of similar altitude (200-300 m) in northern, central and southern Greece. Data from Mikra Volvi were collected from 17-20 April 1989. More data were available for the other two sites, but only those collected at about the same time are considered here: 24 April - 17 May 1989 at Meteora and 20-23 May 1989 at Sparta. Active tortoises were measured at the time and place of capture, throughout the daily activity period. Temperatures were measured with a mercury thermometer to the nearest 1°C: T_b in the cloaca, and T_s at the surface near the tortoise.

RESULTS

Individual and Sexual Variation

There was continuous variation in the area of dark plastral pigmentation between individuals within all

TABLE 1. Altitude, effective temperature (ET) and pigmentation of *T. hermanni* at different sites. Values are the mean±SD area of dark pigmentation as a percentage of the area of plastron or costal scutes of the carapace, with sample size in parentheses.

	Altitude (m)	ET (°C)	Area dark (%)	
			Plastron	Carapace
Sparta	300	14.9	56.0±14.6 (156)	41.6±14.2 (112)
Kalamata	0	15.4	60.2±19.1 (77)	35.4±11.2 (69)
Arfai	150	15.1	60.2±16.3 (7)	38.7 ±7.4 (8)
Langadia	1250	13.0	39.3±13.8 (47)	44.3±13.6 (44)
Olympia	200	15.0	51.3±16.2 (120)	42.4±14.7 (171)
Antirrion	50	15.0	37.4 ±7.3 (8)	34.7±13.9 (7)
Kymi	150	14.8	41.9±10.1 (12)	50.9±11.1 (8)
Parga	300	14.3	29.7 ±8.1 (38)	49.7±11.1 (35)
Igoumenitsa	0	14.8	36.6±12.7 (104)	46.9±14.4 (93)
Meteora	250	14.3	26.3±15.7 (130)	38.5±16.8 (187)
Deskati	650	13.6	23.6±13.1 (116)	32.8±16.4 (108)
Agios Dimitrios	600	13.6	22.1±14.2 (89)	35.5±19.8 (73)
Litochoron	450	13.9	34.6±17.5 (49)	49.5±15.4 (49)
Kastoria	800	13.1	27.4±10.6 (100)	39.0±14.4 (94)
Kilkis	250	14.0	22.5 ±9.8 (45)	24.8±11.4 (40)
Mikra Volvi	200	14.2	21.8±13.4 (34)	36.7±16.1 (34)

populations, with no evidence of separate classes (i.e. polymorphism); examples of two populations from northern and southern Greece are shown in Fig. 2. There was a significant tendency for males to have a larger area of dark plastral pigmentation than females (Fig. 3). The regression equation is: M=-4.0+1.30F (n=16, r^2=91.4%), where M and F are the mean areas of dark plastral pigmentation for males and females, respectively. The slope is significantly different from 1.0 (t=2.77, P<0.02), but the intercept is not significantly different from 0 (t=1.07, P=0.303). The area of dark plastral pigmentation was thus about 1.3 times greater in males than in females, across all populations.

The effect of body size on plastral pigmentation was examined separately in males and females, as there is sexual size dimorphism in Greek populations of *T. hermanni* (females being larger; Willemsen & Hailey, 1999). The correlation coefficient between carapace length and the area of dark pigmentation was calculated for all samples with 30 or more individuals. The mean value of r was -0.020 (SE=0.043, n=15), not significantly different from 0 (t=0.46, P=0.65). There was thus no consistent effect of body size on the area of dark plastral pigmentation among these populations.

The final question on variation within populations concerns the independence of pigmentation on the plastron and carapace. The correlation coefficient between the area of dark pigmentation on the plastron and on the carapace was calculated for all samples with 30 or more individuals with both measurements. The mean value of r was +0.297 (SE=0.045, n=12), which differs significantly from 0 (t=6.66, P<0.001), showing that

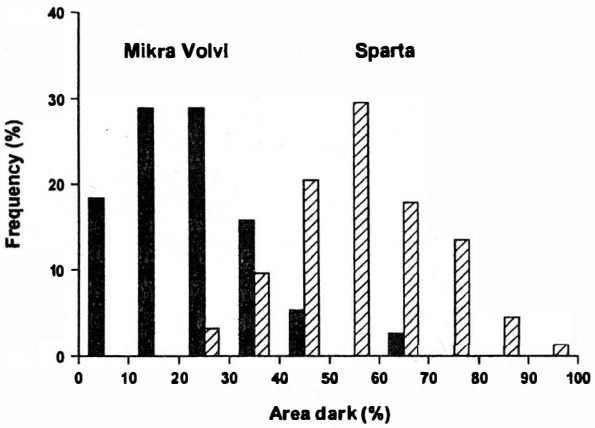

FIG. 2. Individual variation in the area of dark pigmentation on the plastron at the extremes of the cline: Mikra Volvi in northern Greece (solid bars), and Sparta in southern Greece (hatched).

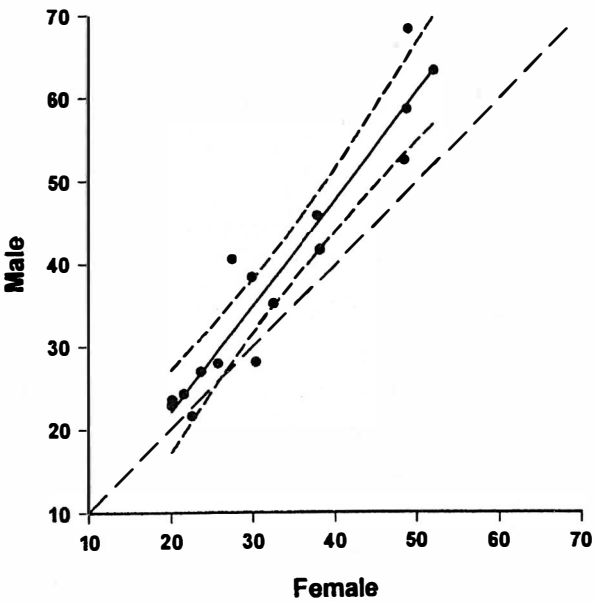

FIG. 3. Sexual differences in the area of dark plastral pigmentation among Greek populations of *T. hermanni*. Each point shows one site, with the fitted regression (unbroken line) and its 95% confidence interval (short dashes). The long dashes show male=female pigmentation.

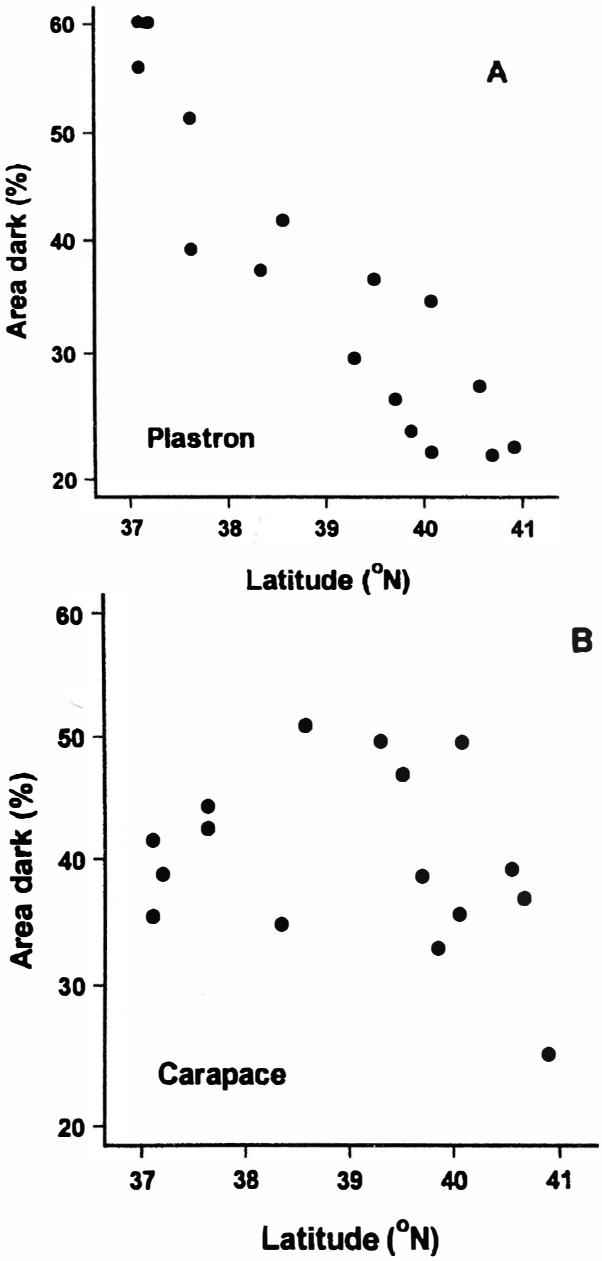

FIG. 4. Latitudinal variation in the area of dark pigmentation on (A) the plastron and (B) the carapace.

TABLE 2. Correlations of dark pigmentation with environmental variation among sites. r is the correlation coefficient, P_r is the probablility that r=0, and P_b is the probability that the coefficient in a multiple regression, b=0. n=16 sites for each analysis, of arcsine-transformed percentages.

	Area of dark pigmentation (%)					
	Plastron			Carapace		
	r	P_r	P_b	r	P_r	P_b
Latitude	-0.922	<0.001	<0.001	-0.243	0.365	0.386
Longitude	0.026	0.925	0.772	-0.156	0.564	0.597
Altitude	-0.318	0.229	0.087	0.036	0.894	0.814
ET	0.687	0.003	-	0.087	0.749	-

there was a positive association between pigmentation on the upper and lower body surfaces within populations, which was consistent across sites. Nevertheless, the mean value of r^2 was only 11%. Only a small proportion of the within-population variation in the area of dark plastral pigmentation could therefore be explained by the area of dark pigmentation on the carapace.

VARIATION AMONG POPULATIONS

The mean area of dark pigmentation varied widely between sites (Table 1) on both plastron (range 22-60%) and carapace (range 25-51%). Table 2 shows an analysis of dark pigmentation in relation to environmental variables. The area of dark pigmentation on the plastron was significantly negatively related to latitude, with a trend over about 400 km between northern and southern Greece (Fig. 4A). Plastral pigmentation was also significantly correlated with ET, but less strongly than with latitude (Table 2); there was no significant effect of altitude. A multiple regression of the three primary environmental variables showed a highly significant effect of latitude, but not of altitude or longitude (Table 2). (ET was excluded from the multivariate analysis as this was a secondary variable, itself calculated from latitude and altitude; rainfall was excluded for the same reason). In contrast, neither latitude (Fig. 4B) nor any other environmental variable had a significant effect on the area of dark pigmentation on the carapace (Table 2).

BODY AND SUBSTRATE TEMPERATURES

Mean T_b varied significantly between the three sites (Table 3), being lowest at the hot, southern site and

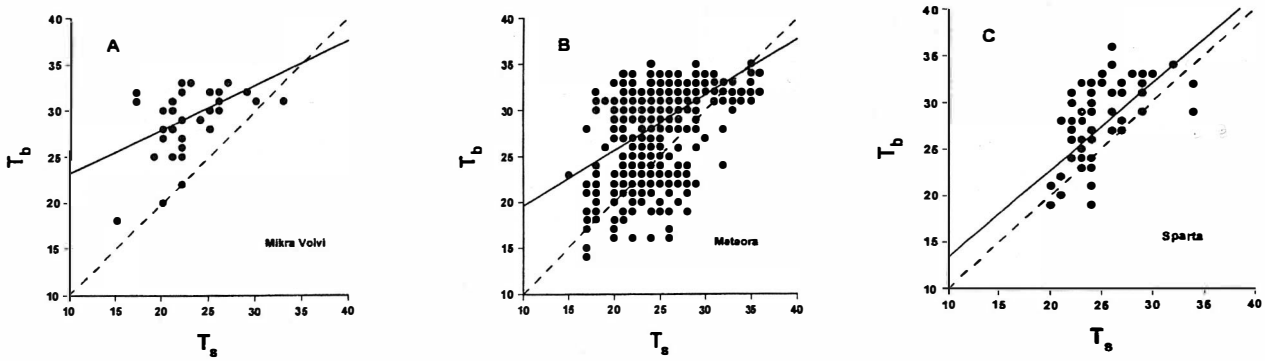

FIG. 5. The relationship between body temperature (T_b) and substrate temperature (T_s) at three sites in northern (A, Mikra Volvi), central (B, Meteora), and southern Greece (C,Sparta). The dashed line shows T_b=T_s, and the continuous line shows the regression fit. Note that T_b is more elevated above T_s and the slope is lower at Mikra Volvi than at Sparta, with Meteora intermediate in both respects. Regression equations are: (A) T_b = 18.5 + 0.474 T_s (n=42, r^2=21.4%); (B) T_b=13.5 + 0.605 T_s (n=603, r^2=22.5%); (C) T_b=4.0 + 0.933 T_s (n=72, r^2=41.9%).

TABLE 3. Body temperature (T_b), substrate temperature (T_s), and the difference T_b-T_s at three sites of similar altitude in northern, central, and southern Greece. Data (°C) shown ±SD or with sample size in parentheses. The F and P values are for ANOVAs comparing the sites or the sexes.

					T_b-T_s			
Site	n	T_b	T_s	T_b-T_s	Males	Females	F	P
Mikra Volvi	42	29.3±3.5	22.7±3.5	6.6±3.6	6.8 (33)	5.7 (9)	0.67	0.42
Meteora	603	28.5±4.6	24.8±3.6	3.7±4.2	4.0 (316)	3.4 (287)	2.52	0.11
Sparta	72	26.9±4.6	24.5±3.2	2.4±3.5	2.0 (42)	2.9 (30)	1.02	0.32
F		4.73	6.71	13.4				
P		0.009	0.001	<0.001				

highest at the cool, northern site. Tortoises achieved high T_b at Mikra Volvi in spite of environmental temperatures being lower there, both in terms of ET (Table 1) and T_s (Table 3). Body temperature at Mikra Volvi was substantially elevated above T_s, especially in cool environmental conditions (i.e. at low T_s; Fig. 5A). The mean difference T_b-T_s varied significantly between the three sites (Table 3), being greatest at Mikra Volvi and lowest at Sparta. Mean T_b exceeded T_s at all three sites, so that on average heat would be lost from the plastron to the substrate during activity. The difference between T_b and T_s, and thus the potential rate of heat loss, increased further north. There were no consistent or significant differences between the sexes in the elevation of T_b above T_s (Table 3).

The regression of T_b on ambient temperature (T_a) may be used as a measure of thermoregulation (Huey & Slatkin, 1976); a slope of 1.0 indicates that T_b depends on ambient temperature, while a slope of 0 indicates that T_b is independent of ambient temperature. This measure of thermoregulation may sometimes be misleading (Hailey & Coulson, 1996*a*), but remains useful in comparisons between populations. The regression slope of T_b on T_s was 0.474 at Mikra Volvi (Fig. 5A), significantly different from 1.0 (SE=0.144, t=3.65, P<0.001), showing that T_b was particularly elevated at lower T_s. The regression slope was 0.933 at Sparta (Fig. 5C), not significantly different from 1.0 (SE=0.131, t=0.51, P>0.5). The pattern at Meteora was intermediate between the other two sites in both the mean elevation of T_b above T_s (Table 3), and in the slope and intercept of the regression of T_b on T_s (Fig. 5B). The regression slope of 0.605 at Meteora was also significantly different from 1.0 (SE=0.046, t=8.59, P<0.001). The slope of T_b on T_s was significantly different from 0 at all three sites: P=0.002 at Mikra Volvi and P<0.001 at Meteora and Sparta. These data therefore show greater thermoregulation in the north, both in terms of the elevation of T_b above T_s and the slope of T_b on T_s.

DISCUSSION

Effect of Incubation Conditions?

A cline (a gradient in a measurable character; Huxley, 1938) of plastral pigmentation clearly occurs in *T. hermanni* in Greece along a north-south axis. Variable pigmentation occurs within many other, perhaps most, reptile species. Such variation usually occurs, however, within populations (polymorphism; Forsman & Shine, 1995), between habitats (Gibbons & Lillywhite, 1981), at a microgeographic scale (Thorpe, Black & Malhotra, 1996), or between races or subspecies (Fritz, 1992). We know of no other well-documented example of a cline of pigmentation of a reptile species over a distance of hundreds of kilometres. The first hypothesis to be examined is whether the cline represents a phenotypic response to incubation conditions. Low incubation temperature causes a decrease in the area of dark plastral pigmentation in emydid turtles (Etchberger *et al.*, 1993). A similar effect might explain the cline of pigmentation in *T. hermanni*, if nest site selection was unable to compensate for differences in climate among populations.

Pigmentation was more strongly correlated with latitude than with ET and was not related to altitude, suggesting that differences in pigmentation were not a purely phenotypic response to incubation temperature. Two other lines of evidence support this conclusion. First, sex is environmentally determined in *T. hermanni* (Eendebak, 1995) as in many other chelonians (Janzen & Paukstis, 1991), with females being produced at higher temperatures. Pigmentation was greater at warmer sites; if this was due to incubation temperature, then females should be more highly pigmented than males. In fact, males had consistently greater plastral pigmentation than females (Fig. 3). Second, *T. hermanni* hatched in captivity show no noticeable effects of incubation temperature (over a wide range, from 24-34°C) on pigmentation (B. T. Eendebak, personal communication). Although it is not possible to rule out some effect of incubation temperature on plastral pigmentation in *T. hermanni*, this will certainly be too small to explain the wide variation of mean pigmentation among field populations (Fig. 4B). The hypothesis that variation of pigmentation between sites is a purely phenotypic effect of incubation temperature can therefore be rejected.

Adaptive or Random Variation?

Although differences in pigmentation are probably genetic, this still leaves the question of whether they are adaptive or due to chance events. Random differentiation along a cline depends on the appropriate level of genetic exchange (of individuals and genes) between adjacent populations: too high, and the populations become uniform; too low, and a mosaic of local differences is produced rather than a broad geographic trend (Endler, 1977). Home ranges of tortoises are generally small in relation to the scale of the clines observed here: home ranges of *T. hermanni* are of a few hectares (Hailey, 1989), implying movements of a few hundred metres in any direction. Nevertheless, longer movements by a few transient individuals (Kiester, Schwartz & Schwartz, 1982) could be enough to promote gene flow between populations.

Random differentiation along a cline may occur through secondary contact between populations which have differentiated when separate, or among contiguous populations as a result of genetic drift or recurrent mutation in certain areas (Endler, 1977). Any of these three models could apply to *T. hermanni* in Greece. The distribution of this species is currently continuous over the whole country, as required by the latter two models. Analysis of mitochondrial DNA suggests, however, that animals from the Peloponnese are distinct from those from other parts of Greece (A. C. van der Kuyl, J. T. Dekker, J. Goudsmit, D. Ballasina & R. E. Willemsen, in preparation), so that the first model could also apply.

Random differentiation is usually assumed as a hypothesis of last resort, where selective differences are unknown and cannot be conceived (Endler, 1977). The possibility that the cline in *T. hermanni* is due to random differentiation is therefore provisionally rejected, in favour of adaptation to some environmental variable, although this cannot be disproved.

Adaptation to Soil Type or Rainfall?

Adaptive differentiation of populations along a cline may occur along a continuous environmental gradient, across an abrupt change in the environment, or as a result of adaptation by populations when allopatric and subsequently merging (Endler, 1977). The cline of dark plastral pigmentation was not steep, but occurred over about 400 km. This pattern suggests that any environmental influence was along a continuous rather than a step gradient (for example, a sharp transition of soil types). The direction of the cline also suggests that soil type is not involved, because the geology of Greece varies along an east-west rather than a north-south axis. The tortoise sites fell in two major geological regions; the western sites 1-10 in the Hellenides (Greek fold-mountains), and the eastern sites 11-16 in the northern crust block (Newbigin, 1943). Geological variation within the western mountains also occurs along an east-west axis perpendicular to the coast (Newbigin, 1943).

Rainfall is related to longitude in Greece (P=0.002) and does not vary significantly with latitude (P=0.983; Willemsen & Hailey, 1999). Rainfall data were not available for most tortoise sites and, although these could be calculated from longitude, the resulting secondary variable could not be included in the multivariate analysis of pigmentation. Nevertheless, the east-west axis of rainfall shows that this variable is unable to explain the north-south cline of pigmentation.

Pleiotropic Effect of Carapace Pigmentation?

The environmental factor most likely to explain a north-south cline of pigmentation is thermoregulation. The radiation balance (rather than ET) will be particularly important in tortoises because their T_b's are not closely related to air temperature (Meek, 1988); light intensity is the most important physical factor in determining heating rates of terrestrial turtles (Boyer, 1965). Colour affects the absorbance of visible radiation (Mount, 1979), and this effect has been shown in comparisons of lizards from different areas (Hutchison & Larimer, 1960). Visible light may make up about half of the total radiant heat load on an animal (Finch, 1972), the remainder being infra-red; body colour may thus have a major effect on thermal balance. Nevertheless, there was no cline of pigmentation on the carapace: the cline of plastral pigmentation is therefore not a pleiotropic effect (i.e. a multiple effect of a single gene) of selection for carapace colour.

Selective Advantage of Plastron Colour

The plastron may be an important route of heat loss from tortoises (Mackay, 1964; Lambert, 1981), by conduction/convection or emission of infrared radiation. Most heat exchange through the plastron will be by conduction/convection, especially at rest when the plastron is in contact with the substrate, but this will be independent of colour. Heat exchange through the plastron will also occur by infrared radiation when the body is raised above the ground during activity, by emission from the body and absorption of infra-red re-radiated from the ground. Coleman & Livezey (1968) found small decreases in infrared reflectance (and thus increases in emissivity) of dark compared to light areas of skin in the lizard *Sceloporus occidentalis*. Plastral pigmentation may therefore have a slight effect on the infra-red balance; and even a slight thermal advantage may be important as the colour of the plastron is not under strong selection for camouflage.

The rate of heat loss from the plastron to the substrate depends on the difference between T_b and T_s, which was greatest in the north. This heat loss would be minimized by reducing the amount of dark pigmentation on the plastron. Body temperature was much less elevated above T_s in the south, so that there would be little heat loss or thermal disadvantage from a high level of dark pigmentation. The sexual difference in plastral pigmentation remains unexplained, since there was no consistent sexual difference in the elevation of T_b above T_s. It is possible that the sexual difference in pigmentation is not functional, but due to the difference in the shape of the plastron between males and females.

A functional explanation for the cline of dark pigmentation in *T. hermanni* based on thermoregulation would explain why similar clines are absent, or at least uncommon, in other reptiles. Many lizards use changes in skin pigmentation as a means of physiological thermoregulation, with increasing reflectance of both visible and near infra-red radiation at higher body temperature (Bartholomew, 1982). Pigmentation in the scutes of tortoises cannot be changed behaviourally, so that adaptation must occur at the level of the population (producing a cline) rather than the individual. Pigmentation of tortoises is in this respect more similar to that of insects, in which geographical variation of colour for thermoregulation is well-documented, than to other reptiles. For example, dark pigmentation varies within and between species of *Colias* (clouded yellow butterflies) with latitude (Watt, 1968), and has significant effects on their thermoregulation (Watt, 1969).

Thermoregulation and Latitude

Thermoregulation of *T. hermanni* in northern Greece is similar to the pattern found further north in Yugoslavia (Meek, 1988), with T_b elevated above ambient temperature, particularly in cool conditions (Fig. 5A) as a result of basking. Thermoregulation at Sparta appears to be more similar to that of the tropical tortoise *Kinixys spekii*, in which there was little elevation of T_b above ambient temperature and a slope of T_b on T_a close to 1.0 (Hailey & Coulson, 1996*a*). It is likely that the avoidance of critically high T_b is more important than gaining or conserving heat in southern Greece, similar

to *K. spekii* in which thermoregulation consists of avoiding high T_b by choice of activity time and microhabitat (Hailey & Coulson, 1996*a,b*).

Mean T_b at Sparta was lower than that further north, although data were collected slightly later in spring so that differences in the timing of fieldwork should have produced slightly warmer conditions at Sparta, if anything; T_s was higher at Sparta than at Mikra Volvi. Low T_b at Sparta also suggests that thermoregulation there consists of minimizing the risk of overheating, by allowing a wider margin before T_b reaches critical levels. The low mean T_b at Sparta is also similar to that of *K. spekii* (27.0°C), which has the lowest T_b reported for any tortoise species despite occupying a hot tropical environment (Hailey & Coulson, 1996b). It is notable that activity of *Testudo graeca* is apparently limited by high environmental temperature in the far south of Europe; tortoises in southwestern Spain (at about 37°N, the same latitude as Sparta) aestivate in summer (Diaz-Paniagua, Keller & Andreu, 1995).

A question remains over the large area of dark plastral pigmentation in western populations of *T. hermanni* (Guyot & Devaux, 1997). Tortoises in southern France have a similar relation between T_b and T_s to that found at Sparta; the slope of T_b on T_s is about 1 (Fig. 3b of Pulford, Hailey & Stubbs, 1984), and T_b is only 1-3°C higher than T_s (Table 1 of Huot-Daubremont, Grenot & Bradshaw, 1996). These data are consistent with the thermal hypothesis for plastral pigmentation. Nevertheless, it is unclear why thermoregulation in southern France, at a latitude of 43-44°N, is similar to that of Sparta rather than northern Greece or Yugoslavia.

Implications for Conservation

In conclusion, Greek populations of *T. hermanni* show marked variation of dark plastral pigmentation with latitude, but not longitude or altitude. There was a linear cline over more than 400 km, with the area of dark pigmentation being greatest in southern Greece. The cline is unlikely to be due to a purely phenotypic effect of incubation temperature, to variation with rainfall or geology, or to pleiotropic effect of selection for pigmentation on the carapace. Data on body and substrate temperatures are consistent with the hypothesis that the variation is functional, plastral pigmentation being reduced in northern populations to minimize loss of heat from the body to the substrate during activity. An alternative of random genetic variation causing the cline cannot be ruled out, but either of these explanations involves genetic differences between Greek populations of *T. hermanni* .

Greek populations differ widely in adult body size, which is also likely to be due to genetic differences as size was not related to growth rates (Willemsen & Hailey, 1999). Such differences between populations mean that caution must be exercised over the release of tortoises into the wild (Guyot & Devaux, 1997), such as captive-bred individuals or those seized by customs (for example Ballasina, 1992). This problem has so far only been considered at the level of the eastern and western subspecies. The differences demonstrated here suggest that even greater caution is needed; tortoises should not be released unless their site of origin is known to within a few kilometres.

ACKNOWLEDGEMENTS

We are grateful to Bert Eendebak for information on pigmentation of captive-bred tortoises, Riet Beersen, Dimitra Apostolopoulou, Marianne Peppelman, Ben Krudde, and the Tsouvalakas family of the Vrachos camping site near Meteora for help in Greece, and Roger Avery and referees for helpful comments on the paper

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Accepted: 16.2.99

BOOK REVIEWS

Untersuchungen zur Populationsdynamik und zum Ausbreitungsverhalten von Amphibien in der Agrarlandschaft. Stephan Kneitz. 237 pp. Laurenti Verlag, Bochum (Paper).

This book is the integral text of a PhD thesis carried out under the supervision of W. Böhme and W. J. Kloft (Bonn) and the guidance of J. Blab. The study, which translated into English is entitled 'Investigations on Population Dynamics and Dispersal of Amphibians in Agrarian Landscapes', reports on what could be termed a medium to long term study of the ecology of an amphibian breeding community in an area situated south-west of the city of Bonn. From 1992 to 1995 three original ponds, and four newly created ponds established in 1988, were studied with regard to the development of amphibian populations present in the region. By following these seven neighbouring ponds at the same time, the author has chosen a sound approach, albeit a difficult one. The establishment of new populations, and fluctuations over the years, are recorded in meticulous detail. Altogether, the study offers many interesting results that can be applied to issues such as the conservation of amphibians in an agricultural area. Several techniques have been applied to study migration, breeding site fidelity and recruitment: surrounding breeding ponds with fences, capture-recapture and collection of animals marked through toe-clipping, and monitoring movement of individuals by means of transponders. This results in a large database of observations on the population structure and ecology of the amphibians present. During the study period, eleven species of amphibians were recorded: *Bufo bufo*, *Triturus vulgaris*, *T. alpestris*, *T. cristatus*, *Rana temporaria*, *R. dalmatina* and green frogs; with occasional sightings of *Bufo calamita*, *Alytes obstetricans*, *Triturus helveticus* and *Salamandra salamandra*. The study focuses on the seven first-mentioned and more common species.

The book is structured as follows: the lengthy introduction to the study site is followed by a detailed description of methods and the interpretation of data. The chapter presenting the results is long and organized in a rather cumbersome manner; by species, by pond, and by issue for each species, such as dominance structure, population dynamics, composition of breeding communities, return rates and sex ratios. It is interspersed with sub-chapters on the different species in each pond, discussions and summaries. The summaries alone do not do full justice to the data assembled, and do not guide the reader through the book in an easy manner.

I am not an ecologist, and may have missed important information while reading, but I must admit that the outpouring of details occasionally obscures possibly exciting new findings or salient points. What does become clear, and is important in itself, is that the variation found in the different breeding ponds and the fluctuations over the years make it difficult, even after four years of intensive enquiry, to make general statements or predictions on the population dynamics of amphibian breeding assemblages such as this. As it stands, this booklet is a thorough, descriptive report of mid- to long term ecological data that can be of use to the field herpetologist. For the implementation of measures needed to maintain a viable amphibian community in the agricultural environment, it is a valuable reference source, but in its present form probably not practical enough to be an easy guide for those who make the decisions about land use and planning. For interested ecologists and herpetologists, a reorganization of the data into a more concise and accessible form, such as through articles in ecological journals, might be a preferred medium of communication.

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Herpetofauna Worker's Manual. Tony Gent and Steve Gibson (Eds.). (1998). xii + 152 pp. Joint Nature Conservation Committee, Peterborough £20.00 (paperback).

In 1993 published information on the practicalities of carrying out field work on reptiles and amphibians in Britain was meagre. This problem was recognised by the voluntary organisations and government agencies charged with responsibilities for reptile and amphibian conservation. The Joint Nature Conservation Committee therefore set about collating the knowledge and experience of those working with these species into one easily accessible volume, and in 1994 fifteen herpetologists were commissioned to produce contributions for this project. These contributors came from a range of disciplines, and included research scientists, consultants, conservationists, members of statutory bodies and dedicated volunteers who spend much of their free time in the field conserving these animals. Thus began the *Herpetofauna Workers' Manual*.

Between the time manuscripts were submitted in 1994 and the eventual publication of the manual in October 1998, a number of other publications were produced to fill in the gap, including a section on herpetofauna in English Nature's *Species Conservation Handbook*, the *Herp Worker's Guide* (Foster & Barr, 1998), proceedings of meetings and conferences (e.g. Foster & Gent, 1996) and information leaflets produced by Froglife, BHS and English Nature. This manual now brings all this information together, providing an indispensable reference for anyone interested in field herpetology.

The manual contains twelve chapters covering all aspects of the conservation of British herpetofauna. Surveying, habitat management, legal considerations,

site protection, species translocations, and public involvement are among the topics included. There are a number of excellent drawings, illustrating species identification, equipment and habitats. In addition, there is a comprehensive bibliography which points the direction for more detailed study. Much of the conservation work for reptiles and amphibians in the UK is carried out by non-specialists, and this manual provides a comprehensible yet thorough overview for those with no experience or knowledge of reptile and amphibian ecology, as well as a useful reference for experienced herpetologists.

With so many authors writing about similar subjects, the potential for redundancy between chapters was considerable. The inevitable - although slight - overlap indicates how complicated the issue of conservation is, and inadvertently encourages a holistic approach. The editors had a difficult job with this publication, but have succeeded in producing a cohesive and well-integrated manual. However, the time lag between submission of the contributions and publication leaves some of the information out of date or inaccurate. One example can be found in the chapter on translocation, where the authors suggest that it is acceptable to transfer amphibians between ponds. In recent years, the risk of spreading non-native invasive species and diseases through inadequately researched translocations has become an important issue, which has led to the movement of amphibians between ponds being discouraged. In fact, frog mortality, which has become the subject of a major research project in Britain (e.g. Cunningham *et al.*, 1996), was only mentioned in one sentence in the entire manual (in the section on threats to status).

Indeed, I found the entire chapter on translocation to be contentious and rather confusing. Species translocation is a popular, if controversial, concept in conservation, and one which may be appropriate for some species, but inappropriate for others. The chapter did not fully explore the issues of concern as they relate to herpetofauna, such as disease transmission, genetic mixing, viable population numbers, reserve design, etc. Although the guidelines provided for translocation follow those produced by IUCN (1995), they were not adequately framed within a herpetological context, and may be difficult to understand and implement.

I was also disappointed to discover the exclusion of the slow-worm and common lizard from the section on species-specific threats to conservation status. These two species are considered to have little conservation priority in this country, primarily because they are poorly understood and under-recorded. Although their conservation status is largely unknown, due to the fact that they are often missed in environmental assessments and survey, they are in decline (Hilton-Brown & Oldham, 1991). It is a sad indication of the current situation when even herpetologists overlook these species.

On the whole, however, the *Herpetofauna Workers' Manual* is a valuable contribution to the conservation of reptiles and amphibians, easily accessible, yet thorough and concise. It certainly should become a standard reference for conservation officers everywhere. It is also useful for teachers, consultants, professional and voluntary herpetologists and anyone else who might occasionally encounter the odd slow-worm. Although primarily written for a British audience, the information it contains may be useful for the development of conservation programmes elsewhere. The editors do acknowledge in the introduction that as more research is carried out, the information contained within is likely to become outdated. Perhaps a second edition is imminent? An added incentive to purchase the book is that it is available at half-price (i.e. £10) to members of certain organisations involved with herpetofauna conservation, including those belonging to the British Herpetological Society.

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Foster, J. & Gent, T. (Eds). (1996). *Reptile Survey Methods*. English Nature Science Series No. 27. English Nature, Peterborough.

Hilton-Brown, D. & Oldham, R. S. (1991). *The status of the widespread amphibians and reptiles in Britain, 1990, and changes during the 1980's*. Nature Conservancy Council, Peterborough.

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Lizards. Manfred Rogner. (1997). 328 pp. + 318 pp. (2 vols). Krieger Publishing, Florida, USA. £93.00 (cloth).

The last fifteen years or so have seen an enormous increase in interest in the husbandry of captive reptiles. There has also been a corresponding increase in the number of books that inform us on how to keep and breed them. This work by Manfred Rogner - which has been translated from the original German edition by John Hackworth - is the latest of these, but unlike many others it is very good. It is very good mainly because the information it contains is far less repetitive than similarly targeted books and it also provides answers to many questions about the husbandry of lizards that other books do not. Additionally, it describes species that most herpetologists from the UK will be unfamiliar

with. It is a comprehensive account of the captive husbandry of selected species of lizards from all the major families in two volumes. Volume 1 deals with the Gekkonidae, Pygopodidae, Agamidae, Chameleonidae, and Iguanidae with additional chapters on *The Care and Husbandry of Lizards in the Vivarium, Feeding and Vitamins Minerals and Trace Elements*, and a useful chapter dealing with *Reproduction, Egg Incubation and Temperature Dependent Sex Determination.* Volume 2 concerns the Xenosauridae (crocodile lizards) Helodermatidae, Varanidae, Anguidae, Gerrhosauridae, Cordylidae, Lacertidae, Scincidae, Xantusiidae and Teiidae. There are further chapters on reptiles that are not lizards, the amphisbaenids, tuatara (Rhynchocephalia) and the crocodilians. Each volume has a species index and extensive bibliography, mainly derived from German language publications.

Family accounts begin with an introduction to the group as a whole followed by species accounts, with a format of (1) species distribution; (2) description; (3) habitat; and (4) lifestyle, husbandry and reproduction. The latter section invariably forms the largest part of the text. The geckos, lacertids and iguanids receive the most attention but there are substantial chapters on agamas, skinks, monitors and anguids. The section dealing with the African plated lizards is particularly comprehensive and informative. There are many good quality colour photographs, often of unfamiliar forms, although not all the species described have accompanying photographs. As far as I can tell, each photograph shows a typical representative of the species, unlike some other publications where the most wonderfully patterned or coloured individual that could be found is shown - such representations are of limited use.

Several husbandry aspects are discussed, for example UV requirements in lizards and the types of special lights that are available to deal with the problem. The failure of fully developed embryos to break free from their eggshells is dealt with in some detail and several interesting hypotheses as to why this should occur presented. The author points out that such failures are much rarer in the wild (although generally I do not see how one could easily determine this). He refers to studies on the alligator, which indicate that during development, the shells in the eggs of natural nests become increasingly thin and fragile. He goes on to say that corrosion of the eggshell by carbonic acid (a product of exhaled carbon dioxide combined with water), in addition to the metabolic products of bacteria and fungi living in the ground, are important factors in rendering the eggshell thin enough for the hatchlings to break free. Relatively dry captive incubation media with low bacteria counts may lack these influences.

As far as I can see there are no major problems with this impressive work, and any criticisms are relatively minor. However, it does seem that research which may have important implications for animal husbandry, takes far to long to be included in this type of publication. For example, in the chapter on general husbandry no mention is made of the importance of stress in the husbandry of captive lizards (Chizar, Murphy & Smith, 1993), in particular its effects on reproduction and longevity (Greenberg, 1990); or an 'emotional fever' in lizards as are result of being handled (Cabanac & Gosselin, 1993).

The inclusion of the crocodiles, amphisbaenids and the tuatara adds interest to the work, but the frequent reference to the tuatara as a lizard may confuse newcomers to herpetoculture, despite its correct classification as a rhynchocephalian at the beginning of volume 1. Of course unlike lizards, the tuatara is a true diapsid, which retains the jugal - quadratojugal bar in the lower arch of the temporal region of the skull.

Readers looking for detailed ecological information on lizards will be disappointed, but then this is not the primary aim of the book. The author claims that his work fills a gap in the herpetoculture of reptiles, and I agree with him. It is a well balanced, comprehensive guide for anyone who is interested in the husbandry of lizards. Much of the information appears to be based on the authors own experiences, although compiling all the relevant information must have been a daunting task. Manfred Rogner is to be congratulated for a scholarly work which is destined to become the standard reference for the herpetoculture of lizards.

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Cabanic, A. & Gosselin, F. (1993). Emotional fever in the lizard, *Callopistes maculatus* (Teiidae). *Anim. Behav.* **46**, 200 -202.

Chizar, D., Murphy, J. B & Smith H. M. (1993). In search of zoo - academic collaborations: a research agenda for the 1990's. *Herpetologica,* **49**, 488-500.

Greenberg, N. (1990). Behavioural endocrinology of physiological stress in a lizard. *J. Exp. Zool. Suppl.* **4**, 170-173.

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ANNOUNCEMENTS

The following applications were published on 18 December 1998 in Vol. 55, Part 4 of the *Bulletin of Zoological Nomenclature*. Comment or advice on any of these applications 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 (email: izcn@nhm.ac.uk).

Case 3012:

***Coluber infernalis* Blainville, 1835 and *Eutaenia sirtalis tetrataenia* Cope in Yarrow, 1875 (currently *Thamnophis sirtalis infernalis* and *T. s. tetrataenia*; Reptilia, Squamata): proposed conservation of the subspecific names by the designation of a neotype for *T. s. infernalis*.**

Sean J. Barry. *Section of Evolution and Ecology, University of California, Davis, California 95616, USA (Present address: Rowe Program in Genetics, Tupper Hall, University of California, Davis, California 95616, USA; (email: sjbarry@ucdavis.edu).*

Mark R. Jennings. *National Biological Service, California Science Center, Piedras Blancas Research Station, PO Box 70, San Simeon, California 93452, USA* and *Research Associate, Dept. of Herpetology, California Academy of Sciences, Golden Gate Park, San Francisco, California 94118, USA (email: mark_jennings@nbs.gov.).*

Abstract. The purpose of this application is to conserve the usage of the subspecific names of *Thamnophis sirtalis infernalis* (Blainville, 1835) for the California red-sided garter snake (family Colubridae) which is found along the Californian coast, and from the restricted area of the San Francisco Peninsula. It is possible that the holotype of *T. s. infernalis* is a specimen of *T. s. tetrataenia*, formally rendering the name *tetrataenia* a junior synonym of *infernalis*. It is proposed that the holotype of *infernalis* be set aside and a neotype designated in accord with accustomed usage.

Case 3005:

***Crotalus ruber* Cope, 1892 (Reptilia, Serpentes): proposed precedence of the specific name over that of *Crotalus exsul* Garman, 1884.**

Hobart M. Smith, Lauren E. Brown, David Chiszar, L. Lee Grismer, G. Scott Allen, Alex Fishbein, Bradford D. Hollingsworth, Jimmy A. McGuire, Van Wallach, Peter Strimple and Ernest A. Liner.

Abstract. The purpose of this application is to conserve the long used and well known specific name of *Crotalus ruber* Cope, 1892 for the red diamond rattlesnake (family Viperidae) of southern California, the peninsula of Baja California and some offshore islands, by giving it precedence over the less widely used name *C. exsul* Garman, 1884. The latter name refers to the rattlesnake of the Isla de Cedros, Baja California, Mexico, which some authors now consider to be conspecific with *C. ruber*.

HERPETOLOGY OF SRI LANKA PUBLICATIONS

The following publications have been donated to the *British Herpetological Society* library, but are also available to BHS members who wish to purchase their own copies at 30% discount:

Lyriocephalus (Journal of the Amphibia and Reptile Research Organisation of Sri Lanka - ARROS) volume 3(2).

Amphibia of Sri Lanka: a checklist and an annotated bibliography (1996).

Snakes of Sri Lanka: a checklist and an annotated bibliography (1998).

Sauria (lizards) of Sri Lanka: a checklist and an annotated bibliography (1998).

Testudines and crocodilians of Sri Lanka: a checklist and an annotated bibliography (1998).

All of the above are available at US $10.00 each (payment also acceptable in pounds Sterling). Airmail postage costs will be notified once the number of copies to be ordered is known. Cheques or international money orders should be payable to 'K. A. L. de Silva'. Orders to: Anslem de Silva, Faculty of Medicine, University of Peradeniya, Sri Lanka.

EDITOR'S NOTE

The Editor is grateful to the following for reviewing manuscripts submitted to the *Herpetological Journal*:

S. Adolph, R. Aldridge, R. Andrews, B. Arano, J. Arntzen, J. Austin, E. Baard, G. Baggot, F. Baharona, J. Baker, E. Balletto, R. Ballinger, E. Barratt, D. Bauwens, T. Beebee, K. Berven, C. Bishop, L. Brady, R. Brown, C. Bull, S. Bush, J. Castanet, A. Castilla, W. Cooper, P. Corn, J. Davenport, T. Dellinger, J. Diaz, C. Diaz-Paniagua, J. Dixon, P. Donohue, J. Downie, J. Fa, M. Faria, V. Ferri, J. Foster, D. Frost, D. Galbraith, A. Gardner, M. Gaywood, A. Gent, C. Giacoma, R. Gibson, A. Green, H. Greven, W. Hanke, S. Hecnar, S. Hitchings, R. Inger, P. Joly, R. Jehle, U. Joger, J. Loman, J. Loveridge, M. Kalezic, C. Keller, M. Klemens, H. Kobel, R. Marquez, J. Mateo, L. Maxson, R. Meek, C. Miaud, G. Nilson, R. Nussbaum, M. Packard, V. Perez-Mellado, J. Petranka, B. Pierce, R. Platenberg, J. Pleguezuelos, S. Poe, C. Reading, C. Richards, K. Richter, R. Schabetsberger, U. Sinsch, F. Slater, H. Strijbosch, A. Stumpel, G. Tattershall, M. Thompson, J. Thorbjarnson, R. Tinsley, C. Tracy, F. Trillmich, L. Trueb, G. Underwood, V. Waights, V. Wallach, P. Watt, J. Wiens, C. Williams, W. Wüster, J. van Wyk.

THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS

(revised January 1999)

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

2. *Three* copies of all submissions, and illustrations, should be sent to the Scientific Editor. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigour, and the degree of general interest in the subject matter. The Editor's decision will be final.

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer (at least near-letter quality, avoid worn ribbons), and double-spaced with wide margins all round. The journal is typeset direct from the author's computer diskette, so all manuscripts should be prepared using a wordprocessor (preferably on a PC-compatible microcomputer). It is not necessary to submit a computer diskette with the initial manuscript, but this will be required in the event of the manuscript being accepted for publication.

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

5. The usual rules of zoological nomenclature apply.

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

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

8. References in the text should be given as in the following examples: "Smith (1964) stated —"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the 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. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal.

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

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

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

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

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

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

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

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

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Herpetological Journal vol. 9, no. 2 was published on 15 April 1999