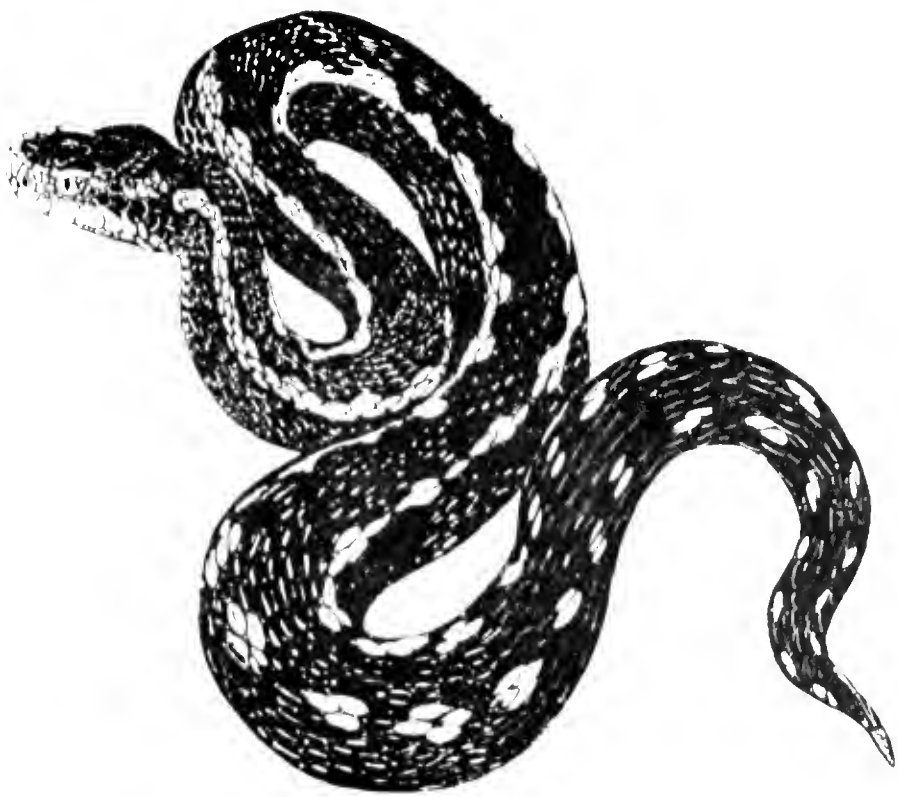
ASIATIC HERPETOLOGICAL RESEARCH



VOLUME 3 1990

ISSN 1051-3825

CONTENTS

ASIATIC HERPETOLOGICAL RESEARCH

VOLUME 6 1995

Asiatic Herpetological Research

Asiatic Herpetological Research is published by the Asiatic Herpetological Research Society (AHRS) and the Chinese Society for the Study of Amphibians and Reptiles (CSSAR) at the Museum of Vertebrate Zoology, University of California. The editors encourage authors from all countries to submit articles concerning but not limited to Asian herpetology. All correspondence outside of China and requests for subscription should be sent to AHR, Museum of Vertebrate Zoology, University of California, Berkeley, California, USA 94720, or by **email** to ***asiaherp@uclink.berkeley.edu***. All correspondence within China should be sent to Ermi Zhao, Editor, Chengdu Institute of Biology, P.O. Box 416, Chengdu, Sichuan Province, China. **Authors** should consult *Guidelines for Manuscript Preparation and Submission* at the end of this issue. **Subscription and membership** are $25 per year ($45 for libraries). Postage outside of the USA and China, please add $5 per issue for surface mail or $10 per issue for air mail. Make checks or money orders payable in US currency to AHRS. If you do not have access to US currency, please notify us, and we will make other arrangements.

Asiatic Herpetological Research Volume 6 succeeds Volume 5 published in 1993, Volume 4 published in 1992, Volume 3 published in 1990 and ***Chinese Herpetological Research*** Volume 2, which was published at the Museum of Vertebrate Zoology, 1988-1989 as the journal for the Chinese Society for the Study of Amphibians and Reptiles. Volume 2 succeeded ***Chinese Herpetological Research*** 1987, published for the Chengdu Institute of Biology by the Chongqing Branch Scientific and Technological Literature Press, Chongqing, Sichuan, China. ***Acta Herpetologica Sinica*** ceased publication in June, 1988.

Cover: *Batrachuperus pinchonii* Elev. 2270 m, 53.2 km north of Hanyuan (29° 21' N 102° 43' E), on the Ya'an to Hanyuan Rd., Liba Shan (mountain), Ya'an Prefecture, Sichuan Province, China. Photo by J. Robert Macey.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 1-26

Systematics of the Vipers of the Caucasus: Polymorphism or Sibling Species?

GÖRAN NILSON[1], BORIS S. TUNIYEV[2], NIKOLAI ORLOV[3], MATS HÖGGREN[4] AND CLAES ANDRÉN[1]

[1]*Department of Zoology, Göteborg University, Sweden*
[2]*Caucasian State Biosphere Reserve, Sochi, Russia*
[3]*Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia*
[4]*Department of Genetics, University of Uppsala, Russia*

Abstract. -Inter- and intramorphological variation were examined in sympatric and allopatric polymorphic and monomorphic populations of the *Vipera ursinii* and *Vipera kaznakovi* complexes. The alpine *Vipera dinniki* populations in upper Great Caucasus show a pronounced, and to a certain extent geographical, polymorphism. Color patterns include among others 'kaznakovi', 'tigrina', 'berus', 'bronze', and 'ursinii' types. Several of these patterns can be represented within the same litter in certain populations. *Vipera dinniki* is sympatric with the Caucasian representative of the *Vipera ursinii* complex in some areas. This last taxon shows a similar degree of polymorphism, which is unique for this complex, and due to morphological and molecular distinction, we consider it to be a Caucasian evolutionary species within the *ursinii* complex - *Vipera lotievi* sp.n.

Key Words: Reptilia, Squamata, Viperidae, *Vipera dinniki, V. kaznakovi, V. ursinii, V. renardi, V. lotievi* sp.n., Caucasus, Russia, Georgia, taxonomy, morphology, polymorphism.

Introduction

The taxonomy of the vipers of Caucasus has for a long time been confusing and contradictory. According to the traditional view a single species, *Vipera kaznakovi*, is distributed in the moist and warm lowlands of the western Caucasus as well as in the mountain valleys towards the east. In the east the habitat is drier and along the range the vipers gradually change toward *Vipera ursinii* in appearance. In the east Caucasus only this last viper was supposed to occur. Thus there seemed to be a somewhat clinal transformation from "pure" *V. kaznakovi* in the west to "pure" *V. ursinii* in the east. Vipers from the intermediate region could be difficult to determine. Within the same locality some specimens look like *V. kaznakovi*, other ones are more like *V. ursinii*, while still some can be intermediate.

Nikolsky (1913) separated the alpine populations into the taxon *Vipera berus dinniki*, which was based on alpine specimens of the conventional *V. kaznakovi* from high altitudes in the western Caucasus (Malaya Laba River- terra typica restricta and Svanetia) as well as from other places. The name *dinniki* was long considered as a synonym of *V. kaznakovi* until Vedmederja et al (1986) recognized it as a separate species inhabiting alpine and subalpine meadows in the Caucasus, and thus restricting *V. kaznakovi* to lower altitudes in western Caucasus and adjacent moist lowland habitats along the eastern Black Sea coast. Thereby the problem of the gradual transformation from *V. kaznakovi* in the west of the Caucasus to *V. ursinii* in the east is restricted to the high Caucasus populations, now including *V. dinniki* and *V. ursinii*. *Vipera kaznakovi* is well defined and restricted in distribution, and geographically separated from all the other viper species in the region.

The complex history of nomenclature and taxonomy has been clarified to a great part in some recent publications (see Orlov & Tuniyev, 1986; 1990), together with hypotheses about the phylogeny of this group. Concurrently the need of genetic studies was stressed, and this has led to the present work where we in a series of papers intend to clarify the taxonomy and evolution of the vipers of this region. The work is planned to have a broad perspective including phenetic and phylogenetic analyses, habitat choice, niche-breadth, and

reproduction. Different methods take different time and in this first paper the morphology is reexamined, based on available material in Museum collections and freshly collected material from a number of new places, as well as on inheritance of color pattern. Genetic structures based on phenetic analyses of allozyme data are also presented.

The morphological distinction between *Vipera dinniki* and *V. kaznakovi* has been presented elsewhere (Orlov and Tuniyev, 1986; 1990) and will not be repeated in this study. In the present paper we are focusing on patterns of morphological and molecular variation within and between the different populations in the Caucasus, and the taxonomy of these populations.

Material and Methods

The work has mainly been a study of variation in external morphology, allozymes and reproduction in order to reveal patterns of sympatry and sibling species. Additional genetic studies will follow when material becomes available in suitable samples (presently delayed due to political reasons). Thus live and preserved museum material has been examined concordantly with studies of reproduction in the laboratory.

Additional preserved material used in this study originates from the Natural History Museum in Göteborg (GNM); Dipl.-Biol. F. J. Obst, Staatliches Museum für Naturkunde, Dresden (MTKD D); Aram Agasian, Zoological Institute, Academy of Sciences, Eriwan, Armenia. Abbreviations for museums as used in the text are: CNR- Caucasian State Biosphere Reserve, Collection of Boris Tuniyev at Yew-box Groove, Sochi; GNM- Göteborg Natural History Museum, Göteborg; MTKD- Staatliches Museum för Naturkunde, Dresden; ZIEr- Zoological Institute, Academy of Sciences, Eriwan; ZIG- Department of Zoology, University of Göteborg; Göteborg- (authors' collection, which later will be incorporated in GNM); ZIN- Zoological Institute, Academy of Sciences, St. Petersburg.

Altogether about 300 preserved or live specimens of vipers from the Caucasus and adjacent regions have been seen during the study. Joint field trips were made in different parts of the Caucasus in 1990 and 1992, but two of us (Tuniyev and Orlov) have performed extensive research in the region prior to that. For morphometric studies 183 preserved snakes within the *ursinii* and *kaznakovi* complexes have been examined more carefully, and for most of these specimens 30 different items of data have been collected. This information was used, down to population level, in morphological descriptions, taxonomical analyses and conclusions about zoogeography and range overlap. Inheritance of color pattern was studied based on 23 pregnant females and their offspring.

Data collected were: total length and tail length; number of preventrals, ventrals, subcaudals, anterior and mid-body dorsal scale rows, apical plates, supralabials, sublabials, circumocular scales, loreals, second chinshields, mentals, crown scales (=intercanthals + intersupraoculars), and zig-zag windings in dorsal band. Further rostral index (height/breadth) and head index (breadth/length) were calculated. Division of parietals, frontal, and nasalia was noted, as was the color of dorsal and ventral sides, and iris (in live specimens). Further, upper preocular size; and head, labial and lateral body patterns, as well as distinctiveness of canthus rostralis were examined. Details about these methods are found in Nilson and Andrén (1986).

Morphology/phenetics

Standard errors accompanying mean character ratios were used as relative measurement of dispersion. For the analysis of intra- and interpopulational morphological variation (phenetic analysis) the samples were divided into subsamples depending on questions raised. Thus besides an analysis of morphological variation also a pattern confirming or rejecting the present taxonomic pattern could be achieved. This pattern could also be

TABLE 1. Number of specimens used in the genetic analyses and localities (Russia if nothing else is stated) for the examined taxa.

kaznakovi:
1. Dagomys, north of Sochi. Six specimens.
2. Rudorova, inland locality, 900 m alt. Four specimens.

dinniki:
3. Fisht/Oshten, the westernmost locality of the main Caucasus range. Seven specimens.
4. Lake Impsi, 1,980 m. alt., at a tributary to the Little Laba River on the northern slope of the main range. Seven specimens.
5. Aishkha-II on the southern slope of the main range. Three specimens.
6. Lake Kardyvach at upper Mzymta River on the southern slope of the main range Seventeen specimens.

lotievii:
7. Armkhi, Checheno-Ingushetia. 2,000 m altitude. Seven specimens.

berus:
8. Uppsala (terra typica), Sweden. Eleven specimens.

eriwanensis:
9. pooled sample from Asbua and Cildir, Kars, east Turkey; and Sevan, Armenia. Six specimens.

supported or rejected by the parallel biochemical studies. Thereby it is possible to state or reject the occurrence of convergent or parallel evolution, i.e. sibling species.

Estimation of the different color pattern frequencies in local populations was based on observations during the field work. Small museum samples collected by others were not included in this analysis due to uncertainty of randomness in sampling (unusual morphs might have been collected and preserved at a higher degree).

Biochemical data

Enzyme electrophoresis.— Sixty-eight specimens representing different taxa of Caucasus vipers, and *Vipera berus* from Sweden were examined. The samples were treated as nine independent operational taxonomic units (OTUs) in the genetic analysis, in order to avoid a priori assumptions of taxonomic relationships among the eight Caucasus populations studied (see Table 1, for locality data and sample sizes). A potential risk of sampling error due to syntopic occurrence of two taxa may be avoided by testing observed genotype distribution within a locality against Hardy-Weinberg expectations (see results). Fresh or frozen tissues (-75°C) from liver and skeletal muscle were homogenized in distilled water. The extracts were centrifuged for 10 min at 10,000 rpm and 4°C and the supernatants were then stored at -75°C until used. Standard horizontal starch gel electrophoresis was carried out, as described by Harris and Hopkinson (1976) and Murphy et al. (1990). Gels (11% w/v) were prepared from Sigma starch (Sigma Chemical Co., St. Louis, Mo). Two buffer systems were used: (A) Gel: 0.03 M tris-0.005 M citric acid; Electrode: 0.06 M lithium hydroxide-0.03 M boric acid, pH 8.0 (Ridgway et al., 1970). (B) Gel: 0.002 M citric acid, pH 6.1; Electrode: 0.04 M citric acid, pH adjusted with N-(3-amino-propyl)morpholin

TABLE 2. Enzymes and electrophoretic conditions of the polymorphic loci scored in this study. Nomenclature and commission numbers following the International Union of Biochemistry, Nomenclature Committee (1984). Abbreviations for tissue sources are: L=liver and M= skeletal muscle.

Enzyme	Commission no.	Locus	Tissue	Buffer system	Reference
Alcohol dehydrogenase	1.1.1.1	Adh-1	L	B	1
Glucose-6-phosphate isomerase	5.3.1.9	Gpi-1	M, L	B	5
Hexokinase	2.7.1.1	Hk-1	L	B	1, 3, 4
Isocitrate dehydrogenase	1.1.1.42	Idh-1	L	B	1
		Idh-2	L		
L-Lactate dehydrogenase	1.1.1.27	Ldh-2	M, L	B	3
Phosphoglucomutase	5.4.2.2	Pgm-2	L	B	1
Superoxide dismutase	1.15.1.1	Sod-1	L	A	1, 2

1) Harris and Hopkinson (1976)
2) Johnson et al. (1970)
3) Shaw and Prasad (1970)
4) Murphy et al. (1990)
5) De Lorenzo and Ruddle (1969)

A) Tris-citrate/lithium hydroxide, boric acid, pH 8.0, 10V/cm, 4h (Ridgway et al., 1970)
B) N-(3-amino-propyl)morpholine/citrate, pH 6.1, 10V/cm, 6h (Clayton and Tretiak, 1972)

(Clayton and Tretiak, 1972). Enzymes assayed, tissues, modified electrophoretic conditions and staining references (Table 2) follow Nilson et al. (1994).

Reproduction

Inheritance of color morphs within broods was studied by keeping pregnant females in the laboratory until giving birth. By this, various hypotheses of polymorphism and its inheritance patterns could be evaluated.

Fieldwork

Substantial information has been gathered during several years in the field in Russia, Georgia, Armenia and Turkey. A more intensive field survey was performed in the western Caucasus in July, 1990, in order to obtain information of color morphs in natural populations. Together with preserved material, this information was the base for the study of morphological intrapopulational variation in *V. dinniki*. Material was also used for reproductive studies (see above).

The *V. dinniki* localities, situated in the Caucasian State Biosphere Reserve and in the Sochi Nature State National Park and listed below were included in this study:

Fisht/Oshten— The westernmost high mountain area of the main Caucasus range, and of the Reserve, characterized by the peaks Mt. Fisht (2868 m. alt.) and Mt. Oshten (2804 m. alt.). This region is separated and isolated from the main range (with Mt. Chugush 3237 m. alt.) by the forested, moist and warm "Colchis Gate" at low altitude (not more than 1500 m).

Loyub— Mt. Loyub (2990 m. alt) at the uppermost part of the Mzymta River in the eastern part of the Reserve, situated on the southern slope of the main range.

Aishkha-II (2858 m. alt)— in the same mountain massif, a little bit further to the west, and situated on the southern slope of the main range.

Lake Kardyvach (1850 m. alt)— the eastern border of the Park, close to Mt. Loyub but a little further down the Mzymta

FIG.. 1. The typical "dinniki" pattern type of *Vipera dinniki*, with unicolored lateral sides typical for Fisht/Oshten (ZIG).

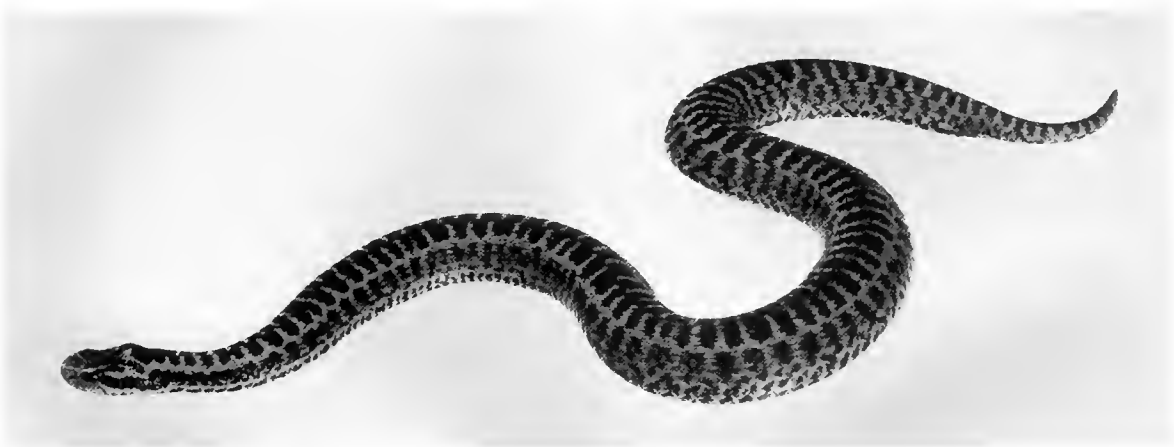


FIG. 2. The "dinniki" pattern type of *Vipera dinniki*, with a tendency towards the "tigrina" morph. From Kardyvach (ZIG).

River. Also situated on the southern slope of the main range.

Lake Impsi— at 1980 m. alt., situated at the Tsahvoa River, a tributary of the Little (Malaya) Laba River. The locality is mainly on the slopes of the Damhorts Range at the northern part of the Reserve, and partly on Akaragvarta Mountain situated on the northern slope of the main range.

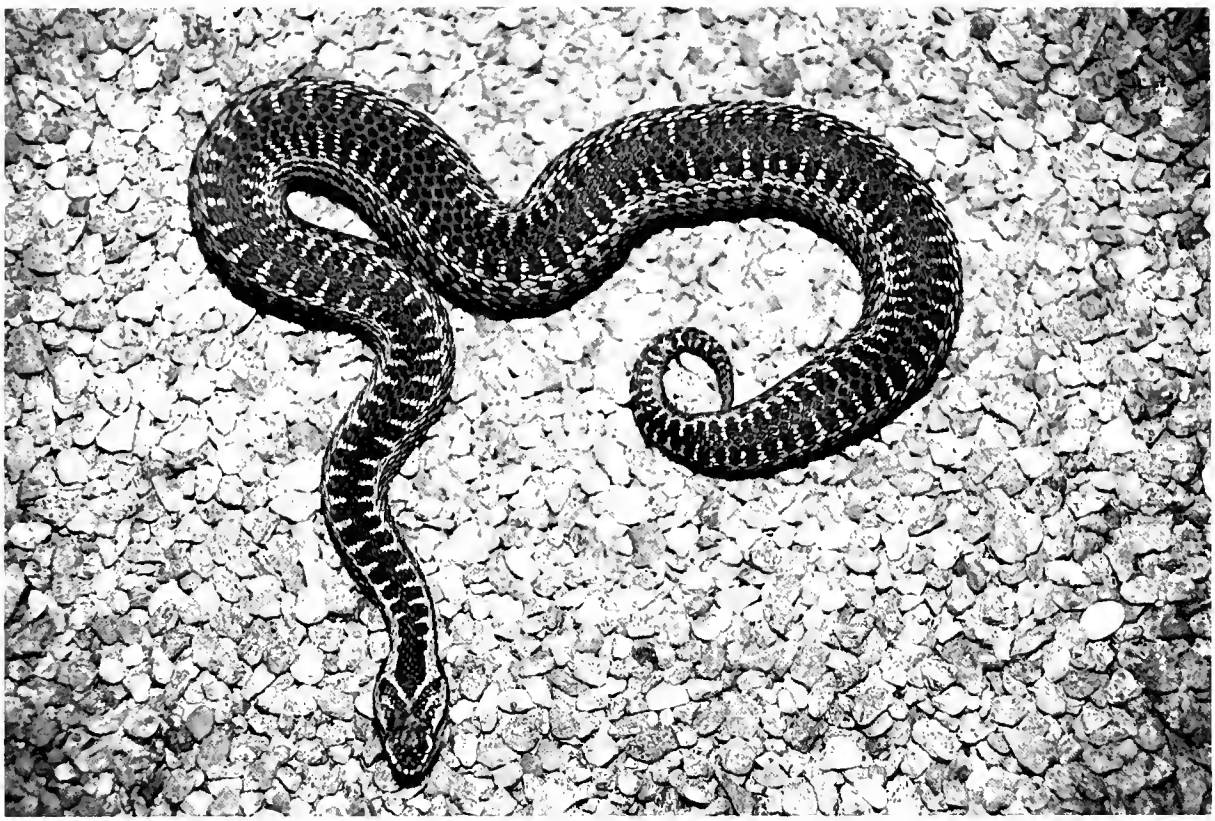
In addition much information on *Vipera kaznakovi* was gathered at the lowland Black Sea coast localities of Dagomys, north of Sochi (Russia) and Hopa, Artvin Province (Turkey).

Results

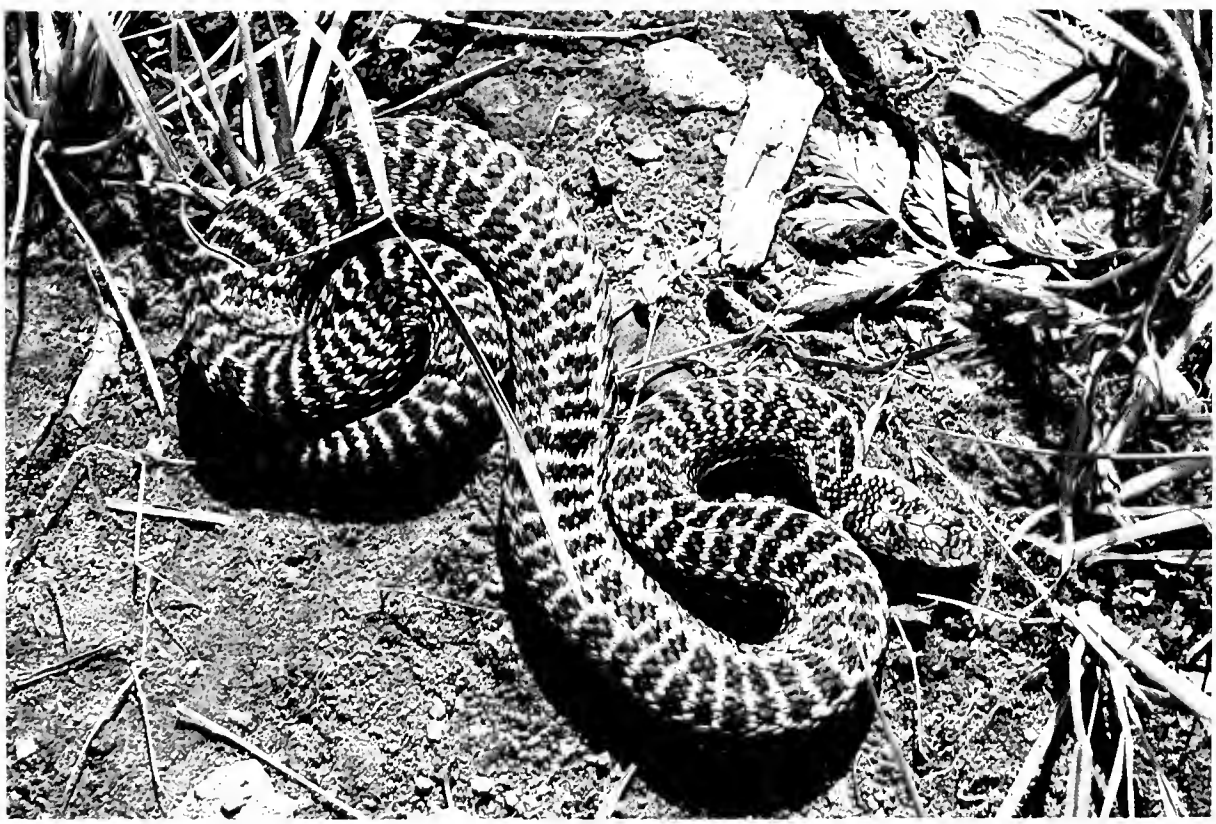
The results of the analyses of morphometrics and enzyme electrophoresis are presented separately.

Intra- and interpopulational variation in morphology

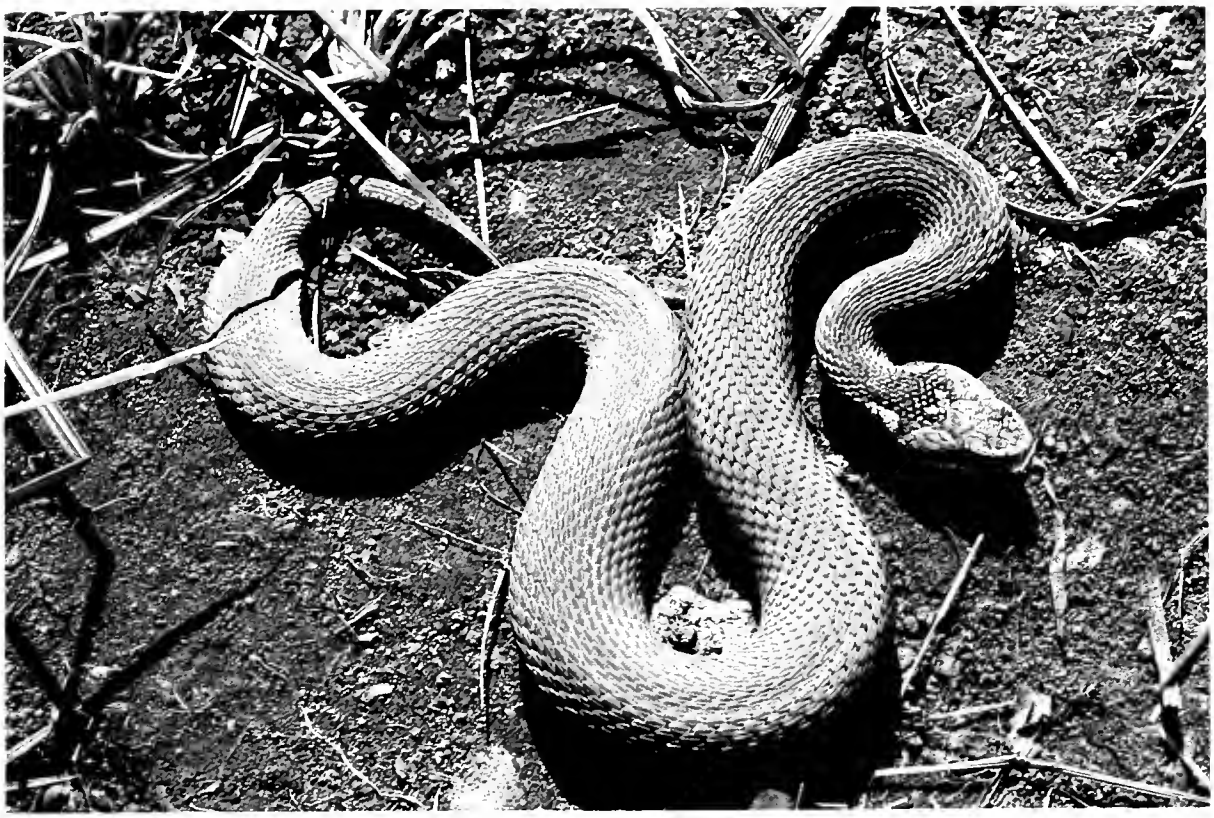
A great number of different color morphs are expressed in the Caucasian vipers. Although several stages of overlapping and intermediate forms could be seen, we define the following major pattern types:



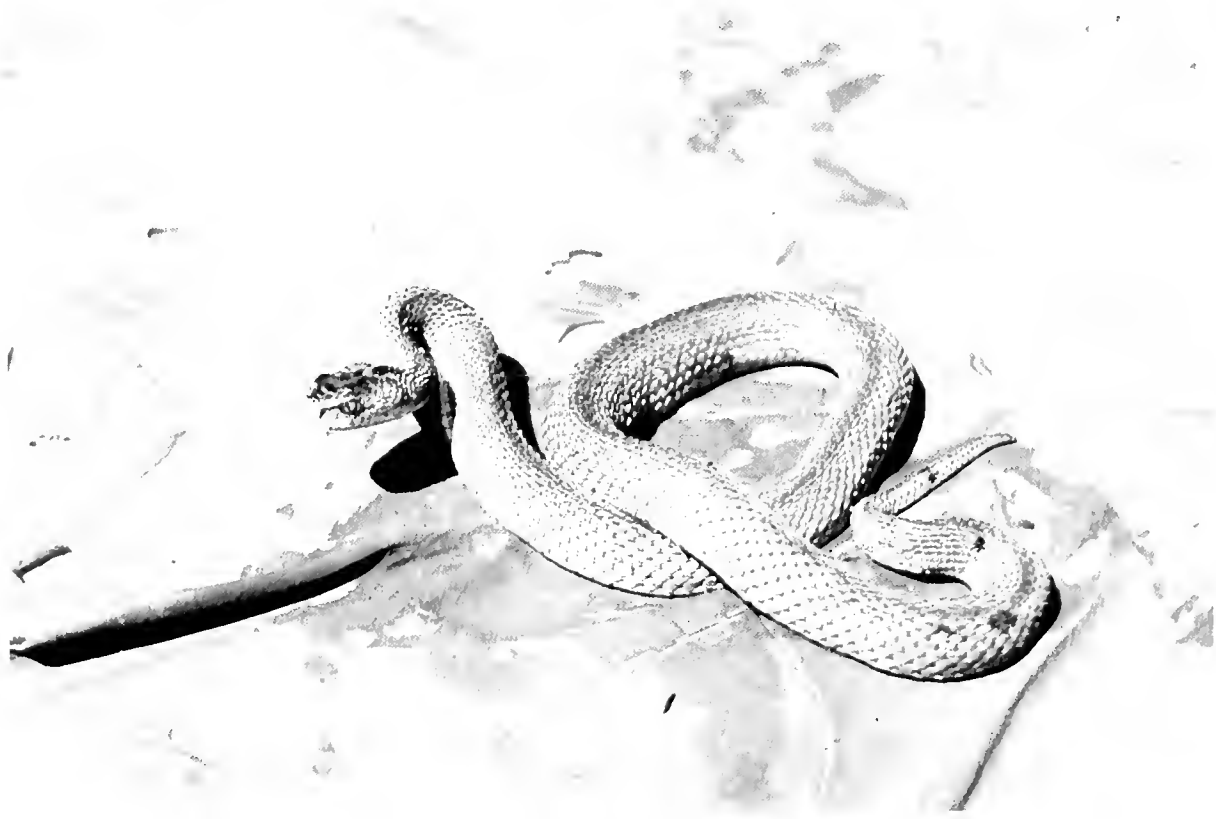
a. The "ursinii" pattern type of *Vipera dinniki*, from Kardyvach (ZIG).



b. The "tigrina" morph of *Vipera dinniki*, from Impsi with partly divided transverse bars (ZIG).



c. The "bronze" morph of *Vipera dinniki*, from Impsi (ZIG).



d. The "bronze" morph of *Vipera lotievi* from Itum Kali, Checheno-Ingushetia.

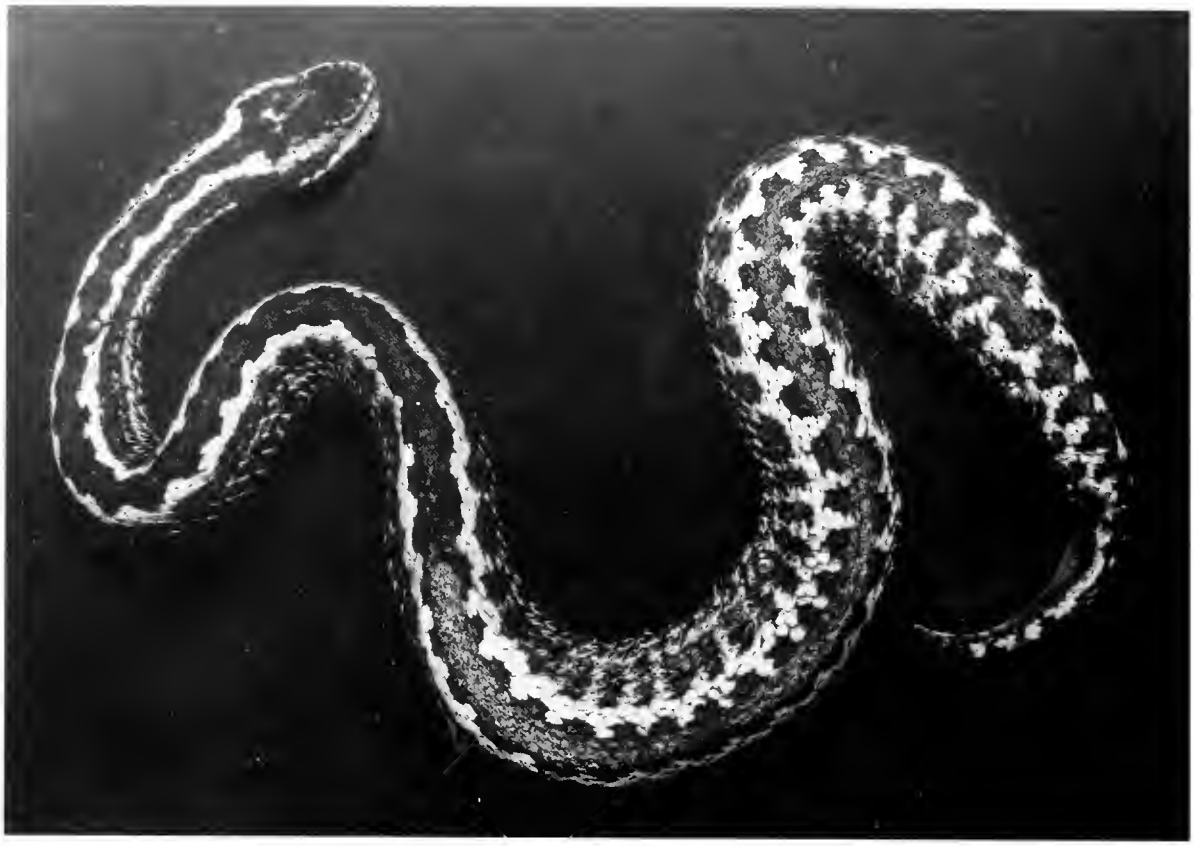


FIG. 3. The "kaznakovi" morph of *Vipera dinniki*, from Fisht/Oshten (Mt.Oshten - Armenian pass).

A. "dinniki": a more or less continuous zig-zag band, and pronounced lateral blotches. Sometimes nebulous in pattern. Sometimes rather *Vipera berus* like (Figs. 1, 2).

B. "kaznakovi": pronounced black lateral and dorsal longitudinal bands, and yellow or orange ground color. The dorsal band is waving or expressed as a straight band resulting in a contrasting more or less bilineated pattern. Besides these we could define a "bilineated" morph which is similar to the "kaznakovi" type of pattern but much lighter. In the analysis below it is included in the "kaznakovi" type (Fig. 3, compare Fig. 4).

C. "ursinii": a black-edged continuous dark brown zig-zag band on a paler ground color and lighter sides of body (Fig. 5, Plate 1a).

D. "tigrina": dorsal pattern fragmented into broad or narrow transverse bands. Also spotted pattern could be seen in some populations. This pattern type is most close to "tigrina", but differ by being divided along the vertebral line thus resulting in two rows of dark spots along the dorsal side of the body (Fig. 6, Plate 1b).

E. "bronze": a uniform greyish to brownish or blackish ground color covering all parts of body except the head. Sometimes with a darker narrow or broad vertebral stripe (Figs. 7, 8, Plate 1c).

F. "melanism": black, with a high production of melanin covering all other color patterns.

The different morphs were represented in different frequencies at the different localities examined, and some morphs seemed to be restricted to one or a few

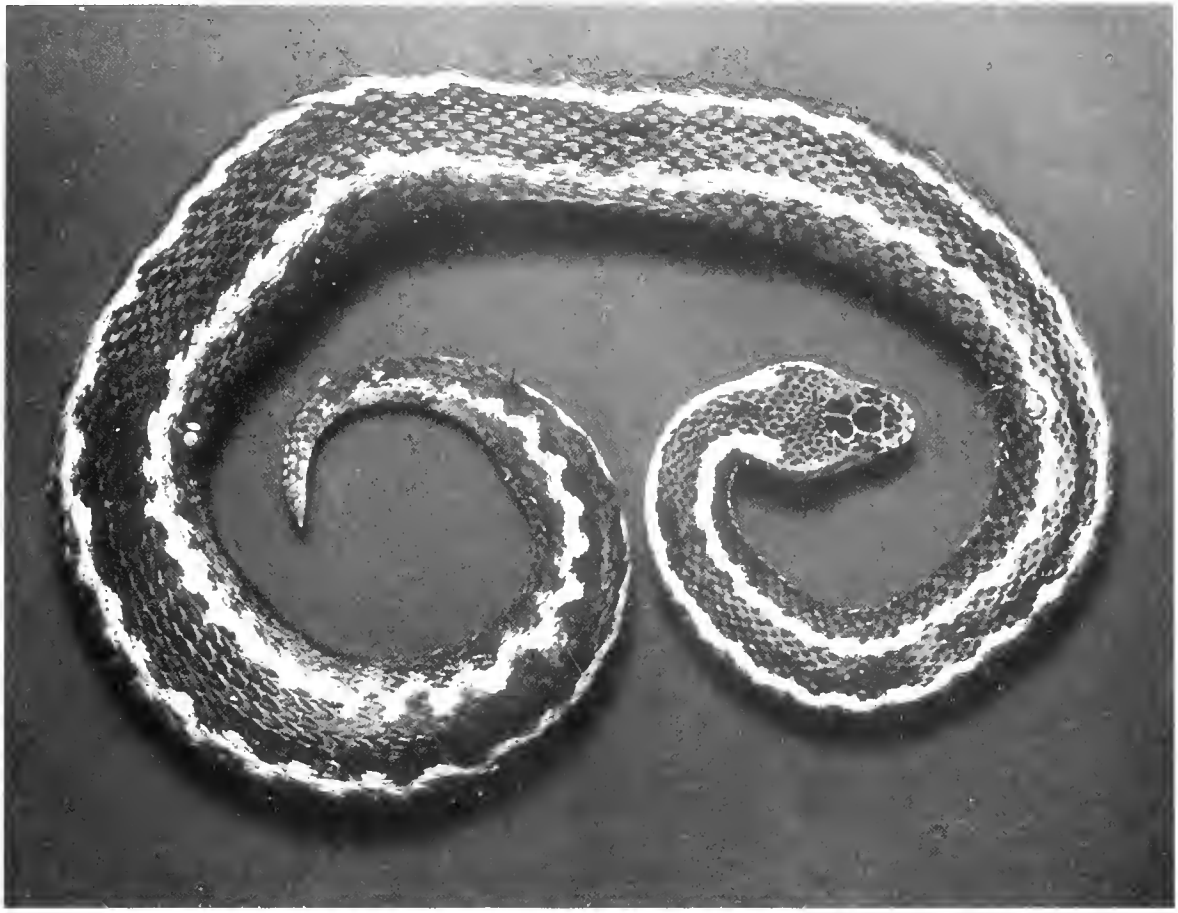


FIG. 4. A typical *Vipera kaznakovi* from the inland locality cordon Babuk-Aul at the foothills of Mt. Fisht (ZIG).

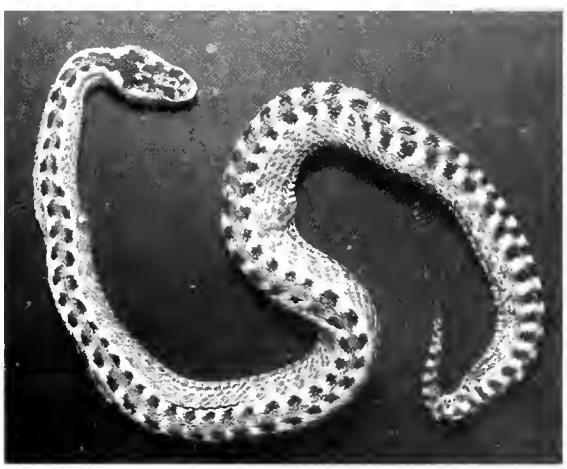


FIG. 5. The "ursinii" pattern type of *Vipera dinniki*, with more unicolored lateral sides from Mt. Fisht (ZIG).

localities (Table 3). Also variation in other color characteristics was obvious when comparing populations. The number of windings in the dorsal zig-zag band varied markedly with low number in the western isolated Fisht population and high in the more eastern populations (Fig. 9). This was especially pronounced when comparing Fisht with Kardyvach, characterized by a high frequency of "tigrina" morphs (Table 3). Almost no overlap was detected as in Kardyvach the vipers have 68 or more windings while in the Fisht population the corresponding figures are 69 or less (Table 4). In general the west Caucasian samples (except Fisht) have higher number of dorsal windings or transverse bars than central and east Caucasian *dinniki* vipers. Also *V. kaznakovi* has fewer windings.

FIG. 7. The "bronze" morph of *Vipera dinniki*, from Mt. Loyub (ZIG).

TABLE 3. Distribution of color morphs in samples of *V. dinniki* and *V. kaznakovi* (Dagomys) in absolute numbers and in percentage (in some cases) in 1991. Sample size in parenthesis.

	Impsi (16)	Kardyvach (33)	Aiskha-II (6)	Fisht (11)	Dagomys (8)
"dinniki"	9 (56%)	4	3	2	0
"tigrina"	3	11 (33%)	1	3	0
"ursinii"	2	9 (27%)	0	2	0
"kaznakovi"	1	1	2	2	7
"bronze"	1	8 (24%)	0	0	0
"melanistic"	0	0	0	2	1

Inheritance of color morphs

The different color morphs can be seen in broods from different types of females (Table 5), verifying that at these localities a single polymorphic species is involved. The sample is not big enough for allowing definite conclusions about inheritance, but some indications can be obtained. The three "bronze" females only gave birth to "bronze" juveniles (one brood) or mixture of "bronze" and narrow banded "tigrina" juveniles (two broods). No other female than these three (in the total sample of 23 pregnant females) gave birth to "bronze" juveniles. Further, the "tigrina" pattern seems dominant as it shows up in several broods, and is always expressed when the female has a pattern towards "tigrina". In the three pure "tigrina" females that gave birth all juveniles were of "tigrina" type (N=8). Further (although not seen from the table) eleven females with other pattern types than "tigrina" also produced "tigrina" juveniles together with other morphs. Thus "tigrina" and "bronze" were the most frequent juvenile morphs in the *dinniki* material.

FIG. 6. The narrow-banded "tigrina" morph of *Vipera dinniki*, from Kardyvach (ZIG).

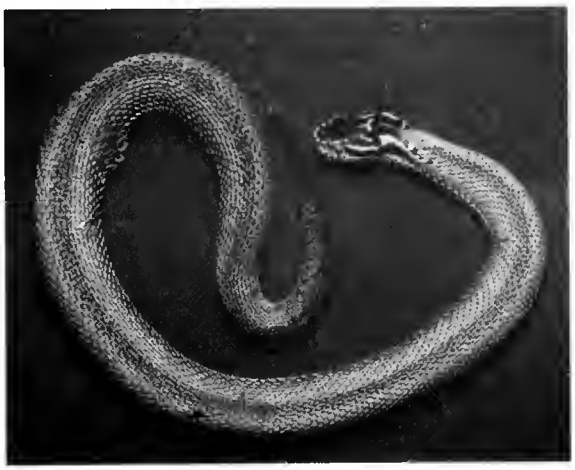


FIG. 8. The "bronze" morph of *Vipera dinniki*, from Mt. Loyub (ZIG).

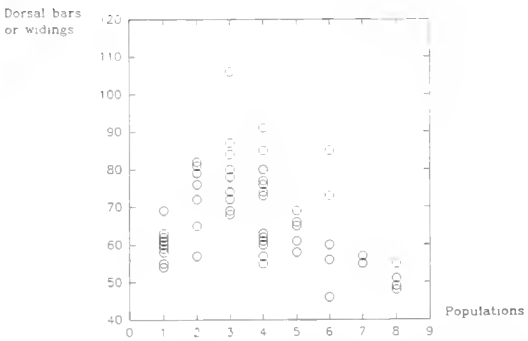


FIG. 9. Distribution of number of bars and/or windings in the dorsal zig-zag band in the different *Vipera dinniki* populations, running from west towards east in high Caucasus (1: Fisht, 2: Loyub, 3: Kardyvach, 4: Impsi, 5: "central Caucasus" (=Elbrus and surroundings), 6: "east Caucasus" (=mountains above Lagodechi, Georgia), and *V. kaznakovi* populations (7: Sochi-Adler, 8: Hopa, Artwin).

Of the juveniles taken together half (50%) were "tigrina". When considering the Kardyvach material alone (10 broods with 29 juveniles) 52% were "tigrina" while 24% were "bronze". Of 47 adults observed in the field in July 1991 at this locality 24% were "bronze". The number of adult "tigrina" observed (33%) was lower than the frequency of juvenile "tigrina" produced while the number of adult "ursinii" was rather high (27%). This slight reduction of the "tigrina" pattern between juveniles and adults can have an ontogenetic explanation as pattern often fades with age.

In other populations examined the combinations of morphs were different, with other patterns dominating. The number of windings is much lower in Fisht compared to the Kardyvach sample (with "tigrina" predominating). "Melanism" was only observed at Fisht, but it is known also from Aishkha-II and the Bezymyanka River. "Bronze" was only observed at Kardyvach and Impsi. When comparing field observations and all available specimens in collections, this morph was not documented from any other locality along the entire area. At Impsi the typical "dinniki" pattern was dominating (56%).

Analysis of scalation characters in the different dinniki *populations*

Geographic variation in color morph frequencies is also reflected in scalation characters (Table 4). Going from the west Caucasus towards east, certain changes could be observed. The central and east Caucasus samples of *dinniki* have higher mean number of preventral plates, lower

TABLE 4. Variation in scalation and color pattern characters between different isolated populations of *Vipera dinniki* The localities include a series of isolates at close distance in the Caucasus State Biosphere Reserve (1: Mt Fisht/Oshten; 2: Mt. Loyub; 3: Lake Kardyvach; 4: Lake Impsi), and 5: central Caucasian population (Mt Elbrus region) and 6: east Caucasian population (the mountain region of Lagodekhi) sample. Given as Mean value, S.E. and range (except for preventrals and apicals). Number of specimens in parentheses.

	1 (13)	2 (9)	3 (16)	4 (16)	5 (7)	6 (6)
Pre-ventrals	1.4±0.2	1.7±0.3	1.9±0.2	1.7±0.2	2.0±0.2	2.2±0.5
Ventrals	135.3 ±1.05 129-141	135.3 ±1.0 131-140	132.8 ±1.1 126-141	134.4 ±0.7 129-139	133.3 ±0.9 130-136	131.2 ±1.2 127-134
Rostral index	1.1±0.1 1.0-1.4	1.1±0.0 1.0-1.3	1.1±0.0 0.9-1.3	1.1±0.1 0.9-1.1	1.0±0.0 0.9-1.3	1.1±0.1 1.0-1.3
Apicals	1.46 ±0.14	1.33 ±0.17	1.47 ±0.13	1.50 ±0.13	1.00 ±0.00	1.17 ±0.17
Circum-oculars*	18.3 ±0.5 15-21	19.1 ±1.0 14-23	18.4 ±0.5 14-22	17.8 ±0.6 13-22	17.3 ±0.9 12-19	19.2 ±0.8 18-23
Loreals*	8.23 ±0.6 5-12	8.44 ±0.8 5-12	9.0 ±0.6 6-12	8.13 ±0.4 6-11	6.86 ±0.6 5-9	7.17 ±0.9 3-9
Crown scales	12.46 ±0.8 9-19	14.78 ±1.6 9-23	14.33 ±0.7 10-18	15.19 ±0.9 10-22	12.43 ±0.5 10-14	16.67 ±1.3 12-21
Zig-zag wind-ings **	60.8 ±1.3 54-69	73.14 ±3.5 57-82	79.78 ±3.9 68-106	70.07 ±2.8 55-91	63.57 ±1.4 58-69	64.0 ±6.8 46-85

* Counted as sum of both sides.
** Unicolored "bronze" specimens as well as completely bilineate and melanistic specimens excluded.

TABLE 5. Phenotypic expression of inheritance of color patterns in 23 clutches of *Vipera dinniki*. Given as taxa, female morph, number of clutches, numeric distribution of morphs in group of juveniles of each female morph.

Taxa (LOCALITY)	Female morph	No. of clutches	No. of juvenile morphs
kaznakovi (DAGOMYS)	"kaznakovi"	3	8 "kaznakovi"
dinniki (FISHT)	"melanism"	1	6 "dinniki" 1 "ursinii"
dinniki (KARKYVACH)	"dinniki"	3	4 "dinniki" 2 "tigrina"
	"bronze"	3	7 "bronze" 4 "tigrina" (narrow-banded)
	"tigrina"	2	4 "tigrina"
	"dinniki/ursinii"	1	2 "tigrina" 2 "ursinii"
	"tigrina/ursinii"	1	2 "tigrina" 1 "tigrina/ursinii" 1"dinniki/bilineate"
dinniki (IMPSI)	"dinniki"	6	7 "dinniki" 4 "spotted-divided" 4 "tigrina 2 "bilineate"
	"ursinii"	1	2"bilineate/tigrina 1 "bilineate"
	"ursinii/tigrina"	1	1"bilineate/tigrina" 1 "tigrina/ursinii"
	"tigrina"	1	4 "tigrina"

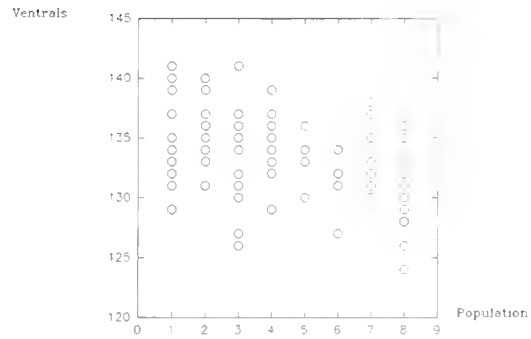


FIG. 10. Distribution of ventral numbers in the different *Vipera dinniki* populations, running from west towards east in high Caucasus (1: Fisht, 2: Loyub, 3: Kardyvach, 4: Impsi, 5: "central Caucasus" (=Elbrus and surroundings), 6: "east Caucasus" (=mountains above Lagodechi, Georgia), and *V. kaznakovi* populations (7: Sochi-Adler, 8: Hopa, Artwin).

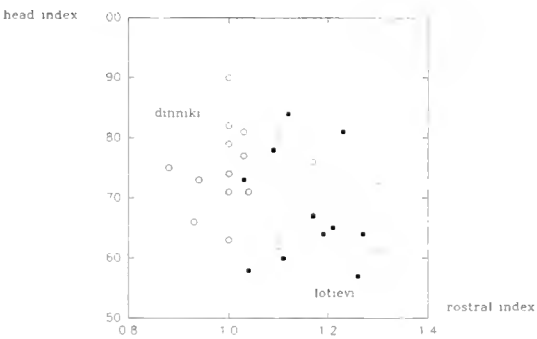


FIG. 12. Head index (breadth/length) and rostral index (height/ breadth) in sympatric sibling species of east Caucasus. White circles= *V. dinniki* ("east-dinniki"); black squares= *V. lotievi*.

mean number of apical scales, and lower number of loreal scales. Also the number of ventrals showed a slight decrease towards the east, a pattern also observed between northwestern and southern populations of *Vipera kaznakovi* (Fig. 10).

Analysis of scalation characters in the different *dinniki* morphs

In subalpine and alpine mountain belts of the west Caucasus (eastward to the basin of the Big Laba River) the "ursinii" morph belongs to the same species as the rest of the

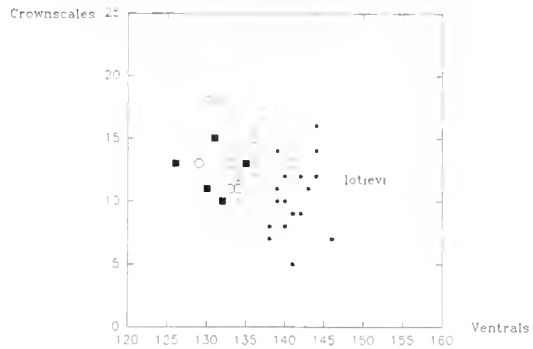


FIG. 11. Numbers of crown scales and ventral plates in "ursinii/ bronze" and "dinniki" morphs and sympatric sibling species of west and east Caucasus. The dotted line indicate the main separation between *V. lotievi* and the *V. dinniki* from different regions. Big white circles= the "dinniki" morph of western Caucasus (*V. dinniki*); big black squares= the "ursinii/bronze" morphs of western Caucasus (*V. dinniki*); small white circles= the "dinniki" morph of eastern Caucasus (*V. dinniki*="east-dinniki"); small black squares= the "ursinii/bronze" morphs of eastern Caucasus (*V. lotievi*)

mountain vipers: *Vipera dinniki*. But in the extreme eastern part of the west Caucasus, and the central and east parts of the high Caucasus, the "ursinii" and "bronze" morphs belong to a different species. An examination of the morphology of the different morphs clearly indicate that in the eastern half of high Caucasus there are two sympatric species (Figs. 11 and 12).

Vipera kaznakovi (Fig. 4).

This species also shows some regional variation, although not so pronounced as in *V. dinniki*. When comparing the southern (Turkish) populations with the northern (Russian) ones, differences in color pattern as well as in scalation could be detected. Specimens from the southernmost population in Hopa (Turkey) are more yellowish in ground color compared to the snakes in the northern parts (Dagomys). To the contrary, specimens in the north often have more black areas on the body and often the orange or reddish ground color is expressed only as two dorsolateral rows of spots. Melanism is frequent in this northern

TABLE 6. Variation in scalation and color pattern characters between the northern and southern *Vipera kaznakovi* populations. The samples are from northeastern Black Sea regions in Russia (Sochi-Adler); and from northeastern Turkish Anatolia (Hopa, Artvin province). Given as Mean value, S.E. and range (except for preventrals and apicals). Number of specimens in parentheses.

	Sochi - Adler (17)	Hopa (13)
Preventrals	1.41±0.19	1.54±0.18
Ventrals	133.8±0.6, 130-138	130.4±0.9, 124-136
Rostral index	1.1±0.02, 1.0-1.27	1.1±0.06, 0.87-1.5
Apicals	1.50±0.13	1.64±0.14
Circumoculars*	20.0±0.48, 16-23	19.31±0.43, 15-21
Loreals*	11.06±0.76, 7-16	8.69±0.60, 5-12
Crown scales	14.94±0.92, 10-23	17.33±1.08, 11-22
Zig-zag windings**	56.33±0.67, 55-57	50.75±1.55, 48-55

* Counted as sum of both sides.
** Four completely bilineate and melanistic specimens are excluded.

TABLE 7. Allele frequencies of polymorphic loci (see Table 2 for locus abbreviations). For taxon abbreviations, sample sizes and localities, see Table 1.

Locus	Allele	KA 1	KA 2	DI 1	DI 2	DI 3	DI 4	LOT	ERI	BER
Adh-1	-100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	-110									1.00
Gpi-1	-100	0.75	1.00	1.00	1.00	1.00	1.00			
	-50	0.25						1.00	1.00	
	-75									1.00
Hk-1	-100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	-120									1.00
Idh-1	-100	1.00	1.00	0.93	0.86	0.83	0.97	1.00	1.00	
	-10			0.07	0.14	0.17	0.03			
	-130									1.00
Idh-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
	130								0.10	
	180								0.90	
Ldh-2	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.77
	120									0.23
Pgm-2	100	1.00	1.00	0.71	1.00	1.00	0.88	1.00	1.00	
	103									1.00
	105			0.29			0.12			
Sod-1	100			0.71	0.21	1.00	0.71			
	33	0.50	0.12	0.29	0.79		0.29	1.00	1.00	
	95	0.50	0.88							
	103									1.00

population, a phenomenon never observed in the southern one. It can be added that melanistic specimens have been found in more or less all Russian populations. During a stay 1992 at an inland locality along the Psou River we observed ten adult specimens of which all but three were melanistic.

In scalation characters there are some minor differences: e.g. the Hopa population has a lower mean value in number of ventrals and crown scales (Table 6).

TABLE 8. Genetic distances (above diagonal) and genetic identities (below diagonal) of eight OTUs of Caucasus vipers and *Vipera berus*. 1- *kaznakovi*, Dagomys; 2- *kaznakovi*, Rudorova; 3- *dinniki*, Fisht; 4- *dinniki*, Impsi; 5- *dinniki*, Aishka; 6- *dinniki*, Kardyvach; 7- *lotievii;* 8- *berus;* 9- *eriwanensis*.

OTU	1	2	3	4	5	6	7	8	9
1		0.01356	0.03323	0.01501	0.04121	0.02912	0.03759	0.25418	0.07680
2	0.98653		0.04061	0.03826	0.03849	0.03810	0.07696	0.25325	0.11778
3	0.96731	0.96020		0.01767	0.00558	0.00125	0.06515	0.25325	0.10549
4	0.98511	0.96246	0.98249		0.02769	0.01358	0.03962	0.25325	0.07892
5	0.95962	0.96224	0.99443	0.97269		0.00448	0.07774	0.25325	0.11859
6	0.97130	0.96262	0.99875	0.98651	0.99553		0.06021	0.25325	0.10034
7	0.96311	0.92593	0.93692	0.96115	0.92521	0.94156		0.25325	0.03774
8	0.77555	0.77627	0.77627	0.77627	0.77627	0.77627	0.77627		0.30214
9	0.92607	0.88889	0.89989	0.92412	0.88817	0.90453	0.96296	0.73924	

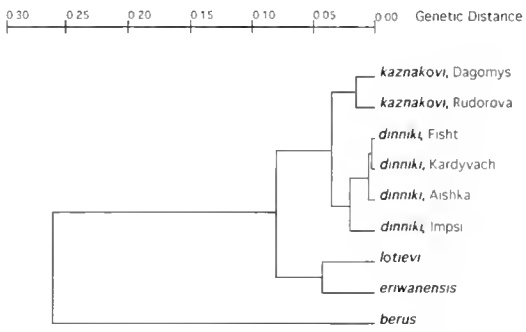


FIG. 13. UPGMA phenogram clustering modified Nei genetic distances (Hillis 1984) among eight OTUs of Caucasus vipers and *Vipera berus*.

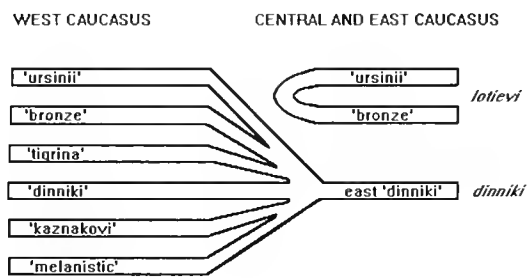


FIG. 14. The different types of morphs and species found in west and east high Caucasus.

Phenetic analysis of electrophoretic data

Of twenty-seven presumptive gene loci scored, from 14 enzyme systems and one general protein, only eight were polymorphic. Allelic products (allozymes) for each locus were designated numerically in order of increasing anodal mobility to identify genotypes (Table 7), with the relative mobility of the most common allele in each locus as a standard of reference ("100"). Despite the fact that average sample sizes were rather small for obtaining significant results of frequency analysis we elected not to pool different localities (except for *eriwanensis),* for reasons of objectivity discussed above, although we recognize the obvious risks of missing rare alleles through this procedure.

Modified Nei genetic distances (Nei, 1978; Hillis, 1984) between OTUs were computed from allele frequencies (Table 8). A UPGMA phenogram (Sneath and Sokal, 1973) was constructed from the distance data (Fig. 13). This phenogram illustrates only the relative degree of genetic differentiation among OTUs and should not be considered as a phylogenetic tree. Observed genotype distribution of the most polymorphic locus (Sod-1) in the largest individual sample (Kardyvach; N=17) was tested against Hardy-Weinberg expectations to investigate homogeneity and possible biased sampling due to potential within-locality sympatry of taxa (i.e. Wahlund effect). A chi-square test performed to obtain the goodness-of-fit between observed and expected distributions (X^2=0.060, 1 df, P=0.90) strongly support unbiased sampling. The genetic analysis confirms that the four populations from the western Main Caucasus (Fisht, Kardyvach, Aishka-

II and Impsi) belong to one polymorphic taxonomic unit *(V. dinniki)*.

Discussion

The original question, whether polymorphism or sibling species are the prevailing phenomenon in the Caucasian populations of vipers, must be answered with yes in both cases. In the western Caucasus a large number of morphs can be recognized. We separate six different ones in that region while the number is restricted to three in the eastern and central parts of the mountain range (Fig. 14). As seen in the reproductive studies all six morphs ("melanism", "bilineat-kaznakovi", typical "dinniki" (including "nebulosa"), "tigrina", "bronze", and "ursinii") in the mountain habitats in the western Caucasus belong to a single species. This is also verified by cladistic analyses of biochemical and morphological data (Nilson et al., 1994). The different morphs can be seen sympatric and syntopic in suitable rocky, vegetation-rich, and moist habitats. There seems to be a certain inheritance pattern, but in principle most morphs can occur in the same brood (Table 5). Certain areas seem to have a certain range of frequency of the various morphs, which might be unique for that particular area. It must be kept in mind that only a random number of populations have been investigated, and it is likely that additional morphs will be described from other isolated localities. The isolation of the different mountains in the Caucasus is much comparable with e.g. the Andes of Ecuador, or the Galapagos islands, and a high degree of isolation has obviously taken place between the different mountain peaks.

How could this particular polymorphic pattern in the subalpine *Vipera dinniki* populations have evolved? The present warm and wet subtropical Colchis area of western Transcaucasia at the Black Sea coast has, as indicated by botanical evidence, served as a refuge for animals and plants during the whole of Pliocene and Pleistocene (Tuniyev, 1990). The total region has varied much in size during the Pleistocene glacial and interglacial periods but remained a permanent relict key area. The warm and wet adapted *Vipera kaznakovi* is distributed in this area today, and it has been considered that this species has had an occurrence in this region for a long time (Orlov and Tuniyev, 1990; Tuniyev, 1990) due to the climatic stability.

A scenario for the arise of various polymorphic *V. dinniki* populations could have taken place in two steps and been like this:

1. First, the evolution of *Vipera dinniki*. If the Pliocene Colchis region decreased in range during a Pleistocene glacial period there are two alternatives for the species in that part of the old range that have turned cold: the viper could disappear or it could adapt. If it disappears it can be done in two ways: the snake becomes extinct or it is forced down to the remaining warm zone. In both these two cases the net result will be a reduced range for the single species (*kaznakovi*) in the remaining warm and wet Colchis refuge. In the second case, if the viper is adapted to the new climate, "the cold zone", it would be a new, physiologically different race.

In an interglacial period when the climate becomes warmer again the warm and wet Colchis zone expands and "the cold zone" is forced upwards to higher altitudes in the mountains. Now, if there is a cold-adapted physiological race, again two alternatives are open: first, it could adapt, or second, it could disappear. If it is adapted back to the new warm environment, it can either go back into and unite with the old species (*kaznakovi*) which under the new climatic conditions can expand its range, or it could form a sympatric but physiological distinct taxon. In the case of sympatry there is a good possibility that it would disappear due to competition (if not ecological distinct). In both these last situations there is a great probability that the original species (*kaznakovi*) would return and again cover its original range.

If the new physiological race disappears from the region it could again be done in two ways: it simply becomes extinct due to the new "severe" climatic conditions, or it

migrates upwards following the pushed up "cold zone". In the first case again only the original species (*kaznakovi*) will remain in its new expanded (=original) warm and wet range. In the second case there will be a number of cold-adapted populations at higher altitudes (the present different *dinniki*, "east-dinniki", *darevskii* populations). This would mean a number of isolated populations as the mountains of the Caucasus are rather steep and subalpine/alpine habitats are in many cases climatologically isolated. Further the geology is very complex reflecting a high degree of local endemism among plants and animals. One can postulate that the different populations, or groups of populations, must have become adapted to local conditions.

2. This may have been the prerequisite for an evolution of a polymorphic *Vipera dinniki*. During Pleistocene there were several glacial and interglacial periods, and the scenario postulated above would have been repeated several times, and during each interglacial the "cold zone" and its cold adapted viper were forced downwards with the result of a secondary contact with neighboring populations. Unique morphs could by this be spread to adjacent populations etc.

Today we can see a polymorphic color-pattern in the cold adapted *Vipera dinniki* that to a high degree is unique for one or a small number of populations in close connection, e.g. the "bronze" and "tigrina" morphs, but not seen in all populations. *Vipera dinniki* could be a polymorphic species, constituent of a number of populations that during periods are isolated from each other, but irregularly have secondary contact. In some cases the isolation could also have been more permanent resulting in a number of sister species along the range, a possible phylogenetic pattern we currently are trying to solve with genetic studies. But overall, the relative genetic differentiation between examined taxa and degree of genetic polymorphism were low, indicating a rather recent divergence.

The present geographical distribution and morphological pattern of *Vipera dinniki*, as well as the fact that in all subalpine mountain regions where *V. dinniki* is located today, there also are fragmented more or less subtropical Colchian refugia at lower altitudes, inhabited by *V. kaznakovi* (Tuniyev, 1990). This supports the evolutionary pattern postulated above.

Further east in the mountains, the habitats get drier with moist areas restricted to stream surroundings and lake shores. In central Caucasus (Mt. Elbrus) the number of morphs decreases to two ("ursinii" and "dinniki") or three in the eastern Caucasus (where again a form of "bronze" morph appears) (Fig. 14).

Now, in the region of the central and eastern Caucasus two different sympatric species are involved. As shown in the results section above, at several localities the two morphs "dinniki" and "ursinii" actually represents two sympatric species from the *kaznakovi* line and the *ursinii* line respectively. It is obvious that these two species are sympatric (but not necessarily syntopic) in a large number of places in these eastern and central parts of the main range of the Caucasus. We have in our material such records of sympatry from Mt. Elbrus in the central Caucasus (Figs. 15-18); mountains north of Lagodechi in the eastern Caucasus; at Itum- Kali, Chechena-Ingushetia (Fig. 19); and various records from Dagestan, besides several isolated records of both species from the entire eastern and central Caucasus range.

Vipers of the *kaznakovi* group are known from subalpine meadows, and snakes of the *ursinii* group have been found in the semiarid hollows between the main range and the Skalisty (Rocky) range. At several places with connection of subalpine meadows and semiarid hollows the two species have a sympatric occurrence (and syntopic along the ecotones of both types of landscapes). The *ursinii* line has probably never been widely represented in the perpetually humid western Caucasus, as this taxon is adapted to dry environments, but as stated earlier, in the extreme eastern part of

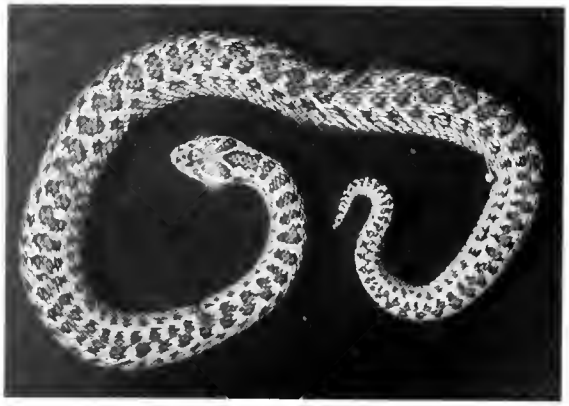


FIG. 15. Male of the east *Vipera dinniki* ("east-dinniki") at the sympatric locality, Mt Elbrus, central Caucasus. This specimen was found together with the *Vipera lotievi* on figures 17 and 18 (ZIG).

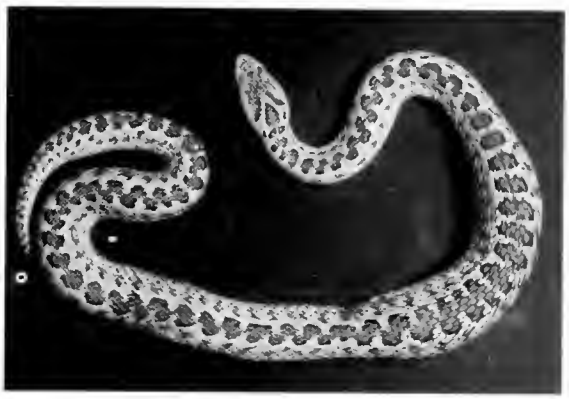


FIG. 17. Female of the *Vipera lotievi* at the sympatric locality, Mt Elbrus, central Caucasus. This specimen was sympatric with the *Vipera dinniki* ("east-dinniki") on photo 15 and 16 (ZIG).

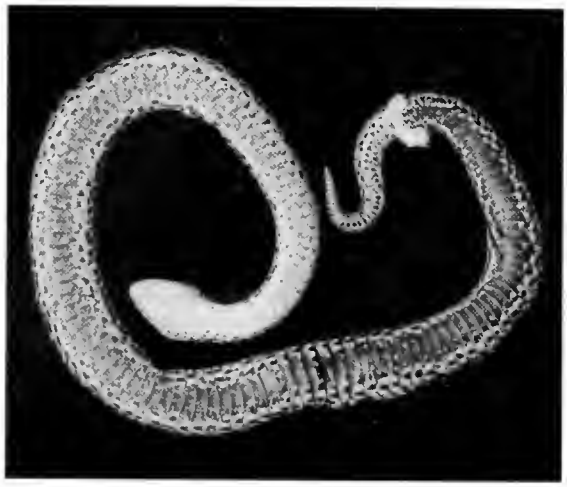


FIG. 16. Ventral side of the male of the east *Vipera dinniki* ("east-dinniki") at the sympatric locality, Mt Elbrus, central Caucasus from figure 15 (ZIG).

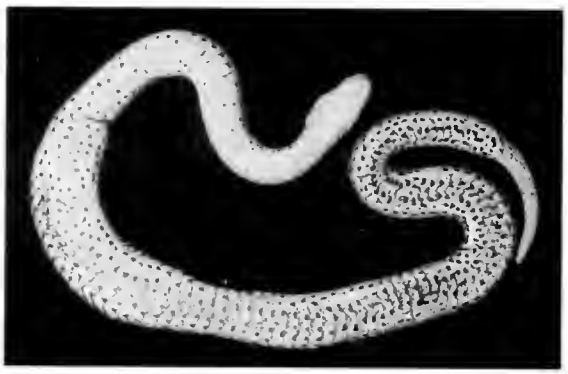


FIG. 18. Ventral side of the female of the *Vipera lotievi* at the sympatric locality, Mt Elbrus, central Caucasus from photo 17 (ZIG).

the west Caucasus there are some isolated populations of the *ursinii* complex (from the Abishiz-Akhuba Range to Mt. Elburs).

The eastern form of *dinniki* is not polymorphic in the same way as the western populations. Rather the main color pattern is the typical "nebulosa-dinniki" kind of pattern. In sympatric areas the *ursinii* taxon is more or less typical "mountain ursinii" in color pattern although with a certain similarity to the type of bilineate pattern seen in the southwest European *V. seoanei* (Fig. 20). In some populations of this taxon in Checheno-Ingushetia, a certain fraction of the snakes are also "bronze" colored (Plate 1d). This pattern type has not been observed in the sympatric populations. The "nebulosa-dinniki" pattern of eastern *dinniki* and the "seoanei-ursinii" pattern of Caucasian *ursinii* taxon shows great similarities, and can in some specimens be difficult to separate. This is certainly the reason for much of the confusion in earlier studies of these vipers.

However, at a closer examination, the two taxa are possible to identify (Table 9). In *ursinii* the belly is lighter and the snout more concave with raised canthus. The preocular is large and in contact with the nasal, and the apical is always single. Also the crownscales are less fragmented. There

TABLE 9. Main morphological characteristics separating the sympatric *Vipera lotievi* and "east-dinniki".

	lotievi	"east-dinniki"
White belly	+	-
Preocular in contact with nasal	+	-
Snout concave	+	-
Mean no. of ventrals	>140	<136
Always a single apical	+	+/-
Mean no. of crown scales	<11	>12
Parietal ocellated spot	+	-
Iris gold-edged in life	-	+

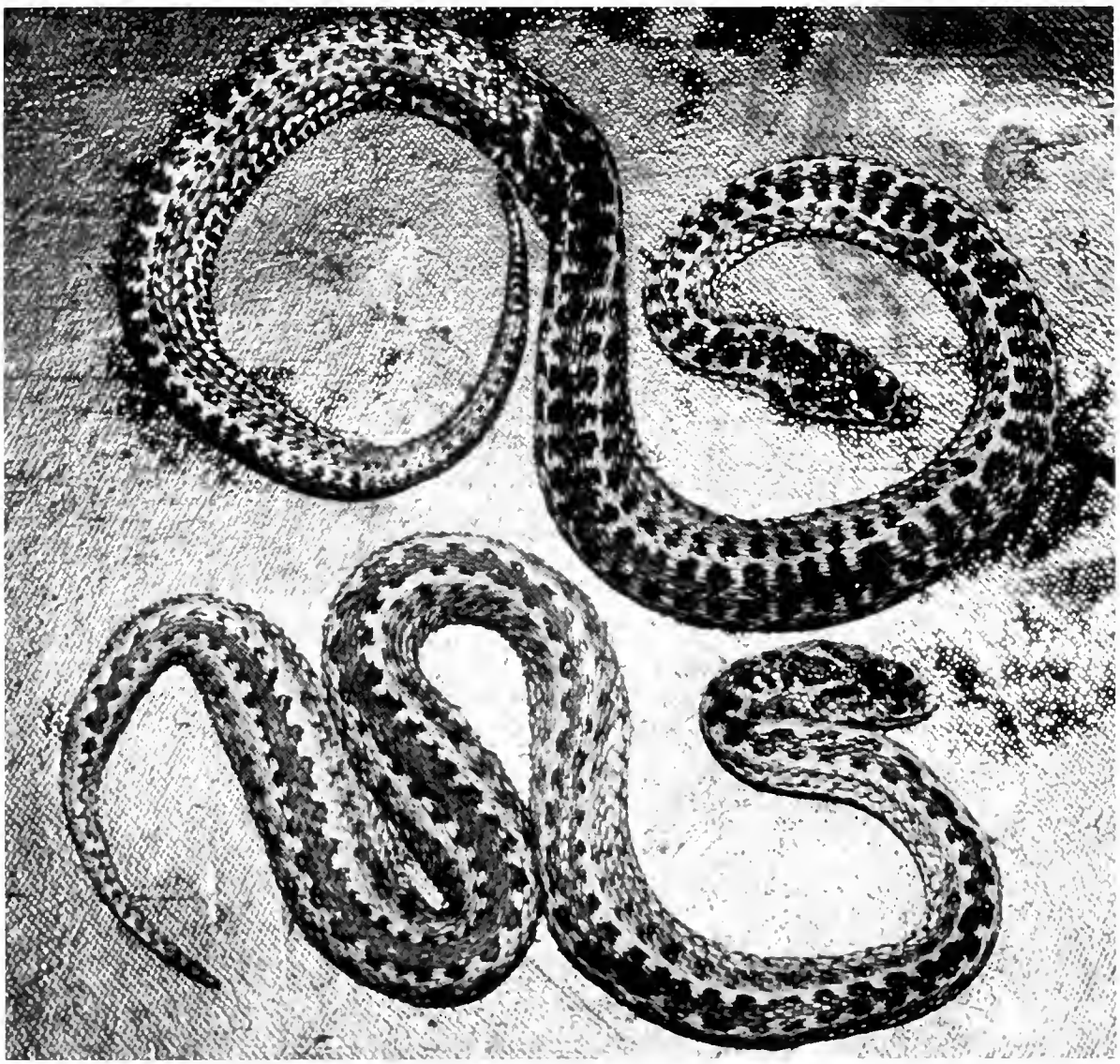


FIG. 19. *Vipera dinniki* ("east-dinniki")(upper) and *Vipera lotievi* (lower) from Itum Kali, Chechena-Ingushetia. These specimens were found together at the same time (ZIEr).

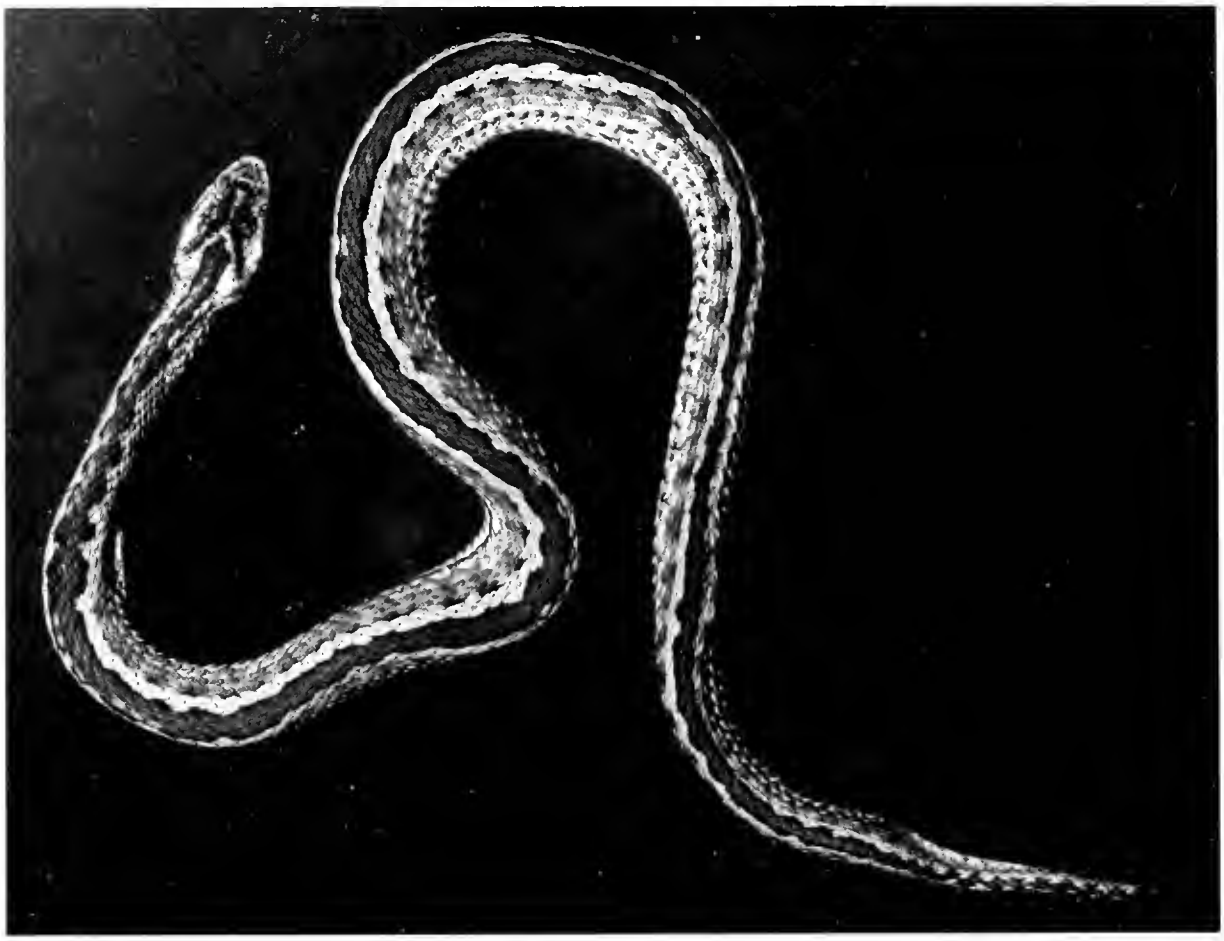


FIG. 20. Female of *Vipera lotievi* sp.n. from the type locality, the surroundings of Armkhi Village, Checheno-Ingushetia, Nazranovskiy District.

always seems to be an ocellated spot present on the parietal plate, and the ventral number is high. In the "east dinniki" taxon the belly is blackish and the snout more flat, the preocular is always separated from the nasal, and there is a higher fragmentation of the crown scales. The iris always seems to be gold-edged in live specimens (as is the case for the entire *V. kaznakovi* complex), and this is specially distinct in younger specimens. The ventral number is lower.

Although in many ways similar in pholidosis the *kaznakovi* lineage and the *ursinii* lineage are genetically well separated and paraphyletic. Immunological comparisons of blood serum albumins indicate that *Vipera kaznakovi* and related taxa belongs to the *berus-aspis* branch while *ursinii* constitute a distinct evolutionary lineage (Herrmann et al., 1987; 1992). The genetic comparisons of the west Caucasian *dinniki* and the *ursinii* taxon from Checheno-Ingushetia point in the same direction (this study; Nilson et al., 1994) except that the closer relation between *kaznakovi* and *aspis* was not supported. Thus this morphological similarity between the two lineages in Caucasus might be a case of convergent adaptation towards a similar habitat, although Muellerian mimicry might be involved.

A number of nominal taxa related to these populations are recognized from this geographical region (Russian Republic, Georgia, Azarbaidjan and Armenia): *renardi*,

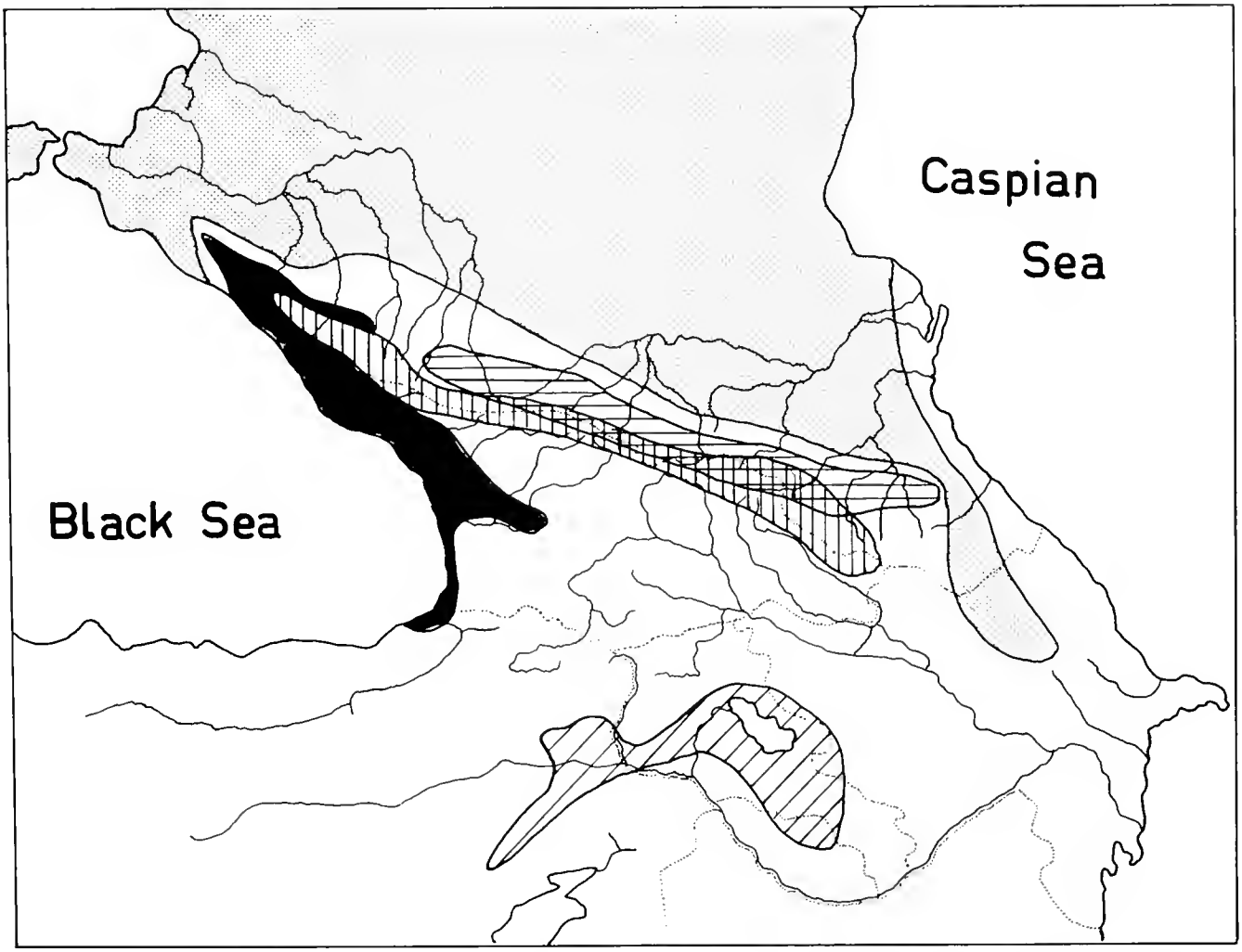


FIG. 21. Distribution of the vipers of the Caucasus and adjacent areas discussed in this paper. Light stippled= *Vipera renardi;* dark stippled= *Vipera kaznakovi;* cross-hatching= *Vipera (u.) eriwanensis;* horizontal hatching= *Vipera lotievi;* vertical hatching= *Vipera dinniki.* Due to environmental reasons the distribution of all taxa are only fragmented within the depictured ranges, a situation especially pronounced in *renardi.* Also occurring in the region and related to the vipers discussed are the north Iranian populations of the *ursinii* complex *('ebneri')* that penetrates into southeastern Azarbaidjan in the Talysh mountains, *Vipera darevskii* (of the *kaznakovi* complex) which has its known distribution restricted to northwestern Armenia (Mt. Legli), and *V. pontica* from the Artwin province in Turkey. Other species of vipers not discussed here occur sympatrically in the region.

kaznakovi, *darevskii*, *eriwanensis*, *dinniki* (Fig. 21).

What names are then available for these two different sympatric central and east Caucasian taxa? *Vipera dinniki* was originally described from Malaya Laba and Svanetia, localities situated on the western side of the upper parts of Little Laba River and the high-mountain basin of the Inguri River, respectively (Orlov and Tuniyev, 1986). The type locality has been restricted to Malaya Laba (by selection of 'The Museum of Natural History of Kharkov State University specimen no. 26044' as lectotype; Vedmederja et al., 1986). The type locality is situated in the western Caucasus and the polymorphic western

TABLE 10. Variation, given as Mean ±S.E. and range (for apicals in % of specimens with two plates) of selected morphological characters in *Vipera eriwanensis* (N=44), *Vipera lotievi* (N=14, if not otherwise stated), and *Vipera renardi* (N=42).

	eriwanensis	*lotievi*	*renardi*
Ventrals	137.66±0.34, 133-143	141.29±0.54, 138-144	142.67±0.54, 135-150
Subcaudals - males	35.0±0.4, 32-39 (N=25)	35.5±0.7, 33-38 (N=6)	34.7±0.4, 31-38 (N=25)
Subcaudals - females	26.6±0.4, 23-30 (N=19)	25.3±0.6, 23-27 (N=8)	26.8±0.4, 24-29 (N=17)
Preventrals	2.00±0.10, 1-3	2.43±0.14, 2-3	2.27±0.10, 1-3
Scale rows on neck	21.07±0.05 21-23	21.00±0.00, 21	21.24±0.095, 21-23
Midbody scale rows	21.02±0.02, 21-22	20.64±0.20, 19-21	20.98±0.02, 20-21
Ventral level of scale row reduction****	94.70±0.94, 82-109	87.50±6.49, 17-106	96.38±1.50, 71-127
Supralabials*	18.02±0.08, 17-20	17.36±0.29, 16-20	17.81±0.11, 14-19
Sublabials*	19.93±0.22, 17-25	19.64±0.49, 15-22	20.26±0.18, 18-24
Circumoculars*	18.61±0.35 (6-),15-22	18.50±0.56, 14-22	18.57±0.23, 16-21
Loreals*	10.18±0.42, 5-18	7.93±0.52, 5-12	8.76±0.32 , 4-12
Crown scales	13.07±0.36, 9-19	10.93±0.68, 7-16	10.43±0.40, 6-16
Chinshields	4.07±0.05, 4-6	4.64±0.25, 4-6	4.21±0.11, 4-8
Gulars	4.50±0.11, 3-6	3.71±0.13, 3-4	4.31±0.10, 3-6
Zig-Zag windings	65.77±0.80, 56-79	65.50±2.88, 50-81 ***	59.31±0.70, 51-72
rostral index (height/width)	1.23±0.02, 0.79-1.67	1.11±0.03, 0.92-1.27	1 04±0.01, 0.91-1.20
Apicals	1.00±0.00	1.27±0.07	1.07±0.04
CV?	0**	27.3	7.1

* Counted as sum of both sides. ** No specimen with two apicals, but one with three. Also two specimens with no apical; thus 6.8% total with more than one or without apical. *** N=16. Four unicolored "bronze" specimens not included. **** reduction from 21 to 19 dorsal scale rows (at ventral number)

dinniki populations must be referred to this name. The complex picture of separation and similarities between all the different isolated mountain populations in the western Caucasus demands parallel genetic studies. The name *Vipera kaznakowi orientalis* (Vedmederja, 1984; non *Vipera orientalis* Seba, 1734-1735; Daudin, 1801-1803; Shaw, 1802) was given by Vedmederja (1984) for eastern vipers at Lagodechi. As stated above at this locality the two species are sympatric, but the name *orientalis* is not available (nomen nudum - Orlov and Tuniyev, 1986). Besides the color pattern, the eastern *dinniki* is as well somewhat morphologically distinguished compared to western *dinniki* (Table 4). It is geographically separated from the western *dinniki* and it might be justified to treat it as a taxon of its own. The genetic distance has

TABLE 11. Frequency of certain characteristics in the populations (in percentage of investigated specimens)

	eriwanensis N=44	*lotievi* N=14	*renardi* N=42
Divided parietals	20.5	14.3	45.2
Divided frontal	4.5	14.3	14.3
Preocular(s) in contact with nasal	22.7	78.6	81.0
Without upper nasal split	71.4	43.8	61.5
Supralabial dark sutures absent	46.5	71.4	0.0
Lateral body blotches absent	38.6	28.6	0.0
Snout concave on dorsal side	51.2	78.6	78.6
Belly whitish (not dark)	71.8	78.6	17.5

however not yet been calculated as fundamental material still is lacking. Thereby we prefer not to draw any taxonomic conclusion about the "east-dinniki" populations. We preserve the terra typica restricta for *Vipera dinniki* to Malaya Laba.

The other question is to what taxon does the viper of the *ursinii* complex belong. In principle north of the Caucasus on the dry steppes *renardi* occurs while in the Armenian highlands south of the Caucasus *eriwanensis* is found (the species status of *renardi* is analyzed and discussed in Joger et al., 1992). We consider also *eriwanensis* as an evolutionary species (Höggren et al., 1993; Nilson et al., 1994). The Caucasian form is geographically separated from these two taxa, and morphologically distinguished (Tables 10 and 11, Figs. 17, 19-20, Plate 1d), also supported by genetic distinction (Tables 7, 8; Nilson et al., 1994). Taxonomically it does not fit in with these two allopatric *ursinii* s.l. taxa, although morphological similarity with *eriwanensis* can be noted. As there is no reason to believe reduced reproductive cohesion- all populations traditionally referred to *ursinii* would belong to a single species (albeit divided in several subspecies) according to the biological species concept (BSC). However, all here (and elsewhere- Nilson & Andrén, 1994) recognized taxa in this complex are allopatric and apomorphic in characters (morphological and/or genetic), and as we are interested in a taxonomy that reflects the phylogeny, we find the evolutionary and phylogenetic species concepts preferable (Frost and Hillis, 1990; Frost et al., 1992; see Nilson, 1993, for application on vipers). We therefore recognize it as a separate taxon in this group of vipers.

Taxonomic Account

Vipera lotievi sp.n. (Fig. 22)

Holotype and Terra typica: ZIN 20309, Fig. 22, female, Armkhi, Checheno-Ingushetia, Russia, below Mt. Stolovaya, 2000 m. altitude, 1986-07-20-23. leg. K. Lotiev.

Paratypes: ZIN 20305, Itum-Kali, Checheno-Ingushetia, 1990-08, leg. K. Lotiev; ZIN 20310, Armkhi, Checheno-Ingushetia, below Mt. Stolovaya, 2000 altitude, 1986-07-20-23, leg. K. Lotiev; ZIN 20304, vicinity of village Armkhi, Checheno-Ingushetia, 1988-07, leg. Gizatulin; ZIG 298-306, river Chanty-Argun w. Itum-Kali, Checheno-Ingushetia,

FIG. 22. The female holotype of *Vipera lotievi* (ZIN 20309), Armkhi, Checheno-Ingushetia, below Mt. Stolovaya, 2000 m altitude.

1986-05-28, leg. B.Tuniyev; ZIN 20307, Itum-Kali, Checheno-Ingushetia, 1987-08, leg. Lotiev; ZIN 20312, Armkhi, Checheno-Ingushetia, 1987-09, leg. Lotiev; ZIN 20313, Armkhi and Mt. Stolovaya, Checheno-Ingushetia, 1986-07-20, leg. Gizatulin; ZIG 297, Mt. Elbrus, 1986, leg Filippov, coll. Tuniyev; ZIN 18203, Teberda, State Reserve, Mt. Bolshaya Hatipara, 1969, leg. Zalslavsky; ZIN 18226, Kabardino-Balkaria, vicinity of village Terskol, 1970-08-19, leg. Kireev; ZIN 11996, Caucasus, Gunib, Dagestan, 1909-05-29, leg. Berg; ZIN 20303, Lagodechi, 1988-07, leg. Bakradze.

Diagnosis and definition: A species of the *Vipera ursinii* complex characterized by polymorphism in color-pattern, including "bilineate pattern" of the same kind as in *V. seoanei*, and "bronze" unimorphs. External morphology evolved as typical for mountain taxa of the *ursinii* complex but not similar to any of the other in color pattern.

From the sympatric "east-dinniki" it differs in several scalation characters and in color of the belly (Table 9, Fig. 11). In *lotievi* the belly is generally white, preocular in contact with nasal, snout concave, 138 or more ventrals, always a single apical, less fragmentized crown scales (7-16), parietal ocellated spot present, iris not gold-edged in life. In "east-dinniki" the belly is black, preocular separated from nasal, snout not concave, 136 or less ventrals, apical single or divided, more fragmentized crown scales (10-21), no parietal ocellated spot, iris gold-edged in life.

From the allopatric *renardi* it differs besides color pattern in morphology by having light supralabials (sutures heavily colored in black in *renardi*), a higher rostral index, smaller size, white belly (dark in *renardi*), and a different niche by being alpine (*renardi* is a lowland steppe inhabitant). No future reproductive cohesion can be postulated.

It is separated from the likewise allopatric *eriwanensis* in the Armenian highlands by the semidesert lowland of the Kura River Valley, that separates the Big Caucasus from the Small Caucasus. No connection can be postulated in an evolutionary time frame. Besides color pattern there is a differentiation in morphology by *eriwanensis* having a higher number of crown scales and a somewhat lower ventral count, and preocular separated from nasal to a higher degree (Tables 10 and 11).

Description of holotype (Fig. 22): An adult female, total length 422 mm, tail 41 mm, latter equal to 10.8 % of total length. Length of head, from posterior border last supralabial to tip of snout 16.8 mm, from posterior border of parietals to tip of snout 12.2 mm, breadth of head at broadest part of head 9.5 mm, at level of the eyes 8.0 mm, size of eye horizontally 2.5 mm and vertically 2.0 mm, distance between eye and lip 2.6 mm. Head covered with rather large scales or plates. Two large supraoculars and 1 large frontal plate on top of head, parietals large, frontal separated from supraoculars by 3 and 2 smaller scales on right and left side respectively, 1 canthal and 1 supranasal scale on each canthus rostralis, but the two supranasals are partly united with the apical; 3 intercanthals and 6 intersupraoculars. Height/depth of rostral 3.4/2.7 mm (=1.26), it is bordered by 2 supralabials, 2 internasals and the broad "apical"; eye surrounded by 8 circumoculars on each side, 5 loreals on each side, upper preocular in contact with nasal on both sides, nasal partly divided at upper edge, 8 supralabials, with forth below eye, and 9 sublabials on each side, anterior supralabials not much enlarged compared to posterior ones, 6 second chinshields bordering the anterior ones and 4 scales in the gular row. Dorsal side of snout concave resulting in a pronounced and raised canthus rostralis. Two preventrals and 141 ventrals, 24+1 subcaudals, 21 dorsal scale rows at midbody and on neck one head-length behind the head, 17 dorsal scalerows one head-length anterior to anal. Reduction from 21 to 19 dorsal scale rows at level of ventral number 89. Dorsal pattern consisting of a weakly winding zig-zag band with 48 windings, lateral body pattern dark weakly contrasting towards the lighter dorsal groundcolor. Head pattern consists of 2 dark oblique bands which do not unite, and a posterior band from eye to corner of mouth and somewhat further back along the lateral sides of neck, no dark pattern on chin or in labial region although a very weak dotted pattern at the supralabial sutures can be imagined, ground color light brown with dorsal pattern dark brown and black edged, ventral side light, throat light. Ocellated spot on frontalia.

Variation: See Tables 5 and 6. Besides the variation in scalation a pronounced variation in color and pattern is expressed. Most striking, and unique for the entire *ursinii* complex, is the bronze morph, which is found in 25 % of the investigated specimens (N=40) (Plate 1d).

Distribution: *Vipera lotievi* is distributed in the semiarid 'hollows' between the northern slope of the main Caucasian range and Skalisty range from the upper part of the Kyafar River (range Abishir-Akhuba) eastward to the interior of

Daghestan (see map). Altitudinal span in this region goes from 1200 m up to 1600 m (1800m). Further it is recorded from Mt. Elbrus in the central Caucasus, and mountains north of Lagodechi in the eastern Caucasus, besides several isolated records from the eastern and central Caucasus range.

Habitats: Typical habitats are oreoxerophytes landscapes with semiarid light-forests (like Shibliak), phrygana (with 'tragakant' astragalus) which are very similar to east-Mediterranean types of vegetation. On the upper elevation of the distribution *V. lotievi* reaches the subalpine mountain belt.

"Refuge History": The development of the xerophilous vegetation has taken place since Pliocene in the eastern part of the Caucasian Isthmus. Four main refuges are known: two humid (Colchis and Talysh-Hyrkanian) and two arid-xerophilous (Armenian and Dagestanian). The north Caucasian refuge of oreoxerophits, including shibliak and phryganas are situated along the shale-depression between the main Caucasian Range and the Skalistiy (Rocky) Range. There are several semiarid hollows from central Dagestan (Gunibskoe Plateau) and westward to the beginning of the Kuban River (at the Mt. Elbrus region). The vegetation is composed of *Juniperus oblonga*, *Paliurus spina-christi*, *Cerasus incana*, *Colutea orientalis*, *Berberis vulgaris*, *Astragalus denudatus*, *Celtis glabrata*, *Ephedra procera* and others. This vegetation superficially is very close to the vegetation of the Armenian highland and the mountains of the Near East, but the regions share relatively few species (3-5%). The major part of the plants of these hollows has an east Caucasian origin. For example, 25% of the flora of the Itum-Kali hollow (Checheno-Ingushetia) has east Caucasian origin. Altogether, more than 200 species of plants are endemic to these hollows (Galushko, 1974).

There have been different interpretations about the age of the vegetation in these hollows. Most botanists have been of the opinion that this vegetation has a Pliocene origin (Grossgeim, 1948; Krasnov, 1894; Kuznetsov, 1890), while Galushko (1974) has the opinion that the semiarid hollows of Checheno-Ingushetia are younger than the hollows of Kardino-Balkaria and Osetia in the west and Dagestan in the east, and perhaps not older than Holocene. But the remains of xerophilous flora on the crests between the semiarid hollows are the witness of the existence of a united enormous xerophilous (Mediterranean) refuge, running from Dagestan to the region of Mt. Elbrus. Later, in Pleistocene, this refuge disintegrated to several micro-refuges which have persisted to different extent until present. However, it must be pointed out that although the xerophilous vegetation (including mountain-steppe) had a wide distribution along the shale-depression, it also had the possibility to disperse up to the mountains along the river valleys. Both ways could be used by representatives of the "*ursinii*-species group" of vipers. Besides the "*ursinii*-group" also thermophilous species like *Lacerta strigata*, *Coluber najadum*, and *Elaphe hohenackeri* are present as isolates in these hollows. One should also pay attention to the occurrence of relicts of the xerothermal epoch in the western Caucasus near the mountains Jatyrgvarta and Magisko (Altukhov, 1966), but the xerophilous vegetation did never have any wide development in that area.

Literature Cited

ALTUKHOV, U. D. 1966. [About the high mountain flora of the limestone of Tryu-Yakirgvart]. Problems of botanik, vol. 8, Leningrad. (In Russian).

CLAYTON, J. W. AND D. N. TRETIAK. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. Journal of the Fisheries Research Board of Canada 29:1169-1172.

DE LORENZO, R. J. AND F. H. RUDDLE. 1969. Genetic control of two electrophoretic variants of glucosephosphate isomerase in the mouse. Biochemical Genetics 3:151-162.

FROST, D. R. AND D. M. HILLIS. 1990. Species in concept and practice: Herpetological applications. Herpetologica 46(1):87-104.

FROST, D. R., A. G. KLUGE AND D. M. HILLIS. 1992. Species in contemporary herpetology: Comments on phylogenetic inference and taxonomy. Herpetological Review 23(2):46-54.

GALUSHKO, A. I. 1974. [On the flora to arid slopes of vicinity of Itumkale (Chechено-Ingushetia)]. Pp. 5-22. *In* Flora and vegetation of the Eastern Caucasus. Ordjonikidze. (In Russian).

GROSSGEIM, A. A. 1948. [Vegetation of the Caucasus]. Publishing House of the Moscow Society of Researcher of Nature. (In Russian).

HARRIS, H. AND D. A. HOPKINSON. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland, Amsterdam.

HERRMANN, H.-W., U. JOGER, G. NILSON AND C. G. SIBLEY. 1987. First steps towards a biochemically based reconstruction of the phylogeny of the genus *Vipera*.. Pp. 195-200. *In* J.J. van Gelder, H. Strijbosch and P.J.M. Bergers (eds.), Proceedings of the Fourth Ordinary General Meeting of the Societas Europaea Herpetologica, Nijmegen 1987.

HERRMANN, H.-W., U. JOGER AND G. NILSON. 1992. Molecular phylogeny and systematics of viperine snakes I. General phylogeny of European vipers (*Vipera* sensu strictu). Pp. 219-224. *In* Z. Korsós and I. Kiss (eds.), Proceedings of the Fifth Ordinary General Meeting of the Societas Europaea Herpetologica, Budapest 1991.

HILLIS, D. M. 1984. Misuse and modification of Nei's genetic distance. Systematic Zoology 33:238-240.

HÖGGREN, M., G. NILSON, C. ANDRÉN, N. L. ORLOV AND B. S. TUNIYEV. 1993: Vipers of the Caucasus: Natural History and Systematic Review. Herpetological Natural History 1(2):11-19.

JOGER, U., H.-W. HERRMANN AND G. NILSON. 1992. Molecular phylogeny and systematics of viperine snakes II. A revision of the *Vipera ursinii* complex. Pp. 239-244. *In* Z. Korsós and I. Kiss (eds.), Proceedings of the Fifth Ordinary General Meeting of the Societas Europaea Herpetologica, Budapest 1991.

JOHNSON, A. G., F. M. UTTER AND H. O. HODGINS. 1970. Interspecific variation of tetrazolium oxidase in *Sebastodes* (rockfish). Comparative Biochemistry and Physiology 37:281-285.

KRASNOV, A. N. 1894. [Caucasian chains of mountains, paralleled to Main Ridge, and its role in formation of forest and steppe flora of the western Caucasus]. Transactions of Naturalist Society of the Kharkov University. (In Russian).

KUZNETSOV, N. I. 1890. [Geobotanical investigation of the northern slope of the Caucasus]. Reports of the Russian Geographical Society. Vol. 26. (In Russian)

MURPHY, R. W., J. W. SITES, D. G. BUTH AND C. H. HAUFLER. 1990. Proteins I: Isoenzyme electrophoresis. Pp. 45-126. *In* D.M. Hillis and C. Moritz (eds.), Molecular Systematics. Sinauer, Sunderland, Massachusetts.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from small number of individuals. Genetics 89:583-590.

NIKOLSKY, A. M. 1913. [Reptiles and Amphibians of the Caucasus]. The Caucasus Museum Press, Tiflis, Georgia. 272 pp. (In Russian).

NIKOLSKY, A. M. 1916. Fauna of Russia and adjacent countries, Reptiles, Vol. 2. (1964: Israel Program for Scientific Translations, Jerusalem, vi+247 pp.).

NILSON, G. 1995. Venomous snakes in west Asia: -Applicability of current species concepts. Proceedings of the Asiatic Herpetological Congress. In Press.

NILSON, G. AND C. ANDRÉN. 1986. The mountain Vipers of the Middle East- The *Vipera xanthina* complex (Reptilia, Viperidae). Bonner Zoologische Monografien 20:1-90.

NILSON, G. AND C. ANDRÉN. 1994. The meadow and steppe vipers of Europe and Asia, the *Vipera ursinii* complex. Submitted.

NILSON, G., M. HÖGGREN, B. S. TUNIYEV, N. L. ORLOV AND C. ANDRÉN. 1994. Phylogeny of the vipers of the Caucasus (Reptilia, Viperidae). Zoologica Scripta. (In press).

ORLOV, N. L. AND B. S. TUNIYEV. 1986. [The recent areas, their possible genesis and the phylogeny of three viper species of Eurosiberian group of the *Vipera kaznakowi* complex in the Caucasus]. Pp. 107-135. *In:* N. Ananjeva and L. Borkin (eds.), Systematics and Ecology of Amphibians and Reptiles. Proceedings of the Zoological Institute, Leningrad, 157. (In Russian).

ORLOV, N. L. AND B. S. TUNIYEV. 1990. Three species in the *Vipera kaznakowi* complex (Eurosibirian group) in the Caucasus: their present distribution, possible genesis, and phylogeny. Asiatic Herpetological Research 3:1-36.

RIDGWAY, G. J., S. W. SHERBURNE AND R. D. LEWIS. 1970. Polymorphism in esterase of Atlantic herring. Transactions of the American Fisheries Society 99:147-151.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. R. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse *(Peromyscus polionotus)*. Studies in Genetics VI. University of Texas Publications 7103:49-90.

SHAW, C. R. AND R. PRASAD. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochemical Genetics 4:297-330.

SNEATH, P. H. A. AND R. R. SOKAL. 1973. Numerical Taxonomy. W.H.Freeman and Co., San Francisco. 573 pp.

TUNIYEV, B. S. 1990. On the independence of the Colchis Center of Amphibian and Reptile speciation. Asiatic Herpetological Research 3:67-84.

VEDMEDERJA, V. L., N. L. ORLOV AND B. S. TUNIYEV. 1986. [On the taxonomy of the three viper species of the *Vipera kaznakowi* complex]. Pp. 55-61 *In:* N. Ananjeva and L. Borkin (eds.), Systematics and Ecology of Amphibians and Reptiles. Proceedings of the Zoological Institute, Leningrad, 157. (In Russian).

Calotes versicolor nigrigularis Auffenberg and Rehman 1993 a Junior Primary Homonym

[1]WALTER AUFFENBERG AND [2]HAFIZUR REHMAN

[1]7008 NW 67th Ave, Gainesville, FL. 32653 USA
[2]Zoological Survey Dept., Karachi 1, Pakistan

Key Words: Reptilia, Sauria, Lacertilia, Agamidae, *Calotes*.

We recently described a new subspecies of *Calotes versicolor,* which we named *C. v. nigrigularis* (Auffenberg and Rehman, 1993, Studies on Pakistan Reptiles. Pt. 3. *Calotes versicolor*. Asiatic Herpetological Research 5:14-30). Immediately after publication, Dr. Hidetoshi Ota brought to our attention that the same name has recently been used for a new species of *Calotes* from Borneo (Ota and Hikida, 1991, Taxonomic review of the lizards of the genus *Calotes* Cuvier 1817 (Agamidae: Squamata) from Sabah, Malaysia. Tropical Ecology 4:179-192). Thus *Calotes versicolor nigrigularis* Auffenberg and Rehman is a junior primary homonym of *Calotes nigrigularis* Ota and Hikida 1991.

The name we propose for *Calotes versicolor nigrigularis* Auffenberg and Rehman is

CALOTES VERSICOLOR FAROOQI

It is named in honor of Farooq Ahmed, Director, Zoological Survey Department, Pakistan. Our extensive herpetological exploration in Pakistan would have been impossible without his generous logistic support.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 28-29

Simplified Field Technique for Obtaining Blood from Freshwater Turtles

RESHMA BATRA AND SANT PRAKASH

Department of Zoology, D.E.I., Dayalbagh Agra, 282 005, India

Brief Communication

Studies on the biochemical and molecular aspects have now been recognized as essential components of the conservation program of species. The choice of the tissue in such studies have invariably been the blood and this has necessitated researchers to look for the best procedure for field sampling without harming or sacrificing the animal.

Several methods have been proposed to obtain uncontaminated blood, each having its merit restricted to the species under study or to the specific experiment. The most common method of collection of samples of blood in reptiles has been by cardiac puncture (Gandal, 1958; Stephens and Creekmore, 1983) but has been less popular in turtles because of their thick plastron. Cutting off the end of the tail (Duguy, 1970), toenail clipping (Frye, 1991) or collecting blood from the major veins and arteries (Maxwell, 1979) have been some of the other proposals. Each of them has at least one disadvantage; for example, intricate dissection of veins/arteries is required (Avery and Vitt, 1984). The procedure for obtaining blood samples from the ventral caudal vein, as suggested by Galbraith (pers. comm.) and described in alligator snapping turtles (Powell and Knesel, 1992), has been initially utilized in our procedure but we had to discard it as the amount of blood obtained was not enough for multiple analyses. Falling back on the oldest method of heart puncture by inserting a long needle laterally through the soft tissue between the plastron and the carapace, we found that the front leg provides the safest and the shortest way to reach the heart, thus avoiding the drilling of the plastron. In addition, our technique does not require elaborate equipment and can be used easily in the field.

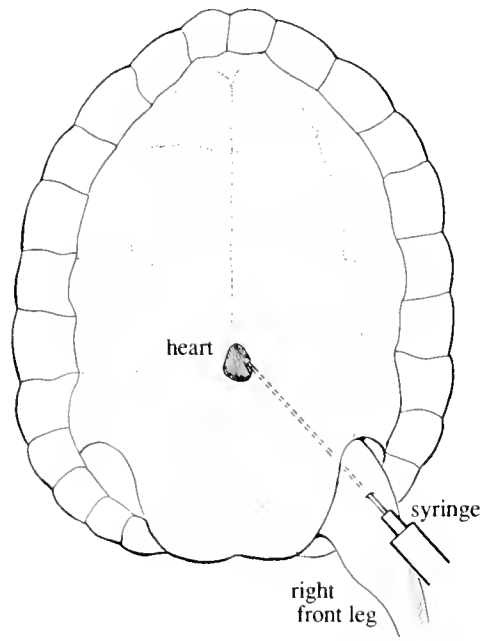


FIG. 1. Method for obtaining blood. h: heart, rfl: fight fore-leg, s: syringe.

We have applied this technique in the turtles of the genus *Kachuga, K. tentoria* and *K. dhongoka*. These are primarily medium sized turtles with males ranging between 4-8 inches and females between 9-18 inches in length. Presumably this technique can be applied to many other turtles of similar size.

Handling of the turtles, to keep them docile, is the skill of the field worker and no standard procedure can be described for it. However, the turtle has to be suspended in a manner that the head hangs freely downward and the foreleg remains unrestrained. The weight of the body forces the foreleg to stretch, but this may need some time. In this position, the left foreleg can be stretched at an angle of 35° from the head. The skin joining the leg with the

carapace is dabbed with 95% alcohol in order to sterilize the area (Fig. 1).

A 2-inch long 32-gauge hypodermic needle attached to a 5 ml syringe is inserted (as shown in the figure) parallel to the stretched foreleg. The needle is gently inserted until it reaches the ventricle. The depth of the needle penetration is often between 1-1.5 inches. Gentle suction is applied until the blood spurts into the syringe and withdrawal pressure is then slowly increased, until the syringe is at its full suction capacity. The needle is then slowly pulled out, with full syringe suction still being applied. About 2-3 ml of blood is drawn per sample. No pressure is applied for the control of bleeding as no visible bleeding occurs in this procedure. However, germicidal powder is immediately sprinkled at the point of the insertion of the needle, before marking and releasing the turtles.

Blood samples have successfully been collected from over 50 freshwater turtles and several of them have been utilized for repetitive blood lettings and maintained in captivity for over 4 months with no apparent ill-effects. For field sampling, this procedure provides a safe, practical and simple technique for obtaining blood in turtles.

Acknowledgments

We thank Mr. Dayal Prashad Gupta and Mr. Akash Mathur for their help in turtle collection.

Literature Cited

AVERY, H. W. AND L. J. VITT. 1984. How to get blood from a turtle. Copeia 1984:209-210.

DUGUY, R. 1970. Numbers of blood cells and their variation. Biology of reptiles. Pp. 93-109. *In* Gans and Parson (Eds). Academic Press, New York.

FRYE, F. L. 1991. Biomedical and surgical aspects of captive reptiles husbandry, Vol. 1. Kreiger Publishing Co., Inc., Florida.

GANDAL, C. P. 1958. Cardiac puncture in anaestized turtles. Zoologica 43:93-94.

MAXWELL, J. H. 1979. Anaethesia and surgery. Pp. 127-152. *In* Harless and Morlock (Eds) Turtles: perspective and research. John Wiley and Sons, New York.

POWELL, S. C. AND J. A. KNESEL. 1992. Blood collection from *Macroclemys temmincki* (Troost). Herpetological Review 23(1):19.

STEPHENS, G. A. AND J. S. CREEKMORE. 1983. Blood collection by cardiac puncture in conscious turtles. Copeia 1983:522-523.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 30-35

The Systematic Relationships of *Dravidogecko anamallensis* (Günther 1875)

AARON M. BAUER[1] AND ANTHONY P. RUSSELL[2]

[1]*Biology Department, Villanova University, Villanova, Pennsylvania 19085, USA*

[2]*Vertebrate Morphology Research Group, Department of Biological Sciences, University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4*

Abstract. -The relationships of the monotypic gekkonine genus *Dravidogecko* are assessed by comparative evaluation of its external and internal morphology. A suite of shared-derived features is possessed by *Hemidactylus* and a variety of satellite genera, including *Dravidogecko*. These similarities are advocated as being so compelling, and the ostensible defining features of *Dravidogecko* to be so weak that the latter is subsumed as a junior synonym of *Hemidactylus*. The biogeographic consequences of this taxonomic shift are considered.

Key words: Dravidogecko, Hemidactylus, Teratolepis, digits, scansors, phalanges, paraphalangeal elements, muscles, biogeography, India.

Introduction

Dravidogecko is a monotypic genus of gekkonid lizards endemic to south India. The single species, *D. anamallensis,* was originally described as a member of the genus *Hoplodactylus* (Günther, 1875; Strauch, 1887), but following the work of Smith (1933), it was assigned to a new genus, primarily on the basis of differences in the distal scansors and in preanal pore arrangement. Subsequently it has been demonstrated that *Dravidogecko* is a gekkonine gecko, whereas *Hoplodactylus sensu stricto* is a diplodactyline (Underwood, 1954; Kluge, 1967). The relationships of *Dravidogecko* have remained obscure, and the systematic status of the species has never been investigated adequately. It is known from only a few specimens from the Anaimalais, Palnis and Tirunelveli Hills (Satyamurti, 1962; Murthy, 1985) but is reportedly widely distributed throughout forested areas of southern peninsular India (Daniel, 1983).

Russell (1972) considered *Dravidogecko* to belong, on morpho-functional grounds, in the *Hemidactylus* group, along with *Hemidactylus, Briba, Teratolepis* and *Cosymbotus*. Kluge (1983) placed it, along with the other gekkonine genera previously mentioned, in the tribe Gekkonini on the basis of the absence of the second ceratobranchial arch. Russell (1976: 238; Fig. 14) suggested that *Dravidogecko* had a digital structure that was most closely approached by that of *Hemidactylus* and its close allies. While external form of the digits is particularly sensitive to functional demands and thus prone to exhibiting convergence and parallelism (Russell, 1979), details of the internal anatomy are much more helpful at indicating true homology and, therefore, affinity (Russell, 1976, 1979; Russell and Bauer, 1990). We herein present the results of a comparative survey of both the external and internal anatomy of the feet and digits in *Hemidactylus* (and its close relatives) and use these to demonstrate both the wide range of variation present and the shared derived features that circumscribe this cluster and help clarify the relationships of the enigmatic *Dravidogecko*. We further relegate the generic name *Dravidogecko* into the synonymy of *Hemidactylus* as there are no derived features of *Dravidogecko* that are not also shared by at least some *Hemidactylus*. It is probable that *H. anamallensis* is a primitive hemidactyl.

Materials and Methods

Specimens of *Dravidogecko* were examined or borrowed from the collections of The Natural History Museum, London (BMNH) and the Institute Royal des Sciences Naturelles de Belgique, Brussels

(IRSNB). Comparative material of other gekkonines, especially *Hemidactylus*, were borrowed from the BMNH and the California Academy of Sciences, San Francisco (CAS). Observations on toe structure were made using a Nikon SMZ-10 microscope. The specimens examined are listed below. All numbers refer to BMNH specimens unless otherwise identified.

Dravidogecko anamallensis 82.5.22.79-84; IRSNB 1194.
Briba brasiliana 1971.1045.
Cosymbotus craspedotus 1926.12.7.7, 1930.10.9.2
C. platyurus xxi.36a, 97.6.21.4, 97.12.28.10, CAS 18565, CAS 18567
Hemidactylus albopunctatus 1946.8.22.75; *H. ansorgii* 1901.1.28.22; 1966.337; *H. barodanus* 1905.11.7.1-6; 1937.12.5.215-216; 1958.1.6.29; 1970.1437-38; *H. bouvieri* 66.4.12.3; 75.4.26.10; *H. bowringii* 1929.12.1.7-10; 1940.4.26.2-3; 1956.1.11.15-16; *H. brookii* 1918.11.12.2-10; 1930.10.6.6; 1931.12.10.6-7; 1970.2196-98; 1971.242; *H. citernii* 1931.7.20.114-119 and 128-130; 1937.12.5.202-204; *H. curlei* 1946.8.25.41; *H. depressus* 52.2.19.21; 61.2.21.5; 1948.1.7.35; *H. echinus* 89.7.6.1; 1903.7.28.1-2; *H. fasciatus* 1919.8.16.48; 1956.1.11.37-40; 1971.253; *H. flaviviridis* 1931.7.20.153-155; 1971.1378-1382; *H. forbesii* 1946.8.25.43-47; *H. frenatus* 1938.10.2.1; 1952.1.4.30-31; 1970.1879-1895; *H. garnotii* 95.11.7.1; 1903.2.21.1-2; 1940.6.3.24-29; *H. giganteus* 1908.12.28.27; 1969.828-829; *H. gracilis* 74.4.29.1388; 80.11.10.47; *H. granti* 1957.1.9.52-66; *H. greeffii* 93.12.7.1; 98.3.30.21-22; *H. homeolepis* 99.12.5.38; 1953.1.7.84-85; 1967.485-489; *H. isolepis* 1952.1.7.79-80; *H. jubensis* 1946.8.23.66; *H. karenorum* 68.4.3.88-89; 91.11.26.13-14; *H. laevis* 1946.8.25.42; *H. leschenaulti* 70.5.18.70-71; 74.4.29.233-236 (six specimens); *H. longicephalus* 1936.8.1.287-305; *H. mabouia* 1923.11.9.46-50; 1964.1429-35; 1970.2209-15; *H. macropholis* 1931.7.20.109; 1937.12.5.250-258; *H. maculatus* 69.8.25.15; 1956.1.11.44; *H. megalops* 1946.8.25.67; *H. mercatorius* 1930.7.1.84-90; 1938.8.3.11-15; *H. muriceus* 1926.9.24.13; 1966.283; *H. modestus* 1946.8.25.37; *H. ophiolepis* 1937.12.5.324-325; *H. oxyrhinus* 99.12.5.170-175; 1967.491-494; *H. persicus* 1970.250; 1972.716; *H. prashadi* 1946.8.14.66-69; *H. pumilio* 1946.8.20.1; 1946.8.25.58-61; *H. reticulatus* 1901.3.8.1-3; *H. richardsoni* 1916.5.29.1; 1919.8.16.49; *H. ruspolii* 1937.12.5.228-229; 1937.12.5.239-246; *H. sinaitus* 97.10.28.83-86; 1937.2.5.293; 1953.1.6.97-98; *H. smithi* 1931.7.20.85-89; 1972.745; *H. somalicus* 1946.8.25.77-78; *H. squamulatus* 98.1.8.2-3; 1902.5.26.2; 1923.10.9.2; 1923.10.9.14-15; *H. subtriedrus* 74.11.11.1; *H. taylori* 1946.8.23.48; *H. triedrus* xxi.19a-b; *H. tropidolepis* 1937.12.5.322-323; *H. turcicus* 1934.11.8.10- 14; 1971.1143-45; *H. yerburii* 99.12.13.43-44; 1903.6.26.3-4; 1945.12.18.12.
Teratolepis fasciata 69.8.28.32; 1933.7.8.37; 1963.1019; 1964.930-931; *T. albofasciatus* 1963.613-621

Results

A considerable range of variation in digital form and subdigital scansor design exists among members of the genus *Hemidactylus* (Fig. 1). This variation is evident in such aspects as the number of divided scansors (lamellae), the extent of their division, the extent of the undivided lamellar series at the base of the digits, and the length, form and degree of separation of the free, distal, claw-bearing segment of the digits. Figure 2 illustrates the general form of the ventral aspect of the right pes of *Dravidogecko* and provides comparison with the ventral aspects of the fourth pedal digit of *Hemidactylus reticulatus* and *Teratolepis fasciata.* While some species of *Hemidactylus*, such as *H. garnotii* and *H. smithii* (Fig. 1), have digits with a large number of completely divided scansors, and an elongate, free distal, claw-bearing portion, this is not so for other species, such as *Teratolepis albofasciatus* (see Grandison and Soman, 1963), *Hemidactylus somalicus* and *H. bouvieri* (Fig. 1). In the latter three cases the number of scansors is small, only the distal most ones are notched, and the distal, free, claw-bearing portion of the digit

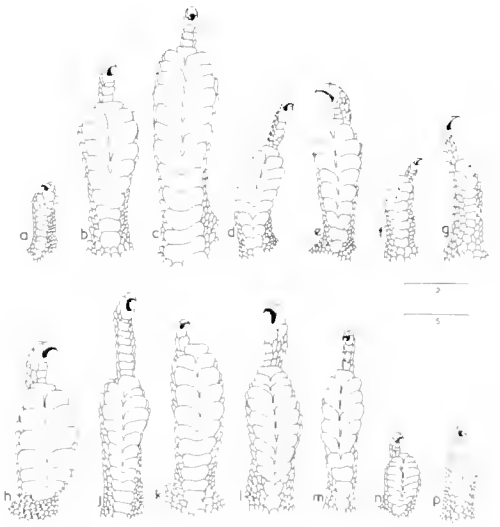


FIG. 1. The array of digital form in the genera *Hemidactylus* and *Teratolepis*. All illustrations are of the fourth digit of the pes; a-e, j are of the right pes, f-h, k-p are of the left pes. The 2 mm scale bar refers to all specimens except n, to which the 5 mm scale bar applies. All catalogue numbers refer to the Natural History Museum, London (BMNH). a. *Teratolepis albofasciatus* 1963.617; b. *Hemidactylus bowringii* 1929.12.1.6; c. *H. garnotii* 95.11.7.1; d. *H. barodanus* 1970.1438; e. *H. turcicus* 1971.1144; f. *H. somalicus* 1946.8.25.77; g. *H. ophiolepis* 1937.12.5.324; h. *H. mabouia* 1964.1431; j. *H. forbesii* 1946.8.25.47; k. *H. smithii* 1931.7.20.85; l. *H. fasciatus* 1919.8.16.48; m. *H. ansorgii* 1901.1.28.22; n. *H. richardsonii* 1916.5.29.1; p. *H. bouvieri* 66.4.12.3.

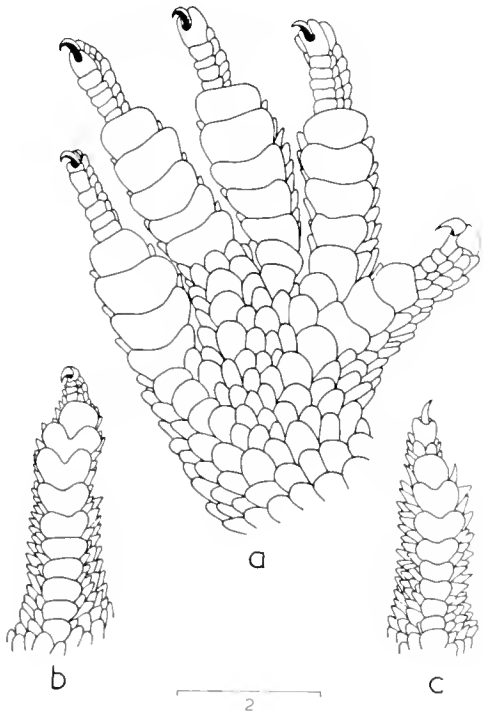


FIG. 2. a. Ventral aspect of the right pes of *Dravidogecko anamallensis*, BMNH 82.5.22.79. b. Ventral aspect of the fourth digit, right pes of *Hemidactylus reticulatus*, BMNH 1901.3.8.1. c. Ventral aspect of the fourth digit, left pes of *Teratolepis fasciata*, BMNH 1933.7.8.37. Scale bar in millimeters.

is relatively short. This situation is also seen in *Hemidactylus reticulatus* and *Teratolepis fasciata* (Fig. 2, b, c). The almost continuous range of variation in external digital characters, especially among the west Asian and Somali species of the *Hemidactylus* group of geckos has long been recognized, and has resulted in the establishment of several different, largely arbitrary, generic arrangements (see Parker, 1942 for a discussion). Thus, while division of the scansors is generally characteristic of the genus *Hemidactylus*, there are many species that express this trait only marginally.

Russell (1976: Fig. 14) indicated this potential continuity in scansor form, from undivided to completely divided, by comparing *Dravidogecko* with *Cyrtodactylus brevipalmatus*, *Hemidactylus reticulatus* and *H. barodanus*. While this was simply a depiction of change of form assembled as a morphotypic series, it was also implied that there may be deeper underlying anatomical clues that are indicative of the closeness of relationship of *Dravidogecko* to *Hemidactylus*. The superficial comparisons of the digits(Figs. 1, 2; see above) provide some idea of the potential range of variation, but should be treated with caution when being implicated in arguments about relationship because of the extreme plasticity of external digital form. [Such aspects are well exemplified by the taxonomic history of the taxon that is the subject of this contribution.] Detailed examination of the internal anatomy of the digits provides more convincing evidence about the affinities of *Dravidogecko*.

Russell (1976) presented a mechanistic diagram of the main features of digital design in *Hemidactylus*. The chief aspects of note here are the unusual form and relationships of the antepenultimate phalanx of digits III-V of the pes (Russell, 1977), the distal extent of the dorsal interossei muscles along the digit, and the means of tendinous insertion of these muscles onto the scansors. The pattern of digital characteristics of *Hemidactylus* is essentially repeated in *Dravidogecko* and is restricted to only a few other genera *(Briba, Cosymbotus* and *Teratolepis*). This suite of shared-derived digital features of these taxa (the short, erect nature of the antepenultimate phalanx of pedal digits III-V, the distal extension of the dorsal interossei muscles as far as the distal end of the antepenultimate phalanx, and the tendinous insertion of the dorsal interossei muscles onto the distal margin of each scansor in turn) unites them as a distinctive evolutionary unit. Apart from *Hemidactylus*, all of the other genera in this cluster are either monotypic *(Briba* and *Dravidogecko*) or include only two species *(Cosymbotus* and *Teratolepis).*

Dissection of the digits of *Dravidogecko* reveals that the dorsal interossei muscles are well-developed and robust and extend as fleshy bellies as far distally as the digital inflection (the point of emplacement of the reduced, erect antepenultimate phalanx onmanual digits III and IV and pedal digits III-V). The dorsal interossei muscles send individual tendons to the distal borders of the scansors as they do in *Hemidactylus* (see Russell, 1976) and *Cosymbotus*. This situation also pertains in *Teratolepis* and *Briba* (Russell, 1972). *Dravidogecko* also shares with *Hemidactylus, Briba* and *Cosymbotus* the particular morphology and placement of paraphalangeal elements (Russell and Bauer, 1988).

The above comparisons indicate that *Dravidogecko* shares with other members of the *Hemidactylus* radiation (*Hemidactylus, Briba, Cosymbotus, Teratolepis*) all of the derived digital features that distinguish these taxa from all other geckos. However, apomorphic features characteristic of many *Hemidactylus* species, such as those associated with the complete division of the scansors, are lacking in *Dravidogecko.* It is therefore likely that *D. anamallensis* is a relatively plesiomorphic member of this radiation. As such, it is probable that the recognition of *Dravidogecko* renders *Hemidactylus* as presently construed paraphyletic. In order to maintain monophyletic generic units we hereby place *Dravidogecko* into the synonymy of *Hemidactylus* Gray, 1825. The correct designation for the single known species formerly referred to this genus thus becomes *Hemidactylus anamallensis* (Günther 1875), new combination.

Discussion

Many lizard families include monotypic genera. Although in some cases these represent independently evolving lineages, in most they are relatively primitive or highly derived members of other lineages, and their recognition renders the latter groups paraphyletic. *Hemidactylus* is the most specious genus in the Gekkonidae, with 75 species currently recognized (Kluge, 1991). Relationships within the genus are very poorly understood (Parker, 1942; Loveridge, 1947; Kluge, 1969; Bastinck, 1981) and a general uniformity among most forms (Russell, 1976) has rendered casual attempts at investigating its phylogeny unsuccessful. The placement of *Dravidogecko anamallensis* into this morass, of course, does nothing to aid this confusion. It does, however, ensure that *Hemidactylus anamallensis* is taken into account if and when a generic revision of all *Hemidactylus* is accomplished.

It is not only in the interest of maintaining monophyletic groups that the revaluation of monotypic genera is undertaken. Current nomenclatural usage has implications for non-systematists. As an endemic Indian subcontinent form, *Dravidogecko* might be used to support arguments about the uniqueness and antiquity of the Indian biota. The use by biogeographers of classification schemes that do not adequately reflect phylogenetic patterns has been shown to lead to the erection of demonstrably false hypotheses

(Bauer, 1989). Clearly, biogeographic interpretations must be based upon the phylogenetic relationships of the organisms considered. Some other *Hemidactylus* group geckos sharing with *H. anamallensis* at least partially undivided scansors are also Indian forms (e.g., *Teratolepis albofasciatus* from the Ratnagiri District, Maharashtra, *Hemidactylus gracilis* from the Madhya Pradesh, Maharashtra and Andhra Pradesh (Smith, 1935;Murthy, 1985), and *H. reticulatus* from Tamil Nadu, Andhra Pradesh and Karnataka (Smith, 1935; Murthy, 1985)). *Teratolepi fasciata* is also from the Indian subcontinent (Anderson, 1964; Minton, 1966) and it appears likely that the hemidactyls, as a group, have undergone a long period of evolution and diversification within the region.

Although the geographic ranges of some forms of *Hemidactylus* are indicative of relatively recent expansions (Kluge, 1967, 1969), most Indian species are moderately to highly circumscribed in their distribution and hold the promise of contributing substantially to biogeographic hypotheses of area relationships within peninsular India. However, both biogeographic analyses and meaningful studies of the evolution of the pedal characteristics that have made *Hemidactylus sensu lato* so successful in India (and elsewhere) must await the ultimate resolution of phylogenetic relationships within the genus. In subsuming *Dravidogecko* within *Hemidactylus* we concur with the sentiments expressed by Loveridge (1947: 97) in discussing the African members of this radiation, "Any arrangement that would break up so unwieldy a genus as *Hemidactylus* is worthy of careful attention . . ." Such an arrangement must be phylogenetically based, and at present insufficient data are at hand to attempt this. However, we regard the identification of all members belonging to the *Hemidactylus* clade as a necessary first step in the process.

Acknowledgments

We thank E. N. Arnold, Mathias Lang and Jens Vindum for access to specimens. E. Prickley and S. F. Milone provided general assistance in the laboratory.

Literature Cited

ANDERSON, J. A. 1964. A report on the gecko *Teratolepis fasciata* (Blyth 1853). Journal of the Bombay Natural History Society 61(1):161-171.

BASTINCK, J. 1981. Phyletische analyse van dertien kenmerken verspreid over degeslachten der Gekkoninae. Unpublished thesis, University of Amsterdam, The Netherlands.

BAUER, A. M. 1989. Reptiles and the biogeographic interpretation of New Caledonia. Tuatara 30:39-50.

DANIEL, J. C. 1983. The Book of Indian Reptiles. Bombay: Bombay Natural History Society. x + 141 pp.

GRANDISON, A. G. C. AND P. W. SOMAN. 1963. Description of a new geckonid lizard from Maharashtra, India. Journal of the Bombay Natural History Society 60(2):322-325.

GÜNTHER, A. 1875. Second report on collections of Indian reptiles obtained by the British Museum. Proceedings of the Zoological Society of London 1875:224-234 + 5 pls.

KLUGE, A. G. 1967. Higher taxonomic categories of gekkonid lizards and their evolution. Bulletin of the American Museum of Natural History 135:1-59.

KLUGE, A. G. 1969. The evolution and geographical origin of the New World *Hemidactylus mabouia-brooki* complex (Gekkonidae: Sauria). Miscellaneous Publications of the Museum of Zoology, University of Michigan 138:1-78.

KLUGE, A. G. 1983. Cladistic relationships among gekkonid lizards. Copeia 1983:465-475.

KLUGE, A. G. 1991. Checklist of gekkonid lizards. Smithsonian Herpetological Information Service 85:1-35.

LOVERIDGE, A. 1947. Revision of the Africn lizards of the family Gekkonidae. Bulletin of the Museum of Comparative Zoology, Harvard University 98(1):1-469.

MINTON, S. A. 1966. A contribution to the herpetology of West Pakistan. Bulletin of the American Museum of Natural History 134:27-184.

MURTHY, T .S. N. 1985. A field guide to the lizards of Western Ghats. Records of the Zoological Survey of India Miscellaneous Publications, Occasional Paper 72:1-51.

PARKER, H. W. 1942. The lizards of British Somaliland, with an appendix on topography and climate by Capt. R. H. R. Taylor, O. B. E. Bulletin of the Museum of Comparative Zoology, Harvard University 91:1-101.

RUSSELL, A .P. 1972. The foot of gekkonid lizards: a study in comparative and functional anatomy. Unpubl. Ph. D. thesis, University of London, England.

RUSSELL, A. P. 1976. Some comments concerning interrelationships amongst gekkonine geckos. Pp. 217-244. *In* A. d'A. Bellairs and C. B. Cox (Eds.), Morphology and Biology of Reptiles. London, Academic Press.

RUSSELL, A. P. 1977. The phalangeal formula of *Hemidactylus* Oken, 1817 (Reptilia:Gekkonidae): a correction and a functional explanation. Zentralblatt für Veterinar Medizin, Reihe C. Anatomia, Histologia Embryologia 6:332-338.

RUSSELL, A. P. 1979. Parallelism and integrated design in the foot structure of gekkonine and diplodactyline geckos. Copeia 1979(1):1-21.

RUSSELL, A. P. AND A. M. BAUER. 1988. Paraphalangeal elements of gekkonid lizards: a comparative survey. Journal of Morphology 197:221-240.

RUSSELL, A. P. AND A. M. BAUER. 1990. Digit I in pad-bearing gekkonine geckos: alternate designs and the potential constraints of phalangeal number. Memoirs of the Queensland Museum 29(2):453-472.

SATYAMURTI, S. T. 1962. Guide to the lizards, crocodiles, turtles and tortoises exhibited in the reptile gallery, Madras Government Museum. pp. 1-45. Government of Madras.

SMITH, M. A. 1933. Remarks on some Old World geckos. Records of the Indian Museum 35:9-19.

SMITH, M.A. 1935. The Fauna of British India, including Ceylon and Burma. Reptilia andAmphibia, vol. II, Sauria. London, Taylor & Francis.

STRAUCH, A. 1887. Bemerkungen über die Geckoniden-Sammlung im zoologischen Museum der Kaiserlichen Akademie der Wissenschaften zu St. Petersburg. Mémoires de l'Academie Imperial des Sciences, St-Pétersbourg. (7)35(2):1-38.

UNDERWOOD, G. 1954. On the classification and evolution of geckos. Proceedings of the Zoological Society of London 124:469-492.

The Ceylonese Tree Frog *Polypedates cruciger* Blyth, a New Record for India

R. J. RANJIT DANIELS[1] AND M. S. RAVICHANDRAN[2]

[1]*M. S. Swaminathan Research Foundation, 3rd Cross Gtreet, Taramani Institutional Area, Madras, India 600113*
[2]*Zoological Survey of India, 100 Santhome High Road, Madras, India 600028*

Abstract. -The Ceylonese tree frog *Polypedates cruciger* was considered endemic to the island of Sri Lanka. However, recent surveys in the Western Ghats, India has revealed that the species is widespread outside its originally reported range. *P. cruciger* differs from both the Indian species *P. maculatus* and *P. leucomystax* in morphology and ecology. *P. cruciger* is an addition to the amphibian fauna of India.

Key words: Amphibia, Anura, *Polypedates cruciger*, India, Western Ghats, distribution.

FIG. 1. *Polypedates cruciger* from the Western Ghats of India.

Introduction

During a recent survey of amphibians in the Western Ghats and southwestern India, three specimens of tree frogs were obtained in June 1990 from a private estate in the hills of Kanyakumari district (c. 8°15' N; 77°25' E). Later in the year, two more specimens of the same species were observed in parts of Karnataka State around 14° N; 75° E. The specimens have been deposited in the ZSI (Madras) and BNHS (Bombay) museums.

Discussion

Based on the extent of webbing between the toes and fingers, the specimens were assigned to the genus *Polypedates* (Liem, 1970; Daniel and Sekar, 1989). In India, only two specimens of *Polypedates* have been hitherto reported. These are *P. leucomystax* (Gravenhorst) and *P. maculatus* (Gray) (Inger and Dutta, 1986). The examples from the Western Ghats differed from both *leucomystax* and *maculatus* in the skin of the head adhering to the nasal and frontoparietal bones, the tympanum being two-thirds the diameter of the orbit, the three-fourth webbing on toes and in the hour-glass shaped marking on the dorsum. These characters agree well with *Rhacophorus (=Polypedates) cruciger* of Boulenger (1890) described from Sri Lanka. Further, the hour-glass marking on the dorsal side commencing from the middle level of eyes to mid-body terminating in the form of a trident is unmistakably similar to that in *Rhacophorus cruciger* illustrated by Kirtisinghe (1957). Considering the above characteristics, we confirm the identity of

our specimens as *Polypedates cruciger(*Fig. 1).

These specimens mark the first record of this species in India outside its range (Sri Lanka). The first three animals were located within a clove plantation at an elevation of 400 m above MSL. These hills receive an average annual rainfall of about 2000 mm. Several males were observed calling on a rainy night in June, perched on low branches (c. 1 m above ground). The calls consisted of a harsh *tre...chuck* repeated by rival males followed by a series of *chucks* of low intensity. In captivity, the males continued to call during overcast and rainy days. They fed well on a variety of live insects including grasshoppers and cockroaches.

The specimens observed in southwestern Karnataka were in evergreen forests in regions of 3000-6000 mm rainfall. One individual was observed on October 1990 crossing a forest road and moving towards a stream at midday. A second individual was observed on November 1990 in a patch of dense forest, resting on a tree trunk at c. 2 m above ground in the company of a few smaller *Philautus* spp. Specimens of *Polypedates cruciger* observed in Karnataka were found at lower elevations between 50 and 250 m above MSL.

The habitat preference, altitudinal and geographical range, of this species in southwest India suggest that this species may be widespread in the Western Ghats and has been overlooked by surveys until recently. This species seems to be most active during the rains and much of the Western Ghats are inaccessible at this time. This partly explains why the species has not been reported from nearly 500 km of intervening hills in its known range in India.

Acknowledgments

The authors wish to thank the Director, Zoological Survey of India, Calcutta, Dr. K. V. Lakshminarayana, Officer-in-charge, Southern Regional Station, ZSI, Madras and the Centre for Ecological Sciences, Indian Institute of Science, Bangalore for the facilities provided. Thanks are due to Dr. R. S. Pillai, Scientist Emeritus (ZSI, Madras), Mr. Romulus Whitaker, and Mr. Harry Andrews of the Madras Crocodile Bank for critically reviewing the manuscript.

Literature Cited

BOULENGER, G. A. 1890. Fauna of British India: Reptilia and Batrachia, Taylor and Francis. London, 541 pp.

DANIEL, J. C. AND A G. SEKAR. 1989. Field Guide to the Amphibians of Western India: Part Four. Journal of the Bombay Natural History Society 86:194-202.

INGER, R. F. AND S. K. DUTTA. 1986. An overview of the amphibian fauna of India. Journal of the Bombay Natural History Society 83 (supplement):135-146.

KIRTISINGHE, P. 1957. The Amphibia of Ceylon. Colombo. 112 pp.

LIEM, S. S. 1970. The morphology, systematics and evolution of the old world tree frogs (Rhacophoridae and Hyperoliidae). Fieldiana Zoology 57:1-145.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 38-44

Size-gradation in Syntopic Frogs in South India

INDRANEIL DAS

Animal Ecology Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, England
Present address: Centre for Herpetology, Madras Crocodile Bank, Post Bag No. 4, Mamallapuram-603 104, Tamilnadu, S. India

Abstract. -An assemblage of eight metamorphosed anuran amphibians were studied at a seasonal locality in South India to examine patterns of body size-gradation. In general, body shape was found to be closely correlated to ecological characteristics of the species. The mean ratio of linear dimensions of the body in adjacent species, when arranged in a size-series was 1.3 (range 0.83-1.88), as predicted by Hutchinson (1959) for closely-related sympatric species. It was concluded that competition may be at work in producing these ratios.

Key words: Amphibia, Anura, India, body size, competition.

Introduction

The body forms and sizes of organisms have been considered, since the time of Darwin (1859), to be determined by the powerful forces of natural selection, and may mirror a wide range of ecological interactions often too complex to comprehend in their entirety. The concept of a form-function relation appears to have stood the test of time, and is central to the problem of organismic evolution (Gans, 1988). Animal size, for example, may be an evolutionary response to demands of the immediate environment, related to key life history traits, such as fecundity, foraging, locomotion, and reduction of predation, desiccation, heating and cooling. Some workers (e.g. Hutchinson, 1959; Schoener, 1986a) have argued that smaller species are generally more specialized, as reflected in their restricted diets and geographical ranges. Gould (1966), however, considered both large and small species to be viable strategists, optimal size, according to Phillipson (1981) being dependent on competitive abilities and survival probabilities of the various size and age classes. However, large species tend to appear later in a group's evolutionary history, an exception being the Amphibia (Peters, 1983).

Within ecological communities, if environmental resources are partitioned according to dimension, as documented by Schoener (1965) and subsequent workers (reviewed by Schoener, 1974; 1986b), differences in the comparative sizes of the organisms are to be expected. In fact, such apparent differences were reported much earlier by Hutchinson (1959), who found constant differences in the ratios of linear dimensions of the trophic (feeding) apparatus among closely-related sympatric species in his now controversial "Homage to Santa Rosalia, or why are there so many kinds of animals?" paper. Hutchinson argued that similar species could coexist if the ratios of linear dimensions and weights of presumed competing species are around 1.3 and 2.0 respectively. While possible causal factors remain unclear, such ratios have been found in a wide range of both invertebrate and vertebrate communities, including beetles, spiders, frogs, lizards and birds (reviewed by Schoener, 1986a).

In this paper, I report patterns of body size and shape, and of size-gradation observed in metamorphosed anurans from a locality in South India. Specifically, I searched for patterns of size gradation within the eight syntopic frog species.

Materials and Methods

Eight species of anuran amphibians occur in sympatry in the coastal scrublands of Chengai Anna District, Tamil Nadu State, South India. These, along with their mean snout-vent lengths have been listed below:

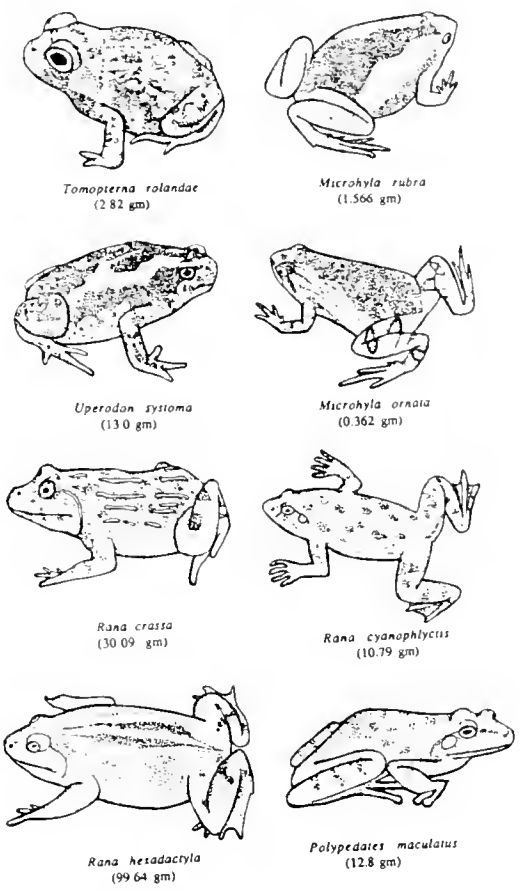


FIG. 1. Shapes and masses of the species studied. Figures in brackets are mean weights.

Microhyla ornata (4.1 mm), *M. rubra* (7.2 mm), *Tomopterna rolandae* (10.9 mm), *Uperodon systoma* (12.4 mm), *Polypedates maculatus* (20.9 mm), *Rana cyanophlyctis* (16.1 mm), *R. crassa* (22.9 mm) and *R. hexadactyla* (33.4 mm).

Abbreviations used include SVL (snout-vent length), HW (head width at the angle of the jaws, perhaps better defined as the gape), TBL (tibia length, the distance from the convex surface of knee to the convex surface of heel, with both tibia and tarsus flexed) and WT (wet body weight). Linear measurements were taken to the nearest 0.1 mm with a Mitutoyo Dial Vernier Caliper, weights were taken to the nearest 0.1 gm with an Acculab Electronic Balance (Model 333).

To interpret shape changes, logarithmic transformations of the dimensions of the organ of each species were used in the function $\log y = b \log x + \log a$ (where x and y are the morphological variates), which has been considered to approximate shape change in most organisms (see Gould, 1966, for justification).

Observations

The assemblage of eight anuran species studied display a range of body forms (Figure 1) and sizes (Table 1). Except for the diminutive species of the genus *Microhyla* (maximum SVL 17.4 and 26.5 mm) and the large ranid, *Rana hexadactyla* (maximum SVL 132.2 mm), all species were small to medium, with maximum SVL between 43.2 to 93.1 mm (mean 68.2).

A general impression is that some species are comparatively short and squat, while others are long and thin. To quantify differences, WT was divided by the cube of the SVL for individuals of each species. Three morphological groups along a continuum were recognized based on the patterns of body form (length-weight data in Table 2), which comprise species with similar ecological preferences, including heavy-bodied, terrestrial forms; light bodied aquatic forms; and an arboreal form of intermediate body mass.

The relationships of untransformed values of WT/SVL^3 (Figure 2.1), as well as the arcsine-transformed data (Figure 2.2) can be described as linear, the slope b being 0.38 and 1.89 respectively, not differing significantly (t-test, $P>0.05$) from isometry, indicating that large frogs are not likely to be comparatively lighter.

The ratios of differences in linear dimensions (SVL, HW, ED and TBL) and weight (WT) of frogs from this study, arranged in a size series (mean dimensions divided by corresponding figures for *Microhyla ornata,* the smallest member of the assemblage) are shown in Figure 3. The range of ratios of SVL (1.57-6.17) appear greater than shown by Neotropical *Anolis* lizards, 1.01-1.46 (Duellman, 1978), or

TABLE 1. Morphometric data on the eight species of anurams studied. References to species: MO, *Microhyla ornata;* MR, *Microhyla rubra;* TR, *Tomopterna rolandae;* US, *Uperodon systoma;* RC, *Rana cyanophlyctis;* PM, *Polypedates maculatus;* RCR, *Rana crassa;* RH, *Rana hexadactyla.* References to body parts: SVL, snout-vent length; HW, head width; TBL, tibia length; WT, weight. Linear dimensions in cm; wights in gm.

Species	SVL range ($\bar{x}$±SE) (N)	HW range ($\bar{x}$±SE) (N)	TBL range ($\bar{x}$±SE) (N)	WT range ($\bar{x}$±SE) (N)
MO	1.27-1.74 (1.48±0.098) (4)	0.37-0.50 (0.41±0.030) (4)	0.63-0.87 (0.75±0.052) (4)	0.2-0.6 (0.362-0.099) (4)
MR	1.68-2.65 (2.32±0.120) (8)	0.53-0.83 (0.723±0.033) (8)	0.95-1.27 (1.41±0.038) (8)	0.80-3.00 (1.566±0.276) (8)
TR	1.72-4.32 (2.70±0.100) (44)	0.76-1.88 (1.09±0.037) (44)	0.77-1.55 (0.99±0.034) (41)	0.50-9.09 (2.82±0.420) (33)
US	3.46-5.47 (4.38±0.320) (5)	1.05-1.49 (1.24±0.084) (5)	1.16-1.64 (1.40±0.139) (3)	3.00-20.00 (13.00±3.719) (4)
RC	2.40-7.07 (4.435±0.111) (74)	0.82-2.43 (1.61±0.331) (74)	1.16-3.42 (2.30±0.106) (32)	1.20-42.80 (10.79±0.940) (68)
PM	2.95-7.92 (5.97±0.510) (11)	1.05-2.72 (2.09±0.17) (11)	1.75-3.58 (2.80±0.235) (9)	1.40-28.00 (12.80±3.195) (10)
RCR	2.33-9.31 (6.32±0.233) (63)	0.85-3.36 (2.29±0.080) (63)	0.74-4.11 (2.33±0.190) (28)	1.00-77.77 (30.09±2.574) (62)
RH	1.51-13.22 (9.13±0.099) (461)	0.56-5.24 (3.34±0.040) (432)	0.62-5.66 (4.65±0.080) (164)	0.30-280.00 (99.64±2.454) (461)

Philippines scincid lizards, 1.41-4.33 (Auffenberg and Auffenberg, 1987). Ratios of HW (here considered the measure of the trophic apparatus) which may be more meaningful for understanding differences in morphology that may be the result of differences in feeding patterns, in the present study were somewhat greater than SVL ratios, the mean 4.3 (range 1.61-8.15). Mean values of the SVL, HW and WT ratios appear to be arranged in pairs that do not reflect phylogenetic affinities, and the implications, if any, are unclear. The incremental increase in SVL from the smallest to the largest species varied between 2.93-60.99, and no regular pattern of increment between adjacent species seems evident, although Auffenberg and Auffenberg (op. cit.) found that the greatest size differences exist among the smallest species in the skink community they studied.

TABLE 2. Weight to cube of snout-vent length in the eight species of anurans studied.

Species	range	($\bar{x}$±SE)	N
Microhyla ornata	0.047-0.115	(0.079±0.017)	4
Microhyla rubra	0.146-0.403	(0.221±0.033)	8
Uperodon systoma	0.289-1.297	(0.933±0.229)	4
Tomopterna rolandae	0.097-0.701	(0.295±0.030)	33
Polypedates maculatus	0.158-1.22	(0.646±0.127)	10
Rana cyanophlyctis	0.167-2.209	(0.745±0.049)	68
Rana crassa	0.079-2.903	(1.394±0.092)	62
Rana hexadactyla	0.064-7.147	(3.355±0.062)	461

Ratios of linear dimension of the body in adjacent species, when arranged in a size-series (Table 2) appear close to the Hutchinsonian ratio, mean values for all species ranging between 0.83-1.88 (mean 1.32± SE 0.09). Weight ratios were, however, 0.83-4.60, the mean 2.63 (±SE 0.56) substantially larger than 2.0 as predicted by Hutchinson.

Discussion

Morphologically similar species tend to display similar ecological need, and if they occur in sympatry, it is assumed that they compete.

Duellman (1978), in his study of the Ecuadorian herpetofaunal communities discovered no size groupings among frog and lizard species, using SVL ratios of differences between sympatric species, showing that there is, instead, a steady increase in SVL. Auffenberg and Auffenberg (1987) found that Philippines skinks show greater ratios of differences between species, and presumed predation on a significantly greater size range of prey by the scincids.

With *Microhyla ornata,* the smallest species in the area, was used as a standard against which all others were compared, the range of ratios of SVL (1.57-6.17) was found to be considerably greater than figures reported from other studies on tropical herpetological assemblages, including those of Duellman (1978) and Auffenberg and Auffenberg (1987), suggesting that members of the assemblage of South Indian amphibians under investigation feed on a greater size range of prey.

For coexisting frog species, the ratio of mean head width among species pairs appear close to the ratio for closely-related, sympatric and presumably competing species described by Hutchinson (1959) in studies from Canada (McAlpine and Dilworht, 1989) and Peru (Toft, 1980), indicating the potential for competition. The mean values (±SE) of the ratios of SVL, HW and WT for adjacent species in the South Indian amphibians were 1.31 (±0.09), 1.37 (±0.09) and 2.63 (±0.56), respectively.

The 1.3 ratio concept has recently come under close scrutiny, leading some workers (e.g. Strong et al., 1979; Simberloff and Boeklen, 1981) to doubt its constancy. In fact, Simberloff and Boecklen, op cit.) attempted to show that the methods utilized by workers reporting the constancy of the

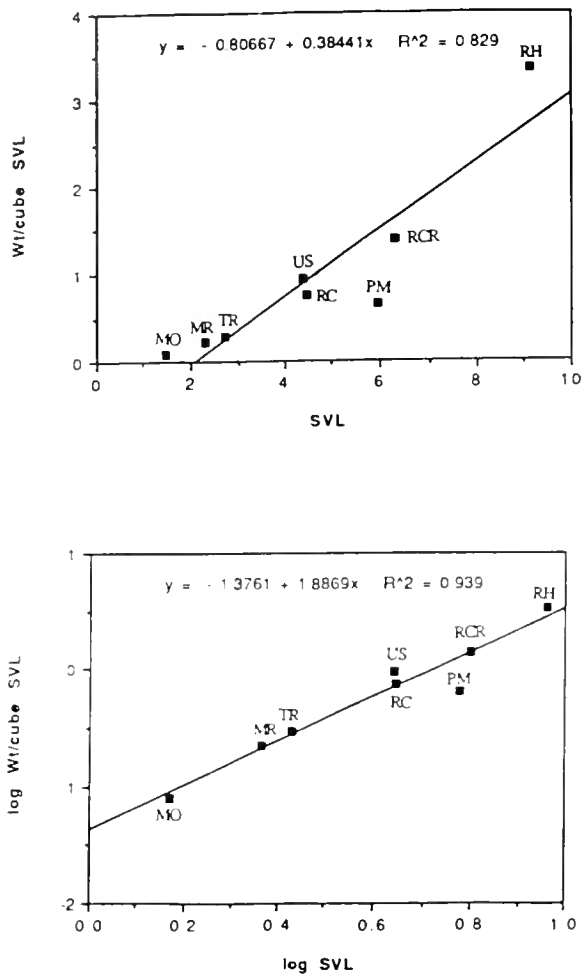


FIG. 2. Relationship between snout-vent length (SVL) and the ratio of weight to cube of snout-vent length (Wt/cube SVL) in the species studied. 2.1. Untransformed data; 2.2. Log-transformed data. Species abbreviations as in Table 1.

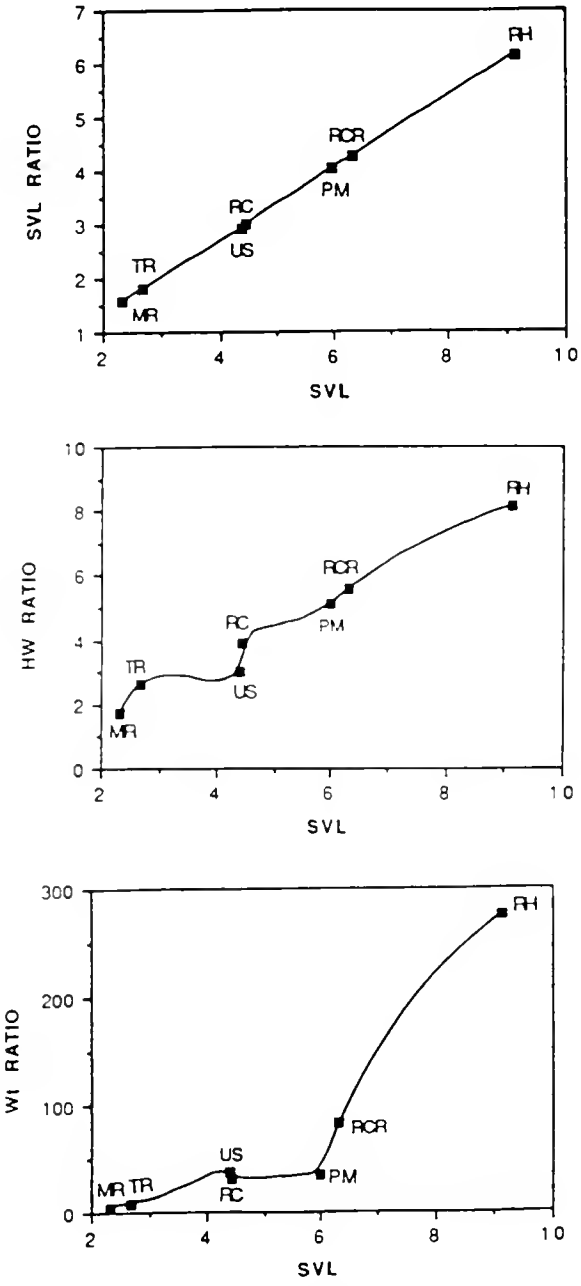


FIG. 3. Size-gradation in the species studied (mean dimensions divided by corresponding figures for the smallest species in the assemblage, *Microhyla ornata*. 3.1. Snout-vent length (SVL); 3.2. Head width (HW): 3.3 Weight (Wt).

ratios have been erroneous, although subsequent workers have demonstrated that the evidence is, in many cases, quite strong on examination of fresh data (e.g. Schoener, 1984) or even on the basis of the tests conducted by Simberloff and Boecklen (see Losos et al., 1989). Maiorana (1978) suggested that if ecological segregation of two species (or age classes) requires a minimum level of overlap in their degree of morphological variability, the linear displacement in mean size will also be relatively constant, and argued that the presence of similar ratios in many man-made objects, as discovered by Horn and May (1977) may be associated with human perceptional abilities derived from the natural world.

The constancy of Hutchinsonian ratios has been shown for a large number of

invertebrate and vertebrate groups (reviewed by Schoener, 1984; 1986a), which is suspected to be indicative of interspecific competition. To complicate matters further, ratios have been shown to be size-dependent, a function of allometry, and may thus change with growth (Roth, 1981). These are also thought to vary geographically, assemblages in the tropics show smaller ratios than temperate ones (Klopfer and MacArthur, 1961), suggestive of greater niche overlap in tropical assemblages. In the present study, HW, considered a measure of the trophic apparatus of frogs, showed negative ontogenetic allometry, the slopes *b* of the regressions of SVL and HW (on log paper) scaling allometrically in all eight anuran species that comprise the assemblage under investigation, indicating differences in the relative size and shape of the trophic apparatus among the different size-classes and sexes within a species may be influenced by food size.

Acknowledgments

The staff of Madras Crocodile Bank Trust helped with field work, and provided laboratory space. In particular, I would like to thank Romulus Whitaker, Harry Andrew, Romaine Andrews, Manjula Tiwari and Aurofilio Schiavina. My research on the ecology of frogs in South India was supported by Inlaks Foundation, Madras Crocodile Bank Trust, Trinity College, Oxford and an Overseas Research Student (ORS) Award. I thank R. Avery, M. Coe, and C. Perrins for comments.

Literature Cited

AUFFENBERG, W. AND T. AUFFENBERG. 1987. Resource partitioning in a community of Philippine skinks. Florida State Museum Bulletin 32:151-219.

DARWIN, C. 1859. On the origins of species by means of natural selection. Murray, London.

DUELLMAN, W. E. 1978. The biology of an equatorial herpetofauna in Amazonian Ecuador. Miscellaneous Publications of the Museum of Natural History, University of Kansas 65:1-352.

GANS, C. 1988. Adaptation and the form-function relation. American Zoologist 28:681-697.

HORN, H. S. AND R. M. MAY. 1977. Limits to similarity among coexisting species. Nature 270:660-661.

HUTCHINSON, G. E. 1959. Homage to Santa Rosalia, or why are there so many kinds of animals? American Naturalist 93:145-159.

KLOPFER, P. H. AND R. H. MACARTHUR. 1961. On the causes of tropical species diversity: Niche overlap. American Naturalist 95:223-226.

LOSOS, J. B., S. NAEEM AND R. K. COLWELL. 1989. Hutchinsonian ratios and statistical power. Evolution 43:1820-1826.

MCALPINE, D. F. AND T. G. DILWORTH. 1989. Microhabitat and prey size among three species of *Rana* (Anura: Ranidae) sympatric in eastern Canada. Canadian Journal of Zoology 67:2244-2252.

MAIORANA, V. C. 1978. An explanation of ecological and developmental constants. Nature 273:375-377.

PETERS, R. H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

PHILLIPSON, J. 1981. Bioenergenetic options and phylogeny. Pp. 20-45. *In* C.R. Townsend & P. Calow (Eds). Physiological ecology: An evolutionary approach to resource use. Blackwell Scientific Publishers, Oxford.

ROTH, V. L. 1981. Constancy in the size ratios of sympatric species. American Zoologist 118:394-404.

SCHOENER, T. W. 1965. The evolution of bill size differences among sympatric congeneric species of birds. Evolution 19:189-213.

SCHOENER, T. W. 1974. Resource partitioning in ecological communities. Science 185:27-39.

SCHOENER, T. W. 1986a. Kinds of ecological communities-- Ecology becomes pluralisitic. Pp. 467-479. *In* J. Diamond & T.J. Case (Eds). Community ecology. Harper & Row, Publishers, New York.

SCHOENER, T. W. 1986b. Resource partitioning. Pp. 91-126. *In* J. Kikkawa & D.J. Anderson (Eds).

Community ecology: Patterns and processes. Blackwell Scientific Publications, Melbourne.

SIMBERLOFF, D. AND W. BOECKLEN. 1981. Santa Rosalia reconsidered. Evolution 35:1206-1228.

STRONG, D. R., L. A. SZYSKA, AND D. S. SIMBERLOFF. 1979. Tests of community-wide character displacement against null hypothesis. Evolution 33:897-913.

TOFT, C.A. 1980. Feeding ecology of thirteen syntopic species of anurans. Oecologiia 45:131-141.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 45-48

Observations on Arboreality in a Philippine Blind Snake

MAREN GAULKE
Muhliusstrasse 84, 24103 Kiel 1, Germany

Abstract. -Five blind snakes were observed in June 1990 in the rain forests of Sibutu Island in the Sulu Archipelago, Philippines. Contrary to the usually fossorial habits of typhlopids, *Ramphotyphlops suluensis* (Taylor, 1918) shows arboreal habits. It climbed through trees at night using the prehensile tail and hindbody. When caught they extruded a strong smelling liquid from their cloaca. Relatively long tails are found in some other rain forest dwelling typhlopids, which may also have arboreal habits.

Key words: Reptilia, Ophidia, Typhlopidae, *Ramphotyphlops suluensis*, Philippines, ecology.

Introduction

Little is known of the behavior of blind snakes (Typhlopidae). Information is normally generalized and consists of little more than that typhlopids are small, burrowing snakes, which live in decaying logs, humus and leaf litter, and feed mainly on ants and termites, especially their grubs, pupae and eggs (e. g. Taylor, 1922; Loveridge, 1946; Gruber, 1980).

This gap in observations is certainly due to a number of different factors. Typhlopids are very inconspicuous and rather dull looking, and as such, arouse the interest of few people, even among herpetologists. About 168 species are known (Hahn, 1980). Many are found infrequently, and often are known from one or a few specimens only. Due to their size, coloration, and secretive habits, they are hard to observe. However, observations reported here on a rain forest dwelling blind snake in the Philippines, indicate that at least not all of them are as secretive as generally assumed.

Methods

In June 1990 a three week field survey was conducted on Sibutu, a small island in the Sulu-Archipelago, a few miles off the northeast coast of Sabah, Borneo (04° 46.4'N, 119° 28.8'E). Observations and collections of amphibians and reptiles were made within a forested area (primary and secondary lowland forest of the molave type *sensu* Dickerson, 1928). Short, but heavy, rains fell every two to three days, the temperature ranged between 25 and 32°C, and the relative humidity between 70 and 95%.

The nomenclatural history and taxonomy of the typhlopids observed and caught on Sibutu is discussed in Gaulke (in press), where the species, previously synonymized with *Ramphotyphlops olivaceus* (Gray, 1845), is revalidated. *Ramphotyphlops suluensis* reaches a length of approximately 40 cm, the eyes are distinctive, and the tail is more than twice as long as broad. The dorsal side is gray, the ventral side is cream, with bright white scales along the median row.

Observations

Although a considerable amount of time was spent turning and splitting decaying logs, and digging in humus and leaf litter in search of blind snakes, all efforts were unsuccessful. However, a few days before I had to leave Sibutu Island, the luck turned. While looking for geckos with a flashlight between 2200 and 0200 hours, the first blind snake was observed, not on the ground as expected, but on a tree on an almost leafless twig approximately 3 m above the ground. While trying to reach it, the disturbed animal let itself drop to the ground, and vanished into the leaf litter. During the following three nights, four more specimens were observed, all on branches and twigs above my head. Being more careful now, it was quite easy to catch them. All four reacted to the capture with the excretion of a pungent musk from their cloaca, the stench of which adhered to the skin for some time.

Before capture, the mode of locomotion of the climbing blind snakes was observed

FIG. 1. *Ramphotyphlops suluensis* climbing in an avocado tree.

FIG. 2. *Ramphotyphlops suluensis* making searching movements with its stiffened forepart while climbing.

for some time. While the tail and hindbody is tightly coiled around a twig, they crawl forward with the free part of their body. Depending on the thickness of the twig, they may use protrusions as resistance and hold, or make serpentine movements, with parts of their body hanging loosely down on both sides of the twig. After the forepart is secured, the tail/body grip is released and then dragged forward, and anchored further along. Compared to typical arboreal snakes, like whip snakes or flying snakes, they move very slowly, but are nevertheless skilled and effective climbers. During their movements, they stop relatively often and demonstrate a conspicuous behavior. While the hind part is coiled in the tree, the stiffened forepart is stretched into the air, making slow circular movements. When they discover another twig or branch within their reach with this searching movements, they might climb over to it. All the while they are tongue flicking. (Fig. 1, 2).

Two of the snakes captured were preserved and transferred to the Forschungsinstitut und Naturmuseum Senckenberg (SMF 74247/8). The stomach of the larger snake (total length 357 mm, weight 11.5 g) contained part of an unidentified earthworm, with a surprisingly large girth in relation to the tiny mouth of the snake. The two other specimens were kept alive for further observation. They are strictly nocturnal. Larvae of the moth, *Galleria mellonella,* were offered as food, but they were never observed feeding. Nonetheless, their good condition after several months in captivity indicated some food uptake. For video records they were placed on a small avocado tree during daytime. Here the same movements could be observed and recorded, as described above. When released in the middle of the avocado stem, they more often climbed up than down, searching for a resting place within the branches. However, sometimes they climbed down and started to burrow into the soil of the flower pot, proving that they are as effective in digging as in climbing.

Discussion

Characteristic features of typhlopid snakes are: a cylindrical body, smooth small scales throughout the entire body, a small narrow head with a solid cranium, a short broad tail ending in a sharp spine, and reduced eyes covered by much larger scales. These adaptations for fossorial life are in almost complete contrast to the characteristics of typical arboreal snakes, such as a laterally compressed or triangular body, a thin prehensile tail, and medium sized to large eyes. However, as shown by *R. suluensis,* it can be erroneous to interpret the mode of living from the habitus alone. Only the relatively longer tail compared to other typhlopids (in most typhlopids the tail is about as long as wide) might be interpreted as an adaptation towards arboreality.

The question remains, why are they climbing in trees, as the disadvantage is obvious. They are more exposed to predation from nocturnal animals, such as owls or cat snakes, than their relatives which only seldomly leave their burrows. Few blind snakes were observed climbing on trees before. A *Ramphotyphlops nigrescens* was found 5 m above the ground in a tree (Shine and Webb, 1990), arboreality is discussed for *R. braminus* (Swanson, 1981), and it is reported for some Leptotyphlopidae (Vanzolini, 1970). Shine and Webb (1990) discuss arboreality in scolecophidians as a feeding strategy. They assume that there may be little difference for them to follow ant-trails underground or on trees. However, the observations on *R. suluensis* indicate that this species is not incidentally climbing up trees, but might be more or less specialized on an arboreal life. All specimens on Sibutu were found climbing, and none on the ground. It can be assumed that *R. suluensis* is not the only blind snake specialized on arboreality. Taylor (1922) collected several Philippine typhlopids in the root balls of aerial ferns, on felled trees. He concluded that they are living and hunting within these root balls. I assume it is much more likely that they are using epiphytes, etc., only as daytime retreats, actively searching for food

in the twigs and branches of the tree during night time. Those blind snakes found in epiphytes have unusually long tails for typhlopids, being four to seven times as long as broad. In view of the skilled way *R. suluensis* uses its much shorter tail for climbing, they should be even better equipped for an arboreal live.

The function of the cloacal sac substance, which *R. suluensis* used as a defense mechanism against capture, was investigated in the leptotyphlopid *Leptotyphylops dulcis*. Gehlbach et al. (1968) found that it repelled attacking army ants, upon which these snakes feed. Furthermore the substance was found to repel sympatric ophiophagous and insectivorous snakes, a much more serious danger to the blind snakes. On the other hand, *L. dulcis* are attracted to their own colacal sac substance (Watkins et al., 1969), so it has different functions, as interspecific repellent, and as intraspecific attraction. It can be assumed that it has similar complex functions in *R. suluensis*.

Acknowledgments

The field study was supported by the Senckenbergische Naturforschende Gesellschaft, Frankfurt a. Main. Video records were done in cooperation with Dr. A. Altenbach, Kiel University. I thank Dr. W. R. Branch, Port Elizabeth, for reviewing the manuscript.

Literature Cited

DICKERSON, R. S. 1928. Distribution of life in the Philippines. Philippine Bureau of Science, Manila, Monograph 21:1-322 pp.

GAULKE, M. (in press). Die Herpetofauna von Sibutu Island (Philippinen), unter Berücksichtigung zoogeographischer und ökologischer Aspekte. Senckenbergiana biologica

GEHLBACH, F. R., J. F. WATKINS, AND H. W. RENO. 1968. Blind snake defensive behavior elicited by ant attacks. Bioscience 18:784-785.

GRUBER, U. 1980. Blindschlangen, Wühlschlangen und Warzenschlangen. Pp. 362-366 *In* B. Grzimek (Ed), Grzimeks Tierleben. Band 6. Kriechtiere. Deutscher Taschenbuch Verlag, München.

HAHN, D. E. 1980. Liste der rezenten Amphibien und Reptilien. Anomalepidae, Leptotyphlopidae, Typhlopidae. Das Tierreich 101:1-93. Walter de Gruyter, Berlin.

LOVERIDGE, A. 1946. Reptiles of the Pacific world. Macmillan Co., New York. 259 pp.

SHINE, R., AND J. K. WEBB. 1990. Natural history of Australian typhlopid snakes. Journal of Herpetology 24(4):357-363.

SWANSON, S. 1981. *Typhlina bramina*, an arboreal blind snake? Northern Territory Naturalist, Darwin, N. T. 4:13.

TAYLOR, E. H. 1922. The snakes of the Philippine Islands. Philippine Bureau of Science, Manila. 312 pp.

VANZOLINI, P. E. 1970. Climbing habits of Leptotyphlopidae (Serpentes) and Wall's theory of the evolution of the ophidian eye. Pap. Avul. Zool. 23:13-16.

WATKINS, J. F., F. R. GEHLBACH, AND J. C. KROLL. 1969. Attractant-repellent secretions of blind snakes *(Leptotyphlops dulcis)* and their army ant pray *(Neivamyrmex niggrescens)*. Ecology 50(6):1098-1102.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 49-52

On the Distribution of Emydid Turtles and the Anuran Genus *Microhyla* in the Philippines

MAREN GAULKE

Muhliusstr. 84, 24103 Kiel, Germany

Abstract. *-Cuora amboinensis kamaroma* Rummler and Fritz and *Microhyla annectens* Boulenger are reported from the Sulu Archipelago in the southwestern Philippines. The Philippines range of *Cyclemys dentata* (Gray) is extended to the Sulu Archipelago. The distribution of emydids and the genus *Microhyla* in the Philippines is discussed.

Key Words: Reptilia, Testudines, Emydidae, *Cuora amboinensis amboinensis, Cuora amboinensis kamaroma, Cyclemys dentata,* Amphibia, Anura, Microhylidae, *Microhyla annectens,* Philippines, distribution.

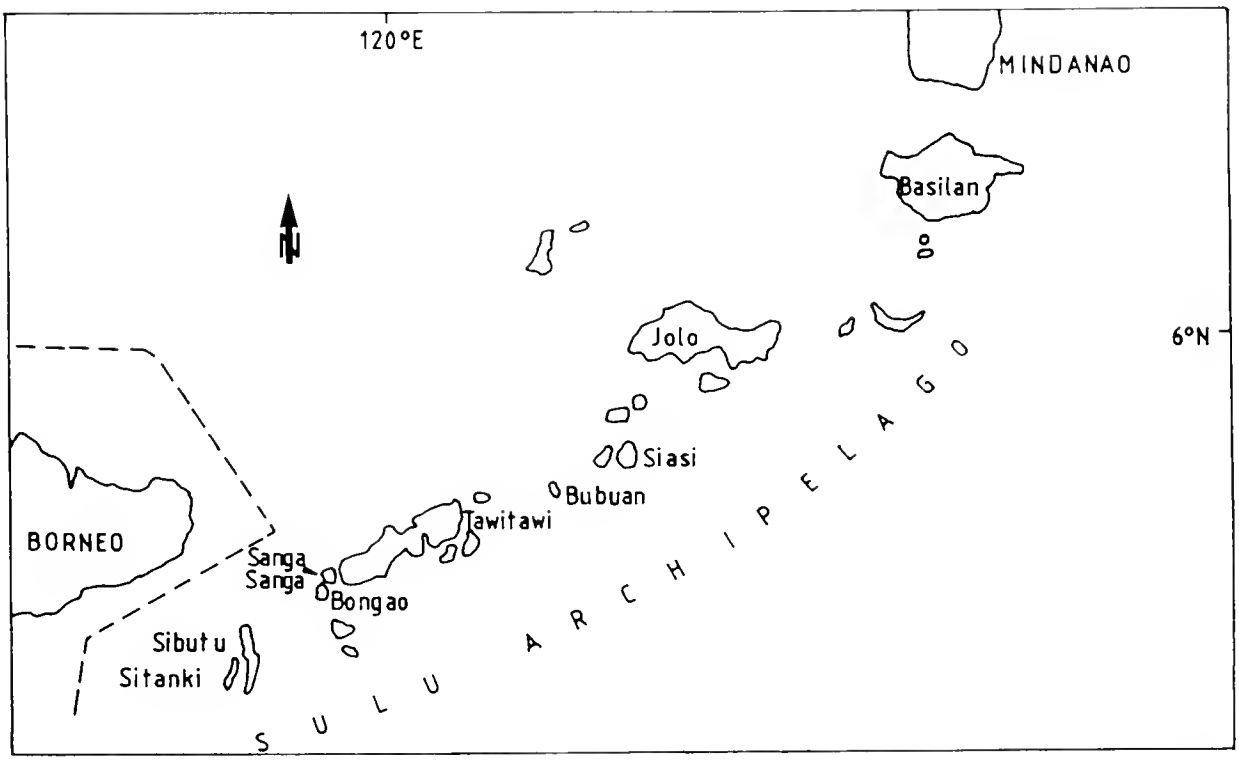


FIG. 1 Generalized map of the Sulu Archipelago.

Introduction

The Philippines are separated into different faunistic regions (Brown and Alcala, 1970, 1980; Dickerson, 1928; Inger, 1954; Leviton, 1963). The Sulu Archipelago, an island chain situated between Basilan and Mindanao to the east, and Sabah, Borneo, to the west, is one of these regions. Faunistically it is more closely related to Borneo than to the Philippines. The only comprehensive work on the herpetofauna of the Sulu Archipelago is from Taylor (1918). Additions to it's herpetofaunal record include for example Gaulke (1993, 1994), Leviton (1963) and Taylor (1922a, 1922b). Almost nothing is known of the emydid turtles of the region,

and knowledge on the anurans is also scarce. This work contributes to both of these subjects, and discusses the distribution of taxa in the groups in other parts of the Philippines.

Material and Methods

The author has conducted field surveys in different regions of the Philippines since 1984. In the years 1990, 1991 and 1992 the following islands of the Sulu Archipelago were visited: Bongao, Sanga Sanga, Siasi, Sibutu, Tawitawi and Jolo (Fig. 1). Most of the turtles found were photographed and released after examination.

Three turtles (one *Cuora amboinensis kamaroma,* two *Cyclemys dentata)* are kept alive by the author, the microhylid frog (SMF 74908) and one *C. dentata* shell (SMF 74909) are deposited in the herpetological collection of the Forschungsinstitut und Naturmusem Senckenberg, Frankfurt am Main, Germany. The *C. amboinensis* subspecies were identified after Rummler and Fritze (1991), and the frog after Inger (1966).

Results and Discussion

Emydidae

Cyclemys dentata.- On June 9, 1990, an adult specimen of *C. dentata* was caught in an undisturbed swampy lowland forest at Languyan on Tawitawi. It's faeces showed that it had fed on ripe figs before capture.

In 1991 and 1992 several, mainly juvenile, specimens of *C. dentata* were found in swamps and small creeks in different areas of Tawitawi (Batu Batu, Magsaggaw), proving that it is not a rare turtle on the island. It has not yet been found on any other island of the Sulu Archipelago.

Previously the known range of *C. dentata* in the Philippines included only the Palawan Province (Balabac, Palawan, Calamian Islands) and Leyte (Alcala 1986; Taylor 1921). While it is common throughout the Palawan Province, records from Leyte are rare. Palawan and Leyte belong to different faunistic regions, and were never connected by a land bridge. Therefore it is surprising that *C. dentata* is known from both regions, but from nowhere in between. Three explanations can be offered for this disjunct distribution: 1. *C. dentata* exists on other Philippine Islands, but has not been recorded (or reported) until now. 2. *C. dentata* previously occurred on other Philippine islands, but became extinct subsequently on most of them, with a relict population surviving on Leyte. 3. *C. dentata* never reached the more eastern Islands of the Philippines, and the Leyte records rely on specimens introduced by man. This is not unlikely, since people often keep turtles as pets and for medicinal purposes (as rheuma remedy) leading to their translocation. *C. dentata* kept in captivity on Negros and Cebu were obtained on Palawan (pers. obs.).

The disjunct Philippine distribution of *C. dentata* is remarkably echoed by the third emydid turtle from the Philippines, the endemic *Heosemys leytensis* (Taylor) which is also reported from Leyte and Palawan, but no in between island. Since both distribution records of this rare turtle rely on single individuals (Timmermann and Auth 1988; Taylor 1921), no assumptions on the reasons for the disjunct distributions can be made.

The Sulu Archipelago and Palawan lie in both Philippine entryways from Borneo, suggesting that the *C. dentata* populations have their origin on Borneo, where this turtle is widespread.

Cuora amboinensis.- In 1990 the author found *Cuora amboinensis* on the islands of Sanga Sanga and Tawitawi, and in 1992 on Jolo. During this period *C. amboinensis* was reported from Jolo (Rummler and Fritz 1991), who identified two specimens from Jolo (MNHN 6152:1-2, Musium Nationale d'Histoire Naturelle Paris) as *C. a. amboinensis,* as they did all Philippine

FIG. 2. Plastral view of three *Cuora amboinensis* from Jolo, showing that almost no dark markings are present on the plastron, as is typical for the subspecies *C. a. kamaroma* Rummler and Fritz.

material they studied. However, the specimens found during my trips on Sanga Sanga, Tawitawi and Jolo do not belong to the nominate form, but to the recently described *C. a. kamaroma* Rummler and Fritz. They have none, or very few, dark patches on their plastron (Fig. 2) and a high carapace, as is typical for this subspecies. U. Fritz, who kindly examined photos of some of the turtles captured on Jolo, mentioned that they are slightly flatter than other *C. a. kamaroma* he had seen, and the marginal scutes were slightly more visible from above. Nevertheless he confirms the determination as *C. a. kamaroma*, assuming that some interbreeding with Philippine *C. a. amboinensis* may have occurred.

C. a. kamaroma, the most widespread of the three subspecies of *C. amboinensis,* is known from Borneo, the Southeast Asian mainland, and now the Sulu Archipelago. *C. a. amboinensis* has a wide distribution over the Philippines, being reported from Bacoo, Cebu, Dinagat, Leyte, Luzon, Mindanao, Mindoro, Negros, Polillo and Samar (Boettger 1886; Rummler and Fritz 1991; Taylor 1921), but occurs on other Philippine Islands as well. The author, for example, has collected a few specimens on Masbate (currently being kept alive). Outside the Philippines, *C. a. amboinensis* is known from Sulawesi and the Moluccas. The Palawan Province is presently the only known Philippine Province not inhabited by any subspecies of *C. amboinensis*.

Rummler and Fritz (1991) consider the high domed *C. a. kamaroma* and the flat *C. a. amboinensis* as the evolutionary extremes of *C. amboinensis,* whilst the third subspecies *C. a. couro* (Schweigger), which occurs on Sumatra and Java, shows intermediate characteristics. They believe it possible that in the future high domed and flat forms of *C. amboinensis* may be found sympatric on Borneo, proving that they belong to different species. The record of *C. a. kamaroma* from the Sulu Archipelago shows that the Bornean form could reach the outskirts of the Philippines, but does not resolve the question of their phylogenetic affinities. It is surprising that within the Philippines two significantly different forms are found. *C. a. amboinensis* must either have developed from *C. a. kamaroma*, or reached the Philippines, via Mindanao, from the Moluccas or Sulawesi.

Microhylidae

On March 18, 1991, the author collected one specimen of *Microhyla annectens* Boulenger (SMF 74908) in leaf litter beside a small pond in a lowland forest at Magsaggaw, Tawitawi. The species has a wide distribution on Borneo, Malaysia and Thailand, but was previously unknown from the Philippines.

Only one other member of the genus *Microhyla* has been recorded from the Philippines. Fischer (1885) reported *Microhyla achatina* (Boie) from Southern Mindanao, based on a single specimen. Boettger (1886) listed this frog, but mentioned that the specimen, according to a comment by G. A. Boulenger, most probably is not *M. achatina* but a *Kaloula* species. Taylor (1921) listed *M. achatina* without questioning the determination. However, he doubted the accuracy of the locality record, assuming that it might have come from another country. He also assumed that it might be from one of the high, little explored mountains on Southern Mindanao and therefore had not subsequently been found. Inger (1954) and Alcala (1986) finally do not include *M.*

achatina in the Philippine amphibian fauna anymore.

Whether the record of *M. achatina* from the Philippines is true or not (the specimen is no longer available) must remain open, however, the new record shows that the genus *Microhyla* has reached the Philippines.

Acknowledgments

I am grateful to Mr. R. Miller and Mr. Th. Borromeo, who were helpful in finding turtles on the Sulu Archipelago, and to the Provincial Department of Environment and Natural Resources in Bongao for allowing me to conduct surveys in their area, and to collect a few specimens. Dr. W. Branch kindly revised the manuscript, giving many helpful comments.

Literature Cited

ALCALA, A. C. 1986. Guide to Philippine flora and fauna. Vol. X. Amphibians and reptiles. JMC Press, Quezon City. 195 pp.

BOETTGER, O. 1886. Aufzählung der von den Philippinen bekannten Reptilien und Batrachier. Senckenbergische Naturforschende Gesellschaft 1886:91-134.

BROWN, W. C., AND A. C. ALCALA. 1970. The zoogeography of the herpetofauna of the Philippine Islands, a fringing archipelago. Proceedings of the Californian Academy of Science, fourth series 38(6):105-130.

BROWN, W. C., AND A. C. ALCALA. 1980. Philippine lizards of the family Scincidae. Silliman University Press, Dumaguete City. 264 pp.

DICKERSON, R. S. 1928. Distribution of life in the Philippines. Philippine Bureau of Science Monograph 21:1-322.

FISCHER, J. G. 1885. A list of reptiles and batrachians of Mindanao. Jahrbuch der Wissenschaftlichen Anstalt Hamburg 1885(2):80-81.

GAULKE, M. 1993. First record of the polydont snake *Sibynophis geminatus geminatus* (Boie, 1826) from the Philippines, with a discussion of *Sibynophis bivittatus* (Boulenger, 1894). Herpetological Journal (3):151-152.

GAULKE, M. 1994. Contribution to the snake fauna of the Sulu Archipelago, with the description of a new subspecies of *Dendrelaphis caudolineatus* (Gray, 1834). Herpetological Journal.

INGER, R. F. 1954. Systematic and zoogeography of Philippine amphibia. Fieldiana: Zoology 33(4):183-531.

INGER, R. F. 1966. The systematics and zoogeography of the amphibia of Borneo. Fieldiana: Zoology 52(1966):1-402.

LEVITON, A. E. 1963. Remarks on the zoogeography of Philippine terrestrial snakes. Proceedings of the Californian Academy of Science, fourth series 31(15):369-416.

RUMMLER, H.-J., AND U. FRITZ. 1991. Geographische Variabilität der Amboina-Scharnierschildkröte *Cuora amboinensis* (Daudin, 1802), mit Beschreibung einer neuen Unterart, *C. a. kamaroma* subsp. nov. Salamandra 27(1/2):17-45.

TAYLOR, E. H. 1918. Reptiles of Sulu Archipelago. Philippine Journal of Science 13(5):233-269.

TAYLOR, E. H. 1921. Amphibians and turtles of the Philippine Islands. Philippine Bureau of Science Monograph 15-1-193.

TAYLOR, E. H. 1922a. Additions to the herpetological fauna of the Philippine Islands, 2. Philippine Journal of Science 21(2):161-206.

TAYLOR, E H. 1922b. Additions to the herpetological fauna of the Philippine Islands, 3. Philippine Journal of Science 22(5):515-557.

TIMMERMANN, W. W. AND D. L. AUTH. 1988. Geographic distribution. *Heosemys leytensis*. Herpetological Review 19(1):21.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 53-61

Ecology and Conservation of *Onychodactylus fischeri* (Caudata, Hynobiidae) in the Russian Far East

PAUL C. GRIFFIN[1] AND VASILI A. SOLKIN[2]

[1]Department of Forestry and Natural Resource Management, University of California at Berkeley, Berkeley, California, 94720, USA

[2]Pacific Institute of Geography, Far East Branch of Russian Academy of Sciences, Vladivostok, 690032, Russia

Abstract. *-Onychodactylus fischeri* (Boulenger, 1886) is a lungless salamander with a larval development time of over four years and lifespan that may extend over 18 years. *O. fischeri* develops and spawns only in cold torrential brooks. In the Russian Primorski Krai of the Russian Far East *O. fischeri* lives in undisturbed Ussuriland montane taiga forest. Adults migrate annually to breed. In homing experiments adults which were ready for breeding migrated over 800m of land to breeding sites. Homing experiments showed that sexually mature adults may demonstrate stream fidelity. Males were more frequently encountered at surface waters than females. There are seasonal peaks to breeding-adult activity. High-impact forest harvesting is now typical in headwater forests and river valleys across the range of *O. fischeri*, causing disturbance and siltation in spawning brooks and surrounding forest habitat. *Pinus koreansis* trees comprised a dominant component of the forest canopy in spawning drainages.

Key Words: Amphibia, Caudata, Hynobiidae, *Onychodactylus fischeri*, Russian Far East, Ussuri taiga, conservation, ecology.

Introduction

This study set out to discover aspects of *Onychodactylus fischeri* biology by collecting standardized morphological and color pattern data during the course of adult and juvenile mark-recapture surveys, by documenting microhabitat location, by determining ages of adults and larvae, by moving marked sets of salamanders and recording their return to home brooks, and by continuing early attempts at inducing captive breeding. and to assess movement of individuals between spawning streams. Ussuri taiga has been heavily harvested from plains and river valleys in this century to the point that headwaters comprise some of the least disturbed examples of this forest type. High-impact, wasteful forest harvesting is now occurring in the elevational range of *O. fischeri* range in an inefficient manner, causing catastrophic disturbance and siltation to its spawning brooks and surrounding forest habitat. *O. fischeri* is an endangered species in the Red Data Book of the USSR but is afforded no protection under current Russian environmental law.

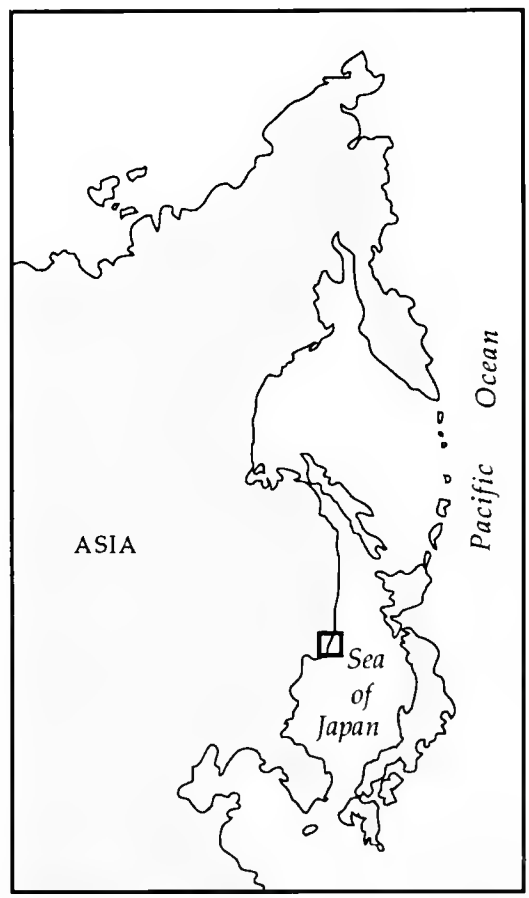


FIG. 1. Map of Asia and the Russian Far East showing study location in boxed area.

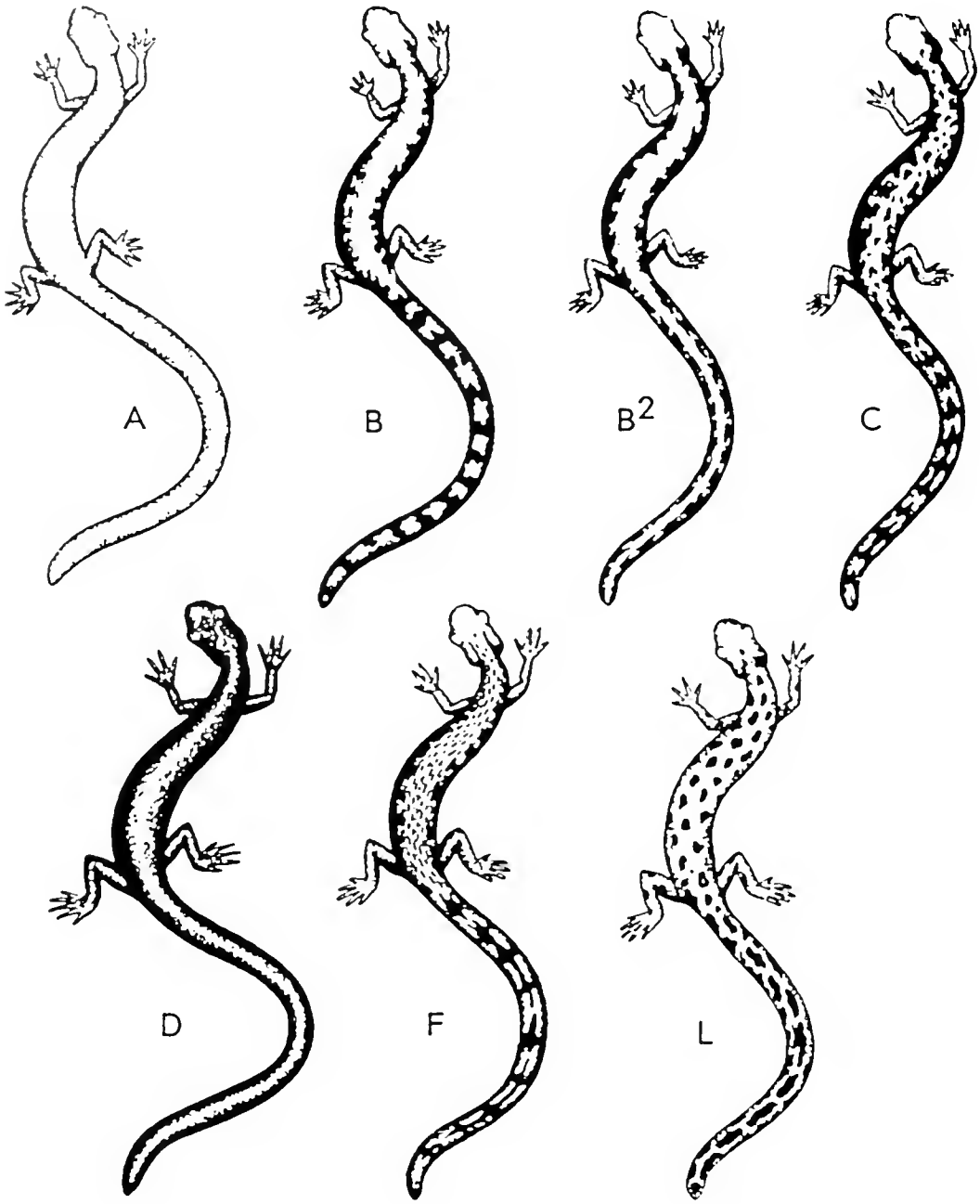


FIG. 2. Dorsal Color Patterns of *O. fischeri*.. Percent frequencies out of 351 salamanders in "Chinese" brook were: A, 1%; B and B2, 36.4%; C, 46.7%;D, 5%; F, 4.8%; L, 0.3%

Methods and Materials

Our studies were within the range of *Onychodactylus fischeri* in the Sixote-Alin Mountain Range of the Primorskii Krai, Russia. These mountains are forested by Usuriland taiga. We conducted our surveys and experiments at elevations between 500 m and 1000 m., at a site 10 km west of the Pacific Ocean in headwater brooks of the Mysovka River (Lat. 135°, Long. 41°). This river flows into the Tinfura River, a tributary of the Ol'ga Bay (Fig. 1). Climate is typical of the maritime Russian Far East,

with humid summer days averaging 25° C in July and cold winters down to -30° C. The summer monsoon and winter snows provide approximately 120 cm of annual precipitation (Fullard, 1972).

We surveyed the brook tributaries at the headwaters of the upper Mysovka watershed and gave them the following arbitrary names: "Chinese," "Zapovednik," "Mutnii," "Himalayan," "Burned," and "Tetkin." We characterized the forest canopy cover in the entire study site in terms of dominant tree species and also inventoried subdominant trees of the forest canopy in "Chinese" drainage. In 1993 we noted changes in soil and forest structure that resulted from forest harvest activity taking place in the Mysovka watershed.

To estimate the sex ratio and age structure in a breeding population we marked and recorded data from all individuals encountered in a one kilometer stream transect of "Chinese" brook, divided into 20 sections, during measurements from 1990 to 1993. Between one and three people with headlamps walked the transect once every night for a two to three hour observation period in June 1990, May and June 1991, September 7-14, 1991, and June 3-13 1993. We began each transect at a downstream gauging station where we measured relative humidity, brook depth and temperature. Each individual yielded data for the following categories: sex, maturity class, biotope, transect section, fat condition, skin pattern, tail wounds, and morphometric lengths for snout-vent, tail, head length and head width. We distinguished adult sex and reproductive stage by the secondary sexual characters discussed in Serbinova and Solkin (1992). We assigned salamanders unique identification numbers by toe-clipping, and reclipped regenerating toes only when we were sure of the original mark. We designated the color pattern for individuals according to our categorization of light-colored spot placement on the neck, back and tail (Fig. 2). To determine exact ages for some individual salamanders we sent a random subset of 118 clipped adult and larval toe bones to Moscow for skeletochronological analysis (Smirina, 1972).

FIG. 3. Timber harvest operations in the Mysovka floodplain downstream from the study site.

To examine stream fidelity we released 20 breeding adults from "Zapovednik" brook into "Chinese" brook in 1991. We released 100 salamanders with a range of secondary sexual characters from "Zapovednik" brook into "Burned" brook. In 1992 we released 31 breeding males, 43 breeding females, and 52 non-breeding adults from "Chinese" brook into "Zapovednik" brook. We searched for marked individuals in "Zapovednik" and "Burned" brooks on nights when we were not surveying the "Chinese" brook transect.

In 1993 we collected three males and females that were ready for breeding from "Chinese" brook. We injected synthetic leutenizing and releasing hormone into them to induce courtship, egg-laying and fertilization. Their aquaria were plastic, with rocks, moss, and 3 to 5 cm of standing water. Air and water temperature in the aquaria were dependent on ambient stream temperature.

Results

Onychodactylus fischeri spawn in "Mutnii", "Zapovednik", "Himalayan", and "Chinese" brooks. "Chinese" brook flows over a substrate of 1.5 meter deep rock cobble which is covered by moss and shallow rooted plant growth. "Zapovednik" brook has much less rock cobble, with smooth

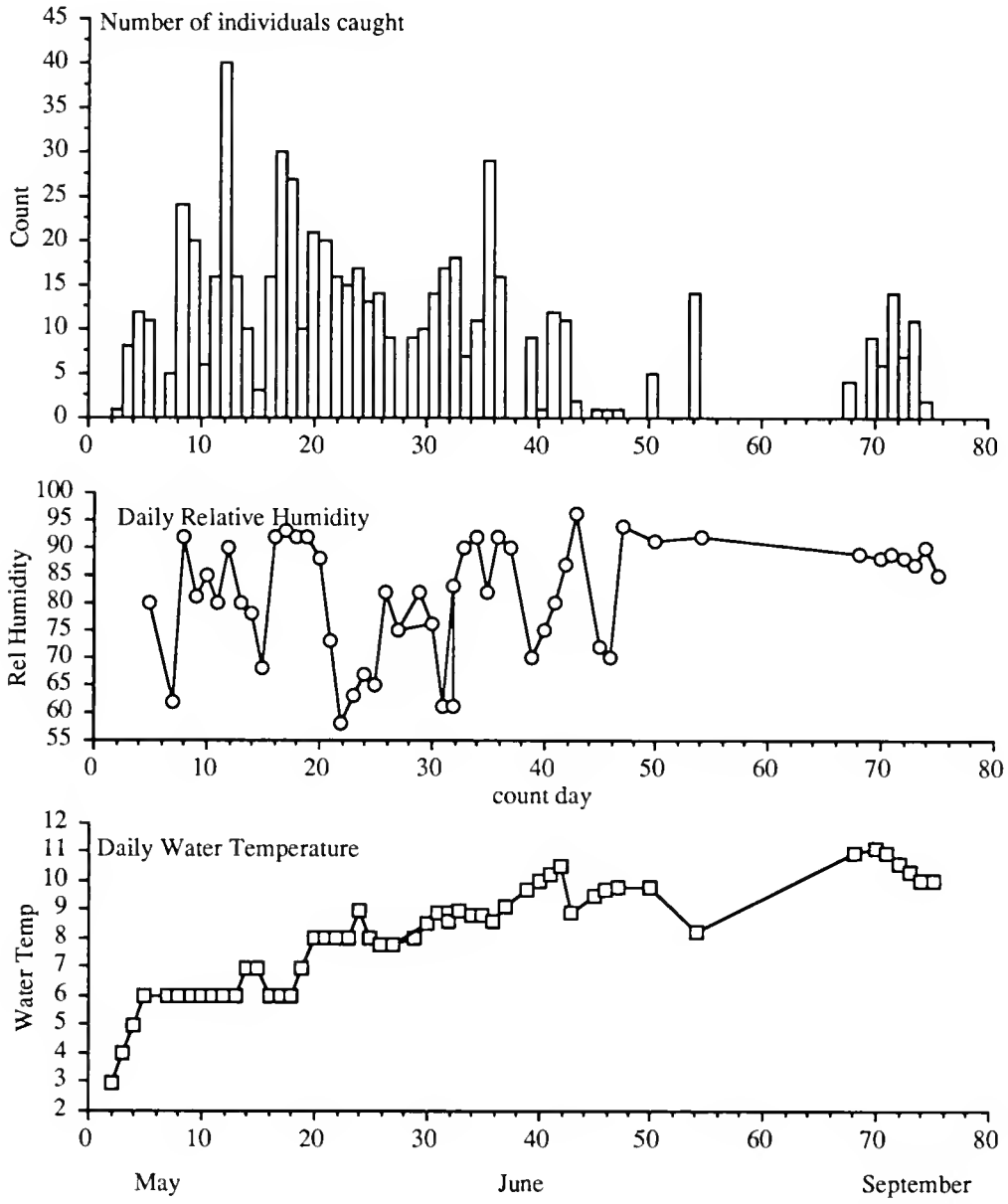


FIG. 4. Daily number of salamanders captured, relative humidity and stream temperature in "Chinese" brook during May, June and September 1991.

bedrock as the substrate for some sections. In the study area predaceous fish only occurred in "Burned" and "Tetkin" drainages, which were both affected by a forest canopy-removing wildfire 40 years ago and by ensuing landslides.

Pinus koreansis accounts for a significant portion of the dominant canopy cover in the brook drainages where *O. fischeri* does occur. Dominant forest trees in the drainage of "Chinese" brook reflects a strong slope effect; Manchurian oak (*Quercus mongolica*) and *P. koreansis* dominate the southeast-facing slope while *P. koreansis*, fir (*Abies* sp.), birches (*Betula* spp.), maples (*Acer* spp.), elms (*Ulmus* spp.), and linden (*Tilia* spp.) comprised much of the canopy of the north-facing slope.

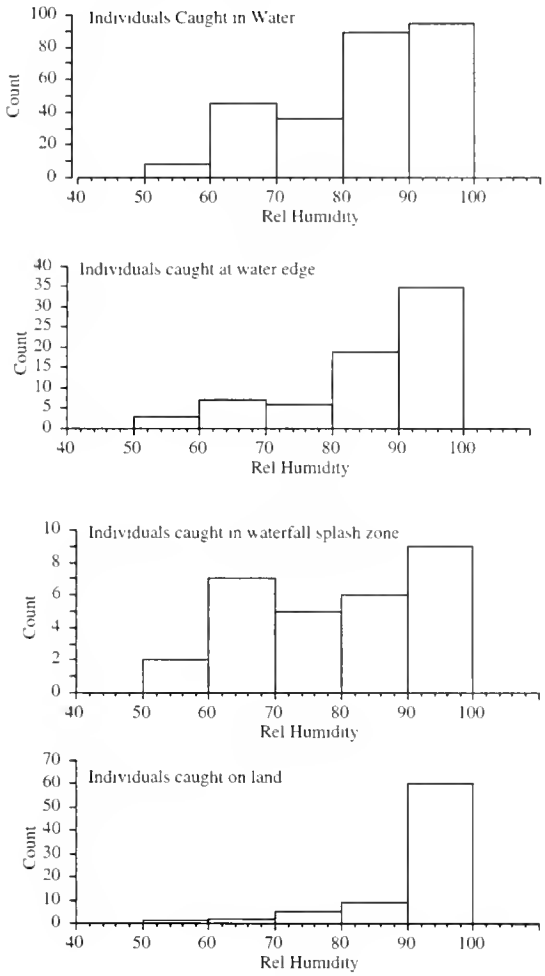


FIG. 5. Histograms divided according to ten-percent atmospheric relative humidity classes showing number of salamander captures in water, water edge, splash zone and land biotopes. Note that few salamanders were active on land when relative humidity was below 90%.

A six man work team from the local forest production ministry harvested timber downstream in 1993 with one tank-treaded bulldozer and one rubber-tired loader servicing a logging truck (Fig. 3). Their road building often crossed the small river in the floodplain. The bulldozer skidded logs down slopes of greater than 35 degrees. There was severe disturbance to soil structure caused by these activities. Soils were brown podbels with an illuvial B horizons ending less than 50 cm below the litter surface (Ivanov, 1976). *Pinus koreansis* was being harvested from the river plain and hills despite current regulations outlawing harvest of that species. Other harvested species included *Abies sp., Tilia sp.* and *Populus tremula.* The river channel was widened in 1992, apparently as a subsequent outcome of harvest activities.

We first encountered breeding adults at spawning brooks during snowmelt in late April and early May. Adult males captured in surface waters outnumbered adult females in all seasons. Breeding adult activity was highest in May and June, when stream temperatures were low (Serbinova and Solkin, 1992). In September 1991 the breeding population rose during a small peak composed of adults aged 6 to 9 years; at that time 11 out of 20 males and 5 out of 9 females exhibited secondary sexual characters and juveniles represented 45.3 % of all captures.

Around spawning brooks we found *O. fischeri* in pools and riffles, on dry and wet rocks in the watercourse, on rocks in the misty zone near waterfalls, and on the shore. Salamanders were most active at the surface during evenings of high relative humidity (Fig. 4). 535 captures provided data for biotope location. We found salamanders most often in the water (54.0 % of total) and on the streambank (16.4 % of total). On nights of low relative humidity salamanders were often located on rocks in the misty splash zone near waterfalls (6.0 % of total). On land salamanders were most often within 50 cm of the water (11.3 % of total). Salamanders were rarely active on land if relative humidity was lower than 90 percent (Fig. 5). Juveniles were active on land markedly less than adults. In the brook channel we uncovered 1 gravid female when we dismantled a 0.5 cubic meter rock matrix where brook water seeped through moss-covered rocks. We occasionally observed *O. fischeri* on land between streams and on ridges during times of rainfall and high relative humidity.

We found abundant larvae in surface waters during nocturnal transect surveys. Larvae were also active diurnally. During two one-hour periods in June 1993 we observed *Onychodactylus fischeri* larvae

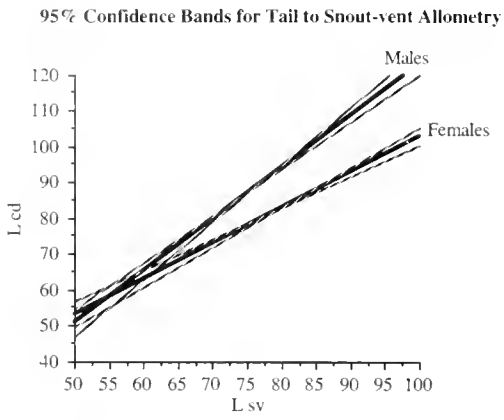


FIG. 6. Allometric relationship showing relatively longer tail size (L cd) in adult males at given snout-vent length (L sv). The difference in the slopes is significant (ANCOVA; F = 18.21; df = 1; p < 0.0001).

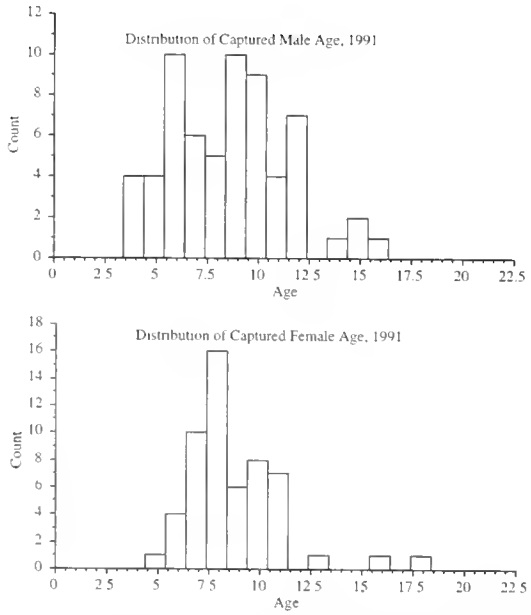


FIG. 7. Histogram showing relative abundance of ages in a subset of captured males and females.

hunting on flat rocks under 3 cm of water in the late afternoon. By means of their limbs the larvae locomoted to approach aquatic Gammarid casings and lunged when the invertebrates exposed their legs and head. It appeared that larvae used their forelimbs in conjunction with a gape-and-suck feeding technique to apprehend the prey.

The number of transect captures was an index of active adult population size, generally decreasing over the course of the summer except for a small increase in early fall. Analysis of our mark-recapture data by the Jolly-Seber stochastic method (Donnely and Guyer, 1994) yielded standard errors too large for individual population estimates of males, females, and juveniles.

Males had significantly longer tails than did females once the effect of snout to vent length was accounted for (ANCOVA; F = 8.99; df = 1; P = 0.003). Also, tail length increased more rapidly with body size in males than in females (ANCOVA; F = 18.21; df = 1; p < 0.0001) (Fig. 6). We observed tail wounds on 23 out of 350 males and 19 out of 166 females. It appeared that females in "Chinese" brook had a thinner fat layer than females in "Mutnii" brook, where the brook substrate includes more bedrock. According to our designation of color pattern 351 individuals in "Chinese" brook, the dominant patterns of bright flecking on grey to brown backgrounds were C (46.7 % of total) and B (36.4% of total).

Our data on age and development times support the hypothesis that *Onychodactylus fischeri* is a slow developing, long-lived salamander. Cross sections of larval bones indicate that larvae may develop up to four years before metamorphosis to terrestrial juveniles. The mean age of breeding males in 1991 was 8.65 years (Std Dev. 2.87); mean breeding female age was 8.75 years (Std. Dev. 2.34) . Maximum ages were at least 16 years for males, and 18 years for females (Fig. 7). Ages approximated by counting winter rings may underestimate true age because of endosteal resorption (Leclair 1990).

In 1991 at "Zapovednik" brook we recaptured one of the 20 salamanders that we had taken from there for release into "Chinese" brook; we recaptured none of these in "Chinese" brook. In 1991 we recaptured none of the 100 salamanders which we had released into "Burned" brook from "Zapovednik" brook. We also

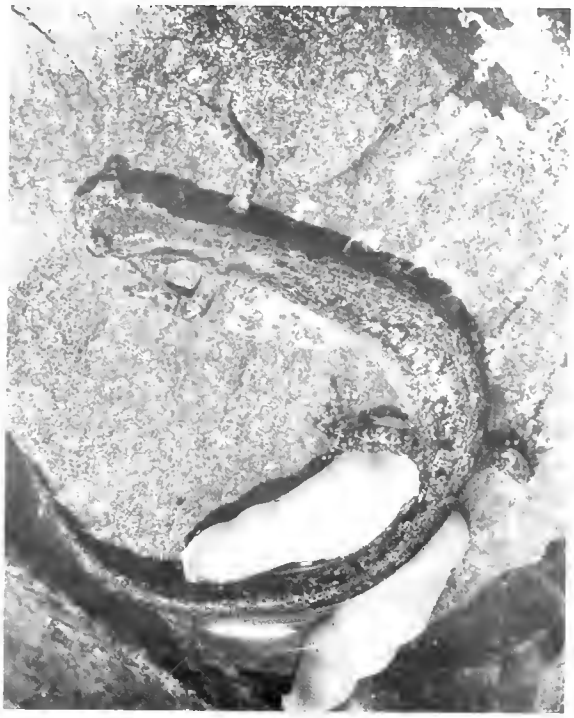


FIG. 8. *Onychodactylus fischeri* female lays eggs in two gelatinous sacs.

FIG. 9. *Onychodactylus fischeri* male grasps and fertilizes egg sacs with hind limbs.

recaptured none of these in their original stream. In 1992 at "Chinese" brook we recaptured 8 of 31 breeding males, 15 of 43 breeding females and none of the 52 non-breeding adults out of the total 126 salamanders which we moved in 1991 from "Chinese" brook to "Zapovednik" brook. The breeding adults which we caught had returned within a month to "Chinese" brook over 1 km of land or by a longer route along the Mysovka river.

Hormone injection in captives successfully induced courtship, egg-laying (Fig. 8) and fertilization (Fig. 9). One female produced a clutch of two egg sacs in 1993. Eggs were 6 to 8 mm in diameter, with 5-10 eggs in each sac. We observed deflation of female cloacal labia in the six days after egg-laying. Egg death probably resulted from a severe rise in pH of available water.

Discussion

Onychodactylus fischeri is limited by the availability of suitable torrential streams for reproduction and development. The seasonal activity pattern which we observed in adults seems limited by water temperatures over 10° C. The burst of breeding activity observable in late spring may signify the most strategic time for egg-laying in view of the short warm season in the Russian Far East; it is logical that eggs laid in the late spring develop faster than eggs laid in early fall because water temperatures drop below 5° C in the winter. The higher proportion of males caught in our surveys suggests that males may wait on the surface rocks and waters to seek females. Egg sacs and larvae seem to be located most often in intersticial rock habitat, sheltered from surface predators in areas of continuous water flow (Regel and Epshtein, 1975). Iwasawa and Kira observed that *O. japonicus* eggs developed slowly for 143 days in water of 10° C (1980). Longer winters may cause the larval stage in *O. fischeri* occasionally to extend longer than the three years observed in *O. japonicus* (Hayase and Yamane 1982).

Lunglessness is a synapomorphy of salamanders in the *Onychodactylus* clade, which have a long larval period in torrential streams as a requisite portion of their ontology. Bruce et al. (1992) supported the hypothesis that lunglessness in amphibians is a larval adaptation to torrential streams by showing that experimentally lungless salamander larvae were displaced

downstream less when released in fast-flowing water than were control larvae with lungs.

The reproductive potential of an individual *Onychodactylus fischeri* seems low, despite their long lifespan, considering that a female living 12 years may only produce 140 eggs. Additionally, many of the adults we found did not have secondary sexual traits indicative of reproduction. Akita (1989) notes that not all adult *O. japonicus* breed every year. The low clutch size and large egg mass suggest that each egg is a significant reproductive investment relative to other hynobiids. Kuzmin showed that the sympatric hynobiid *Salamandrella keyserlingii* is more general in its feeding and breeding habitat preferences (1990).

Our 1992 experiments suggest that successful homing was only by adults which were ready for breeding, indicating that adults may have a preference for particular spawning brooks. It is also possible that non-breeding adults may also have returned to their original brooks and not been recaptured, or that the breeding adults that did not return may have spawned in a different stream. *Onychodactylus fischeri* may migrate over land through forested corridors. We speculate that the connectedness of suitable habitat between large watersheds is important for dispersal after landscape level disturbances.

The pressure to harvest trees in the fragile taiga is promoted by an international wood and fiber market that devalues natural capital by not factoring in negative externalities such as anthropogenic fire, landslides and species loss. Repeated highgrading is the most widespread harvest method in the Russian Far East and has lasting effects on forest ecology. Forested slopes and floodplains are exposed to catastrophic erosion for years after tractors are used to skid out widely spaced trees. Reduction of canopy cover to levels to below 40% alters the evapotranspiration regime in a way which allows catastrophic wildfire. The hard currency that goes to the Primorskii krai in return for the Ussuri taiga logs does not compensate for the permanent loss of seasonal employment in fur, mushroom, honey, berry, ginseng, and game meat gathering and preparation (Schumacher, 1973). The best conservation strategy for *Onychodactylus fischeri* appears to be exclusion of high-impact timber harvest activity from key spawning watersheds, given that regional timber harvesters, Hyundai and other multinational timber interests generally ignore existing rules of the Russian Federation's Ministry of Forests. It would be informative to delineate metapopulation boundaries in order to assess the adequacy of existing preserves.

Onychodactylus fischeri is an indicator species of high-quality old growth forest habitat in upper slopes of Ussuri taiga. Because eggs and larvae develop in cold running water year round under the cover of rocks, undisturbed stream substrate and a specific hydrological regime are critical for quality spawning habitat. Long-term stability of stream hydrology, which is needed to support *O. fischeri* breeding and development, is dependent on undisturbed complex forest structure. Shade and large woody debris affect stream temperature and riffle-pool ratio. Extensive root systems retain soil structure and decaying logs on the forest floor regulate soil moisture. The deep roots of conifers provide more soil stability on slopes than shallow-rooted deciduous trees. Acid soils locally associated with *Pinus koreansis* litter may regulate stream pH and nutrient availability. We observed that catastrophic disturbance to spawning brooks occurred through natural processes of wildfire and landslides and through anthropogenic road building and logging in the watercourse, upslope, and downstream. Tree harvest on slopes above watercourses causes stream sedimentation of fine materials and organics. Such increased sediment loads associated with logging have negative effects on aquatic amphibians (Corn and Bury, 1989). As is the case on "Burned" creek, siltation eliminates the spaces between rocks which are critical for egg deposition and larval development. Disturbed brooks may be suitable to *O. fischeri* spawning only after adequate forest structure regenerates and interstitial spaces filled with silt and sand are cleared.

Acknowledgments

The authors would like to thank Vladimir Aramilev for his invaluable assistance in Vladivostok and in the field; Dmitri Pikunov for support from the Laboratory of Wild Animals of the Pacific Institute of Geography; Irina Olenicheva for her assistance with the skeletochronological work; and David Wake and Mario Garcia-Paris for their constructive criticism of the manuscript.

Literature Cited

AKITA, Y. 1989. Breeding Cycle of *Onychodactylus japonicus* on Mt. Hodatsu, with Special Reference to Biannual Spawning. P. 305. *In* M. Matsui, T. Hikida and R. C. Goris (Eds), Current Herpetology in East Asia. Herpetological Society of Japan, Kyoto.

BRUCE, R. C., C. K. BEACHY, P. G. LENZO, S. P. PRONYCH AND R. J. WASSERZUG. 1994. Effects of Lung Reduction on Rheotactic Performance in Amphibian Larvae. The Journal of Experimental Zoology 268:377-380.

CORN, P. S. AND R. B. BURY. 1989. Logging in Western Oregon: Responses of Headwater Habitats and Stream Amphibians. Forest Ecology and Management 29:39-57.

DONNELLEY, M. A. AND C. GUYER. 1994. Estimating Population Size: Mark Recapture. *In* Heyer, W.R. et al. (Eds.), Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonion Institution Press, Washington and London.

FULLARD, H. (ED.). 1972. Soviet Union In Maps. George Philip and Son, Ltd., London.

HAYASE, N. AND S. YAMANE. 1982. [Life History During the Aquatic Life Period of a Salamander, *Onychodactylus japonicus* (Houttuyn), at Mts. Tsukuba, Ibaraki, Japan]. Japanese Journal of Ecology 32(3):395-403. (In Japanese).

IVANOV, G. I. 1976. [Soil Formations in the south of the Far East]. Moscow. Science. 200 pages. (In Russian).

IWASAWA, I. AND Y. KIRA. 1980. [Normal Stages of Development of he Japanese Lungless Salamander, *Onychodactylus japonicus* (Houttuyn)]. Japanese Journal of Herpetology 8(3):73-89. (In Japanese).

KUZMIN, S. L. 1990. [Feeding of Sympatric Species of Hynobiidae in the Primorye]. Zoological Journal 69(5):71-75. (In Russian).

LECLAIR, R. 1990. Relationships between relative mass of the skeleton, endosteal resorption, habitat and precision of age determination in ranid Amphibians. Annales des Sciences Naturelles Zoologie et Biologie Animale 11:205-208.

REGEL, E. D., AND S. M. EPSHTEIN. 1975. [Some Patterns of Biology in *Onychodayctulus fischeri*]. Zoological Journal 63(7):1515-1524. (In Russian).

SCHUMACHER, E. F. 1975. Small is Beautiful: Economics as if People Mattered. Harper and Row. New York. 305 pages.

SERBINOVA, I. A. AND SOLKIN V. A. 1992. Reproduction of the Ussuri Lungless Triton *(Onychodactylus fischeri)]*. Zoological Journal 71(7):68-74. (In Russian).

SMIRINA, E. M. 1972. [Annual Layers in bones of *Rana temporaria*]. Zoological Journal 51:1529-1534. (In Russian).

Studies on the Distribution of Trace Elements in *Agkistrodon blomhoffii brevicaudus* Stejneger

JIN LIN[1], KE-MIN XÜ[1] AND DONG-GEN LIU[2]

[1]*Department of Biology, Liaoning Normal University, Dalian, Liaoning, 116022 China*
[2]*Kunshan Snake Museum, Suzhou, Jiangsu, 215334 China*

Abstract: -The distribution of trace elements in various organs and tissues of *Agkistrodon blomhoffii brevicaudus Stejneger* was studied by ICP-AES. The results show that there are more than 17 kinds of trace elements in the snake, namely, Cu, Zn, Fe, Mn, Se, Sr, Al, Si, Al, Ni, Ba, Co, Pb, Ge, Cd, Mo, La, etc. The amount of each kind of trace element is quite different in the various organs and tissues. The contents of Zn, Fe, Al and Cr have the highest value; Cu and Mn have the medium value; Se, Sr, Si, Ba, Pb, Ge and Mo have the lower value; and Ni, Cd, Co and La have the lowest value. The results suggested that the distributional characteristics of trace elements in *Agkistrodon blomhoffii brevicaudus* Stejneger correspond with the snake's physiological and biochemical functions.

Key words: Reptilia, Serpentes, *Agkistrodon blomhoffii brevicaudus* Stejneger, China, trace elements.

Introduction

According to recent literature, there are four species which belong to the genus *Agkistrodon* in northeast China. They are *A. blomhoffii brevicaudus* Stejneger, *A. saxatilis* Emelianov, *A. shedaoensis* Zhao, and *A. ussuriensis Emelianov* (Zhao and Adler, 1993). Xü et al. (1993) reported the distribution of trace elements in *A. shedaoensis* Zhao and *A. ussuriensis* Emelianov and the results showed that there existed interspecific differences. This paper reports the quality and quantity observations of trace elements in the various organs and tissues of *A. b. brevicaudus* Stejneger. It will provide experimental data for comparative physiology and contribute also to the classification of pit-vipers.

Materials and Methods

Four specimens of *Agkistrodon blomhoffii brevicaudus* were provided by Kunshan Snake Museum at Kunshan County, Jiangsu Province, China in December, 1993. The snakes were decapitated and various organs and tissues were freshly removed. All samples, except serum, plasma and bile, were stored in an incubator at 105^{0}C for 5 hrs., cooled down and weighted after incubation. Then, the samples were immersed in mixed acid (HNO3 : HCl=4 : 1) for 24 hrs, then heated continuously until clear and transparent, and transferred into a 10 ml graduated tube by adding double distilled water.

The trace elements of all samples were measured through ICP-AES (Leeman Co., USA). The data was analyzed with a micro-computer, then they were randomly arranged as a trace elements graph.

Results

The results show that there are more than 17 kinds of trace elements, such as Cu, Zn, Fe, Mn, Se, Sr, Al, Si, Ni, Ge, Cd, Ba, Mo, Co, Pb, Cr and La in various organs and tissues of *A. b. brevicaudus*. The contents of each kind of trace elements in the various organs and tissues are as follows:

Skin, Muscle and Skeleton.

The trace element graphs of the skin, muscle and skeleton are very similar, but they still have their own characteristics. The contents of Zn, Fe and Al are higher in skin, the order is Al > Fe > Zn, and the Al content in skin is the highest among all organs and tissues. The contents of Cr, Mn, Cu, Pb and Ba have a medium value, and Se, Sr, Ni, Ge, Cd, Mo and La have a lower value in the skin. The trace elements showed different contents in the different segments of the skin (Figs. 1a, 1b, and 1c).

The skeletal muscle has more Fe and Zn and less Al, compared with those of the skin. Other elements have no more differences. Ge can not be tested out (Fig. 2).

There are more Zn, Sr and Ba and less Fe, Al and Cr found in the skeleton than in the skin and skeletal muscle. The skeleton has more Sr than in other organs and tissues. But, Ge and La can not be tested out in the vertebrae (Figs. 3 and 4).

Cardio-Vessel System, Lung and Trachea.

The similarity of elements distribution in the cardio-vessel system, lung and trachea is the presence of more Zn, Fe, Al and Cr, then Cu and Mn, and less Ni, Ge, Cd, Mo, Co, and La (Figs. 5-10).

There are more contents of Cu, Zn, Fe and Al found in the heart, vessel and lung than in the blood and trachea. But, the contents of Ge and Se are the opposite. The Cu content in the heart is ahead of the other organs, and La and Ge can not be tested out in the lung and trachea.

It reveals that most trace elements are found in bile, e.g., Cu, Mn, Se, Sr, Al, Ge, Ba, Mo, Pb, Cr, especially Cu, Ba, Mo, Pb, and Cr are much more than those in the serum. The Cr content is the highest among all samples. The La and Cd can not be tested out in the serum (Figs. 6 and 10).

Digestive Tract, Liver, and Pancreas.

The contents of Zn, Fe, Al, Mo and Cr found in the digestive tract and mesentery have a medium value, then the Cu, Mn, Se, Sr, Ba, and the other elements are very small (Figs. 16-21). The Al content is obviously less than that of the skin.

The liver, spleen and gall-bladder have similar contents of trace elements. They contain considerable amounts of Zn and Fe. The other elements, like Se, Sr, Si and Ge, are more than those of the digestive tract, mesentery and other organs. There are no difference of Cu, Mn, Se, Ni, Ba, Co, Pb and La among them (Figs. 11-15).

Besides, the contents of Fe and Mo in the liver and Zn in the pancreas are the highest among all the samples. It suggests that the liver is the important place to store Fe.

Kidney and Reproductive Organs.

There is a large amount of Mn, Se, Ba and Cr in these organs, but very little of Si, Ni, Cd, Mo and Co (Figs. 22-25). Furthermore, there are more Zn, Fe, Al, Cu and Pb in the oviduct, Fallopian tube and uterus than in the kidney.

The Al content of the kidney is similar to that of the digestive tract, but clearly less than that of the skin.

Brain, Spinal Cord and Poisonous Gland.

The graph of the brain resembles that of the spinal cord; both contain abundant elements in the order of Zn > Fe > Cr > Cu > Al > Mn > Se > Sr > Ni > Ba. The Fe content of the brain is just lower than that of the liver, but the Ge and La in the brain can not be tested out (Figs. 26 and 27).

The Zn content in the poisonous gland is higher than that of the brain and spinal cord, and the La content is the highest among all samples. Se, Ge and Mo can not be examined (Fig. 28).

Discussion

There are 17 kinds of trace elements found in *Agkistrodon blomhoffii brevicaudus* through ICP-AES. The contents of each element are quite different in the various organs and tissues. The results showed that the trace elements are the important parts of life substances in the snake. And, the distribution characteristics of trace elements correspond with the animal's physiological and biochemical functions.

There are abundant amounts of Zn, Fe, Al and Cr, then Cu, Mn, Se and Sr found in the organs and tissues, especially in the cardio-vessel system, lung, liver, spleen, brain, oviduct, etc. It suggests that these

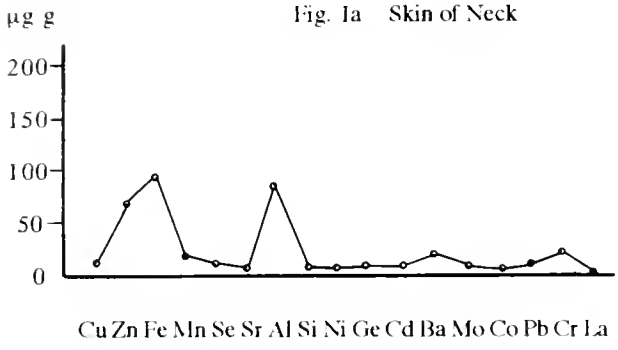


Fig. 1a Skin of Neck

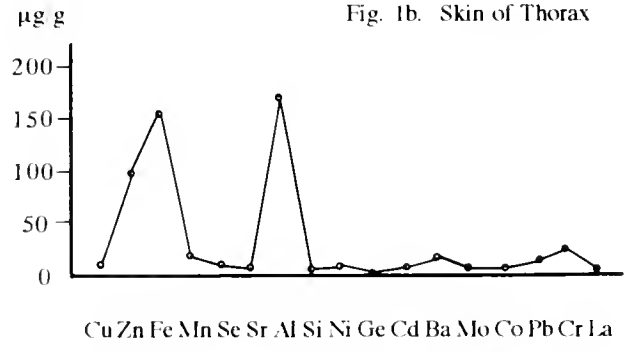


Fig. 1b. Skin of Thorax

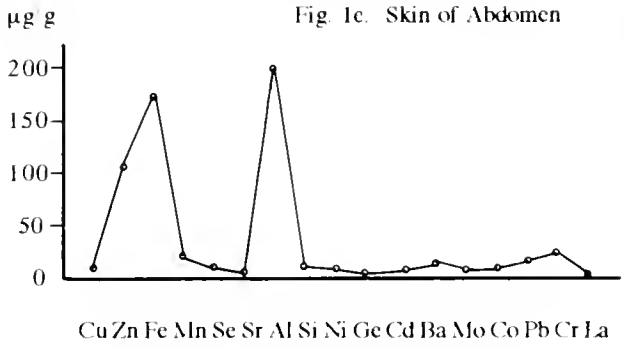


Fig. 1c. Skin of Abdomen

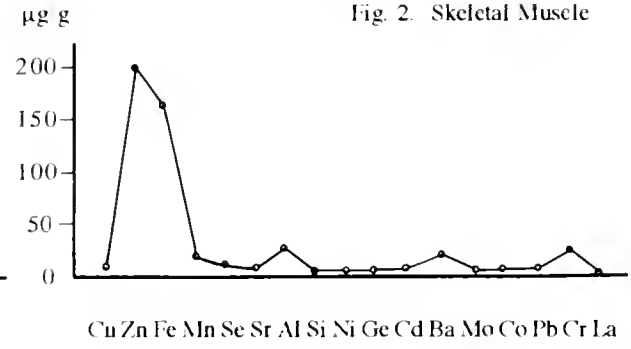


Fig. 2. Skeletal Muscle

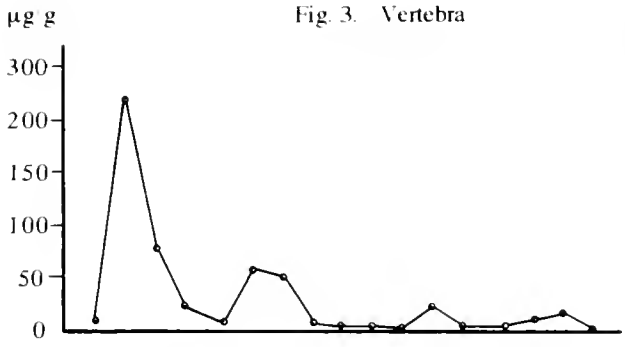


Fig. 3. Vertebra

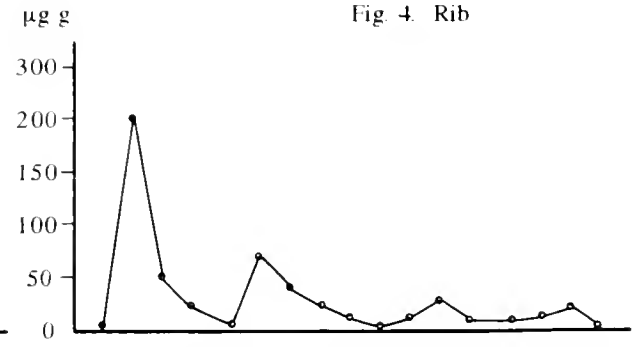


Fig. 4. Rib

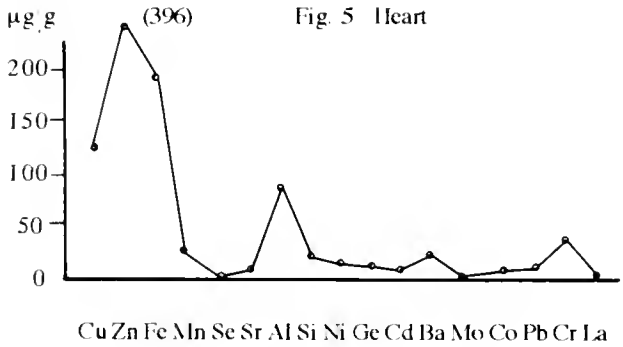


Fig. 5 Heart

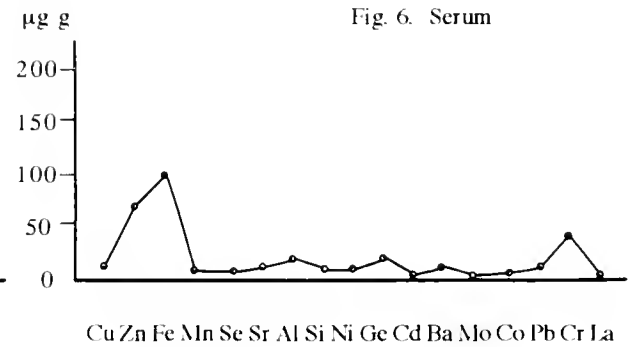


Fig. 6. Serum

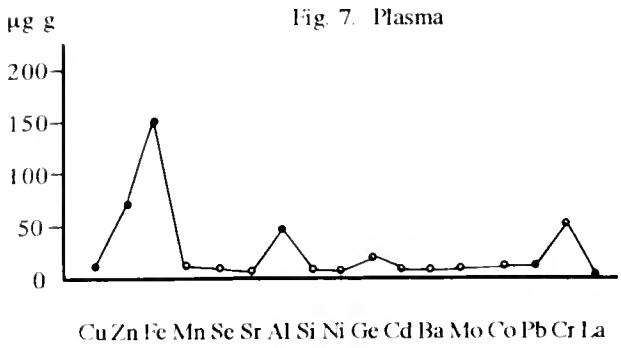


Fig. 7. Plasma

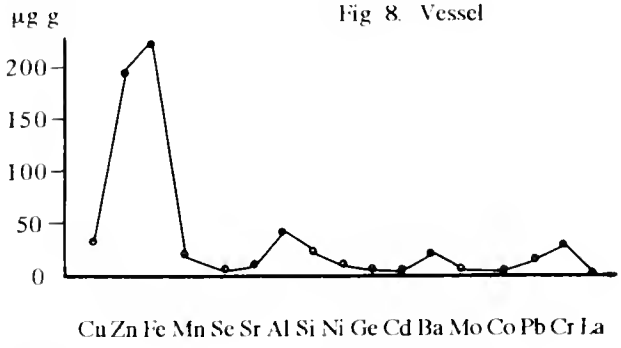


Fig. 8. Vessel

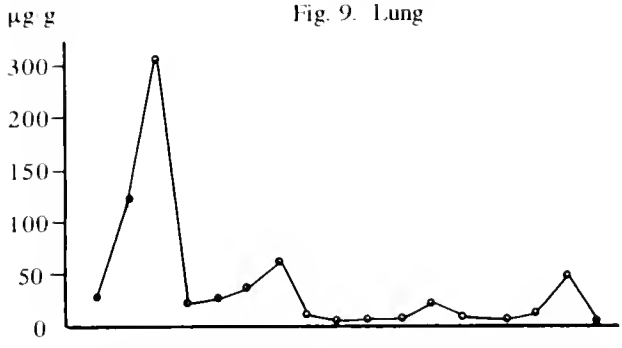


Fig. 9. Lung

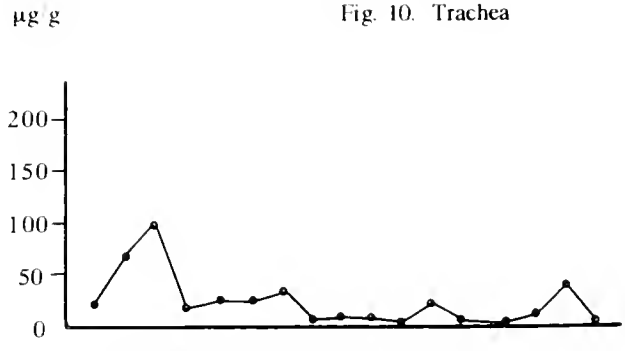


Fig. 10. Trachea

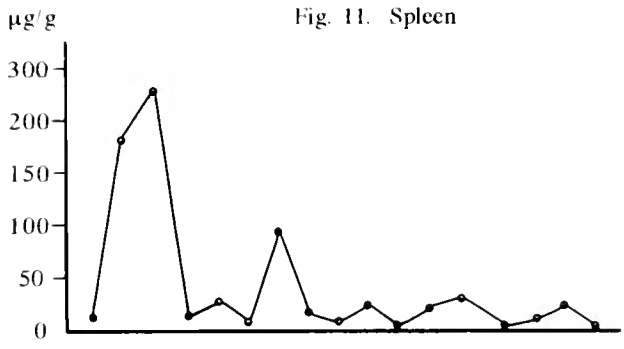


Fig. 11. Spleen

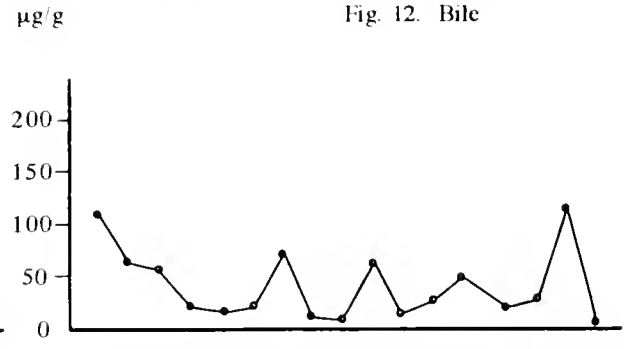


Fig. 12. Bile

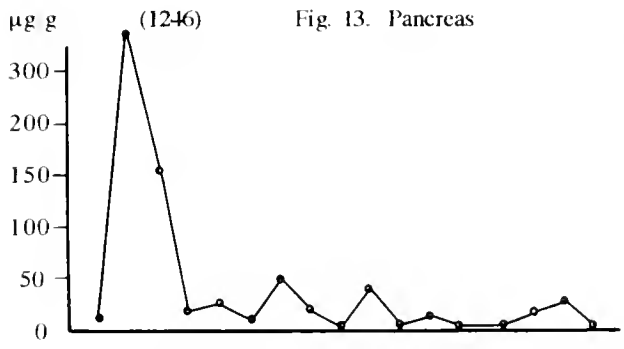


Fig. 13. Pancreas

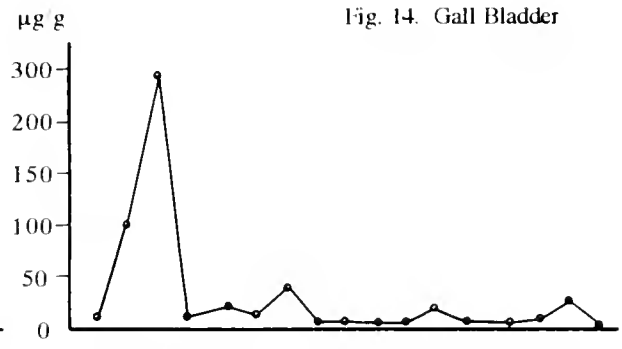


Fig. 14. Gall Bladder

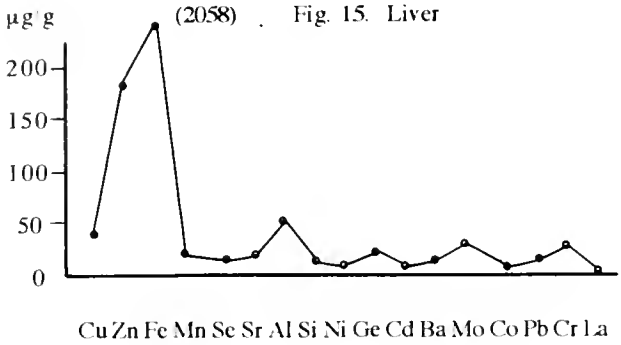


Fig. 15. Liver

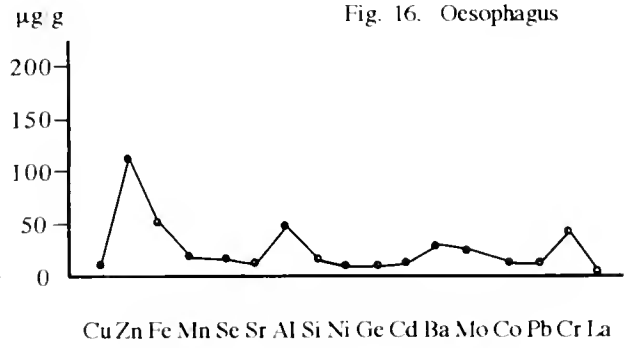


Fig. 16. Oesophagus

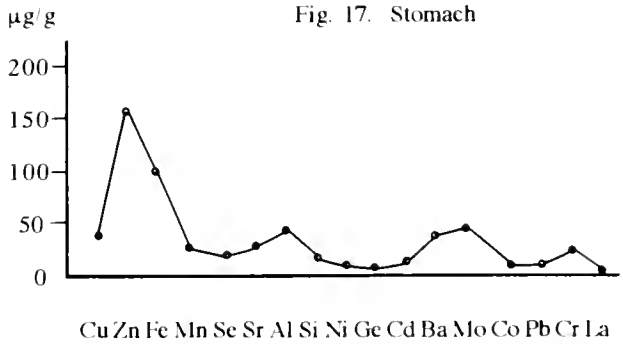


Fig. 17. Stomach

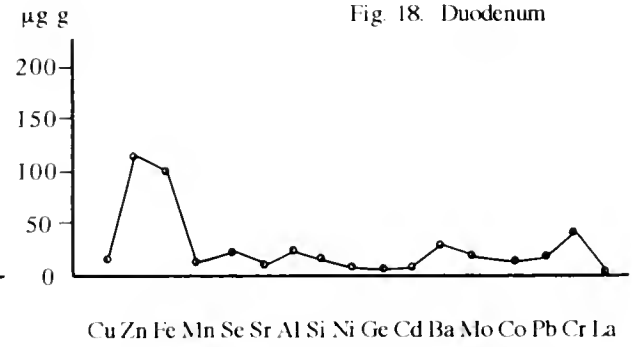


Fig. 18. Duodenum

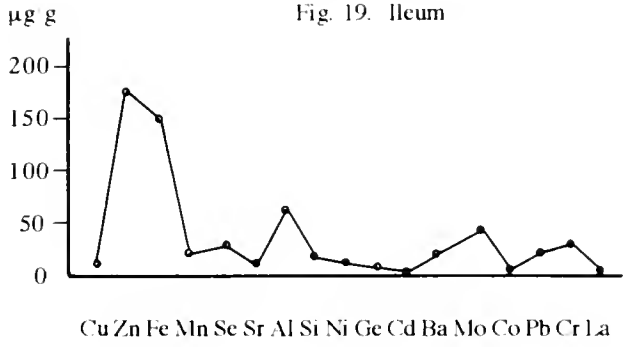


Fig. 19. Ileum

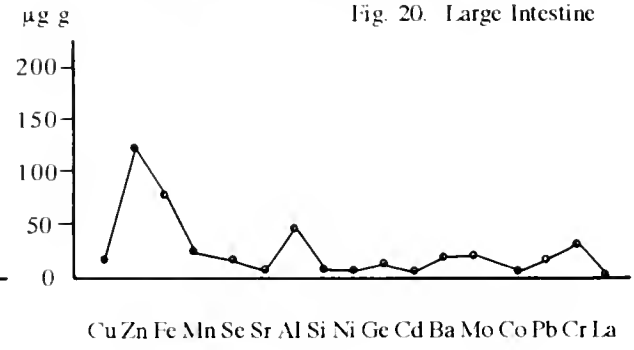


Fig. 20. Large Intestine

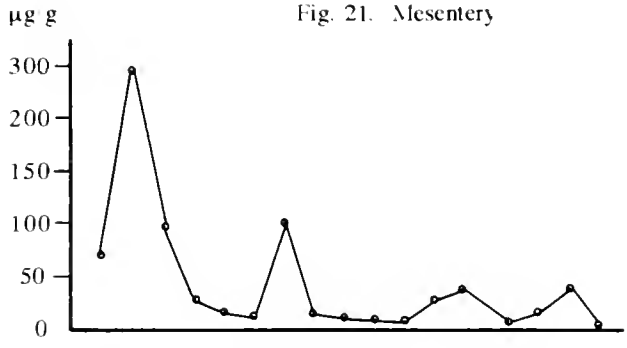


Fig. 21. Mesentery

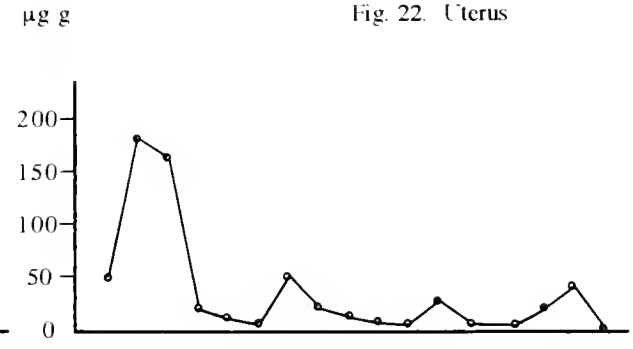


Fig. 22. Uterus

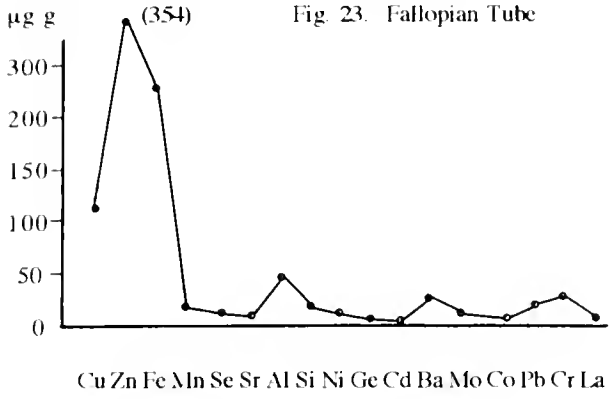


Fig. 23. Fallopian Tube

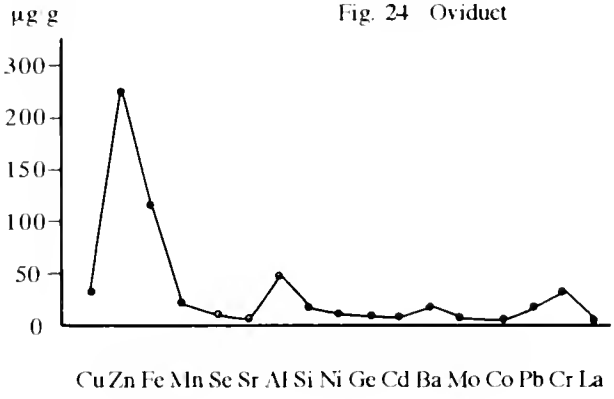


Fig. 24 Oviduct

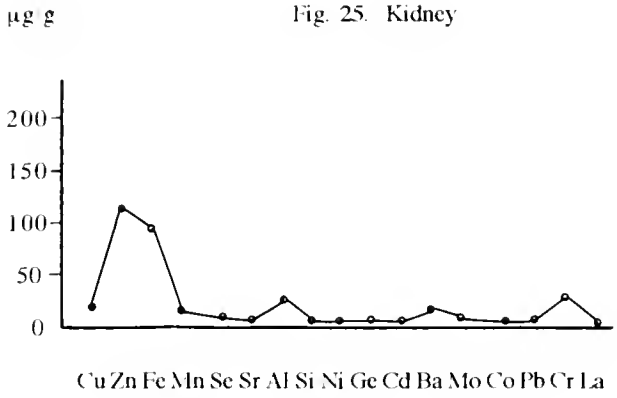


Fig. 25. Kidney

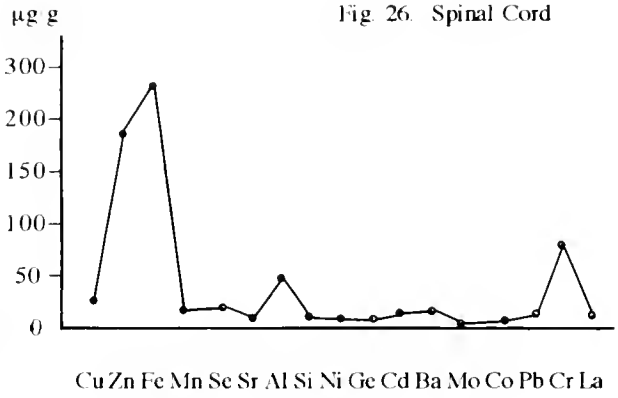


Fig. 26. Spinal Cord

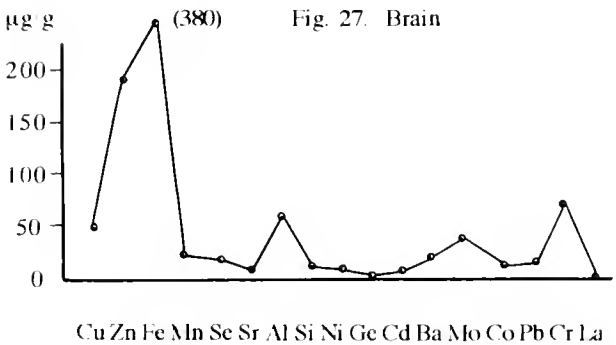


Fig. 27. Brain

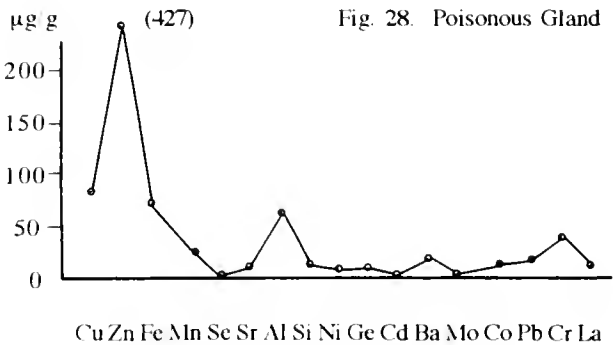


Fig. 28. Poisonous Gland

elements have a close relationship to the growth and development, the metabolic process, the enzyme synthesis and reproduction. But, how the trace elements take part in the regulation of physiological and biochemical functions and which is the essential or non-essential element needs to be further studied.

There contains much more Al (just less than Zn and Fe) in most organs and tissues, especially in the skin; and the Al contents in the heart, lung, spleen etc. are higher than those in the kidney and digestive tract. It show that the skin can collect Al. The authors thought that the skin of *A. b. brevicaudus* may be the place to store and excrete Al. It is known that Al is harmful to humans and mammals, but what is the reason for the high Al content in the body of the snake is still a question to be solved.

The characteristics of element graphs in bile is the balanced content of most elements, and there contain more Cu, Cr, Ge, Pb, Sr, Cd and Mo than those in other the organs and tissues. The ratio of Cu/Zn (2.00) is the highest among the organs. The results may be due to the concentrating mechanism of bile.

In addition to morphological differences, there are also changes in metabolic activity between species due to the divergence of the genetic constitution, ecological surroundings and living habits. So, the authors suggest that trace element detecting is a way to help classify the pit-vipers.

Literature Cited

XU, K. M., Y. K. ZHAO, J. LIN, AND J. L. LI. 1993. Studies on the interspecific differences in the distribution of trace elements in various organs between *Agkistrodon shedaoensis* Zhao and *Agkistrodon saxatilis* Emelianov. Pp. 68-74. *In* Proceedings of the First Asian Herpetological Meeting (eds. Zhao, E. M., B. H. Chen, and T. J. Papenfuss). China Forestry Press, Beijing. [In Chinese].

ZHAO, E. M. AND K. ADLER. 1993. Herpetology of China. SSAR in cooperation with CSSAR, Contributions to Herpetology 10. 522 pp.

A Study on Morphological Similarity between the Genera *Nanorana* and *Altirana* (Amphibia, Anura, Ranidae)

SHUN-QING LU AND DA-TONG YANG

Kunming Institute of Zoology, Academia Sinica, Kunming, China

Abstract. -Through Wilk's stepwise discriminant analysis, 16 of 18 indices of *Nanorana ventripunctata, N. pleskei*, and *Altirana parkeri* were selected and used in a numerical taxonomy study with their weights given as the following formula: W=Cx1/U. The result of clustering analysis of the Euclidean Distances between the three species reveals that *N. ventripunctata* is more similar to *A. parkeri* than to *N. pleskei* in morphology.

Key words: Amphibia, Anura, Ranidae, *Nanorana, Altirana*, China, Transhimalaya Mountains, stepwise discriminant analysis, numerical taxonomy.

TABLE 1. Number, locality, and altitude of species used.

Species	Groups	Number		Altitude
N. ventripunctata	1	10 M, 10 F	Zhongdian, Yunnan	3350 m
N. pleskei	2	10 M, 10 F	Kangding, Sichuan	3260 m
A. parkeri	3	10 M, 10 F	Bashu, Xizang	4100 m

Introduction

Nanorana ventripunctata, N. pleskei, and *Altirana parkeri* are three species of frogs in two genera that are distributed in the Transhimalaya Mountains of China. Except for morphological identification and chromosome research, we know of no other studies on these frogs that has been published.

Nanorana and *Altirana* have a close relationship (Su et al, 1985: Hu et al, 1986), and some distinguishing characters between them are vague since the discovery of *N. ventripunctata* (Fei and Huang, 1985). It is necessary to reexamine the two genera. In this paper, based on 18 external morphological indices, the authors use stepwise discriminant analysis and numerical taxonomy to compare the three species.

Materials and Methods

The number, locality, and altitude of the specimens used are shown in Table 1.

Nineteen external morphological characters were measured from each specimen, and changed to eighteen ratios, i.e. 18 indices: HEL (head length)/SVL (snout vent length), HEW (head width)/SVL, SNL (snout length)/HEW, BND (distance between noses)/HEL, BED (distances between eyes)/HEL, ELW (eyelid width)/HEL, EYD (eye diameter)/HEL, FED (distance between front angles of eyes)/HEL, AHL (hand and front arm length)/SVL, ARW (front arm width)/HEL, HAL (hand length)/SVL, SVL/LFL (leg length, TIL (tibia length)/HEL, TIW (tibia width)/HEL, TFL (tarsalia and foot length)/TSVL, FOL (foot length)/SVL, NSL (length from nose to the top of snout)/HEL, and SPN (snout process length)/HEL.

Results

After stepwise discriminant analysis, sixteen of the 18 indices were selected, their Wilk's statistic measure U (from the first step of the stepwise discriminant analysis) are shown in Table 2.

The weights of the 16 indices are given by the following formula:

$$\frac{W=Cx1}{U}$$

TABLE 2. Selected indices and their U after discriminant analysis.

Indices	TIL/SVL	FED/HEL	HAL/SVL	HEL/SVL	HEW/SVL	SPN/HEL	NSL/HEL	ELW/HEL
U	0.1903	0.2766	0.4200	0.7509	0.7937	0.5534	0.8152	0.6283
Indices	ARW/HEL	SVL/LEL	TFL/SVL	BED/HEL	EYD/HEL	AHL/SVL	FOL/SVL	SNL/HEL
U	0.8488	0.2948	0.5800	0.3349	0.9677	0.5032	0.7438	0.9453

TABLE 3. The weights of the 16 indices.

Indices	TIL/SVL	FED/HEL	HAL/SVL	HEL/SVL	HEW/SVL	SPN/HEL	NSL/HEL	ELW/HEL
W	0.5254	0.3615	0.2381	0.1332	0.1260	0.1807	0.1227	0.1592
Indices	ARW/HEL	SVL/LEL	TFL/SVL	BED/HEL	EYD/HEL	AHL/SVL	FOL/SVL	SNL/HEL
W	0.1178	0.3392	0.1724	0.2986	0.1033	0.1987	0.1344	0.1058

TABLE 4. The matrix of weighted measures of the 16 indices.

Indices	TIL/SVL	FED/HEL	HAL/SVL	HEL/SVL	HEW/SVL	SPN/HEL	NSL/HEL	ELW/HEL
1	0.2051	0.1666	0.0552	0.0356	0.389	0.0297	0.0370	0.0465
2	0.2155	0.1449	0.0600	0.0389	0.406	0.0209	0.0371	0.0389
3	0.2128	0.1480	0.0622	0.0367	0.417	0.0227	0.0364	0.0398
Indices	ARW/HEL	SVL/LEL	TFL/SVL	BED/HEL	EYD/HEL	AHL/SVL	FOL/SVL	SNL/HEL
1	0.0461	0.2492	0.1131	0.0637	0.0460	0.0785	0.0644	0.0556
2	0.0451	0.2396	0.1144	0.0590	0.0451	0.0821	0.0652	0.0535
3	0.0455	0.2448	0.1161	0.0683	0.0457	0.0858	0.0627	0.0548

TABLE 5. The Euclidean Distances among the three species. Unit: 10^{-3}

	Nanorana ventripunctata	*Nanorana pleskei*	*Altirana parkeri*
1	0	0.8868	0.1707
2	0.8868	0	0.8787
3	0.1707	0.8787	0

Here C is a coefficient used to regulate the size of weight, which is given according to the condition, and U is the Wilk's statistic measure. Its calculating formula is: $U = |W|/|T| = |W|/(|W| + |B|)$, where W is the variance in group, B is the variance between groups, T is the total variance. This formula reveals that the smaller the U, the more important the index. So the weighting formula used in this paper is agreeable with the weighting principles. In addition, it has some merits when compared to other weighting formulas used in the literature: 1) No negative weights appear; 2) As a measure of the importance of characters, U has been accepted commonly, and as a measure of weights, it may be accepted easily; 3) Convenient for calculation.

The calculated weights of the 16 indices are shown in Table 3.

Multiplying the measures of the 16 indices with their weights, a numerical matrix is given as shown in Table 4.

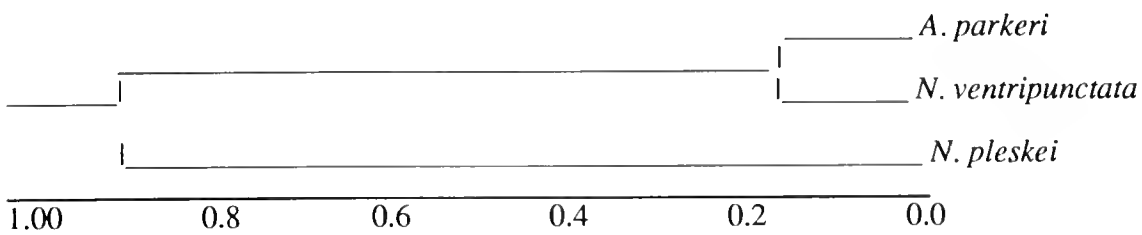


FIG. 1. The UPGMA phenogram of the three species based on Table 5.

Table 6. Some identification characters between the three species.

Nanorana pleskei	*N. ventripunctata* and *Altirana parkeri*
tympanum under skin, but visible; columella exists	tympanum and columella absent
nasals separate, not connected with frontal-parietal	nasals connected with each other and connected with frontal-parietal
precoracoid ossified incompletely	precoracoid ossified completely
clavicle short, not attach epiconicoid	clavicle long, attach epiconicoid
the first low labial teeth of tadpole shorter than the second obviously	the first low labial teeth of tadpole slightly shorter than the second

The Euclidean Distance is selected in this paper to measure the morphological differences between the three frogs. The formula is: $D_{ij}= \sqrt{\Sigma(X_{ik}-X_{jk})^2}$. The calculated Euclidean distances among the three frogs are shown in Table 5.

Figure 1 detects that the distance between *N. ventripunctata* and *A. parkeri* is the shortest. The two frogs meet together at the distance 0.1703, then they meet with *N. pleskei* at the distance of 0.8827. The morphological similarity of *N. ventripunctata* and *A parkeri* is closer than that of *N. ventripunctata* and *N. pleskei*.

Discussion

Up to the present, the differences between the genera *Nanorana* and *Altirana* reported on by Tian and Jiang (1986) contained the most details. But the genus *Nanorana* as they meant, did not contain *N. ventripunctata*, so it was just the differences between *N. pleskei* and *A. parkeri* that they noted.

The characters of *N. ventripunctata* show that this species is more similar to the genus *Altirana* than to the genus *Nanorana* as shown in Table 6. The numerical taxonomy research of this paper and the biochemical systematic study (Lu and Yang, 1994) show the same results. It seems logical to take *ventripunctata* out of genus *Nanorana* and place it in genus *Altirana*, but the biochemical systematic study reveals that the Nei's (1972) genetic distances between the three frogs are 0.30, 0.57, 0.57, respectively, smaller than 1.05 obviously, but larger than 0.15. We feel that these differences are at the species level, not the generic level (Thorpe, 1983). Thinking of the principle: in order to avoid more monogenera, the interruption of a genus with other genera should be anti-relative

with the size of the genus, i.e. the number of species contained in this genus, the authors suggest that the genus *Altirana* be cancelled and the species *parkeri* be placed in the genus *Nanorana*..

Acknowledgments

We are grateful to Mr. Dingqi Rao for collecting specimens together with one of us (Lu). Thanks also due to Mr. Ruliang Pan and Fahong Yu for offering us the computer program for stepwise discriminant analysis.

Literature Cited

FEI, L. AND Y. Z. HUANG. 1985. A new species of the genus *Nanorana* (Amphibia Ranidae) from northwestern Yunnan, China. Acta Biologica Plateau Sinica 1985(4):71-75. (In Chinese).

HU, Q. X., Y. M. JIANG, AND E. M. ZHAO. 1985. Studies on the influence of the Hengduan Mountains on the evolution of the amphibians. Acta Herpetologica Sinica 1985, 4(3):225-233. (In Chinese).

LU, S. Q. AND D. T. YANG. 1994. A study of relationships among ranid frogs of the genera *Nanorana* and *Altirana* in the Transhimalaya Mountains of China. Asiatic Herpetological Research. (In Press).

SU, C. Y., D. T. YANG, AND S. Q. LI. 1986. Studies on vertical distribution of Amphibians in the middle section of the Hengduan Mountains. Acta Herpetologica Sinica 1986, 5(2):134-144. (In Chinese).

THORPE, J. P. 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. Pp. 131-152. *In* G. S. Oxford and D. Rollonson (Eds.), Protein polymorphism: Adaptive and taxonomic significance. Academic Press, London.

TIAN, W. S. AND Y. M. JIANG (EDITORS), ASSISTED BY G. F. WU, Q. X. HU, E. M. ZHAO, AND Q. Y. HUANG. 1986. Identification manual for Chinese species of amphibians and reptiles. Science Press, Beijing. 164 pp. (In Chinese).

A Study of Relationships among Ranid Frogs of the Genera *Nanorana* and *Altirana* in the Transhimalaya Mountains of China

SHUN-QING LU AND DA-TONG YANG

Department of Vertebrate Zoology, Kunming Institute of Zoology, Academia Sinica, Kunming, Yunnan, China

Abstract. *-Nanorana ventripunctata, N. pleskei* and *Altirana parkeri* were examined electrophoretically to investigate the intraspecific genetic relationships. Twelve isozyme loci were assayed and their allele frequencies were calculated. The result of UPGMA clustering, when corrected by the Present-Day Ancestor Method and based on the allele frequencies, detected that the genetic relationship between *N. ventripunctata* and *A. parkeri* is closer than that between *N. ventripunctata* and *N. pleskei*. The authors suggest that the genus *Altirana* should be canceled and that *A. parkeri* be placed in the genus *Nanorana*.

Key words: Anura, Ranidae, *Nanorana, Altirana*, genetic relationships, isozyme, China, Transhimalaya Mountains.

TABLE 1. The location, altitude, date and number of specimens collected.

Species	Locality	Altitude	Date	Number
N. ventripunctata	Zhongdian, Yunnan	3100 m	July 11, 1990	15
N. pleskei	Kangding, Sichuan	3260 m	August 7, 1990	16
A. parkeri	Bashu, Xizang	4100 m	July 4, 1990	15
R. shuchinae	Deqing, Yunnan	3950 m	July 7, 1990	1
R. chensinensis	Mangkang, Xizang	3700 m	July 1, 1990	15

Introduction

The genera *Nanorana* and *Altirana* include three species, which are distributed in the Transhimalaya Mountains of China. They are considered to be closely related, and some identification characters between the two genera have been vague since the description of *N. ventripunctata* (Fei and Huang, 1985). Except for some morphological identification and chromosome studies, there are no other studies published on these genera. In order to re-study the two genera, the authors here use starch gel electrophoresis on extracts from liver and muscle to determine genetic distances in order to better understand the genetic relationships among the three species of frogs.

For comparison, the species of *Rana shuchinae* and *R. chensinensis* were selected as out-groups. Part of the distribution of these two frogs is the same as *Nanorana* and *Altirana*.

Materials and Methods

The collecting locality, altitude, date and number of living specimens of the five species are shown as in Table 1.

The specimens were killed in the field, the liver and thigh muscle of each specimen were taken and placed in 1.5 ml plastic micro centrifuge tubes with several drops of physiological saline, then preserved in liquid nitrogen, and taken back to the laboratory.

Tissues were washed with distilled water and physiological saline, the volume ratio is tissue: physiological saline = 1:1.5, homogenized and centrifuged. These samples were run in horizontal starch gels using gel buffers described by Pasteur et al (1988) as follows: Tris-Borate-EDTA (pH 8.6), Tris-Citrate (pH 6.7). Isozyme stains used were also described by Pasteur et al (1988). The following enzyme systems were stained: alcohol-dehydrogenase

TABLE 2. Allele frequencies detected at polymorphic isozyme loci in the five species.

Locus	Allele	*N. ventripunctata*	*N. pleskei*	*A. parkeri*	*R. shuchinae*	*R. chensinensis*
ADH-1	a		1.0000		1.0000	1.0000
	b			1.0000		
	c	1.0000				
ADH-2	a			0.1667		
	b	0.1000		0.1250		
	c	0.4333	0.5000	0.2917		0.1667
	d	0.0667	0.3125	0.4167		0.8333
	e	0.2667	0.1875		0.5000	
	f	0.1333			0.5000	
EST-2	a					0.6250
	b		0.5714	0.1818	1.0000	0.2500
	c	0.3077	0.3571	0.8182		0.1250
	d	0.6923	0.0715			
EST-3	a		0.4286	0.5000	0.5000	0.2000
	b	0.5000	0.5714	0.5000	0.5000	0.4333
	c	0.5000				
	d					0.3667
EST-4	a	0.5667	0.0667	0.1786		0.4000
	b	0.4333	0.9333	0.8214	1.0000	0.6000
GLC-1	a			0.1250		
	b	0.0357	0.7778	0.6250		0.3750
	c	0.5714	0.2222	0.2500	1.0000	0.3333
	d	0.3929				0.2083
	e					0.0833
LDH-1	a	1.0000	1.0000	1.0000		0.0833
	b				1.0000	0.4167
	c					0.5000
LDH-2	a	1.0000	1.0000	1.0000		
	b				1.0000	
	c					1.0000
MDH-1	a	1.0000	0.0333	0.0667		
	b	0.4333	0.2667	0.6000	0.5000	0.3125
	c	0.3667	0.3000	0.1333		0.3125
	d	1.0000	0.3333	0.1000	0.5000	0.3750
	e		0.0667	0.1000		
MDH-2	a			0.3636		0.5000
	b		0.2333	0.0808	0.5000	0.1250
	c	0.0769	0.4333	0.5000	0.5000	0.3333
	d	0.8077	0.3333	0.0808		0.0417
	e	0.1154		0.0808		
MOD-1	a	0.1250	0.6250	0.2500		
	b	0.0833	0.3750		1.0000	0.4000
	c	0.7917		0.2500		
	d			0.5000		0.6000
SDH-1	a	0.2500	0.2500	0.6250		
	b	0.1000	0.5000	0.3750	0.5000	0.3333
	c	0.1500	0.2500		0.5000	
	d					0.5000
	e	0.4000				0.1667
	f	0.1000				

TABLE 3. Genetic distances and similarities among the five frogs, based on allozyme data in Table 2. Nei's (1972) genetic distances above diagonal, and genetic similarities below diagonal.

	N. ventripunctata	*N. pleskei*	*A. parkeri*	*R. shuchinae*	*R. chensinensis*
1		0.5709	0.5693	1.4414	1.3817
2	0.5650		0.2979	0.5793	0.6224
3	0.5659	0.7424		1.1775	0.8730
4	0.2366	0.5603	0.3080		0.6559
5	0.2512	0.5367	0.4178	0.5900	

TABLE 4. The corrected distances with *R. shuchinae* as the present-day ancestor.

	N. ventripunctata	*N. pleskei*	*A. parkeri*
N. ventripunctata		-1.4498	-2.0496
N. pleskei			-1.4589
A. parkeri			

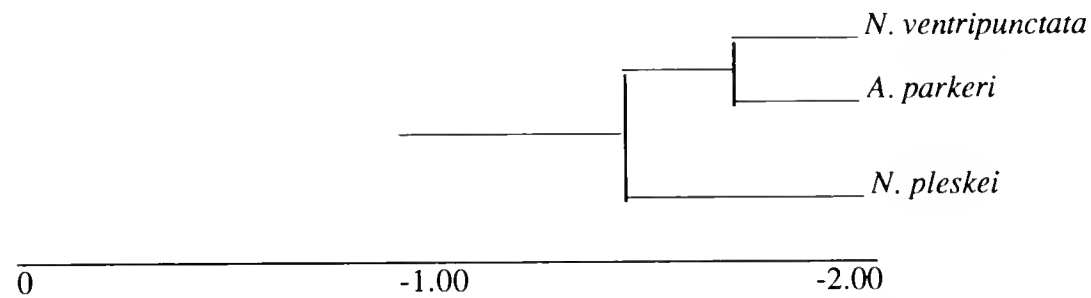


FIG. 1. The UPGMA phenogram of corrected distances with *R. shuchinae* as the present-day ancestor.

(ADH, Ec 1.1.1.1). esterase (EST, Ec 3.1.1.1), NAD-glucose-dehydrogenase (GLC, Ec 1.1.1.1), lactate-dehydrogenase (LDH, Ec 1.1.1.27), malate-dehydrogenase (MDH, Ec 1.1.1.37) malic enzyme (MOD, Ec 1.1.1.40), sorbitol-dehydrogenase (SDH, Ec 1.1.1.14).

Results

Twelve isozyme loci were resolved and scored. Their allele frequencies are shown as in Table 2.

From Table 2, the Nei's (1972) genetic distances and similarities among the five species was calculated and is shown in Table 3.

It is obvious from Table 3 that the evolutionary rates of the five species are unequal, so it is necessary to make a correction before UPGMA clustering. The Present-Day Ancestor Method (Li, 1987) was selected in this paper, the correcting formula is: $D'ij=Dij-Djx$, here $D'ij$ is the corrected distance, the Dij is the original distance, x represents the supposed present-day ancestor. At first, with *R. shuchinae* from the out-group selected as the present-day ancestor, the corrected distances among the three frogs in the genera *Nanorana* and *Altirana* are are shown in Table 4.

The UPGMA clustering phenogram of the corrected distances among the three species in Table 4 is shown in Figure 1.

When *R. chensinensis* is selected as the present-day ancestor, the corrected distances are shown in Table 5.

TABLE 5. The corrected distances with *R. chensinensis* as the present-day ancestor.

	N. ventripunctata	*N. pleskei*	*A. parkeri*
N. ventripunctata		-1.4332	-1.6854
N. pleskei			-1.1975
A. parkeri			

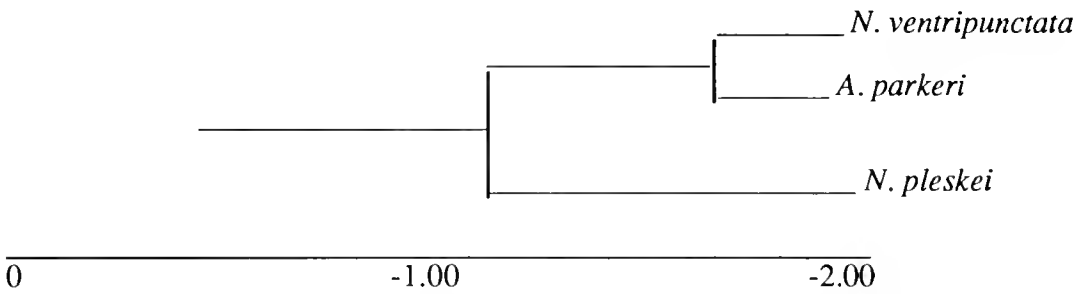


FIG. 2. The UPGMA phenogram of corrected distances with *R. chensinensis* as the present-day ancestor.

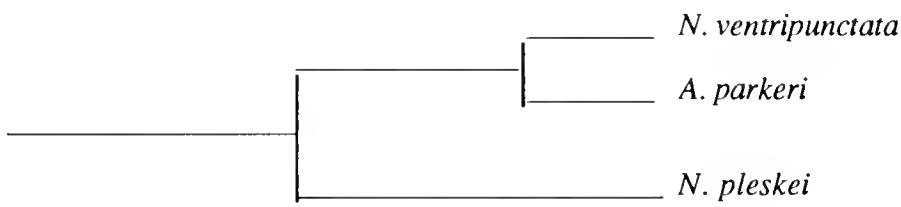


FIG. 3. The genealogical phenogram among the three species.

Based on Table 5, we prepared a UPGMA phenogram which is shown in Figure 2.

Not minding the difference of distances among the frogs, we find that they are similar in both Figure 1 and Figure 2, though they are based on the different present-day ancestors, so we synthesized and simplified them as shown as in Figure 3.

Figure 3 shows that the genetic relationship between *N. ventripunctata* and *A. parkeri* is closer than that between *N. ventripunctata* and *N. pleskei*.

Discussion

Up to the present, the differences between *Nanorana* and *Altirana* described by Tian and Jiang (1986) are the most detailed, but the genus *Nanorana* of their meaning does not contain *N. ventripunctata*. It is just *N. ventripunctata* that confuses the distinction between the two genera, and the study of morphological similarities among the three species of the two genera shows the same result that *N. ventripunctata* and *A. parkeri* are more similar than *N. ventripunctata* and *N. pleskei* (Lu and Yang, 1994). Both of the results of biochemical systematics and morphological similarity studies do not support the presently recognized generic assignments and we suggest that *N. ventripunctata* should be taken out of the genus *Nanorana* and placed in the genus *Altirana*.

From Table 3, we know Nei's (1972) genetic distances among the three species are 0.5709, 0.5693 and 0.2979. This is larger than 0.15, but much smaller than 1.05. These differences are at the species level,

but not the generic level (Thorpe, 1983. Also, we know that there is a principle: in order to avoid more mongenera, the interruption of a genus with other genera should be anti-relative with the number of species in this genus. Thinking of these and the vague line between *Nanorana* and *Altirana*, according to the principle of priority of the International Code of Zoological Nomenclature, the authors suggest that it is perfect to cancel the genus *Altirana*, and that the species *parkeri* should be placed in the genus *Nanorana*.

Acknowledgments

We thank Mr. Dingqien Rao for collecting specimens with one of us (Lu) and his help in the laboratory. Thanks also are given to Professor Jingyan Li for his suggestions concerning the Present-Day Ancestor Method.

Literature Cited

FEI, L. AND Y. Z. HUANG. 1985. A new species of the genus *Nanorana* (Amphibia Ranidae) from northwestern Yunnan, China. Acta Biologica Plateau Sinica 1985(4):71-75. (In Chinese).

HU, Q. X, Y. M. JIANG, AND E. M. ZHAO. 1985. Studies on the influence of the Hengduan Mountains on the evolution of the amphibians. Acta Herpetologica Sinica 1985, 4(3):225-233. (In Chinese).

LI, J. Y. 1987. The essence of the Present-Day Ancestor Method for constructing evolutionary trees from sequence data and the puzzle encountered in applying this method. Zoological Research 9(2):141-150. (In Chinese).

LU, S. Q. AND D. T. YANG. 1994. A study on morphological similarity between the genera *Nanorana* and *Altirana* (Amphibia, Anura, Ranidae). Asiatic Herpetological Research. In Press.

PASTEUR, N. 1988. Manual technique de genetrique par electrophorese des proteines. Lavoisier TEC & DOC., Paris. 215pp.

THORPE, J. P. 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. Pp. 131-152. *In* G. S. Oxford and D. Rollonson (eds.), Protein polymorphism: Adaptive and taxonomic significance. Academic Press, London.

TIAN, W. S. AND Y. M. JIANG (EDITORS), ASSISTED BY G. F. WU, Q. X. HU, E. M. ZHAO, AND Q. Y. HUANG. 1986. Identification manual for Chinese species of amphibians and reptiles. Science Press, Beijing. 164 pp. (In Chinese).

Fibrinogenase from the Venom of *Trimeresurus mucrosquamatus*

JIAN-PING MAO[1], WAN-YU WANG[1], YU-LIANG XIONG[1] AND LIANG LU[2]

[1]Kunming Institute of Zoology, Academia Sinica, Kunming, 650223 China
[2]Yunnan Provincial Hospital, Kunming, 650021, China

Abstract. -A new fibrin(ogen)olytic protease (FP) was purified from the venom of *Trimeresurus mucrosquamatus* by DEAE-Sephadex A50, Sephadex-G75, CM-Sepharose CL-6B and mono-s (FPLC) column chromatography. The molecular weight was 22,000 Da and the isoelectric point was 9.2. It was a glycoprotein composed of 194 amino acid residues. FP could hydrolyze casine, finbrin, fibrinogen and also showed hemorrhagic subcutaneously, no phospholipase A activity, arginine esterase activity which existed in the crude venom. The enzyme could be inhibited by ethylenediamine tetra-acetate (EDTA) and cysteine, but not by phenylmethyl sulfonyl fluoride (PMSF). FP cleaved the Bß-chain of fibrinogen first following the Aa-chain. In vivo, thrombolytic activity was tested on artificial thrombus placed in the cerebral artery of rabbits. Thrombolysis was then characterized by angiographic techniques over several intervals. The fibrinolytic activity resulted in thrombolytic recanalization of two dosage groups. Of four rabbits of 0.2 mg/kg, one achieved recanalization in 12 hrs. and three in 24 hrs. Of another four under the dosage of 0.4 mg/kg, three recanalized successfully in 5 hrs. and one in 9 hrs.

Keywords: Venom, Fibrin(ogen)olytic Protease, Thrombolysis.

Introduction

Fibrinolytic and fibrinogenolytic activity had been described in the venoms of a number of snake species, including members of the Crotalinae, Viperinae, and Elapidae families (Ouyang and Teng, 1976; Willis et al., 1988; Daoud et al., 1987; Evans and Barrett, 1988). The fuibrin(ogen)olytic enzymes in snake venoms had also been reviewed previously (Seegers and Ouyang, 1979; Hellmann, 1968; Markland, 1988; Markland, 1991). Several fibrin(ogen)olytic enzymes were isolated from the venom of *Trimeresurus mucrosquamatus*: two fibrinogenases (Ouyang and Teng, 1976), two hemorrhagic principals HT-a and HT-b (Nikai et al., 1985) and three proteinases (Sugihara and Mori, 1985). Willis, et al. (1989) evaluated the thrombolytic potential of anticoagulant proteases in *Crotalus atrox* venom using rats. In the present study, we purified a new fibrinogenase from the venom of Chinese habu snake and studied its characteristics.

Materials And Methods

Lyophilysed Trimeresurus mucrosquamatus venom was obtained from Yuanlin Farm (Hunan, China) and stored at -20°C. Human thrombin was purchased from Shanghai Hospital. Fibrinogen, BAEE (N- benzoyl- L- arginine ethyl ester), PMSF were from the Shanghai Institute of Biochemistry, Academia Sinica. Urokinase (UK) came from Nanjing University. DEAE-Sephadex A50, Sephadex-G75, CM-Sepharose CL-6B and mono-s HR5/5 (FPLC) were purchased from Phrmacia Fine Chemicals (made in Uppsala, Sweden). The other chemicals used were analytical grade from commercial sources.

Isolation Procedure: Isolation of FP was achieved by a combination of gel filtration and ion-exchange chromatography at 4°C (Fig. 1). One gram of crude venom was dissolved in 5 ml of 50 mM Tris, pH 8.8. The insoluble material was removed by centrifugation (2000 g) for 10 min. The supernatant was fractionated thus: first, on DEAE-A50 (3 X 100 cm), 50 mM Tris-HCl pH 8.5; second, Sephadex -G75 (2 X 100 cm), 20 mM Tris-HCl pH 7.5; third, CM-Sepharose CL-6B (2 X 30 cm), 10 mM

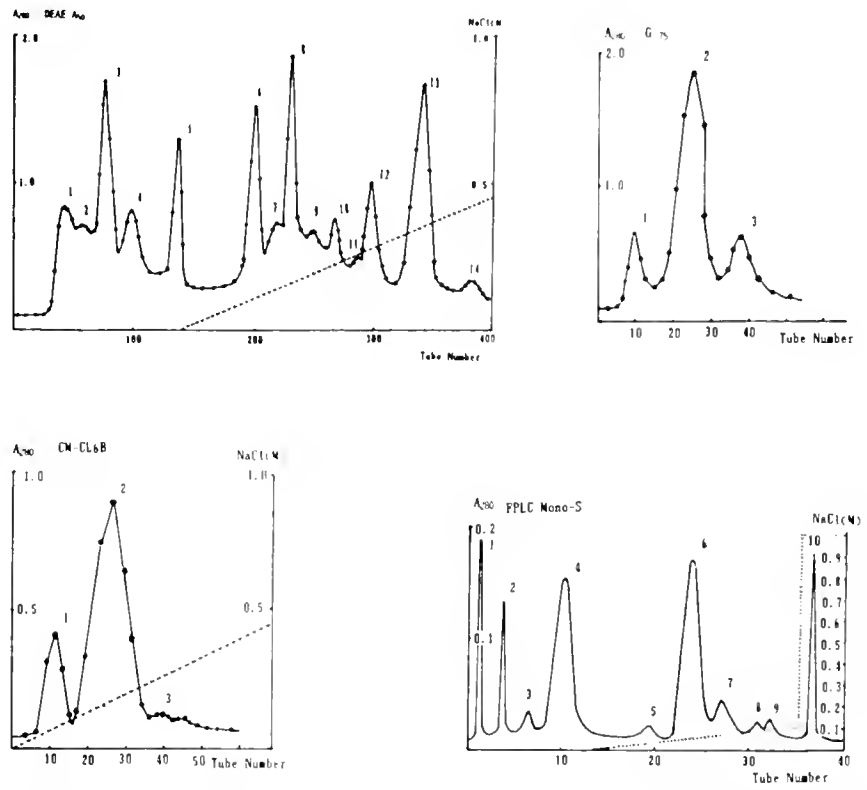


FIG. 1. Fractionation of *Trimeresurus mucrosquamatus* venom. 1st, DEAE-A50 (3X100 cm) anion exchanging, 50mM Tris-HCL pH 8.5; 2nd, Sephadex G-75 gel filtration (2X100 cm), 10mM ammonium acetate pH 7.0; 4th, mono-s cation exchanging, 10mM sodium acetate pH 5.8.

ammonium acetate pH 7.0; and fourth, on mono-s HR 5/5, 10 mM sodium acetate pH 5.8.

Characterization of FP: Assay for hemorrhagic activity assay for gross observation was performed as reported previously (Bjarnason and Fox, 1983). Proteolytic activity was assayed by a method using casine of Kunitz (1947). The inhibition of EDTA, Cysteine, and PMSF was also tested with this method.. Fibrinogenase activity was measured by the method of Ouyang and Huang (1979). Fibrinolytic activity was tested with the fibrin plate method of Astrup and Mullertz (1952), and also with fibrin clot from fibrinogen with thrombin. Arginine ester hydrolytic activity was assayed using BAEE as a substrate. BAEE 50 mM (containing 1 mM CaCl2) was prepared with 50 mM Tris-HCl (pH 8.0) buffer. Trypsin was taken as the control. The phospholipase activity was qualitatively assayed with the substrate of yolk. The pH of the substrate was modulated to 8.0; after the fraction was added, the pH decreased by the phospholipase activity, and was adjusted to that of the original by 10 mM NaOH. The values of sodium hydroxide was taken to present the activity. Amino acid compositions were carried on a Model 835-50 Hitachi high speed automatic analyzer by the method of Simpson et al. (1976). Twenty-four h, 48 h hydrolysates were used. Phanolanaline was used as the minimum residue to calculate the number of amino acid residues.

Thrombosis assay by angiography: For FP dosage determination, 0.2 ml rabbit plasma made clot with 3U thrombin, 50μg FP was used to test in vitro activity. In 4.5-5.5 hours, the milky white

TABLE 1. Summary of purified FP from *T. mucrosquamatus* venom (n=6).

	Fraction in step				
	Crude venom	DAEA-A50 3	G-75 2	CMCL-6B 2	mono-s 6
Recovered protein (mg)	1000	107	93.1	72.6	19.1
Hemorrhage (mm X mm) (25±3 gm mice 100 μg sample)	16.3	6.25	7.20	7.80	8.64
Caseinolytic activity Units/mg.min	0.64	0.42	0.46	0.58	0.48
Fibrinolytic activity (Fibrin heated plate mm^2)	270	225	240	288	216
Arginie esterase activity	+	-	-	-	-
Phospholipase A activity	+	-	-	-	-

TABLE 2. Effects of chemical factors on FP proteolytic activity (n=6).

%	Na+	Ca++	Mg++	Cu++	Zn++	Al+++	Fe+++	Co6+	EDTA	Cyst.	PMSF
Acti.	100	180	115	230	335	305	310	390	0.1	0.1	98

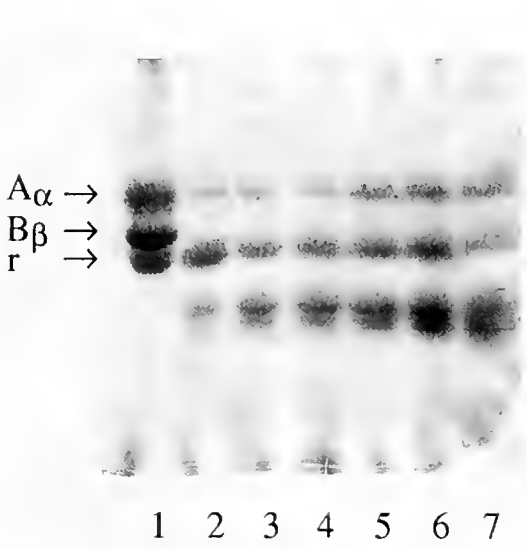


FIG. 2. SDS-PAGE of reduced fibrinogen after incubation with FP (10ug) lane 1; fibrinogen; lane 2-7; fibrinogen with FP for 5, 10, 20, 30; 45; and 60 min. respectively.

clot could become clear. Thrombolytic activity by FP was tested on artificial thrombus catheterized in the rabbit. Sixteen rabbits, ranging from 2.2 to 2.7 kg, were used. 0.5 ml of blood was drawn from the ear vein of the rabbits and made thrombus as 20 mm (long) X 1.00 mm (diameter). The rabbits were then anesthetized with an intravenous injection of a ketamine at a dosage of 2 mg/kg weight. The left common carotid artery was isolated, and a polythylene catheter (1.00 ID X 1.40 OD) was inserted into the artery. By digital subtraction angiology (DSA, Angiotran cmp, Siemens, Germany), normal angiographic was recorded with angiografin solution (meglucamine diatrizoate 65% 30 ml X "Schering AG", made in Germany) of 3 ml. The thrombus was catheterized into the artery, then the thrombosis graph was recorded. Thirty minute after the thrombus induction, the 16 rabbits, separated into four groups, were injected with saline, U.K. 1,000 IU/kg weight, 0.2 mg FP/kg weight, 0.4 mg FP/kg weight transcatheterically respectively, then the four angiogrames were taken after 5, 9, 12 and 24 hours.

Results

Fourteen fractions were achieved after DEAE-Sephadex A50 chromatography, and three fractions, which contained fibrinolytic activity, were further fractionated. Fraction 3 was equilibrated with 20 mM Tris-HCl buffer (pH 7.5) by dialysis and loaded onto the Sephadex-G75 column. Fraction 2

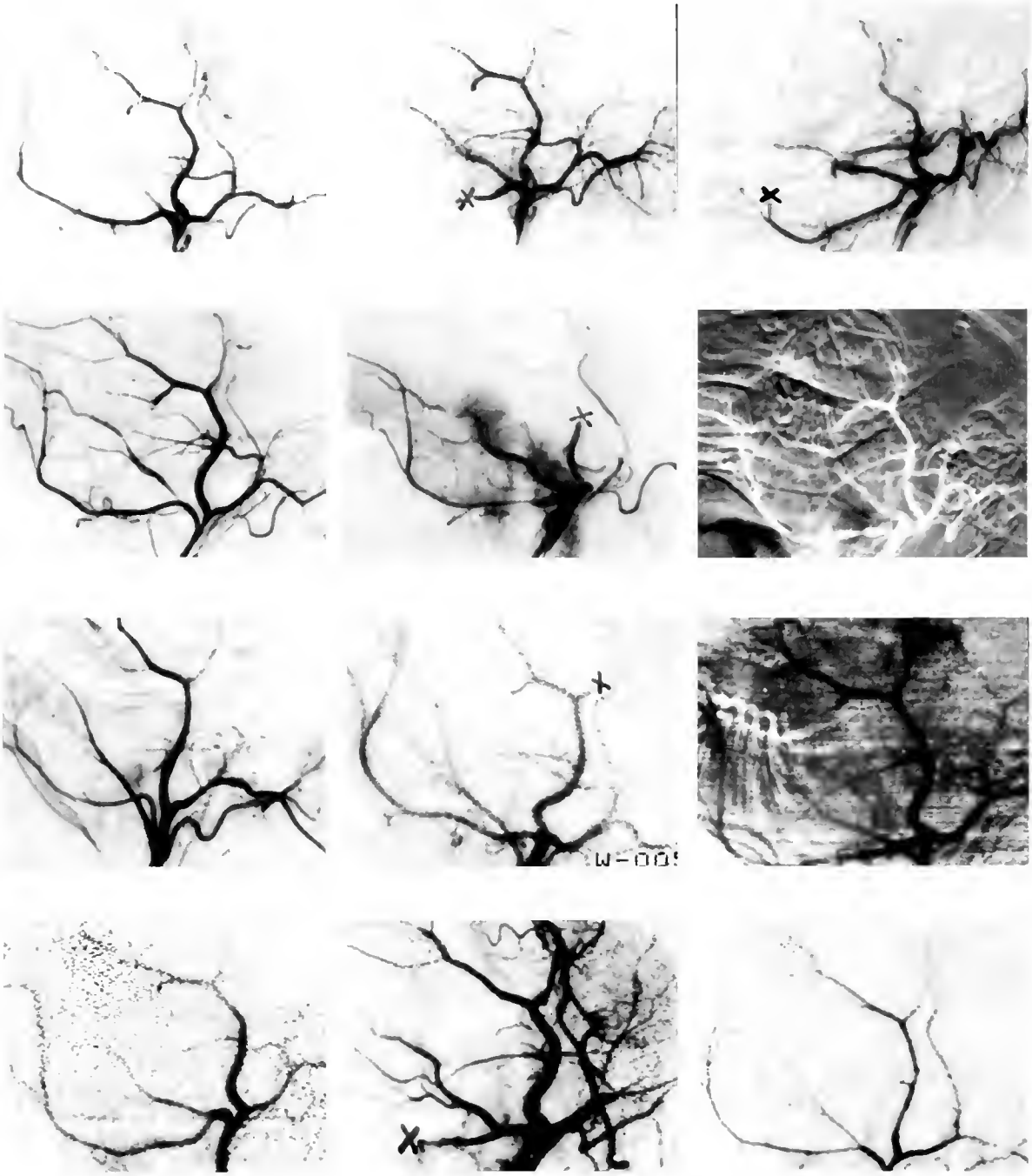


FIG. 3. Aniographs of in vivo thrombolysis (from left). Control group: A., Normal graph. B, Thrombosis performed (X). C, Treated with saline, 24 hours. FP group: D and G, Normal graphs. E and H, Thrombosis performed (X). F, Treated with FP at 0.2 mg/kg dosage, recanalization occured in 24 hrs. I, Treated with FP at 0.4 mg/kg dosage, recanalization occured in 5 hrs. U. K group (positive control): J, Normal graph. K, Thrombosis performed (X). L, Recanalized within 9 hours of U. K administration (1,000 IU/kg).

obtained in this step yielded fibrinolytic activity. This fraction was dialyzed against 10 mM aminium acetate (pH 7.0) and loaded on the CM-Sepharose CL-6B column and was separated into three fractions, the fibrinolytic activity was located in the 2nd, this fraction was rechromatographied on FPLC mono-s column and eluted with three phases of gradient: 0-30 ml (0 M NaCl); 30-80 ml (0-0.1 M NaCl) and 80-100 ml

TABLE 3. Proteases from the venom of *T. mucrosquamatus*.

Amino Acid Resid.	Proteases								
	MuA	ßFP	*a*FP	HTa	HTb	P-1	P-2	P-3	FP*
Asx									28
Asp	86	26	28	12	25	28	29	29	
Thr	36	12	14	4	11	16	14	12	12
Ser	94	13	14	14	28	16	17	15	11
Glx	110	15	21	19	33	15	17	17	21
Pro	27	18	7	2	7	5	1	2	6
Gly	56	24	9	10	25	11	14	13	8
Ala	48	12	9	12	12	8	10	11	6
Val	69	16	16	8	1	16	14	15	14
Met	15	3	6	2	5	6	3	3	0
Ile	29	11	9	3	8	8	9	10	10
Leu	42	19	17	6	15	18	16	16	17
Tyr	41	8	6	6	8	5	8	7	9
Phe	42	6	5	8	12	6	5	5	9
His	20	5	7	4	6	8	7	8	8
Lys	62	11	15	7	16	14	11	12	15
Trp	16	14	8	3	2	2	2	2	2
Arg	30	7	6	5	10	5	7	7	9
$CysSO_3$	39	9	6			8	12	10	9
CmCys				6	15				
NH_4									3
Total	862	229	203	131	239	195	196	194	194

TABLE 4. Proteases from the venom of *T. mucrosquamatus*.

Name	pI	M.W.	A.A.	res. on fibrinogen	Author	Date
*a*fibrinogenase	8.1	22,400	203	A*a*	Ouyang et al	1976
ßfibrinogenase	5.7	26,000	229	Bß	Ouyang et al	1976
Mucrotoxin A	4.3	94,000	862	A*a*, Bß	Sugihara et al	1983
HT-a	4.72	15,000	131	Bß	Nikai et al	1985
HT-b	8.9	27,000	239	A*a*	Nikai et al	1985
P1	8.1	23,000	195	Bß, A*a*	Sugihara et al	1985
P2	9.2	23,500	196	A*a*, Bß	Sugihara et al	1985
P3	9.8	23,000	194	A*a*, Bß	Sugihara et al	1985
FP*	9.2	22,000	194	Bß, A*a*	This study	

(1 M NaCl). The fibrinolytic activity was in the 6th fraction and the principal was qualified as FP.

Properties of FP: The molecular weight of the purified FP was determined to be 22,000 Da by SDS-polyacrylamide slab gel electrophoresis. The isoelectric point obtained by isoelectric focusing disc polyacrylamide gel was 9.2. The enzyme is a glycoprotein as shown by periodic acid-Schiff's agent staining after low pH (pH 4.3) polyacrylamide gel electrophoresis. The amino acid residues of FP was 194, the combined number of Glx and Asx were 49. Yet, the isoelectric point of the proteinase was basic. This enzyme possessed proteolytic activity hydrolyzing casine, fibrin and fibrinogen, but did not have BAEE hydrolase,

phospholipase A activity. The enzyme also had hemorrhagic activity (Table 1).

Heat and pH stability: The proteinase, at a concentration of 40 μg/ml in 10 mM acetate buffer (pH 5.8) containing 10 mM NaCl, were incubated for 30 minutes at various temperatures and then quickly cooled down to room temperature. The caseinolytic activity was then determined. The enzyme was fully active at 37°C, showed little activity at 50°C, and almost lost complete activity at 55°C. Incubation of FP at pH values below 5.0 and above 10.0 for 30 min lead to a sudden decrease in proteolytic activity.

Biological activity: The effects of some reagents on the proteolytic activity of FP were examined. This activity was inhibited by ethylenediamine tetraacetic acid (EDTA), cysteine but not by PMSF. The effects of some divalent and trivalent ions on the proteolytic activity of FP were also assayed. The enzyme (40 μg/ml) and ions at a concentration of 10 mM in 10 mM acetate buffer were first incubated at 37°C for 30 min. before the proteolytic activity was assayed. The proteolytic activity increased in the presence of bivalent ions in the following order: Ca++ < Cu++ < Zn++ < Co++. The increase by Fe+++ and Al+++ were lower than that of Zn++ but higher than that of Cu++, Ca++ (Table 2). When fibrinogen was incubated with FP, this enzyme cleaved the Bb-chain of fibrinogen and followed the Aa-chain as shown in Figure 2. The degrading of fibrinogen by FP was measured as 36.5 mg per mg enzyme in one minute.

Thrombolysis: None of the four rabbits of saline administration reached recanalization. Of the FP 0.2 μg/kg group, one achieved recanalization in 12 hrs. and three in 24 hrs.; of the other four of 0.4 mg/kg, three recanalized successfully in 5 hrs. and one in 9 hrs. Of the four rabbits treated with 1,000 IU/kg of U.K., thrombolytic recanalization occurred in two in 6 hrs. and two in 9 hrs. (Fig. 3).

Discussion

The venom of the Crotalinae species contained much proteinases, which had proteolytic and esterase activities. Several enzymes were isolated from *Trimeresurus mucrosquamatus* venom (Table 3 and 4). Our results showed that FP is a new fibrinogenase existing in *Trimeresurus mucrosquamatus* venom. Compared with the others, this enzyme, is a metallo-proteinase which attacks the Bb-chain of fibrinogen preferentially. As we know, the enzymes which had fibrinogenolytic activity were classified as a-fibrinogenases and b-fibrinogenases; most of the a-fibrinogenases can degrade Aa-chain of fibrinogen and usually are metallo-proteinases. Of most of the b-fibrinogenases degrading the Bb-chain of fibrinogen, little of them could also degrade the Aa-chain inhibited by DFP or PMSF. They are serine proteinases. HT-a, the first example of which hydrolyzed Bb-chain of fibrinogen and was inhibited by EDTA. The followed FP was the second report of these enzymes. Three proteinases from the *Agkistrodon halys blomhoffii* were activated by Ca++ and Co++ (Satake et al., 1963). Ca++ and Zn++ were also needed for the proteolytic activity of protein G from *Bothrops asper* (Ortiz and Gubensak, 1987). FP was activated by Co++ and Zn++. For these metallo-proteinases, these bivalent cations were important for their stability and their activities. FP had marked activity on the plasma clot in vitro, also 0.4 mg FP/kg dosage occurred thrombolysis in 3/4 in vivo. It was a good trial for FP thrombolytic potential. Hemorrhage was caused when injected subcutaneously, but did not occur within the heart, liver, kidney and lung after FP injection in rabbits at the dosage of 1 mg/kg in mice.

Literature Cited

ASTRUP, T. AND S. MULLERTZ. 1952. The fibrin plate method for estimating fibrinolytic activity. Archives of Biochemistry and Biophysics 40:346-349.

BJARNASON, J. B. AND J. W. FOX. 1983. Proteolytic specificity and cobalt exchange of hemorrhagic toxin e, a zinc protease isolated from the venom of the western diamondback rattlesnake *(Crotalus atrox)*. Biochemistry 22:3770-3778.

DAOUD, E., H. Y. HALIM AND F. M. EL-ASMAR. 1987. Further characterization of the anticoagulant proteinases, cerastase F-4, from *Cerastes cerastes* (Egyptian sand viper) venom. Toxicon 25:891-897.

EVANS, J. H., AND A. J. BARRETT. 1988. The action of protease F 1 from *Naja nigricollis* venom on the Aa-chain of human fibrinogen. Pp. 213-222. *In* Pirkle H. and Markland F. S. (eds.). Hemostasis and Animal Venoms. Marcel Dekker, New York.

HELLMANN, K. 1968. Naturally occurring anticoagulants and fibrinolysis. Pp. 254-265. *In* The Scientific Basis of Medicine Annual Reviews. Oxford University Press, New York.

MARKLAND, F. S. 1988. Fibrin(ogen)olytic enzymes from snake venoms. Pp. 149-172. *In* Pirkle H. and Markland F. S. (eds.). Hemostasis and Animal Venoms. Marcel Dekker, New York.

MARKLAND, F. S. 1991. Inventory of *a* and ß-fibrinogenases from snake venom. *In* Hemostasis and Animal Venoms. F. K. Schattauer Verlagsgesellschaft mbH (Stuttgart), 65(4):438-443.

NIKAI, T., N. MORI AND M. KISHIDA. 1985. Isolation and characterization of hemorrhagic factors *a* and ß from the venom of the Chinese habu snake *(Trimeresurus mucrosquamatus)*. Biochimica et Biophysica Acta 838:122-131.

ORTIZ, F. A. AND F. GUBENSAK. 1987. Characterization of a metallo-proteinase from *Bothrops asper* (Terciopelo) snake venom. Toxicon 25:759-763.

OUYANG, C. AND C. M. TENG. 1976. Fibrinogenolytic enzymes of *Trimeresurus mucrosquamatus* venom. Biochimica et Biophysica Acta 420:298-308.

SEEGERS, W. H. AND C. OUYANG. 1979. Snake venoms and blood coagulation. Handb. Exp. Pharm. 52:648-750.

SIMPSON, R. J., M. R. NEUBERGER AND T. Y. LIU. 1976. Complete amino acid analysis of proteins from a single hydrolysate. Journal of Biological Chemistry 25:1936-1940.

SUGIHARA, H. AND N. MORI. 1985. Comparative study of three proteinases from the venom of the Chinese habu snake *(Trimeresurus mucrosquamatus)*. Comparative Biochemistry and Physiology 82B(1):29-35.

WILLIS, T. D. AND A. T. TU. 1988. Purification and characterization of Atroxase. A non-hemorrhagic fibrinolytic protease from western diamondback rattlesnake venom. Biochemistry 27:4769-4777.

WILLIS, T. D., A. T. TU AND C. W. MILLER. 1989. Thrombosis with a snake venom protease in a rat model of venous thrombosis. Thrombosis Research 53:19-29.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 85-96

Digital Pad Morphology in Torrent-living Ranid Frogs

ANNEMARIE OHLER
Laboratoire des Reptiles et Amphibiens, Muséum national d'Histoire naturelle, 25 rue Cuvier, 75005 Paris, France

Abstract. -Digital pads of 24 species of ranoid frogs (Raninae, Dicroglossinae, Ranixalinae, Rhacophorinae, Hyperoliinae) were studied by scanning electron microscopy. In many species of Raninae the cells of the adhesive pad are differentiated (elongated and wearing projections). Functional aspects of cell morphology and digital pad expansion are discussed in relation with sticking condition in aquatic medium.

Key words: Amphibia, Anura, morphology

Introduction

Digital pads occur in most of advanced anuran families. This organ seems to be of multiple origin and of difficult use in systematics (Noble and Jaeckle, 1928; McAllister and Channing, 1982). Digital pads occur in arboreal anurans (*Hyla*), but they can also be observed in torrent-living frogs (*Amolops*), and in some fossorial species (*Kaloula*). In Asian and African frogs of the family Ranidae, several genera and species groups belonging to different subfamilies have fingers and toes bearing digital pads.

There exists no strong hypothesis of phylogeny of ranids as a whole. Phylogenetic analyses were undertaken only for geographic and taxonomic limited groups (Liem, 1970; Clarke, 1981; Hillis, 1985; Emerson and Berigan, 1993). The broadly accepted classification (Frost, 1985) is based on Boulenger's works dating from the beginning of this century (Boulenger, 1882; 1920). Recently Dubois (1986, 1992) tried to review the entire group and proposed a tentative classification which he sees as a working hypothesis. In this hypothesis ranids are split into several families, subfamilies and tribes (Dubois, 1992). Species that are enclosed in the genus "*Rana*" in Frost (1985) are in Dubois' classification distributed in several subfamilies (Table 1).

Results from study of skeleton showed several major lines in "*Rana*" (Deckert, 1938; Clarke, 1981). Study of the morphology of the digital pads (Ohler and Dubois, 1989) confirmed that two of these lines could be distinguished by their digit morphology. Ranines have digital pads with a latero-ventral groove, often separated terminally. Dicroglossines have digital pads showing a dorso-terminal groove.

The histological structures of the digital pads were first described by Schuberg (1895) and Siedlecki (1910). Noble and Jaeckle (1928) undertook a comparative histological analysis of 47 species of anurans. The fine structure of the epidermal cells in the digital pad has been observed by transmission electron microscope (Komnick and Stockem, 1969; Ernst, 1973 a-b). Scanning electron microscopy has been used to describe morphology of digital pads, often in view of taxonomic utilisation or functional interpretation (Welsch, Storch and Fuchs, 1974; Green, 1979, 1980, 1981; Emerson and Diehl, 1980; Mc Allister and Channing, 1983; Green and Simon, 1986; Green and Carson, 1988).

The epidermis of anurans has a superficial layer of hexagonal or pentagonal squamosal cells, which are disposed in a regular way (Tyler and Miller, 1985). Differentiation of the pad leads to prismatic epithelial cells. Their surface is usually hexagonal or pentagonal, as is that of generalized cells, but their height is more important than in the latter. They are separated in their distal part forming deep crypts.

In the dermis of amphibians both mucous and venomous glands are present. Their aperture is situated between the epithelial cells of the epidermis. On the pad

TABLE 1. Classification of Dicroglossinae and Raninae as proposed by Dubois (1992) and numbers of species studied here. D: digital pad or expanded digit tip present in some species at least; M: some species at least in the genus *Micrixalus* in the classification given by Frost (1985); R: some species at least in the genus *Rana* in the classification given by Frost (1985); the number indicates the number of species here studied by morphometry, external morphology and/or scanning electron microscopy.

Dircoglossinae			
Ceratobatrachini			
Ceratobatrachus	D		
Discodeles	D		
Ingerana			
Ingerana	D	M,R	1
Liurana	D		
Palmatorappia			
Platymantis	D		1
Taylorana	D	R	
Conrauini			
Conraua	D		
Raninae			
Paini			
Chaparana			
Annandia	D	R	
Chaparana	D	R	
Feirana	D	R	
Ombrana	D	R	
Paa			
Eripaa	D	R	
Gynandropaa		R	
Paa		R	
Quasipaa	D	R	
Ranini			
Amolops			
Amo	D		
Amolops	D	R	6
Huia	D		1
Meristogenys	D		1
Batrachylodes	D		1
Micrixalus	D	M	
Nanorana			
Altirana			
Nanorana			
Rana			
Afrana			
Amerana			
Amietia			
Amnirana	D		3
Aquarana			
Aurorana			
Bourretia	D	R	2
Fejervarya		R	1
Limnonectes		R	2
Dicroglossini			
Euphlyctis		R	
Occidozyga			
Phrynoglossus	D	R	1
Limnonectini			
Hoplobatrachus		R	
Limnonectes			
Babina	D		
Chalcorana	D		1
Clinotarsus	D		
Eburana	D		1
Glandirana			
Humerana	D		
Hydrophylax			2
Hylarana	D		2
Lithobates			
Nasirana	D		
Nidirana	D		
Odorrana	D		1
Pantherana			
Papurana	D		3
Pelophylax			2
Pseudorana	D		
Pterorana	D		
Pulchrana	D		3
Rana			
Rugosa			
Sanguirana	D		
Sierrana			
Strongylopus			
Sylvirana	D		3
Trypheropsis	D		
Tylerana	D		
Zweifelia			
Staurois	D		2

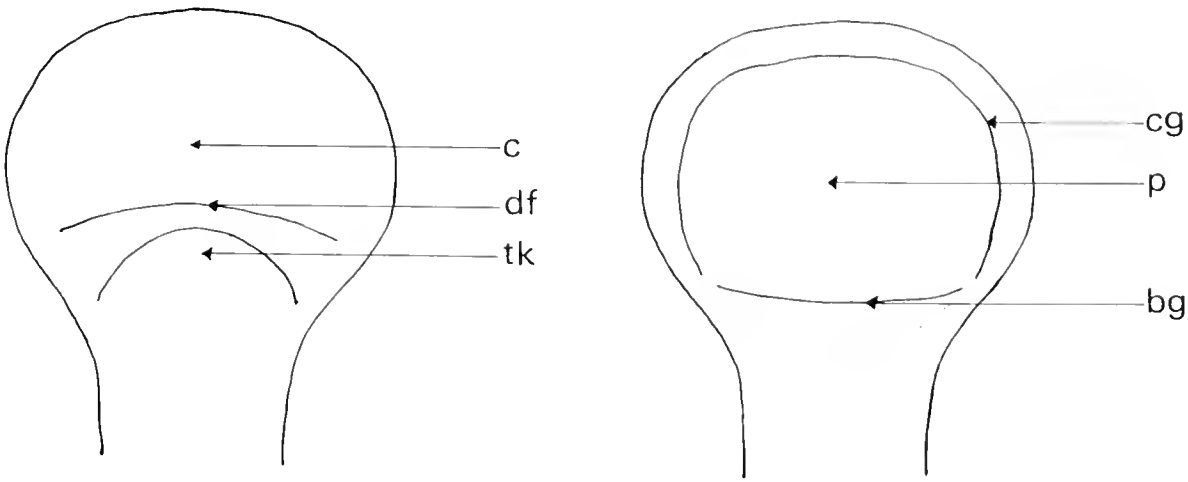


FIG. 1. Generalized plan of digital pad. Left dorsal view; right ventral view. c: cover; df: dorsal fold; tk: terminal knuckle; cg: circumferential groove; p: pad; bg: basal groove.

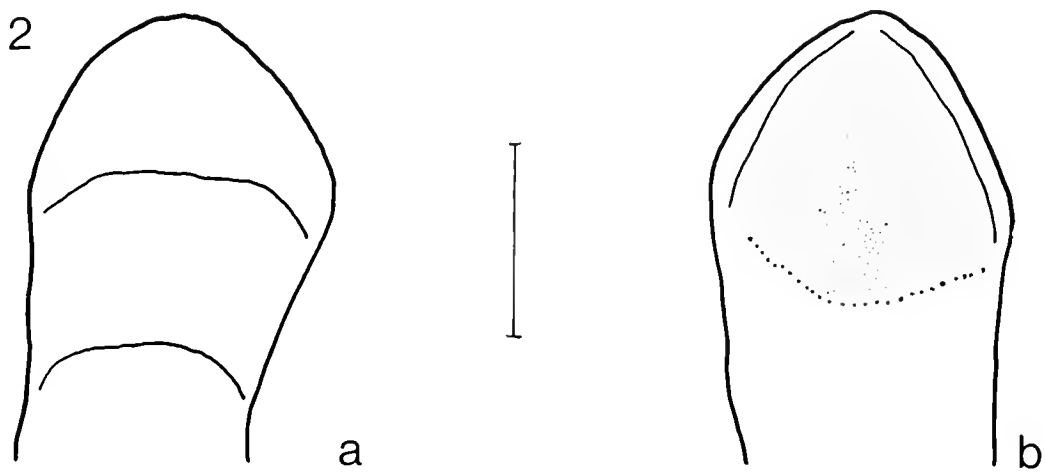


FIG. 2. Digital pad of Raninae with latero-ventral groove (*Rana (Hylarana) erythraea*, MNHN 1987.3343, Thailand). a: dorsal view of finger III; b: ventral view of finger III; stippled area corresponds to the pad with prismatic cells.

only openings of mucous glands can be observed.

The first authors (Schuberg, 1895; Siedlecki, 1910; Noble and Jaeckle, 1928) supposed that the products of the mucous glands were implicated in sticking function. To complete sticking the epidermal cells would allow attachement to natural surfaces that are covered with irregularities (Welsch, Storck and Fuchs, 1974), somehow close to the mechanism of clinging in lizards. But lizards differ substantially from amphibians in having a dry or setal adhesive system (Green and Carson, 1988).

Emerson and Diehl (1980) and Green (1981) independently showed that surface tension was mechanically responsible for the adhesive abilities of treefrog digital pads. As the surfaces of plants have usually a low surface tension, the structure of the pad cells assures humidification responsible for adhesion. The grooves surrounding the pad

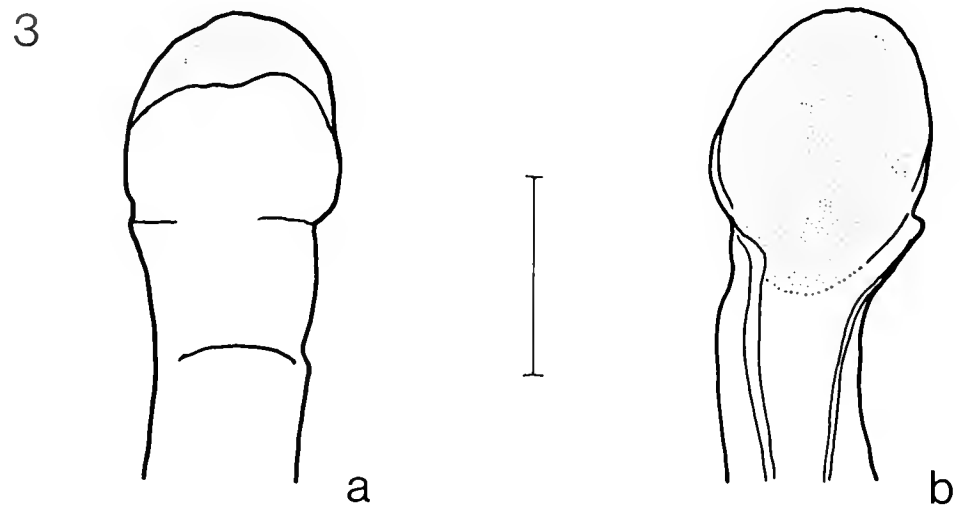


FIG 3. Digital pad of Dicroglossinae with dorso-terminal groove (*Limnonectes (Bourretia) doriae*, MNHN 1987.3130, Thailand). a: dorsal view of toe IV; b: ventral view of toe IV; stippled area corresponds to the pad with prismatic cells.

serve as a reservoir for the fluid wetting agent (McAllister and Channing, 1983).

Numerous frog species with enlarged digital tips have been studied (Hyperoliinae, Hylidae, Telmatobiinae, Rhacophorinae, and others), as well as the digital tips of some species without enlarged digital tips. Only some species of the family Ranidae have been studied in this respect, including only species without digital pad. Here I will present the structure of digital pads and digital pad cells of subfamilies of ranoids according to Dubois' (1992) classification, Ranixalinae, Dicroglossinae, Raninae, Rhacophorinae and Hyperoliinae. They include arboreal ("*Hylarana*") and torrent-living frogs (*Amolops*) that have digital pads with grooves and modified cells. For the torrent-living frogs a mechanism of sticking is proposed and the correlation of cell morphology, digit tip enlargement and biology of these frogs is outlined.

Material and methods

Specimens representing 15 of 34 genera and subgenera, possessing digital pads, as recognised by Dubois (1992) were chosen in the collection of MNHN (see Table 1, Appendix I). They had been generally formalin fixed and all had been stored in 70 % alcohol. Finger II or IV or toe III were cut on the terminal articulation. Cleaned with ultrasonic sounds, they were dehydrated in alcohol. After critical point drying, they were gold covered (2-4 Å). Specimens were observed with the Scanning electron microscope (JSM-840) of the MNHN SEM facilities. Photographs were taken on 120 Ilford FP4 film. Measurements were taken with a slide caliper (SVL) or a binocular microscope (FW): SVL - snout-vent length; FW - third finger width (maximum width of tip of third finger). To eliminate size factor, FW is given as a ratio of SVL (per thousand).

Terminology of digital pad morphology (Fig. 1, 2, 3)

(1) The *circumferential groove* (Green and Simon, 1986) (Fig. 1) surrounds the digit tip latero-terminally and separating a dorsal part from a ventral part. The groove may be complete or open (with a distal zone of contact between the dorsal and ventral part). This is the generalized groove that is modified in various manners according to the group of frogs observed.

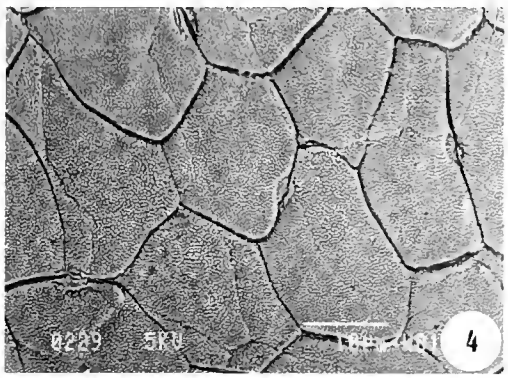


FIG. 4. Squamosal cells with short spinulae, ventral view, proximal of pad of finger III (*Batrachylodes vertebralis*, MNHN 1970.1407, Salomon Islands).

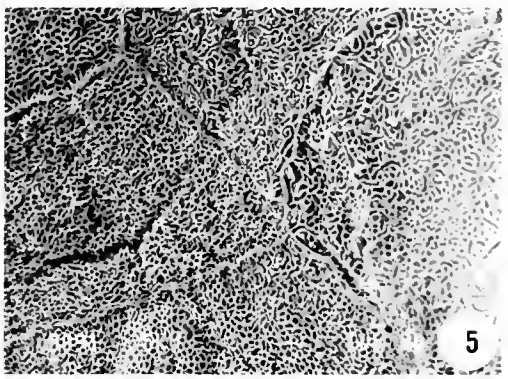


FIG. 5. Squamosal cells with microridges, ventral view, outside the circumferential groove of finger III (*Amolops marmoratus*, MNHN 1988.2787, Nepal).

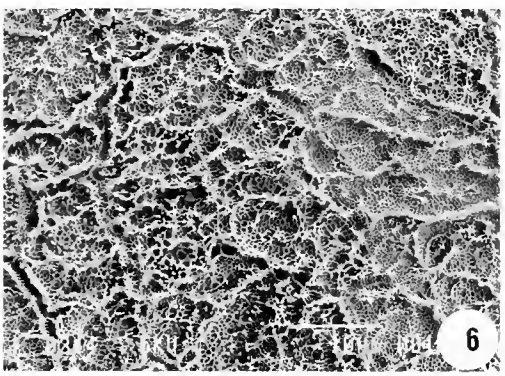


FIG. 6. Squamosal cells with spongious structures, dorsall view, on subunguis close to the dorso-terminal fold of finger III (*Ingerana tasanae*, MNHN 1987.2002, Thailand).

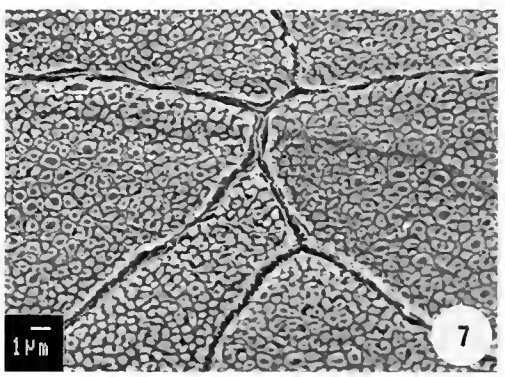


FIG. 7. Sqamosal cells with hallow tubercles, ventral viw, proximal of pad of finger III (*Ingerana tasanae*, MNHN 1987.2002, Thailand).

(a) The *latero-ventral grooves* (Ohler and Dubois, 1989) (Fig. 2) close the pad, that is of triangular shape, laterally. In some species they join distally and close to a unique groove arround an oval or rounded pad.

(b) The *dorso-terminal groove* (Ohler and Dubois, 1989) folds on the dorsal part of the digit. The pad is of oval or rounded form. In species where the groove is more pronounced its lateral parts can be observed ventrally (Fig. 3).

(2) The *basal groove* (Fig. 1) is the basal limit of the digital pad. Fusion of this with the circumferential groove results in a *circumplantar groove*. The latter is not present in all digital pad types.

(3) The ventral part is the proper adhesive organ, the *pad* (Savage, 1987) (Fig. 1). Its latero-terminal limits are usually distinct formed by the groove. Its basal limit is intergrading, and the basal groove, if present, is not the limit of the functional part as indicated by presence of modified cells still beyond this limit distally.

TABLE 2. Distribution of prismatic cell types and relative width of tip of third finger in ranoid frogs. - Cell differentiation: L: elongated prismatic cells; R: regularly outshaped prismatic cells; H: cells of heterogeneous shape; P: projections on proximal border of prismatic cellls; S: small projections on proximal border of prismatic cells; N - no projections on prismatic cells; -: no prismatic cells in the digit tip. - Relative width of tip of third finger, measured by FW/SVL: x: mean; s: standard deviation; n: number of specimens measured; EV: extreme values of ratio FW/SVL in group.

Species studied	Cell differen-tiation	Relative finger tip width			
		x	s	n	Group (EV)
Limnonectes (Limnonectes) kuhlii	-	17.2	1.17	7	
Rana (Hydrophylax) galamensis	-	19.2	0.84	5	A
Phrynoglossus laevis	-	19.3	2.62	10	(17-20)
Platymantis corrugatus	R N	19.9	1.13	2	
Limnonectes (Bourretia) pileatus	R N	22.9	0.71	2	
Limnonectes (Bourretia) doriae	R N	23.7	3.18	2	B
Rana (Hylarana) erythraea	L N	24.9	2.42	9	(22-28)
Rana (Sylvirana) sp.	L N	27.3	2.50	6	
Rana (Odorrana) andersoni	L S	35.7	5.03	31	
Amolops (Huia) nasicus	L S	37.8	3.33	8	
Rana (Amnirana) lepus	L P	41.5	7.78	2	C
Rana (Amnirana) albolabris	L H N	42.2	2.68	5	(35-43)
Amolops (Huia) kinabaluensis	L H P	42.5	4.95	2	
Indirana gundia	L P	42.6	1.96	10	
Amolops (Amolops) sp. 3	L P	47.9	3.42	40	
Rhacophorus leucomystax	R N	51.2	4.26	10	
Amolops (Amolops) sp. 1	L P	52.6	3.83	24	
Hyperolius viridiflavus karissimbiensis	R N	55.0	3.77	10	D
Ingerana tasanae	L P	55.5	1.63	2	(47-60)
Amolops (Amolops) sp. 2	L H S	55.6	3.70	16	
Batrachylodes vertebralis	R S	56.7	9.03	6	
Rana (Chalcorana) chalconota	R S	60.0		1	
Amolops (Amolops) formosus	L H P	68.0	3.20	9	E
Amolops (Amolops) marmoratus	L S	68.7	5.71	12	68-69

(4) The dorsal part, the *cover* (Savage, 1987) (Fig. 1), does not show histological specialisation.

(5) Proximally the cover is limited by the *dorsal fold* (Fig. 1).

(6) Dorsally on fingers and toes a *terminal knuckle* (Lynch, 1979) (Fig. 1) is present in the area of distal articulations.

(7) The pad is generally entirely masked by the cover, but in some cases it projects distally. The part of the pad that is then visible dorsally is called the *subungis* (Lynch, 1979).

Results

The study of digital pads in Ranidae gave interesting results concerning gross morphology and its use for phylogeny already published (Ohler and Dubois, 1989) as well as new results concerning the type of prismatic cells observed in the digital pad. These microstructural results are exposed below and a functional hypothesis is proposed.

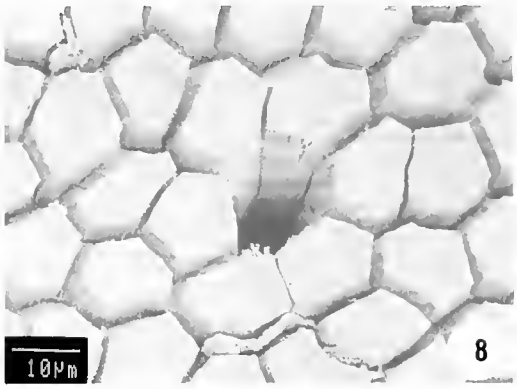


FIG. 8. Regular outshaped prismatic cells with mucous gland pore on pad of finger III (*Hyperolius viridiflavus karissimbiensis*, MNHN 1988.1055, Rwanda).

FIG. 9. Elongated prismatic cells with disatal projections on pad of finger III (*Amolops* sp. 1, MNHN 1987.2163, Thailand).

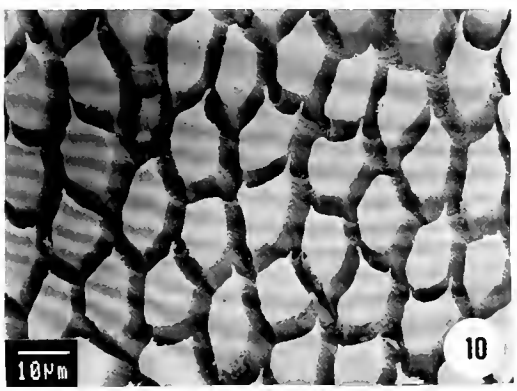


FIG. 10. Elongated prismatic cells with distal projections on pad of finger III (*Amolops* sp. 3, MNHN 1987.2140, Thailand).

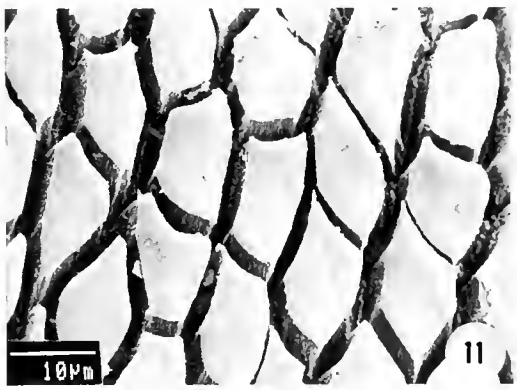


FIG. 11. Elongated prismatic cells with small distal projections on pad of finger III (*Amolops marmoratus*, MNHN 1988.2787, Nepal).

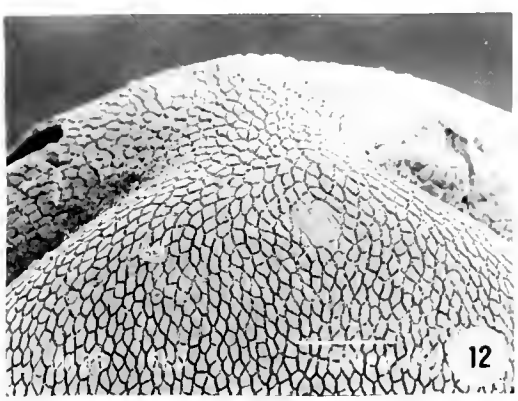


FIG. 12. Orientation and distribution of prismatic cells on distal part of the digital pad of finger III of *Rana (Odorrana) andersoni* (MNHN 1938.57, Vietnam).

FIG. 13. Distribution of prismatic and sqamosal cells on the extreme distal part of the digital pad of finger III of *Rana (Sylvirana)* sp. (MNHN 1987.3471, Thailand).

The epidermal cells

On the tips of the digits one observes two major cell types (squamosal cells and prismatic cells) with intermediary cells that occur in high numbers in the proximal pad zone.

(1) *Squamosal cells.* This is the generalized cell type, covering the body of amphibians (Tyler and Miller, 1985). The cells often show short spinulae (Fig. 4) or structures called microridges (Fig. 5). In *Ingerana tasanae* the surface of the squamosal cells is extremely rough and can show spongious structures (Fig. 6). On other parts of the epiderm the surface of the squamosal cells of *Ingerana tasanae* shows hallow tubercles (Fig. 7). The squamosal cells cover fingers and toes outside the pad. The groove is generally the border, but sometimes the squamosal cells are present on the border of the pad (*Rhacophorus leucomystax*) or in the contrary they are pushed back by the prismatic cells even outside the groove (*Amolops*).

(2) *Prismatic cells.* The prismatic cells are present on the pad. They are of regular outline in all the species already studied (Green, 1979; Green and Simon, 1986; McAllister and Channing, 1982; Richards *et al.*, 1977; Welsch, Strock, and Fuchs, 1974). Among the species studied here, *Hyperolius vividiflavus karissimbiensis* and *Rhacophorus leucomystax* have prismatic cells of regular outline (Fig. 8) like those found by previous authors. Also some other species of ranids (*Limnonectes (Bourretia) doriae, Batrachylodes vertebralis*) have this kind of prismatic cells. However, in most of the ranid species investigated (Table 2) in this study, the prismatic cells are not of regular outline but elongated. Their long axis is oriented in the proximo-distal direction on the digital pad. The ratio of the width to the length of these cells is smaller than 60%, while in normal prismatic cells this ratio is over 80%, often close to 100%. On their narrow distal side, the elongated cells have more or less developed projections.

In the species of the genus *Amolops*, this kind of cells is present with well developed projections (Fig. 9, 10). These were also observed in different "subgenera" of the genus *Rana* (*Odorrana*, *Amnirana*, *Hylarana*, *Chalcorana*) and in *Indirana gundia* (Ranixalinae). The prismatic cells of these species vary in their elongation, in the size of the projection, and in the degree of regularity. They are often rather regularly hexagonal, rounded proximally, with small distal projections, as in *Rana (Chalcorana) chalconota* and in *Ingerana tasanae.* In some species the prismatic cells are elongated, rounded proximally without projections (*Rana (Hylarana) erythraea*). In other species outlines are very variable among neigbouring cells; the cells are elongated forming a somehow triangular outshape wearing a single or two distal projections (Fig. 11). In all species of *Amolops* of this study, this kind of elongated cells with heterogeneous outlines was observed.

The prismatic cells are present outside the latero-ventral grooves in *Rana (Odorrana) andersoni* and in *Amolops* sp. 3. Observation of direction of the channels formed by the prismatic cells shows a generalized alignement in the direction of the space between the pair of lateral grooves (Fig. 12). In other species the border of pad is formed by squamosal cells, but a contact between the ventral and dorsal part of digital tip remains (ex. *Rana (Sylvirana)* sp., Fig. 13).

The development of the toe pad

The measurements of the digital width (Table 2) show an important variation that can be divided in several units. The species *Amolops formosus* and *Amolops marmoratus* show the most enlarged finger pads (FW/SVL = 68 p.m.). Other species of *Amolops*, but also *Rana (Chalcorana)*, *Ingerana tasanae* and *Rhacophorus leucomystax* have very well developed digital pads (FW/SVL = 47-57 p.m.). The frogs of the subgenera *Rana (Amnirana)* and *Rana (Odorrana)* show moderately enlarged digital pads (FW/SVL = 35-43 p.m.). The species of *Rana (Sylvirana)* and *Rana (Hylarana)*, as the species of the subgenus

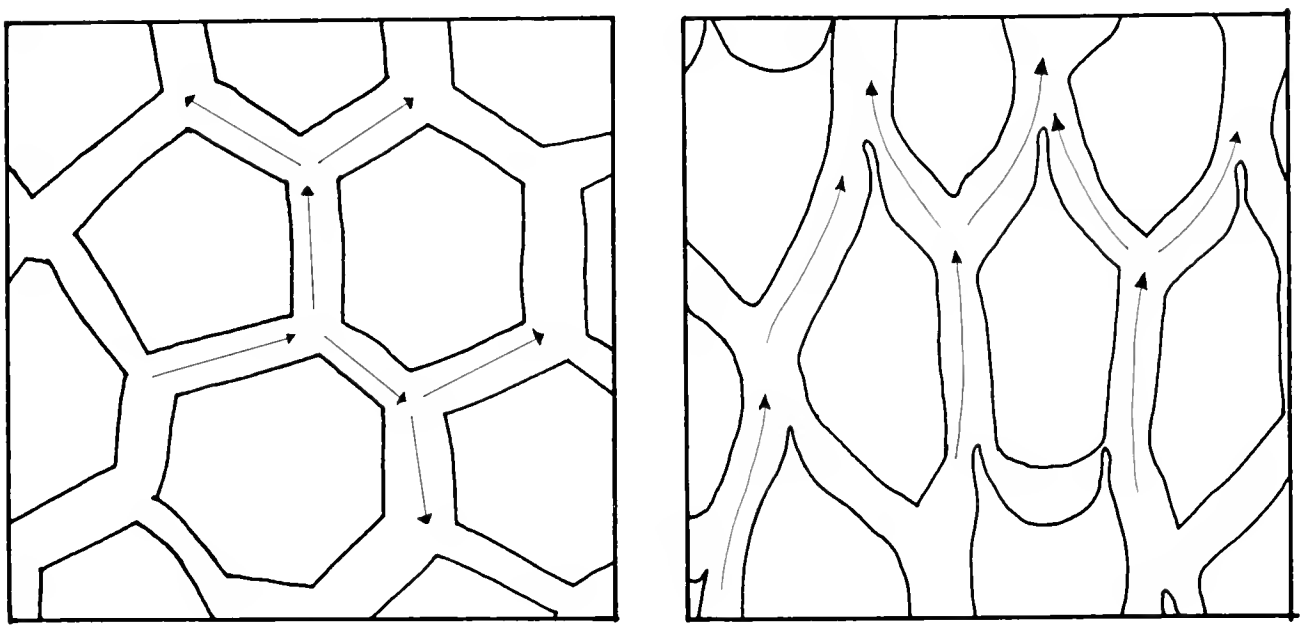


FIG. 14. Scheme of liquid fluid on a digital pad with regular outshaped cells (left) and with elongated cells (right).

Limnonectes (Bourretia) have very little enlarged finger pads (FW/SVL = 22-28 p.m.). The species studied that show no digital pad formation have the lowest ratios (FW/SVL = 17-20 p.m.).

Discussion

The elongated cells here described in some species of Ranidae have not been described in other anuran families. In fact the species that have been studied until now are "treefrogs", and no torrent-living frogs have yet been investigated. Considering the ecology of the studied species, five types can be distinguished: (1) torrent-living frogs of the genus *Amolops*, *Rana (Odorrana)* (the possible sister-group of *Amolops*) and *Rana (Amnirana)*; *Ingerana tasanae* should be placed in this group; (2) aquatic frogs, like *Limnonectes (Limnonectes) kuhlii* and *Phrynoglossus laevis*; (3) terrestrial frogs of the genus *Limnonectes (Bourretia)* and *Rana (Hydrophylax)*; (4) ground/vegetation living frogs of the genera *Rana (Hylarana)*, *Rana (Sylvirana)*, and *Rana (Chalcorana)*; (5) arboreal frogs (*Hyperolius*, *Rhacophorus*).

Actually the Raninae, the Dicroglossinae and the Ranixalinae do not include strictly arboreal species. The closest group of treefrogs are the Rhacophorinae, an other subfamily of Ranidae. *Hyperolius viridiflavus karissimbiensis* is another ranoid treefrog studied. *Rhacophorus leucomystax*, *Hyperolius viridiflavus karissimbiensis* and the species studies by the previous authors (Green, 1979; Green and Simon, 1986; Richards *et al.*, 1977; McAllister and Channing, 1982; Welsch, Strock, and Fuchs, 1974) have prismatic cells of regular outshape. This kind of regular cells was here also observed in *Limnonectes (Bourretia) doriae* and *Limnonectes (Bourretia) pileata*, two terrestial species. Elongation of digital pad cells in *Amolops*, *Rana (Odorrana)*, and *Rana (Amnirana)* might be in relationship with their mode of life. The presence of elongated cells in *Rana (Hylarana)* and in *Rana (Sylvirana)* might indicate

phylogenetic relationship to *Amolops*. The heterogeneous cells in some of these species might indicate a regression in comparison to the elongated cells with projections in *Amolops*. The functional analysis of cell morphology underlines this interpretation.

The major sticking force of tree frogs is surface tension (Emerson and Diehl, 1980; Green, 1981). It is a kind of wet adhesion, where two surfaces are hold together by an interlaying liquid. The prismatic cells, the channels and the mucous glands are required in the humidification mechanism necessary for sticking. For torrent-living frogs the surfaces to stick to are already humid or in a liquid medium. In liquid the force is no more proportional to the surface, but to the squared surface which reduces the sticking force to its square root (Emerson and Diehl, 1980). When sticking to glass at an angle of 90 to 180 , a treefrog is inmerged in water, it will separate almost immediately (Emerson and Diehl, 1980). The force of attachment in liquid medium is inversely proportional to the distance of the two surfaces, separated by the liquid.

To provide a good sticking in water, the surface of the pads should be enlarged. Some of the species of *Amolops*, as *Amolops formosus* or *Amolops marmoratus* have in fact very much enlarged digital pads (Table 2). A correlation between the digital pad development, as defined by the groups A, B, C, D and E see Table 2), and the ecology of the species may be found. The terrestial species and the aquatic frogs belong to the group A. The group B includes ground/vegetation-living frogs. The torrent-living frogs are distributed in three groups: C (*Odorrana*, *Amnirana* and *Huia*), D (*Amolops*, *Ingerana*), E (*Amolops formosus* and *Amolops marmoratus*). The treefrogs (*Rhacophorus*, *Hyperolius*) are all members of the group D, thus not the species with the largest digital pads.

Elimination of the distance between the pad and the surface to stick to will increase attachment force equally and more distinctly. In treefrogs the regular cells guide the fluids in all directions, thus humidifying the whole pad in a regular manner and optimizing the use of liquid (Fig. 14). The elongated cells of *Amolops* guide the liquid in the disto-proximal direction. The digital pad is not closed posteriorly and often also anteriorly by a groove, and prismatic cells are not restricted to the pad surface, but are also present in the groove and outside to it. Water can flow out of the pad and distance from pad to sticking surface is minimized, thus increasing the sticking force inversely.

It would be interesting to compare the cell morphology of torrent-living frogs of other anuran families, like *Ansonia* (Bufonidae), *Petropedetes* (Phrynobatrachidae), some *Litoria* and *Hyla* (Hylidae) and *Heleophryne* (Heleophrynidae) to what is here described in Raninae. A more detailed morphological analysis of surface of digital pads should be undertaken to compare sticking surface in tree and torrent-living frogs.

Acknowledgments

The photos of this work were realized with the precious help of Jean Menier in the facilities of the "Service Commun de Microscopie electronique" of the Museum national d'Histoire naturelle of Paris. I express my gratitude to Alain Dubois for advice and discussion.

Literature Cited

BOULENGER, G. A. 1882. Catalogue of the Batrachia Salientia s.Ecaudata in the collection of the British Museum. London, Taylor and Francis: i-xvi + 1-503, pl. I-XXX.

BOULENGER, G. A. 1920. A monograph of the South Asian, Papuan, Melanesian, and Australian frogs of the genus Rana. Rec. Indian Mus. 20:1-126.

CLARKE, B. T. 1981. Comparative osteology and evolutionary relationship in the African Raninae (Anura Ranidae). Monit. zool. ital. (n. s.) 15 suppl. :285-331.

DECKERT, K. 1938. Beiträge zur Osteologie und Systematik ranider Froschlurche. Sber. Ges. naturf. Freunde Berl. 1938:127-184.

DUBOIS, A. 1987. Miscellanea taxinomica batrachologica (I). Alytes 5:7-95.

DUBOIS, A. 1992. Notes sur la classification des Ranidae (Amphibiens Anoures). Bull. mens. Soc. linn. Lyon 61:305-352.

EMERSON, S. B. AND D BERRIGAN. 1993. Systematics of southeast Asian ranids: multiple origins of voicelessness in the subgenus *Limnonectes* (Fitzinger). Herpetologica 49:22-31.

EMERSON, S. B. AND D DIEHL. 1980. Toe pad morphology and mechanisms of sticking in frogs. Biol. J. Linn. Soc. 13:199-216

ERNST, V. V. 1973 a. The digital pads of the treefrog *Hyla cinerea*. I. The epidermis. Tissue Cell 5:83-96.

ERNST, V. V. 1973 b. The digital pads of the treefrog *Hyla cinerea*. II. The mucous glands. Tissue Cell 5:97-104.

FROST, D. R. (ed.) 1985. Amphibian species of the world. Lawrence, Allen Press and Assoc. Syst. Coll.: (i- iv) + i - v + 1 - 732.

GREEN, D. M. 1979. Treefrog toe pads: comparative surface morphology using scanning electron microscopy. Canadian J. Zool. 57:2033-2046.

GREEN, D. M. 1980. Size differences in adhesive toe-pad cells of treefrogs of the diploid-polyploid *Hyla versicolor* complex. J. Herpet. 14:15-19.

GREEN, D. M. 1981. Adhesion and the toe-pads of treefrogs. Copeia 1981:790 - 796.

GREEN, D. M. AND J. CARSON. 1988. The adhesion of treefrog toe-pads to glass: cryogenic examination of a capillary adhesion system. Austr. J. nat. Hist. 22:131-135.

GREEN, D. M. AND M. P. SIMON. 1986. Digital microstructure in ecologically diverse sympatric microhylid frogs, genera *Cophixalus* and *Sphenophryne* (Amphibia: Anura), from Papua New Guinea. Austr. J. Zoo. 34:135-145.

HILLIS, D. M. 1985. *Evolutionary genetics and systematics of New World frogs of the genus* Rana*: an analysis of ribosomal DNA, allozymes, and morphology*. Thesis, The University of Kansas. i-vi + 1-304.

KOMNICK, H. AND W. STOCKEM. 1969. Oberfläche und Verankerung des Stratum corneum an mechanisch stark beanspruchten Körperstelllen beim Grasfrosch. Cytobiologie 1:1-16.

LIEM, S. S. 1970. The morphology, systematics, and evolution of the Old World treefrogs (Rhacophoridae and Hyperoliidae). Fieldiana: Zool. 57: i-vii + 1-145.

LYNCH, J. D. 1979. A new genus for *Elosia duidensis* Rivero (Amphibia, Leptodactylidae) from Southern Venezuela. Amer. Mus. Novit. 2680:1-8.

MCALLISTER, W. AND A. CHANNING. 1983. Comparison of toe-pads of some southern African climbing frogs. S. Afr. J. Zool. 18:110-114.

NOBLE, G. K. AND M. E. JAECKLE. 1928. The digital pads of the tree frogs. A study of the phylogenesis of an adaptive structure. J. Morphol. Physiol. 45:259-292.

OHLER, A. AND A. DUBOIS. 1989. Démonstration de l'origine indépendante des ventouses digitales dans deux lignées phylogénétiques de Ranidae (Amphibiens, Anoures). C. r. Acad. Sci. Paris 309 (3):419-422.

RICHARDS, C. M., B. M. CARLSON, T. G. NONNELLY, S. L. ROGERS AND E. ASHCROFT. 1977. A scanning electron microscopic study of differentiation of the digital pad in the Kenyan reed frog *Hyperolius viridiflavus ferniquei*. J. Morphol. 153:387-396.

SAVAGE, J. M. 1987. Systematics and distribution of the Mexican and central American rainfrogs of the *Eleutherodactylus gollmeri* group (Amphibia: Leptodactylidae). Fieldiana: Zool. (n. s) 33:i-iv + 1-57.

SCHUBERG, A. 1895. Über den Bau und die Funktion der Haftapparate des Laubfrosches. Arb. zool. zootom. Inst. Würzburg 10:57-118.

SIEDLECKI, M. 1910. Die Haftballen des javanischen Flugfrosches (*Polypedates reinwardtii*). Bull. Anat. Rec. 185:253-257.

TYLER, M. J. AND C. A. MILLER. 1985. Surface architecture of the dorsal epidermis in Australian frogs. Trans. Roy. Soc. South Austr. 109 (2):45-48.

WELSCH, U., V. F. STORCH, AND W. FUCHS. 1974. The fine structure of the digital pads of rhacophorid treefrogs. Cell Tissue Res. 148:407-416.

APPENDIX I

Specimens studied by scanning electronmicroscopy (origin and reference in the catalogue of the Muséum national d'Histoire naturelle of Paris).

Species studied	Origin	Collection Number
Amolops (Amolops) formosus	Namdu Khola, Nepal	MNHN 1994.5559
Amolops (Amolops) marmoratus [1]	Timal, Nepal	MNHN 1988.2787
Amolops (Amolops) sp. 1	Khao Chong, Thailand	MNHN 1987.2163
Amolops (Amolops) sp. 2	Doi Inthanon, Thailand	MNHN 1987.2082
Amolops (Amolops) sp. 3	Phu Kradung, Thailand	MNHN 1987.2140
Amolops (Huia) kinabaluensis	Kina Balu, Borneo	MNHN 1889.240
Amolops (Huia) nasicus	Hanoi region, Vietnam	MNHN 1938.70
Batrachylodes vertebralis	Bougainville, Solomon Islands	MNHN 1970.1407
Rana (Amnirana) albolabris	Liberia	MNHN 1989.3456
Rana (Amnirana) lepus	Central African Republic	MNHN 1968.247
Rana (Chalcorana) chalconota	Khao Chong, Thailand	MNHN 1987.3490
Rana (Hydrophylax) galamensis	"Afrique Orientale Francaise"	MNHN 1920.145
Rana (Hylarana) erythraea	Chiangmai, Thailand	MNHN 1987.3343
Rana (Odorrana) andersoni	Vietnam	MNHN 1938.57
Rana (Sylvirana) sp.	Doi Pui, Thailand	MNHN 1987.3471
Ingerana tasanae	Khao Phra Tiu, Thailand	MNHN 1987.2002
Limnonectes (Limnonectes) kuhlii	Phu Kradung, Thailand	MNHN 1987.3332
Limnonectes (Bourretia) doriae	Khao Chong, Thailand	MNHN 1987.3130
Limnonectes (Bourretia) pileatus	Phu Kradung, Thailand	MNHN 1987.3140
Phrynoglossus laevis	Khao Chong, Thailand	MNHN 1987.2944
Platymantis corrugatus	New Guinea	MNHN 1989.3461
Rhacophorus leucomystax	Khao Chong, Thailand	MNHN 1987.3544
Hyperolius viridiflavus karissimbiensis	Gihirwa river, Rwanda	MNHN 1988.1055
Indirana gundia	Gundia, India	MNHN 1985.607

1. Formerly *Amolops afghanus*: see Dubois (1992: 340).

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 97-110

Social Organization and Demography in the Rock Agama, *Stellio caucasius*

EUGENY N. PANOV AND LARISA Y. ZYKOVA

Severtsov Institute of Animal Evolutionary Morphology and Ecology, Russian Academy of Sciences, Leninsky Prospect 33, Moscow 117071, Russia

Abstract. -We studied the social organization and demography of the rock agama *Stellio caucasius* in a natural population, located in Gobustan (eastern Azerbaijan, approximately 60 km south of Baku) and in an introduced population in the small Karadag Range near Krasnovodsk (western Turkmenistan). We found that these populations are highly stable with low turnover. This appears to be the result of delayed reproduction, longevity and a sedentary life style. Population growth is relatively slow due to high juvenile mortality and low immigration rates from adjacent subpopulations. The age structure of all subpopulations studied was dominated by older age classes. Rock agamas exhibit those natural history and population characteristic of a K selected species.

Key words: Agamidae, age, Azerbaijan, density dependence, K-strategy, Lacertilia, ontogenetic trajectories, polygyny, population dynamics, social behavior, spacing, *Stellio caucasius*, survivorship, mortality, territoriality, translocation, Turkmenistan.

FIG. 1. Adult (10+ years old) male *Stellio caucasius* perched on basking site (left); adult female *S. caucasius* (right).

Introduction

Long-term mark and recapture studies of natural populations have become important in recent decades due to their great power in demonstrating population parameters. These investigations permit the testing of hypotheses concerning the mechanisms governing population parameters. The ability to follow particular individuals through time reveals the scale of behavioral heterogeneity within the local population. It also allows the examination of these population parameters as a function of age or changing social status.

This approach has already gained firm position in the population studies of birds and mammals, but it has only recently been broadly employed in reptile studies.

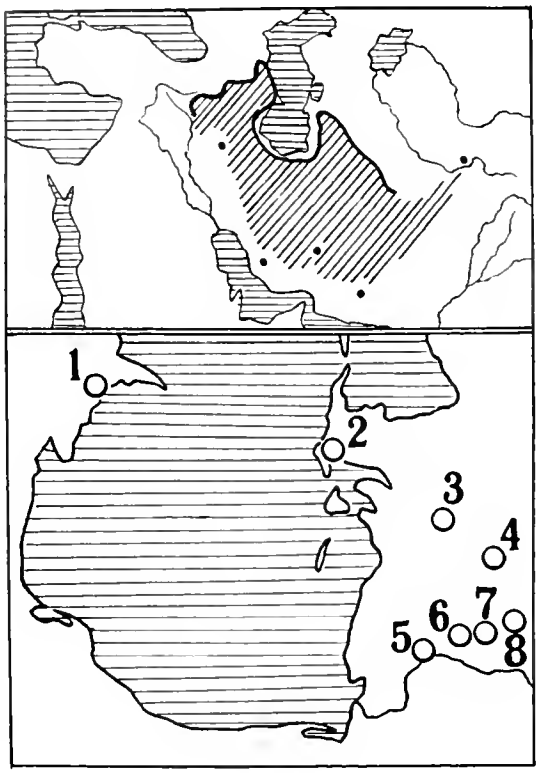


FIG. 2. Regional distribution (cross hatched area) of *Stellio caucasius* (upper plate). Specific study sites in Azerbaijan and the Turkmen Republic: 1) Gobustan; 2) Krasnovodsk; 3) Bol'shoi Balkhan Range; 4) Kyurendag Range; 5) lower Sumbar River; 6) Parkhai Gorge; 7) Kalaligez; 8) Aidere (lower plate).

Although this method is used in modern herpetology rather frequently, it is oriented mainly toward answering the questions of traditional population ecology (dynamics of numbers and sex-age composition of local populations, modes of spacing in the context of resource utilization by communities and species, etc.). In behavioral ecology, and particularly with respect to the fates of particular individuals and their social relationships, the reptiles in general and lizards in particular remain poorly studied (for review see Semenov, 1989).

The rock agama, *Stellio caucasius*, is an ideal subject for the study of behavioral aspects of population organization in reptiles that are K strategists. This is a long-lived lizard attaining an age of ten or more years (Zykova and Panov, 1991). Many populations are characterized by high and very high density. Most individuals demonstrate strong home area fidelity. Adults live in small social groups in which the lizards form long lasting pair bonds (Panov and Zykova, 1985; 1989).

This paper examines the social interactions within local settlements of rock agamas and analyzes the role of social behavior as a regulator of demographic processes.

Methods and Materials

Rock agamas are large lizards with an overall length of up to 30 cm. and weighing up to 160 gm. Males are on average larger than females and have a heavier build. General background color is a mixture of gray, brown and olive with a darker, dull spotted pattern on the back and sides. During the breeding season males differ from females by having black on the breast, contrasting with pale or pinkish-gray on the throat (Fig. 1). In males, there is light gray epidermal holocrine gland in the center of the blackish-gray belly. These lizards are typical inhabitants of stony landscapes, although some populations have become adapted to the life on the steep slopes of clay canyons or even at the margins of the sandy desert (Ananyeva and Ataev 1984; Panov, Zykova, Glauzer and Vasil'ev, 1987).

The bulk of data presented here was obtained during a comparative study of two populations of rock agama: a natural population, located in Gobustan (eastern Azerbaijan, approximately 60 km south of Baku) and in an introduced population in the small Karadag Range near Krasnovodsk (western Turkmenia) where rock agamas were known to be absent earlier (Fig. 2). In the second area, on 17 May 1985, we introduced into an abandoned quarry 13 adult males, 19 adult females, 13 two year old lizards and 25 juveniles born in the preceding year. All these animals were caught in the Blocky Balkan Range situated some 160 km from the introduction site. The latter lies in view of Krasnovodsk Plateau known to be a part of rock agama

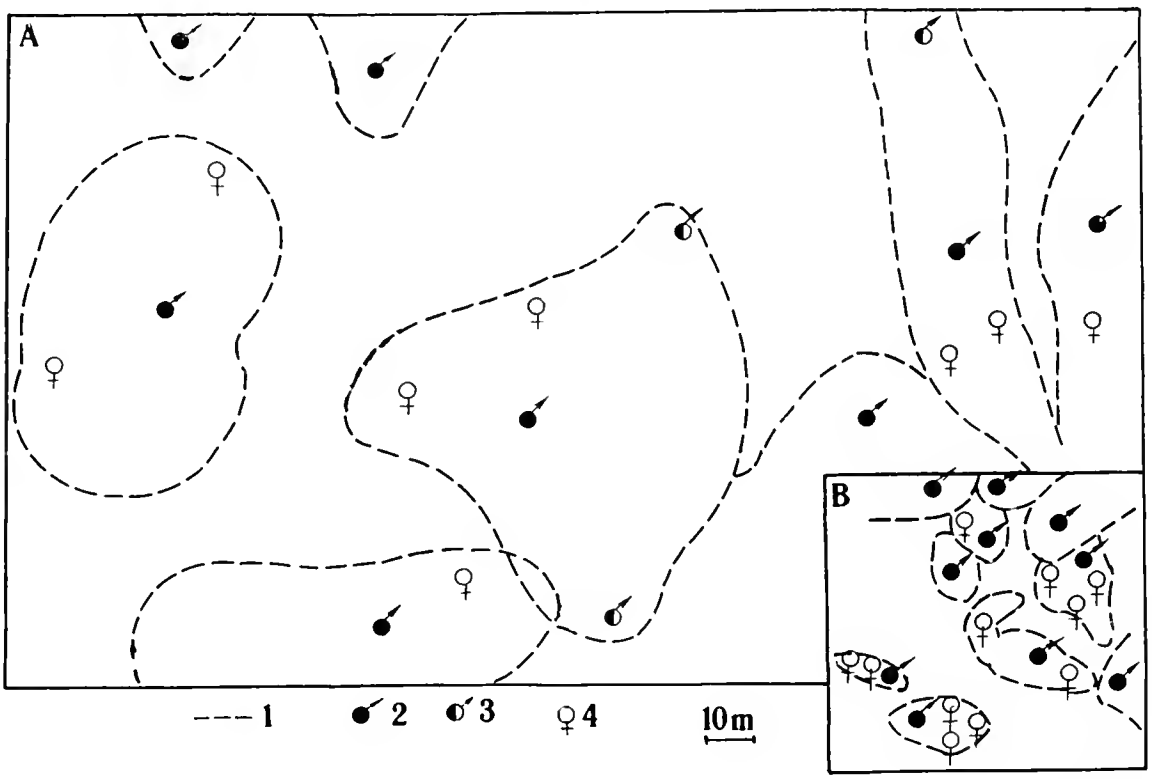


FIG. 3. Territory structure in Gobustan (A) and Aidere (B): 1) territory boundaries; 2) breeding males; 3) subordinate males; 4) females.

breeding range (Ataev, 1985). The introduction site is similar to the dry semidesert habitats of rock agamas found in Gobustan. The predominately stony surface is covered by very sparse grasses, but trees and bushes, in contrast with Gobustan, are wholly absent. The experimental plot was situated in a broad, dry valley with steep slopes broken by narrow ravines and rifts. The numerous cavities and cracks under and between the stones provided abundant temporary and permanent shelters for the lizards. Prior to release all introduced agamas were marked by toe clipping.

In Gobustan, on the natural plot of 0.65 hectares, capturing, marking and observations were conducted in April 1987 and 1988; in spring of 1986 and 1989 we performed censuses and selective capture of lizards. In Krasnovodsk, on the introduced population, field work was carried out 24 April - 6 May 1986, 22 - 25 March and 30 April - 18 May 1987, 26 April - 17 May 1988, 5 - 10 April and 28 April - 23 May 1989, 15 - 25 May and 10 -11 September 1990 (total of 103 days). Some observations were made during short visits in the summer and fall from 1985 to 1989.

Important additional data were obtained during the course of field studies conducted on two marked populations in western Kopetdag near Kara-Kala settlement (Sjunt-Khasardag State Reserve). An observational plot in Parkhai Gorge was inspected in the spring months of 1986 and 1989 and in September - October 1986 - 1988; a population in the Kalaligez area was investigated in the fall of 1984 - 1987 (total of 25 days).

In all of the above study plots we carried out total censuses of lizards in the areas under study. Most of the agamas observed on the plots were captured. They were measured with a ruler and calipers according to standard procedures, weighed and

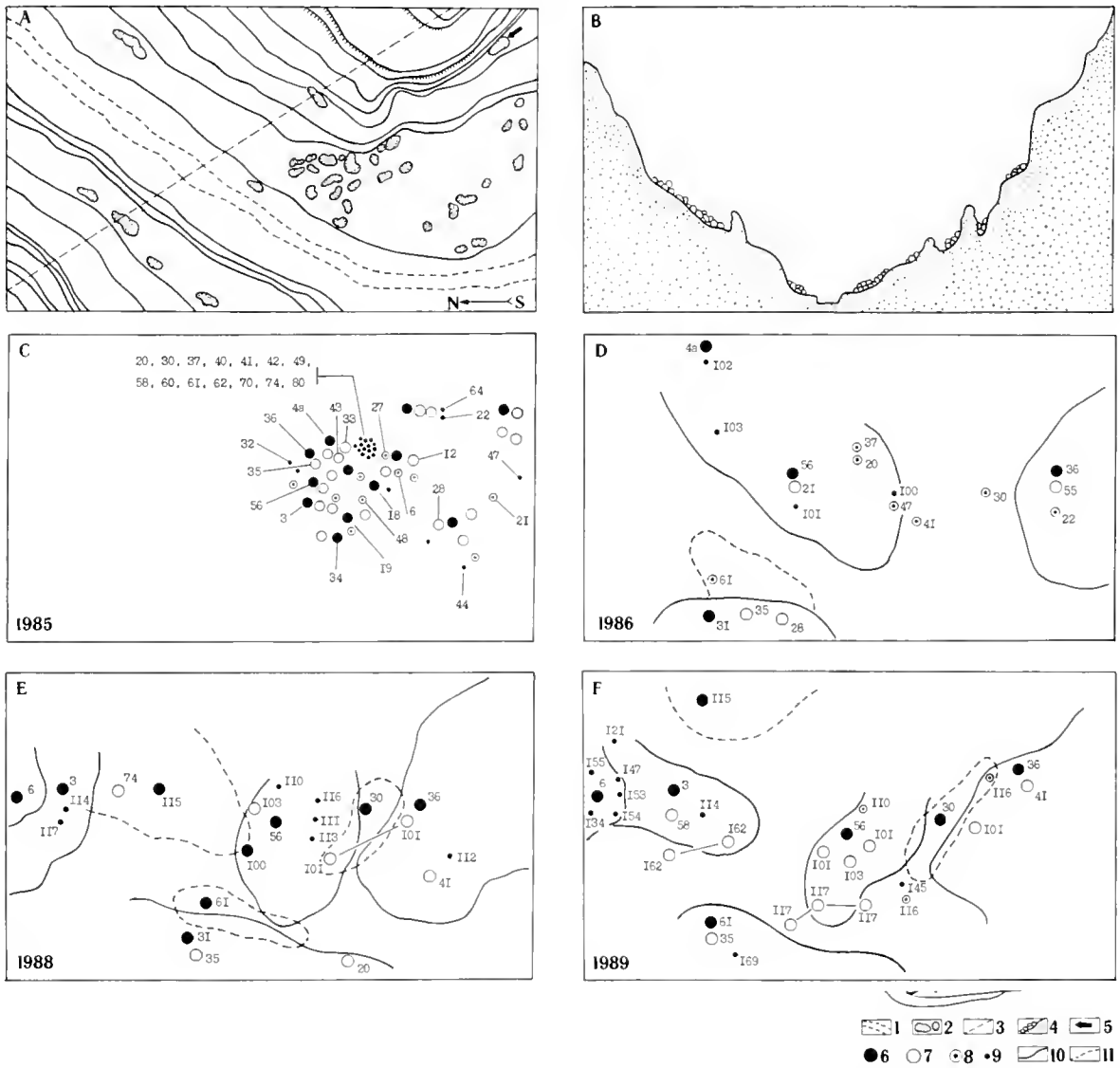


FIG. 4. Krasnovodsk experimental study plot. A. Map of study site. B. Cross-section of the study . C. Distribution of rock agamas in 1985. D. Distribution of rock agamas in 1986. E. Distribution of rock agamas in 1988. F. Distribution of rock agamas in 1989. 1. Dry creek bed. 2. Large rocks and boulders. 3. Cross-section illustrated in Fig. 4B. 4. Talus. 5. Communal winter shelter. 6. Adult male. 7. Adult female. 8. Second-year lizard. 9. Yearling. 10. Territory boundary. 11. Subordinate male home range boundary. Numbers refer to individual lizards.

photographed. Animals caught for the first time were marked by toe clipping. Prior to release all agamas received individual color marks made with dye. The locations of all individuals on the plots were mapped.

The ages of agamas were estimated using standardized size criteria (Zykova and Panov, 1991). We assumed that the age of individuals corresponds to the number of winter hibernations. So, the ages of agamas captured in spring will be slightly overestimated. Our "yearlings" in May are actually about 10 months old; "two year old" lizards in May are approximately a year and 10 months old, etc.

Altogether 426 agamas were captured and marked, 144 of them were observed a total of 273 times during subsequent years.

Results and Discussion

Spacing Patterns in Rock Agama Settlements

The baseline of spacing pattern of a whole social organization in Rock Agama is a mosaic of mutually exclusive territories owned by mature adult males. Mature females have either mutually exclusive or overlapping home ranges situated within territories of those males with which the given female is tied by the family bonds. The adult female, as a rule, does not leave the territory of "her" male. Therefore, the territory of adult breeding male, if there is female(s) living in it (which is not necessarily the case) in the same time the territory of pair or a family group defended as a whole by the breeding male only.

The home ranges of immature lizards one and two years of age may lie both within the territory of the family group or outside in the neutral zones separating such territories. The home range of an immature male during the first years of life within an adult male territory, shifts to the periphery of this territory as the immature male becomes older. In contrast, the home range of an immature female adjacent to that of an adult male shifts with time toward the male's territory.

In saturated habitats all suitable space is shared among adult males, so that neighboring territories have common boundaries. The size of territories in such saturated habitats depends on the substrate and local food abundance (Fig. 3). In barren habitats of Gobustan with low microrelief (absence of talus mounds, in particular) the size of adult male territories was found to vary from 100 to 210 m^2 (140.0 15.8 m^2 on average). In the middle altitudes of the Western Kopetdag Mountains (Canyon Ai-Dere, see Fig. 3B) where the climate is more humid and the vegetation is rich and diverse and the substrate includes jumbles of fallen rocks and boulders, the size of territories was found to range from 28 to 136 m^2 (94.0 $\pm$ 16.3 m^2 on average), for details see Panov and Zykova (1985).

Formation of Territorial Structure

We followed the establishment of territorial structure in the course of our long-term observations on the Krasnovodsk introduced population. Here 13 male "founders" were released into an area that would have supported a population of a density comparable to that in natural colonies of rock agamas. Males were released into deep holes and crevices which seemed to us to be similar to hollows normally used by rock agamas as their permanent dens.

However, contrary to our expectations, the majority of introduced males left the area where they had been released, and moved away at distances ranging from 60 to 500 m. Only three males remained in proximity to the release site by the next spring. At that time the territory of only one male (N 56) overlapped the release site. The boundaries of two other males (NN 36 and 31) territories were 60 and 100 m respectively from the release site (Fig. 4 c, d).

The process of territory establishment adjacent to the release site is shown in Fig. 4 c-f. During 1986, the year following introduction, adult male territories were large and had no common boundaries (Fig. 4d). Since male rock agamas do not patrol the borders of their territories (as, for example, males of steppe agama, *Trapelus sanguinolentus*, do (see Panov and Zykova, 1986), it is difficult to locate precisely the boundary between territories and, therefore to estimate exactly the real territory size. The greatest territory diameter was estimated in 1986 on the experimental plot as 140 m, with the width of the neutral zones separating neighboring territories as some 20-60m. The small, indistinctly demarcated home ranges of three immature lizards (males NN 30 and 61, and female N 41, all less than two years old) were situated in these neutral zones (Fig. 4e). Other immatures approximately two years old established their home ranges within the territories of mature males, as well as four

lizards born already in place of introduction in preceding year.

In 1988, the third year after introduction, most of the area that in 1987 was neutral zone was shared among relatively young males of about four years of age (NN 30 and 61) and three years of age (N 115 already born on the introduction site). Although these males apparently had attained maturity, they seemed to be bachelors at that time. Three year old male N 115 and four year old female N 74 were repeatedly seen in 1988 in close proximity (10-15 m) to each other, but we did not witness immediate contacts between them.

In 1989, the fourth year after introduction, slight changes in territorial structure were observed (Fig. 4e). There was some tendency toward clumping of territories toward each other, possibly because of the increasing density of lizards. Male N 61 (about five years old) left his adolescent bachelor home range and occupied the territory of deceased male N 31 and established a bond with his former mate, a female of eight or nine years of age. The corpse of male N 31 indicated that he had died between June 1988 and April 1989. Relatively young male N 115 occupied the territory of five to six year old male N 4a after he disappeared. Female N 74 moved into the territory of 10 to 11 year old male N 3.

Dynamics of Space Utilization Within a Home Area

During the hot periods of the year, each individual had at least one permanent den and one to several observational posts (used also as basking sites) where it spent considerable time, except during periods of foraging. In individuals about one year old or younger the den and basking site were the same or immediately adjacent. Usually the shelter was situated under a boulder or rock on which the lizard basked. Foraging activities of juveniles and yearlings take place within a radius of several meters of the individual dens. Many subadults of both sexes and some adult females behave in a similar way, although during foraging they often move greater distances from the den, up to several tens of meters.

During the day adult males range more widely. The several posts of a male are connected by a network of relatively permanent pathways. The territory is utilized unevenly with some points located along the pathways receiving regular use while others, situated at some distance from the pathways being visited only occasionally or not used at all during a given season.

This pattern of territorial use can result in changes in territorial boundaries. For example, by comparing the positions "c", "d", and "e" in Fig. 4, one can see that in 1988 the border of the territory of male N 36 shifted 30-40 m eastward from its position in 1986. Such shifts are possible in non-saturated habitats only. In established rock agama settlements with dense populations the boundaries remain constant from year to year.

In relatively sparse Krasnovodsk population the cases of mutual intrusions by neighboring males into neutral zones separated their territories and even into peripheral parts of these territories themselves are possible. The above said does not hold in respect to male-"pretenders", or "satellites". Their home ranges may broadly overlap the peripheral parts of two or more territories of adult "resident" males (see, for example, home ranges of male-pretenders NN 30 and 61 in Fig. 4). In saturated habitats home ranges of satellites are practically always situated within the territories of resident males.

By the winter the agamas leave their summer home areas and migrate to communal hibernation shelters. Migrations begin when air temperatures are relatively high (25^0 C and above). Communal winter dens may be situated up to 500 m from an individual's home area. On 23 March 1987 on the Krasnovodsk experimental plot, when only a few agamas had returned to their summer areas from the communal hibernation shelter (daily temperatures ranged from 2^0 C to 16.5^0 C) we found in that shelter males NN 21, 27, 36, 61,

females NN 22 and 58 (ages from two to seven years) and subadult male N 105. Summer residences of these lizards are shown in Fig. 4, home areas of others were located 100 - 500 m from the communal winter den.

Individual Ontogenetic Trajectories

An ontogenetic trajectory is defined as a sequence of changes in social status and of social roles of an individual during its life (Wiley 1981). In rock agamas social behavior and social status of individuals of both sexes appear to be similar during the first two years of their lives. But thereafter ontogenetic trajectories of males and females become progressively divergent.

It is not known if lizards remain in the vicinity of their birth place during their lives. Among those juveniles (n=61) that were captured on the two plots in the western Kopetdag at the end of September and the beginning of October (i.e. at the age of two to three months), only five (8.2%) were observed near the places of their first capture (within a radius of 25 m) in next year. The proportion of such recaptures on one of the two plots was as high as 25%, while on another plot none of 41 juveniles caught during preceding fall was observed later on. Unfortunately of all those juveniles that were captured during the first months of their lives, it remains unknown whether they remained near their birthplaces or if they dispersed by the time of capturing (dispersion of juveniles just after hatching has been described for *Anolis aeneus* ; Stamps, 1988).

More definitive results were obtained from recaptures of those first year lizards that were initially captured soon after their first winter hibernation, in April and May. Of those lizards 64.5% (20 of 31) were observed regularly within a radius of 10 to 50 m from the place of their first capture, in some cases over a period of several years. First year animals initially occupy small home ranges of about 10 m in diameter both outside the territories of adult males (5 males and 5 females in Krasnovodsk population in 1985) and within such territories (4 males and 5 females). When the home ranges of first year lizards were immediately adjacent their interactions appeared to be agonistic. In some cases between adjacent home ranges a well defined boundary was established and both neighbors displayed pronounced territorial behavior toward each other. In other cases the home ranges overlapped and a stable rank order was formed so that one lizard appeared to dominate the other in the overlap zone.

Generally, the mature members of the settlement behave indifferently toward yearlings. However, in periods of high sexual activity adults may chase the yearlings short distances.

After the second winter the young agamas returned from communal den to their original summer home area where they knew the topography of the place and their foraging routes became longer and pioneering of new feeding places and new temporary shelters began. Apparent differences between social behaviors of young males and females began to emerge only after their third winter, at an age of more than 30 months.

Male Ontogenetic Trajectories

Males participate in reproduction only after they have acquired a territory. Males continue to reproduce until the end of their lives. For example, on the Krasnovodsk plot male N 3 in 1989 at an age of approximately 12 years had a large territory that included four adult females of different ages and two immature females. In addition the same year we observed interactions between this male and immature female N 161 in the border zone between his territory and that of male N 61.

If, on the given territory, the only one female lives, in the case of her disappearance a holder of territory becomes a widower. He however, subsequently does not try to search for females outside his territory. In Gobustan, such case of widowhood was observed in about 6 years old male. A more young male, evidently, may also turn out to be a widower.

TABLE 1. Composition and age structure of family groups of the experimental introduced population at Krasnovodsk and the natural population at Gobustan.

	Krasnovodsk					Gobustan			
Year	1986	1987	1988	1989	1990		1986	1987	1988
Individual	Age in years					Present (+)/Absent (-)			
♂ N 36	5*	6	7	8	9	♂ N 41	+	+	+
♀ N 55	4-5*	-	-	-	-	♀ N 38	+	+	_
♀ N 22	2	3	-	-	-	♀ N 45	+	+	+
♀ N 41	2	3	4	5	6	♀ N 66		2	-
♀ N 119	-	-	1	2	3	♂ N 42	+	+	+
Juv N 148	-	-	-	1	2	♀ N 51		+	-
♂ N 56	6*	7	8	9	10	♂ N 52		+	+
♀ N 101	1	2	3	4	5	♀ N 47		+	+
♀ N 103	1	2	3	4	-	♀ N 48		-	+
♀ N 117	-	-	-	2	?	♀ N 81		2	3
Juv N 110	-	-	1	2	-	Juv N 90		-	+
♂ N 6	3	4	5	6	7	♂ N 56		+	+
♀ N 121	-	-	1	2	3	♀ N 49		+	+
♀ N 154	-	-	-	2-3	-	♀ N 50		+	+
Juv N 157	-	-	-	1	-	♂ N 63		+	+
♂ N 30	2	3	4	5	-	♀ N 64		+	+
♂ N 37	-	-	-	5	6	♀ N 65		+	+
♀ N 116	-	-	1	2	3	♂ N V		+	+
Juv N 145	-	-	-	1	2	♀ N 40	+	+	+
♂ N 18	7	8	-	-	-	♀ N 44		-	+
♂ N 27	-	-	5	6	7	♀ N 87		2	3
♀ N 43	-	4*	5	6	-	Juv N 80		-	+
♀ N 80	-	3	4	5	6				
♀ N 107	-	2	3	4	5				
♀ N 106	-	1	2	3	4				
Juv N 144	-	-	-	1	-				
Juv N 158	-	-	-	1	2				
Juv N 159	-	-	-	1	-				

* = minimum estimated age.

Female Ontogenetic Trajectories

Female rock agamas are able to reproduce at about three years of age. By this age to be a successful breeder, a female should establish a stable bond with a male territory holder and obtain constant shelters within his territory. For a female that had a juvenile home area within a male's territory the process of assimilation into a family group and establishment of a bond with the male is very direct. An example of this was female N103 who was observed for the first time in 1986 as a first year juvenile living on the territory of male N 56 where she continued to stay at least until 1989 (see Fig. 4d-f and Table 1A).

Females that had juvenile home areas on the periphery of an adult male's territory, in the home range of a non-territorial satellite male, or in a neutral zones where sexually active males were totally absent usually left and attempted to establish a bond with a territory holding male. This process of selection of a male and territory may take several years and involve short stays in the areas of several males. For example, female N 101, originally having frequented the territory of male N 56,

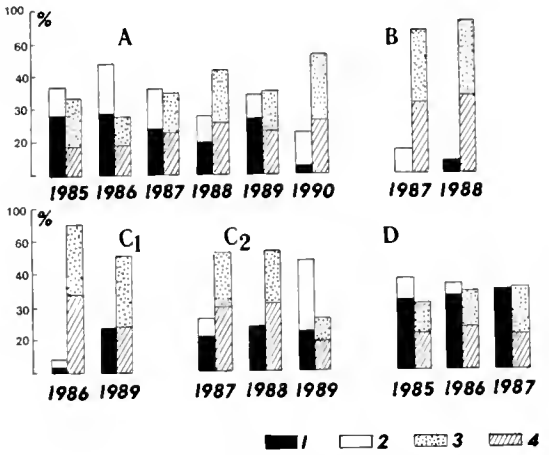


FIG. 5. Population structure of study populations. A. Krasnovodsk. B. Gobustan. C_1. Parkhai Gorge (spring). C_2. Parkhai Gorge (fall). D. Kalaligez. 1. Yearlings. 2. Second-year lizards. 3. Adult males. 4. Adult females.

subsequently over a period of 3 years (1988-1990) was resident of the neutral area visited at different time by two adult territorial males (NN 56 and 36). Female N 74 resided at ages of a little less than 4 years temporal home range of 3 year old male N 115 in next year moved on to territory of male N 3 (Fig. 4 e,f) who
became a permanent target for her courtship displays.

It is useful to describe shortly a peculiar courtship behavior of females addressed by them towards males, which we regard as a very important mechanism contributing to establishment and maintenance of personal bonds between mating partners. At sight of a male young female moves to him and at once tries to climb onto his back. A male, as a rule, during first minutes of contact tolerates these actions of female who is crawling over him in different directions and makes insistent attempts to crawl under him. After that male behaves as if he is inclined to retire, while a female pursues him and repeats her actions. Such a behavior is quite characteristic of females younger age classes, even of those lesser than 2 years old. The behavior retains in older puberal females, although, contrary to expectation, it almost never occurs prior to actual sexual interactions, i.e. copulations. Once a female has selected a male she begins to co-habit his shelters. Where there are several females on a territory usually the oldest female cohabits with the male.

The home ranges of adult females overlap broadly, especially if the territory is large. However, some older females exhibit territorial behavior in the vicinity of their shelters, basking sites and foraging areas when approached by other older female.

Females leave the territory of their family groups only for egg laying and to migrate to communal winter shelters. We did not observe emigration or dispersal of females. Over the five year study of the Krasnovodsk population the time of residence of adult females introduced in 1985 ranged from one to five years (mean = 2.5 ± 0.6, n=6).

Family Groups

The mode of sexual relations in rock agama settlement may be defined as a territorial facultative polygyny. As many as four females may establish bonds with a territorial male. In the natural Gobustan population there were 1.73 females per male on territories. In the introduced Krasnovodsk population the average number of females per male was increasing as population structure matured. Over the duration of our study the average number of females within the family groups was 1.33 (1988), 1.86 (1989) and 2.43 (1990).

The term "family group" is not quite precise since each female enters into such a unit independently from other females. Any personal or functional bonds between female members appear to be absent. Only relations between the territorial male and each of the females may be regarded as bonds.

The stability of such breeding units is determined primarily by an association its

TABLE 3. Survival of rock agamas introduced as adults in the Krasnovodsk experimental plot in May 1985. The number of lizards alive is presented; the number is parentheses is the per cent surviving since the previous year.

Year	1985	1986	1987	1988	1989	1990
Males	13 (100)	8 (61.5)	7 (87.5)	6 (85.7)	5 (83.3)	4 (80.0)
Females	19 (100)	6 (31.6)	4 (66.7)	3 (75.0)	3 (100)	1 (33.3)
Total	32 (100)	14 (43.8)	11(78.6)	9 (81.8)	8 (88.9)	5 (62.5)

TABLE 2. Life table of a cohort of rock agama yearlings introduced into the experimental population at Krasnovodsk.

Age x	Frequency f_x	Survival l_x	Mortality d_x	Mortality Rate q_x	Survival Rate p_x
1	25	1.000	0.280	0.280	0.720
2	18	0.720	0.120	0.167	0.833
3	15	0.600	0.120	0.200	0.800
4	12	0.480	0.080	0.167	0.833
5	10	0.400	0.080	0.200	0.800
6	8	0.320			

$l_x = \frac{f_{xi}}{f_{xl}}$; $d_x = l_{xi} - l_{xi+1}$; $q_x = \frac{d_x}{l_x}$; $p_x = 1 - q_x$ (Caughley, 1977)

TABLE 5. Sex ratios in different rock agama populations (n).

	Year					
Location	1985	1986	1987	1988	1989	1990
Gobustan (spring)	-	-	1:1.1 (31)	1:0.9 (33)	-	-
Western Kopetdag						
Parkhai Gorge						
spring	-	1:0.9 (21)	-	-	1:1.6 (13)	-
fall	-	-	1:0.7 (10)	1:0.8 (16)	1:0.7 (10)	-
Kalaligez (fall)	1:1 (12)	1:0.8 (18)	1:1.2 (11)	-	-	-
Krasnovodsk (spring)	-	1:0.8 (20)	1.1 (30)	1.1 (40)	1:0.9 (47)	1:1.2 (41)

Grand sex ratio for all localities summed = 1:0.98 (393).

member to certain shelters and to its home range (or territory) as a whole. All this seemingly explains why females accept so readily a new male partner after the death of a previous mate. For example, male N 36 established bonds with a single adult female and two immature females (all from the initial cohort of lizards simultaneously introduced to the study plot) immediately upon introduction (Table 1). Subsequently, upon the deaths of these females replacement females were recruited from those subsequently born in the colony.

Population Dynamics

Rock agamas are long lived and predominately sedentary. Populations are characterized by stable membership with recruitment as the primary source of new members. Emigration and immigration are of minor importance. Each of these factors were examined in the Krasnovodsk population.

The general picture of changes in the relative proportions of immatures, matures and sex ratio is shown in Fig. 5. It is necessary to repeat that in 1985 the original colonizing cohort was composed primarily of immatures, this may have resulted from capture sampling error. In 1986 the proportion of immatures to adults was similar but in 1987 and 1988 the ratio of immatures to adults was nearly equal. Since 1988 adults have predominated. Comparison with natural populations (Fig. 5) shows that adults predominate though relative proportions may vary significantly among areas and years. Sex ratio, males to females, is generally equal (Fig. 5; Table 5).

Recruitment

Surveys of juveniles were conducted during the spring (Fig. 5) since few lizards were active during the hottest weather in summer and early fall. Differences in the numbers of over wintering juveniles may be attributable to embryonic mortality or hatchling mortality in the first months after emergence or over the first winter.

Post Hatching Mortality and Survivorship

The survivorship of 25 juveniles introduced into the Krasnovodsk experimental plot are presented in Table 2. We assumed that those lizards that disappeared, that is, those not recaptured, had died. Of the initial 25 hatchlings, 15 (10 females, five males) survived to sexual maturity (three years) and of this cohort four males (80%) and four females (40%) survived six years, to the end of the study. The annual survival rate (p_x) ranged from 0.72 to 0.83.

We believe the high survivorship of lizards in the Krasnovodsk experimental plot during the first three years of life are comparable to natural populations. We tested this by comparing survivorship in a cohort of 20 hatchlings, hatched in 1986, 1987, and 1988, that were first captured in 1987, 1988 and 1989 as yearlings. Among these lizards 15 (75%) survived to two years and 13 (65%) to three years of age. Of those surviving to three years were three males and 10 females.

Since the duration of this study was approximately half the life span of a rock agama, survivorship in animals older than six years is based on indirect evidence. We estimated the age, based on standardized size criteria (Zykova and Panov 1991) of adult lizards captured for introduction onto the Krasnovodsk experimental plot. Such age estimates are at best imprecise but the adults in this group ranged from three to 11 years with 78% being four to six years old (Table 3).

The maximum rate of disappearance (which we attribute to mortality, not emigration) occurs in the first year (Table 3) after introduction. From the second year after introduction adult male survivorship is nearly constant and comparable to survivorship during the first six years of life (Table 2). The more variable mortality rates for females may be due to small sample size.

We combined cohorts of lizards of four, five and six years of age and calculated survivorship and estimated survivorship to the 9-11 year age class (Table 4).

In general, survivorship in rock agamas after the first winter following hatching is relatively constant until the eighth year. After the eighth year mortality increases. The maximum estimated age of for males was 12-14 years (n=4) and for females it was 9-10 years (n=2).

Sex Ratio

Although the rock agama social system is territorial polygyny, the sex ratio among adults does not differ significantly from even one (Table 5). This relationship was found at several localities throughout the range and for all localities taken together.

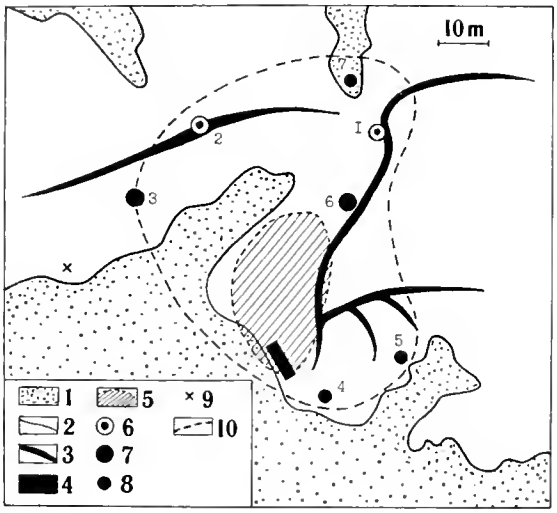


FIG. 6. Expansion of the introduced colony at Krasnovodsk from 1985 to 1990. 1. Unsuitable habitat. 2. Boundary of unsuitable habitat. 3. Principal ridges. 4. Initial introduction site. 5. Principal study area under regular observation. 6. Subpopulation established by an introduced adult male. 7. Subpopulation established by an introduced immature male. 8. Subpopulation established by offspring of introduced lizards. 9. Single sighting of rock agama. 10. Boundary of populated area in 1990.

Movement and Dispersion

Rock agamas display a high level of home area fidelity. Juveniles that were marked after their first winter were found to remain within 50 m of that location up to two years or until sexually mature (at two to three years of age). Some data on the capture of juveniles before their first winter suggests that home site fidelity may extend from the age of three or four months to the end of life. Four of six juveniles (66%) marked in October 1988 in the Parkhai Gorge (Western Kopetdag) were found in the same place the next spring. The maximum movement of immature lizards was not greater than 300 m. A juvenile male first captured in October 1985 before his first winter was recaptured in 1987, 200 m from the first capture location. He was recaptured in the spring of 1988 as a mature male with a territory 250 m from the previous capture location and 100 m from the initial capture point 31 months before.

As the population increases, new areas will be pioneered, mainly by young dispersing lizards. Given the sedentary habits of these agamas we would expect such expansion to be relatively slow. Such an expansion occurred in the introduced population at Krasnovodsk (Fig. 6). From 1985 to 1990 the initial introduced population occupying an area of approximately 300 m^2 dispersed into adjacent areas of approximately 25 ha. Of the seven subpopulations formed during this period two (Fig. 6, points 1 and 2) were founded by introduced mature adult lizards after the initial introduction. Two other subpopulations (Fig. 6, points 3 and 6) were founded by lizards that were immature when introduced. Finally, three subpopulations were established by the offspring of the original introduced lizards. In 1988 (the fourth year after introduction) there were 33 individuals (13 adult males, 14 mature females and 10 immatures) in these seven subpopulations.

Individual lizards were observed to leave their home areas only for collective winter shelter. Females may leave their home areas looking for places appropriate for egg laying (Danieljan and Grigorjan 1975; Ananyeva and Danieljan 1987).

Conclusions

As it can be seen, the characteristic features of Rock Agama social organization and demography are high stability of breeding individuals' contingent and low population turnover. This is evident consequence of sedentary way of life characteristic for males of all age classes, longevity of these lizards, and postponed onset of breeding in early lifetime of young agamas.

The latter may be especially applied to males. Although they are capable to reproduce already at the age of a little under three years (after their third wintering), most of them begin to breed, actually, only at the age of four or five

years. How it may be seen, a male attains the status of a breeder only after he had taken possession of territory of his own. So each maturing male faces with obvious difficulties since many features of species' social organization of settlement (high density together with strong territoriality of males retaining control over his home area until his death) lead to deficiency of vacancies which might be used by young male-recruits. Temporary exclusion of part of mature (non-territorial) males from process of reproduction may, in principle, decrease the whole reproductive potential of the local population.

Besides such social regulators of population growth, rapid increase of population size is retarded also by rather slow recruitment of new deme members. Although breeding productivity of Rock Agama is relatively high (from seven to ten eggs per mature female during breeding season-- see Ataev, 1985), only a small number of new-born agamas die. Even if these losses (especially the latter figure) is overestimated, the analysis of demographic structure of all demes under study shows the numerical preponderance of mature individuals over immature ones. This is in good agreement with the general conclusion about the low rate of population growth in Rock Agama. Another argument in favor of this conclusion is a quite slow expansion of growing population into new, early unoccupied areas.

To conclude, it may be stated that Rock Agama give us a good example of lizard species practicing a typical K-strategy. It was to be expected providing large size of individuals and the ecological peculiarity of the species-- in particular, its pronounced omnivorousness with prevalence in diet (at least in respect to biomass) of diverse plant objects. It is noteworthy that in Rock Agama, like in many species of higher vertebrates (birds and mammals) practicing K-strategy, among deme members there are considerable number of mature male being excluded from reproduction by density-dependent social factors.

Acknowledgments

We are indebted to Drs. Mira E. Gauzer, Vladislav I. Vasiljev (Krasnovodsk State Reserve) and Nikolaj Andreev (Sunt Khasardag State Reserve) for their help in organization of field work and for participation in catching lizards. We also wish to express our sincere thanks to Prof. Ilya S. Darevsky, Drs. Natalia B. Ananjeva (Zoological Institute, Russian Academy of Sciences, St. Petersburg) and Valentina F. Orlova (Zoological Museum, Moscow University) for their assistance and help during our work with museum collections. The final stage of our research was supported by the Soros Foundation.

Literature Cited

ANANYEVA, N. B. AND C. ATAEV. 1984. [*Stellio caucasius triannulatus* ssp. nov. - a new subspecies of the Caucasian agama from southwestern Turkmenia]. Trudy Zoologicheskogo Instituta Akademii Nauk SSR 124:4-11. (In Russian).

ANANYEVA, N. B. AND F. D. DANIELJAN. 1987. [Seasonal migrations of rock agama, *Stellio caucasius* Eichwald, 1981, in Armenia.]. Trudy Zoologitcheskogo Instituta Akademii Nauk SSR 158:33-38. (In Russian).

ATAEV, C. 1985. [Reptiles of the mountains of Turkmenistan]. Ylym Publishing House, Ashkabad. 343 pp. (In Russian).

DANIELJAN, F. D. AND E. S. GRIGORJAN. 1975. [On the population number and migrations of the rock agama *Stellio caucasius* Eichwald in Armenia]. Pp. 62-63 *In* [Fauna and its protection in the Transcaucasian republics]. Erevan. (In Russian).

PANOV, E. N. AND L. Y. ZYKOVA. 1985. [Comparative biology the steppe and rock agamas (*Agama sanguinolenta, A. caucasica*) in the Sumbar River basin (Western Kopetdag)]. Pp. 185-204 *In* [Vegetation and animal world of the western Kopetdag]. Ashkhabad. (In Russian).

PANOV, E. N. AND L. Y. ZYKOVA. 1986. [Notes on the behavior of the steppe agama, *Agama sanguinolenta*. I. General features of the biology, spatial structure of the population and

social behavior]. Zoologichesky Zhurnal, Moscow 65:99-109. (In Russian).

PANOV, E. N. AND L. Y. ZYKOVA. 1989. [Dynamics of social relations in a population of rock agamas, *Stellio caucasius* Eich.]. Pp. 184-185 *In* [Questions of Herpetology. Abstracts of the Reports at the Seventh All Union Conference of Herpetology, Naukova Dumka, Kiev]. (In Russian).

PANOV, E. N., L. Y. ZYKOVA, M. E. GLAUZER AND V. I. VASIL'ER. 1987. [Zone of intergradation of different forms of *Stellio caucasius* compex in South-western Turkmenia]. Zoologichesky Zhurnal, Moscow 66: 402-411. (In Russian).

SEMENOV, D. V. 1989. [Spatial organization in populations of reptiles. Achievements of science and technology, ser. Vertebrate Zoology]. VINITI 17: 101-134. (In Russian).

STAMPS, J. 1988. Conspecific attraction in territorial species. American Naturalist 131:329-347.

WILEY, R. H. 1981. Social structure and individual ontogenesis: problem of description, mechanism and evolution. Perspectives in ethology 4:105-133. Plenum Press, New York.

ZYKOVA, L. Y. AND E. N. PANOV. 1991. [Long-term study of growth in the rock agama, *Stellio caucasius*]. Zoologichesky Zhurnal, Moscow 70: 81-90. (In Russian).

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 111-113

A Study on the Comparative Cytology of Some Endocrine Glands in *Rana plancyi* between Hibernation and Post-hibernation

JI QING

Department of Biology, Xuzhou Teachers College, Xuzhou, Jiangsu, 221009 China

Abstract: -This paper studies the ultrastructure of the pituitary gland cell and the adrenal cortex cell in *Rana plancyi* between hibernation and post-hibernation. The results show that the two kinds of cells mentioned above are much less inactive during hibernation than during post-hibernation. The significance of those results is also discussed.

Key words: Amphibia, Anura, *Rana plancyi*, pituitary gland, adrenal cortex, comparative cytology.

Introduction

Hibernation is an adaptive strategy of frogs for keeping out of the cold during the winter. Studying the hibernation biology of frogs is beneficial to protecting the frogs and making use of frog resource. No paper related to the comparative cytology of the endocrine glands in frogs has been reported in China for many years. This paper reports the results of studying the comparative cytology of the pituitary gland cell and the adrenal cortex cell in *Rana plancyi* between hibernation and post-hibernation by using a transmission electron microscope.

Methods

According to the regular pattern of hibernation in the locality, many specimens of *Rana plancyi* were collected from a little river in October 1992, in the suburbs of Xuzhou City, put into a box, and then laid outdoors during the winter. Some specimens, which represent the hibernation group, were fetched from the box and used for the experiment on January 8, 1993. Some specimens representing the post-hibernation group were collected from the same little river mentioned above and were used to do the experiment on May 17, 1993.

Four specimens were collected from the two groups, regardless of their sex. The specimens were killed and dissected so that the pituitary gland and the adrenal gland were obtained. The two glands were fixed with 4% glutraldehyde and embedded in Epon-812. Ultra-thin sections were doubly stained with uranyl acetate and lead citrate by the standard method, and the specimens were examined with a Hitachi 600-A-II electron microscope.

Results

Pituitary gland cell

The pituitary gland cell of the hibernation group has plenty of glycogen particulates (Pl. I:1) but few rough endoplasmic reticulum and mitochondrium (Pl. I:2), while the pituitary gland cell of the post-hibernation group has plenty of rough endoplasmic reticulum and Golgi bodies but no glycogen particulates (Pl. I:3). The pituitary gland cell of the post-hibernation group also has plenty of secretory granules (Pl. I:4), and some secretory granules can sometimes be seen moving to blood capillary (Pl. I:5).

Adrenal cortex cell

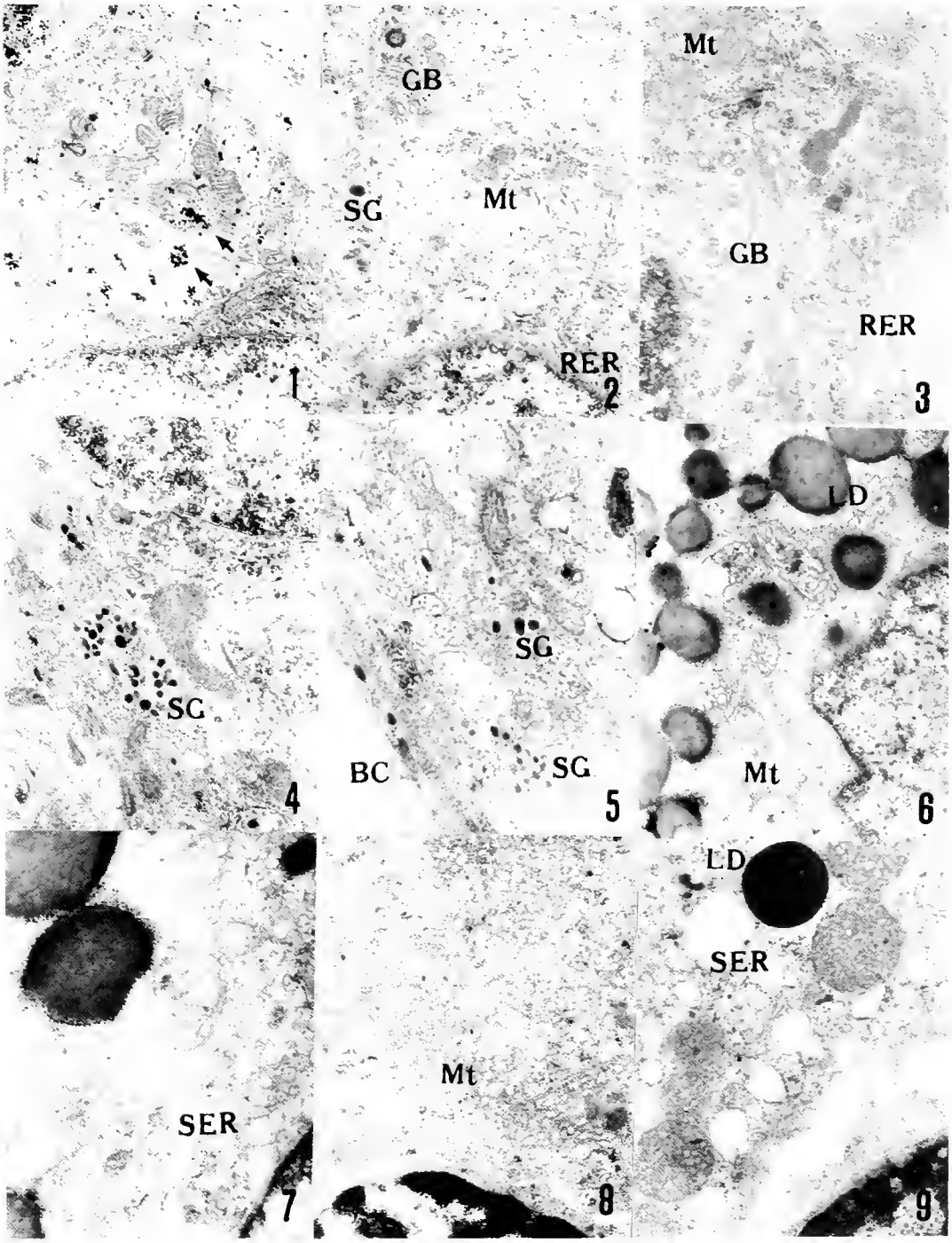
According to the result of the examination of the semi-thin sections, the adrenal cortex in the adrenal gland was defined. The adrenal cortex cell has tubular cristate mitochondrium as its marking. The adrenal cortex cell has plenty of lipid drops, some smooth endoplasmic reticulum and few mitochondrium (Pl. I:6, 7) during hibernation, but has plenty of mitochondrium (Pl. I:8), few lipid drops and plenty of expanded smooth endoplasmic reticulum (Pl. I:9).

Discussion

The pituitary gland is the most important endocrine gland in the frog and plays an

Plate I

1. Pituitary gland cell showing glycogen particulates during hibernation (arrows). X 12 000
2. Pituitary gland cell showing mitochondrium (Mt), rough endoplasmic reticulum (RER), Golgi bodies (GB) and secretory granules (SG) during hibernation. X 15 000
3. Pituitary gland cell showing Mt, RER and GB during post-hibernation. X 20 000
4. Pituitary gland cell showing SG during post-hibernation. X 17 000
5. Pituitary gland cell showing SG near the blood capillary (BC) during post-hibernation. X 20 000
6. Adrenal cortex cell showing lipid drops (LD) and Mt during hibernation. X 20 000
7. Adrenal cortex cell showing smooth endoplasmic reticulum (SER) during hibernation. X 20 000
8. Adrenal cortex cell showing Mt during post-hibernation. X 20 000
9. Adrenal cortex cell showing expanded SER and LD during post-hibernation. X 20 000



important role in its hibernation. It can secrete, releasing hormone which can regulate the activity of the other endocrine glands. The studies on frog cytology have not concerned the pituitary gland for many years. Previous work (Daguy, 1963; Saint Girons, 1975) has showed that the pituitary gland in some reptiles increase in activity several weeks before the end of hibernation. This investigation shows that the pituitary gland cell is inactive during hibernation, but active during post-hibernation. It also suggested that the behavior of the pituitary gland in frogs is similar to the same gland in reptiles during hibernation and post-hibernation.

The adrenal cortex can secrete glucocorticoid and mineralocorticoid. The two kinds of hormones can regulate glucometabolism and mineralometabolism. These two kinds of hormones are both synthesized in the smooth endoplasmic reticulum, so that the number of smooth endoplasmic reticulum can indicate the secreting level of the two kinds of hormones. Previous work (Robertson et al., 1959; Chan et al., 1971) have shown that the adrenal cortex is inactive during the winter and active in the summer. This investigation also shows that the adrenal cortex cell is active during post-hibernation and inactive during hibernation. According to this investigation, it is considered that the activity of the adrenal may begin from the end of hibernation.

Acknowledgments

The author is deeply indebted to Professor Shou-chang Zou for having supplied the materials and for his helpful advice throughout the research. The author also wishes to thank Professor She-chao Chen for his excellent technical assistance.

Literature Cited

CHAN, S. W. C. AND J. G. PHILIPS. 1971. Seasonal variations in production in vitro of corticosteroids by frog (*Rana rugulosa*) adrenal. Journal of Endocrinology 5:1-17.

DAGUY, R. 1963. Biologie de la latence hibernale chez *Vipera aspis*. Vie Milieu 1963 14:311-443.

ROBERTSON, O. H. AND B. C. WEXLER. 1959. Histological changes in the organs and tissues of migration and spawning Pacific salmon (Genus *Oncorhynchus*). Endocrinology 66:222-239.

SAINT GIRONS, H. 1975. Le cycle annuel des glandes endocrines chez les serpents des regions temperas. Bulletin Zoology of France 100:654-655.

Reproductive Behavior in the Long-tailed Salamander (*Onychodactylus fischeri* Boulenger).

I. A. SERBINOVA[1] AND V. A. SOLKIN[2]

[1]*Moscow Zoo, B. Gruzinskaya 1, Moscow 12342 Russia*
[2]*Pacific Ocean Institute of Geography, Far East Branch of Russian Academy of Sciences, Vladivostok, 690032, Russia*

Abstract. - We studied reproductive behavior in wild and captive *Onychodactylus fischeri*. Reproductive behavior and physiology is triggered by warming water temperatures in the spring. Pair formation begins at 7° C and spawning at 9° C water temperature. Fertilization is external. The development of distinctive femoral musculature and skin on the posterior surface of the hindlimbs in the male is for grasping the eggs during fertilization. Pairs of salamander experimentally injected with a synthetic analogue of gonadotropin releasing hormone exhibited typical mating behavior and produced fertile clutches of eggs.

Key words: Onychodactylus fischeri , salamander, reproduction, external fertilization, courtship, gonadotropin releasing hormone.

Introduction

The long-tailed salamander, *Onychodactylus fischeri* , is one of the most poorly known amphibians in Russia. Emeliyanov (1947) noted that spawning takes place from the time salamanders appear in the spring until the middle of July. Regel and Epshtein (1975) concluded that reproduction in this species is not restricted to a specific season. The discovery of a clutch with some hatched larvae indicated that oviposition probably occurred in the spring (Kozik, 1991). It is difficult to establish the precise time of oviposition since the length of embryonic development for this species is unknown. The length of embryonic development for the related species *O. japonicus* is 120 days (Hayase and Oseki, 1983) and it breeds in the winter (Akita, 1989). The absence of precise information on the timing and method of reproduction of the long-tailed salamander prompted us to undertake this study.

Methods and Materials

This study was conducted from May through July, 1991 on the Primorsky krai a tributary of the Mineral'naya River. Daylight observations of the location and reproductive state of salamanders in nature were made along a 1000m transect along Kitaisky Spring. The search effort was directed to the time of maximum diurnal activity. All captured salamanders were individually numbered by toe-clipping using standard techniques. A total of 601 salamanders were captured of which 102 were recaptured. All salamanders were measured and weighed on a triple beam balance. We noted the degree of development of such male secondary sexual characters as the distinctive femoral musculature and skin development on the posterior surface of the hind limbs. We recorded such female characteristics as the relative size of oocytes observed through the abdomen, their passage into the oviducts and the condition of the ventral opening to the cloaca.

The long-tailed salamander has secretive habits and we determined that observation of natural oviposition was unlikely. Therefore, we stimulated reproductive behavior hormonally in captive individuals. Salamanders were housed outdoors in the shade of an awning in 8 liter aquaria with 3-5 cm of running water and shelters. Aquarium water was changed daily. Each of seven males and seven females were injected with 1-5 µg/individual/day of a synthetic analogue of gonadotropin releasing hormone for 7-12 days. All individuals were determined to be ready for breeding before injection. Males and females were kept separate until ovulation began; then they were paired.

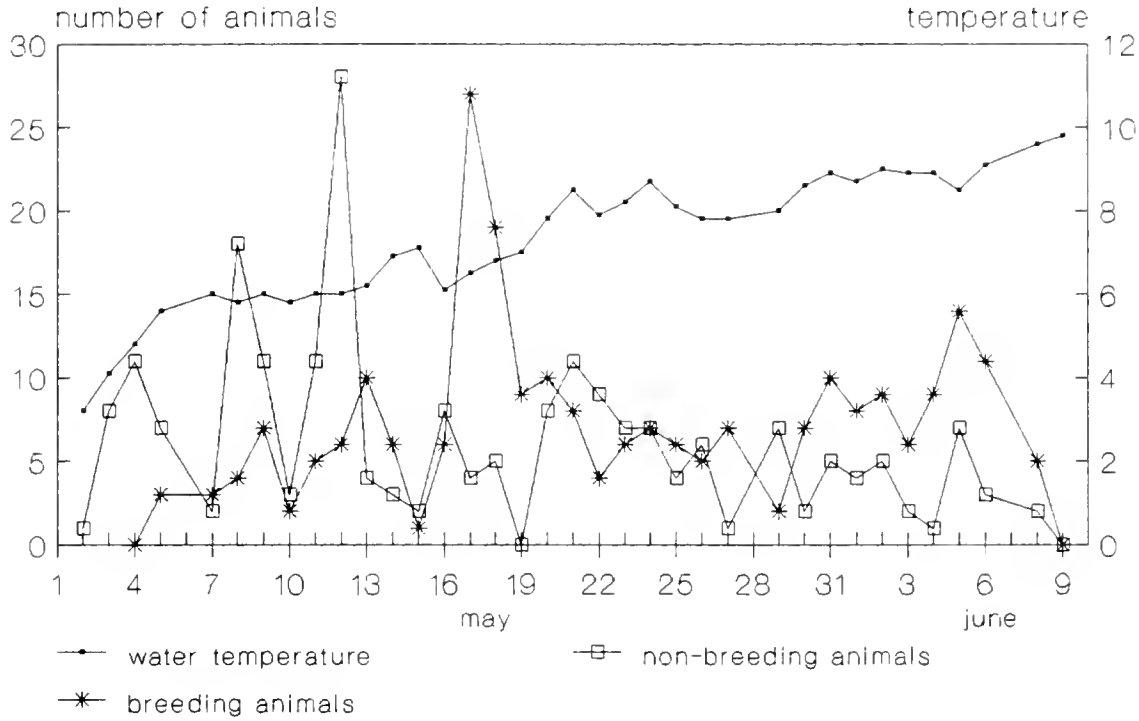


FIG. 1. Pattern of *Onychodactylus fischeri* activity and water temperature during the spring..

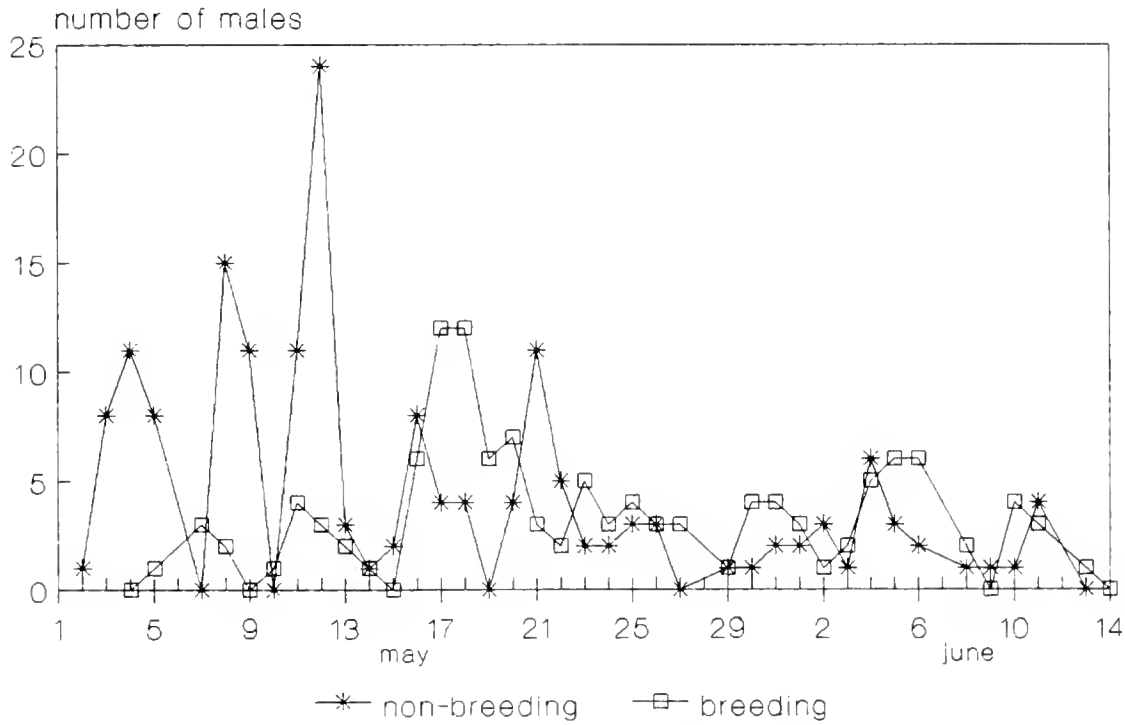


FIG. 2. Frequency occurrence of breeding and non-breeding male *Onychodactylus fischeri*.

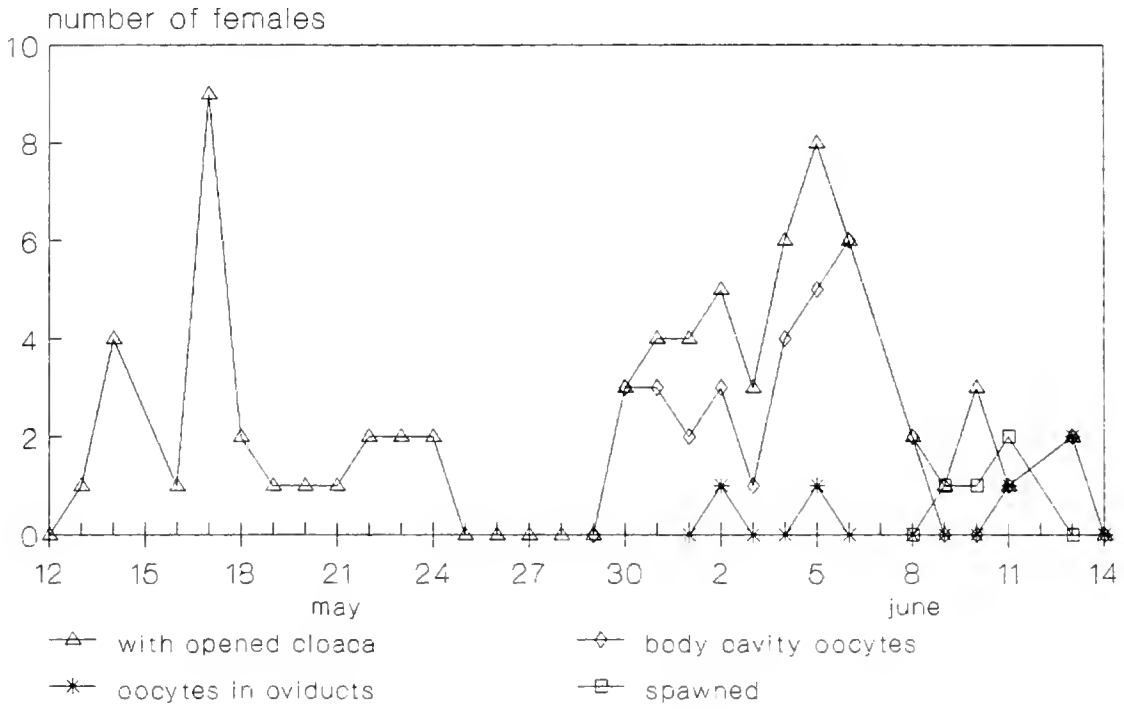


FIG. 3. Frequency occurrence and reproductive state of female *Onychodactylus fischeri*.

Results and Discussion

The first observations of active salamanders were made in late April to early May at a water temperature of approximately 3° C and while sections of the stream were still covered with ice. The number of animals active each day increased as temperatures warmed during the spring (Fig. 1). The first salamanders observed did not exhibit readiness for breeding. Oocytes were not visible through the abdomen wall of females and males did not exhibit hindlimb muscle and skin development. All animals were in a poor nutritional state. The peak number of non-reproductively ready salamanders occurred on 12 May when water temperature reached 6° C. Not all salamanders leave their winter shelters simultaneously due to differential warming rates along the slopes and this is reflected in Fig. 1. After 12 May the number of non-reproductive salamanders decreased to a constant level by 20 May.

The peak number of reproductively ready salamanders was encountered on 17 May when the last stretches of the stream were ice free. These animals appeared to be well nourished. From 15 May to 15 June a majority of the males encountered were in breeding condition (Fig. 2).

By 20 May post-hibernation aggregations of salamanders began to disintegrate and the individuals dispersed along the stream. This marked the beginning of the breeding season.

By 10 May we began encountering females with opened cloacas (Fig. 3). By the end of May females with ovulated oocytes in the body cavity were observed and by 10 June we found salamanders with oocytes in the oviducts. We first observed a female that had spawned or oviposited her eggs on 9 June. In early June we observed 3 male-female pairs of salamanders with at least one member in reproductive readiness. These observations agree with previous accounts (Regel and Epshtein, 1975).

In the hormonally treated females the caudad displacement of oocytes usually took

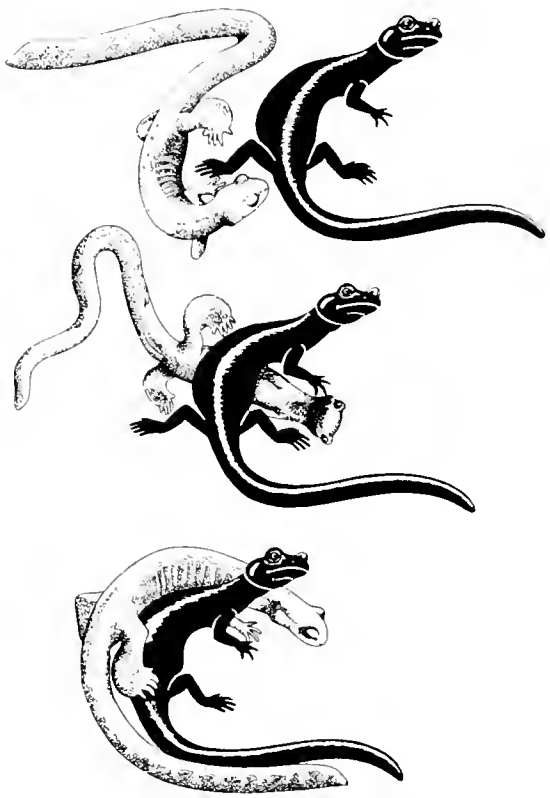


FIG. 4. Courtship behavior of *Onychodactylus fischeri* with the male (light salamander) approaching the female's (dark salamander) vent, rubbing against the female's abdomen and straddling the female.

two to four days after injection. In another day oocytes appeared in the body cavity and three days later they filled the oviducts. From hormone injection to readiness for spawning usually took from six to 10 days. We observed 6 cases of spawning, two of which were followed by spermatophore deposition.

In the hormonally treated animals approximately one to two days before spawning the female salamander ceased most activity and assumed a typical pre-spawning posture on a stone with the rear third of the body in the water. At this time the male moves about the aquarium, periodically approaches the female touching his snout to her vent, rubbing his body on hers and resting beside her (Fig. 4). The

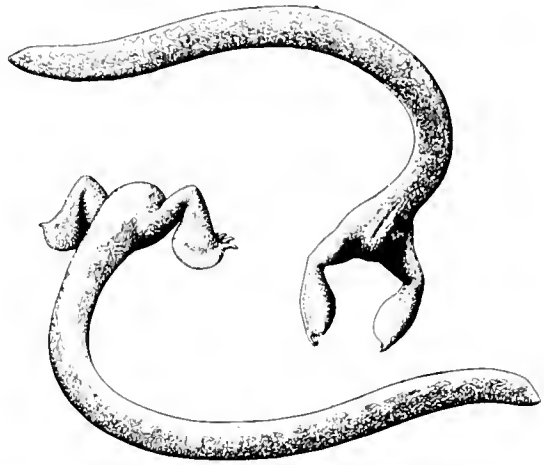


FIG. 5. Comparison of the male *Onychodactylus fischeri* hindlimbs and tail in normal posture and in the pre-fertilization posture with the legs extended. Note the distinctive heavy femoral musculature and skin development on the posterior of the hindlimbs.

male then extends his rear legs and holds this position (Fig. 5).

When spawning begins the female attaches a mucous cord to the stone with a pair of egg sacs each containing one to eight eggs. The eggs are five to six mm in diameter. The mucous capsule is thick and strong unlike that of *Salamandrella keyserlingii* and *Ranodon sibiricus*. During egg deposition (30-40 min.) the male remains sitting beside and in contact with the female. When the egg sacs appear the male enters the water and approaches the female's vent with his snout. In this position the male's body begins to undulate. When the male nudges the egg sac with his snout this causes a burst of excited thrashing from side to side by his snout. This dislodges the egg sac from the female. The male moves immediately over the egg sac with his legs extended and grasps the egg sac with his forelimbs, positioning it between his hindlimbs. The male then grasps the egg sac with the hindlimbs and pulls the base of the sac against his vent and deposits the spermatophore (Fig. 6).

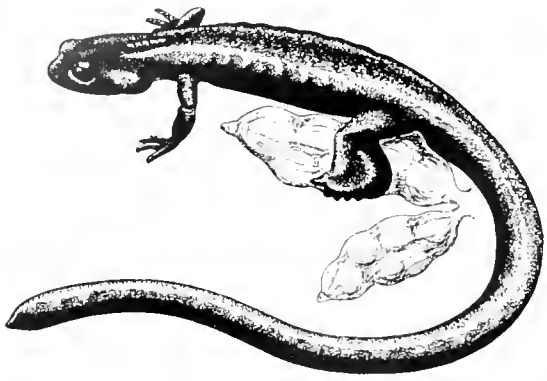


FIG. 6. Male *Onychodactylus fischeri* grasping the egg sac with the hindlimbs in apposition with the vent during spermatophore deposition. Note the distinctive heavy femoral musculature and skin development on the posterior of the hindlimbs.

Those individuals that are ready for breeding emerge from hibernation later than those that are not reproductively ready. We believe that the triggering mechanism for breeding is the elevation of the stream temperature as can be seen in the increased activity of reproductively ready salamanders when the water temperature reaches 6 to 7° C (Fig. 1). At this time females with open cloacas begin to appear. When water temperatures have reached 8° C most females have opened cloacas and many have oocytes in the body cavity. At 9° C water temperature some females have oocytes in the oviducts. By the time water temperature reaches 10° C most females have oocytes in the oviducts and some are beginning to spawn (Fig. 3). We believe increasing temperature is the primary triggering mechanism for reproductive readiness, courtship behavior and spawning.

We found very few post reproductive females on the surface (n=3 in 1990; n=5 in 1991). Very few males were observed on the surface with their legs extended in the reproductive posture. Spawning activity coincided with the period of optimum temperature for activity and low water levels. Salamanders generally remain near the area of spawning until the next reproductive season.

These salamanders are sparsely distributed and do not form breeding aggregations. Male salamanders seem to seek out females well before spawning (i.e. 15-20 days), perhaps by olfaction. The males initiate courtship behavior at this time and it may continue for up to 10 days thus assuring that the male is likely to be in attendance to fertilize the eggs when the female spawns. This lengthy courtship period is the longest known of our native amphibians. This pattern contrasts with that of *Mertensiella caucasica*, the Caucasian salamander, a species with a similar ecology but different reproductive pattern from that of *O. fischeri*. The Caucasian salamander has internal fertilization that induces ovulation. Courtship and copulation take much less time and the pair is together for a much shorter time than are long-tailed salamanders. These represent two very different reproductive strategies for two distantly related species living in similar habitats.

Summary

Our studies have demonstrated that reproduction in *O. fischeri* is triggered by warming water temperatures in the spring. Pair formation begins at 7° C and spawning at 9° C water temperature. Fertilization is external. The development of distinctive femoral musculature and skin is for grasping and holding the eggs during fertilization. Pairs of salamander experimentally injected with a synthetic analogue of gonadotropin releasing hormone exhibited typical mating behavior and produced fertile clutches of eggs.

Literature Cited

AKITA, Y. 1989. Breeding cycle of *Onychodactylus japonicus* on Mt. Hodatsu, with special reference to biannual spawning. Pp. 305 *In* Proceedings of the Second Japan-China Herpetological Symposium, Kyoto, Japan, Herpetological Society of Japan.

EMELIYANO A. A. 1947. [On the biology of the long-tailed salamander]. Zoologichesky Zhurnal 56(1): 65-70. (In Russian).

HAYASE, N. AND K. OSEKI. 1983. [Spawning and embryology of the salamander *Onychodactylus japonicus* (Houttuyn). Collect. and Breed 45(3):122-123. (In Japanese).

KOZIK, S. S. 1991. [The discovery of the long-tailed salamander clutch]. Zoologichesky Zhurnal 70(5):159-160. (In Russian).

REGEL, E. D. AND S. M. EPSHTEIN. 1975. [Some peculiarities of the long-tailed salamander biology]. Zoologichesky Zhurnal 54(10):1515-1524. (In Russian).

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 120-131

On the Inheritance of the Mid-dorsal Stripe in the Iranian Wood Frog (*Rana macrocnemis*)

DAVID N. TARKHNISHVILI

Department of Biology, Tbilisi State University, University Str. 2, Tbilisi 380077, Georgia

Abstract. -The frequency of the phenotype *Striata* (presence of a light mid-dorsal stripe) is often used in population investigations of anurans. Simple hereditary nature of this character was established experimentally. Nevertheless, animals with a poorly developed stripe can be found in populations of some species, together with non-striped specimens and clear *Striata*. in the frog, *Rana macrocnemis*, the expression of the mid-dorsal stripe varies continually. Poorly striped frogs prevail in some populations. We examined morph distribution among froglets obtained from seven *R. macrocnemis* pairs whose eggs and larvae developed at 18, 25, and 29°C. Non-striped froglets predominate in the offspring of non-striped parents and striped in the offspring of striped frogs. Non the less, all phenotype spectra, from specimens with a clear stripe to non-striped animals, are represented in almost all groups. The morph distribution is quite continual and the character varies as a quantitative one. When the developmental temperature increases, the frequency of the morph predominating in optimal conditions (18°C) declines with the increasing frequency of poorly striped froglets. Modifications of the character under the influence of thermal conditions shadows genetic specifics of sibling groups. Similar influence of conditions on the morph distribution in froglets was found in natural populations. Morph distribution among newly emerged froglets affects morph distribution of adults in the populations two years later

Key words Amphibia, Anura, Ranidae, *Rana macrocnemis*, inheritance, Georgia

Introduction

Specimens with a high mid-dorsal stripe can be found among a lot of anuran species. This character (phenotype *Striata*) appears in representatives of systematically distant anuran families. Sometimes a light stripe appears as a specific character (e.g. in the frog *Rana amurensis* or among some boreal toads). In other species, *Striata* appears as a morph and within the same population non-striped as well as striped animals can be found. Prevalence of this morph is species-specific. For instance, if among *Discoglossus pictus* or some *Eleutherodactylus* striped animals predominate (Duellman and Trueb, 1986), in *Rana temporaria* they are sparse (Istchenko, 1978) and in *Occidozyga laevis* only a few striped juveniles could be found (our unpublished data).

Anuran species among which a mid-dorsal stripe appears can be divided into two groups independently of their systematic position. The first group includes species in which the mid-dorsal stripe appears as a very clear and discrete character, and striped animals can be divided from non-striped ones easily. There are, for example, some representatives of the genus *Rana*:: *Rana cf. esculenta*, *R. nigromaculata*, brown frogs *R. sylvatica*, *R. arvalis* (Istchenko, 1978). The second group includes species having often or always softened stripe, characterized by the absence of a clear border between the stripe and ground color of dorsal surface. These include some brown frogs (*R. temporaria*, *R. chensinesis*, *R. macrocnemis*) among others. Specimens with a clear mid-dorsal stripe, as well as with a softened stripe, can be found in *R. macrocnemis* only (Istchenko, 1978). Moreover, in populations of these species, the degree of development of the mid-dorsal stripe varies continually. If numerous specimen samples are taken, a range from quite unstriped to very clear *Striata*-s may be constructed. These extreme phenotypes may be connected by animals with softened stripe (*Pseudostriata* phenotype group).

Inheritance of the Striata phenotype was examined experimentally. It was established that, in different anuran families, the hereditary mechanism may be similar and

dependent on the effect of the fully-dominant autosomal gene. It was shown, for instance, on *Eleutherodactylus ricordii* (Goin, 1947), *Rana nigromaculata* (Morya, 1952), *R. sylvatica* (Browder et al., 1966), *R. arvalis* (Stchupak, 1977; Stchupak and Istchenko, 1981), *R. ridibunda* (Berger and Imietowski, 1982). Simple hereditary nature was established for the coloration of the mid-dorsal stripe of *Acris crepitans*, as well (Pyburn, 1961). That is the reason of wide exploitation of the character in population-genetic investigations, especially of frogs from the genus *Rana* (Stugren, 1966; Fishbeck and Underhill, 1971; Istchenko, 1978; Pikulik, 1985; Gogoleva, 1985; Shibata, 1988; Kubantsev and Peskova, 1989 etc.). Nonetheless, the frequency of the *Striata* morph has been used as the index of isolation degree (Stugren, 1966; Pikulik, 1985; Shibata, 1988) or as the instrument of the natural selection investigations (Fishbeck and Underhill, 1971; Istchenko, 1978; Vershinin, 1987 etc.). Presence of a poorly expressed stripe in some species made us use mid-dorsal stripe in the phenetical investigations with care. Inheritance of *Pseudostriata* is not clear. For *R. limnocharis*, Moriwaki (1953) supposed polyallelic inheritance; there are no data for other species. In any case, connections between characters *Pseudostriata* and *Striata* must be present. Continual connections between morphs, e.g. in *R. macrocnemis* makes possible modifications of stripe development under environmental conditions, like most other quantitative characters. Variations of mid-dorsal stripe expression during the life span of individual *Rana temporaria* specimens was noted (Heran, 1986). Earlier (Tarkhnishvili and Mamradze, 1989; Mamradze, 1990) we showed that *Striata* and *Pseudostriata* phenotypes frequency in *R. macrocnemis* sibling groups can be modified as a result of temperature variability by which larval development takes place. This present article describes the results of the more detailed experiments and field observations on the Iranian wood frog populations.

Materials and Methods

Experiment. Adults of *R. macrocnemis* (7 pairs) were obtained in 1989 from a hibernation site in Satovle Mountain ridge (eastern Georgia, surroundings of Tbilisi). Mid-dorsal stripe expression differs in different specimens. We divided frogs conditionally in non-striped ones *(Maculata; M)*, specimens with poor enlightment in the middle-- or hind part of the dorsal surface *(Pseudo-pseudostriata; PPS)*, with poorly developed *(Pseudostriata; PS)* or clear *(Striata; S)* mid-dorsal stripe. The description of the parents (male phenotype/female phenotype) is: pair 1-M/M; 2-M/M; 3-M/PPS; 4-M/PPS; 5-PS/M; 6-M/PS; 7-PS/S. We selected 3 groups of 40 eggs from each of the 7 clutches obtained. Groups were placed in 40-l aquaria with aeration and stable temperature of 18, 25 and 29°C. Tadpoles were fed boiled spinach. Half of the water volume was renewed every second day. After metamorphosis, the complete phenotype of each froglet (M, PS or S) was described. Therefore, 20 experimental sites were elaborated (tadpoles from the 7th clutch developed under 29°C died before metamorphosis had begun).

Field Data. During 1989, 1990 and 1991 in a local population of *R. macrocnemis* from Borjomi Canyon (central Georgia), the ratio of phenotypes M, PPS, PS and S were investigated. We separately analyzed the distribution of different morphs in adult frogs, juveniles, and in 16 groups of froglets emerged from different ponds and pools (each sample included 20-44 froglets). There are more than 60 spawning sites in the population investigated, but every year 2 or 3 water bodies ensure about 90% of the whole generation. Comparative part of each water body was established as well as the number of clutches deposited in each of them (Tarkhnishvili, in press).

For the comparison of morph distribution between different samples, we

TABLE 1. Significance of the differences in morph distribution between experimental groups (values of λ -test).

At 18°C Group No.	Sibling group number 1	2	3	4	5	6
2	-					
3	1.56+	-				
4	2.19+++	2.63+++	-			
5	3.00+++	1.83+++	1.43+	-		
6	3.62+++	3.26+++	2.32+++	1.47+	2.10+++	
7	4.55+++	4.36+++	3.33+++	2.40+++	3.08+++	-
At 25°C	1	2	3	4	5	6
2	-					
3	1.53+	1.42+				
4	2.18+++	2.13+++	-			
5	2.53+++	2.42+++	-	-		
6	2.97+++	2.80+++	1.49+	-	-	
7	3.63+++	3.40+++	2.15+++	-	1.79++	-
At 29°C	1	2	3	4	5	6
2	-					
3	-	-				
4	-	-	-			
5	-	1.52+++	-	-		
6	1.99+++	2.07+++	1.61++	-	1.86++	

Note: + = P 0.05
++ = P 0.001
+++ = P 0.001

used the Kolmogorov-Smirnov λ-test. We compared the frequency of M, PS (PPS+PS) and S phenotypes in different groups using Fisher's F-test (Zaitsev, 1984).

Results

Experimental data. The distribution of different morphs in the 20 experimental froglet groups, obtained from 7 clutches and developed under the different conditions, is shown in Figure 1. Some main results of the experiment must be noted. Dominance of the S-gene excludes clear-striped animals' appearance among the offspring of non-striped parents; but independently of developmental conditions in offspring of the pair 1 froglets of the S-phenotype present. Morph PS appears in each sample. There are a few non-striped froglets among group 7 (male of S-phenotype) but their frequency is significantly lower than 25 or 50% that could be expected if, in the genotype of parents, the recessive gene M is present. Besides that, the inheritance ability of a mid-dorsal stripe is obvious. After development under the optimal thermal conditions (18°C), differences in the morph distribution between most of the samples, excluding froglet groups with the same or very similar parents' phenotype, are significant (Table 1). Among the offspring of the pairs 1 and 2 (M/M), non-striped specimens predominated; their frequency was significantly higher than even the 3rd or 4th pairs (M/PPS) offspring. Correspondingly, among group 7 (parents PS/S), non-striped morph was significantly lower than in other groups. There is shown the significance of

TABLE 2. Inter-group differences in the frequency of each morph separately (F-test).

Groups Compared	Phenotypes								
	M			PS			S		
	18°C	25°C	29°C	18°C	25°C	29°C	18°C	25°C	29°C
1 and 2	-	-	-	+	-	-	-	-	-
1 and 3	+++	++	-	++	-	-	++	++	-
1 and 4	+++	+++	+	+	-	-	+++	+++	++
1 and 5	+++	+++	+	+++	++	+	++	+++	-
1 and 6	+++	+++	++	+	+	-	+++	+++	+++
1 and 7	+++	+++	?	-	-	?	+++	+++	?
2 and 3	++	++	++	-	-	-	+++	+++	++
2 and 4	+++	+++	++	-	-	-	+++	+++	+++
2 and 5	+++	+++	++	++	+++	+++	+++	+++	-
2 and 6	+++	+++	+++	-	-	-	+++	+++	+++
2 and 7	+++	+++	?	-	-	?	+++	+++	?
3 and 4	-	-	-	-	-	-	-	-	-
3 and 5	++	+	-	+	-	-	-	-	-
3 and 6	+++	+++	-	-	-	?	+++	-	+++
3 and 7	+++	+++	?	-	-	-	+++	+++	-
4 and 5	-	-	-	++	+	+	-	-	+
4 and 6	++	+	-	-	-	-	++	-	+
4 and 7	+++	+++	?	-	-	?	+++	+	?
5 and 6	-	-	-	++	+	++	+++	-	+++
5 and 7	+++	++	?	+++	++	?	+++	+++	?
6 and 7	+	-	?	-	-	?	-	+	?

Note: - = insignificant
+ = P 0.05
++ = P 0.01
+++ = p 0.001

inter-sample differences in Table 2. In all, along the road of 1-7 groups, frequency of M-phenotype decreases and frequency of S-phenotype increases significantly but gradually. Frequency of (PS)-morphs increases gradually from the 1st to the 5th group (PS/M) and decreases again among the 6th and 7th groups.

Developmental conditions of larvae affect the ratio of different morphs. When temperature increases, especially from 25 to 29°C, in pair 3-6 offspring (information of group 7 absent) frequency of S-morph decreases significantly. These groups are established by frogs with mid-dorsal stripe, even very pale or lightened. This time, a few S-morph froglets among groups 1 and 2 appear independently of the thermal conditions. On the other hand, frequency of non-striped morph in group 1 decreases significantly as a result of high developmental temperature. Inversely, among groups 5 and 6 (parents PS/M and M/PS) part of the non-striped morph increases, especially between 25 and 29°C. At least among most of the groups frequency of PS+PPS morph group slightly increases under high developmental temperature (see Fig. 1 and Table 3). Thus variation of the developmental conditions during the larval period can affect, but in different ways, the ratio of the phenotypes in the froglet groups. We can not conclude

TABLE 3. Significance of the phenotype frequency changes under the thermal conditions.

Sibling Group	Difference of thermal conditions between 18°C to 25°C M	(PS)	S	25°C to 29°C M	(PS)	S	18°C to 29°C M	(PS)	S
1	-	-	-	-	-	-	++	++	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	++	-	-	-
4	-	-	-	-	-	+	-	-	-
5	-	-	-	+	-	+++	-	-	++
6	-	+	-	++	+	-	+	-	+
7	-	-	-						

Note: -, +, ++ and +++ are significance levels.

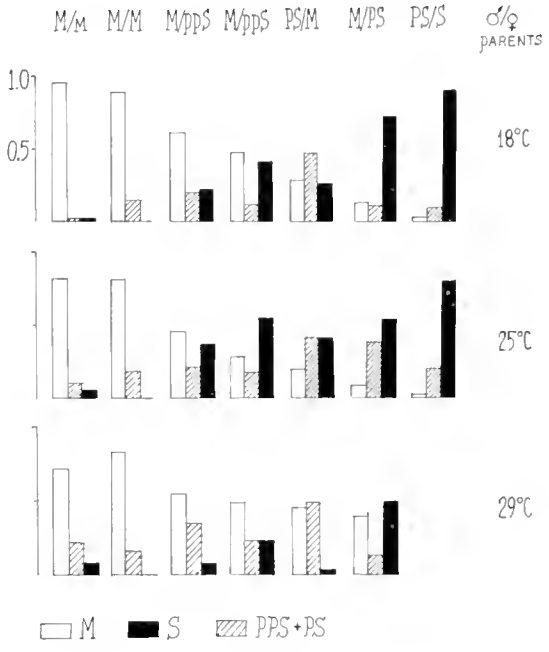


FIG. 1. Frequency distribution of morphs in experimental froglet groups.

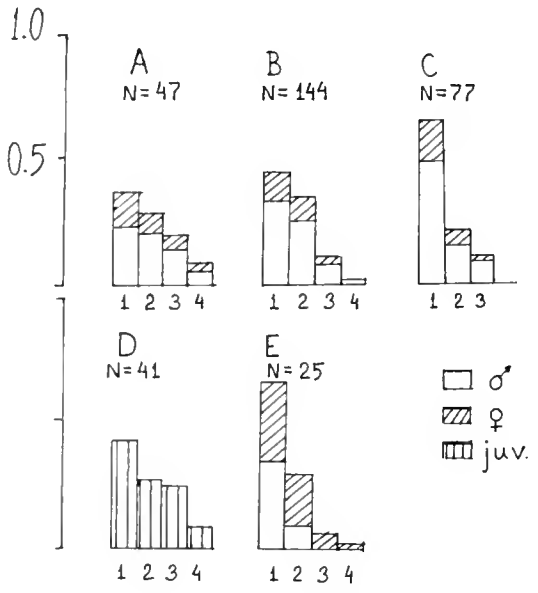


FIG. 2. Frequency distribution of morphs in natural populations. A- adults from Borjomi Canyon in 1989; B- adults from Borjomi Canyon in 1990; C- Adults from Borjomi Canyon in 1991; D- juveniles from Borjomi Canyon in 1989; E- adults from Satovle Mountain ridge in 1989. 1- *Maculata;* 2- PPS; 3- PS; 4- S.

that the rise of temperature always increases or decreases the percentage of striped frogs. Rather it declines frequency of display of the genotype predominating. In other words, the comparative part of heredity in determining the morphological features of the group decreases. This is illustrated in Table 1. Inter-group differences so clear in optimal conditions, after development in warm water often became non-significant. Perhaps the reason for this phenomena is the changing in expression of the stripe. In warm water, the part of genetically striped specimens processes determining the appearance of stripe are delayed and only non-clear stripes develop. Inversely, the

TABLE 4. Significance of the differences in morph distribution between groups emerged from the sites 9, 11 (1989) and 12 (1990), and that which emerged from another site (values of the λ-test).

	1a	2a	3a	4a	5a	6a	7a	8a	9a	10a	11a	12b	13b	14b	11c	12c	15c
	+++	+++	-	++	-	++	-	-	-	-	+++	-	-	+	+	-	+
9a	2.00	1.97		1.90		1.88					2.39			1.60	1.46		1.86
	-	-	-	-	+	-	-	+	+	-	-	++	-	-	-	-	-
2a					1.60			1.58	2.39			1.82					
	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+
12b	1.44	1.45		1.36			1.43										1.42

Note: Numbers 1-15 are the pond number from which the froglets emerged.
a = generation of 1989
b= generation of 1990
c = generation of 1991
+, ++, and +++ are significance levels

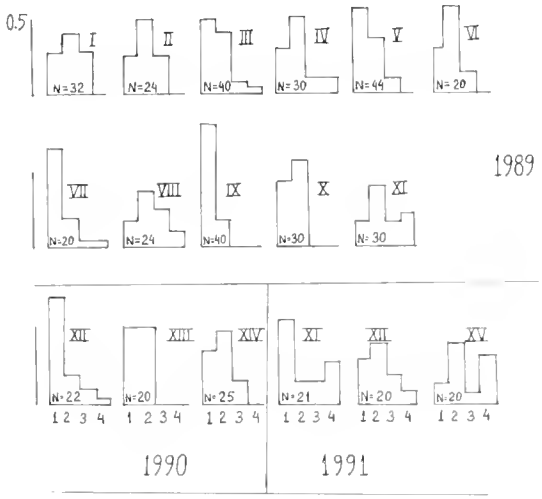


FIG. 3. Frequency distribution of morphs in frogs that emerged from natural ponds and pools in Borjomi Canyon. I-XV- number of the water body; N- sample size; Phenotypes: 1- M; 2- PPS; 3- PS; 4- S.

part of genetically non-striped frogs can be turned into the PS-category.

Field data. Morph frequency distribution among the adult frogs from the natural population is shown in Fig. 2. The sexual differences are not significant. The differences between these samples are the sample collected in 1991: λ= 1.51 (1989-1991) - 1.41 (1990-1991) ($P<0.05$). It correlates with an increase of non-striped frogs to 65% in this year ($F=10.11^{xx}$) and the disappearance of the froglets with a clear mid-dorsal stripe ($F=13.34^{xxx}$). Interestingly, morph distribution in the investigated population became similar to morph distribution in the population from the Satovle Mountain ridge (this is a drier habitat distant from the former by 100-150 km) (Fig. 2e). Note that in both population, S-morph frequency is lower than in other populations of *R. macrocnemis* investigated earlier (Istchenko, 1978), but the frequency of the frogs with poorly developed stripes remain very high. Phenotypic distribution of sub-adults and juveniles in 1989 (Fig. 2d) is similar to those of adults during 1989-1990.

A great part of animals in brown frog populations begins to breed after the 2nd to 3rd hibernation (Istchenko and Ledentsov, 1987; Ledentsov, 1990). We can not exclude the fact that froglets which emerged in 1989 could participate in the formation of the generation that emerged during 1991. Therefore, analysis of their phenotypic distribution appears interesting. During 1989, froglet groups that emerged from 11

small water bodies were described. Results are shown on Fig. 3. Because of the small volume of sample differences between them, it appears non-significant in most cases. Only froglets from the water bodies 9 and 11, moreover from 12 (1990) are significantly different from most other groups (Table 4). Spawning sites 9 and 12 are well warmed pools, filled with vegetation. Daily temperature during the developmental period often exceeds 30°C. Among the froglets that emerged from these pools, the non-striped morph predominates. The percent of the S-morph (in site 9, this morph is absent), as well as PS and even PPS, is low. Inversely, site 2 is self-flowing and shadowed. Vegetation is absent and the water temperature do not exceed 20°C, even during the warmest days. PS and PPS morphs predominate here, and the percent of the S-morph exceeds 20.

There is the question of whether the differences noted above can reflect non-random distribution of the parent's morphs along the different spawning sites. On the one hand, the displacement of warmed and shadowed sites are irregular in the habitat examined, and striped frogs have equal possibility of spawning in different water bodies. On the other hand, only a few clutches (4 and 7 correspondingly) were deposited in sites 9 and 11 during 1989. Morph distribution in the froglets could be the consequence of random processes as a result. But, as many as 93 clutches were deposited in site 12 (1990). It is quite a representative sample of the population, numbering 1037 adult females in all (1990). This time, the frequency of non-striped froglets emerged from site 12 (68%) is instantly higher than M-morph frequency among adult frogs (45%). Moreover, during 1991 in site 2, already 37 clutches were deposited, but the frequency of S-morph among froglets even slightly increased. Therefore, we can suppose non-random reasons of the morph distribution in the groups of froglets emerged from different spawning sites. The frequency of one or another morph depends on the physical conditions under which larval development takes place. This is argued by the significant differences in phenotypic structure between froglets that emerged from different water bodies as well as between the young generation and adult animals. The stability of the morph composition of groups emerging from the same site, but in different waters, is additional evidence.

Above, we told about the increase of the non-striped morph in 1991 to 65%. It may be connected with the increasing of the comparative part of warmed pools during previous years. For instance, spawning site 5 produced at least 45% of the total number of the 1989 generation (about 20,000). In that time, the frequency of M-morph approached 55% and not one froglet with a clear stripe was found. Most of the other sites producing metamorphosis in 1989 were warmed. Shadowed site 2 (17% M and 23% S) produced not more than 25% of the generation. In 1990, the situation changed in *Maculata's* favor still more. There were 68% of froglets of this phenotype that emerged from site 12 (site 12 produced about 70% of the generation numbering 44,000 froglets). Perhaps in the future, we can suppose still more increase of non-striped morph's comparative part and a decline of the striped animals' frequency in the investigated population.

Discussion

Two directions of the S-morph frequency investigations in amphibian population can be noted. The first is the analysis of geographic variations of morph frequency in populations of different species. The second one is to study the dynamics of polymorphism of separate populations including age-dependent changes of morph distribution. The summarizing bibliography following must be noted.

Geographic variation. There is no good evidence of clear clinal variations of frequency of the morph *Striata* in a single species. Stugren's conclusion (1966) about increasing of frequency of striped frogs in *Rana arvalis* toward the east was refuted by Istchenko (1978). In some cases (i.e. *R. arvalis* and *R. temporaria* in Belorussia), the

percent of striped frogs increase toward the north (Istchenko, 1978; Pikulik, 1985), but in the populations of *R. arvalis* from the Urals or of *R. semiplicata* from the Russian Far East, the inverse situation was noted (Toporkova, 1965; Istchenko, 1978). On the other hand, irregular but clear interpopulational differences in S-morph frequency are common in different species' populations (Masalikin, 1985; Panchenko, 1985; Shibata, 1988 etc.). The frequency of the S-morph in *R. arvalis* may increase under the hardening of antropogenous pressing (Vershinin, 1987). Therefore, frequency distribution of phenotypes in the separate populations of some species depend highly on the climatic or microhabitat conditions but not on historical reasons. Interestingly, when the coexistence of *R. temporaria* and *R. arvalis* populations takes place (in the Middle Volga) the same environmental conditions ensure parallel variations in population morphology (Lebedinski et al., 1989)

Intrapopulation dynamics.
Comparative frequency of different morphs may vary between years and separate generations (Istchenko, 1978). Influence of the pond conditions on the morph distribution in *R. macrocnemis* froglet groups was described by this author. Besides, morph distribution in the generation could be changed in relation to animal age. For some *R. arvalis* populations, decreasing of S-morph frequency in adult animals was established in comparison to juveniles (Pikulik, 1985; Vershinin, 1987). Inversely, in the same species, increasing of the comparative part of striped animals was recorded for the older age groups (Istchenko, 1978). Therefore, the morphological features of the population may be changed rapidly and instantly by environmental conditions. There is the mean adaptation on the population level. Istchenko (1978) supposes that rapid reactions of morph distribution on the environmental conditions take place because of the elective elimination of animals. Rapid modifications of the genotypes' distribution is the result of this process. Selection may be connected not only with preferences of striped or non-striped frogs but also with elective elimination of larvae accordingly to their genotypes. For instance, larvae of *Striata* genotype are more vulnerable to the high density and shortage of the oxygen because of the abnormally high metabolic rates (Schvarz and Istchenko, 1968). On the other hand, the adult *Striata*-s are less susceptible than other frogs to drying and are characterized by high migration ability. Correspondingly, in some ecosystems with unfavorable conditions, striped specimens predominate (Istchenko, 1978; Vershinin, 1987).

Therefore, the influence of the developmental conditions on the ratio of striped and non-striped morphs in the generation may be considered well-grounded. The role of the developmental sites' type is obvious and our data are in accordance with this fact. All the researchers agree that the phenomena depends on the elective elimination.

It is not possible in practice to only conduct field investigations. What is the part of individual modifications in the process of morph distribution changing? High natural mortality, especially during the larval stages, does not allow us to exclude the effect of natural selection. Thus, each explanation depends on a prior opinion of the concrete researcher. For instance, in field conditions, information principally similar to our experimental data was obtained by Stchupak (1975). Increasing the frequency of genotype S or M has inverse reaction of larval grouping as the result: the comparative part of the dominant phenotype decreases by the completion of metamorphosis. The author explains this fact as the result of frequency-dependent selection.

Part of the phenotypic modification during the early development can be established only in experiments where larval mortality can be neglected. The experiment described above illustrates the main part of modifications taking place during larval development. We conclude that in some specimens displacement of the characters in the road of alternative variants could be changes as a result of temperature changes.

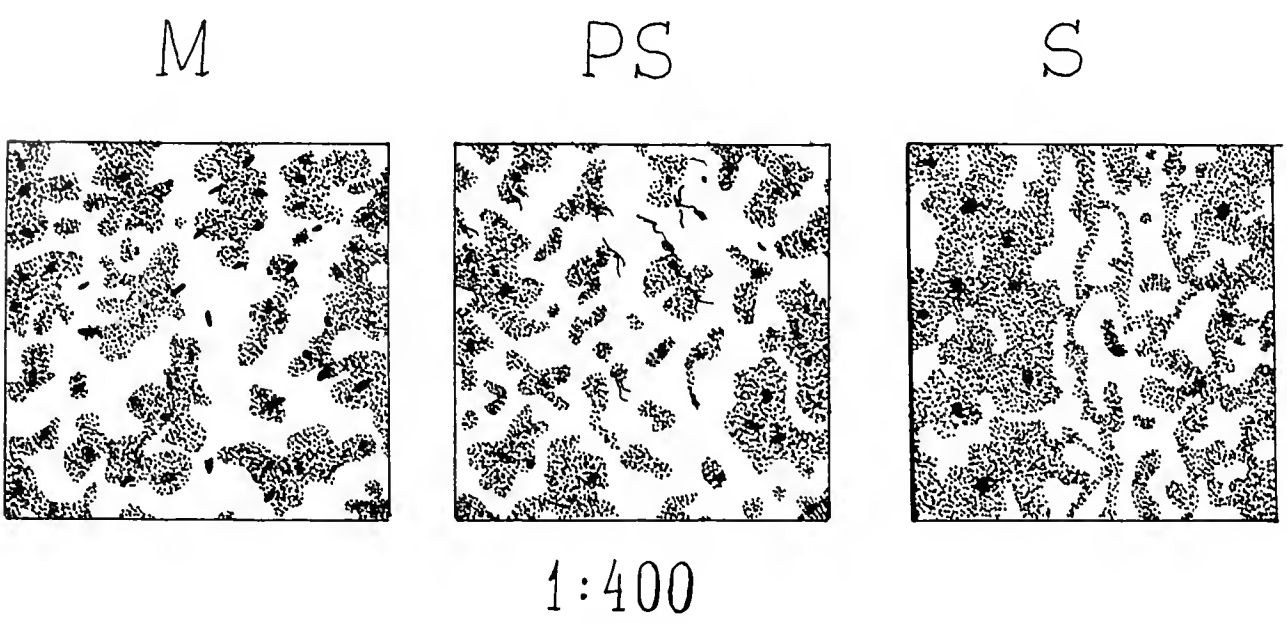


FIG. 4. Distribution of the pigmental cells in the mid-dorsal area of froglets that have different phenotypes. Magnification is X400.

It brings us to suppose that morphological specifics of froglet groups is provided not only by elective elimination but by phenotypic modifications as well. What is the comparative part of this latter could be shown by further investigations.

Experimental results described here and evidence in favor of the main part of phenotypic modifications are obtained namely for *R. macrocnemis*, not surprisingly. This is the only boreal brown frog in which non-striped and striped morphs are connected by continual intergradations (Istchenko, 1978) and practically the character "presence of the mid-dorsal stripe" varies as the quantitative one. On the one hand, it may be connected with the specific nature of the character inheritance in this species; but it is less possible. More realistic is taking in our opinion that every character, both quantitative and qualitative, are determined by quantitative morphogenetic processes. Thus development of coloration of the mid-dorsal stripe in *Acris crepitans* is determined by the size of pigmental cells and genetical specifics of morphs may be shaded (Gray, 1972). We observed the dorsal surface of *R. macrocnemis* froglets with different coloration patterns under a binocular. Presence of the mid-dorsal stripe depends on the rate of pigment concentration declining toward the dorso-medial line (Fig. 4). The lower density of the pigmental cells along the mid-dorsal line in all froglets can be observed. But to distinguish the stripe with the naked eye, the gradient of pigmental cells concentration must be expressed enough or exceed a certain threshold. Morphogenetically, the presence of the gradient is determined by the kinds of melanophorisation of the dorsal surface before metamorphosis: the mid-dorsal surface is filled in by pigmental cells at the end. The phenotype of the froglet: S, PS, PPS or M which appears to correspond with the degree of filling in the mid-dorsal surface by melanophores just before metamorphic climax has begun. The hereditary mechanisms keep the main part of this process but regulation of morphogenesis by the environment is important, too. Perhaps comparative significance of the environment increases in non-optimal conditions.

Perhaps in the base of phenotype formation in species characterized by severe alternative variation of the character (e.g. *R. arvalis* or *R. ridibunda)* the same morphological processes occur. But, in these species, inter-gradations between S- and M- morphs are absent. It may depend either on greater part of hereditary in stripe determination among these species or on a more expressed threshold level. It must be explained. Developmental processes which form the ground of coloration pattern formation may finish in some developmental stage before metamorphosis has began. One or another phenotype could be formed depending on if the process exceeds some threshold level or not. Depending on the expression of this threshold or prolongation of final stage of color formation (when basic processes are completed) takes place either in continual distribution of morphs (as in *R. macrocnemis*) or in bi-modal distribution (as in *R. arvalis*).

The scheme described above, as we can see, does not exclude identity of the inheritance nature of the *Striata* in *R. macrocnemis* to those in *R. arvalis* or other anurans. But, it does not allow us to neglect the modification ability of this character in the population investigations, especially in species with continual distribution of the stripe expression. Perhaps in some cases, the quantitative value of pigment cell density along the mid-dorsal line can be a more adequate index of the mid-dorsal-strip development.

We took into consideration the evidence data of differential survival ability of S- and M- genotypes (Schvarz and Istchenko, 1968). In part, a higher mortality of tadpoles with S-genotype was established when a high density or an oxygen shortage takes place. Increasing the developmental temperature could have the same effect. But, not only the death of S- tadpoles can be a result of the worsening of environmental conditions: they can modify their genotype without lacking of survival ability. Perhaps, the character *Striata* correlates with the intensification of metabolism, not at a genetical but rather at an epigenetical level. Processes which take part in the mid-dorsal stripe formation also affect intensification of energetic exchange. If intensity of these processes change, neither their morphological nor physiological results appear. There is an interesting example of the phenomena when modifications during the separate animal's life results similarly to a short-term selection at the population level. It appears as one of the hard moments in micro-evolutional investigations but would not be neglected.

Acknowledgments

I greatly appreciate my wife, R. Mamradze, for her help in the elaboration of the experiment. I also appreciate J. Manukjan and S. Tzabadze for their help in the experimental and field work, and to J. Ilieva for reading the manuscript.

Literature Cited

BERGER, J. AND J. IMIETOWSKI. 1982. Inheritance of vertebral stripe in *Rana ridibunda* Pall. Amphibia-Reptilia 3,(2-3):145-151.

BROWDER, L. W., J. C. UNDERHILL AND D. J. MERREL. 1966. Mid-dorsal stripe in the wood frog. Journal of Heredity 57(2):65-67.

DUELLMAN, W. E. AND L. TRUEB. 1986. Biology of amphibians. McGraw-Hill Book Company. i-xvii + 1-670.

FISHBECK, D. W. AND J. D. UNDERHILL. 1971. Distribution of stripe polymorphism in wood frogs, *Rana sylvatica*. Le Conte, from Minnesota. Copeia 1971(2):253-259.

GOGOLEVA, N. P. 1989. [Polymorphism in anuran populations]. Pp. 26-29. *In* N.N. Szczerbak (Ed.) (Proc. 7th Sov. Herpetol. Symp.). Kiev, Naukova dumka. (In Russian).

GOIN, C. J. 1947. Studies in the life history of *Eleutherodactylus ricordii planirostris* (Cope) in Florida. Univ. Florida publ. Biol. Sci. 4(2):1-56.

GRAY, R. H. 1972. Metachrosis of the vertebral stripe in the cricket frog *Acris crepitans*. American Midland Naturalist 87(2):549-551.

HERAN, I. 1986. Comments on coloration and color changes in *Rana temporaria* L. Pp. 267-270.

In Z. Rocek (Ed), Proc. Eur. Herpetol. Meet, Prague.

ISTCHENKO, V. G. 1978. [Dynamic polymorphism of brown frogs of the USSR fauna]. Moscow, Nauka. 148pp. (In Russian).

ISTCHENKO, V. G. AND A. V. LEDENTSOV. 1987. [Environmental influence on the age structure dynamics in the moor frog populations]. Pp. 40-51. *In* L. M. Suzumova (Ed), Vlijanie sredi na dinamiku structuri I chislennosti populacij zhivotnih. Sverdlovsk, Ural Sci. Cent. Acad. Sci. USSR. (In Russian).

KUBANTSEV, B. S., T. J. PESKOVA AND I. GORODNICHEVA. 1989. [On the variation of some tailless amphibian species' coloration]. Pp. 126-127. *In* N.N. Szczerbak (Ed.), Voprosi gerpetologii. Kiev, Naukova Dumka. (In Russian).

LEBEDINSKI, A. A., T. B. GOLUBEVA AND V. I. ANISIMOV. 1989. [Polymorphism among brown and green frogs under the antropogenic pressure]. Pp. 139-140. *In* N.N. Szczerbak (Ed.), Voprosi gerpetologii. Kiev, Naukova Dumka. (In Russian).

LEDENTSOV, A. V. 1990. [Dynamics of the age structure and number of the reproductive part of the moor frog *(Rana arvalis* Nilss.) population]. Reph. Dissertation Cand. Biol. Sci. Sverdlovsk, Ural Sci. Center Acad. Sci. USSR. 23pp. (In Russian).

MAMRADZE, R. G. 1989. [Effect of the thermal conditions on the frequency of the phenotype *Striata* in Iranian wood frog]. Pp. 151-152. *In* N.N. Szczerbak (Ed.), Voprosi gerpetologii. Kiev, Naukova Dumka. (In Russian).

MASALIKIN, A. I. 1985. [Populational-phenetic analysis of the fire-bellied toad in the Voronezh reserve]. P. 169. *In* Phenetica populacij (Proceedings of the 3rd Soviet Phenetic meeting, Saratov). Moscow, Nauka. (In Russian).

MELKUMJAN, L. S. AND K. E. VARDANJAN. 1985. [Polymorphism in the transcaucasian populations of lake frog]. Biol. Journ. Armenia 38(3):267-270. (In Russian).

MORIWAKI, T. 1953. The inheritance of the dorso-madian stripe in *Rana limnocharis* Wiegmann. J. Sci. Hiroshima Univ., Ser. Biol. 1(14):159-164.

MORIYA, K. 1952. Genetical study of the pond frog *Rana nigromaculata*.. 1. Two types of *Rana nigr. nigromaculata* found in Tokata district. J. Sci. Hiroshima Univ., Ser. Biol. 1(13):189.

PANCHENKO, I. M. 1985. [Phenotypic variation of moor frog coloration in the flood-lands of Oka River]. Pp. 171-173. *In* Phenetica populacij (Proceedings of the 3rd Soviet Phenetic meeting, Saratov). Moscow, Nauka. (In Russian).

PIKULIK, M. M. 1985. [Amphibians of Belorussia]. Minsk, Nauka i technica. 192pp. (In Russian).

PYBURN, W. F. 1961. The inheritance and the distribution of vertebral stripe color in the cricket frog. Pp. 235-261. *In* Vertebrate speciation. University of Texas Press, Austin.

SCHVARZ, S. S. AND V. G. ISTCHENKO. 1968. [Dynamics of the genetic structure of the moor frog population]. Bull. Mosc. Soc. Nat. Res. Ser. Biol. 73(3)127-134. (In Russian).

SHIBATA, Y. 1988. [Mid-dorsal stripe type of a frog, *Rana limnocharis*, in northwestern islands of Kyushu, Japan (Amphibia: Ranidae)]. Shizenzhi-Kenkyu. (Proc. Osaka Mus. Nat. Hist.) 2(4):69-71. (In Japanese).

STCHUPAK, E. L. 1975. Populational mechanisms of conservation of the genetical structure in the *Rana arvalis* Nilss. population. Bull. Acad. Sci. USSR 222(1):220-222. (In Russian).

STCHUPAK, E. L. 1977. Inheritance of the mid-dorsal stripe in moor frog. Pp. 36-37. *In* Inform. Materials Inst. Plant and Anim. Ecol. Sverdlovsk. (In Russian).

STCHUPAK, E. L. AND V. G. ISTCHENKO. 1981. On the hereditary base of color polymorphism in moor frog *(Rana arvalis* Nilss.). 1: light mid-dorsal stripe. Pp. 128-132. *In* Herpetological researches in Siberia and Far East. Leningrad, Nauka.

STUGREN, B. 1966. Geographic variation and distribution of the Moor frog, *Rana arvalis* Nilss. Ann. Zool. Fenn. 3(1):29-39.

TARKNISHVILI, D. N. In press. Embryonic and larval mortality of the caucasian brown frog in the Borjomi Canyon: peculiarities and the main reasons. Varan, St. Petersburg.

TARKNISHVILI, D. N. AND R. G. MAMRADZE. 1989. Modification of caucasian brown frog's phenotype under the influence of high temperature. Bull. Acad. Sci. Georgia. 135(2):437-440.

TOPORKOVA, L. J. 1965. Geographical variation of the morphological features in amphibians. I. *Rana terrestria* Andrz. Nauchnie dokladi visshey scholi, Ser. Biol. 1:31-36.

VERSHININ, V. L. 1987. Some features of the phenetical structure of the moor frog groupings in the industrial city. Pp. 74-79. *In* L. M. Süzümova (Ed), Vlijanie sredi na dinamiku structuri I chislennosti populacij zhivotnih. Sverdlovsk, Ural Sci. Cent. Acad. Sci. USSR.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 132-150

A New Genus for the *Ramphotyphlops subocularis* Species Group (Serpentes: Typhlopidae), with Description of a New Species

VAN WALLACH

Center for Vertebrate Studies, Department of Biology, Northeastern University, Boston, MA 02115, USA

Abstract.—A new genus, *Acutotyphlops*, is established for McDowell's *Ramphotyphlops subocularis* species group (minus *R. willeyi* which is transferred to the *R. flaviventer* group) based on a parietal bone projection, head shield fragmentation, and a V-shaped lower jaw. Two species are revalidated, *A. infralabialis* and *A. solomonis*, and a new species is described from Bougainville Island, *A. kunuaensis*. *Acutotyphlops kunuaensis*, represented by more than 250 specimens, is distributed throughout Bougainville, both in the coastal lowlands and the interior highlands. Sexual dimorphism is present in certain scutellation and proportional characters. At least five species of typhlopids are now known to inhabit Bougainville Island.

Key words: Ramphotyphlops subocularis group, *Acutotyphlops*, *A. kunuaensis*, *A. subocularis*, *A. infralabialis*, *A. solomonis*, *Typhlops adamsi*, *T. bergi*, Papua New Guinea, Bougainville

Introduction

McCoy (1970), McDowell (1974), and Hahn (1980) listed three species of *Typhlina* (= *Ramphotyphlops*) inhabiting Bougainville Island in the Solomons: *Ramphotyphlops braminus*, *R. flaviventer* (= *R. depressus fide* Wallach, in prep.), and *R. subocularis*. In his thorough review of the typhlopids of New Guinea and the Solomon Islands, McDowell (1974) defined the *Ramphotyphlops subocularis* species group as lacking a rectal caecum and exhibiting a wedge-shaped lateral snout profile. He recognized two species in the group, *Ramphotyphlops subocularis* (Waite, 1897) and *R. willeyi* (Boulenger, 1900), considering the latter to be the most primitive member, scarcely differing from *R. flaviventer* (Peters, 1864) of the *Ramphotyphlops flaviventer* species group except in the wedge-shaped snout and absence of a caecum. *Ramphotyphlops subocularis* was described as differing from *R. willeyi* in the more specialized fragmentation of the lateral head shields (multiple preoculars and suboculars vs. a single preocular and no subocular), increased number of midbody scale rows (26-36 vs. 20-22), and the acute mandibular symphysis (V-shaped vs. U-shaped). *Ramphotyphlops willeyi* also differs from *R. subocularis* in the presence of a unicameral right lung (vs. multicameral), a convoluted and multisegmented liver (vs. straight and unsegmented), and absence of frontorostral and paired prefrontals on the dorsum of the head (pers. obs.).

McDowell (1974) placed five nominal taxa in the synonymy of *Ramphotyphlops subocularis*, stating that the number of valid species remained to be determined but based upon published data there was no evidence to suggest that more than one variable species was involved. The taxa synonymized with *R. subocularis* were *Typhlops infralabialis* Waite, 1918, *Typhlops solomonis* Parker, 1939, *Typhlops bergi* Peters, 1948, *Typhlops keasti* Kinghorn, 1948, and *Typhlops adamsi* Tanner, 1951 (see McDowell, 1974, for complete synonymy). An examination of the types of all of the above taxa, plus the majority of *Ramphotyphlops subocularis* material in museum collections indicates that the *R. subocularis* complex consists of at least four valid species (*R. subocularis*, *R. infralabialis*, *R. solomonis*, and an undescribed species that forms the topic of this paper). A description of the cranial osteology, internal anatomy, and geographic variation in the *Ramphotyphlops subocularis* group, with emphasis on the new species, will be the subject of a future paper (Wallach, Wong and Meszoely, in prep).

Methods

All traceable museum specimens of the *Ramphotyphlops subocularis* group (> 325 specimens) were examined, including the

types of all nominal taxa. Due to misidentifications in collections, some specimens may have been overlooked. Specimens were examined with an Olympus binocular dissecting microscope and measurements were made to the nearest 0.5 mm, including total length (LOA), tail length (TL), midtail diameter (MTD), anterior (ABD), midbody (MBD) and posterior (PBD) body diameters, and diameter in the nuchal region (ND). Total middorsals or transverse scale rows (TSR) were counted between the rostral and terminal spine, dorsocaudals (DC) along the midline in a perpendicular plane to the anterior ventrolateral edge of the vent to the apical spine, and subcaudals (SC) between the vent and the spine. Five longitudinal scale row (LSR) counts were made: postcephalic or anterior (ASR) scale rows were counted at the level of the 20th scale caudad of the mental, midbody (MSR) rows at midbody, and precloacal or posterior (PSR) rows were counted at the level of the l0th scale craniad of the anals; two further values were calculated by adding the midbody and posterior counts (MPSR) and also the anterior, midbody and posterior counts (AMPSR). Relative tail length ratio (TL/LOA) is the length of the tail from posterior border of vent to tip of apical spine divided by overall length, body proportion ratio (LOA/MBD) is overall length divided by midbody diameter, and tail proportion ratio (TL/MTD) is length of tail divided by the midtail diameter.

Due to state of preservation and injection of preservative (either lack thereof or overinjection), not to mention health of the animal at time of preservation, both body and tail proportion figures only approximate the condition in life and were thus rounded to the nearest integer (except in the case of type specimens); carrying out the calculations to one decimal point infers a precision that is unrealistic. Care must be taken especially in interpreting the tail proportion ratios as injection of tail with preservative probably leads to a distortion of the true values but the data are presented in the hope that all tails have been similarly biased and therefore of comparative value. Head width (HW) is diameter of head at midocular level; head length (HL) is distance from tip of snout to midocular level. All diameter measurements were made in either dorsal or ventral view.

Statistics were calculated with the Macintosh Statview program. Mean values are presented with their standard errors (SE) and ranges (r); CV represents the coefficient of variation. Probability values in the Tables refer to the student's *t*-test of sample means. In Tables 4-5 data are presented by sex and both sexes combined; when a statistically significant difference exists between the means of the male and female samples, the probability value is given in place of the data for the sexes combined. Tables 6-7 summarize only the combined sex samples as no statistically significant sexual difference was found to be present.

Due to inconsistencies in the literature, clarification is given for terminology of the head shields in the *Ramphotyphlops subocularis* group as the fragmentation of cephalic scutes is a diagnostic character. As discussed by Parker (1939) and Kinghorn (1948), the proliferation of head shields in the *R. subocularis* group has led to confusion and uncertainty as to correct homologies. Kinghorn (1948) proposed the most logical system of nomenclature for these shields and his system is followed here with minor changes. Proliferation of the lateral head shields is the result of division of the ocular and preocular shield of typical typhlopids. The *Ramphotyphlops subocularis* group exhibits two patterns of preocular (PR) arrangement: a single large preocular (*R. infralabialis* and new species) or a longitudinally divided shield with a large superior preocular and a smaller inferior preocular (*R. subocularis* and *R. solomonis*). Division of the typical typhlopid ocular shield has produced an anterior (AO) and posterior ocular (O) and suboculars (S). The suboculars are arranged in one or two horizontal rows between the oculars and supralabials (L) and termed superior and inferior, and considered vertically in one to three columns as anterior, middle and posterior. Postoculars (T) are defined as all scales in contact with

TABLE 1. Variation in the holotypes of the *Ramphotyphlops suboculars* species group [1]

Character	*subocularis*	*keasti*	*solomonis*	*infralabialis*	*adamsi*	*bergi*	*kunuaensis*
MUS	AMS	AMS	IRSNB	AMS	MVZ	UMMZ	MCZ
NO	R2202	R12856	2029	R4609	40753	95445	76964
S	M	M	F	F	F	M	M
LOA	361	373	427	305	150.5	171	221
TL	19	21	15	6	4.5	9	10.5
TSR	472	403	366	476	493	418	385
LSR	40-34-30	40-34-30	36-32-26	32-26-24	34-26-22	32-26-25	38-32-28
MPSR	64	64	58	50	48	51	60
AMPSR	104	104	94	82	82	83	98
SC	24	21	19	15	17	24	21
DC	25	22	20	17	18	26	22
TL/LOA	5.3	5.6	3.5	2.0	3.0	5.3	4.8
TL/MTD	2.1	3.0	1.7	1.0	1.0	2.0	2.3
LOA/MBD	30.1	35.5	34.2	50.8	43.0	34.2	36.8
PROC	2	2	2	1	1	1	1
OC	1	1	1 + 1	1 + 1	1 + 1	1 + 1	1 + 1
SOC	2+2+2:3/2	2+2+2	2	1 + 2	1 + 2	1 + 2	1 + 2
PTOC	4	4	4	4	3	3:4	3:4
SNS	0.5	0.5	0.5	0.5	1.0	0.5	0.5
INS	SL2	SL2	SL2	SL1	SL2	SL2	SL2
SIP	T-0	T-0:T-III	T-III	T-III	T-III	T-III	T-III
SL	4	4:5	4	4	4	4	4
IL	6:5	7	6:7	6	7	7	6
AS	5	5	5	5	5	5	5
ABD	11.5	7.5	12	6	4	5	5
ND	13	13	15	7	4.5	5.5	6
MBD	12	10.5	12.5	6	3.5	5	6
PBD	12.5	10	11	7	3.5	5	5
HW/HL	1.50	1.27	1.58	1.50	1.44	1.45	1.00
DOR	uniform	uniform	uniform	lineate	lineate	lineate	uniform
PRD	13-15-15	15-15-14	14-14-12	?	17-17-17	13-15-17	18-18-17
URV	27-19-15	25-19-16	22-18-14	?	5-5-5	5-5-5	20-14-11

[1] MUS = museum, NO = catalogue number, S = sex (M = male, F = female), LOA = total length (mm), TL = tail length (mm), TSR = transverse scale rows, LSR = longitudinal scale rows, MPSR = midbody and posterior scale row sum, AMPSR = anterior, midbody and posterior scale row sum, SC = subcaudals, DC = dorsocaudals, TL/LOA = relative tail length, TL/MTD = tail length/midtail diameter, LOA/MBD = total length/midbody diameter, PROC = preoculars, OC = oculars, SOC = suboculars (colon separates values from left/right side of head), PTOC = postoculars (colon separates values from left/right side of head), SNS = nostril-rostral division by superior nasal suture, INS = supralabial contact of inferior nasal suture, SIP = supralabial imbrication pattern (colon separates pattern on left/right side of head), SL = supralabials (colon separates values from left/right side of head), IL = infralabials (colon separates values on left side from right side), AS = anal shields, ABD = anterior body diameter, ND = nuchal diameter, MBD = midbody diameter, PBD = posterior body diameter, HW/HL = head width/head length, DOR = dorsum pattern, PRD = pigmented rows of dorsum, URV = unpigmented rows of venter

the ocular and/or suboculars between the parietal and fourth supralabial. In addition to the ocular fragmenting into preoculars and suboculars, there are three shields (a median azygous shield bordered laterally by a pair of larger shields) located on the dorsum of the snout between the rostral/postnasals and supraoculars (SO). In reference to the azygous shield typically known as the prefrontal in typhlopids, Kinghorn (1948) termed it the frontonasal while the pair laterally bordering the frontonasal were

TABLE 2. Qualitative characters of the *Ramphotyphlops suboculariS* species group [1]

Species	DP	LP	RS	SN	IP	P	CS	AS
infralabialis	P	P	N	0	0	D	R	S
+ *adamsi*	P	P	N	0	+	D	R	0
+ *bergi*	P	P	M	0	+	E	H	S
kunuaensis n. sp.	P	P	M	0	+	D	R	S
solomonis	R	R	N	0	0	D	H	S
subocularis	R	W	B	+	+	E	H	T
+ *keasti*	R	P	B	+	0	D	H	T

[1] DP = dorsal snout profile (R = rounded, P = pointed), LP = lateral snout profile (R = rounded, W = wedge-shaped, P = pointed), SN = supranasals, IP = interparietal, P = parietals (E = enlarged, D = divided), CS = costal shape (R = rounded, H = subhexagonal), AS = apical spine (T = thornlike, S = spinelike, 0 = absent), RS = rostral size (N = narrow, M = moderate, B = broad)

called prefrontals (PF). Preference is here given to the term frontorostral (FR) for the azygous shield, a term that better describes its position as it is located between the rostral (R) and frontal (F). Peters (1948) erroneously suggested that the prefrontals of *Typhlops bergi* were the first of three pairs of supraoculars. The shields that Kinghorn (1948) termed the internasals in *Typhlops keasti* (also present in *R. subocularis*) are here referred to as supranasals (SN), following Brongersma (1934) and McDowell (1974). The median shield that Kinghorn (1948) referred to as a parietal in *T. keasti* is here called the postfrontal (FL) as some species in the *R. subocularis* group retain paired, enlarged parietals (P) in addition to a median postfrontal and interparietal (IP). See Fig. 1 for identification of the head shields in the *Ramphotyphlops subocularis* species group.

Supralabial imbrication patterns (SIP) follow Wallach (1993a) with the addition of the following prefixes for multiple preocular, ocular, and subocular shields: A = anterior, M = median, and P = posterior. *In situ* hemipenes were observed to determine the number of coils in the retracted organ but this was difficult to objectively evaluate as, in addition to simple coils, all manner of twists, partial loops, and zig-zag folds occur. Single folds or zig-zags and half loops were scored as half coils. All counts were made on the left organ. Museum acronyms follow Leviton et al. (1985). Catalogue entries for Bougainville localities with different spelling from those on recent maps include Melilup (= Melelup), Mutahi (= Mutuhai), Topanas (= Topanos), and Torakina (= Torokina). Data from specimens of the new species from the following localities were combined and analyzed as single units: Kieta and North Nasioi (= Kieta); Buin, Malabita and Turiboiru (= Buin); Torokina, Cape Torokina, Piva and Empress Augusta Bay (= Torokina); Mutuhai and Melelup (= Mutuhai). Elevations determined from government topo maps with 50 m contour lines prefaced with "ca."; NSL represents an elevation near sea level.

Results

Redescription of holotypes

Comparative data for the types of all nominal taxa in the *Ramphotyphlops subocularis* group are presented in Tables 1-2. Only those features not listed in Table 1 or previously mentioned in the literature are discussed below.

Typhlops subocularis.—The holotype of *T. subocularis* (AMS R2202; Figs. 1a-c, 2a-b) was erroneously reported by Waite (1897) to have 36 midbody scale rows (34 in the paratype, which is now missing *fide* Cogger, 1979, and not available for examination) but there are 34 rows at midbody. The apical spine is large with a

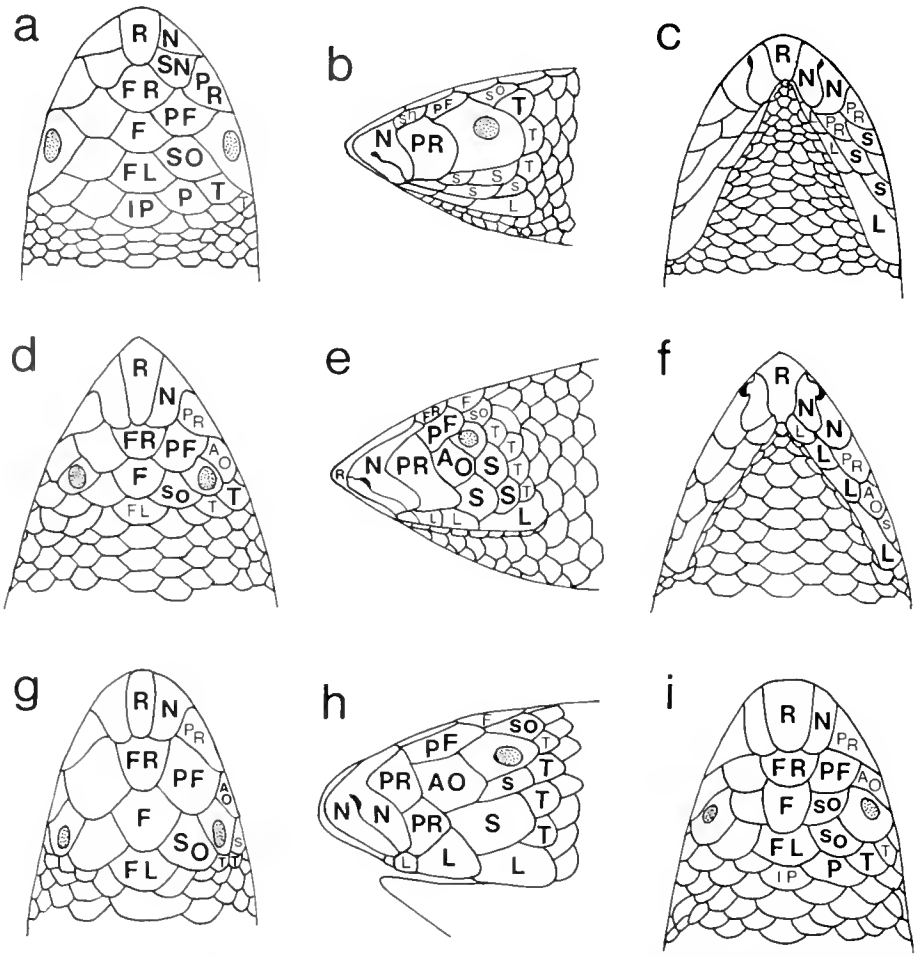


FIG. 1. Head shield terminology of the *Ramphotyphlops suboculariss* species group. Head of holotype of *T. subocularis* (after Waite, 1897): a) dorsal view, b) lateral view, c) ventral view; head of holotype of *T. infralabialis* (after Waite, 1918): d) dorsal view, e) lateral view, f) ventral view; head of holotype of *T. solomonis* (after Parker, 1939): g) dorsal view, h) lateral view; head of holotype of *T. bergi* (after Peters, 1948): i) dorsal view. AO = anterior ocular, F = frontal, FL = postfrontal, FR = frontorostral, IP = interparietal, L = supralabial, N = nasal, P = parietal, PF = prefrontal, PR = preocular, PT = postfrontal, R = rostral, S = subocular, SN = supranasal, SO = supraocular, T = postocular, stippled eye shield = ocular (*T. subocularis*) or posterior ocular (*T. infralabialis*, *T. solomonis*)

broad base (aptly described as thorn-like by Waite) and it points upward as the tail is flexed dorsally. Whether this is an artifact of preservation (injection with preservative) or a characteristic of the *R. subocularis* group is unknown, but this dorsal flexure of the tail tip was commonly observed in other specimens. The nostril is half-moon shaped, oriented at 45° to the vertical, and directed laterally. The SIP is T-0 (Nl/SLl, PrOc/SL2, ASOc/SL3, PtOc/SL4). The first three supralabials are subequal in size and length while the fourth supralabial is more than twice as deep and long as any of the other three. The dorsum is uniformly dark brown while the venter is golden-yellow. A sharp demarcation separates the two colors with only an occasional brown scale appearing in the uppermost yellow scale row.

Typhlops keasti.—The status of *T. keasti* (AMS R12856) is uncertain. Other than the distinctive depression of the head with its laterally pointed snout and strongly tapered head in dorsal aspect there is nothing to separate *T. keasti* from *R. subocularis*. Kinghorn (1948) erroneously reported the

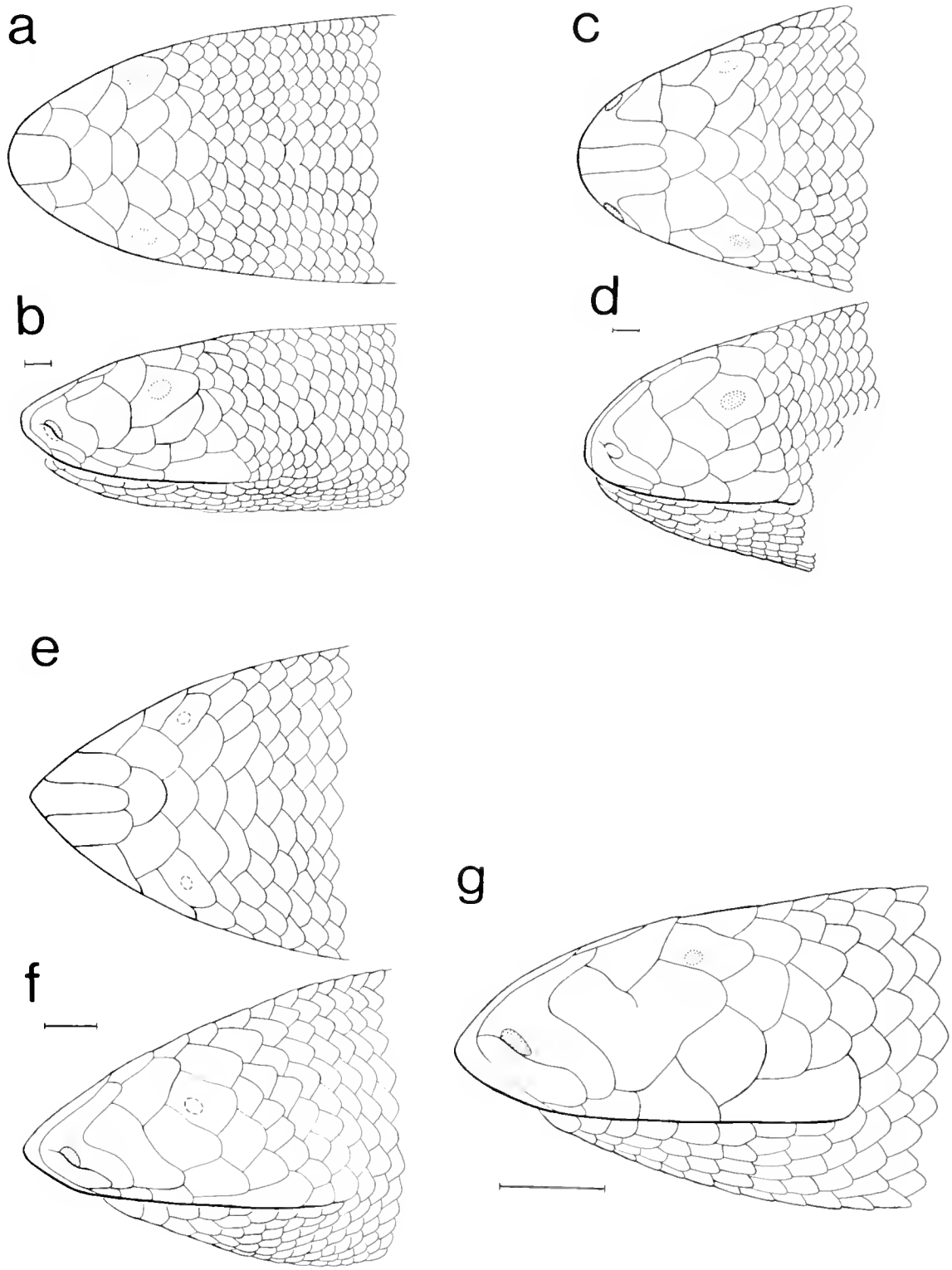


FIG. 2. Head of holotype of *Typhlops subocularis* (AMS R2202): a) dorsal view, b) lateral view; head of holotype of *Typhlops solomonis* (IRSNB 2029): c) dorsal view, d) lateral view; head of holotype of *Typhlops infralabialis* (AMS R4609): e) dorsal view, f) lateral view; head of holotype of *Typhlops adamsi* (MVZ 40753): g) lateral view. Bar = 1 mm.

type of *T. keasti* to possess 32 midbody scale rows but it has 34 rows with an identical formula (40-34-30) to that of the type of *Typhlops subocularis*. Also, he reported an overall length of 285 mm with a midbody diameter of 5 mm but the specimen now measures 373 mm overall with a 10.5 mm diameter. The apical spine of the tail flexes dorsad. On the left side of the head, the SIP is T-0 (N1/SL1, PrOc/SL2, MSOc/SL3, PtOc/SL4) with four supralabials. On the right side the second supralabial is divided, resulting in five supralabials, and the third supralabial overlaps the inferior anterior subocular, forming a T-III SIP (N1/SL1, PrOc/SL2a, PrOc/SL2b/ASOc, MSOc/SL3, PtOc/SL4). This is clearly an anomalous condition: five supralabials occur only rarely in the *R. subocularis* group and the more primitive T-III SIP is characteristic of the three other species. The dorsum is reddish-brown and the venter is gold with occasional scales pigmented in brown. A wide nuchal collar (6-10 scales) is confluent with the light color of the chin and venter. In addition to the type of *Typhlops keasti* I have examined three additional specimens from Papua New Guinea referrable to this taxon (NMBA 11705-06, 11708). Several specimens of the undescribed species resemble *T. keasti* in the acute depression of the head. It seems preferable to consider *Typhlops keasti* a synonym of *Ramphotyphlops subocularis* as suggested by McDowell (1974). Robb (1966) reported *T. keasti* to have a *Ramphotyphlops*-like hemipenis but did not mention the number of coils. If further material should substantiate other differences between *T. keasti* and *R. subocularis*, then *T. keasti* may be regarded as a separate sympatric species.

Typhlops solomonis.—The type of *T. solomonis* (IRSNB 2029; Figs. 1g-h, 2c-d) is in a premolting condition with a milky appearance and numerous sloughing scales along various portions of the body, a factor making an accurate middorsal count difficult. Prominent gland depressions are present along the head shield margins. A large circular nostril is obliquely oriented in a semidivided nasal and just visible from above. The eye is dimly visible with a pupil and the SIP is T-III (N1/SL1, PrOc/SL2, PrOc/SL3/SOc, PtOc/SL4). The dorsum is uniformly dark brown (with a grayish overcast due to premolting condition) while the venter is gold with a sharp demarcation between them. Several anals are white and the terminal spine is directed slightly ventrad and orange.

Typhlops infralabialis.—The type of *T. infralabialis* (AMS R4609; Figs. 1d-f, 2e-f) has a T-III SIP (N2/SL1, PrOc/SL2, PrOc/SL3/ASOc, PSOc/SL4) and the tail curves ventrally. The half-moon shaped nostril is directed laterally and inclined at 45° to the body axis. The dorsum and venter are severely faded but a lineate pattern is faintly visible; the central portions of each scale are brown with lighter margins. Pigmentation decreases ventrally so that the lower scale rows are light with a small dark central spot.

Typhlops adamsi.—The type of *T. adamsi* (MVZ 40753; Fig. 2g) is similar to *T. infralabialis* except that the suture dividing the preocular from the anterior ocular is incomplete (as in two specimens of the undescribed species) and the dorsal head profile is bluntly rounded rather than pointed. The SIP is T-III (N1/SL1, PrOc/SL2, AOc/SL3/ASOc, PtOc/SL4); the tail tip is straight and terminates in a soft protuberance (possibly an apical spine was present but is missing due to damage). The color pattern consists of a brown dorsum with lineate effect (only central half of each scale pigmented), fading to pink ventrally. Six specimens of the new species from Bougainville exhibit a condition similar to that seen in the type of *Typhlops adamsi* with the preocular semifused to the anterior ocular. The type of *Typhlops adamsi* appears to be an anomalous *Ramphotyphlops infralabialis* and is placed in the synonymy of that species.

Typhlops bergi.—The type of *T. bergi* (UMMZ 95445; Fig. 1h) exhibits a T-III SIP (N1/SL1, PrOc/SL2, PrOc/SL3/ASOc, PtOc/SL4). The half-moon shaped nostril is inclined at 45° and directed laterally. The inferior nasal suture contacts SL2 near its

TABLE 3. Variation in the ocular shields of the *Ramphotyphlops suboculuris* species group [1]

Shield	*subocularis* (n = 58) no.	freq.	*solomonis* (n = 62) no.	freq.	*infralabialis* (n = 24) no.	freq.	*kunuaensis* n. sp. (n = 510) no.	freq.
PR	2	100.0%	2	100.0%	1	100.0%	1	100.0%
0	1	100.0%	1 + 1	82.8%	1 + 1	100.0%	1 + 1	98.2%
0			1 + 2	14.1%			2 + 1	1.6%
0			1 + 3	3.1%			1 + 2	0.2%
S	3/2	43.1%	2	84.4%	1 + 2	100.0%	1 + 2	98.0%
S	2 + 2	27.6%	1 + 2	6.2%			1 + 3	1.2%
S	2/3	6.9%	1 + 2	6.2%			1 + 1	0.2%
S	3 + 3	5.2%	2/1 + 2	3.1%			1 + 2/1	0.2%
S	2 + 2 + 2	3.5%	3/2	3.1%			1 + 1/2	0.2%
S	2/2	3.5%	2 + 3	1.6%			2	0.2%
S	3 + 2 + 2	1.7%	1/2	1.6%				
S	1 + 2 + 2	1.7%						
S	1 + 2/2	1.7%						
S	1/2 + 2	1.7%						
S	2 / 1 + 1 + 2	1.7%						
S	2 / 1 + 1+ 1+ 2	1.7%						
PT	3	5.2%	3	7.8%	3	37.5%	3	40.2%
PT	4	75.8%	4	78.1%	4	50.0%	4	56.1%
PT	5	19.0%	5	14.1%	5	12.5%	5	3.7%

[1] no. = number or formula, freq. = frequency (each side counted separately), PR = preoculars, O = oculars, S = suboculars, PT = postoculars

TABLE 4. Transverse scale rows of *Acutotyphlops* [1]

Species	S	n	TSR	SC	DC
infralabialis	F	6	469.0±13.084 (422-511)	15.0±0.577 (13-17)	16.8±0.946 (14-18)
infralabialis	M	6	484.8±15.294 (418-526)	22.0±0.683 (20-24)	24.8±0.872 (23-28)
infralabialis	B	12	476.9±9.888 (418-526)	0.0005	0.005
kunuaensis	F	141	421.6±3.190 (360-542)	13.9±0.172 (10-20)	15.0±0.141 (11-19)
kunuaensis	M	114	405.7±3.039 (360-532)	21.6±0.190 (17-28)	23.1±0.240 (19-31)
kunuaensis	B	255	0.0005	0.0005	0.0005
solomonis	F	22	388.1±3.453 (362-424)	18.5±0.353 (16-22)	20.7±0.361 (18-24)
solomonis	M	9	363.4±5.762 (334-381)	24.8±1.051 (19-29)	28.0±0.732 (25-30)
solomonis	B	31	0.025	0.005	0.0005
subocularis	F	12	398.8±6.028 (363-428)	16.4±0.802 (12-22)	20.3±1.382 (14-23)
subocularis	M	17	399.6±6.392 (363-472)	24.3±0.541 (21-28)	25.6±0.833 (22-31)
subocularis	B	29	399.3±4.427 (363-472)	0.0005	0.005

[1] mean ± SE (range), S = sex (F = female, M = male, B = both sexes combined or *p* value when significant difference exists between means of each sex), TSR = transverse scale rows, SC = subcaudals, DC = dorsocaudals

TABLE 5. Proportional characters of *Acutotyphlops* [1]

Species	S	n	TL/LOA	LOA/MBD	TL/MTD
infralabialis	F	6	2.1±0.292 (1.0-3.1)	44.5±3.611 (33.4-50.8)	1.3±0.126 (1.0-1.7)
infralabialis	M	6	4.2±0.293 (3.2-5.3)	45.7±2.674 (34.2-53.1)	2.1±0.095 (1.7-2.3)
infralabialis	B	12	0.005	45.1±2.151 (33.4-57.4)	1.7±0.140 (1.0-2.3)
kunuaensis	F	141	2.7±0.032 (1.8-3.8)	37.1±0.494 (22.4-57.6)	1.5±0.021 (1.0-2.1)
kunuaensis	M	114	4.9±0.054 (3.4-6.7)	36.5±0.494 (26.8-52.1)	2.1±0.031 (1.2-3.3)
kunuaensis	B	255	0.0005	36.9±0.351 (22.4-57.6)	0.0005
solomonis	F	22	3.9±0.117 (2.9-4.9)	32.4±1.321 (18.2-42.5)	2.1±0.079 (1.3-3.1)
solomonis	M	9	6.8±0.280 (5.2-7.7)	29.3±0.841 (23.6-33.0)	2.7±0.160 (2.0-3.0)
solomonis	B	31	0.0005	31.5±0.993 (18.2-42.5)	0.01
subocularis	F	12	3.7±0.146 (3.0-4.5)	32.0±1.524 (23.2-39.7)	1.7±0.097 (1.2-2.3)
subocularis	M	17	5.5±0.205 (3.0-6.3)	32.3±1.240 (25.6-43.8)	2.5±0.127 (1.8-3.7)
subocularis	B	29	0.0005	32.2±0.945 (23.2-43.8)	0.005

[1] mean ± SE (range), S = sex (F = female, M = male, B = both sexes combined or *p* value when significant difference exists between means of each sex), TL/LOA = tail length/total length, LOA/MBD = total length/midbody diameter, TL/MTD = tail length/midtail diameter

TABLE 6. Longitudinal scale rows of *Acutotyphlops* [1]

Species	n	ASR	MSR	PSR
infralabialis	12	34.0±0.492 (32-36)	27.0±0.302 (26-28)	25.0±0.369 (22-26)
kunuaensis	255	37.5±0.103 (34-42)	32.4±0.074 (30-36)	28.4±0.083 (26-33)
solomonis	31	33.7±0.246 (30-36)	32.0±0.182 (29-34)	26.6±0.190 (24-28)
subocularis	29	40.0±0.314 (36-44)	34.5±0.251 (32-36)	30.8±0.250 (28-34)

[1] mean ± SE (range), ASP = anterior scale rows, MSR = midbody scale rows, PSR = posterior scale rows

TABLE 7. Miscellaneous characters of *Acutotyphlops* [1]

Species	n	MPSR	AMPSR	LOA
infralabialis	12	52.0±0.640 (48-54)	85.8±1.006 (82-90)	254.0 (115-372)
kunuaensis	255	60.7±0.127 (56-68)	98.2±0.210 (90-110)	237.0 (104-373)
solomonis	31	58.5±0.274 (55-61)	92.3±0.447 (85-96)	357.6 (164-487)
subocularis	29	65.3±0.442 (60-70)	105.3±0.659 (98-111)	270.0 (191-394)

[1] mean ± SE (range), MPSR = midbody and posterior scale row sum, AMPSR = anterior, midbody and posterior scale row sum, LOA = total length

junction with SL1 and partially covers an inferior nasal pit. The dorsum is brown with light scale margins forming a lineate pattern. The inferior nasal pit is not unique to *T. bergi* as nearly 10% of the sample of the undescribed Bougainville species possesses it. The paired supraoculars are distinctive although aberrantly present in three individuals (1.2%) of the new species. Scale counts, body proportions, and color pattern suggest that the type of *T. bergi* is an anomalous *T. infralabialis*. Should further material display the paired supraoculars, *T. bergi* might be considered subspecifically distinct.

Revalidation of Taxa

The summarized data in Tables 1-7 demonstrate the distinctness of the four species here considered valid (*R. subocularis*, *R. solomonis*, *R. infralabialis*, and the new species). The *R. subocularis* group is characterized by six uniquely derived features unknown among other typhlopids. An osteological synapomorphy of all species is the presence of an acuminate parietal projection separating the posterior frontals. This median middorsal "spike" of the parietal bone, wedged between the posterior portions of the frontals, has not been reported in any other scolecophidian. Examination of six skulls reveals the parietal spike to extend for the following distances along the interfrontal suture: 0.20-0.25 in *R. solomonis*, 0.33 in *R. subocularis* and the undescribed species, and 0.40 in *R. infralabialis*. Multiple preocular, ocular and/or subocular shields (Table 3), a V-shaped lower jaw with 5-7 infralabials (Figs. 1c, f), and a frontorostral shield bordered by a pair of enlarged prefrontals (Figs. 1a, d, g, i) are other synapomorphies of the group as here defined with the exclusion of *Ramphotyphlops willeyi*. Additional derived characters present in the *R. subocularis* group (but not exclusively so) include lack of a rectal caecum, a uropeltid-like nuchal expansion such that the greatest diameter of the body is behind the head, a high number of longitudinal scale rows (≥ 26 midbody rows present in 27 species of African and Asian *Typhlops* and 15 species of *Rhinotyphlops*), sexual dimorphism in relative tail length and number of subcaudals and dorsocaudals (i.e., Perry, 1985, for *Typhlops vermicularis*), a multicameral right lung (present in *Rhinotyphlops* and some *Typhlops*), and a straight unsegmented liver (present also in *Rhinotyphlops*). *Ramphotyphlops willeyi* is thus transferred to the *R. flaviventer* species group, which possesses a unicameral right lung. It is considered a derived member of that group based upon the wedge-shaped snout and absence of a rectal caecum (McDowell, 1974).

Due to the uniqueness of the *R. subocularis* group, which is distinguished from all other typhlopids by no fewer than six synapomorphies and has been previously suggested as worthy of separate generic status (Dunn and Tihen, 1944; McDowell, 1974), a new genus is established to contain the four species discussed herein. The removal of these species, having 26-36 midbody scale rows, from *Ramphotyphlops* leaves all members of that genus but one with 16-24 midbody scale rows, the sole exception being the Philippine *Ramphotyphlops cumingii* (24-28 rows). The new genus may be known as

***Acutotyphlops* gen. nov.**

Type species.—*Acutotyphlops kunuaensis* sp. nov.

Diagnosis.—Distinguished from all other typhlopid genera by any of the following characters: a middorsal parietal spike partially separating the frontal bones, a V-shaped lower jaw, two or more subocular shields, a frontorostral shield bordered by a pair of prefrontals, five or more infralabial shields, and sum of preocular and ocular shields three or more.

Etymology.—From the Latin *acutus*, meaning pointed, in reference to both the parietal spike of the skull and the symphysis of the lower jaw of the four included species, plus the dorsal and lateral head profiles of the type species.

Description.—Acuminate projection of parietal bone separating posterior frontals along 0.2-0.4 of the interfrontal suture; 4-5 maxillary teeth (4 in *A. subocularis* and *A. solomonis*; 5 in *A. infralabialis* and undescribed species); 2-4 dorsal foramina in nasal bone; cephalic glands confined to bases of shields beneath sutures; widest part of body in nuchal region due to expanded axial musculature, presumably adaptive for burrowing and similar to condition in the Uropeltidae *fide* Gans, 1976, and Gans et al., 1978; rostral narrow and short, extending halfway to the level of the eyes; median azygous frontorostral bordered by paired prefrontals; superior nasal suture

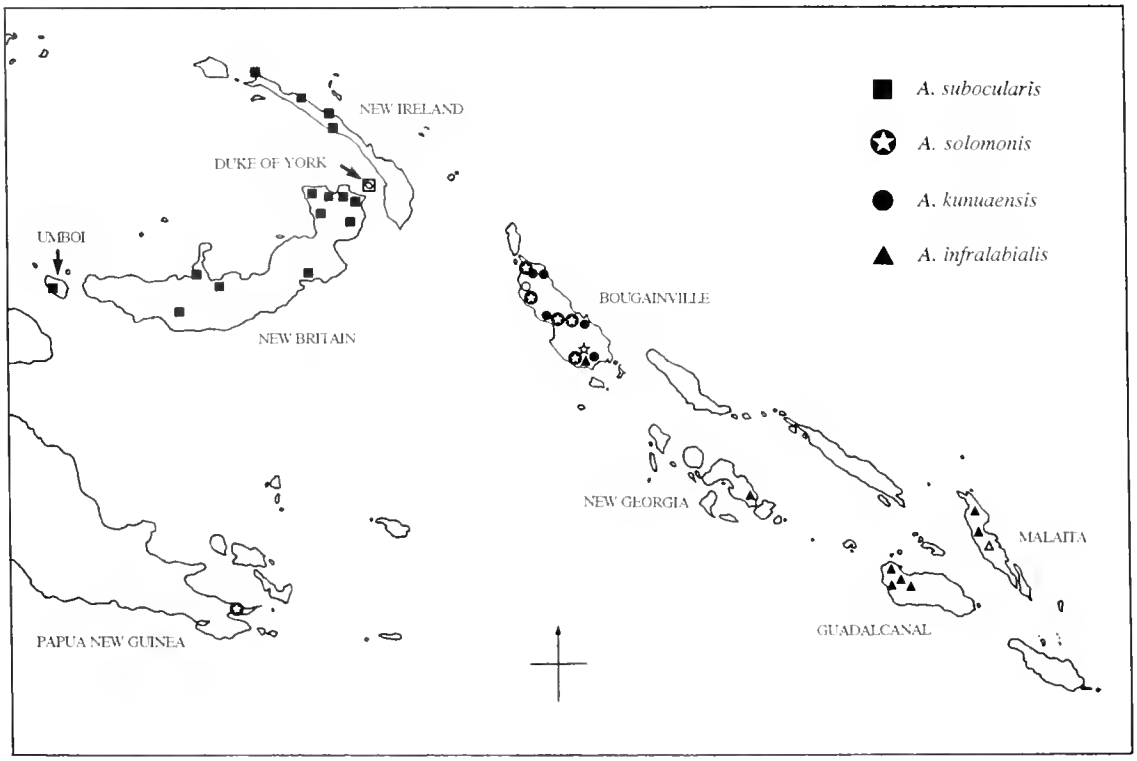


FIG. 3. Distribution of *Acutotyphlops* n. gen.. Solid symbols represent specimens examined, open symbols denote type localities.

incomplete; caudal border of nasal concave; inferior nasal suture contacting second supralabial; preocular single or divided longitudinally; ocular single or divided transversely (if divided, small eye with visible pupil present beneath posterior ocular); sum of preocular and ocular shields 3-6; 2-7 suboculars; 4 supralabials with fourth 2-3 times as long as deep and twice as long as any of the other three; 5-7 infralabials; longitudinal scale rows with anterior and posterior reductions; 26-36 midbody scale rows; sum of anterior, midbody and posterior scale rows 88-110; lower jaw V-shaped; right lung multicameral; liver straight and unsegmented (as in Alethinophidia); rectal caecum absent (as in most Alethinophidia); lateral tongue papillae absent; *Ramphotyphlops*-like hemipenis with 3-9 coils in retracted position; sexual dimorphism in relative tail length and both subcaudal and dorsocaudal scale counts; moderate-sized scolecophidians with maximum length of 400 mm (except *A. solomonis* at 500 mm) and moderate length/width ratios of 25-50.

Content.—Four recognized species: *Acutotyphlops subocularis* (Waite, 1897), including its synonym *Typhlops keasti* Kinghorn, 1948; *Acutotyphlops infralabialis* (Waite, 1918), including its synonyms *Typhlops bergi* Peters, 1948, and *Typhlops adamsi* Tanner, 1951; *Acutotyphlops solomonis* (Parker, 1939); and *Acutotyphlops kunuaensis* sp. nov.

Distribution.—Eastern Papua New Guinea and the Solomon Islands (Fig. 3). *Acutotyphlops subocularis*, the northern form, is known from eastern Papua New Guinea (one record from Morobe Province) and the Bismarck Archipelago, NSL-1065 m. The two central forms, recorded from NSL-915 m, include *Acutotyphlops kunuaensis* n. sp., endemic to Bougainville Island, and *A. solomonis*, recorded from eastern Papua New Guinea (one record from Milne Bay Province) and Bougainville.

Artificial key to the genus Acutotyphlops

1a.	Preocular single, anterior suboculars paired, dorsal snout profile rounded	2
1b.	Preocular divided, anterior suboculars single, dorsal snout profile pointed	3
2a.	Supranasals present, ocular single, supralabial imbrication pattern T-0	*A. subocularis*
2b.	Supranasals absent, ocular divided, supralabial imbrication pattern T-III	*A. solomonis*
3a	Midbody scale rows 26-28	*A. infralabialis*
3b.	Midbody scale rows 30-36	*A. kunuaensis*

Acutotyphlops infralabialis, the southern form, has the widest distribution, being known from Bougainville, New Georgia, Malaita, and Guadalcanal in the Solomon Islands, 15-245 m.

Specimens of *Acutotyphlops* have not been reported from some of the large islands in the Solomons (Choiseul, Florida, San Cristobal, and Santa Isabel). Thus its distribution in the southern Solomons is poorly known. More collecting is urged, not only in the southern Solomons, but also in eastern Papua New Guinea as *Acutotyphlops* may occur in other localities along the eastern coast.

Type Species of Acutotyphlops

The species to be designated as the type of *Acutotyphlops* has been recognized as a novel taxon for 25 years and is represented in the MCZ collection by more than 220 individuals collected by Fred Parker from August 1960 to May 1966, 180 of them from the type locality of Kunua. Several workers have borrowed the MCZ material to study but it has never been described. This blind snake was mentioned by Parker (1970) as being one of 13 new species from Bougainville that "either have been described or soon will be," based upon nine years of collecting by himself and natives. The new form is finally christened

***Acutotyphlops kunuaensis* sp. nov.**

Figs. 4a-b, 5

Holotype.—MCZ 76964, an adult male collected by Fred Parker (field no. X-4688) on 19 August 1963.

Type locality.—Kunua, coastal northwestern Bougainville Island, North Solomons Province, extreme eastern Papua New Guinea, 5°46'S, 154°43'E, elevation ca. 30 m.

Paratypes (n = 180).—Same locality and collector as that of the holotype (date of collection in parentheses following catalogue number): MCZ 72067-74 (21.vi.62), 72075-77 (22.vi.62), 72078 (27.vi.62), 72080 (22.vii.62), 72130 (25.xii.62), 72131-32 (24.v.62), 72133-36 (13.vi.62), 76714, 76716-26 (24.vii.63), 76926-27, 76929-30 (28.vii.63), 76931-32, 76935-39 (11.viii.63), 76950, 76955-57, 76959, 124473 (16.viii.63), 76960 (21.viii.63), 76961-65, 76967 (19.viii.63), 76968-74, 76977, 76979-80, 76982-83, 76986-89 (28.viii.63), 76990-96, 76998-7007 (29.viii.63), 77008-13 (31.viii.63), 77016-22 (5.ix.63), 77023-33, 77036, 77038 (8.ix.63), 77037 (15.ix.63), 77267-79, 77282-90 (1.ix.63), and 77292-306 (12.ix.63). Collection date unknown for the following paratypes: MCZ 76206, 76682-87, 76690-700, 76704-12, 76948.

Etymology.—The specific epithet is derived from the type locality, Kunua, where the entire type series originated.

Diagnosis.—Distinguished from all other typhlopids by the following combination of characters: snout pointed in dorsal and lateral aspects, mandibles V-

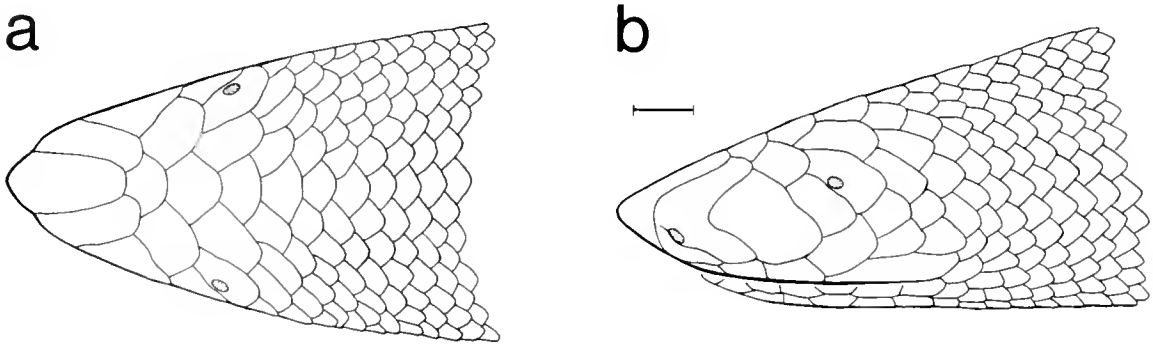


FIG. 4. Head of holotype of *Acutotyphlops kunuaensis* n. sp. (MCZ 76964): a) dorsal view, b) lateral view. Bar = 1 mm.

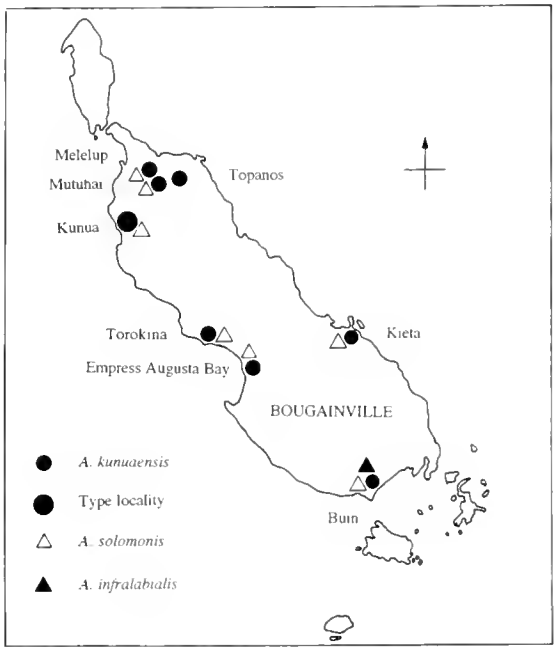


FIG. 5. Distribution of *Acutotyphlops* on Bougainville showing sympatric localities of *A. kunuaensis*, *A. solomonis*, and *A. infralabialis*.

shaped in ventral view, 30-36 midbody scale rows, and a T-III supralabial imbrication pattern. On Bougainville Island, *Acutotyphlops kunuaensis* can be identified by its pointed snout in conjunction with 30 or more midbody scale rows.

Description of holotype.—Total length 221 mm, tail length 10.5 mm, midtail diameter 4.5 mm, relative tail length 4.75%, tail length/width ratio 2.3. Postcephalic and precloacal body diameters 5 mm, midbody diameter 6 mm, latter contained in overall length 37 times. Widest portion of body is expanded nuchal region with diameter of 6.5 mm. Transverse scale rows 385, subcaudals 21, and dorsocaudals 22. Longitudinal scale row formula 38-32-28. Tail tip straight with spine-like apical spine.

Head much narrower than neck and body, tapering to acute point. Rostral twice as long as broad in dorsal view, bordered on either side by pair of postnasals that extend slightly beyond rostral border. Postnasals separated on midline by frontorostral, followed by slightly larger frontal, and then somewhat smaller postfrontal and interparietal, subequal in size and broader than long. Two pairs of larger shields, lateral to this series, are the anterior prefrontals and posterior supraoculars, followed by smaller parietals.

Head obtusely pointed in lateral view with wedge-shaped rostral. Nasal divided into small prenasal and much larger postnasal that extends onto dorsum of head. Nostril's axis oriented at 45° angle to vertical. Inferior nasal suture contacts SL2. Superior nasal suture incomplete, extending across half the nostril-rostral distance. Preocular is large and single; ocular transversely divided into anterior and posterior shields of subequal size. Eye small with large pupil, located beneath the posterior ocular near border of anterior ocular. Three smaller suboculars present between oculars and SL4, arranged as

anterior subocular plus superior and inferior posterior suboculars. Bordering posterior ocular and suboculars, between the supraocular and SL4, are three small postoculars on left side and four postoculars on right. Supralabial imbrication pattern T-III (N1/SL1, APrOc/SL2, APrOc/SL3/PPrOc, PtOc/SL4). Fourth supralabial elongated and equal to length of first three combined. All head shields possess tiny convex tubercles irregularly scattered over their surfaces.

In ventral view snout acutely pointed and slightly trilobate, and mandibles of lower jaw acutely angled or V-shaped with slightly bulbous symphysis. Six infralabials present on each side, separated by median mental shield.

Overall coloration is dark brown dorsum and pale yellow venter with strong demarcation between the two colors. Only occasionally are there a few lightly pigmented scales in uppermost yellow row. Each dorsal scale pigmented centrally with very narrow light margin around periphery. Wide yellow nuchal collar 4-5 scales long middorsally and 8 scales midlaterally. Middorsal 18 scale rows (17 posteriorly) heavily pigmented while yellow rows occupy 20-14-11 ventral scale rows.

Distribution.—Endemic to Bougainville Island, Northern Solomons Province, Papua New Guinea (Fig. 4). Apparently widespread over the island as it is known from the northwestern (Kunua), west-central (Cape Torokina, Torokina, Torokina Bay, Empress Augusta Bay), east-central (North Nasioi, Kieta) and southern (Buin) coastal regions plus the northern interior (Melelup, Mutuhai, and Topanos).

Natural history.—The population structure of *Acutotyphlops kunuaensis* is such that juveniles and immature individuals appear to have total lengths less than 180 -220 mm. The smallest gravid females are 222 mm in length. Adults average 220-300 mm in length. Mean female length is greater than that for males. Only 3 males (2.7% of sample) have total lengths greater than 300 mm but 16 females (11.3%) have lengths between 300 and 380 mm. Females appear to lay one (n=6) or two (n=5) eggs per cycle, although one female (MCZ 72136) has four developing ova on the left side with a large egg on the right. Females with large eggs were collected on 24 July, 28-29 August and 5 September 1963, indicating that egg deposition probably occurs in August and/or September.

Variation.—Lateral tongue papillae are absent in nine specimens with protruded tongues (AMS 121957, 123396, 123402-03, MCZ 76695-96, 76722, 76972, 76986). An inferior nasal pit, similar to that in the type of *Typhlops bergi*, is visible in at least 21 specimens (8.2% of sample).

The mean number of helical coils in the retracted hemipenis is 5.9±0.176 SE in 68 specimens with a range of 3-9 coils and modal values of 6.5 and 7 (9 specimens each). There is a positive but weak correlation between the number of coils and total length, with regression formula for number of hemipenis coils = 0.02 LOA + 1.38 (R^2 = 0.29, CV = 21.2, F = 27.25 with 67 df, $p < 0.0001$). The large intraspecific variation in the number of coils in the retracted hemipenis is surprising and urges caution in using the number as a systematic character (McDowell, 1974; Wallach, 1993b). Variation in hemipenial coils should be examined in species of *Ramphotyphlops* as it may be more stable in that genus. If not, then the number of coils would appear to have little taxonomic value. The mean number of coils in the hemipenis of other members of *Acutotyphlops* is as follows: *A. solomonis* – 4.7 (r = 4.5-5, n = 6, CV = 16.1), *A. subocularis* – 5.5 (r = 4-8, n = 8, CV = 24.8), and *A. infralabialis* – 8.2 (r = 7.5-9, n = 3, CV = 9.4). In juvenile specimens the hemipenis is folded in a zig-zag configuration rather than coiled in loops. One specimen (MCZ 77306), with a tail length of 9.5 mm, has both hemipenes fully everted, the right organ measuring 43 mm in length and the left one 30 mm. The hemipenis is nude, 0.5 mm in diameter, and exhibits a *sulcus spermaticus* extending the length of the organ that is V-shaped in cross-section with a lateral flange or flap-like extension. Another specimen (UPNG

TABLE 8. Sympatric female *A. kunuaensis* and *A. solomonis* scale counts [1]

Species	locality	n	TSR	DC	AMPSR
kunuaensis	Kunua	100	404.4±1.746 (360-442)	14.5±0.138 (11-19)	98.2±0.258 (91-104)
solomonis	Kunua	5	399.8±6.328 (387-424)	20.6±0.678 (19-23)	91.8±1.020 (88-94)
kunuaensis	Torokina	20	483.5±5.486 (441-542)	16.5±0.380 (13-19)	101.8±0.593 (96-107)
solomonis	Torokina	1	390	20	94
kunuaensis	Kieta	10	415.4±6.695 (390-453)	15.6±0.427 (14-18)	99.5±1.147 (93-106)
solomonis	Kieta	6	393.3±4.602 (380-409)	21.3±0.558 (19-23)	93.0±0.894 (90-96)
kunuaensis	Mutuhai	3	528.7±1.453 (526-531)	18.3±0.667 (17-19)	107.7±1.453 (105-110)
solomonis	Mutuhai	3	398.7±3.844 (393-406)	21.0±1.528 (19-24)	93.7±1.202 (92-96)
kunuaensis	Buin	1	525	16	95
solomonis	Buin	5	364.8±0.860 (362-367)	19.4±0.510 (18-21)	93.2±0.490 (92-94)

[1] mean ± SE (range), TSR = transverse scale rows, DC = dorsocaudals, AMPSR = anterior, midbody and posterior scale row sum

TABLE 9. Sympatric male *A. kunuaensis* and *A. solomonis* scale counts [1]

Species	locality	n	TSR	DC	AMPSR
kunuaensis	Kunua	81	393.3±1.579 (360-442)	22.0±0.160 (19-25)	96.6±0.298 (90-104)
solomonis	Kunua	4	366.0±4.564 (356-376)	27.8±1.031 (25-30)	91.3±1.493 (88-95)
kunuaensis	EAB [2]	1	532	21	104
solomonis	EAB [2]	1	339	22	85
kunuaensis	Kieta	11	387.3±2.413 (372-402)	26.6±0.453 (24-29)	98.0±0.660 (95-101)
solomonis	Kieta	3	364.0±15.044 (334-381)	27.7±1.453 (25-30)	92.3±1.202 (90-94)
kunuaensis	Mutuhai	2	487.5±4.500 (483-492)	25.0±1.000 (24-26)	96.6±3.000 (104-110)
solomonis	Mutuhai	1	376	30	90

[1] mean ± SE (range), TSR = transverse scale rows, DC = dorsocaudals, AMPSR = anterior, midbody and posterior scale row sum, EAB = Empress Augusta Bay

1101), with a tail length of 13 mm, has the right organ incompletely (?) everted to a length of 18 mm and a diameter of 0.2 mm throughout. The partially everted organ of AMS 121699 shows the terminus of the hemipenis to be slightly bulbous and containing a shallow teardrop-shaped expansion of the sulcus.

Sympatric populations.—Acutotyphlops kunuaensis is sympatric with *A. solomonis* at seven localities throughout the island: Kunua, Torokina, Empress Augusta Bay, Kieta, Buin, Melelup, and Mutuhai (Fig. 5). At each of these localities the two species are distinctly different in head shape, body form, and scutellation (Tables 8-9). Since sexual dimorphism is present in scale counts and tail proportions, each sex is discussed separately. At Kunua, the two species have similar TSR counts, but there are fewer DC and more AMPSR in *A. kunuaensis* than in *A. solomonis*. At Torokina, Mutuhai and Melelup, *A. kunuaensis* has a significantly higher number of TSR and AMPSR in conjunction with fewer DC than *A. solomonis*. At Kieta *A. kunuaensis* has higher TSR and AMPSR counts but lower DC counts than *A. solomonis*. In fact, *A. solomonis* is more homogeneous with respect to TSR count throughout its range than *A. kunuaensis*, and in all localities except Kieta, it has fewer TSR than *A. kunuaensis*. However, the DC number is

higher in *A. solomonis* than in *A. kunuaensis* at all sympatric localities. All three Bougainville species of *Acutotyphlops* are sympatric at Buin; unfortunately, the *A. infralabialis* sample is composed entirely of males. Nevertheless, the three species (*A. infralabialis*, *A. solomonis*, and *A. kunuaensis*, respectively) are easily distinguishable on head shape, head scutellation, coloration, and longitudinal scale rows (MSR = 28, 32, 30; AMPSR = 90, 92-94, 95), while *A. solomonis* (x = 364.8) exhibits significantly fewer middorsals than either *A. kunuaensis* (525) or *A. infralabialis* (x = 508). Because the three species retain their integrity throughout their ranges and in areas of sympatry, without any evidence of hybridization, they are justifiably recognized as valid species.

Anomalies of scutellation.—In *Acutotyphlops subocularis*, Hediger (1934) and McDowell (1974) reported a specimen lacking supranasals (NMBA 11704). Although they are absent bilaterally, all of the other characters of this female are within the range of variation of *A. subocularis*. In number of anterior scale rows (40), subcaudals (14), and suboculars (2 + 2), plus the presence of a single shield between nasal and ocular, it differs from *A. solomonis* so there can be no doubt about its identity. NMBA 11709 has the supraocular fused to the ocular on the left side and NMBA 11707 has the postocular fused to the superior posterior subocular on the right side. AMS 41254 has five supralabials on the right side (resulting from division of SL3); PNGM 24601 exhibits five supralabials on both sides of the head (from division of SL4); and PNGM 24603 has the frontal divided into two shields plus five supralabials on each side of the head (from division of SL3).

In *Acutotyphlops solomonis*, NMV 10108 has five supralabials on both sides, with the second to fourth occupying positions of typical second and third; MCZ 65992, 65998, and 72138-39 have five supralabials on both sides; and MCZ 72138 has both the nasal and prefrontal semidivided on the left side.

In *Acutotyphlops infralabialis*, MCZ 72129 has the prefrontal, preocular and anterior ocular partially fused into a single shield; NMBA 10155 has the third and fourth supralabials fused on the right side and the supraocular fused to the prefrontal on the left; and AMS 71360 exhibits one supranasal on the right side.

In *Acutotyphlops kunuaensis*, five supralabials are present in two individuals (USNM 120936 and both sides of MCZ 65990), a T-V SIP in five specimens (both sides of MCZ 72067, 77295; right side only in MCZ 76957, 77003, 77298), and a T-VI SIP on both sides of MCZ 77036; two prefrontals are present in MCZ 72133, 76719, two frontals are present in MCZ 76994, a suprarostral in three specimens (MCZ 76694, 76714, 76994), and paired supraoculars in three specimens (both sides of MCZ 76994, 77006; right side only in MCZ 76697). Fusion of the preocular and anterior ocular occurs in six individuals (both sides of MCZ 76693, 76708, 76710, 77038; left side only of MCZ 76969; right side only in MCZ 76683), fusion of the preocular and anterior subocular occurs on the left side of MCZ 76683, and fusion of the preocular and postnasal occurs on the right side in MCZ 76686. The prefrontal is semidivided in three specimens (left side only in MCZ 72133, 76697; right side only in MCZ 76719) and the preocular and anterior ocular are each semidivided on both sides in MCZ 64236. The preocular is divided on the right side only in MCZ 76961 while the preocular and anterior ocular are semifused in two specimens (left side only in MCZ 77267 and 121909). Due to an apparent injury, MCZ 175085 exhibits a concavity and lacks a nostril on the right side in addition to possessing extra scales in that region. Based upon a sample of 510 (counting condition on each side of the head separately), the above cephalic scutellation anomalies occur in frequencies of 0.2-1.6% and may therefore be considered of rare occurrence. However, the increased aberrations may be related to the conditions that led to the original fragmentation of head shields within the group.

Summary

A new genus, *Acutotyphlops*, is proposed to contain four species of highly derived blind snakes from McDowell's (1974) *Ramphotyphlops subocularis* species group (with the exclusion of *R. willeyi* and its transfer to the *Ramphotyphlops flaviventer* species group). In addition to *Acutotyphlops subocularis* (with its synonym *T. keasti*), two species are revived from synonymy (*Acutotyphlops solomonis* and *Acutotyphlops infralabialis*, the latter with its synonyms *T. bergi* and *T. adamsi*) and a new endemic species is described from Bougainville (*Acutotyphlops kunuaensis*). Unique characters for the group include a parietal spike between the frontal bones, multiple ocular, preocular and/or subocular shields, a V-shaped lower jaw, five or more infralabials, and a frontorostral shield with paired prefrontals. *Acutotyphlops* is also known for its wedge-shaped or pointed head, a high number of longitudinal scale rows, a multicameral right lung, a straight unsegmented liver, lack of a rectal ceacum, *Ramphotyphlops*-like hemipenis with 3-9 coils in retracted organ, and prominent sexual dimorphism in tail proportions and caudal counts. Five species of typhlopids are now known from Bougainville Island: *Acutotyphlops infralabialis*, *A. kunuaensis*, *A. solomonis*, *Ramphotyphlops braminus*, and *R. flaviventer* (= *R. depressus*).

Acknowledgements

Appreciation is due to the curators and staff of the following institutions who loaned material for this study: Charles W. Myers (AMNH), Alan E. Greer (AMS), Carla H. Kishinami (BPBM), Jack W. Sites, Jr. (BYU), Harold K. Voris, Robert F. Inger, and Alan Resetar (FMNH), J. Govaere and Mathias Lang (IRSNB), José P. Rosado and Ernest E. Williams (MCZ), Harry W. Greene (MVZ), Eugen Kramer (NMBA), A. J. Coventry (NMV), Ilaiah Bigilale (PNGM), Arnold G. Kluge and Greg Schneider (UMMZ), James I. Menzies (UPNG), W. Ronald Heyer and Addison H. Wynn (USNM), Rainer Günther (ZMB), and Hans-Wilhelm Koepcke (ZMH). Laszlo Meszoly illustrated the holotypes of *Typhlops subocularis*, *T. infralabialis*, *T. solomonis*, *T. adamsi* (Fig. 2), and *Acutotyphlops kunuaensis* (Fig. 4). Thanks are due to Roxane Coombs for executing the illustrations in Fig. 1 and proofreading the manuscript and to Kevin Wong for transverse scale counts of *A. kunuaensis* and statistical analyses of some of the data. Charles Meszoely and the Biology Department of Northeastern University provided funding for the illustrations of the type specimens.

Material Examined

Acutotyphlops kunuaensis (excluding type material listed above).— BOUGAINVILLE IS.: No specific locality: USNM 120211; Buin (6°50'S, 155°44'E, ca. 60 m), MCZ 65990; Cape Torokina (6°15'S, 155°02'E, NSL), USNM 120949; Empress Augusta Bay (6°25'S, 155°05'E, NSL), FMNH 44800-01; Kieta (6°13'S, 155°38'E, NSL), AMNH 87360-62; MCZ 64226-36, 72104-05; NMV 10109; Melelup (5°37'S, 154°55'E, ca. 915 m), MCZ 175089; Mutuhai (5°38'S, 154°57'E, ca. 820 m), MCZ 87605, 174754-55, 174759; North Nasioi (ca. 6°10'S, 155°30'E), MCZ 6601014; Topanos (5°38'S, 155°00'E, 150 m), MCZ 87606-07, 88049, 175082-88; Torokina Bay (6°14'S, 155°03'E, NSL), USNM 120931, 120933-34, 120935-48; Torokina: Piva (6°14'S, 155°03'E, NSL), AMS 121582-84, 121698-700, 121769, 121909, 121956-57, 123393-99, 123402-03. Skull: MCZ 76699.

Acutotyphlops infralabialis.— BOUGAINVILLE: Malabita (6°46'S, 155°43'E, ca. 150 m), MCZ 65991, Buin, MCZ 72129, Turiboiru (6°44'S, 155°41'E, ca. 50 m), MCZ 92504; GUADALCANAL: BYU 7040; Visale (9°15'S, 159°42'E), AMS 71360, Mt. Austen (9°29'S, 159°59'E, 245 m), AMS 77116; Makaruka (9°30'S, 160°04'E, 60 m), MCZ 110249; Nalimbu River, 1 mi. inland (ca. 9°24'S, 160°09'E, 15 m), MVZ 40753 (holotype of *T. adamsi*); MALAITA: AMS 4609 (holotype of *T. infralabialis*), vic. of Mbita'ama (8°24'S, 160°36'E), AMS 87396; Buma (8°56'S, 160°47'E), NMBA

10155; NEW GEORGIA: Segi Point, Horseshoe Reservation (8°34'S, 157°55'E), UMMZ 95445 (holotype of *T. bergi*). Skull: MCZ 64226.

Acutotyphlops solomonis. — BOUGAIN-VILLE: IRSNB 2029 (holotype of *T. solomonis*); Buin, AMS 11451-52, MCZ 65999, 72084; Empress Augusta Bay, FMNH 44802; Kieta, MCZ 64225, 65992-98, NMV 10108; Kunua, MCZ 72083, 72085-86, 72138-39, 72938, 73766, 76688, 175099; Melelup, MCZ 175090; Mutuhai, MCZ 174756-58, 174760; Torokina, USNM 120932, 120934. PAPUA NEW GUINEA: Alotau (10°18'S, 150°25'E, NSL), MCZ 145955. Skulls: MCZ 65597, 65993, 72084.

Acutotyphlops subocularis.—PAPUA NEW GUINEA: Bismarck Archipelago: ZMB 38612, 50458; NEW IRELAND: Fissoa (2°55'S, 151°27'E, NSL), NMBA 11709-10; Lemkamin (3°20'S, 151°55'E), ZMUC 5269; Medina (2° 54'S, 151° 22'E, <100 m), UPNG 5652; Radina (? = Medina), AMS 41253-54; Yalom (4°25'S, 151°45'E, 1000 m), ZMUC 5265-68; DUKE OF YORK IS. (4°10'S, 152°28'E, <50 m), AMS 2202 (holotype of *T. subocularis*); NEW BRITAIN: Iambon, S slope Whiteman Range (ca. 5°50'S, 150°00'E, 1065 m), AMNH 82317; Jacquinot Bay (5°34'S, 151°30'E), AMS 12856 (holotype of *T. keasti*), NMBA 11704; Keravat (4° 21'S, 152° 02'E, ca. 25 m), UPNG 1101; Kokopo (4°21'S, 152°16'E, NSL), ZMH 3968; Mosa, West Nakanai (5° 38'S, 150° 17'E, ca. 50 m), PNGM 24600-03; Talasea (5°17'S, 150°02'E, NSL), MCZ 175091; Wunung, Jacquinot Bay (5°37'S, 151°27'E, NSL), NMBA 11705-08; UMBOI IS.: Awelkon (5°38'S, 147°50'E, 600 m), BPBM 5457; "New Guinea," ZMB 24341. Skull: NMBA 11704. Unexamined literature record: Toma, NEW IRELAND (4°23'S, 152°10'E, 400 m).

Literature Cited

BOULENGER, G. A. 1900. On a new blind snake from Lifu, Loyalty Islands. Pp. 603-604. *In* A. Willey, Zoological results, based on material from New Britain, New Guinea, Loyalty Islands and elsewhere collected during the years 1895, 1896 and 1897. Vol. 5. Cambridge University, Cambridge.

BRONGERSMA, L. D. 1934. Contributions to Indo-Australian herpetology. Zoologische Mededeelingen 17(3-4):161-251.

COGGER, H. G. 1979. Type specimens of reptiles and amphibians in the Australian Museum. Records of the Australian Museum 32(4):163-210.

DUNN, E. R., and J. A. TIHEN. 1944. The skeletal anatomy of *Liotyphlops albirostris*. Journal of Morphology 74(2):287-295.

GANS, C. 1976. Aspects of the biology of uropeltid snakes. Pp. 191-204. *In* A. d'A. BELLAIRS and C. B. COX (eds.), Morphology and biology of reptiles. Linnean Society Symposium Series No. 3, London.

GANS, C., H. C. DESSAUER, and D. BAIC. 1978. Axial differences in the musculature of uropeltid snakes: the freight-train approach to burrowing. Science 199:189-192.

HAHN, D. E. 1980. Liste der rezenten Amphibien und Reptilien: Anomalepididae, Leptotyphlopidae, Typhlopidae. Das Tierreich 101:1-93.

KINGHORN, J. R. 1948. A new species of *Typhlops* from New Britain. Records of the Australian Museum 22(1):67-69.

LEVITON, A. E., R. H. GIBBS, JR., E. HEAL, and C. E. DAWSON. 1985. Standards in herpetology and ichthyology: part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985(3):802-832.

MCCOY, M. 1970. Reptiles of the Solomon Islands. Wau Ecology Institute Handbook (7):1-80.

MCDOWELL, S. B., JR. 1974. A catalogue of the snakes of New Guinea and the Solomons, with special reference to those in the Bernice P. Bishop Museum. Part I. Scolecophidia. Journal of Herpetology 8(1):1-57.

PARKER, F. 1970. Collecting reptiles and amphibians in New Guinea. Australian Natural History 16(9):309-314.

PARKER, H. W. 1939. Reptiles and amphibians from Bougainville, Solomon Islands. Bulletin de Musée royal d'Histoire naturelle de Belgique 15(60):1-5.

PERRY, G. 1985. Sexual dimorphism in *Typhlops vermicularis* (Reptilia: Ophidia). Israel Journal of Zoology 33:11-13.

PETERS, J. A. 1948. A new snake of the genus *Typhlops* from the Solomon Islands. Occasional Papers of the Museum of Zoology, Uiversity of Michigan (508):1-5.

PETERS, W. 1864. Über neue Amphibien (*Typhloscincus*, *Typhlops*, *Asthenodipsas*, *Ogmodon*). Monatsberichte der Königlich preussischen Akademie der Wissenschaften zu Berlin 1864:271-276.

ROBB, J. B. 1966. The generic status of the Australasian typhlopids (Reptilia Squamata). Annals and Magazine of Natural History, series 13, 9:675-679.

TANNER, V. M. 1951. Pacific Islands herpetology, no. V. Guadalcanal, Solomon Islands: a check list of species. The Great Basin Naturalist 11(3-4):53-86.

WAITE, E. R. 1897. A new blind snake from the Duke of York Island. Records of the Australian Museum 3(3):69-70.

WAITE, E. R. 1918. Description of a new blind snake from the Solomon Islands. Records of the South Australian Museum 1(1):35-38.

WALLACH, V. 1993a. The supralabial imbrication pattern of the Typhlopoidea (Reptilia: Serpentes). Journal of Herpetology 27(2):214-218.

WALLACH, V. 1993b. A new species of blind snake, *Typhlops marxi*, from the Philippines (Serpentes: Typhlopidae). Raffles Bulletin of Zoology 41(2):263-278.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 151-156

Studies on the Physiological Ecology of Incubation in *Chinemys reevesii* Eggs

PEI-CHAO WANG, WEI MA, BO LU AND WEN-HUI YOU

Department of Biology, East China Normal University, Shanghai 200062, China

Abstract: -The length of incubation period in *Chinemys reevesii* eggs is 66.91±3.70, 62.29±9.00 and 56.57±2.85 days at 28°C, 30°C and 33°C, respectively. The values of effective accumulative temperature approximate to a range of constant (1871 to 1903 C-day) during different incubation temperatures. The mass of eggs buried in wet sand through incubation increased slightly about an average 0.48% to 3.66%. The mass of turtle hatchlings just after hatching at 28°C, 30°C, and 33°C averaged 59.76±6.85%, 59.12±5.33% and 56.31±5.36% of pre-incubation egg mass, respectively. The total lost rate of energy substances increased with the temperature of incubating and lost 25.92±9.67% at 28°C, 32.56±6.77% at 30°C, and 34.35±5.67% at 33°C. The metabolic rate of *C. reevesii* eggs was measured through incubation at 28, 30 and 33°C. The pattern of metabolic rate of embryonic development in *C. reevesii* is peaked, similar to the conditions of some other fresh water turtles. Maximum VO_2 occurred when its incubation is 65% to 80% of total incubation times. Total VO_2 of *C. reevesii* eggs was 94.61 mL/g at 28°C, 112.88 mL/g at 30°C and 152.22 mL/g at 33°C.

Key Words: Reptilia, Testudines, *Chinemys reevesii*, incubation period, hatchlings, effective accumulative temperature, oxygen consumption.

Introduction

The metabolic rate of developing embryos in reptiles reflects the energetic demands of both growth and maintenance (Wang et al., 1988). The ontogeny of embryonic metabolic rate in reptiles has been shown to have three patterns (Thompson, 1989). The metabolic rate during most of the incubation period has been reported in seven species of snakes (Clark, 1953; Dim'el, 1970; Black et al., 1984), ten chelonians (Lynn and von Brand, 1945; Ackerman, 1981a; Thompson, 1989; Gettinger et al., 1984), three crocodilians (Thompson, 1989; Whitehead 1987), and one lizard (Wang et al., 1988). So far, that of the common turtle (*Chinemys reevesii)* has not been reported.

Ecological and heat energy metabolic studies on adult turtles (*Chinemys reevesii)* have been reported before (Wang and Lu, 1985; Wang et al, 1988). In this paper, we will attempt to deal with the length of the incubation period and the metabolic rate of embryonic development inside the eggs of *Chinemys reevesii* in relation to ambient temperature from July to September, 1988 and 1989.

Methods and Materials

Fresh eggs of the turtle (*Chinemys reevesii)* were collected in the morning, after being laid in the sandbox of the turtle farm near our University. When each fresh egg was removed from the sandbox on the day of laying, it was marked and weighed with a torsion balance (±0.01 g) to determine the fresh egg mass. A total of 350 eggs were examined and divided into three groups in which the eggs of each group were buried in a dish of moist sand and incubated at temperatures of 28, 30 and 33°C, respectively. The relative humidity of sand in the dish was maintained at a range of 98 to 100%.

The determination of oxygen consumption of eggs in *C. reevesii* during the period of incubation was carried out with a small, simple and closed system respirometer that was described by Wang (1986). During determination, the ambient temperature also identified with the incubation temperature of each group. The experimental period was limited at 8:30 to 10:30 in the morning and each experiment lasted an hour with recording every five minutes. The carbon dioxide (CO_2) was absorbed by 10% NaOH solution. The

TABLE 1. T-test on differences of mean times from incubation to hatching in *C. reevesii* eggs at 28°C, 30°C, and 33°C

$T_{incub.}$ = C	Different values (days)	t	P
28 v. 30	4.63	18.37	<0.001
28 v. 33	10.34	11.37	<0.001
30 v. 33	5.71	8.66	<0.001

TABLE 2. Total temperature-day of requirement for embryonic development inside the egg of *C. reevesii*.

$T_{incub.}$ = C	28°C	30°C	33°C
N	98	103	54
Total temperature-day (C-day)	1882.61 (SD= 168.99)	1903.80 (SD= 116.62)	1871.27 (SD= 94.88)

TABLE 3. T-test on the different values of total temperature-day for embryonic development of *C. reevesii* at 28, 30, and 33°C.

$T_{incub.}$ = C	Different values (days)	t	P
28 v. 30	21.19	0.73	<0.005
28 v. 33	11.34	0.40	<0.005
30 v. 33	32.53	1.27	<0.005

TABLE 4. The changes of egg masses in *C. reevesii* throughout incubation period at 28, 30, and 33°C.

Days of	28°C			30°C			33°C		
incubation	N	M±SD	%	N	M±SD	%	N	M±SD	%
0	7	7.73±1.96	100	7	6.89±0.76	100	7	7.56±1.17	100
5	7	7.58±1.80	98.1	7	7.02±0.72	101.9	7	7.71±1.12	102
10	7	7.56±1.63	97.8	7	7.27±0.61	105.5	7	7.73±1.13	102.2
15	7	7.60±1.63	98.3	7	7.23±0.56	104.9	7	7.61±1.24	100.7
20	7	7.60±1.63	98.3	7	7.33±0.50	106.4	7	7.99±1.31	105.7
25	7	7.68±1.61	99.4	7	7.16±0.53	103.9	7	8.05±1.42	106.5
30	7	7.71±1.61	99.7	7	6.98±0.70	101.3	7	7.67±1.14	101.5
35	7	7.92±1.61	102.5	7	7.21±0.39	104.6	7	7.65±1.23	101.2
40	7	7.99±1.53	103.3	7	7.24±0.33	105.1	7	7.78±1.43	102.9
45	7	8.09±1.63	104.7	7	6.98±0.45	101.3	7	8.05±1.94	106.5
50	7	7.95±1.86	102.8	7	6.92±0.24	100.4	7	7.82±1.73	103.4
55	7	7.86±2.14	101.7						
Total (M±SD)		7.27±0.18	100.6 ±2.4		7.11±0.16	103.2 ±2.3		7.79±0.17	103 ±2.3

TABLE 5. A comparison between the mass of pre-incubation eggs and one of *C. reevesii* hatchlings during incubation at 28, 30, and 33°C.

Incubation Temp.	Pre-incubation			Just after hatching						
	Eggs, g	Standard rate %		Egg Shell			Hatchlings			Total lost rate, %
		Egg Shell	Egg Content	g	% of egg	>SRes %	g	% of egg	>SRc %	
28°C	8.23 SD=1.10 N=10	14.6 N=20	85.4 N=20	1.17 SD=1.17 N=10	14.31 SD=2.56 N=10	0.29	4.83 SD=0.74 N=10	59.76 SD=6.85 N=10	25.64	25.92 SD=9.67 N=10
30°C	7.86 SD=0.94 N=10	14.6 N=20	85.4 N=20	0.75 SD=0.30 N=10	9.44 SD=2.97 N=10	5.16	4.66 SD=0.78 N=10	59.12 SD=5.33 N=10	26.28	32.56 SD=9.68 N=10
33°C	7.28 SD=1.01 N=10	14.6 N=20	85.4 N=20	0.7 SD=0.17 N=10	9.51 SD=1.60 N=10	5.09	4.12 SD=0.83 N=10	56.37 SD=5.36 N=10	29.03	34.35 SD=9.67 N=10

Note: Standard rate (%) shows both eggshell % of and egg contents % of whole egg mass according to the average value of twenty fresh eggs.
SRes shows standard rate of egg shell.
SRc shows standard rate of egg content.
Total lost rate equals the mass of pre-incubation egg subtracting both masses of eggshell and of hatchlings just after hatching.

oxygen consumption was expressed as mL $O_2/h^{-1}g^{-1}$ or mL $O_2/day^{-1}g^{-1}$.

Results

Length of Incubation Period

The mean time of incubation to hatching for *C. reevesii* eggs was 66.91 days (SD=3.70, N=98), 62.29 days (SD=9.00, N=103) and 56.57 days (SD=2.85. N=54) during 29°C, 30°C and 33°C of incubation temperature, respectively. These differences of the mean values were compared by a t-test and the results indicate the significant differences (Table 1).

The length of the incubation period in turtle eggs decreased as the incubation temperature increased, that is a negative correlation of linear regression with the following equation: For Days = 124.2550 - 2.0550 (°C), r = -0.9985 (P>0.05).

Total Temperature-day of Requirement for Hatching

The total values of temperature-day for embryonic development in *C. reevesii* eggs during 28°C, 30°C and 33°C, is shown in Table 2. The different values of total temperature-day for embryonic development inside egg from Table 2 were compared by a t-test and the results of those show no significant differences in Table 3.

Changes of Egg Mass through Incubation

During the incubation of turtle eggs buried in wet sand, the average mass of those eggs increased slightly from 0.48% to 3.66% (Table 4).

Mass of Hatchlings

The mass of turtle hatchlings (*C. reevesii)* just after hatching at 28°C, 30°C and 33°C averaged 4.83 g (SD=0.74, N=10), 4.66 g (SD=0.78, N=10) and 4.12 g (SD=0.83, N=10) or 59.76% (SD=5.36,

TABLE 6. Equations relating VO_2 (ml/g-^{1}day^{-1}) to incubation days, total VO_2, and VO_2 of peak during incubation to hatching in *C. reevesii* eggs.

T-incub.	Before Peak in VO_2 = a +b days	r	After Peak in VO_2 = a +b days	r	Peak	Total O_2 ml/g
28°C	VO_2 = -2.0888+0.0356	0.944	VO_2 = 1.2524-0.0074	-0.931	2.06	94.61
30°C	VO_2 = 2.7922+0.0546	0.979	VO_2 = 1.3576-0.060	-0.695	2.52	112.88
33°C	VO_2 = 1.75+0.043	0.974	VO_2 = 1.7529-0.115	-0.811	2.72	152.22

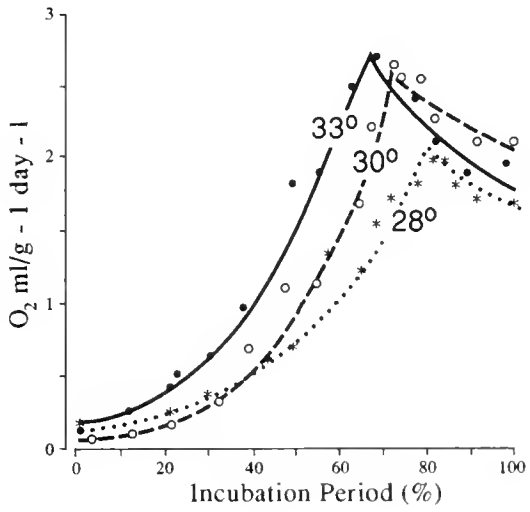


FIG. 1. Relationship between oxygen consumption of eggs and percentage of incubation period in *Chinemys reevesii*.

N=10), 59.12 % (SD=5.33, N=10) and 56.31% (SD=5.36, N=10) of pre-incubation egg mass, respectively. These values are 25.64%, 26.28% and 29.03% less than the standard rates of substance contents inside eggs during pre-incubation eggs of *C. reevesii* (Table 5).

Oxygen Consumption

The pattern of change in oxygen consumption (VO_2) of *C. reevesii* eggs was similar at 28°C, 30°C and 33°C. The VO_2 increased approximately exponentially and peaked to 65% of incubation period at 33°C, to 70% of that at 30°C, and to 80% of that at 28°C, and then declined to the time of hatching (Fig. 1). Total amount of O_2 consumed was calculated by integration of the equations (Table 6) over the interval from the first VO_2 measurement to the mean total incubation period at relevant temperature. The total VO_2 was 94.61 mL/g at 28°C, 112.88 mL/g at 30°C, and 152.22 mL/g at 33°C.

Discussion

Length of Incubation Period and Thermal Constant

Table 1 shows that the results of t-test for the average lengths of incubation period in *C. reevesii* eggs at 28°C, 30°C and 33°C exhibit the significant differences ($P<0.001$). The average lengths of those decreased with incubation temperature increased. This may be a characteristic of embryonic development in oviparous ectothermic animals. The thermal demands of embryonic development inside eggs is provided by the surroundings, so that, the developing velocity (days) of embryo in *C. reevesii* is affected by its surrounding temperature.

The relationship between length of incubation period and effective temperature of ectothermic embryonic development may be presented in an equation of effective accumulative temperature as follows: $K = D(t_1-t_0)$, where K = effective accumulative temperature , it is a constant or total temperature-day (C-day), t_0 = developmental temperature, D= total time (days) of embryonic development. On developmental zero (t_0) of embryos in *C. reevesii*, it is supposed by 0°C, so that , the effective accumulative temperature amounted to 1882.61±168.99 C-day during incubation to hatching in the *C. reevesii* eggs at 28°C, 1903.80±116.62 C-day at 30°C, and 1871.27±94.88 C-day at 33°C (Table 2).

The average values of those were taken by t-test and the results of those exhibited no significant differences (Table 3), in other words, the values of effective accumulative temperature for the embryonic developing inside the egg of *C. reevesii* are approximately a range of constant during different incubation temperature.

Changes of Incubating Egg Mass

During incubation, *C. reevesii* eggs buried in wet sand (RH, 98-100%) increased slightly in mass (Table 4). This may be due to the intake of water through the egg shell from the substrate (wet sand) and egg shell type.

On changes of egg mass (weight) in *C. reevesii* during incubation which are considered due to a net water intake from surroundings or of export. For an egg to absorb water, the potential of water in substrate must exceed the algebraic sum of the pressure potential and the osmotic potential of the egg contents (Packard et al., 1977). So that the viable egg contacting wet substrates experienced net increases in mass during incubation, that reflects on net fluxes of water across its egg shell (Packard et al., 1977, 1982, 1985; Gutzke and Packard, 1987). The egg shell type of *C. reevesii* is a hard shell, so the water contents of intake from its wet substrates must be controlled or limited to its egg shell type.

The Ratio between Mass of Pre-incubation Egg and of Hatchlings

The mass of *C. reevesii* hatchlings just after hatching at 28°C, 30°C and 33°C averaged for 59.76%, 59.12% and 56.37% of pre-incubation egg mass (Table 5), respectively. The values of those were less 25.64%, 26.28% and 29.03% less than standard rate of pre-incubation egg mass (Table 5). However, the algebraic sum for mass of egg shell and of hatchling just after hatching in *C. reevesii* is also less than one of pre-incubation egg (Table 5). This suggests that a part of energy substances in egg is lost or consumed through incubation. The total lost rate of energy substances increased with the temperature of incubation. The losses were 25.92±9.67% at 28°C, 32.56±6.77% at 30°c and 34.75±5.67% at 33°C (Table 5).

Pattern and Rate of Metabolism

The ontogeny of embryonic metabolic rate has been reported with three patterns: peaked, sigmoid and exponential. The eggs of *C. reevesii* during incubation at three different temperatures had an extreme peaked pattern of oxygen consumption, similar to the conditions of some fresh water turtles (Gettinger et al., 1984; Lynn and von Brand, 1945; Thompson, 1989; Webb et al., 1986), *Crocodylus* (Whitehead, 1987), *Alligator* (Thompson, 1989), and some birds (Vleck et al, 1980) but different from many other reptiles (Ackerman, 1981; Black et al., 1984; Clark, 1953; Dmei'el, 1970; Wang et al., 1988).

The patterns of embryonic metabolic ontogeny appear to be associated with different patterns of growth, egg shell types and environmental conditions of incubation (Thompson, 1989; Whitehead and Seymour, 1990). However, peaked or sigmoid pattern of embryonic metabolic ontogeny may be due to the fact that the energy expenditure for embryonic growth is decreased as the growth rate of embryo in late incubation period declines. This can possibly facilitate synchronous hatching in clutches.

Acknowledgments

This work was supported by the Scientific Fund of the State Educational Committee of China and East China Normal University. We would like to thank Dr. Allan Muth and Dr. Gary C. Packard for providing some literature, and also thank Dr. M. B. Thompson for comments on the manuscript and Professor Ermi Zhao for helpful discussion and encouragement.

Literature Cited

ACKERMAN, R. A. 1981. Oxygen consumption by sea turtle eggs (Chelonia, *Caretta)* during devlopment. Physiological Zoology 54:316-24.

BLACK, C. P., G. F. BIRCHARD, G. W. SCHUETT, AND V .D. BLACK. 1984. Influence of incubation water content on on oxygen uptake in embryos of the Burmese Python *(Python molurus).* Pp. 137-45. *In* R. S. Seryour (ed.), Respiration and metabolism of embryonic vertebrates. Dr. W. Junk Publishers, Dordrecht.

CLARK, H. 1953. Metabolism of the black snake embryo. II. Respiratory exchange. Journal of Experimental Biology 30: 502-505.

DMI'EL, R. 1970. Growht and metabolism in snake embryos. Journal of Embryology and Experimental Morphhology 23:761-772.

GETTINGER, R. D., G. L. PAUSKTIS, AND W. H. N. GUTZKE. 1984. Influence of hydric environment on oxygen consumption by embryonic turtles, *Chelydra serpentina* and *Trionyx spiniferus.* Physiological Zoology 57:468-473.

GUTZKE, W.H.N. AND G.C. PACKARD. 1987. Influence of the hydric and environments on eggs and hatchlings of bull snakes, *Pituophis melanoleucus.* Physiological Zoology 60(1):9-17.

LYNN, M. G. AND T. VON BRAND. 1945. Studies on the oxygen consumption and water metabolism of turtle embryos. Biological Bulletin of the Maryland Biological Laboratory, Woods Hole 88:112-125.

PACKARD, G. C., C. R. TRACY AND J. J. ROTH. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. Biol. Review 52:71-105.

PACKARD, G. C.,. M. J. PACKARD, T. J. BOARDMAN, K. A. MORRIS, AND R. D. SHUMAN. 1982. Influence of water exchanges by flexible-shelled eggs of painted turtles *Chrysemys picta* on metabolism and growth of embryos. Physiological Zoology 56(2):217-230.

PACKARD, G. C.,. M. J. PACKARD, AND W. H. N. GUTZKE. 1985. Influence of hydration of the environment on eggs and embryos of the Terrapene ornata. Physiological Zoology 58(5):564-575.

THOMPSON, M. B. 1989. Patterns of metabolism in embryonic reptiles. Respiration Physiology 76:243-256.

VLECK, C. M., D. VLECK, AND D. F.. HOYT. 1980. Patterns of metabolism and growth in avian embryos. American Zoologist 20:405-416.

WANG, P. C. AND H. LU. 1985. Thermometabolism and thermoregulation of *Chinemys reevesii* (Gray). Acta Herpetologica Sinica 4(1):61-62. (in Chinese).

WANG, P., H. F. XU, W. MA, AND X. JI. 1988. The influence of ambient temperature on the body temperature and energy metabolism in *Chinemys reevesii.* Acta Herpetologica Sinica 7(2):122-127. (In Chinese with English Abstract).

WANG, P. 1986. A simple, small and closed-system respirometer. Sichuan Journal of Zoology 5(1):28-20. (In Chinese).

WHITEHEAD, P.J. 1987. Respiration of *Crocodylus johnstoni* embryos. Pp. 473-497. *In* G. J. W. Webb, S. C. Manolis, and P. J. Whitehead (eds.), Wildlife Management: Crocodiles and alligators. Surrey Beatty & Sons Pty Limited, Australia.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 157-160

Research on the Sex Sensitive Period During the Incubation of Chinese Alligator Eggs

ZHENG-DONG ZHANG

Anhui Research Center of Chinese Alligator Reproduction, Xuanzhou, Anhui, China

Abstract. -The sex of Chinese Alligators (*Alligator sinensis)* is determined by the temperature that incubating eggs are exposed to. There is a sex sensitive period between the 14th and 27th day of incubation. Eggs treated at temperatures above 34° C produce males.

Key words: Reptilia, Crocodylia, Alligatoridae, *Alligator sinensis*, China, incubation, sex sensitive period.

Introduction

It is generally understood that the sex distinction of the majority of reptiles (including crocodiles and alligators) is determined by the environmental temperature during incubation. We have conducted several experiments on the incubation of Chinese Alligator *(Alligator sinensis)* eggs at different temperatures.

At present, there has not been a study which reports the existence of the "sex sensitive period" in the incubation of alligator eggs. Ferguson (1982) reported on the sex exchange during the entire period of incubation for *Alligator mississipiensis* eggs at different temperatures. In regard to the "sex sensitive period" of *Alligator mississipiensis* (Fergusion called it "temperature sensitive period"), it is believed that the sex determination occurs in the period between the second and third week of incubation. In 1988, we visited the United States and learned that an approach had been in progress to jointly study the "sex sensitive period" of *Alligator mississipiensis* by American and British scientists in London using more accurate means. Obviously such an approach in China still remains blank at the time of writing.

For the purpose of initiating an approach to determine whether there really exists a "sex sensitive period" during Chinese Alligator egg incubation, the Anhui Research Center of Chinese Alligator Reproduction has been conducting experimental studies since 1988 in search of factual understanding. The results of the two-year study (1988-1989) are reported here.

Materials and Methods

The eggs for the experiment were selected from captive reproduction at the Anhui Research Center. It is imperative to know the exact time when the eggs are laid. The time difference for the laying of the experimental eggs should not be more than 6 hours, nor remain in the natural environment over 12 hours. It is essential that the entire brood be collected immediately and then the eggs divided into separate groups to be hatched under artificial temperature control.

Each experimental group is made up with a corresponding number of eggs from each pre-determined brood to erase the influence which may originate from the different broods.

In 1988, three experimental groups were organized: 88-1, 88-2 and 88-3 together with a control group. The time to undergo the high temperature treatment was pre-arranged with Group 88-1 from the 2nd to the 15th day after the eggs were laid, Group 88-2 from the 16th to the 29th day, and Group 88-3 from the 30th to the 43rd day. All the high temperature treatments were conducted in a constant temperature box. For the rest of the time, each group was taken to the incubation room under the normal temperature. The average temperature received by the groups at the various stages are listed in Table 1 (The temperature appearing on the list and all lists hereafter is in Centigrade).

TABLE 1. The incubation temperature of experimental groups and control group at various time periods in 1988.

Group No.	No. of eggs	In incubation, the average temperature of various time periods			
		2nd-15th day July 2-15	16th-30th day July 16-30	31st-46th day July 31-Aug. 15	47th-54th day Aug. 16-23
88-1	20	34.5±0.8°C	39.5±0.2°C	31.7±0.2°C	32.2±0.2°C
88-2	20	31.6±0.2°C	35.0±0.8°C	*	
88-3	20	31.6±0.2°C	31.5±0.2°C	31.7±0.2°C	32.2±0.2°C
Control group	20	31.6±0.2°C	31.5±0.2°C	31.7±0.2°C	32.2±0.2°C

*At 2nd day of the high treatment, the constant temperature box was bad and the embryos were all dead.

TABLE 2. The incubation temperature of experimental groups in 1989.

Average temperature and time periods for high temperature treatment			Average temperature of stages in incubation room	
Group No.	Time period of treatment	Average Temperature	Incubation stages	Average Temperature
89-1	14th-18th day	34.5±0.3°C	1st-9th day (July 7-15)	30.5±0.1°C
89-2	15th-19th day	34.5±0.3°C		
89-3	17th-21st day	34.3±0.6°C		
89-4	19th-23rd day	34.5±0.6°C	10th-25th day (July 16-31)	31.0±0.4°C
89-5	21st-25th day	34.6±0.4°C		
89-6	23rd-27th day	34.6±0.1°C		
89-7	16th-20th day	34.3±0.6°C	26th-40th day (Aug. 1-15)	31.5±0.1°C
89-8	18th-22nd day	34.5±0.6°C		
89-9	20th-24th day	34.6±0.4°C		
89-10	24th-31st day	34.7±0.2°C	41st-54th day (Aug. 16-29)	31.3±0.1°C

Attention must be paid to comparing the growth of young alligators in the first 8 months after hatching.

Based on the experiments achieved from Group 88-2, 9 groups were established in 1989. The time for the high temperature treatment of various groups was shortened to 96 hours. The time threshold for the treatment of each group was to be alternated from one to two days. Because of some unanticipated causes, the experiment on Group 88-3 was not satisfactorily accomplished. Another group, No. 10, was then set up, to pass through the prolonged alternate high temperature treatment for 7 days. The average value of the treatment group to various groups and the temperature at various stages at other times are listed in Table 2.

Care is also required to note the comparison among the hatching results of various groups in 1989, and also the cause of mortality of young alligators in the first ten months of growth.

Tissue-section tests were used for sex identification in order to determine the correct sex without error.

TABLE 3. The incubation results, growth comparisons and sex ratio of the 1988 groups (weight in grams and length in centimeters).

Incubation results				Growth comparison of the first 8 months					
				(7th day)				(hibernation begins)	
Group	No.	No.	No.	Aug. 30, 1988		Nov. 25, 1988		Dec. 13, 1988	
No.	eggs	dead	hatched	weight ($\bar{x}$)	length ($\bar{x}$)	weight ($\bar{x}$)	length ($\bar{x}$)	weight ($\bar{x}$)	length ($\bar{x}$)
88-1	20	14	6	21.1±0.6	21.2±0.6	45.8±6.2	25.1±1.3	48.0±5.0	25.5±1.4
88-2	20	1	19	24.1±1.9	22.2±0.6	40.1±5.1	23.7±0.9	43.7±5.6	23.5±1.2
Comp.	20	0	20	23.3±1.1	22.2±0.5	43.3±4.7	25.1±0.8	45.6±5.9	24.9±0.9
Sex ratio			(hibernation finishes)					No. dead or weak	
	No.	No.		Mar. 22, 1989		April 30, 1989		No.	No.
	?	!		weight ($\bar{x}$)	length ($\bar{x}$)	weight ($\bar{x}$)	length ($\bar{x}$)	Dead	Weak
88-1	1	5		44.9±6.1	25.2±1.9	65.2±8.0	27.9±1.5	0	0
88-2	19	0		40.7±6.2	23.4±1.2	53.0±11.5*	35.4±1.7	2	0
Comp.	5	15		43.7±6.0	25.4±0.9	54.7±9.2**	27.0±1.5	1	2

Note: * n=17, ** n=19

TABLE 4. The incubation results, growth comparisons and sex ratio of the 1989 groups.

Incubation result					Cause of mortality		Sex proportion		
Group No.	No. of eggs	No. of embryos	No. hatched	Artificial Midwifery	No. dead	No. growing badly	No. probed	No. ?	No. !
89-1	10	0	10	7	0	4	10	10	0
89-2	10	0	10	5	0	4	3	3	0
89-3	10	0	10	7	0	4	10	10	0
89-4	10	0	10	8	0	3	2	2	0
89-5	10	1	9	6	1	1	8	8	0
89-6	10	1	9	5	0	4	9	9	0
89-7	10	1	9	7	0	3	9	9	0
89-8	10	1	9	7	1	3	9	9	0
89-9	10	1	9	7	1	2	9	9	0
89-10	16	1	15	6	0	1	15	13	2

Results

The main results from the 1988 experiment are shown in Table 3, and the main results from the 1989 experiments are shown in Table 4.

Discussion

According to the incubation results from Group 88-1, it is understood that in the first 14 days of incubation was the "high temperature sensitive period" of the alligator's embryo. The embryo is easily damaged and even death occurs in the high temperature for experimental purposes.

It is worthy to note that in another group which underwent the experimental high temperature simultaneously, all the tested embryos were found dead in the first 14 days, because of the application of

36±0.5°C to the temperature environment (The data relative to this test is not presented here). Therefore, the range of temperature control at this stage for Group 88-1, in fact, reached the high temperature limit. In comparison, the average temperature applied to Group 88-2, which was raised 0.5°C higher than that of Group 88-1, safety could be ensured at that time threshold.

From data gathered from various groups in 1988 on the growth of young alligators, it indicated that although among the three groups, the weight of young alligators in Group 88-2 was the largest, those in Group 88-1 had the smallest weight. However, after 90 days (Nov. 25), the young alligators of Group 88-1 overtook the other two groups, and the weight of Group 88-2 became smallest among the three groups. Such condition was maintained until the conclusion of the experiment. The average length of the young alligators in the three groups showed a similar phenomenon (See Table 3). This suggests that the young alligators, which were under the treatment of the special temperature at the "sex sensitive period", have become comparatively weak.

The sex proportion of Group 88-1, 88-2 and the control group revealed that Group 88-1 was almost the same as the sex proportions of wild Chinese Alligators which we have studied (M:F=5:1). The time threshold of high temperature treatment for Group 88-2 was just at the "sex sensitive period". All of them were males. Those in the control group showed sex differences somewhere between the other two groups. The cause of the latter condition is still unknown (Table 3).

In the experimental test in 1989, the sex proportion from the various groups was much beyond expectation. It provided the understanding that the time threshold for the "sex sensitive period" in the incubation of the Chinese Alligator is quite wide in range. Direct study on experiments and Group 88-2 pointed out that the time threshold must be from the 14th to the 27th day during the time of incubation.

Based on the incubation results of young alligators in various groups as well as the cause of mortality in the first 10 months, it was determined that the physical condition of young alligators in Groups 89-1 to 89-9 was considerably weak, corresponding with the result obtained in 1988. Comparatively speaking, those in Group 89-10 proved to be better. The materials relative to their sex distinction revealed that the time threshold for high temperature in this group has somewhat deviated from the "sex sensitive period". Therefore, during the time of the "sex sensitive period", the unisexual offspring that we obtained from treatment of artificial temperature control were considerably weak. At the initial stage of growth, they require particular care.

Direct study has discovered that there may exist some other factors which influence the "sex sensitive period" of the Chinese Alligator. At present, we are deep in our research on this field.

Literature Cited

CHENG, B. H., Z. H. HUA, B. H. LI. 1985. [Chinese Alligator]. Anhui Science and Technology Publishing House, Hefei, 1st edition. (In Chinese).

FERGUSON, M. W. J. AND T. JOANEN. 1982. Temperature of egg incubation determines sex in *Alligator mississipiensis*. Nature 296:850-853.

WANG, P. C. 1989. [Progress of studies on environmental sex determination in reptiles]. Acta Ecologica Sinica 9(1):84-90. (In Chinese).

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 161-166

Karyotypes of Four Microhylid Frogs from Xishuangbanna, Southern Yunnan, China

XIAO-MAO ZHENG AND GUAN-FU WU

Chengdu Institute of Biology, Academia Sinica, Chengdu, Sichuan, 610041 China

Abstract: -The karyotypes of *Microhyla butleri*, *ornata* and *pulchra* and *Kaloula pulchra pulchra* from Xishuangbanna, southern Yunnan are reported. The karyotype of *Microhyla butleri* is reported for the first time. Its diploid number of chromosomes (2n=22) differs from the other species investigated in the same genus which is 24. The results of *M. ornata* and *M. pulchra* are also different from those obtained by the previous authors. The karyotype of *Kaloula p. pulchra* (2n=28) had a slight difference from the result obtained by the previous authors. The further C-banding analysis of this species revealed that an amount of heterochromatin is located in the centric, terminal and interstitial position of chromosomes.

Key Words: Anura, *Microhylidae*, *Microhyla*, *Kaloula*, cytotaxonomy, China

Introduction

The karyotypes of *Microhyla ornata* from Sichuan and Fujian were reported by Chen (1983) and Gao et al. (1985) respectively. The karyotypes of *Microhyla pulchra*, *Kaloula pulchra pulchra* from Guangzhou were reported by He (1986). In the present study, the karyotypes of those species from Xishuangbanna, southern Yunnan, are reported, and they are analyzed by means of C-banding and silver-staining NORs techniques. In addition, the karyotype of *Microhyla butleri* from the same locality is reported for the first time.

Materials and Methods

Microhyla ornata (4 males, 4 females), *M. pulchra* (5 males, 5 females), *M. butleri* (5 males, 1 female) and *Kaloula p. pulchra* (2 males, 1 female) were captured in Xishuangbanna, southern Yunnan, China in May 1991. Chromosome preparations were made from the bone marrow cells by the method of Wu et al. (1981). C-banding and silver-staining NORs were carried out following the methods of Sumner (1972) and Tan et al. (1986).

Results

The karyotypes for the four species are separately shown in Figs. 1-3 and the chromosome measurements in Table 1. The secondary constrictions and results of Ag-NORs are listed in Table 2.

The diploid chromosome number of *M. ornata* and *M. pulchra* is 24, with 18 m, 4 sm and 2 m or 2 sm chromosomes, whereas that of *M. butleri* is 22 with 18 m and 4 sm chromosomes. *Kaloula p. pulchra* had 28 including 20 m, 6 sm and 1 sm or 1 st. In four species, the chromosome length decreased gradually, not forming distinct groups in size. The conspicuous secondary constrictions (SC) were found on the long arm of No. 5 of *K. p. pulchra*, No. 8 of *M. butleri* and Nos. 8, 10 of *M. pulchra*, whereas the unremarkable one can be sought on the long arm of No. 11 of *M. ornata* in a few mitotic metaphases. No consistent heteromorphic pairs were observed in all four species.

The C-banding were successfully obtained in *K. p. pulchra*. The centric positive bands were discovered on all chromosomes, especially present on smaller ones; terminal bands were shown on Nos. 2-4; interstitial bands, as well, can be observed on both the short and long arm of No. 1 and the short arm of No. 4. The result still revealed the highly heterochromatic region possesses two-thirds of the length of No. 5 (Fig. 3). The prominent heterochromatinization are observed in individuals of both sexes, and there are no difference between both sexes, indicating the existence of sex-differentiation.

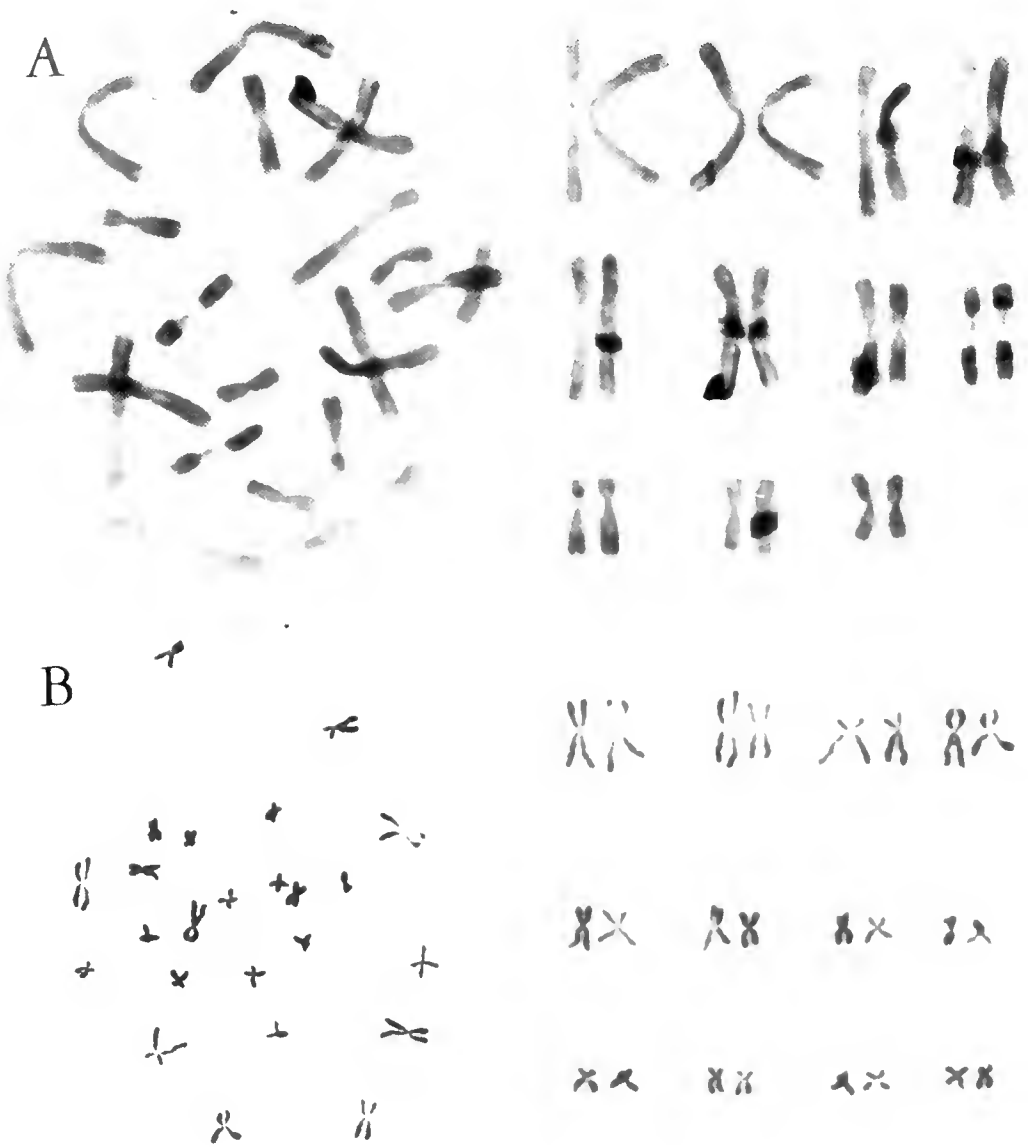


FIG. 1. Karyotypes of *Microhyla butleri* (A) and *M. ornata* (B).

The silver-staining NORs revealed that NOR is located on No. 10, associated with the secondary constriction in *M. pulchra* (Fig. 2). *M. butleri* and *K. p. pulchra* are also treated with the silver-staining NORs technique. Although no perfect spread of silver-staining NORs are in the two species, NOR associated with SC can be sought in some cells.

Discussion

Karyotypes

The karyotypes of *M. ornata* and *M. pulchra* from Xishuangbanna were different from those of the other localities (Table 2). Firstly, all 12 chromosome pairs, except No. 3 in two species, are metacentric, but Nos. 7-9 from Xishuangbanna changed to, or close to, submetacentric. Next, the

FIG. 2. Karyotype and Ag-NORs of *Microhyla pulchra*.

numbers and positions of SC on the chromosomes are distinctly varied between the same species from Xishuangbanna and the other localities (Table 2). These results seem to show that the karyotypic type of the species are gradually altered due to slowly fitting for the various environments.

There are about 20 species described in the genus *Microhyla* that range over Asia only. Up to now, seven species have been analyzed karyologically. The karyotypic character proved their obvious interspecific differentiation. In *M. nornata* and *mixtura*, st chromosomes can be observed. The positions of SC on the chromosomes are quite different between these species: *ornata* on Nos. 3, 9 and 11; *heymonsi* on No. 2 (Gao et al., 1985; Guo et al., 1987); *pulchra* on several pairs; *inornata* (Zhao, 1988) and *mixtura* (Guo et al., 1991) on No. 9 and *butleri* on No. 8. Moreover, this evident differentiation is reflected on the various diploid number in the genus. Most of them have 2n=24 except for *inornata* and *rubra* with 2n=26 and *butleri* with 2n=22 (the present study). Diploid number of 22, 24, 26 and 28 are known for *Microhylidae*. Usually, most species in the same genus have the same diploid number in anurans. On the other hand, if we supposed the 24 was the diploid number of the genus *Microhyla*, it would be possible to consider whether *butleri*, *inornata* and *rubra* might be separated from the genus.

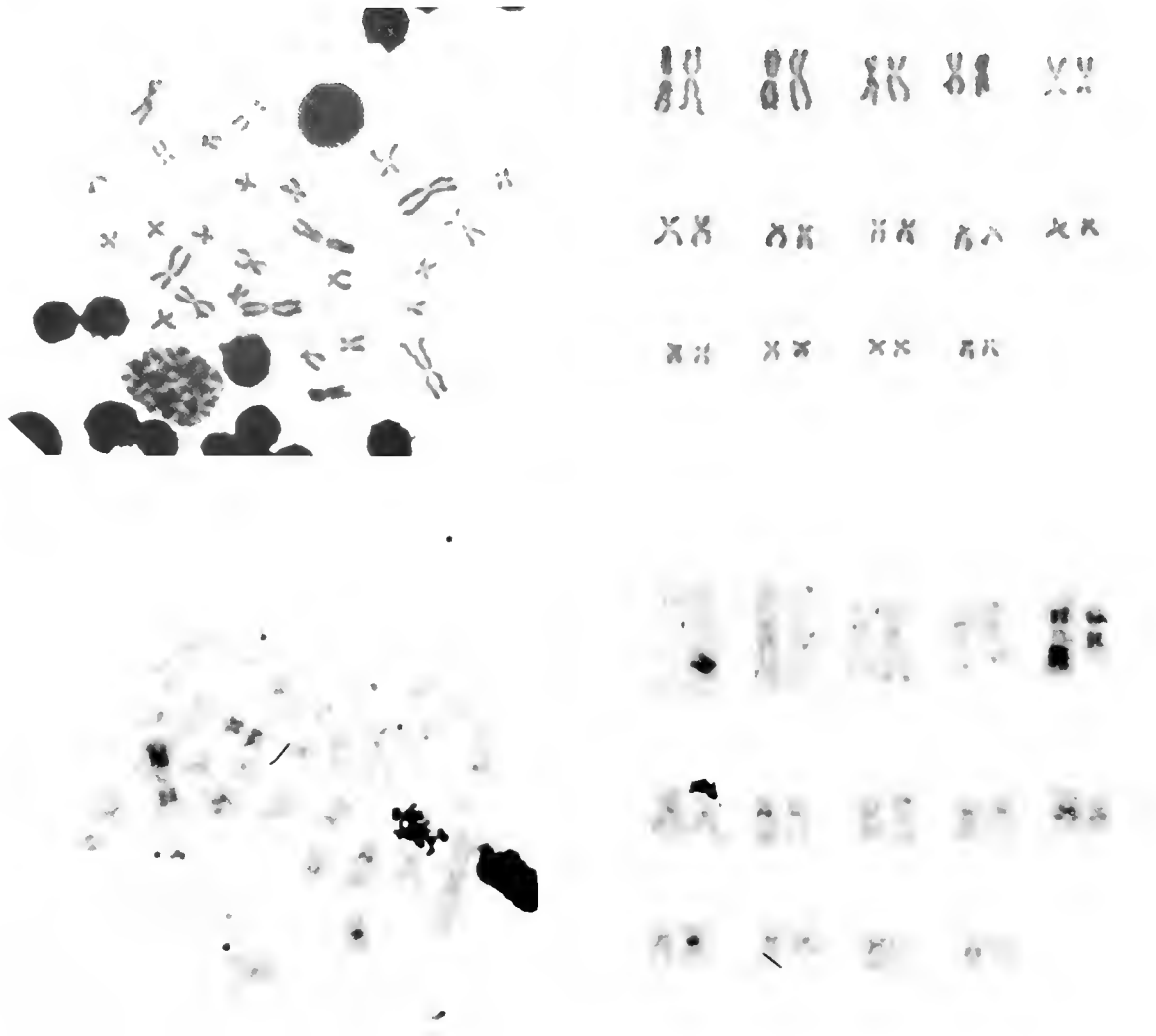


FIG. 3. Karyotype and C-bands of *Kaloula p. pulchra*.

The karyotypes of *K. p. pulchra* from Xishuangbanna and Guangzhou are compared (Table 2) and the difference between them are not obvious. Unlike in *Microhyla*, five species in *Kaloula* whose karyotype are known have 2n=28, and the conspicuous SC located on No. 5 except *K. picta* (Kuramoto, 1980). The interspecific differentiation is less clear. The differences between these species are shown on the variety of centromere type of a few corresponding chromosome pairs.

C-bands and NORs

Although many *microhylids* are analyzed karyologically, their C-banding is rarely reported. The C-bands of *K. rugifera* are even mentioned (Zeng et al., 1989) in which the interstitial and terminal bands except centric are observed. In *K. p. pulchra*, not only interstitial and terminal but also centric bands are easily seen (Fig. 3). In the two species, the interstitial bands associated with the main SC on No. 5 are enhanced, which indicates the genetic stability in this genus. The C-bands of the two species present the

TABLE 1. Chromosome measurements of four microhylid species from Xishuangbanna, southern Yunnan, China.

No	*Microhyla ornata* Arm Ratio	Relative Length	Type	*Microhyla pulchra* Arm Ratio	Relative Length	Type
1	1.34± 0.10	13.71± 0.75	m	1.21± 0.13	13.68± 0.92	m
2	1.49± 0.25	11.71± 0.51	m	1.42± 0.18	11.69± 0.59	m
3	2.36± 0.35	11.06± 0.64	sm	2.24± 0.38	10.38± 0.47	sm
4	1.54± 0.31	10.24± 0.78	m	1.34± 0.12	10.01± 0.41	m
5	1.28± 0.25	9.10± 0.43	m	1.24± 0.21	9.25± 0.57	m
6	1.18± 0.09	8.58± 0.48	m	1.33± 0.33	8.47± 0.46	m
7	1.87± 0.40	7.05± 0.56	sm	1.60± 0.39	7.40± 0.61	sm
8	1.42± 0.26	6.48± 0.23	m	1.65± 0.35	6.73± 0.31	sm/m
9	1.64± 0.43	5.94± 0.28	m/sm	1.32± 0.28	6.19± 0.48	m
10	1.39± 0.23	5.61± 0.26	m	1.55± 0.30	5.61± 0.48	m
11	1.37± 0.22	5.26± 0.39	m	1.45 - 0.27	5.46± 0.42	m
12	1.24± 0.10	5.06± 0.45	m	1.25± 0.14	5.13± 0.22	m
	Microhyla butleri			*Kaloula p. pulchra*		
1	1.29± 0.15	14.18± 0.92	m	1.16± 0.13	14.52± 0.84	m
2	1.34± 0.25	12.63± 0.85	m	1.23± 0.09	12.07± 0.71	m
3	1.90± 0.26	11.07± 0.45	sm	1.60± 0.18	10.21± 0.38	sm
4	1.40± 0.18	10.47± 0.42	m	1.15± 0.10	9.04± 0.38	m
5	1.19± 0.12	9.68± 0.52	m	1.28± 0.25	8.28± 0.40	m
6	1.22± 0.15	8.81± 0.43	m	1.35± 0.16	7.01± 0.33	m
7	1.39± 0.28	8.31± 0.55	m	2.56± 0.51	6.10± 0.25	sm/st
8	1.32± 0.16	7.24± 0.44	m	1.53± 0.19	5.82± 0.16	m
9	1.96± 0.36	6.54± 0.34	sm	1.95± 0.48	5.24± 0.35	sm
10	1.41± 0.21	6.00± 0.36	m	1.34± 0.26	4.87± 0.26	m
11	1.23± 0.20	5.07± 0.66	m	1.37± 0.27	4.58± 0.41	m
12				1.19± 0.17	4.46± 0.37	m
13				1.28± 0.33	4.11± 0.40	m
14				2.28± 0.34	2.28± 0.34	sm

TABLE 2. Karyotypes of four microhylid species from different localities

Species	Locality	1	2	3	4	5	6	7	8	9	10	11	12	13	14	S.C.	Ag. NOR's
M. ornata	Sichuan	m	m	sm	m	m	m	m	m	m	m	m	m				
	Fujian	m	m	sm	m	m	m	m	m	m	m	m	m			Nos. 3, 9q	
	Xishuangbanna	m	m	sm	m	m	m	sm	m	m/sm	m	m				No. 11q	
M. pulchra	Guangzhou	m	m	sm	m	m	m	m	m	m	m	m	m			Nos. 1,3, 6, 10, 11q	
	Xishuangbanna	m	m	sm	m	m	m	sm	sm/m	m	m	m				Nos 8, 10q	No. 10q
M. butleri	Xishuangbanna	m	m	sm	m	m	m	m	m	sm	m	m				No. 8q	No. 8q
K. pulchra	Guangzhou	m	m	sm	m	m	m	m	sm	m	m	m	m	m	m	Nos. 5, 14p	
	Xishuangbanna	m	m	sm	m	m	m	sm/stn		sm	m	m	m	m	sm	No. 5q	No. 5q

heterochromatin of *microhylids* are widely spread on the centric, interstitial and terminal positions of chromosomes. The results are very similar to those in the higher anurans. It suggests that the evolutionary level of *microhylids* correspond to that of the higher anurans from cytogenetics. The obvious heterochromatinization of No. 5 in *K. pulchra* does not show sex differentiation, and it acts as part of a special sign to distinguish it from other species.

The stable and conspicuous SC is always the location of NORs. In fact, silver-staining NORs shows that NORs of *K. pulchra*, *K. rugifera*, *M. pulchra*, *M. butleri*, *M. ornata*, *M. mixtura*, and *M. heymonsi* are just located in the position of their main SC. Tymowska (1977) concluded these species in the genus show a close relationship due to having the same NORs. From this point, the close relationship exist between species in the genus *Kaloula* for they have the same SC on No. 5. On the contrary, those species in the genus *Microhyla* reveal their higher interspecific differentiation level because of their different NOR association with the main SC.

Literature Cited

GAO, J. M., X. ZHAO AND H. B. DING. 1985. A comparative study on the karyotypes of two species of *Microhyla*. Acta Herpetologica Sinica, Chengdu, new ser. 4(3):163-165, 2 plates. [In Chinese with English abstract].

GUO, C.W. AND Y. W. DONG. 1987. Studies on the karyotype and Ag-NORs of *Microhyla heymonsi* Vogt. Hereditas, Beijing 9(6):12-14. [In Chinese with English abstract].

GUO, C. W., Y. W. DONG AND S. H. ZHAN. 1991. Studies on the karyotype and Ag-NORs of *Rana tientaiensis* and *Microhyla mixtura*. Hereditas, Beijing 13(2):6-8, 2 plates. [In Chinese with English abstract].

HE, H. Y. 1986. Comparative studies on the karyotypes of nine anuran species of Guangdong Province. Acta Herpetologica Sinica, Chengdu, new ser. 5(3):171-175, 2 plates. [In Chinese with English abstract].

KURAMOTO, M. 1980. Karyotypes of several frogs from Korea, Taiwan and the Philippines. Experientia, Basel, CHE 36:826-827, 2 plates.

SUMNER, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75:304-306, 3 plates.

TAN, A. M., Z. A. WU, E. M. ZHAO AND H. X. OUYANG. 1986. A handy one-step method for silver-staining NORs. Acta Herpetologica Sinica, Chengdu, new ser. 5(1):72-74, 1 plate. [In Chinese].

TYMOWSKA, J. 1977. A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing a 36-chromosome complement. Cytogenetics and Cell Genetics, Basel, CHE 18:165-181.

WU, G. F., W. M. YANG AND E. M. ZHAO. 1981. Studies on genus *Vibrissaphora* (Amphibia: Pelobatidae). 3. A preliminary observation on karyotype of *Vibrissaphora liui* (Pope). Acta Herpetologica Sinica, Chengdu, old ser. 5:139-142. [In Chinese with English abstract].

WU, Z. A. AND H. Y. YANG. 1981. The karyotype of *Kaloula borealis*. Acta Zoologica Sinica, Beijing 27(1):106. [In Chinese].

ZENG, X. M. AND G. F. WU. 1990. Studies on the karyotype of *Kaloula rugifera* Stejneger. Pp. 169-172. *In* Er-mi Zhao (ed.): From Water onto Land, China Forestry Press, Beijing. [In Chinese with English abstract].

ZHAO, E. M. 1988. *Microhyla inornata* Boulenger, 1890 found in mainland China, with its karyotypic report. Acta Herpetologica Sinica, Chengdu, new ser. 1988(2):119-121. [In Chinese with English abstract].

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 167-171

Cytotaxonomic Studies on Chinese Pelobatids VI. The Karyotypes, C-bands and Ag-NORs of *Megophrys minor* and *Oreolalax major*

XIAO-MAO ZHENG AND GUAN-FU WU

Chengdu Institute of Biology, Academia Sinica, Chengdu, Sichuan, China

Abstract: -Chromosome preparations were successfully stained for C-bands and Ag-NORs in two Chinese pelobatids, *Megophrys minor* and *Oreolalax major*. The results were analyzed and compared. The karyotype formula of *O. major* was 6+7 like most species of Chinese Oreolalaxinae whereas *M. minor* was 5+8 just as in most species of Chinese Megophryinae. The SC on the long arm of chromosome No. 6 associated with the C-band-positive was speculated the Standard NORs of genus *Oreolalax*. The NOR just in the conspicuous SC was not emerged firmly in the genus *Megophrys*.

Key words: Anura, Pelobatidae, *Megophrys*, *Oreolalax*, cytotaxonomy, China

Introduction

Chinese pelobatids are attributed to two subfamilies with about 50 species (Tian et al., 1986). The karotypes of 15 species among those 50 species, with the majority distributed in the Heng Duan Mountains region where the karyotypic characteristics of some anurans are unusual (Zeng and Wu, 1989), were reported. In this paper, two other species, *Oreolalax major* and *Megophrys minor* were analyzed by means of C-banding and silver-staining NORs techniques.

Materials and Methods

Megophrys minor (3 males and 1 female) were collected on Mt. Emei, Sichuan Province, China and in Maowen County, Sichuan Province, China, respectively. *Oreolalax major* (3 males) were captured from Mt. Emei in 1989-90. Chromosome preparations were performed by centrifugal air-drying method (Wu et al., 1981) using 0.4 M KCl as hypotonic solution for 40 minutes. C-banding was made following Sumner (1972), for 5 minutes with Barium Hydroxide treatment at 54° C. Silver-staining NORs was prepared following Tan et al. (1986), $AgNO_3$ acting time for about 5 minutes at about 55° C.

Results

The measurements of chromosomes for the two species are shown in Table 1.

All specimens of *M. minor* from two places, Mt. Emei and Maowen had 2n=26 and the complement included five pairs of large (Nos. 1-5) and eight pairs of small (Nos. 6-13) chromosomes. Nos. 1, 4-5 and 8-12 were metacentric (m), whereas Nos. 2-3 and 6-7 were submetacentric (sm) chromosomes. The last pair was telocentric (t). The highly differentiated heteromorphic sex chromosomes as seen in *Pyxicephalus adspersus* (Schmidt, 1980a) were not found when males and females were compared.

Three males of *O. major* also had 2n=26 with the complement of six pairs of large (Nos. 1-6) and seven pairs of small (Nos. 7-13) chromosomes. The chromosomes were metacentric except for Nos. 3-5 and 9 with submetacentric and No. 6 with sub- or metacentric. Whether or not highly differentiated heteromorphic sex chromosomes are present is not known, due to the lack of female animals.

The conspicuous secondary constriction was found in a pericentric position on the long arm of chromosome No. 6 of *O. major* whereas the small inconspicuous one can be seen in a proximal position on the short arm of chromosome No. 6 of *M. minor* (Figures 1 and 2).

The result of silver-staining NORs revealed that Ag-NORs were present on chromosome No. 6 associated with the secondary constriction in both species (Figs. 1, 2). The strongly C-band-positive was in a

TABLE 1. The arm ratio and relative length of *Megophrys minor* and *Oreolalax major*.

	Megophrys minor				*Oreolalax major*		
	Arm Ratio	Relative length	Type		Arm Ratio	Relative length	Type
1	1.32±0.10	17.91±1.39	m	1	1.35±0.12	16.75±0.77	m
2	1.74±0.07	14.10±0.90	sm	2	1.54±0.15	13.53±1.28	m
3	2.12±0.20	12.52±0.52	sm	3	2.09±0.24	11.90±0.84	sm
4	1.65±0.05	11.41±0.36	m	4	1.98±0.25	11.11±0.48	sm
5	1.53±0.10	9.89±0.49	m	5	1.77±0.08	9.38±0.50	sm
6	1.72±0.03	5.81±0.48	sm	6	1.61±0.21	7.63±0.70	m/sm
7	2.24±0.31	5.13±0.35	sm	7	1.38±0.21	5.52±0.63	m
8	1.51±0.15	4.72±0.41	m	8	1.44±0.14	5.03±0.35	m
9	1.31±0.15	4.35±0.44	m	9	1.87±0.22	4.75±0.39	sm
10	1.35±0.15	3.93±0.30	m	10	1.41±0.19	4.18±0.53	m
11	1.50±0.19	3.71±0.40	m	11	1.36±0.18	3.97±0.55	m
12	1.33±0.19	3.47±0.34	m	12	1.10±0.12	3.60±0.63	m
13	*	3.06±0.39	t	13	1.28±0.17	3.17±0.39	m

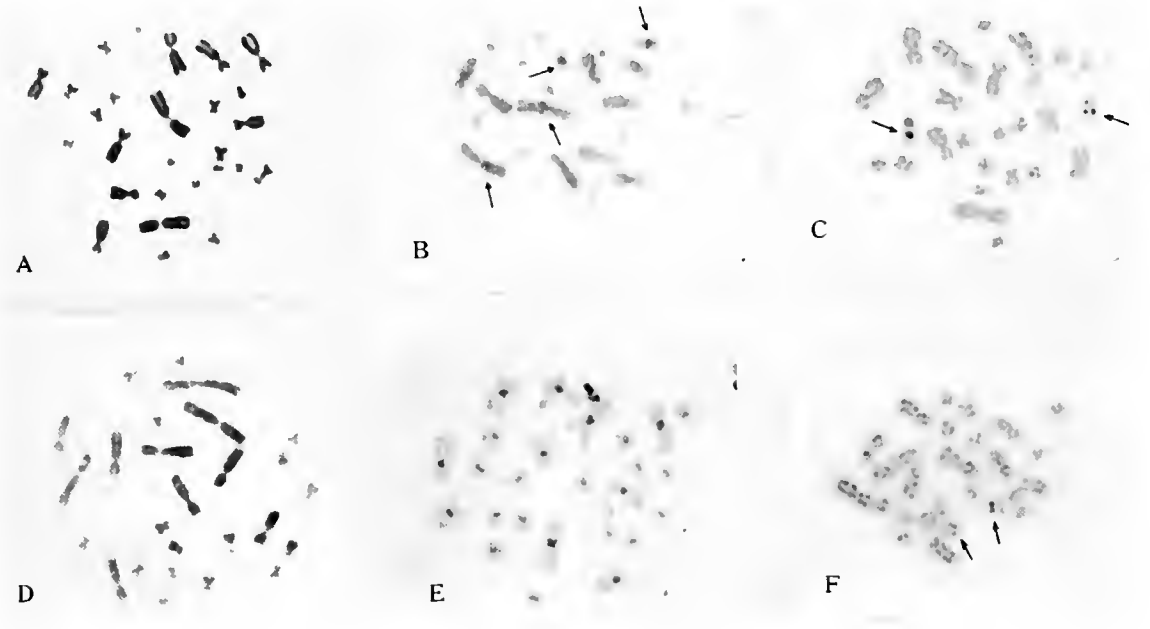


FIG. 1

centric position on each chromosome pair of *M. minor*, and only two weakly positive C-bands were discovered in *O. major*. One was associated with the secondary constriction of chromosome No. 6, and another was in the proximal position of the long arm of chromosome No. 1 which was not related to the secondary constriction (Figs. 1, 2).

Unfortunately, we cannot get the C-bands from females from the two species. Moreover, it is also impossible to know whether the early stage of ZW/ZZ sex chromosomes differentiation like *Poecilia shenops var. melanistica* (Haaf and Schmidt, 1984) and *Leiopelma hamiltoni* (Green, 1988) exists or not.

Discussion

Karyotypes

There is no doubt that *M. minor* has the second karyotype formula (5+8) of Morescalchi (1973) as shown in Table 1.

FIG. 2

TABLE 2. The karyotypes of three *Megophrys* species and six *Oreolalax* species.

Species	Centric Type												
	1	2	3	4	5	6	7	8	9	10	11	12	13
M. lateralis	m	m	sm	m/sm	m	m	m	st	m	m	m	m	st
M. omeimontis	m	m/sm	sm	m	m	m	sm	m	t	m	m	sm	t
M. minor	m	sm	sm	m	m	sm	sm	m	m	m	m	m	t
O. omeimontis	m	m/sm	sm	sm	m	m	m	m/sm	m/sm	m	m	m	m
O. pingii	m	m	sm	sm	m/sm	m	m	sm	m	m	m	sm	m
O. popei	m	sm	sm	m/sm	m	m	m	m	m	m	m	m	m
O. rugosa	m	m/sm	sm	sm	sm	sm	m/sm	m/sm	m/sm	m	m	m	m
O. schmidti	m	m	sm	m	m	m	m	m	m	m	m	m	t
O. major	m	m	sm	sm	sm	m/sm	m/sm	m	m	m	m	m	m

This result corresponded to that of most species of Chinese Megophryinae. The karyotype of *M. minor* was roughly similar to those of the other two species of *Megophrys*, *M. lateralis* (Wu, 1987) and *M. omeimontis* (Zeng and Wu, 1989). All of them had several pairs of sm, st and t chromosomes, and chromosome No. 3 was sm (Table 2). The difference among species of the genus were mainly shown on the position of secondary constriction (SC): on the short arm and long arm of chromosome No. 6 for *M. minor* and *M. omeimotis*; on the short arm of chromosome No. 5 for *M. lateralis*. Besides, these three species can be distinguished from each other by the number of sm, st and t chromosome pairs.

As shown in Table 1, the karyotype of *O. major* was classified into the first formula (6+7) of Morescalchi. The result was the same as the other 5 species of genus *Oreolalax*, *O. pingii*, *O. rugosa*, *O. popei*, *O. omeimontis* (Wu, 1988) and *O. schmidti* (Zeng and Wu, 1989). The karyotype of *O. major* only consists of m and sm chromosomes like the other 5 species of the same genus (Table 2). Moreover, 6 *Oreolalax* species had a conspicuous SC present on the long arm of chromosome No. 6. The differences among the *Oreolalax*

species were only reflected on the arrangement of m and sm chromosomes.

Compared with the genus *Megophrys*, the *Oreolalax* species had no st chromosomes and all chromosomes in the 6 known species were m or sm except chromosome No. 13 of *O. schmidti* (Table 2) which was t chromosomes. That their SC always appeared firmly on the long arm of chromosome No. 6 was different from *Megophrys*, in which 3 species had 3 different positions of SC. Furthermore, the number of the large sm chromosome pairs of the *Oreolalax* species were much more than that of *Megophrys*. *Oreolalax* was a subgenus of the *Scutiger* (Duellman, 1985) and it was considered as a genus (Myers and Leviton, 1962) which was attributed to Megophryniae. The differences of the two subfamilies (most species mentioned above) were present on two different karyotype formulas: the former was 6+7, whereas the latter was 5+8. It was thought that the more primitive karyotypic characteristics the karyotype of pelobatids had, the more t, st and sm pairs of chromosomes in comparison with that of the higher Anura (Duellman, 1985; Morescalchi, 1973). This position is still similar to the point of view on morphological taxonomy.

C-bands and NORs

C-bands in pelobatids were found more weakly and in less number than those in higher Anura (Bufonidae, Ranidae, and Hylidae). The C-banding of *M. minor* was very similar to those of the other two species of *Megophyrs*, *M. nasuta* (Schmidt, 1980b) and *M. omeimontis*. The constitutive heterochromatin emerged on the procentric area of each pair of chromosomes. The C-band associated with the SC on chromosome No. 6 *(M. nasuta, M. omeimontis)* and No. 5 *(M. minor)* was not enhanced to be particularly distinguished with centric C-band. The result of the C-banding treatment to *Oreolalax* species were less active than that to *Megophrys*. The centric C-bands were always weak *(O. omeimontis, O. pingii, and O. rugosa)* or invisible *(O. schmidti, O. major,* and this paper). Most of them had no centric C-bands and only had one interstitial C-band positive on chromosome No. 6 which was just associated with the position of the SC. Apart from one on chromosome No. 6, *O. major* had the other interstitial C-band on chromosome No. 1. This is different from the other species of this genus. If the interstitial C-bands revealed the relics of chromosome rearrangement (Schmidt, 1978a; King, 1980), it should be possible that the karyotype of *O. major* shows more higher evolutionary level in the *Oreolalax*.

It is said that stable and conspicuous SC is always the location of NORs. In respect to the genus *Rana*, Schmidt (1978b) concluded that the Standard NORs were always emerged in this SC on the long arm of chromosome No. 10 and thought it as a sign of *Rana*. The result of silver staining in *O. major* and *O. schmidti* proved that NOR was just in the SC on the long arm of chromosome No. 6. In terms of the other four species of this genus, *Oreolalax* with the same SC, it is speculated that the SC region on chromosome No. 6 should be in the location of the standard NORs of this genus. Furthermore, it is possible the close relationship between species in this genus was shown due to them having the same NORs (Tymoska, 1977). The NOR of *M. minor* and *M. omeimontis* emerged on the place associated with themselves SC. Compared with *Oreolalax*, they did not have the same SC associated with NOR, thus species showing higher interspecific differentiation.

Literature Cited

DUELLMAN, W. E. AND LINDA TRUEB. 1985. Biology of Amphibians. McGraw-Hill, New York. 670 pp.

GREEN, D. M. 1988. Heteromorphic sex chromosomes in the rare and primitive frog *Leiopelma hamiltoni* from New Zealand. Journal of Heredity 79:165-169.

HAAF, T. AND M. SCHMIDT. 1984. An early stage of ZW/ZZ sex chromosome differentiation in *Peocilia sphenops var. melanistica* (Poeciliidae, Cyprinosontiformes). Chromosoma 89:37-41.

KING, M. 1980. C-banding studies on Australian Hylid frogs: Secondary constriction structure and the concept of Euchromatin Transformation. Chromosoma 80:191-217.

MYERS, G. S. AND A. E. LEVITON. 1962. Generic classification of the high-altitude pelobatid toads of Asia *(Scutiger, aelurophryne,* and *Oreolalax).* Copeia 1962(2):287-291.

MORESCALCHI, A. 1973. Amphibia. Pp. 233-248 *In* A. B. Chiarelli and C. Capanna (eds.), Cytotaxonomy and vertebrate evolution. Academic Press. London-New York.

SCHMIDT, M. 1978a. Chromosome banding in Amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla.* Chromosoma 66:361-388.

SCHMIDT, M. 1978b. Chromosome banding in Amphibia. II. Constitutive heterochromatin and nucleolus organizer regions in Ranidae, Microhylidae and Rhacophoridae. Chromosoma 68:131-148.

SCHMIDT, M. 1980a. Chromosome banding in Amphibia. V. Highly differentiated ZW/ZZ sex chromosomes and exceptional genome size in *Pyxicephalus adspersus* (Anura, Ranidae). Chromosoma 80:69-96.

SCHMIDT, M. 1980b. Chromosome banding in Amphibia. IV. Differentiation of GC- and AT- rich chromosome regions in Anura. Chromosoma 77:83-103.

SUMNER, A. T. 1972. A simple technique for demonstrating centrometric heterochromatin. Experimental Cell Ressearch 75:304-306.

TAN, A. M. , Z. G. WU, E. M. ZHAO, AND H. X. OUYANG. 1986. A handy one-step method for silver-staining NORs. Acta Herpetologica Sinica 5:72-74. (In Chinese).

TIAN, W. S. AND Y. M. JIANG (EDITORS), ASSISTED BY G. F. WU, Q. X. HU, E. M. ZHAO, AND Q. Y. HUANG. 1986. Identification manual for Chinese species of amphibians and reptiles. Science Press, Beijing. 164 pp. (In Chinese).

TYMOWSKA, J. 1977. A comparative study of the karyotypes of eight *Xenopus* species and subspecies possessing a 36-chromosome complement. Cytogenetics and Cell Genetics 18: 165-181.

WU, G. F., W. M. YANG, AND E. M. ZHAO. 1981. Studies on genus *Vibrissaphora* . 3. A preliminary observation on karyotype of *Vibrissaphora liui.* Acta Herpetologica Sinica. 5: 139-142. (In Chinese).

WU, G. F. 1987. Cytotaxonomical studies on Chinese Pelobatids III. The analysis of the Karyotypes of *Megophrys lateralis* and *Atympanophyrs shapingensis.* Acta Herpetologica Sinica. 6: 45-48. (In Chinese).

WU, G. F. 1988. Cytotaxonomical studies on Chinese Pelobatids IV. The Karyotypes and C-bands of four species in the genus *Oreolalax.* Acta Herpetologica Sinica. 1: 1-4. (In Chinese).

ZENG, X. M. AND G. F. WU. 1989. Cytotaxonomical studies on Chinese Pelobatids V. The Karyotypes, C-bands and AgNORs of *Megophrys omeitmontis Oreolalax schmidt.* Chinese Herpetological Research 2: 37-45.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 172-180

Amphibians and Reptiles of the Royal Chitwan National Park, Nepal

GEORGE R. ZUG[1] AND JOSEPH C. MITCHELL[2]

[1]Department of Vertebrate Zoology, National Museum of Natural History, Washington, DC, USA, 20560
[2]Department of Biology, University of Richmond, Richmond, Virginia, USA, 23173

Abstract. -The Royal Chitwan National Park encompasses over 900 km^2 of grassland and forest in south-central Nepal. This mixture of habitats provides the home for 11 frog species, two crocodilians, eight turtles, ten lizards, and 24 snakes. A checklist documents species occurrences and habitat preferences; species accounts provide natural history observations for selected species of the park's herpetofauna.

Key Words: Nepal, Amphibia, Salientia, Reptilia, Crocodylia, Testudines, Lacertilia, Serpentes, checklist, natural history

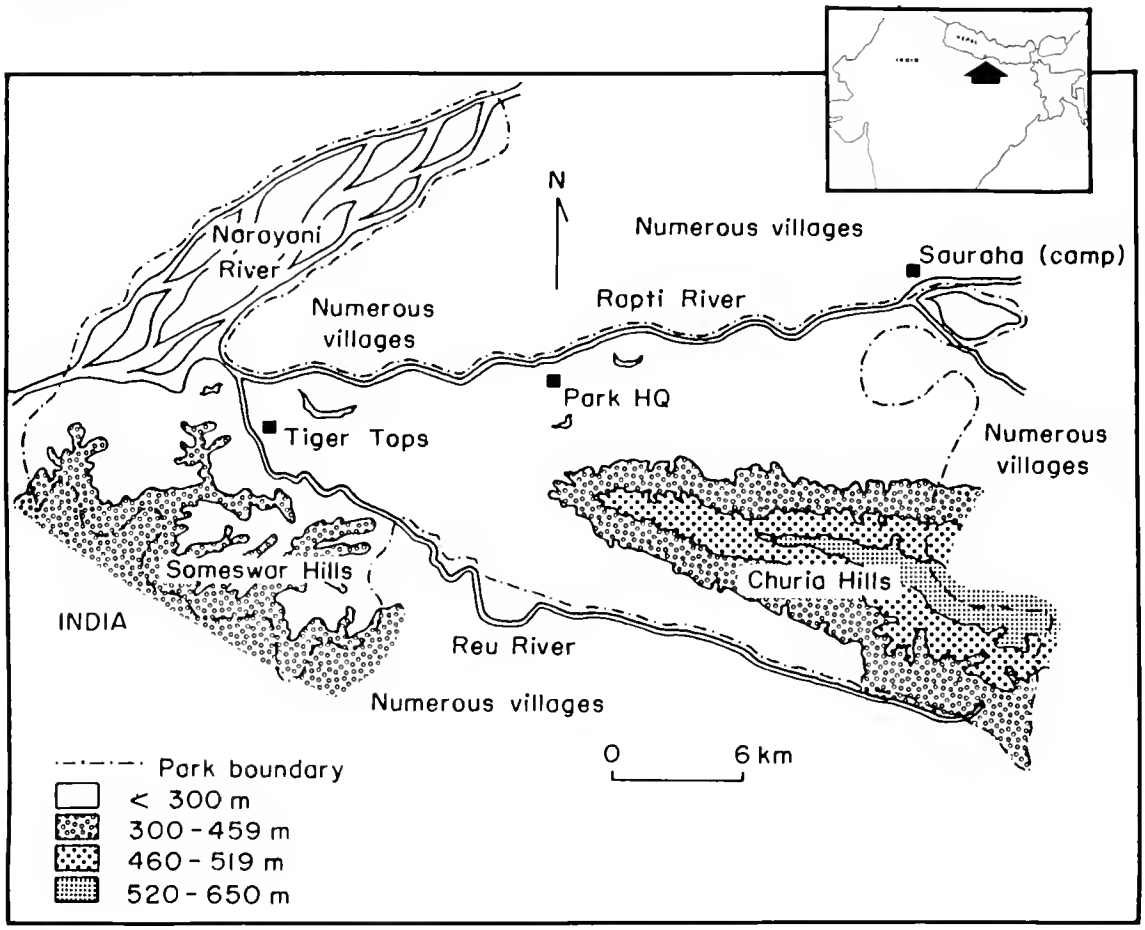


FIG. 1. Map of Royal Chitwan National Park. The Smithsonian camp was on the northeastern edge of the park and adjacent to Sauraha. After Sunquist (1988: figure 1)

Introduction

The Royal Chitwan National Park (RCNP) is a mixed grassland and forested area in south-central Nepal, centered at approximately 27°30'N 84°20'E (Fig. 1). The park lies in the Siwalik Range of low rolling hills in front of the Himalayas and along the Nepal-India border. The park is bordered to the north by the Rapti River, to the west by the Narayani River and Someswar Hills, to the south by the Reu River, and to the east by the Hasta River.

TABLE 1. A list of the known amphibians and reptiles of the Royal Chitwan National Park and neighboring areas. Habitat occurrence is noted by abbreviations within brackets and represents our observations or field notes associated with United States National Museum voucher specimens from the Chitwan area; thus, data are not available for many species and the habitats listed for a species may not encompass all habitats occupied by that species. The habitats and their abbreviations are defined in the Materials and Methods section. The type of voucher supporting each taxon's occurrence in the Chitwan area follows the habitat categories in the brackets: *, specimen in a permanent collection/museum; s, sight and/or photographic record or specimen examined in a nonpermanent collection; l, literature record.

Salientia	
Bufonidae	
Bufo melanostictus	[W/*]
Bufo stomaticus	[Ra,C,A/*]
Ranidae	
Rana crassa	[/*[1]]
Rana cyanophlyctis	[Rgh,Pfgh,Mgh/*]
Rana danieli	[Pf,F/*]
Rana limnocharis	[F/*]
Rana pierrei	[C/*]
Rana syhadrensis	[Pf,W,F,A/*]
Rana tigerina	[Pf,A/*]
Tomopterna breviceps	[/*[1,2]]
Rhacophoridae	
Polypedates maculatus	[Ra,C/*]
Crocodylia	
Crocodylidae	
Crocodylus palustris	[Rg/s[3]]
Gavialidae	
Gavialis gangeticus	[Rg/s[3]]
Testudines	
Testudinidae	
Kachuga dhongoka	[/l[4,5]]
Kachuga kachuga	[/l[4,5]]
Kachuga tecta	[/l[4,5]]
Melanochelys tricarinata	[/l[6]]
Melanochelys trijuga	[Pg/*]
Indotestudo elongata	[/*]
Trionychidae	
Aspideretes gangeticus	[/l[5]]
Chitra indica	[/l[4,5]]
Lacertilia/lizards	
Agamidae	
Calotes versicolor	[G,W,C,A/*]
Gekkonidae	
Hemidactylus brookii	[W,C/*]
Hemidactylus flaviviridis	[/l[3]]
Hemidactylus frenatus	[C/*]
Hemidactylus garnotii	[C/*]
Scincidae	
Mabuya dissimilis	[G/*]
Mabuya macularia	[W,C/*]
Scincella sikimmensis	[W,F/*]
Varanidae	
Varanus bengalensis	[/l[7]]
Varanus flavescens	[A/*]
Lacertilia/snakes	
Colubridae	
Ahaetulla nasuta	[/s[3,8]]
Amphiesma stolata	[Pgh/*]
Boiga ochracea	[/l[9]]
Boiga trigonata	[s[10]]
Chrysopelea ornata	[s[8,10]]
Dendrelaphis tristis	[W/*]
Elachistoon westermanni	[/l[9]]
Elaphe helenae	[/s[8]]
Elaphe radiata	[A/*]
Homalopsis buccata	[/s[10]]
Lycodon aulicus	[W/*]
Oligodon arnensis	[C/*]
Psammophis condanarus	[/l[9]]
Ptyas mucosus	[A/*]
Sibynophis collaris	[/l[3,11]]
Xenochrophis piscator	[Rgh/*]
Elapidae	
Bungarus caeruleus	[/l[9]]
Bungarus fasciatus	[W,A/*]
Calliophis macclellandii	[/l[11]]
Naja naja	[G/s]
Ophiophagus hannah	[/l[7]]
Leptotyphlopidae	
Ramphotyphlops braminus	[C/*]
Pythonidae	
Python molurus	[W,A/s[8,10]]
Viperidae	
Trimeresurus albolabris	[/s[8]]

Nearly a third of the park is a floodplain valley floor (150-250 m elevation; Anon., 1985) covered by a mix of grasslands, patches of hardwood forest, marshes, ponds and small streams. The Churia Hills (250-600 m), occupying the southeastern and central third of the park, are covered largely by sal forest. The climate is monsoonal with heavy rains typically from June through September, then becoming progressively drier and drought-like through April; May is a transitional month with increasingly heavier rains. Total annual precipitation averages about 230 cm. Daily temperatures generally range from 5-30° C in the cool, dry winter season (November-February) through 16-40° in the dry premonsoonal months (March-May) to 20-34° during the monsoonal rains of June-September (Bhatt, 1977; Gurung, 1983; Sunquist, 1981).

The Royal Chitwan National Park was established in the mid 1960s to provide a preserve for large mammals, particularly the Bengal tiger and the Indian rhinoceros (Sunquist, 1981; Laurie, 1982; Sunquist and Sunquist, 1988). Until the mid 1950s, the Siwalik Range and the encompassing terai area were a high malaria area and had low human density. The control of malaria-carrying mosquitoes in the 1950s allowed explosive human colonization. Fertile flood plains became pastures and farmlands, and the hill forests were cut for firewood and local building materials. The park is now totally surrounded by human settlements. The pastures and farmlands abut the park and expose it to daily incursions by an inadequately fed and fueled human population and their domestic animals.

The following observations on the species composition and natural history of the Chitwan herpetofauna derive from a report submitted to the Nepal Department of National Parks and Wildlife Conservation in 1986 (report's checklist used but uncited in Maskey and Schleich, 1992:Table 1, Schleich and Maskey, 1992:254, and Schleich, 1993). The report was designed as a field guide for park visitors and the training of park personnel; it included an identification key, which will be published separately in the Smithsonian Herpetological Information Service series.

Materials and Methods

Our visits to the park included a premonsoonal survey in April 1985 and a postmonsoonal one in November 1985. Episodic collections by the staff of the Smithsonian-Nepal Terai Ecology Project provided additional vouchers and observations. The headquarters' staff of the Royal Chitwan National Park and the guides and naturalist of Gaida Wildlife Camp maintained synoptic collections of amphibians and reptiles, especially snakes, collected in and around the park. The specimens from the preceding sources provide a primary data base for constructing the herpetofaunal list of RCNP. We also include taxa reported in the literature, although we did not confirm the specific identification of these taxa.

To document the habitat occurrence of the herpetofauna, we use the following habitat categories: Aquatic -- river (R), within and along the shore of rivers and major tributary streams; ponds and small streams (P), streams of <2 m width and temporary pools of water, modified by f, g, or h to denote location in forest/woodlands, grasslands, or human-occupied sites; marsh (M), marshes adjacent to rivers or formed in grasslands by small streams. Flood plain -- terai grasslands (G); woodlands (W), canopied forest patches on slightly elevated hummocks scattered throughout the grasslands. Hills -- sal forest (F). Human sites -- commensal (C), living on, in, or immediately adjacent to human and domestic animal buildings; agricultural areas (A), pastures, fields, and fence-row habitats. These habitat occurrences are based exclusively on our observations or field notes associated with voucher specimens. The habitat occurrences are summarized in Table 1.

The following species accounts represent those species for which we can provide new or broader based observations. Snout-vent length (SVL) in frogs, lizards, and snakes is distance (mm) from tip of the

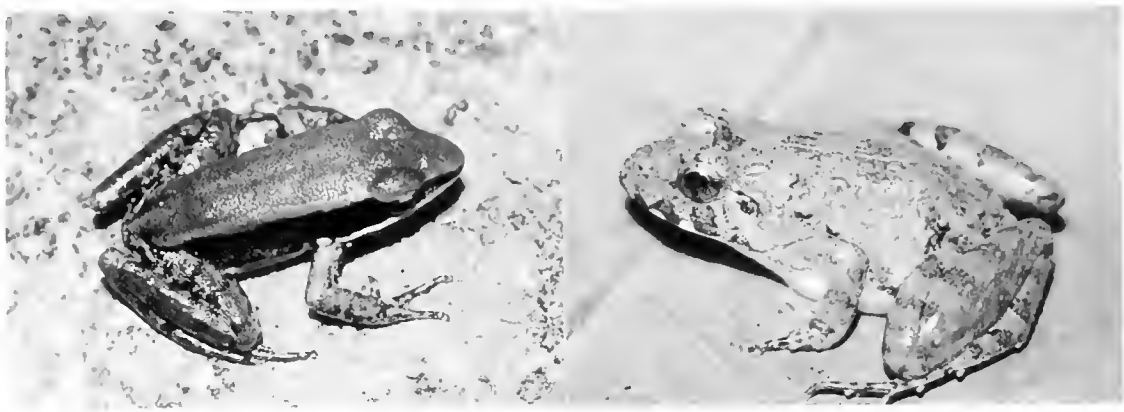


FIG. 2. The Chitwan frogs *Rana danieli* (left; USNM 266838) and *Rana syhadrensis* (right; USNM 266878).

snout to the cloacal opening; carapace length (CL) in turtles is the straight-line distance of the shell on the midline. Body weights (g) were taken with a Pesola scale. Statistics were performed by a PC version of Statistix 4.0. A priori statistical significance was set at $\alpha = 0.05$; means are followed by ± one standard deviation.

Species Accounts

The herpetofauna of the Chitwan area consists of 11 amphibians and 44 reptiles (Table 1). At this time, none of the amphibians or terrestrial reptiles are considered threatened, and only the two crocodilians are officially recognized as endangered or threatened species.

Estimates of abundance are desirable, but our field work did not coincide with the seasons of likely greatest activity for most species. Premonsoonal conditions are drought-like, and these conditions suppress the activity of most species except for a few lizards. The postmonsoonal visit occurred as day and night time temperatures were declining, and over the two week visit, lizards slowly disappeared. Nonetheless, our observations show the herpetofauna of RCNP to be rich and diverse.

Frogs

Bufo. Neither of the two toad species (*B. melanostictus, B. stomaticus*) were common. We captured nine individuals, only one of which was a *B. melanostictus* (32.1 mm SVL, 3 g). This latter individual was discovered (November) beneath a decomposing log in the forest. All *B. stomaticus* occurred in human-modified habitats. Recent metamorphs (9.4 ± 0.9 mm SVL, $n = 5$) were captured (April) on a mud bank of the Rapti River.

Rana cyanophlyctis. The skittering frogs were the most abundant of the Chitwan postmonsoonal amphibians; none were seen in April. They occurred in a wide variety of flood plain habitats from riverside to the small ponds and streams in the terai grassland and farmlands. In late afternoon and at night, numerous individuals sat at the water's edge on the bank and in the water. Human or animal movement along the shore would send the frogs skittering outward on the river in a semicircular path, some frogs landing behind the disturbance, others ahead and then repeating the escape or avoidance reaction.

Of the *R. cyanophlyctis* captured ($n = 27$), only a single female (55.2 mm SVL) was sexually mature. The immature frogs ranged 25.9 - 50.2 mm SVL; presumably they all represent young of the year, although derived from egg clutches laid at different times during the monsoon rains. This sample yields a regression, Mass = -9.0814 + 0.4184 SVL ($r = 0.90$).

Rana danieli(Fig. 2). Three juveniles (31 - 38 mm SVL) were found in the sal forest. The premonsoonal individual occurred beneath a log (with a *R. syhadrensis)* beside a small stream; the two postmonsoonal frogs were beneath logs on the forest floor 10 m or more from a small stream. These individuals represent the first *R. danieli* from Nepal and are a significant westward range extension (700 km) from the Indian type locality in the Khasi Hills, Assam (Pillai and Chanda, 1977).

Rana limnocharis. Three species (*R. limnocharis, pierrei, syhadrensis*) of the *limnocharis* complex occur in Chitwan. Our observations show *R. limnocharis* and *R. syhadrensis* to be forest-floor species and syntopic in sal forest. Two adult male *R. limnocharis* (36.2 & 44.3 mm SVL, 3.0 & 8.5 g) were captured (November) beneath logs; a few other individuals were seen in the sparse ground litter of the sal forest.

Rana pierrei. A single immature male (46.0 mm SVL, 10.8 g) was discovered (November) sitting in the "lawn" of the S1 camp at night.

Rana syhadrensis(Fig. 2). Of the Chitwan frogs, *R. syhadrensis* was abundant during both the pre- and postmonsoonal surveys. Most individuals captured were juveniles (41 of 42) with SVL <31 mm (18 - 30 mm SVL). Two recent metamorphs (18 mm SVL) were captured (April) in the leaf litter along the bank of a small sal forest stream. Most individuals were captured (April & November) beneath forest-floor litter or in tree buttresses.

Rana tigerina. Two individuals, both immature (44.7 & 63.3 mm SVL), were found in or near rhino wallows within riverine forest.

Tomopterna breviceps. We saw no individuals of this species during our pre- and postmonsoonal sampling. Their absence at these time supports Schleich and Maskey's comment (1992) that *Tomopterna* are active on the surface only during the breeding season at the beginning of the monsoon.

Turtles

Turtles are uncommonly seen in the park's waters, grassland, and forest. When they are found, they become food for local inhabitants. Our turtle sightings and vouchers derive mainly from shells on kitchen middens or shells nailed to walls of local tea shops.

Indotestudo elongata. Frazier (1992) showed that this species is the only tortoise confirmed to occur in Nepal. Earlier reports of *Geochelone elegans* and *Testudo horsfieldii* are incorrect, usually misidentifications. We saw in several tea houses tortoise shells nailed to the wall as decoration. Our voucher is a shell salvaged from a dog at the Smithsonian camp. Its anterior end had been sawed off, attesting to tortoises as local food items.

Melanochelys trijuga. An adult female (215 mm CL; 1.2 kg) was found (Oct.) in the grass bordering a rhino wallow next to a patch of riverine forest. She was judged to be 7 yr old and had grown an average of 20.4 mm/yr (PL) since hatching. Dinerstein et al. (1988) provided additional information on this female and on the occurrence of this species in Nepal..

Lizards

Calotes versicolor. The garden lizard was the most common of Chitwan lizards, and the most readily observed reptile, owing to their use of elevated forage and basking sites on shrubs, trees, fence posts, etc. Adult males (87.1 ± 6.8 mm SVL, 71.3 - 97.0 mm. n = 16; 22.5 ± 5.9 g, 8.8 - 30.3 g, n = 16) averaged larger than adult females (77.9 ± 8.5 mm SVL, 66.7 - 87.0 mm, n = 7; 15.2 ± 6.2 g, 9.1 - 22.4, n = 5). Adults (21:1, adults:juveniles) predominated in the premonsoonal sample and juveniles (2:4) in the postmonsoonal one. The presence of gravid females in the premonsoon period and juveniles in the postmonsoonal suggest that most egg-laying occurs at the beginning of the monsoon in Chitwan. The postmonsoonal juveniles averaged 53.7 ± 25 mm SVL (50.0 - 55.7 mm) and presumably represent the size of the

FIG. 3. A possible defense mechanism; neural spines projecting through the median row of dorsal scales in a *Bungarus fasciatus* (USNM 267012) from the Royal Chitwan National Park.

season's cohort at the end of its first growing season.

Body temperatures of adults basking in the mid morning (0800-0950 hr; April) averaged 35.3 ± 1.64° C (33.4 - 38.2, n = 11) compared to an average ambient temperature (in shade) of 33.9 ± 0.86 (30.4 - 32.5, n = 5). The body temperature of a single juvenile, captured in the shade, was 32.2° C, identical to ambient temperature

Hemidactylus. We observed three of the four Chitwan geckos (Table 1). The rarity of *H. flaviviridis*, *H. frenatus*, and *H. garnotii* and their exclusive commensal occurrence suggest that these three species are exotics. In contrast, *H. brookii* is abundant both on human-made structures and in some forested sites. We provided a brief review (Mitchell and Zug, 1988) of *H. brookii* biology around the Smithsonian camp.

Female *H. brookii* mature at 43 mm SVL, males at 42 mm. They are active at night in the forest and on buildings. During the day, forest individuals hide beneath the bark of dead trees, in litter filled tree buttresses, and beneath logs. Four *H. frenatus* (37 - 53 mm SVL) and two *H. garnotii* (52 - 55 mm SVL) were captured on the camp building during the *H. brookii* survey; none were seen in the adjacent forest.

Scincella sikimmensis. Three adult *Scincella* (31 - 35 mm SVL) were found beneath logs or litter in the riverine and sal forest. The two *Mabuya* (*M. dissimilis, M. macularia*) appear to be more open-habitat denizens, e.g., at the forest edge or along trails in the grassland, although a *M. macularis* was found beneath a log with a *S. sikimmensis*.

Snakes

Snakes suffer the same level of persecution in the Chitwan area as in most rural communities, i.e., death when seen. The local population is primarily Hindu, although some of the original Chitwan residents, Tharu, remain. There is no evidence of either culture practicing tolerance of snakes, and the Tharu are

reported to eat pythons (but no other snake species) and varanid lizards.

Amphiesma stolata. Two males (387 & 440 mm SVL, 19 & 22 g) were found dead on unpaved streets in Sauraha in April. Villagers said that smaller one was found in a house and killed and the other one in a drainage ditch. Children had killed both of them and tossed them on the street.

Lycodon aulicus. A female (181 mm SVL, 2 g) was discovered in soil beneath a rotting stump in November. Possibly, she was preparing to hibernate.

Oligodon arnensis. An adult male (662 mm SVL, 17.1 g) was captured at 2100 hr (April) crossing a path in the Smithsonian camp. To avoid capture, it flattened its head by the lateral extension of the proximal ends of the jaws and struck repeatedly with the mouth open. The strike behavior was a bluff, because it did not bite even though its mouth contacted a plastic bag several times. Also it coiled several times in a three minute interval; the head was flattened and held close to the body, and it struck laterally several time from this posture. Its tail was partially coiled but never in the defensive posture described for other *Oligodon* species (Greene, 1973). Daniel (1983) reported body inflation and head flattening in this species. This individual inflated its body only slightly. Its body temperature was 27.8° C, compared to 29.2° C ambient air temperature, suggesting that it had emerged recently from its daytime retreat.

Bungarus fasciatus. An adult female (1570 mm SVL) was killed in the Smithsonian camp (April) one evening. Presumably during the human attack, she voluntarily extruded 36 neural spines through the vertebral scales (Fig. 3). Neural spines are exposed in this individual at vertebral scale 11 (counting posteriorly from the parietal scale), 136-139, 142-157, 172-178, 181, 184-189, and 190-191. Several spines protrude 3 mm above the scale surface, and in other instances, vertebral scales are slit longitudinally but the neural spines do not project presently above or through the scale.

A. H. Savitzky (personal communication & in lecture) called our attention to this novel antipredator mechanism. He has also discovered that several other species of kraits show this specialized behavior and have a specialized epaxial musculature and suture zones in the vertebral scales to effect the extrusion of the sharp neural spines when grasped by a predator.

Naja naja. A single individual was seen at midday (1300 hr) basking (approx. 1.2 m above the ground) on a large clump of elephant grass beside a game trail in the grassland.

Comments

The known Chitwan herpetofauna consists predominantly (>75%) of widespread Indian-Oriental (as defined by Leviton and Swan, 1962) taxa. The exceptions are either Himalayan or Indochinese-Himalayan taxa. *Rana danieli, R. pierri, R. syhadrensis,* and *Scincella sikimmensis* represent the Himalayan element and they are predominantly low-to moderate elevation species confined to the southern face of the Himalayas and its foothills. *Indotestudo, Elachistoon, Boiga ochracea,* and *Trimeresurus albolabris* share a similar Himalayan distribution as the preceding group, but have distributions extending into western Indochina.

One additional frog, *Microhyla ornata,* is a likely resident of Chitwan. It is reported higher in the Rapti drainage system (Nahoe and Ouboter, 1987), as well as being widespread in northern India. We excluded it from the present list, because we found no literature or specimen voucher placing it adjacent or within the RCNP.

In summary, the Chitwan herpetofauna consists of 11 frogs and 44 reptiles. With a few exceptions, the taxa represent a subset of the herpetofauna of northern India. As human population growth continues, the value of RCNP and its resident animals and plants will increase as an essential biological reserve.

Acknowledgments

Our field work was supported by the Smithsonian Research Opportunity fund. The staff of the Smithsonian-Nepal Terai Ecology Project [formerly the Smithsonian "Tiger Camp"] provided daily assistance. We especially wish to acknowledge the support and encouragement of Eric Dinerstein, director of the Terai Project, and Chris Wemmer of the Smithsonian's National Zoological Park Conservation & Research Center. The Nepal Department of National Parks and Wildlife Conservation encouraged and provided permits for our field surveys and for the export of voucher specimens. The original report submitted to the national parks' department was reviewed and improved by A. Dubois, R. F. Inger, T. K. Shrestra. The staffs of the RCNP headquarters and Gaida Wildlife Camp allowed us to examine their synoptic collections. We thank all of the preceding organizations and individuals for their support and assistance.

Appendix

Notes to Table 1

1. MNHN specimens, Dubois (1974: appendix).

2. Schleich and Maskey (1992) state that two species of *Tomopterna* are known from Chitwan, but they do not identify either species.

3. Schleich and Maskey (1992) report voucher specimens.

4. Iverson (1992).

5. Moll (1984) lists 14 turtle species from the Gandak River south of the Gandak Dam to Bettiah. Since the Gandak R. is a continuation of the Narayni R. in India, some of these species (*Geoclemys hamiltoni, Hardella thurji, Kachuga smithi, K. tentoria, Morenia petersi, Lissemys punctata, Aspideretes hurum*) might also occur in Chitwan.

6. Schleich and Maskey (1992) report a voucher specimen. Moll and Vijaya (1986) report a Chitwan occurrence based on a photograph.

7. Gurung (1989); we have excluded other species from Gurung's list because they appear to be misidentifications, e.g., *Enhydris enhydris, Rhapdophis subminata.*

8. Royal Chitwan National Park headquarter's synoptic collection.

9. Kramer (1977).

10. Gaida Wildlife Camp synoptic collection.

11. Swan and Leviton (1968).

Literature Cited

ANON. 1985. Royal Chitwan National Park, Nepal. [printed handout with map] Dept. Natl. Parks & Wildl. Conserv., Kathmandu

BHATT, D. D. 1977. Natural History and Economic Botany of Nepal. Orient Langmann Ltd., New Delhi.

DANIEL, J. C. 1983. The Book of Indian Reptiles. Bombay Natural History Society, Bombay, India.

DINERSTEIN, E., G. R. ZUG, AND J. C. MITCHELL. 1988. Notes on the biology of *Melanochelys* (Reptilia, Testudines, Emydidae) in the terai of Nepal. Journal of the Bombay Natural History Society (1987) 84(3):687-688.

DUBOIS, A. 1974. Liste commentée d'amphibiens récolté au Népal. Bulletin du Museum national d'Historie naturelle, 3rd sér., Zool. (213):341-411.

DUBOIS, A. 1975. Un noveau complexe d'espèces jumelles distinguée par le chan: les grenouilles du Népal voisines de *Rana limnocharis* Boie (amphibiens, anoures). Compte Rendu des Seances de l'Academie Sciences, Paris, sér. D 281:1717-1720.

FRAZIER, J. 1992. The land tortoise in Nepal: a review. Journal of the Bombay Natural History Society 89(1):45-54.

GREENE, H. W. 1973. Defensive tail display by snakes and amphisbaenians. Journal of Herpetology 7:143-161.

GURUNG, K. K. 1983. Heart of the Jungle. The Wildlife of Chitwan, Nepal. Andre Duetsch Ltd., London.

IVERSON, J. B. 1992. A Revised Checklist with Distributional Maps of the Turtles of the World. Privately printed, Richmond, Indiana.

KRAMER, E. 1977. Zur Schlangenfauna Nepals. Revue Suisse Zoologie. 84(3):721-761.

LAURIE, A. 1982. Behavioural ecology of the greater one-horned rhinoceros (*Rhinoceros unicornis*). Journal of Zoology, London 196:307-341.

MASKEY, T. AND H. H. SCHLEICH. 1992. Untersuchungen und Schutzmassnahmen zum Gangesgavial in Südnepal. Natur und Museum 122(8):258-267.

MITCHELL, J. C. AND G. R. ZUG. 1988. Ecological observations on the gecko *Hemidactylus brookii* in Nepal. Amphibia-Reptilia 9:405-413.

MOLL, E. O. 1984. Freshwater turtles in India: their status, conservation and management. Hamadryad 9(3):9-17.

MOLL, E. E. [SIC], AND J. VIJAYA. 1986. Distributional records for some Indian turtles. Journal of the Bombay Natural History Society 83:57-62.

NAHOE, L. M. R., AND P. E. OUBOTER. 1987. The distribution of reptiles and amphibians in the Annapurna-Dhaulagiri region (Nepal). Zoologische Verhandelingen (240):1-105.

PILLAI, R. S. AND S. K. CHANDRA. 1977. Two new species of frogs (Ranidae) from Khasi Hills, India. Journal of the Bombay Natural History Society 74(1):136-140.

SCHLEICH, H. H. 1993. Contribution to the systematics and to a bibliography of the amphibians and reptiles from Nepal. Journal of the Nepal Research Centre 9:141-168.

SCHLEICH, H. H., AND T. M. MASKEY. 1992. Der Chitwan-Nationalpark im Süden Nepals. Natur und Museum 122(8):248-257.

SUNQUIST, F., AND M. SUNQUIST. 1988. Tiger Moon. University of Chicago Press, Chicago.

SUNQUIST, M. E. 1981. The social organization of tigers (*Panthera tigris*) in Royal Chitwan National Park, Nepal. Smithsonian Contributions to Zoology (336):1-94.

SWAN, L. W. AND A. E. LEVITON. 1962. The herpetology of Nepal: A history, check list, and zoogeographical analysis. Proceedings of the California Academy of Sciences 32:103-147.

June 1995 *Asiatic Herpetological Research* Vol. 6, pp. 181-185

GUIDELINES FOR MANUSCRIPT PREPARATION AND SUBMISSION

Summary

Manuscripts must:

1) be written in English.
2) be of letter quality (laser printed or typewritten on bond paper).
3) include camera ready figures (if any).
4) include complete and accurate literature citations.
5) include complete and accurate localities with latitude and longitude.
6) include a camera ready map illustrating regions discussed (when applicable).

Manuscripts failing to meet these criteria will be returned for correction.

Purpose and Content

Asiatic Herpetological Research publishes articles concerning but not limited to Asian herpetology. The editors encourage publications from all countries in an attempt to create an open forum for the discussion of Asian herpetological research.

Articles should be in standard scientific format and style. The following sections should be included:

Title. The title should reflect the general content of the article in as few words as possible. A poor title may cause readers to read no further.

Names and Addresses. The names and addresses of all authors must be complete enough to allow postal correspondence.

Abstract. The abstract should briefly summarize the nature of the research, its results, and the main conclusions. Abstracts should be less than 300 words.

Key Words. Key words provide an index for the filing of articles. Key words provide the following information (when applicable): 1) Taxonomy (e.g. Reptilia, Squamata, Gekkonidae, *Gekko gecko*). 2) Geography (e.g. China, Thailand). 3) Subject (e.g. taxonomic validity, ecology, biogeography). The order of taxonomy, geography, and subject should be observed.

Text. Manuscripts must be in English and spelling must be correct and consistent. Use *Webster's New International Dictionary* for reference. For clarity, use active voice whenever possible. For example, the following sentences in active voice are preferable to those in passive voice.

Active voice:

"Lizards were extremely common on the site." and "I examined three female snakes."

Passive voice:

"Lizards were observed to be extremely common on the site." and "Three female snakes were examined."

Abbreviation. Do not abbreviate unless the full phrase has already appeared. Scientific names may be abbreviated only if they have appeared fully in the same paragraph. Never begin a sentence with an abbreviation of a scientific name.

Standard Format

Manuscripts following standard format should include introduction, methods, results, and discussion sections. While other formats are acceptable, the editors encourage the use of standard format. **Please do not type in all capital letters** (headings, Fig., Table, and authors names in the Literature Cited section).

Introduction. The introduction typically states the significance of the topic and reviews prior research.

Methods. This section should clearly state where, when, and how research was carried out. Include sample sizes. Protocols designed by other investigators must be properly cited. Research materials and their manufacturers should be listed. The reader must be able to replicate the methods of the author(s).

Results. This section states the results and their significance to the investigation. Figures and tables may be used to clarify, but not to replace, results statements in the text. Statistics should be used when applicable. Large amounts of data should be avoided, or included as an appendix at the end of the article.

Discussion. The discussion is a synthesis of the introduction and the results. No new information should be discussed unless it was presented in the results section. New findings should be discussed in relation to prior research. The author(s) should feel free to present several possible interpretations of the results. The editors particularly encourage suggestions of future research in Asian herpetology.

Section Headings

Articles will be published using three section heading styles. Level 1: text is bold and centered Level 2: text is italic and centered. Level 3: text is italic and at the beginning of the first paragraph. Authors should take this style into account when writing manuscripts.

Statistics

Statistics must be accompanied by sample sizes, significance levels, and the names of any tests. Investigators should pay careful attention to independence and applicability of tests, and randomness of samples. One of the most frequent examples of nonindependence is the use of multiple, paired t-tests instead of analysis of variance (ANOVA). **In general, multiple tests on the same data set are not valid.** Descriptive statistics are in many cases more appropriate than inferential statistics.

References

Accurate and standard references are a crucial part of any article. This is especially important when dealing with publications from many different countries. The reader must be able to precisely identify any literature cited. References in the text must be checked for consistency with references in the literature cited section. **All references cited in the text must be in the literature cited section. The literature cited section may not contain any references not mentioned in the text.** Articles containing inaccurate or inconsistent literature citations will be returned for correction.

References in Text

1) References to articles by one or two authors must include both surnames in the order they appear in the original publication. References to articles by more than two authors must include the first author's surname, followed by "et al."
2) The year of article follows the authors, separated only by a space.

3) References with the same author and year are distinguished by the lower case characters "a, b, c, ..."
4) References cited in text are listed in **alphabetical order by first author.**

For example, "My results also incorporate literature records (Marx et al., 1982; Marx and Rabb, 1972; Mertens, 1930; Pope, 1929; Wall, 1909, 1910a, 1910b, 1910c)."

References in Literature Cited

1) References must include all authors, in the order that they appear in the original publication; **"et al." is never used in a literature cited section.**
2) The first author is listed surname first, initial(s) last. All other authors are listed initial(s) first, surname last.
3) References with the same author and year are distinguished by the lower case characters, "a, b, c, ..."
4) References cited are listed in alphabetical order by first author.
5) **Names of journals are not abbreviated.**

See below for examples:

Journal article.

Dial, B. E. 1987. Energetics and performance during nest emergence and the hatchling frenzy in loggerhead sea turtles (*Caretta caretta*). Herpetologica 43(3):307-315.

Journal article from a journal that uses year instead of volume.

Gatten, R. E. Jr. 1974. Effect of nutritional state on the preferred body temperatures of turtles. Copeia 1974(4):912-917.

Journal article, title translated, article not in English.

Ananjeva, N. B. 1986. [On the validity of *Megalochilus mystaceus* (Pallas, 1776)]. Proceedings of the Zoological Institute, Leningrad 157:4-13. (In Russian).

Note that for *Acta Herpetologica Sinica,* the year must precede the volume number. This is to distinguish between the old and new series, and between 1982-1987, Vols.1-6 (new series) and 1988 with no volume number, numbers 1 and 2 (new series).

Cai, M., J. Zhang, and D. Lin. 1985. [Preliminary observation on the embryonic development of *Hynobius chinensis* Guenther]. Acta Herpetologica Sinica 1985, 4(2):177-180. (In Chinese).

Book.

Pratt, A. E. 1892. To the snows of Tibet through China. Longmans, Green, and Co., London. 268 pp.

Article in book.

Huey, R. B. 1982. Temperature, physiology, and the ecology of reptiles. Pp. 25-91. *In* C. Gans and F. H. Pough (eds.), Biology of the Reptilia, Vol. 12, Physiological Ecology. Academic Press, New York.

Government publication.

United States Environmental Data Service. 1968. Climatic Atlas of the United States. Environmental Data Service, Washington, D. C.

Abstract of oral presentation

Arnold, S. J. 1982. Are scale counts used in snake systematics heritable? SSAR/HL Annual Meeting. Raleigh, North Carolina. [Abstr].

Thesis or dissertation.

Moody, S. 1980. Phylogenetic and historical biogeographical relationships of the genera in the Agamidae (Reptilia: Lacertilia). Ph.D. Thesis. University of Michigan. 373 pp.

Anonymous, undated.

Anonymous. Undated. Turpan brochure. Promotion Department of the National Tourism Administration of the People's Republic of China, China Travel and Tourism Press, Turpan, Xinjiang Uygur Autonomous Region, China.

Figures and Tables

Figures and tables should be referenced in order in the text. Each table should be typewritten, double spaced on a separate sheet. See below for instructions for figures.

Figures

Each figure illustration (line art or photograph) submitted must be "camera ready" for publication with **no modifications** necessary other than reduction. Do not write on figure; do not mount more than one figure to a sheet. AHR cannot be responsible for redrawing, touching up, or otherwise modifying figure illustrations for authors. In addition, figure illustrations submitted must be:

1) of publication quality with **typeset text**.

2) mounted on a separate 21.5 x 28 cm (8.5 x 11 inch) sheet with figure number on back.

3) on a separate sheet from figure legend.

Times Roman typeface is preferred. Do not submit figures with poor type or handwriting on the face of the figure. In order to avoid wasted effort, please follow the above instructions carefully. Please note: **AHR will not alter or lay out figures for publication**. Any figure requiring modification will be returned, and may cause significant delay in publication.

Figure Legends

Figure legends should be typed on a separate sheet. Legends should explain the figure without reference to the text. A figure and legend should make sense if separated from the rest of the article. For example:

FIG. 2. Lateral view of live *Psammodynastes pulverulentus* holding a prey lizard (*Anolis carolinensis*). Note buccal tissue surrounding the enlarged anterior maxillary and dentary teeth of the snake.

Copyright

Asiatic Herpetological Research reserves the copyrights to all material published therein, except that excluded by permission of the editors. Any material under a prior copyright submitted to *Asiatic Herpetological Research* must be accompanied by the written consent of the copyright holder.

Submission of Manuscripts

Authors should submit letter quality, double spaced, single-sided manuscripts both in English and in the original language on 21.5 x 28 cm (8.5 x 11 inch) white bond paper. If possible, include a computer diskette containing the manuscript. Macintosh diskettes with Word Perfect, Microsoft Word, Macwrite, Write Now, or text files, or 3.5" MS/PC DOS diskettes with Word Perfect, Microsoft Word, RTF, or ASCII files are preferable. Computerized manuscripts should include italic, bold, and centered text only. Additional formatting is not necessary or desirable. Please indicate author, computer, file format, and file name in writing on the disk.

Manuscripts will be reviewed. The editors will attempt to choose reviewers whose research knowledge most closely matches the content of the manuscript.

Asiatic Herpetological Research requests $25 US per printed page from authors with funds available. Please indicate if funds are available.

Send manuscripts by **postal mail** to Editors, Asiatic Herpetological Research, Museum of Vertebrate Zoology, 3101 Valley Life Sciences Building, University of California, Berkeley CA 94720-3160 USA.

Send manuscripts by Internet **email** to *asiaherp@uclink2.berkeley.edu* as a *MIME* attachment with *binhex* or *uuencode* encoding. If you use email to submit, an editor will acknowledge receipt of your manuscript. Please note that it is possible for your email message to disappear on the Internet without being delivered. If your message is returned, or not acknowledged, you may want to try again or to send your manuscript by postal mail.

ISSN 1051-3825

CONTENTS

(Continued on inside of back cover)