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Cover: *Eublepharus turkmenicus* from vicinity of Temen Spring, 2.5 km west of Danata (39° 07' N 55° 08' E), Krasnovodsk Region, Turkmenistan. Photo by J. Robert Macey.

## *Coluber atayevi* Sp. Nov. (Ophidia, Colubridae) from the Kopet-Dag Mountains of Turkmenistan

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**Abstract.** -An examination snakes, formerly considered to be *Coluber najadum*, from the Kopet-Dag Mountains of Turkmenistan, leads us to believe that this population represents an undescribed species. We here describe this population as *Coluber atayevi*.

**Key words:** Reptilia, Ophidia, Colubridae, *Coluber*, Turkmenistan, systematics.

### Introduction

During field work in the Kopet-Dag Mountains, Turkmenistan, we observed and analyzed many individuals of *Coluber najadum* under natural conditions. Comparison of these individuals with Caucasian material led to the conclusion that the Kopet-Dag snakes belong to a previously unrecognized species. This conclusion is supported by significant morphological divergence from the other representatives of the *najadum-rubriceps* complex.

### Methods

We analyzed 10 specimens of *Coluber najadum* (Eichwald) from various areas of the Caucasian Isthmus and 10 specimens from the Kopet-Dag belonging to the new taxon. Morphometric data were compared with the available information in the literature about *Coluber najadum* in its natural habitat throughout its range (Terentjev and Chernov, 1949; Bannikov et al., 1977) and for separate regions (Ananjeva and Orlov, 1977; Muzskheleshvili, 1970).

The following features and indices have been used: 1) L. = length of body, mm; 2) L. cd. = length of tail, mm; 3) Sq.= number of scales around body; 4) Ventr.= number of ventral scutes; 5) S. cd.= number of subcaudal scutes; 6) Lab.= number of upper labials; 7) Sublab.=

number and size of sublabials; 8) form of mandibular scutes; 9) shape of the head; 10) distribution of scutes on throat; 11) L/L. cd.= body length/tail length; 12) Pr. oc.= number of preorbital scales; 13) Post. oc.= number of postorbital scales; 14) Temp.= number of temporal scales; 15) A.= form of anal scutes.

For numerical features and indices, we have calculated the mean ( $\bar{x}$ ), mean error ( $m$ ), mean square deviation ( $S^2$ ), using the formulas for small samples (Lakin, 1980)

When describing the biotopes, we determined the plant species according to Nitikin and Geldykhonov (1988); the general vegetation type follows Korovin (1934), with some corrections.

### History of the Study of "*Coluber najadum*" in the Kopet-Dag

*Zamenis dahli* Fitzinger was first mentioned from the environs of Sulukli Spring and the Kuchan road by Varentzov (1894). Nikolskij (1905, 1916) observed this species in the vicinity of Ashkhabad. On the basis of these records, *Coluber najadum* (Eichwald) was included in the list of the Turkmenistan herpetofauna (Chernov, 1934; Terentjev and Chernov, 1949; Bogdanov, 1962). All previous collections from the territory of Turkmenistan and the neighboring parts of Iran have been analyzed by Ananjeva and Orlov (1977). They added the localities of

TABLE 1. Morphometry of *Coluber atayevi* paratypes in the collection of the Caucasian Reserve, Sochi, Russia (see text for abbreviations).

No.	L.	L.cd.	Sq.	L./L.cd	Ventr.	S.cd.	Lab.	Preoc.	Postoc.	Temp.	Sublab.
421	533	201	19	2.65	208	102	8/8	1/1	2/2	2+2/2+1	10/11
422	455	143	19	3.18	214	90	8/8	1/1	2/2	2+1/2+2	10/10
423	381	141	19	2.70	210	106	8/8	1/1	2/2	2+1/2+3	10/10
424	392	122	19	3.21	190	90	8/8	1/1	2/2	2+1/1+2	9/9
425	246	78	19	3.15	207	105	8/8	1/1	2/2	2+1/2+3	10/10
426	242	73	19	3.31	209	102	8/8	1/1	2/2	2+1/2+1	10/10
427	238	74	19	3.22	197	96	8/8	1/1	2/2	2+2/2+1	9/9
428	242	73	19	3.31	204	101	8/8	1/1	2/2	2+3/1+3	10/10
429	250	79	19	3.16	203	100	8/8	1/1	2/2	2+1/2+3	10/10

Firuz settlement and, tentatively, Dzhebel Station to the distribution of *Coluber najadum*. The last locality was doubtful (Shcherbak and Golubev, 1981; Ataev 1985). Rustamov and Shammakov (1979) and Shcherbak and Golubev (1981) mentioned it from Dushak Mountain. Recent localities include the Babazon region in the Kopet-Dag Reserve (Shcherbak, et al., 1986), Saivan and Imarat villages and Kara-Kala settlement (Ataev, et al., 1991). During almost a century, less than 20 representatives of this taxon have been recorded, half of them in recent years (Ataev et al., 1991).

The small amount of preserved material, part of which had been lost (Ananjeva and Orlov, 1977), dissociation of time of collection and place of storage of specimens, led to the opinion that *Coluber najadum* was the taxon distributed in the Kopet-Dag. This form was included in the nominate form, and had never even been considered as a separate subspecies (Bannikov et al., 1977). It is interesting to note that Bannikov et al. (1977) included *C. n. rubriceps* Mertens (now recognized as a distinct species [Engelman et al., 1986; Rehak, 1986; Ananjeva et al., 1988]) in the synonymy of *Coluber najadum*.

*Coluber atayevi* Tuniyev and Shammakov, sp. nov.

*Zamenis dahli*: Varentzov, 1894:27; Nikolskij, 1905:233; 1916:92.

*Coluber najadum*: Chernov, 1934:273; Terentjev and Chernov, 1949:240-241 (part); Bogdanov, 1962:167; Bannikov et al., 1977:262-263 (part); Ananjeva and Orlov, 1977:14-16; Rustamov and Shammakov, 1979:144; Shcherbak and Golubev, 1981:70-72; Ataev, 1985:242-243; Shcherbak et al., 1986:98-100; Latifi, 1991:102-103.

We name the new species in honor of the famous Turkmen herpetologist, Chary Ataevich Ataev, who studies reptiles of the mountains of Turkmenistan.

**Holotype:** Collection of the Caucasian Reserve, Sochi, Russia, No. 420, adult male, environs of Saivan Village, Saivan-Nokhur Plateau, western Kopet-Dag, Bakharden Region, Turkmenistan, 12 May 1991, collected by B. S. Tuniyev (Fig. 1).

**Paratypes:** Twenty two specimens. Collection of the Caucasian Reserve, Sochi, Nos. 421-429, 4 adults and 5 juveniles, same data as holotype, collected by C. A. Ataev and B. S. Tuniyev; California Academy of Sciences (CAS) Nos. 182948-182950, same locality as holotype, May 1990; CAS 185185-185194, 5 adults and 13 juveniles, Elev. 1200-1300 m, 38° 30' N, 56° 47' E, 2 km SE (airline) of Saivan, Ashgabad Region Turkmenistan, 21 May 1992, collected by B. S. Tuniyev, S. M. Shammakov, N. B. Ananjeva, T. J. Papenfuss and R. Macey (Plate 1).



Adult *Coluber atayevi*.



Type locality of *Coiluber atayevi*, environs of Saivan Village, Saivan-Nokhur Plateau, western Kopet-Dag, Bakharden Region, Turkmenistan.



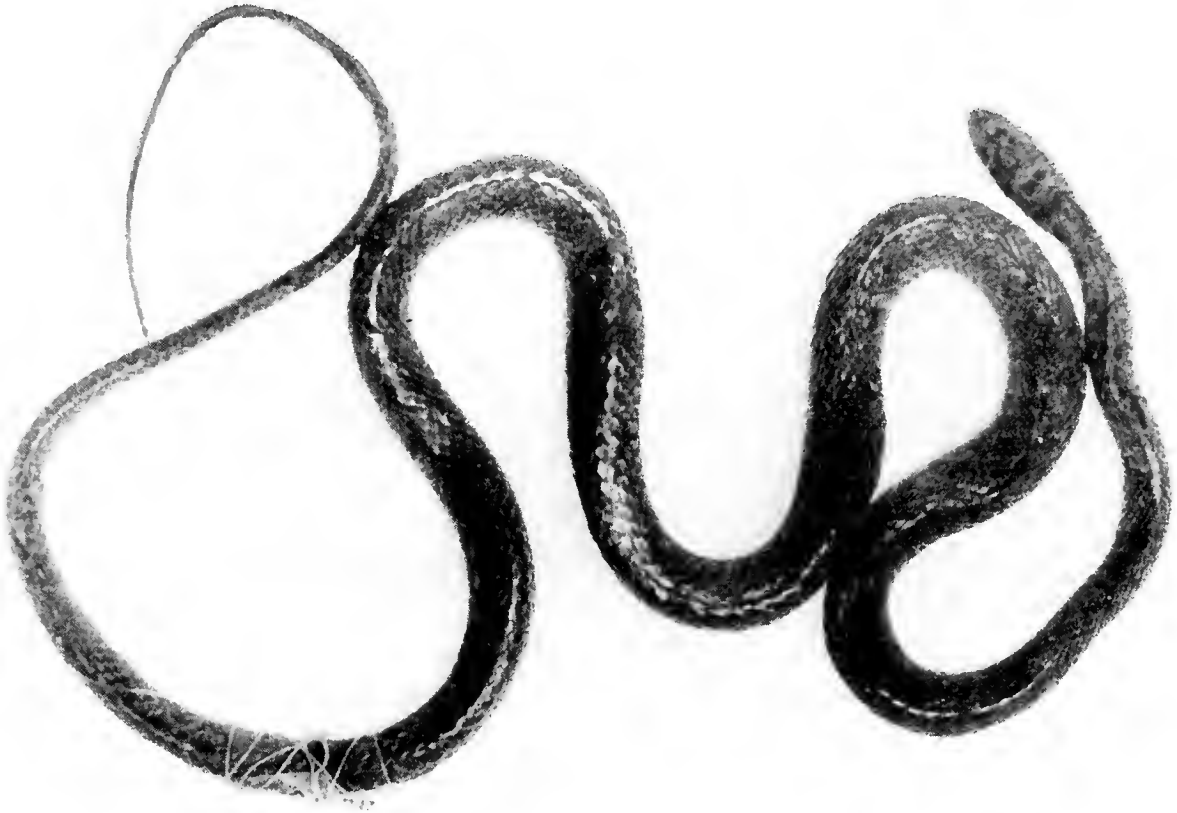


FIG. 1. Holotype of *Coluber atayevi* sp. nov. (Collection of the Caucasian Reserve no. 420).

**Description of holotype:** Snout-vent length 500 mm, tail 172 mm; head length 19.6 mm, head width 8.4 mm, head height 6.2 mm. Head smoothly rounded, narrow, covered with large regular scutes; 8 upper labials, 5th upper labial touches lower postorbital and large lower temporal with its extended upper posterior side; 9 lower labials, 6th largest, last two pairs almost covered by upper labials; a single preorbital on either side, 2 small scutes below; posterior pair of upper temporals slightly larger than anterior one; seen from above, rostral extends slightly between internasals. Narrow genial scutes in contact along mental groove, no space between posterior genials.

Nineteen scale rows at midbody; 206 abdominal scutes; 97 pairs of subcaudals; anal divided. Scales bordering abdominal scutes of same size as other lateral scales; body scales smooth, rhombic; ridge on lateral aspect of abdominal scutes indistinct, almost absent.

**Coloration in preservative:** Dorsum gray, venter grayish; 5 large dark ocelli bordered with light circles on sides of neck; lateral row of small black dots ending abruptly on anterior third of body. Eye outlined with white lines extending anteriorly and posteriorly; lower white stripe extends over upper labials; a narrow black streak running posteriorly and down from eye, situated on 5th upper labial and slightly touching 6th.

**Description of paratypes:** Counts and measurements of the paratypes in the collection of the Caucasian Reserve, Sochi, Russia are given in Table 1.

**Diagnosis:** Comparatively small snake (Fig. 2), smaller than *C. n. najadum*, *C. n. dahli*, and *C. r. rubriceps* in dimensions. It is comparable in size with the European subspecies, *C. rubriceps thracicus*. In contrast to *C. najadum*, whose tail constitutes 1/3 of its total length, *C. atayevi* has a comparatively short tail,





FIG. 2. Representatives of the "najadum-rubriceps"-complex. Left- Largest specimen of *Coluber atayevi* sp. nov. (Collection of the Caucasian Reserve no. 421). Right- Medium-sized specimen of *Coluber najadum* (Sochi environs, Maly Akhun, Collection of the Caucasian Reserve no. 94).

approximately 1/4 of total length, also characteristic of *C. rubriceps*.

Habitus and elements of coloration of *C. atayevi* are intermediate between *C. najadum* and *C. rubriceps*; its narrow, sharp, and flat head with the rostrum beveled downward is closer to the *C. rubriceps* head shape than to that of *C. najadum*, with its comparatively wide, rounded, and high head, where the upper and lower surfaces of head are parallel. Color pattern of *C. atayevi* resembles that of *C. najadum*, but brown colors prevail instead of olive-green ones. Lateral

abdominal ridges are practically absent in *C. atayevi*, in contrast to the above-mentioned species, and consequently the body is round in cross-section and not rectangular as in *C. najadum* and *C. rubriceps*.

Genial scutes of *C. atayevi* contact one another along the mental groove, rarely having a few isolated granules between the posterior pair, whereas between the widely separated posterior genials of *C. najadum* there are always 2-4 rows of well-developed scales (Fig. 3). Posterior upper labials of *C. atayevi* are weakly

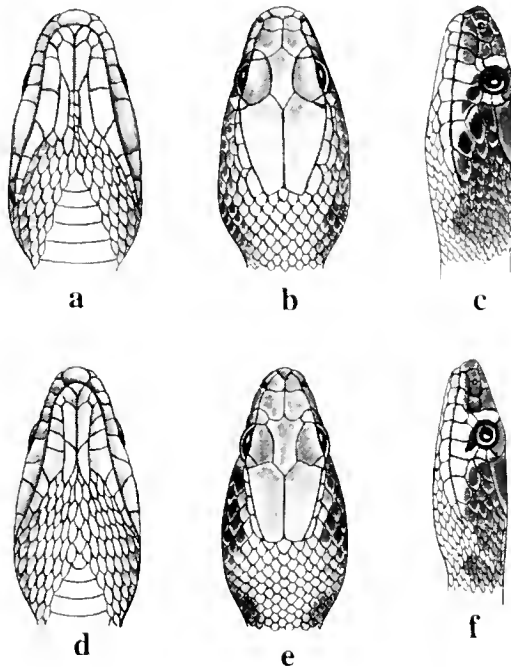


FIG. 3. Distribution of head scales on representatives of the *Coluber najadum*-*Coluber rubriceps*-complex: a, b, c- a young specimen of *C. najadum*; d, e, f- a young specimen of *C. atayevi* sp. nov.

distinguished from the throat scales, whereas all upper labials of *C. najadum* are strongly pronounced.

**Geographic distribution:** The range of *Coluber atayevi* includes the western and central Kopet-Dag, from the surroundings of the Kara-Kala settlement in the west to the Sulukli Spring in the east. This is an upland species, associated with such vegetation types as "prashiblyak," "broad-leaved forest" (Kamelin, 1970) and "phrygana," and in the western part of the uplands, where these plant associations occur at lower elevations, individuals of *C. atayevi* are found at elevations of 400-1600 meters (Shcherbak et al., 1991). At the eastern end of its range (Dushak Mountain), the snake has been found at 2000 meters elevation (Shcherbak et al., 1986).

**Biotores:** According to our observations, *Coluber atayevi* is found on the highest parts of the Saivano-Nokhur Plateau and on the crests of mountains at

900-1400 meters elevation. The most typical biotopes of the species are ecotones of mesophilous derivatives of deciduous forest and meadow-steppe coenosis along the edges of small ravines having deposits of limestone and argillaceous slates. Indicators of the deciduous forest are isolated old trees of Oriental plane (*Platanus orientalis*) and English walnut (*Juglans regia*). Forest plots of "prashiblyak" are typified by *Aceretum fruticans* and *Acer turcomanicum*, a subdominant role played by *Crataegus turcomanica*, *Lonicera floribunda*, *Prunus cerasifera*, *Cotoneaster nummularioides*, *Cotoneaster ovatus*, and *Rubus anatolicus*. In the herb layer, there are such species as *Alliaria petiolata*, *Lamium album*, *Geranium pusillum*, *Arum juquemontii*, and *Allium paradoxum*.

Rocky-shrubby vegetation is usual for the ecotone of forest ravines on the rocky and scree slopes, with isolated trees of *Celtis caucasica* and many different shrubs and semi-shrubs: *Colutea gracillis*, *Ephedra equisitina*, *Thelycrania meyeri*, *Rhamnus coriacea*, *Hymenocrater bituminosus*, and *Artemisia turcomanica*.

Representatives of *Coluber atayevi* are found in *Cousinia smirnovii* associations and *Astragalus piletoclados* groups (phrygana type) in the immediate vicinity of the forest and shrubby communities with steppe wedges, mainly in overgrazed places. The region is comparatively well-watered, because almost every ravine has a spring or stream.

The vegetation of the eastern border of the distribution of *C. atayevi* is described by Korovin (1934): "Dushak mountain is an isolated massif, formed by light limestone. Its steep slopes serve as a home for typical mountainous xerophytes. Here we find juniper, both isolated trees and groups of them. A number of semishrubs and xerophytic herbs form flora of these mountains." Later, the author mentions the domination of shrubs of *Astragalus piletoclados*, as well as groups of different species of *Acantholimon* and here very common gray cushion-like groups of *Onobrychis cornuta*. Korovin concludes

that the cushion-like xerophytes of the Kopet-Dag are better developed on the tops of mountains (about 2000 meters elevation). This is higher than the steppe zone, so phrygana belongs to high-altitude vegetation.

To our regret, absence of data about the biotopes of species from the other places gives us no opportunity to characterize the Cenozoic ties of the species throughout the whole area.

**Population density:** *Coluber atayevi* is the most numerous snake species on the Saivano-Nokhur Plateau. Six specimens were found during a three-hour excursion in the vicinity of Saivan village in May, 1990; 12 specimens were noticed during the same period of time in May, 1991. The largest number of snakes (as many as 5 specimens per 300 meters) was among the shrubs of rocky-scrub plots of ravines. Isolated specimens of snakes were met in stoneless places. The fact that this species is rather common for the western Kopet-Dag is proved by the data of Ataev et al. (1991). All other authors mention only isolated findings, reckoning it among the rarer species of the Kopet-Dag. Apparently, the sporadic distribution of the species and the considerable altitudes at which its habitat occurs, are the reasons why it is rarely met. It is not excluded that its population density in the eastern part of its range is significantly lower than in its western part.

**Seasonal and daily activity:** Presumably, the species' activity begins in the middle of April, considering temperature conditions of this mountain zone (Babaev, et al., 1982) and preferable temperatures of daily activity, noted in May, 1990-1991. *Coluber atayevi* is a diurnal species with two-peak activity in May; the morning peak (9:00-11:00) is strongly pronounced and the evening one is feebly marked. The snakes are active in sunny, windless weather. We have never seen them when it rains, there are strong winds, or heavy overcast. In bad weather the snakes are absent not only on the surface, but from under the plates of slate,

where they are usually met in sunny weather.

**Diet:** Lizards in the habitat of *Coluber atayevi* are *Ablepharus pannonicus*, *Stellio caucasicus*, and *Pseudopus apodus*. *Cyrtopodion caspius* and *Eremias strauchi* are common, though not so numerous, while *Mabuya aurata* and *Eumeces taeniolatus* are rare. Considering the small size of the head and body of *Coluber atayevi*, the bulk of its diet must be formed only of *Stellio caucasicus*, young specimens of *Eremias strauchi*, and possibly *Cyrtopodion caspius*. Evidently, *C. atayevi* is a saurophage exclusively, since the common and abundant *Microtus socialis* and *Saxetania cultricolis* (a micromammal and an orthopteran insect, respectively) are too large to be eaten by this snake.

**Syntopical species of snakes:** Subdominants of *Coluber atayevi* are *C. nummifer*, *C. ravergeri* and *Vipera lebetina*; *Typhlops vermicularis* is common; *Agkistrodon halys caucasicus* and *Natrix tessellata* are rare. At the borders of the species' biotopes *Eirenis meda*, *Psammophis lineolatum*, *Naja oxiana*, and *Eryx miliaris* are met. These species are more characteristic for smaller hypsometric marks.

## Discussion

*Coluber atayevi* is most closely related to *C. najadum* and *C. rubriceps*, possessing features of both species; in habitus (head shape, in particular) it resembles *C. rubriceps*, but it is similar to *C. najadum* in color pattern. We should note that these features are characteristic for juvenile specimens as well as adults; in other words, we cannot distinguish ancestral features that would allow us to unite *C. atayevi* with either of the species mentioned (Table 2, Fig. 3).

Judging from the contemporary distribution of the three species, we propose that the center of the complex is the Eastern Mediterranean region, so-called dry land of Asia Minor or Balkan-Caucasian

TABLE 2. Comparative morphometry of representatives of the "najadum-rubriceps" species complex (see text for abbreviations).

Species	L.	L. cd.	L.+L. cd.	L./L. cd.	Sq.	Ventr.	S. cd.	Lab.	Sublab.	Temp.
<i>Coluber ataevi</i> sp.nov.	381-533 452.2±29.6	122-201 155.8±13.8	514-734 608	2.65-3.31 3.08±0.08	19	197-214 206.7±1.5	90-106 98.9±1.8	8, 8 (100%)	10 (75%) 9 (20%) 11 ( 5%)	2+1 (55%) 2+3 (20%) 2+2 (15%) 1+2 (5%) 1+3 (5%)
<i>C. najadum</i> (Terenjev & Chernov)	to 1000	-	-	2.09-2.9	19	205-238	98-135	8 (7.9)	-	2+3 (2+2, 1+2, 1+3)
<i>C. najadum</i> (Muskhelishvili, 1970)	M to 689 F to 720	M to 282 F to 267	-	2.3-2.6	19	214-229 F 102-119	M 110-130 7 (11.1%)	8 (88.8%)	-	2+3 (88.3%) 1+3 (11.1%)
<i>C. najadum</i> (Sochi area)	578-870 735±28.4	175-320 239.6±27.0	-	-	19 (87.5%) 21 (12.5%)	211-224 216.6±1.7	-	8 (87.5%) 9 (12.5%)	-	-
<i>C. rubriceps rubriceps</i> (Rehak, 1986)	-	-	960-1112 1040	2.43-2.51 2.47	19	207-211 209	104-105 104.5	8/8 (100%)	10/10 11/11	2+2
<i>C. rubriceps thracicus</i> (Rehak, 1986)	-	-	412-710 562	2.53-3.03 2.8	19	186-192 189	84-94 88	7/7 (18.75%) 7/8 (37.5%)	9 (6.25%) 10 (81.25%) 9/10 (12.5%)	1+1 (6.25%) 1+2 (12.5%) 2+1 (6.25%) 2+2 (75.0%)
<i>C. rubriceps thracicus</i> (Rehak, 1986)	-	-	443-625 538	2.81-3.52 3.11	19 (90.9%) 21 (9.1%)	198-203 203	79-91 85	7/7 (27.3%) 7/8 (18.2%) 8/8 (54.5%)	9 (18.2%) 9/10 (18.2%) 10 (63.6%)	1+1 (18.2%) 1+2 (18.2%) 2+2 (63.6%) 3+2 (9.1%)
<i>C. najadum</i> (Arnold & Burton, 1978)	-	-	to 1350	-	19	205-235	-	-	-	-

dry land. The ancestral form could have been widespread in the Upper Miocene-Pliocene over subtropical semiarid regions of Asia Minor united with the Central Asian Massif, Caucasian Island and the Balkan Mountains in the Miocene. Mixed savanna-hyllile landscape, combining alternation of open spaces with subtropical forests were characteristic for the northern parts of the Iranian Plateau in that period. Fossil mammals and birds testify to this fact (Vereshchagin, 1959). In Vereshchagin's opinion, similarity of the Upper Miocene fauna unites Central Asia, the Caucasus, the Crimea, and the Balkan Mountains. Analysis of fossil tortoises testifies to the presence of a bridge between the Balkan-Caucasian dry-land and an Iranian-Pakistanian Peninsula (Chkhikvadze, 1991).

Alpine orthogenesis that has led to the formation of mountain relief over the whole area of study, from the Balkan Mountains to the Kopet-Dag, evidently promoted the breakup of the original area of the ancestral form of the *najadum-rubriceps* complex. A number of populations along the sea coasts of that time (Mediterranean, Black, and Caspian Seas) could already have been isolated in the Pleistocene. The event has been connected with aridization of the continental interior and the formation of new ecological conditions. These conditions have given impetus to the development of the highland xerophytic vegetation of Iran and the formation of the fauna of arid mountains. It should be noted that the vegetation of the central and western Kopet-Dag has more features in common with the vegetation of southern and western territories than with the other mountain massifs of Central Asia. Korovin (1934) emphasizes that the Kopet-Dag forests are the connecting link with Mediterranean macchias. The provenance of highland xerophytes (phrygana), as well as forest vegetation, Korovin stated to be Persia and Armenia.

Pleistocene cataclysms, connected with the glaciation in the Caucasus, Asia Minor, and the Iranian Plateau, as well as the changes in the basins of the Black and

Caspian seas, could have favored the secondary overlapping of the diverged *C. najadum* and *C. rubriceps*. This sympatry, which can be seen today in some areas, served to reinforce the species divergence. In the east, representatives of this complex could survive only in relict populations on the northern (Caspian) slope of the Alborz Range and, in isolated form, in the derivatives of forest coenosis in the western Kopet-Dag that were never subjected to glaciation (Gvozdetskiy and Mikhailov, 1987). In the Iranian part of the Turkmen-Khorasan mountains there are islands of gyrkana forests to the east of Astrabad, reaching the longitude of Gombedezh-Kabus. Some islands of oak forests reach Bodgenurd in Khorasan (Menitskiy, 1984). Anderson (1968) wrote about possible preservation of lizard refuges since the Pleistocene in the Kopet-Dag mountains.

Increasing climatic aridization in the Holocene can be the cause of disappearance of *C. atayevi* in the foothills and severe restriction of its area in the middle-altitude and high-altitude parts of the western and central Kopet-Dag and, possibly, to the breakup of the area into several local refuges. The "primitive" morphological features were preserved in the absence of contacts with closely related forms. In any case, only further study of species variability in specific localities of the Kopet-Dag can throw light on this question.

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On the Status of *Rhacophorus prasinatus* Mou, Risch, and Lue  
(Anura: Rhacophoridae)

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Key Words: Amphibia, Anura, Rhacophoridae, *Polypedates prasinatus*, *Rhacophorus smaragdinus*, Taiwan, New Combination.



FIG. 1. The species of *Polypedates* and *Rhacophorus* on Taiwan: *P. megacehalus* (upper left), *R. taipeianus* (upper right), *R. moltrechti* (lower left), *P. prasinatus* (lower right).

The emerald tree frog, *Rhacophorus prasinatus* Mou, Risch and Lue, 1983 (= *R. smaragdinus* Lue and Mou, 1983), has had an uncertain taxonomic status. When first described, it was thought to be closely related to *R. chenfui*, currently placed in *Polypedates* (Jiang, Hu and Zhao, 1987), on morphological grounds and to *P. leucomystax* on account of genetic similarity. Having examined the external physical structure, muscular structure and

stained skeletons of specimens from the type locality, I conclude that this species should be classified in *Polypedates* in accordance with the definitions and criteria set forth by Liem (1970) and Jian et al. (1987) to distinguish the genera *Rhacophorus* and *Polypedates*. The following are the key features observed:

Dermal fold along outer edges of forearm and above anus in rows of

tubercles, tarsal fold not evident or absent. Fingers half webbed; web of all toes except the fourth extending beyond subarticular tubercles; fourth toe webbed to middle subarticular tubercle or somewhat beyond. Dorsal view of intercalary cartilage heart-shaped. Esophageal and lateral processes of laryngeal apparatus present. M. extensor radialis accessorius lateralis moderately large, originating along lateral side of humerus and inserting on the disto-dorsal end of radio-ulna; M. extensor brevis superficialis of the first digit present. Vomerine teeth present. Parieto-squamosal arch of the fronto-parietal bone absent. Vertebral column procoelous.

*Polypedates prasinatus* (Mou, Risch and Lue 1983) comb. nov.

*Rhacophorus prasinatus* Mou, Risch and Lue, 1983;

*Rhacophorus smaragdinus* Lue and

Mou, 1983

The two junior synonyms share the same holotype and bear the same publication year. However, the *Rhacophorus prasinatus* has priority. It was published on 30 December 1983 in *Alytes* 2(4) which was mailed on that date, as indicated in the journal. Although *R. smaragdinus* has the same publication date in the Journal of Taiwan Museum 36(2), I have learned from the editor, S. F. Hung, that the mailing date of this issue was 17 January 1984. This supports a previous arbitrary adoption of the first junior synonym (Frost, 1985).

In general, *Polypedates* and *Rhacophorus* species can be distinguished from the other treefrogs by the Y-shaped terminal phalanx and absence of anterior horns of the hyoid (Liem, 1970). The species in Taiwan (Fig. 1) can be distinguished by the following key:

#### Key To Species Of *Polypedates* And *Rhacophorus* Of Taiwan

- 1a. Dermal fold along the forearm absent or in rows of tubercles; tarsal fold not evident or absent..... (*Polypedates*)
- 2a. Skin shagreened, ground color green above; supratympanic fold yellowish brown anteriorly..... *P. prasinatus*
- 2b. Skin smooth, ground color light brown or brown, usually with dark spots or stripes..... *P. megacehalus*
- 1b. Dermal fold along the forearm and tarsus present..... (*Rhacophorus*)
- 3a. Skin smooth, with black spots or blotches on flanks and inner side of thighs..... *R. moltrechti*
- 3b. Skin shagreened to granulated, with fine dark dots on inner side of thighs..... *R. taipeianus*

These two genera in Taiwan also exhibit different reproductive modes. Both *Polypedates* species usually deposit foamy masses suspended on low tree branches overhanging pools or on cistern walls above the water. Egg masses become light brown when the outer foamy substance dries. In contrast, *Rhacophorus* species deposit foamy egg masses near puddles in holes burrowed by the males or beneath soil or fallen leaves.

*Specimens examined:* NMNS 01455-01459, 18 specimens.

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## Studies on Pakistan Reptiles. Pt. 3. *Calotes versicolor*

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**Abstract:** -Variation in the scutellation and color of *Calotes versicolor* populations in Pakistan are analyzed, leading to the recognition of a new subspecies (*C. v. nigrigularis*) from the front ranges of the Himalayan Mountain complex in Afghanistan, Pakistan and India. Several variant populations of the same species in other parts of its range are noted, but not given taxonomic recognition at this time.

**Key words:** Reptilia; Sauria; Lacertilia; Agamidae; *Calotes*

### Introduction

*Calotes versicolor*, a large, common, widespread and showy lizard, was described early in the history of reptilian study in the Indian subcontinent (Daudin 1802, as *Agama versicolor*, type loc. "India", restricted to near Pondicherry, India by Kuhl 1820). Variation in color and scalation was also documented early, resulting in the description of several species (now synonyms, see Smith 1935 for review) and races. No subspecies are recognized at the present time, in spite of obvious geographic variation and a wide ecologic and geographic range.). The current study of color and scalation supports the contention of earlier workers that morphologically distinct populations with circumscribed geographic boundaries exist. The latest morphological study is by Tiwari and Aurofilio (1990), though it is restricted to populations from Tamil Nadu, India.

During the collection of new material for a future major publication on the herpetology of Pakistan, populations of *Calotes versicolor* from the mountains of northern Pakistan were noted as being distinctly different from those in other parts of the country. This discovery suggested an analysis of geographic and sexual variation in several scute and color characters, similar to our earlier study of Pakistan *Echis carinatus* populations (Auffenberg and Rehman 1991). The following is the result of this analysis.

### Methods

This study is based on slightly more than 500 specimens located in the 14 institutions listed below. The museum source of those specimens specifically referred to are identified by the abbreviations given.

American Museum of Natural History, New York (AMNH); Natural History Museum, London (BMNH); Bombay Natural History Society (BNHS); California Academy of Sciences, San Francisco (CAS); Field Museum of Natural History, Chicago (FMNH); Museum of Comparative Zoology, Harvard University (MCZ); Pakistan Museum of Natural History, Islamabad (PMNH); Senckenberg Museum, Frankfurt (SMF); Florida Museum of Natural History, University of Florida (FMNH/UF); University of Michigan Museum of Zoology, University of Michigan (UMMZ); National Museum of Natural History, Washington (USNM); Zoological Survey Department, Karachi (ZSD); Zoological Survey of India, Calcutta (ZSI) and Alexander Koenig Museum, Bonn (ZFMK).

All drawings were done by the senior author.

Figure 1 shows the localities from which specimens were examined. Appendix 1 provides data on museum holdings of specimens examined from these geographic locations. No specimens with either general, questionable, or erroneous locality

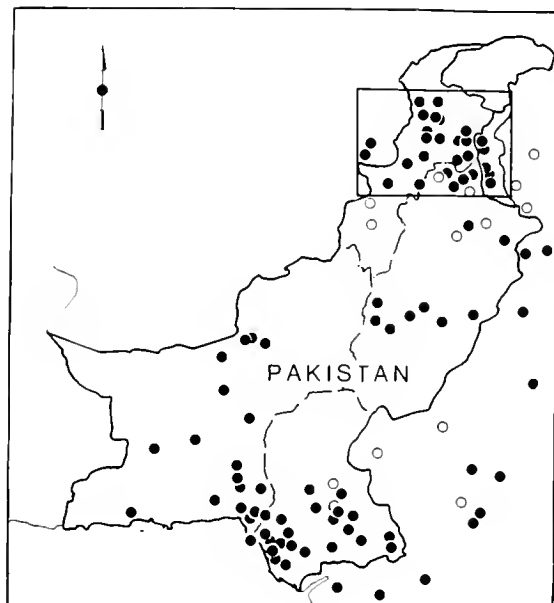


FIG. 1. Localities in Pakistan and adjacent countries from which we examined specimens (dots). Pakistan localities for which we know material is available in museums, but which we have not seen are indicated by circles. The area represented in Fig. 9 is shown with a rectangle.

data have been included. All data used in the analyses were obtained by us - none were drawn from the literature.

The following characters were tabulated for all the specimens listed above: 1) number of subdigital laminae under toe IV, 2) number of scale rows at midbody, 3) snout-vent length (SVL), 4) degree of mucronation of dorsal scales (0 none or very weak, 1 moderate mucronation, 2 strong mucronation; Figure 2), 5) angle of posterior edge of dorsal scale rows (Fig. 3), 6) number of gular scales from just behind mental to a level even with the middle of the eye, 7) number of scales in the dorsal crest that are higher than the length of their base, 8) color pattern of chin, 9) color pattern of belly, 10) color pattern of dorsal body surface, 11) degree of darkening of postorbital stripe (0 none, 1 moderately dark, 2 very noticeable, see Figs. 9, 12), and sex.

Figure 4 shows the locations and general size of sample areas chosen. The geographic limits of the samples were

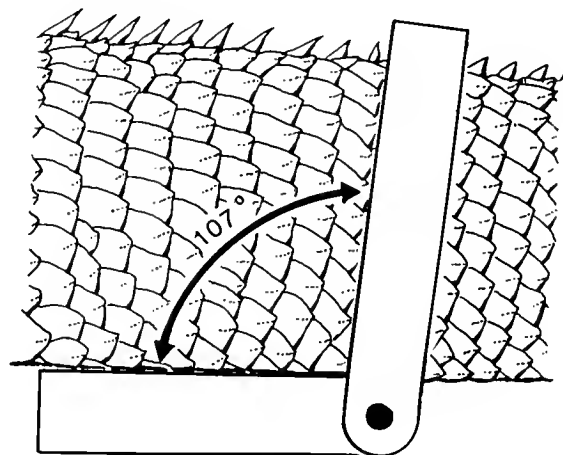


FIG. 2. Side of body of *Calotes versicolor*, showing method of determining the angle of the dorsal scale rows.

selected principally on the basis of sample size (museum material available), but in some cases partly on environmental differences between closely approximated geographic areas (i.e., elevation, major habitat, etc.). Each of these sample areas served as the basis for all calculations and evaluations, so that all specimens available from each area were considered as constituting the same sample for computational purposes.

## Results

Only one species of *Calotes* - *C. versicolor* - has been identified by us in Pakistan, though two others were previously listed or implied as occurring there. Murray 1886 reports *Calotes grandisquamis* (Gunther 1875, a valid species from southern India) from Sindh Province (Karachi and Jerruck), Pakistan. There are no substantiating specimens and none of the several herpetologists who have worked in the Karachi area for extensive periods since have ever found this species. It is distinctly different from *C. versicolor* in its decidedly green body color, the significantly lower number of transverse midbody scale rows (27-35), and in the presence of a short, oblique fold (sometimes called a pit) in front of the shoulder.

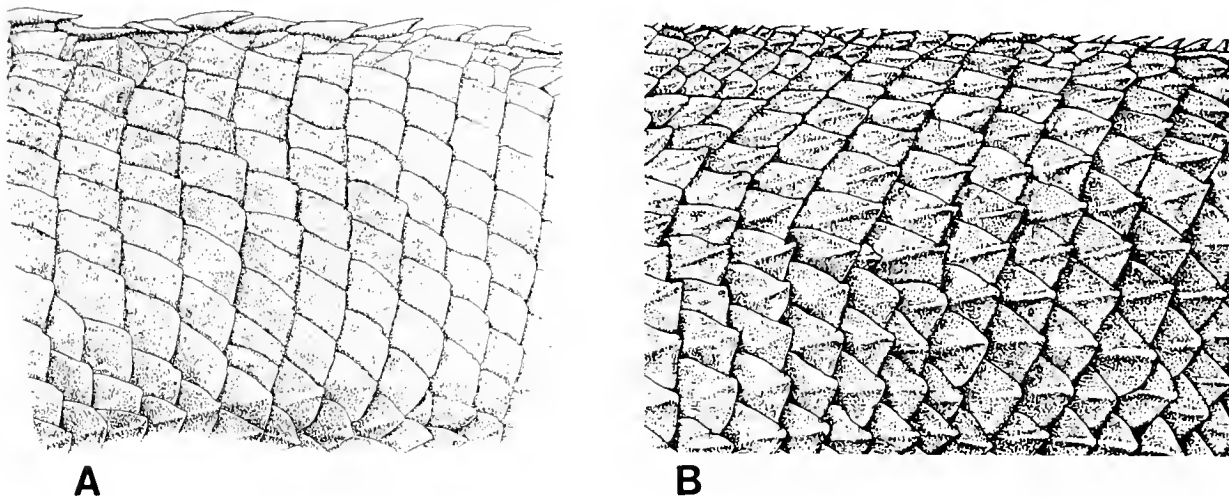


FIG. 3. Geographic variation in degree of lateral body scale mucronation. A, *Calotes v. versicolor*, FMNH/UF 70516, adult male, Karachi, Karachi Dist., Sindh Prov., Pakistan..

The second species is *Calotes jerdoni* (Günther 1870, type loc. Khasi Hills, Assam, India. It is represented in the BMNH by two preserved specimens cited by Boulenger 1885), said to have been taken in Afghanistan. This locality suggests that the species should also be found in the intervening Pakistan area. The Afghanistan data are obviously incorrect, as has already been suggested by Smith (1935). *Calotes jerdoni*. We have examined the specimen in question and confirm it belongs to this species, which is easily distinguished from *C. versicolor* on the basis of its bright green color and the parallel rows of enlarged and keeled scales on top of the head, and in lacking the characteristic pair (usually) of enlarged spines above the tympanum.

We believe that *Calotes versicolor* is the only species of the genus in Pakistan. Between populations of this species in and beyond the borders of Pakistan we are able to demonstrate significant clinal variation (north/south, east/west) beyond that ascribable to race. Such clines occur in at least four scale characters. Sexual variation is demonstrated in color and adult size.

Within Pakistan boundaries, geographic variation suggests the recognition of two races of *Calotes versicolor*, one of which is new. It is described below. Additionally, the populations found essentially east of

India are distinguished on the basis of color and scale characters. However, in this paper we do not recognize them as separate nomenclatorial entities. The solution to the question of their status must await the availability of additional, fresher material.

#### *Individual And Geographic Variation*

Here we discuss the variations in color, proportion, and scutellation which are correlated with geographic locality, sex, environment, or ontogeny.

#### *Clinal Variation*

The term cline has been used to express a condition in which the values of a variable character form a slope or gradient over a geographic area. With increasing knowledge of variation systematists have come to recognize different types of clines. Some are related to gradual changes in environment (including climate) and others are not. The change in character state over distance have varying slopes when the values of the characters being examined are plotted against distance. Two extreme types of clines are recognizable - *narrow* (or steep) and *broad* (or low) slopes. The former is represented by a character-gradient which significantly changes its slope in a step-like fashion, with separate subspecies corresponding to two more or less level character values (flat or slightly

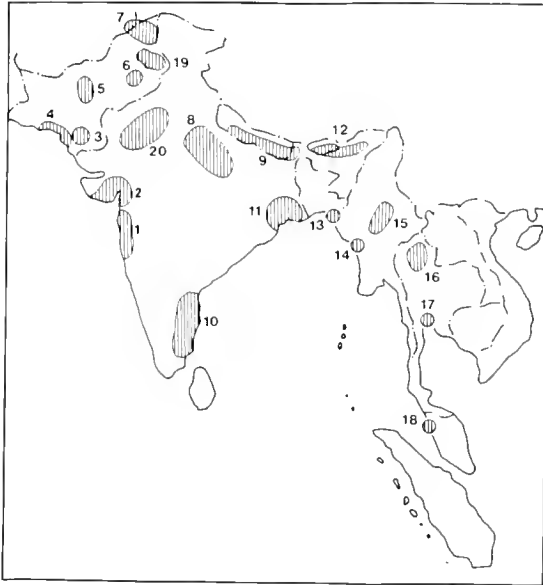


FIG. 4. Location, size, and approximate area of samples used in this study

sloping) on either side of a uniting steeper slope - the zone of character intergradation. The broad cline is one which does not show any steepening of the character gradient in a particular place, but is represented by a continuous slope with no obvious interruptions. There are, of course, all gradations of slope between these two extreme types. Both narrow and broad clines, as well as intermediate types, exist within the pattern of character variation in the species *Calotes versicolor*.

*Number of midbody scale rows* (Fig. 5). —The mean number of midbody scale rows illustrate the narrow type of clinal character change in which there is a distinct and rapid change (= steep slope) from one rather uniform area to another. In *Calotes versicolor* a steep north-south cline exists between the northern Himalayan Mountain and Indo-Gangetic Plain populations. The mean dorsal scale rows of all southern (plains) populations (India and Pakistan combined) is  $43.0 \pm 1.0$  (OR sample means 41.0-44.1); for all northern (upland) populations (India, Pakistan, Afghanistan, Nepal) the mean is  $45.9 \pm 1.5$  (OR sample means 43.0-49.2). The difference in mean number of dorsal scale rows between northern and southern populations is

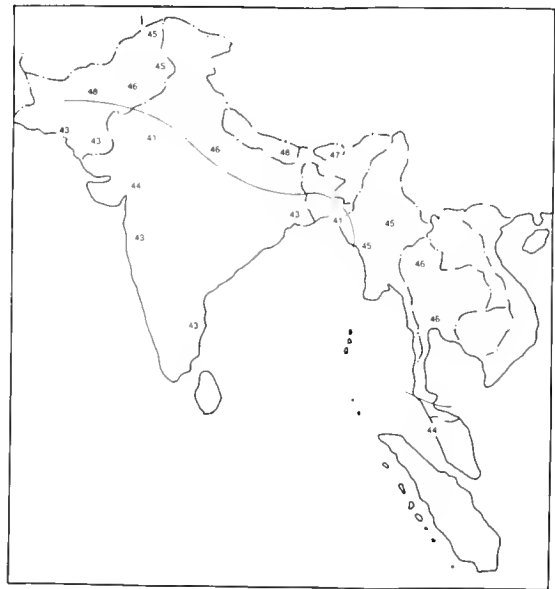


FIG. 5. Geographic variation in mean number of dorsal scale rows of *Calotes versicolor* samples studied. Lines (isophenes) enclose samples of similar value (see text).

highly significant ( $t$  5.83,  $df$  13,  $p < 0.001$ ), with the distinct change in slope of the character gradient occurring along the frontal hills of the northern and northwestern mountains of Pakistan. Similar north-south clines can be demonstrated within this species in other characters as well (see below). However, there is no significant east-west change in the mean character state value in either the plains or the mountain populations, in spite of the fact that other character states do show important east-west changes (see below). Thus the east-west axis of the pattern of character change is independent of the north-south axis. Within southern, plains populations, several samples have graphically different values for this character than all the surrounding ones (Quetta is higher, Rajasthan and the Mekkran Coast are lower). However, in every case, the samples from these sites are small and the differences are not statistically significant.

*Subdigital lamellae under 4th Toe* (Fig. 6). —In this character the pattern of geographic variation is more complex. There is no clear north-south trend. While



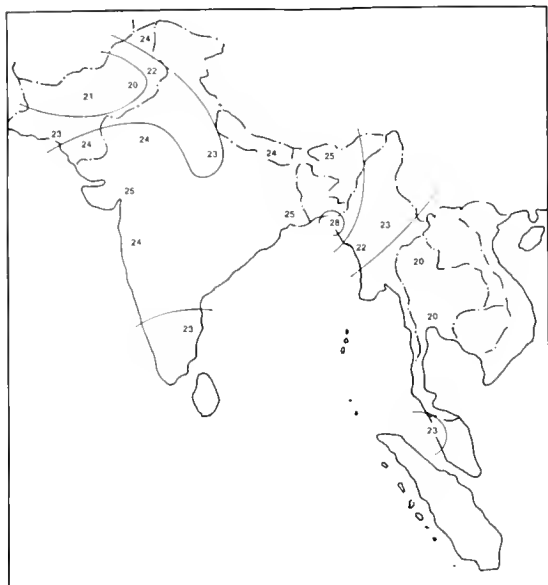


FIG. 6. Geographic variation in the mean number of lamellae under the 4th toe.

equally high values occur in the upland, northwestern part of the species range, the same isophene sweeps down to sea level in the Calcutta area, terminating in the high value for Chittagong (though the latter is statistically insignificant). This tongue of higher values separates the Myanmar-Malaysian populations in the east from the Indo-Pakistan ones in the west. Within Pakistan there is no clear evidence of the even clinal changes witnessed in the mean number of dorsal scale rows (the difference in means between eastern and western Mekran populations is based on small samples and is statistically insignificant). Thus the overall pattern of character change is one of an east-west component in which geographically intermediate populations (West Bengal) have distinctly higher mean values than populations to both the east and west. At the same time, the "isolated" eastern section includes a population in Thailand in which the mean value (low) is statistically distinct from its neighbors (with Arakan  $t$  3.23,  $p$  <0.01; Rangoon  $t$  2.70,  $p$  0.02; Penang  $t$  3.56,  $p$  0.001). Near the western edge of the species range there is an apparent north-south trend, in which the former area (Jalalabad, Peshawar and Taxila combined) has a statistically significant higher mean value than the south

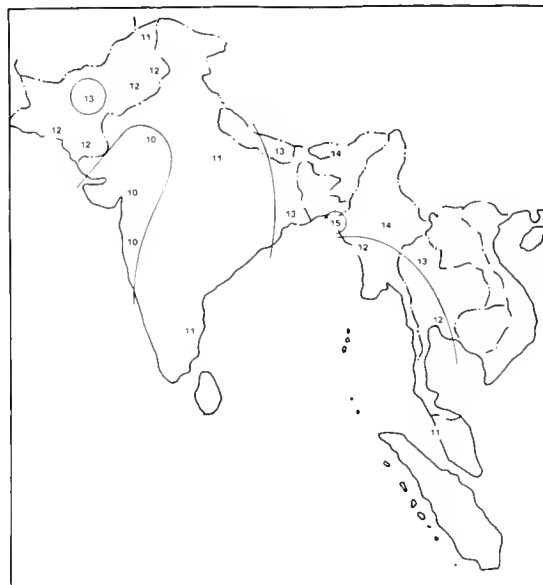


FIG. 7. Geographic variation in the mean number of gular scales.

(Baluchistan) ones ( $t$  3.15  $p$  0.01). This pattern is, however, confused by populations from Azad Kashmir and Swat District, which are more like one another in having statistically similar low mean values than either is to populations from the geographically intermediate area of Manshera District.

*Number of Gular Scales* (Fig. 7). —In this character there is a distinct two-way, east-west cline which proceeds from lowest mean values in peninsular India to higher values to the west (reaching maximum values in those samples in the arid mountains at the eastern edge of the Iranian Plateau) and the east (highest in Chittagong and Mandalay). There is no evidence for a north-south cline anywhere. The Chittagong sample is again distinctive (though sample size is small and the values are not significantly different from neighboring ones).

*Angle of Dorsal Scale Rows* (Fig. 8). —This character illustrates still another clinal pattern—essentially northwest to southeast. Highest mean values (111-119) are found in the former and lowest in the latter (90-98). There are, however, some exceptional points outside this general

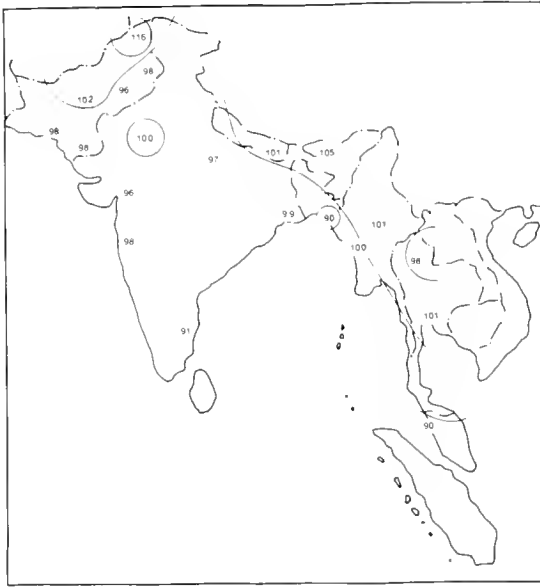


FIG. 8. Geographic variation in the mean angle of the dorsal scale rows.

trend, particularly within Pakistan. Thus the values for the Kirthar, Dadu, Rajasthan samples are significantly lower than all the surrounding ones ( $t$  values 2.56 to 4.60,  $p$  0.02 to 0.001). The differences between the the Khuzdar-Quetta samples and Khuzdar-Panjgur samples are not significant. In the eastern sector, the Penang sample has a significantly higher mean angle of the dorsal scale rows (with Rangoon  $t$  3.06  $p$  0.01; with Thailand  $t$  4.20,  $p$  <0.001). The Madras sample is also significantly different from that from Bombay ( $t$  4.01  $p$  <0.001).

*Head and Body Length (SVL).* —In a short discussion of SVL, Smith (1935) provides data suggesting that the peninsular Indian populations are larger than those of the Indo-Chinese region. Our data confirm this statement, but the larger number of specimens available to us allow a finer-grained breakdown of the size and geographic representation of our sample areas. We find, first of all, that the geographic trends in SVL of males and females parallel one another. Thus, only the males are discussed here (the females follow identical patterns). Adult males from peninsular India and the Indus Valley are significantly larger than those of all

surrounding samples. The mean SVL for the (combined) Indus Valley-peninsular India sample is 94.3 mm. This differs significantly from the combined sample of mountainous Pakistan (Student's  $t$  7.25,  $p$  < 0.001, and is significantly different from a combined sample from mountainous India-Nepal (mean 82.1,  $t$  3.32,  $p$  < 0.01). The sample from Calcutta has a smaller SVL, but the difference in means is not significant at  $p$  < 0.05. However, there is a very significant difference ( $t$  5.6,  $p$  < 0.001) between the Calcutta mean (92.2 mm) and that of the combined Myanmar sample (81.2 mm). Samples from Thailand and Malaysia have still lower values, but the means are not significantly different from that of Myanmar at  $p$  < 0.05. The calculated differences in the means of all of these samples suggest a broad bi-directional clinal in which the central part of the species range has the highest mean values, with gradually lower ones in all directions, rather than in only one. Analysis of additional samples from peninsular India would undoubtedly clarify the shape of this cline better than we are able to do on the basis of our present material.

What is obvious here is that each of the scale characters analyzed from the standpoint of geographic variation is represented by a different pattern of variation. Thus each of these characters show a pattern of geographic variation that is independent of one another. The patterns undoubtedly reflect the complexity of selective factors acting through the clinally changing physical and/or biotic environments found throughout the species range.

#### *Sexual Variation*

As stated above, within all samples examined, males attain a greater SVL than females (though statistically not significant in one, see Table 1). Overall SVL range of all mature males is 70-138, mean 99.3 + 17.2, females 64-121, mean 80.5 + 15.7; Student's  $t$  test for difference in means = 5.6,  $df$  264,  $p$  < 0.001. Our analysis also shows that this dimorphism is geographically variable (Table 1), with the

TABLE 1. Geographic variation in SVL of adult *Calotes versicolor*.

Sample Area	O.R	Mean	t Test	Probability
Mts. Pakistan				
Males	70-114	81.2		
Females	64-79	72.3	1.93	not sig.
Penin. India				
Males	76-138	102.3		
Females	57-121	86.5	4.20	< 0.001
Myanmar				
Males	71-91	76.9		
Females	68-83	72.2	2.14	< 0.05
Thailand				
Males	73-96	81.4		
Females	67-88	76.8	2.05	0.05

strongest divergence in the peninsular Indian sample.

In a recent morphological study of Tamil Nadu, India samples, Tiwari and Aurofilio (1990) report no sexual difference in scalation. While we find this to be true of almost all of the scale characters we studied, we do find significantly different means in the number of midbody scale rows, with females having a higher number than males by a factor of from 9.7 to 11.7 percent. (six of our largest samples were analyzed; mean in males  $41.8 \pm 9.7$  to  $46.7 \pm 11.1$ ; females  $46.7 \pm 3.1$  to  $48.1 \pm 9.9$ ;  $t$  3.5 to 19.5,  $p < 0.05$  to  $< 0.01$ ).

Many workers have described the difference in color and pattern of adult male and female *Calotes versicolor*. In general, the male is lighter, with no, or 4-8 very dim crossbars on the dorsal part of the body. The ground color is usually some shade of tan in the Indian subcontinent and more grayish in the eastern sectors. Adult females are darker, the ground color being brownish to grayish, with the same number, but more obvious narrow brown to black crossbars (or remnants of them).

There is often a faint to quite obvious lighter dorso-lateral stripe on each side of the body. These are missing in adult males. Females usually have a series of circumorbital radiating darker bars which are usually lacking in males. Females lack a dark ventral partial collar at the base of the neck, which is characteristic of the adult males of some populations. Finally, adult males have a remarkable change in color and pattern during the breeding season, which is absent in the females.

#### Ontogenetic Variation

Ontogenetic variation is noted in color and pattern. The belly of juveniles (< 75 mm SVL) possess 5 to 7, dim, longitudinal, grayish stripes or dashes, each one scale wide. These disappear with age, though they are usually represented in the adult by traces of the median member. In neonates the chin is white to dirty gray, laterally streaked in the sub-infralabial area with a series of medium gray to black diagonal stripes, which become dimmer with age. In one-third- to one-half- grown individuals the chin is additionally suffused with light pink. A series of gray to black

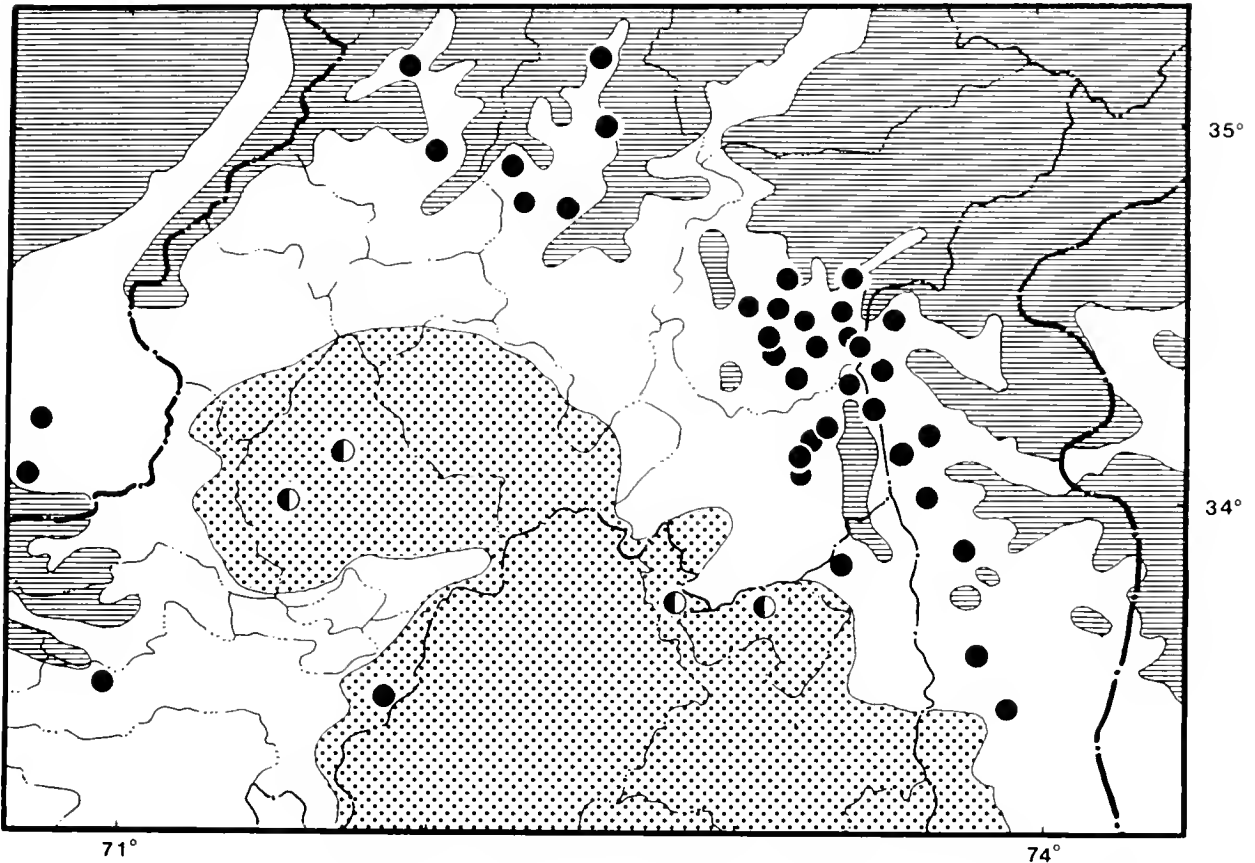


FIG. 9. The geographic distribution of *Calotes versicolor nigrigularis* (solid dots) is restricted to elevations between 300 and 1800 m. The stippled zone is < 300 m, and the cross-hatched one is > 1800 m. Localities of specimens morphologically intermediate between this race and *Calotes v. versicolor* are indicated as half-circles.

radiating circumorbital bars are almost always evident, which also fade with age.

### Taxonomic Considerations

This report recognizes a subspecies if 75 percent of the available individuals from a geographic area can be correctly assigned to that provenance on the basis of one or more characters. Analysis of the data suggests that *Calotes versicolor* is divisible into at least two subspecies. Geographic discontinuities in the diagnostic characters are the basis for the generalized racial distributions shown in Figure 9.

*Calotes versicolor versicolor* (Daudin)

*Agama versicolor* Daudin 1802:395. Type locality "India".

*Agama tiedmanni* Kuhl 1820:109. Type

locality Pondicherry, India.

*Calotes versicolor* (Daudin), Jerdon 1853:470.

? *Calotes viridis* Gray 1846:648. Type locality Madras. (Type specimen lost).

A subspecies of *C. versicolor* distributed from Sri Lanka north through most of peninsular India and Pakistan, west to the Kabul Valley in southeastern Afghanistan, northeast to Hainan Island, China and southeast to Sumatra, Indonesia; replaced in the northern mountains of Pakistan and adjacent Afghanistan and India by *C. v. nigrigularis* (nov. ssp, described below). Other undescribed races probably replace this plains form in northeastern India, and lowland areas of Myanmar, Thailand, Malaysia and Sumatra.

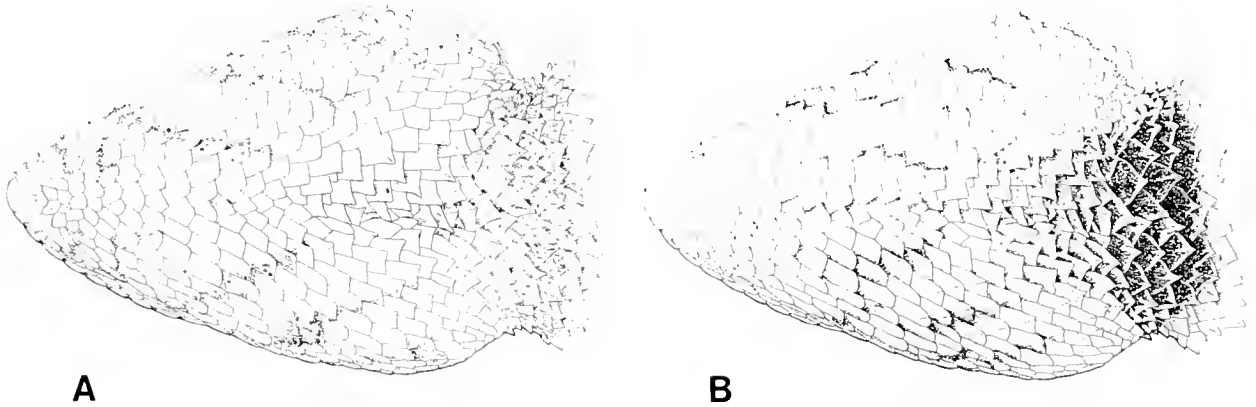


FIG. 10. Chin and throat color pattern in adult *Calotes v. versicolor*. A, FMNH/UF 70511, adult male, Karachi, Karachi Dist., Sindh Prov., Pakistan. B, FMNH/UF 19952, adult male, 8 mi. W. Madras, Tamil Nadu State, India.

*C. v. vesicolor* has the following suite of characters which distinguishes it from *C. v. nigrigularis*: larger adult size, mean angle of dorsal scale rows  $90 - 105^{\circ}$ , mean number two distinct postocular stripes (absent in largest males, Fig. 10A), dorsal body pattern usually indistinct, tending to uniform tan during most of the year, becoming pink to reddish in males during the breeding season; 5-7 crossbars may be present (particularly in juveniles and adult females), each 1-2 scales long at the vertebral line; gulars either uniformly light-colored or marked with narrow, diagonal, faint, dusky or sometimes black stripes; scales of the throat and pre-shoulder areas vary from the same color as the gulars to having dark brown or black bases. Large males from southern India often have a ventrally located black, partial collar (Fig. 11); the belly is always uniformly yellowish- to dirty-white.

Several months before the breeding season, the color and pattern of adult males change. At this time the lateral and dorsal surfaces of the head, neck, and shoulders, and the sides of the body all become suffused with yellow, pink, orange, or even dull red (depending on geographic location, and age of the individual). The throat and chest change to orange or red with black mottling (seasonal adult color changes in Indian subcontinent populations

also described by Murray 1886, Smith 1935, and Minton 1966); the tail and limbs become black.

*Holotype* — Presumably in the Paris Museum, but now lost. The type locality had been simply stated as "India", but this was later restricted to Pondicherry, India by Kuhl (1820).

*Exemplary Material Examined.*—The specimen materials examined by us that best fit the type description are listed below. We do not include any specimens here from the eastern Himalaya Mountains and from West Bengal eastward, as we believe that those populations will eventually be recognized as representing one or more races distinct from the nominate form and that one described below: FMNH/UF 19886, 19955-9, Kanheri Caves, nr. Borivli, Maharashtra State, India; FMNH/UF 70535-7, Khadiji Falls, Dadu Dist., Sindh Prov., Pakistan; FMNH/UF 19949-53, 8 mi. W. Madras, Tamil Nadu State, India; FMNH/UF 79087, 79099, Sujabad, Deri Ghazi Khan Dist., Punjab Prov., Pakistan; FMNH/UF 78932, Sonmiani, Las Bela Dist., Baluchistan Prov., Pakistan; AMNH 39377-8, 5 mi. E. Kalka and AMNH 39382, nr. Kalka, Amballa Dist., Punjab State, India; CAS 94337-8, 3 mi. SE Sirohi, Rajasthan State, India; MCZ 55502-3, Baroda, Gujarat

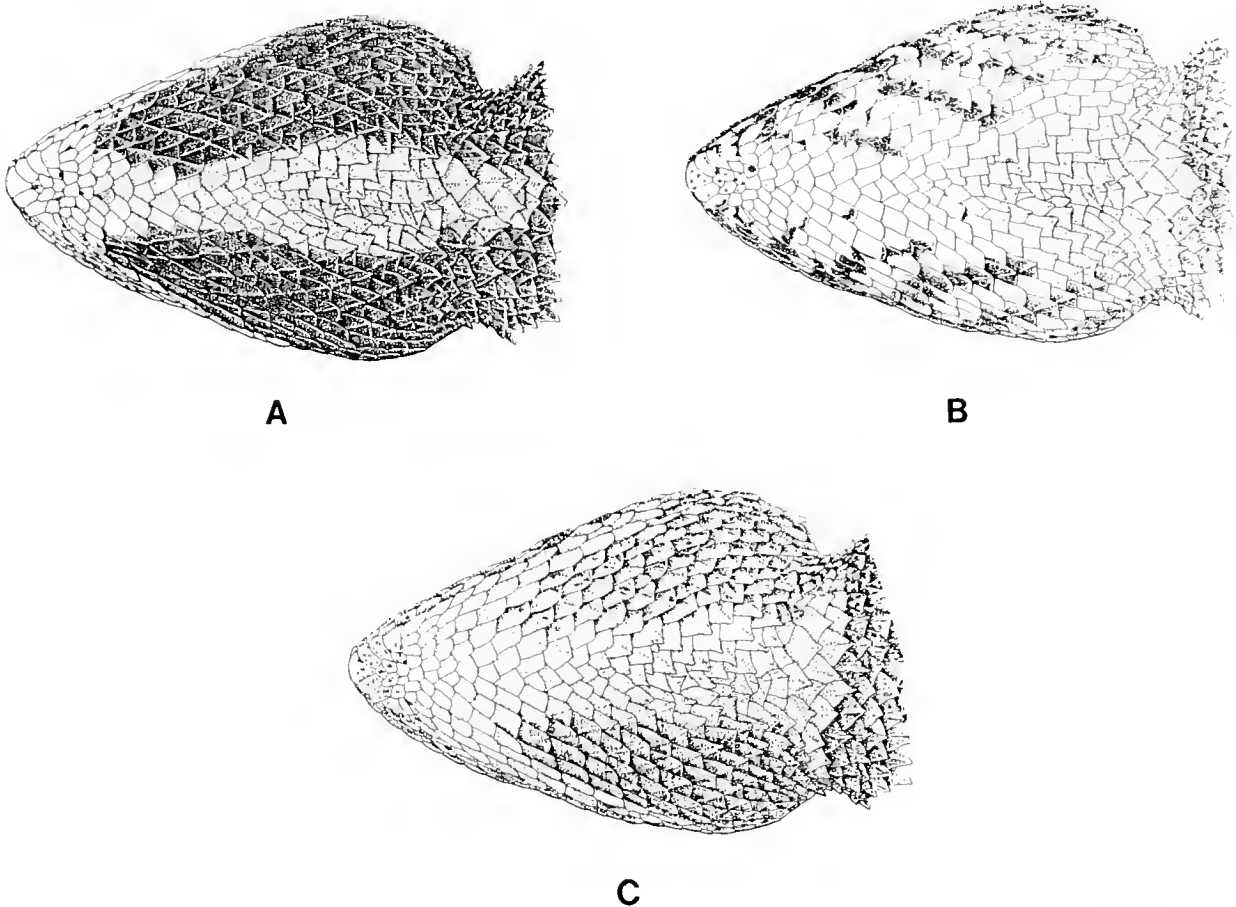


FIG. 11. Chin and throat color pattern in *Calotes v. nigrigularis*. A, FMNH/UF 79470, adult male, nr. Chergal, Manshera Dist., NWFP, Pakistan. B, FMNH/UF, juvenile male, Miandam, Swat Dist., NWFP, Pakistan. C, FMNH/UF 70503, adult female, Abbottabad, Abbottabad Dist., NWFP, Pakistan.

State, India; FMNH/UF 19884-5, New Delhi, India; FMNH/UF 78420, Multan, Multan Dist., Punjab Prov., Pakistan; ZSI 20798, 20800, Pali, Pali Dist., Rajasthan State, India; ZSI 1383, 13486, Ajmer, Rajasthan State, India; ZSI 20796, Jodhpur, Rajasthan State, India; BNHS 325-6, Wanothi, Kutch Dist., Gujarat State, India; BNHS 318 Bhavnagar, Rajkot Dist., Gujarat State, India; SMF 70074 Amritsar, Punjab State, India; SMF 61925, Bangalore, Karnataka State, India; SMF 55444, Meerut, Uttar Pradesh State, India; UMMZ 172083-90, Bhubaneswar, Orissa State, India; and BMNH 1923-3-445, Mirpur Sakro, Thatta Dist., Sindh State, Pakistan.

**Scutellation Characteristics.**—Overall ranges and means for the scale characters of the samples of *Calotes v. versicolor* examined are as follows (Details regarding

geographic variation in these parameters are found in the diagnosis above, in Figures 5-8, and in the text discussions regarding them): Upper head scales unequal, smooth to feebly keeled; two well-separated spines on each side of the back of the head above the tympanum; canthus and superciliary ridge sharp; 11-13 (mean 12.0) infra- and 11-15 (mean 12.3) supralabials; dorsal scales large, distinctly keeled, all pointing backwards and upwards, larger than the ventral scales, which are always strongly keeled and mucronate, in 35-52 scale rows at midbody (mean 44.5); subdigital laminae of 4th toe 20 - 27 (mean 23.1); gular scales behind mental to middle of eye 7 to 15 (mean 11.2).

**SVL and Color Variation.**—Juveniles have a dorsal color pattern like that of adult females, except that the ground color is grayish, rather than the usual brownish,

and the dorso-lateral stripes are usually dirty white, rather than yellow. The chin and throat are the same color and pattern as in adult females, except that the lateral black diagonal stripes are usually better defined (Fig. 11).

*Nomenclature.*—The type localities of both *C. vultuosa* Harlan (1825) and *C. gigas* Blyth (1853) (synonyms of *Calotes versicolor*) are Calcutta, West Bengal, India. Our studies show that *C. versicolor* from West Bengal exhibit a high level of character variation. They are excluded from our synonymy of *C. v. versicolor* on the basis that we cannot confidently place them in any named valid race at the present time, as they are intermediate between surrounding populations in many respects.

Murray (1886) reported *Calotes viridis* Gray (1846) from Upper Sindh, Baluchistan, Punjab, southern India and the Deccan Plateau (this reference not included in *C. versicolor* synonymy given by Smith 1935). Murray's identification of some material from southern Pakistan as *C. viridis* is clearly incorrect, for no specimens referable to this name have been found there by any of the several thorough herpetologists who have worked extensively in Sindh Province since that time. The species has been considered a probable synonym of *C. versicolor*; the species type locality is Madras; the type specimen is lost.

Because the original type locality of *Calotes versicolor* was imprecise, ("India"), Kuhl (1820) re-designated it as Pondicherry, India. It then follows that the peninsular Indian and Indus Valley (*sensu latu*) populations are to be given the name *Calotes versicolor versicolor*. In addition, we note an apparently distinct population of *C. versicolor* which occurs from Thailand, Myanmar, Assam, Sikkim, Darjeeling, and Nepal. However, we believe that taxonomic recognition of this population is not currently warranted until sample sizes are increased and fresher material becomes available for study. Specimens from this area differ from *C. v. versicolor* in having more mucronate dorsal scales, a lower

number of scales under the fourth toe, a higher number of gular scales, wider body crossbands, in having many adults and subadults (in addition to in juveniles) with dusky longitudinal stripes on the belly, and the adults having a smaller SVL. Specimens we recognize as intermediate between these populations and those typical of *Calotes v. versicolor* occur in parts of Nepal and West Bengal.

*Geographic and Vertical Range.*—*Calotes v. versicolor* does not occur above 2000 m in the Indian Ghats (this study). Populations from the Himalaya Mountains (racially not yet defined), from Garwhal, India east through Sikkim and Bhutan are found to 2500 m elevation (this study), but to only 1030 m in Indochina (Smith 1935).

*Calotes v. versicolor (sensu latu)* is distributed from the drier, more open forests of Sumatra and the Malay Peninsula north to near Hong Kong and Hainan, west through the mainland to southeastern Afghanistan, and eastern Iran, including the Andaman Islands and Sri Lanka. Additional study will undoubtedly lead to the recognition of additional races in the eastern parts of the range as here defined. If so, the nominate form *C. v. versicolor* will undoubtedly become restricted to those populations living in the lowlands of the Indo-Pakistan subcontinent.

*Calotes versicolor nigrigularis* ssp. nov.

*Holotype.*—FMNH/UF 79470 (Figs. 11, 12), adult male, on shrub on rocky hillside, Shargal, 20 km S Balakot, Manshera Dist., Northwest Frontier Province (lat. 34.3° N, long. 73.4° E), Pakistan. Pakistan Museum Natural Science field crew, June 15, 1990.

*Paratypes* (N 16, all from Pakistan).—AZAD KASHMIR PROVINCE: FMNH/UF 79049, Gulpur; Kotli Dist., FMNH/UF 79396, 81165, Red Fort, Muzaffarabad, Muzaffarabad Dist.; FMNH/UF 79472, Chalpani, Muzaffarabad Dist.; FMNH/UF 79494, Panyola, Poonch Dist.; FMNH/UF 79495,



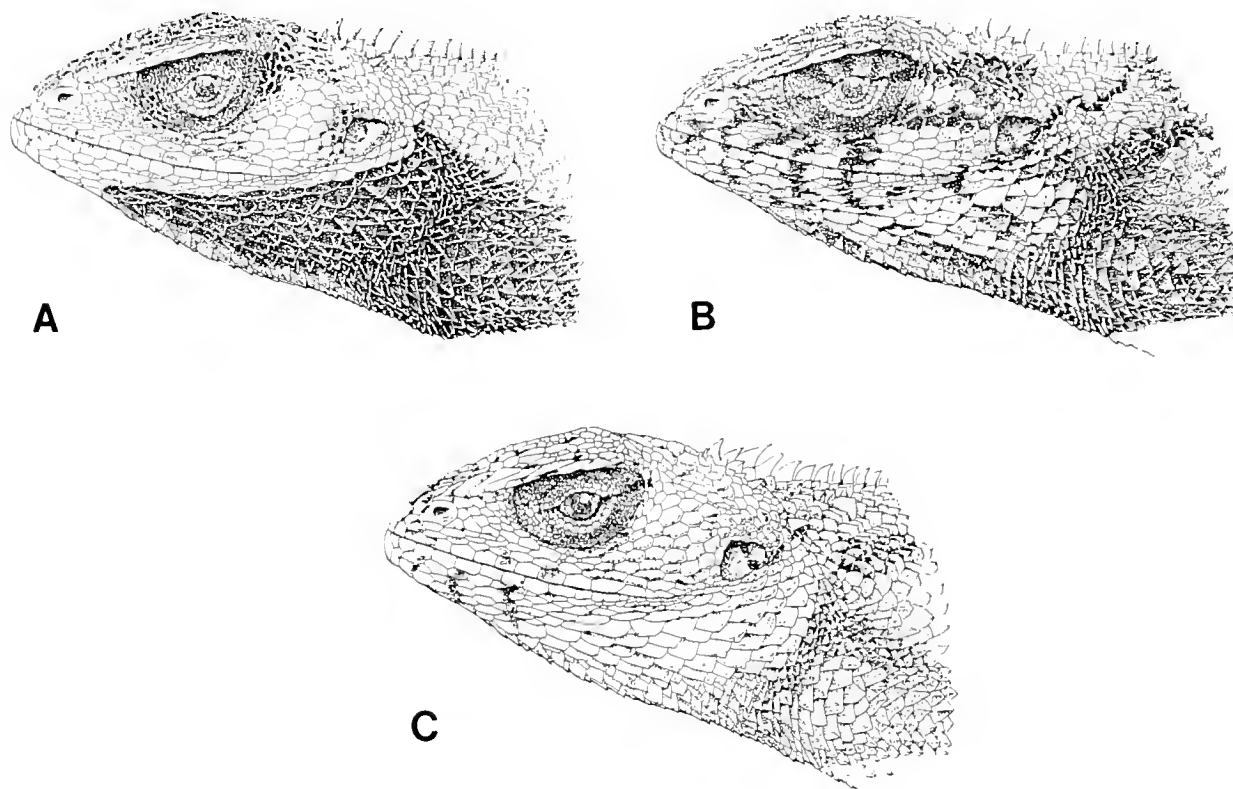


FIG. 12. Side views of head of adult *Calotes versicolor*. A, *C. v. nigrigularis*, FMNH/U 79470, adult male, Chergal, Manshera Dist., NWFP, Pakistan.. B, *Calotes v. versicolor*, FMNH/UF 78926, adult male, Mach, Quetta Dist., Baluchistan, Pakistan. C, *C. v. versicolor*, FMNH/UF 70516, adult male, Karachi, Karachi Dist., Sindh Prov., Pakistan.

Seri, Muzaffarabad Dist.; FMNH/UF 79601, Chela, Muzaffarabad Dist.; NORTHWEST FRONTIER PROVINCE: FMNH/UF 78944, Charsadda, Mandan Dist.; FMNH/UF 79229, Bahrain, Swat Dist.; FMNH/UF 79326, Miandam, Swat Dist.; FMNH/UF 79471 Khakai, Manshera Dist.; FMNH/UF 81133, 11 km W. Hungu, Togh Serai, Kohat Dist.; FMNH/UF 82243, Temargarh, Dir Dist.; FMNH/UF 70503, Abbottabad, Abbottabad Dist.; FMNH/UF 79462 Dhariyal, 4 km SW Balakot. PUNJAB PROVINCE: FMNH/UF 81136, Company Bagh, N. Tret, Rawalpindi Dist.; FMNH/UF 82242, 1.9 km SE Kohala, Rawalpindi Dist.

Other exemplary material (all juveniles, or in poor condition): AZAD KASHMIR: FMNH/UF 82244, Pottri, nr. Bhimber, Mirpur Dist.; PUNJAB PROVINCE: ZSD 1231, Ghora Gali, Rawalpindi Dist.;

FMNH/UF 82656, 11 km S Kohala, Rawalpindi Dist.; NORTHWEST FRONTIER PROV.: FMNH/UF 79138, Miandam, Swat Dist.; BNHS 313, Drosh, Chitral Dist.; FMNH/UF 82821, 1.1 km SW Garh Habibullah, Manshera Dist.; FMNH/UF 82822, 0.6 km SW Garh Habibullah, Manshera Dist.; BNHS 341, Parachinar, Kuram Dist.; FMNH/UF 81218, 13 km NE Abbottabad, Manshera Dist.; FMNH/UF 81093, 2 km W Hungu, Kohat Dist.; and FMNH/UF 82077, 5.8 km NW Khaki, Manshera Dist.

*Diagnosis.*—Conspecific with *Calotes versicolor* on the basis of its short head, the scales on the sides of the body pointing upwards and backwards, and that it lacks a fold or pit in front of the shoulder. It differs from the nominate race in having more strongly keeled (and usually more mucronate) body scales, more transverse scale rows at midbody, generally more

median gular scales from the tip of the jaw behind the mental to a level perpendicular to the middle of the eyes, fewer enlarged vertebral scales composing the in the nucho-dorsal crest, and in the adult state it lacks dark postocular stripes. During the breeding season the skin over the posterior part of the lower jaw in adult males (only) is jet black, except for a longitudinal median ventral band, which varies with season from pink to scarlet. The most vivid red color is found in the largest males during July and August. Each black gular patch extends (during the breeding season) posteriorly along the side of the neck, thence dorso-posteriorly at an upward angle to the vertebral line, including the entire shoulder region (Figs. 10, 12A). At the same time the entire head and dorsal neck surface are pinkish-red. In some individuals, both the red and black pattern may disappear at death.

Adult males of this race lack the greatly swollen jaw muscle mass of the nominate form, resulting in a head that in top view has more parallel posterior borders behind the eyes than that of *C. v. versicolor* (where these edges are clearly divergent). In many individuals the toes are shorter than in those of the nominate population and the brachium is usually as long as the antibrachium; in *C. v. versicolor* the antibrachium is often shorter.

*Description of the Holotype.*—Length of the head 1.48 times its width; snout broad, a little longer than the orbit; top of head from side slightly convex, slightly concave from the front; upper head scales unequal, smooth, to faintly keeled or tuberculate; canthus rostralis and supraciliary ridge sharp; two thin, spinous scales above the tympanum, the anterior one smallest, separated from the tympanum by about 5 scale rows; 13 supra- and infralabials; body somewhat compressed laterally; dorsal scales medium in size, distinctly keeled, most being mucronate, pointing backwards and upwards, larger than the ventrals, which are more strongly keeled and mucronate; 51 scales round the middle of the body. No gular pouch; gular scales like those of the ventrals, but larger. Nuchal

and dorsal crests developed, composed anteriorly of lanceolate spines, gradually decreasing in size to the base of the tail. Limbs moderate; fingers 3 and 4 almost equal in length; toe 4 longer than 3. Tail rounded, covered with more or less equal-sized, strongly keeled, mucronate scales.

The measurements (in mm) are as follows: total length 339; SVL 94; tail length 245; body length (axilla-groin) 47; greatest head length (snout tip to posterior extent of lower jaw) 34; greatest head width (across most posterior part of lower jaw) 23; greatest head height (just behind posterior edge of eye) 18.5; height of ear opening 3.1; length of brachium (axilla to elbow) 15; length of antibrachium (elbow to wrist) 13.6; posterior limb when extending anteriorly nearly reaches posterior edge of eye.

The dorsal ground color is more or less grayish-tan (pinkish-tan in life) over the posterior 2/3 of the body. From the level of the posteriorly extended elbows to and onto the base of the tail. The body is very indistinctly marked with 3 slightly darker cross bars. Anteriorly it is almost completely black, being lightest along the vertebral line. Ventrally it is dark gray from near a line connecting the anterior edges of the shoulder posteriorly to just before the insertion of the hind limbs, where the color changes abruptly to grayish cream. The hind limbs and tail base are more or less uniform above and below, matching the colors of adjacent body surfaces. On the dorsal caudal surface, from about the level of the posteriorly adpressed knee to slightly beyond the claw tips of the hind foot, faint darker cross bars can be discerned, fading posteriorly as the tail becomes suffused with dark gray from its middle to the tip. The front limbs from shoulder to claw tips are uniform grayish black. The sides of the neck are black, continuing anteriorly to the black color of the limbs and the sides of the body. The most intense black on the entire individual occurs from the anterior lateral surface of the neck anteriorly onto the jaws and gular region. This black jaw marking is distinctly set off from the lighter color of

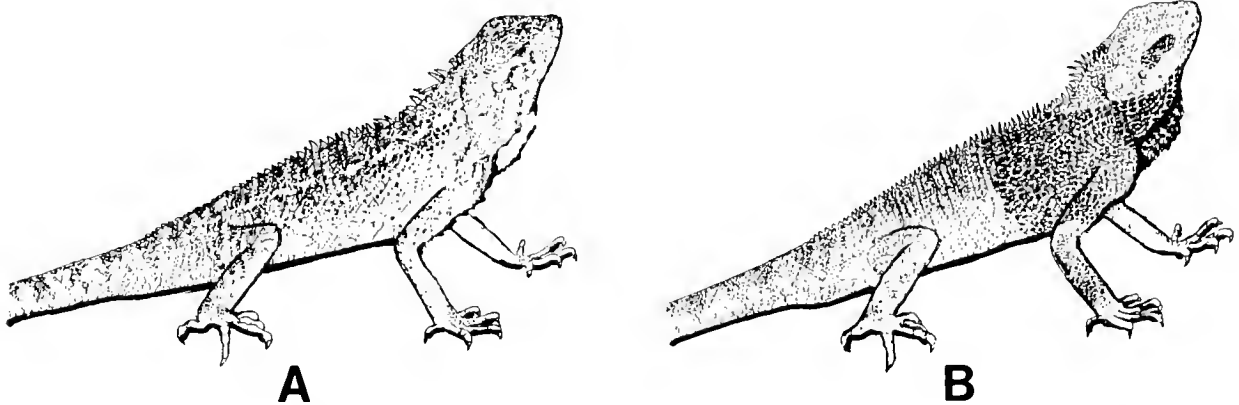


FIG. 13. General body color pattern of *Calotes v. nigrigularis*; A, adult female; B, adult male in breeding coloration.

the head and midgular areas, forming a more or less arrow-shaped black patch on each side (Fig. 10, 12A). Between the two black jaw patches is a dark pink (scarlet in life) median longitudinal band, beginning in the postmental region and extending onto the gular fold. The top of the head is medium gray, slightly mottled with grayish-tan. Laterally and posteriorly this fades into a pinkish-tan which covers all of the temporal areas and extends posteriorly in a V-shaped mark to above the shoulders. The eyelids above and below are light gray, with a nearly black spot in the anterior corner and a larger one in the posterior corner. Both the supra- and infralabials are light grayish-tan with faint grayish stripes radiating from the orbit. The lightest part of the body is in the area of the mental and surrounding shields. The most striking part of the entire color scheme is the black and scarlet gular pattern.

*Sex and Color Variation.*—Adult females lack distinct metachroic color changes (Fig. 13). The dominant dorsal color is gray to grayish-brown, usually with a narrow white to yellow dorsolateral stripe (sometimes represented by a series of dashes) from the neck to above the hind limb insertion. All these markings are variable in intensity and completeness. Along the vertebral area between the light stripes are 5 to 6 darker brown to black blotches or crossbands, 4 to 5 scales long, which in the largest females fade into the ground color. The ground color of the

gular region is white or pale gray to pink (latter during the breeding season only). There are no large jet-black gular patches as found in adult males, though the base of the scales in this area may be dark gray (Fig. 11). Frequently the gular area is also marked with 5 to 7 more or less distinct black lines or dashes running postero-medially from the infralabials toward the midline.

The reddish throat of adult males is first evident in individuals about 50 mm SVL (FMNH/UF 82656), i.e., at the end of the first year of life. The ventral surface of neonates of both sexes (mean ca 37 mm SVL), through nearly the entire first year is uniform dirty white. The smallest male with well defined black jowls with a red median area has a SVL of 76 mm (FMNH/UF 82623).

*Distribution.*—This subspecies is restricted to the foothills and outliers of the Himalaya Mountains, from the Jhellum and Neelam River Valleys of Azad Kashmir, Pakistan, west to the Hindu Kush Mountains and foothills bordering the Kabul River Valley in southeastern Afghanistan, south to include the Safed Koh Range on the Pakistan-Afghanistan border (Fig. 1). It may extend further south to Waziristan or even Quetta, but this will only be proven with fresh material (see below). Within this area the race is apparently restricted to subtropical chir pine (*Pinus roxburghii*) and oak (*Quercus*

*incana*) forests, which are found at elevations between 1000 and 3000 m, depending on exposure and slope conditions.

Individual character states of the plains race *C. v. versicolor* extend into the foothills along several of the larger rivers. Such changes coincide with the general floral change from the plains into the foothills. The Indus Valley (which further specimens may prove completely divides *C. v. nigrigularis* into isolated eastern and western populations) is an example (see remarks of intergradation under *C. v. versicolor*). Such intergrade populations nearly bisect the mountain range of *C. v. nigrigularis* in the Kabul River Valley near Peshawar (specimens in ZSDP, uncat.). We have not yet found any intermediate populations in the valleys of the Neelum or Jhellum Rivers. However, intergrades do occur near the foot of the Himalayan front range (Taxila and Islamabad, PMNH uncat.). The fact that specimens somewhat intermediate between the two races have been found as far south as Khuzdar, Baluchistan (BMNH H 1964-276-8) suggests that *C. v. nigrigularis* may eventually be found throughout the Quetta area and the Central Brahui Range as well, though no fresh specimens are available for study at this time.

The only other intensive study of geographic variation of character states in Pakistan with which these results can be compared is our earlier study of *Echis carinatus* (Auffenberg and Rehman 1991). Like *Calotes versicolor*, this species is found over virtually all of Pakistan except the higher mountains. *Calotes versicolor* does not, however, occur in the sandy deserts of northwest Pakistan.

In the *Echis* study we analyzed 12 characters. While each of these demonstrate a unique pattern of geographic variation, several features common to most of them stand out. These comprise what we believe to be five major centers of adaptive speciation in *Echis carinatus* - Transcaaspia, Iranian Plateau, Astola Island, Indo-Gangetic Plain, Himalayan foothills,

and the Cholistan-Thar Desert. Of these, the first three are essentially extralimital from the standpoint of the current study. The remaining three areas (Himalayan foothills, Indo-Gangetic Plain and Cholistan-Thar Desert) are also recognizable on the basis of distinctive character states, or combination thereof, in the characteristics of *Calotes versicolor* populations studied in this report. Thus, the Himalaya foothills populations are distinguishable from those of the Indo-Gangetic Plain and Cholistan-Thar Desert on the basis of several significantly different scale characters as well as a strikingly different metachroic color and pattern change in the adult males during the breeding season. Likewise, the Desert populations are different at statistically significant levels from those of all the surrounding Indo-Gangetic Plains populations in regard to certain scale characters. Though the recommended nomenclatorial designation for these *Echis* and *Calotes* populations is different in each case, the correspondence of similar geographic patterns of variation is certainly important from the standpoints of both zoogeography and speciation in the subcontinent.

We have not found any evidence for the curious mosaic of mean character states found in the Indus Delta region, as we did for *Echis carinatus*. The reason may be related to the fact that *Calotes versicolor* is often found in riverain forests, so that river and channel changes may be less important as an isolating mechanism in this species than in *Echis carinatus*.

### Acknowledgments

We particularly thank the United States Fish and Wildlife Service (Washington), the Deutscher Akademischer Austauschdienst (Bonn, Germany), and the Office of Sponsored Research, University of Florida for providing funds to conduct this study. To all curators and collection managers of the institutions listed above, we extend our sincere thanks for the many ways in which they have contributed to the success of this project. Finally we wish to

acknowledge the support offered by our respective institutions.

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- ### APPENDIX 1
- Localities (to district only) from which specimens were examined, the museum collections in which they are found, and the number studied (in parentheses).
- AFGHANISTAN: BMNH (3); Jalalabad CAS (3).
- BANGLADESH: Chittagong MCZ (1).
- INDIA: **Assam State:** Chabus AMNH (1), Goalpara Dist., Raimona FMNH (3); **Behar State:** Benares BMNH (8), Patna BMNH (1); **Gujarat State:** Baroda MCZ (2); Bhaunagar BNHS (1), Hingolghad BNHS (2) Rajkot BNHS (1), Kutch BNHS (2); **Himachal Pradesh State:** Amballa MCZ (2); Kulu Valley MCZ (2); **Jammu-Kashmir State:** Jammu BMNH (1); **Karnataka State:** Bangalore SMF (1); **Maharashtra State:** AMNH (1), Bombay FMNH/UF (7); **Orissa State:** Bhubaneswar UMMZ (13); **Punjab State:** BMNH (1), Amritsar SMF (1), Amballa Dist. nr. Kalka AMNH (3); **Rajasthan State:** Ajmer ZSI (2), Bikaner ZSD (1), Pali ZSI (1), Jodhpur BNHS (1) CAS (2), SMF (1), ZSI (3) Mt. Abu/Abu Rd. CAS (1), AMNH (1); Nagaur ZSI (2), Jaipur ZSI (4); **Tamil Nadu State:** Madurai FMNH/UF (7); **Uttar Pradesh State:** nr. Chalti BMNH (1), Delhi FMNH/UF (2), Kanpur AMNH (1); Pitharagah (Kumaon) BMNH (1), Meerut SMF (1), Mussoorie ZSI (1); **West Bengal State:** Calcutta UMMZ (1), FMNH (2), MCZ (6), Darjeeling MCZ (1), Kalimpong Dist. Tarkhala MCZ (1).
- HONG KONG: BMNH (3).
- MYANMAR ("Burma"): Rangoon FMNH/UF (27); Arakan FMNH/UF (4); Mandalay FMNH/UF (2); "at Chinese border" BMNH (1).
- MALAYSIA: **Penang State:** Penang FMNH/UF (8).
- NEPAL: BMNH (10), Katmandu SMF (1), Swayabonath SMF (1), Lapha Kamali Valley BMNH (1), Rasna Dist. BMNH (1), Maewa-Khola BMNH(12).
- PAKISTAN: **Azad Kashmir Prov.:** Muzaffarabad Dist. SMF (1), FMNH/UF (5); Kotli Dist. FMNH/UF (2); Poonch Dist. FMNH/UF (1); Mirpur Dist. FMNH/UF (2); **Baluchistan Prov.:** Kalat Dist., AMNH (1), BMNH (2), ZSI (1); Las Bela Dist. AMNH (4), FMNH/UF (2); Panjgur Dist., MCZ (1); Quetta Dist. FMNH/UF (1); Khuzdar Dist. BMNH (3); Waziristan Dist. BNHS (1); **Northwest Frontier:** Chitral Dist. BNHS (1); Dir Dist. FMNH/UF (1); Abbottabad Dist. ZSD (1), FMNH/UF (1); Kohat Dist. FMNH/UF (1); Kuram Dist. BNHS (1); Manshera Dist. ZSD (2), FMNH/UF (7); Peshawar Dist. BNHS (1), ZSDP (3), Swat Dist. FMNH/UF (6); **Punjab Prov.:** Dera Ghazi Khan Dist. FMNH/UF (2); Lahore Dist. ZSI (1); Kohat Dist. FMNH/UF (1); Multan Dist. ZSDM (4), FMNH/UF (1); Chakwal Dist. ZSI (2); Bahawalpur Dist. ZSDM (3); Rawalpindi Dist. ZSD (1), CAS (1), FMNH/UF (3); **Sindh Prov.:** FMNH (1); Badin Dist. ZSD (1); Dadu Dist. AMNH (1), ZSD (1), FMNH/UF (4); Hyderabad Dist. AMNH (7), BMNH (1), ZSD (1); Karachi Dist. AMNH (4), BMNH (4), CAS (7), FMNH (4), UMMZ (4), SDSNH (1), FMNH/UF (91), ZSD (30), ZSI (2); Thatta Dist. AMNH (7), UMMZ (1), ZSD (6), FMNH/UF (2); Thar Parkar Dist. BNHS (1), ZSD (2).
- REPUBLIC OF THE MALDIVE ISLANDS: Addu Atoll BMNH (2), Baras Isl. BMNH (1); Hulalag Isl. Bmnh (3); RAF BAsE BMNH (1).

SIKKIM: BMNH (1); Mangpu FMNH (34),  
Teesta Valley MCZ (2).

THAILAND: **Bangkok Prov.:** FMNH/UF (2);  
**Chiang Mae Prov.:** FMNH/UF (5); **Mae  
Hong Son Prov.:** FMNH/UF (1); **Udon  
Thani Prov.:** FMNH/UF (1); **Yala Prov.:**  
FMNH/UF (1); **Phattabung Prov.:** FMNH/UF  
(1).

## Holocene anurans from Caucasus

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**Abstract.** -Holocene deposits of the Kudaro I Cave from the vicinity of Ertso Lake (South Ossetia, NW Caucasus) yielded, among others, rather numerous disarticulated anuran bones. Examination of this sample revealed that majority of this material belongs to the genus *Bufo* and to the family Ranidae. This generally corresponds to the composition of the contemporary anuran fauna of that region.

**Key Words:** Anura, Holocene, Caucasus, osteology.

### Introduction

The material described in the present paper was recovered from the deposits of the cave Kudaro I. The cave is on the slope of Mt. Chasavalskaya (1600 m altitude), valley of Dzhordzhoři River, in the vicinity of Ertso Lake, about 90 km NE from the town Kutaisi, South Ossetia, near Rachinsky ridge in the NW Caucasus (approx. 42° N, 43° W; see also Lyubin 1980a). The deposits are of the Holocene age (Lyubin 1980b).

First description of the anuran and reptile material from this cave was published by Darevsky (1980). His taxonomic assignments generally agree with those in the present paper. The material consists of isolated bones; anuran bones described in this paper are deposited in the Zoological Institute of the USSR Academy of Sciences, St. Petersburg, under collection numbers ZIL/EL/1 - ZIL/EL/185.

Anatomical terminology mostly follows that of Bolkay (1919) and Gaupp (1896). It should be noted that most of elements in the sample are postcranial bones and only few cranial bones are present. Fragmentary material bearing no diagnostic characters was excluded from the account below.

### Systematic Paleontology

*Bufo* sp.

Material: Left scapula, ZIL/EL/7 (Fig.

7B). Probably also ZIL/EL/140 (Fig. 7A).

**Description:** The margin of the cavitas glenoidalis is elevated. Although both the pars acromialis and proc. glenoidalis are broken off it is obvious that there was a deep incision between them. There is a moderately prominent and rather pointed outgrowth on the margo anterior. Both scapulae are comparatively big elements corresponding by their size to the below described humeri and ilia.

**Material:** Humeri ZIL/EL/21 (Fig. 2B), ZIL/EL/25, ZIL/EL/55,

ZIL/EL/79, ZIL/EL/112 (Fig. 2A), ZIL/EL/132 (Fig. 2C), ZIL/EL/139, ZIL/EL/153, ZIL/EL/173.

These specimens (except for ZIL/EL/153 that includes also the most distal section of the crista ventralis) are preserved only as distal parts of the humerus. All of them are characteristic by conspicuous degree of development of the epicondylus medialis, the distal margin of which extends almost to the level of the distal margin of the caput humeri. Hence, there is a distinct notch between the both structures that can continue onto the dorsal surface of the distal section of the bone. On the ventral surface of the medial epicondylus one can discern an indistinct ridge running onto its distal surface. The lateral epicondylus is developed in lesser degree, extending laterally from the outline marked by the crista lateralis in some specimens (see Fig.

2A, B). The lateral surface of the caput humeri is worn away in larger specimens, so the ball is not complete. The whole distal end of the bone is bent ventrally; this is correspondingly reflected on its dorsal surface which is convex along its longitudinal axis. Some variation may be observed concerning the extent of the medial and lateral cristae which might be, however, assigned to secondary sex differences. This might be suggested also by small specimen ZIL/EL/55 which may represent an immature individual, and in which both cristae are lacking. On the other hand, all specimens have their crista medialis directed laterally, so its ventral surface meets the medial surface of the diaphysis almost perpendicularly. In ZIL/EL/25 and ZIL/EL/132 the margins of both cristae are rather undulated and thickened.

**Discussion:** These humeri (preserved only as distal sections) are morphologically closest to those of *Bufo*. In large with the determination of size-corresponding ilia. However, some (esp. smaller) humeri may fall into the variation range of the ranids, but the latter assignment lacks reliable foundation if only distal part of the bone is at the disposal. Discoglossids may be excluded because their medial and lateral cristae are confluent with the diaphysis, with no distinct border. The only fossil anuran that is of similar size as ZIL/EL/112 is *Latonia seyfredi* v. Mayer (= *Discoglossus giganteus* Wettstein-Westerheimb). However, its morphology and stratigraphic range are different (see e.g. Mlynarski, 1976, pl.I/4).

**Material:** Radioulnae ZIL/EL/43, ZIL/EL/62 (Fig. 8A), ZIL/EL/106 (Fig. 8B), ZIL/EL/116, ZIL/EL/120 (Fig. 8C), ZIL/EL/162.

**Description:** The margin of the olecranon rimming the articular cavity is formed either by calcified cartilage or ossified tissue lacking periosteal bone. The border between the smooth periosteal bone and rough surface rimming (and also covering) the articular cavity is distinctive. It seems that this most proximal part of the

olecranon may be abraded in large specimens (e.g., ZIL/EL/62).

On the inner surface of the bone, close to the point where the margins of the articulation cavity of the olecranon and capitulum meet with one another, is a small but deep pit. Similar pit is lacking or not so deep in ranids, but regularly present in *Bufo*. It serves as a muscle insertion area and in some specimens may be doubled. The posterior margin of the bone (i.e., of its ulnar part) bears an indistinct crista in some specimens.

**Material:** Iliia ZIL/EL/41, ZIL/EL/66, ZIL/EL/75, ZIL/EL/94 (Fig. 4A), ZIL/EL/97, ZIL/EL/99 (Fig. 4B), ZIL/EL/124, ZIL/EL/129.

**Description:** The ala ossis ilii, if compared with the posterior part of the bone, are stout (ZIL/EL/94) or rather slender (ZIL/EL/99). Their dorsal margin is rounded, only in the mid-part it becomes an edge distinctly bent medially along its whole extent. In its anterior part, the ala is compressed dorsoventrally, ellipsoid in cross-section. On the medial surface of the ala, approximately at the level of the highest point of their arch, there is an orifice of the narrow horizontal canal coming onto the bone surface from the posterior. There is certain variation in the location and morphology of this canal - it may continue as a groove for a short distance anteriorwards, and the orifice may be located on the bottom of a horizontal depression developed below the above-mentioned edge. The depression may terminate anteriorly on the dorsal surface of the ala or, in some specimens (esp. smaller ones), the orifice is located posterior to the depression. The torus superior is extensive, with two to three tubercles continuing (except the most anterior one) as a short and low ridges laterally. The anterior-most tubercle continues as a short rounded ridge anteroventrally, onto the medial surface of the bone. ZIL/EL/124 (and some other ones) is much smaller but except for size its morphology corresponds in all principal features to that described above.



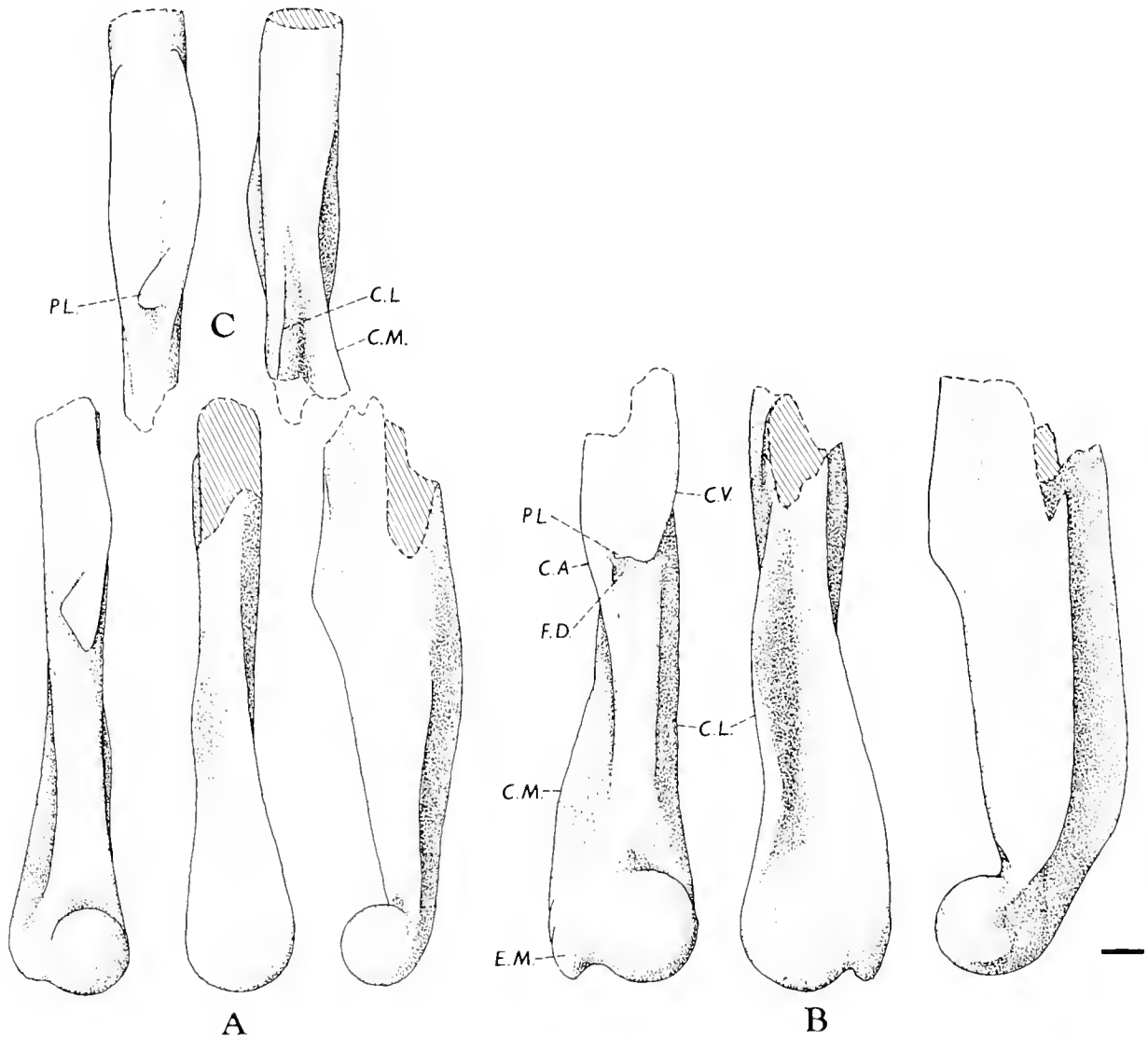


FIG.1. A- *Rana* sp. (ZIL/EL/148). Left humerus in ventral, dorsal and lateral views (from the left to right). B- *Rana* sp. (ZIL/EL/151). Left humerus in ventral, dorsal and lateral view (from the left to right). C- *Rana* sp. (ZIL/EL/74). Ventral (left) and dorsal (right) view of the proximal section of the right humerus (drawing reversed for comparison). Bar equals 1mm. Abbreviations: C. A. - crista adventiva; C.L. - crista lateralis; C. M. - crista medialis; C. V. - crista ventralis; E. M. - epicondylus medialis; F. D. - fossula dividens; P. L. -processus lingualis.

Discussion: The shape of the ilium corresponds to that in contemporary *Bufo bufo* and *B. viridis*. The only difference is that in both latter forms the longitudinal depression on the medial surface of the ala is developed in much lesser degree (due to lesser extent of the edge). It should also be noted that the orifice of the mentioned canal on the medial surface of the bone displays certain variation in contemporary forms (the

orifice may be doubled, and the differences in this respect may be found also between the left and right ilium of a single individual). The same seems to hold for fossil material. Size differences may be ascribed either to interspecific variation or to differences between both sexes (the latter may reach quite a high degree in some contemporary representatives of the genus *Bufo*).

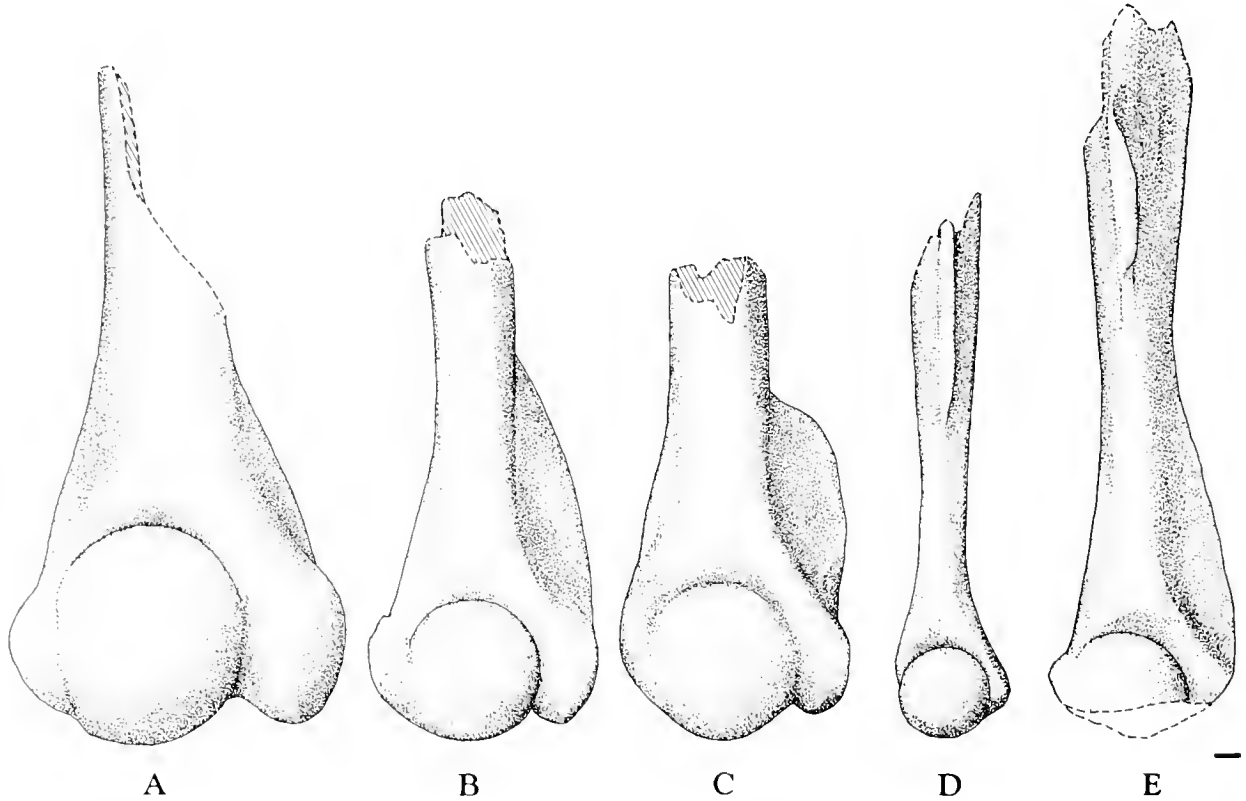


FIG. 2. A- *Bufo* sp. Right humerus (ZIL/EL/112). B- *Bufo* sp. Right humerus (ZIL/EL/21). C- *Bufo* sp. Left humerus, drawing reversed for comparison (ZIL/EL/132). D- Ranidae indet. Right humerus (ZIL/EL/150). E- Ranidae indet. Right humerus (ZIL/EL/110). Bar is 1 mm.

### *Rana* sp.

Material: Humeri ZIL/EL/56, ZIL/EL/74 (Fig. 1C), ZIL/EL/83, ZIL/EL/128, ZIL/EL/148 (Fig. 1A), ZIL/EL/151 (Fig. 1B), ZIL/EL/179.

Description: The crista ventralis and crista adventiva delimit a wide, shallow and rather longitudinal depression for muscle insertion. The latter crista may be developed to various degree, whereas the crista ventralis is well developed in nearly all individuals, with a distinctive lingual process (only in ZIL/EL/128 this process is poorly developed, and the crista ventralis continues distally as a gradually lowering ridge). The crista ventralis has a hollow inside its free margin; consequently, it is thinner along its attachment to the diaphysis. This is manifested externally by grooves along the insertion of the crista, on either side. The crista lateralis and medialis are directed dorsally, forming thus a

longitudinal groove on the dorsal surface of the bone. The proc. lingularis and the outgrowth produced by the crista adventiva may form together a roof over the fossula dividens; this nearly results in a canal. The caput humeri is well prominent ventrally (clearly seen in lateral aspect). Although the distal part of the bone is straight, the crista medialis and lateralis make it seemingly "S" shaped. Lateral epicondylus is entirely absent. Other features may be seen in Fig. 1B.

Some variation may be observed, mainly in the degree of development of the lingual process and in the extent of the medial and lateral cristae, as well as of the crista adventiva.

Anatomical notes: As may be deduced from the condition in *Rana esculenta* (Gaupp, 1896) the depression between the crista ventralis and adventiva could serve as an area of insertion for three heads of the

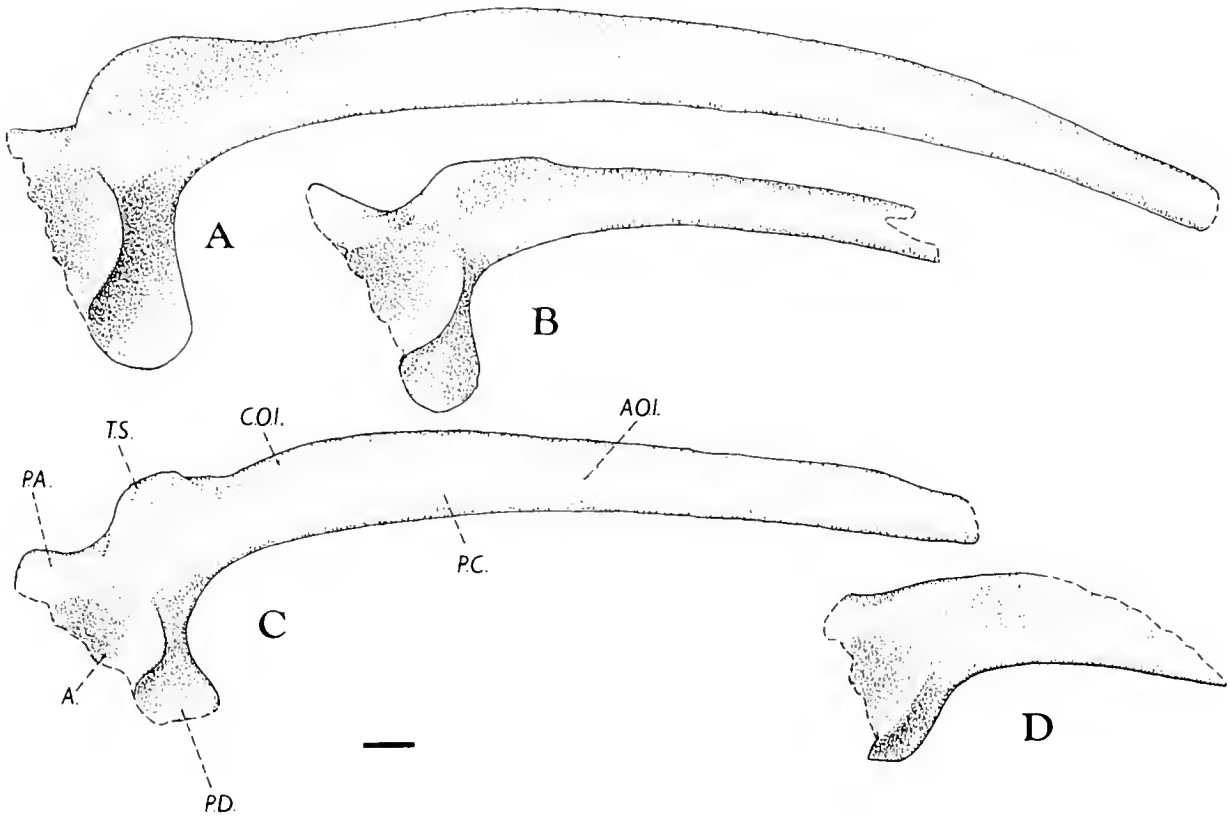


FIG. 3. A- Ranidae indet. Right ilium in lateral view (ZIL/EL/87). B- Ranidae indet. Left ilium in lateral view (ZIL/EL/1). C- Ranidae indet. Left ilium in lateral view (ZIL/EL/14). D- Anura indet. Left ilium in lateral view (ZIL/EL/3). B, C, and D reversed for comparison. Bar equals 1mm. Abbreviations: A. - acetabulum; A. O. I. - ala ossis ilii; C. O. I. - crista ossis ilii; P. A. - pars ascendens; P. C. - pars cylindriciformis; P. D. - pars descendens; T. S. - tuber superius.

m. pectoralis (portio epicoracoidea, sternalis and abdominalis), whereas the proximal part of the crista itself (its edge) could serve for attachment of two heads of the m. deltoideus (pars clavicularis and scapularis). The third head of the deltoideus muscle (pars episternalis) is fixed to the ventral ridge of the medial epicondylus. The fossula dividens probably served for tendon of the m. coracoradialis. The groove between both the crista medialis and lateralis served no doubt for insertion of the caput profundum of the m. anconeus. The medial crista serves in anurans for attachment of the m. flexor carpi radialis and the lateral crista for the m. extensor carpi radialis. The former has its special function in amplexus. Consequently, the crista medialis is usually better developed in males, and the degree of

its development is considered secondary sex character.

Taxonomic notes: Humeri recalling those described above may be found in some individuals of contemporary "brown" frogs, i.e. of *Rana temporaria* *R. arvalis*, *R. dalmatina*, *R. latastei*, and *R. macrocnemis*. I found morphology closely resembling that in ZIL/EL/151 (Fig. 1B) in contemporary *Rana arvalis* (DP FNSP 5830) from S Bohemia (Czechoslovakia), in *R. arvalis* *wolterstorffii* (DP FNSP 6264) from Soroksar (Hungary), and in *R. latastei* (DP FNSP 6419) from Como (Italy). In other individuals, the crista and the lingual process were developed to various degree, similar to the condition in the described fossil material. In all cases these humeri belonged to males. Hence, it

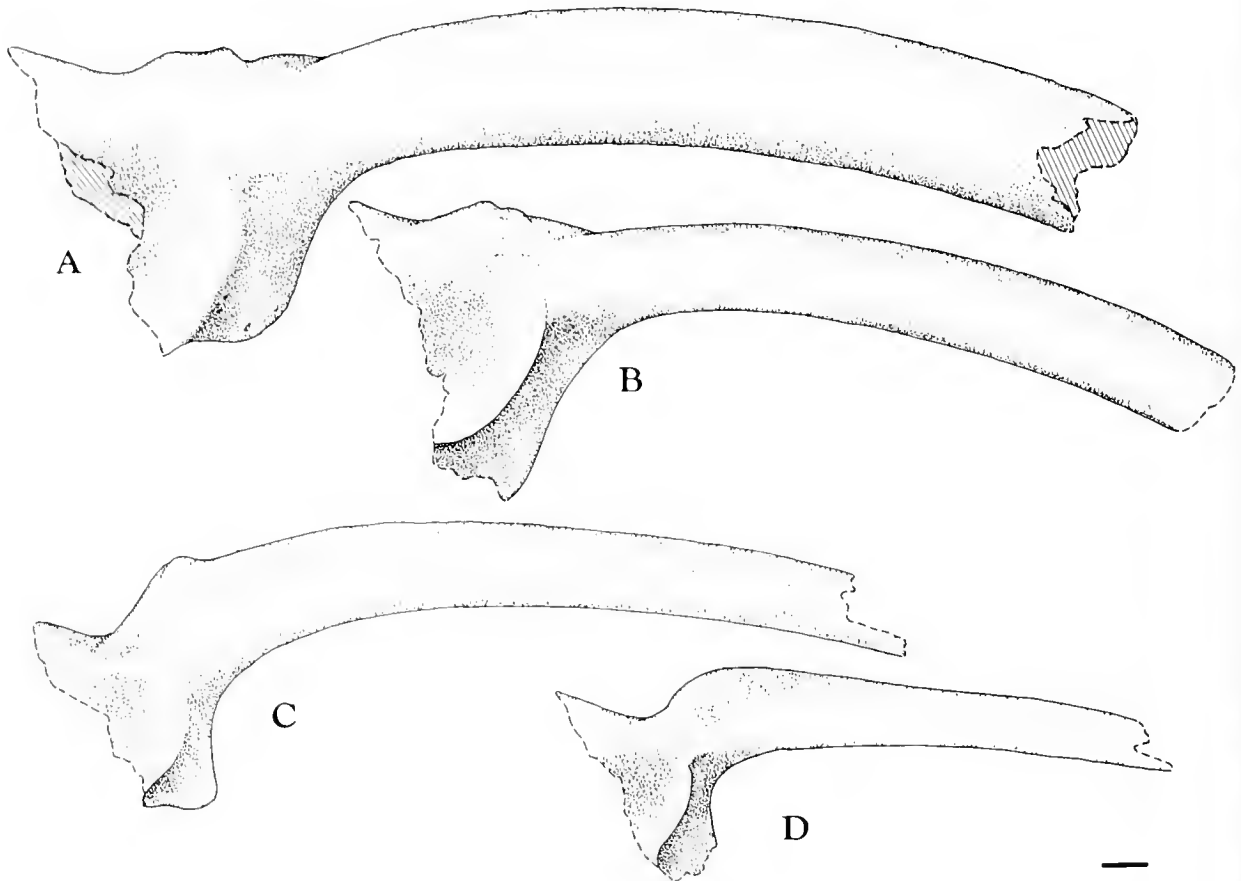


FIG. 4. A- *Bufo* sp. Right ilium in lateral view (ZIL/EL/94). B-*Bufo* sp. Left ilium in lateral view (ZIL/EL/99). C- Ranidae indet. Left ilium in lateral view (ZIL/EL/68). D- Ranidae indet. Right ilium in lateral view (ZIL/EL/111). B and C reversed for comparison. Bar equals 1 mm.

may be concluded that the described characters on the humerus may be ascribed to sexual dimorphism, but they are not present in all males. In any case, relations to contemporary "brown" ranids seems to be beyond any doubt. It is quite possible that the above described humeri assigned to *Rana* sp. and humeri (and other elements) described below as Ranidae indet. might belong to a single form.

#### Ranidae indet.

Material: Right coracoid, ZIL/EL/54 (Fig. 7E).

Description: The intumescencia glenoidalis is circular, with distinct but small fovea acetabuli where the ligament of the humerus is inserted. The fovea is

surrounded by marginal part for the cartilago paraglenoidalis that connects this bone with the proc. glenoidalis scapulae. The pars epicoracoideal is nearly regularly arch-like, exceeding by its antero-posterior diameter the lateral part of the bone.

Material: Humeri ZIL/EL/110 (Fig. 2E), ZIL/EL/150 (Fig. 2D), ZIL/EL/168.

Description: The crista ventralis humeri in ZIL/EL/110 (and in ZIL/EL/168 that is similar) is prominent, gradually lowering distally. Part of its margin is tongue-like bent medially. Only within the proximal section of the crista there is a groove along the medial surface of its basis. The crista medialis is well developed, but only in the distal third of the bone. The lateral crista is developed in lesser degree. The medial

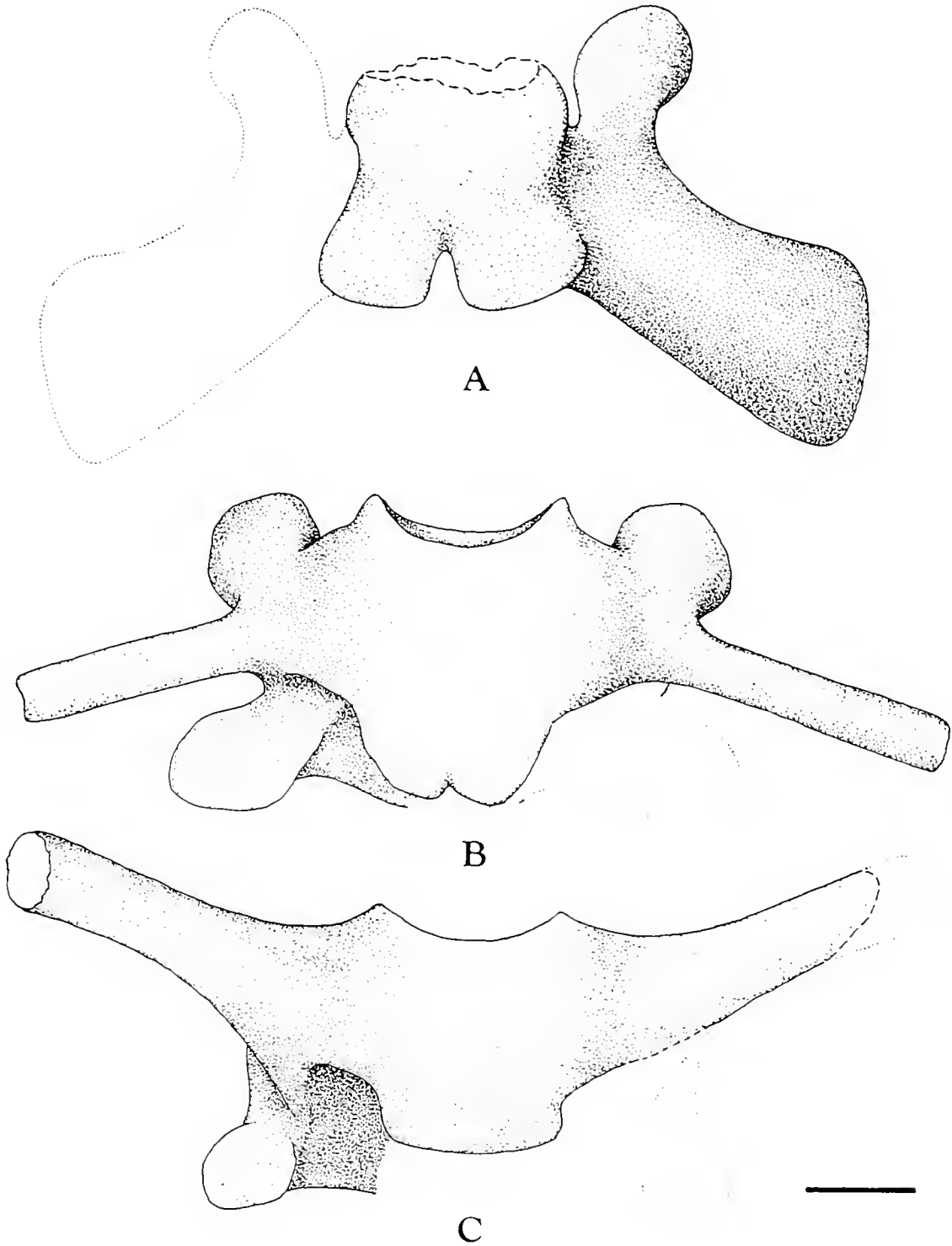


FIG. 5. Ranidae indet. Vertebrae in ventral view. A- Sacral vertebra (ZIL/EL/10). B- V6 (ZIL/EL/70). C- V2 (ZIL/EL/8). Bar equals 1 mm. Fig.6 Ranidae indet. Praesacral vertebrae in dorsal (above) and ventral (below) views. A- V5 (ZIL/EL/17). B- V2 (ZIL/EL/125). Bar equals 1 mm.

epicondylus is well developed, the lateral one is rudimentary. On the medial surface of the diaphysis there is low but discernible crista. Specimen ZIL/EL/150 has its ventral crista well developed, with rounded and almost straight margin. It is thin along its attachment to the diaphysis. This, together with the fact that the diaphysis is oval in cross-section, caused that there is a groove along the basis of the crista, especially well developed on its medial surface. Neither medial nor lateral crista are developed in this specimen, and the lateral epicondylus is absent, too. In spite of these differences, both humeri may be assigned to the Ranidae.

Material: Iliia ZIL/EL/1 (Fig. 3B), ZIL/EL/2, ZIL/EL/14 (Fig. 3C), ZIL/EL/18, ZIL/EL/19, ZIL/EL/28, ZIL/EL/29, ZIL/EL/49, ZIL/EL/68 (Fig. 4C), ZIL/EL/87 (Fig. 3A), ZIL/EL/111 (Fig. 4D), ZIL/EL/135, ZIL/EL/164, ZIL/EL/177. Specimens ZIL/EL/2, ZIL/EL/19, ZIL/EL/29, ZIL/EL/135 and ZIL/EL/177 are similar to ZIL/EL/111 (Fig. 4D) in that the tuber superius is continuous with the crista ilii.

Description: The crista ossis ilii (vexillum of some authors) and the pars cylindriformis can be well distinguished in medial view, whereas only with some difficulties in lateral view. The crista does not reach up to the anterior end of the ala. It is directed dorsally in its section adjacent to the tuber superius, and bent dorsomedially in its anterior portion. Consequently, it forms wide groove on the medial surface of the ala, roofed dorsally by the edge of the crista, and ventrally delimited by the pars cylindriformis. The tuber superius is prominent above the level of the crista (but not in ZIL/EL/111), however, its margin corresponds by its thickness to the edge of the latter. Only posteriorly the tuber has a rounded margin, declined rather laterally. In specimen ZIL/EL/68 the tuber is prominent but not extensive; it has conspicuous ridge running down obliquely from its top. There is a distinct depression between the tuber and pars cylindriformis. The depression extends to the dorsal margin of the ala,

separating thus the tuber from the crista. The acetabulum is with acute and prominent margins, considerably lifted above the pars descendens, but rather slanting down towards the pars ascendens. However, even here the margin of the acetabulum is represented by a distinct ridge. There is a considerable notch between the tuber superius and the dorsal margin of the pars ascendens. The dorsal margin of the pars ascendens continues anteroventrally onto the medial surface of the bone as a rounded ridge that disappears after a short distance. ZIL/EL/1 (Fig. 3B) is essentially the same but the crista is much lower than is the dorsoventral diameter of the pars cylindriformis, in whole its extent anterior to the tuber superius. Anteriorwards it is even getting lower, so its transition into the dorsal margin of the pars cylindriformis is indistinct. The tuber is prominent and extensive anteroposteriorly.

Anatomical notes: The lateral surface of the crista ossis ilii is an insertion area for the m. iliacus externus, the other end of which is fixed by a tendon to the proximal part of the femur (Gaupp, 1896, figs 104, 105). The inner surface of the crista is occupied by the m. coccygeo-iliacus that runs to the urostyle. The iliacus externus muscle is, together with the iliacus internus, one of the most robust pelvic muscles in ranids and perhaps it plays an important role in jumping, despite of the fact that its tendon is fixed close to the proximal end of the femur. The tuber superius serves for attachment of the m. gluteus magnus, m. ilio-fibularis, and m. ilio-femoralis. The first is the most robust muscle of the dorsal side of the thigh, and together with other heads of the m. triceps femoris it stretches the knee joint. All muscles that are inserted on the tuber superius are important for locomotion.

Discussion: Contemporary European ranids mostly have the torus superior ilii continuous with the crista ilii, regardless if they belong to the esculenta or temporaria complexes. However, certain variation exists in this respect, so one can find individuals with prominent torus also in those forms in which it is continuous with

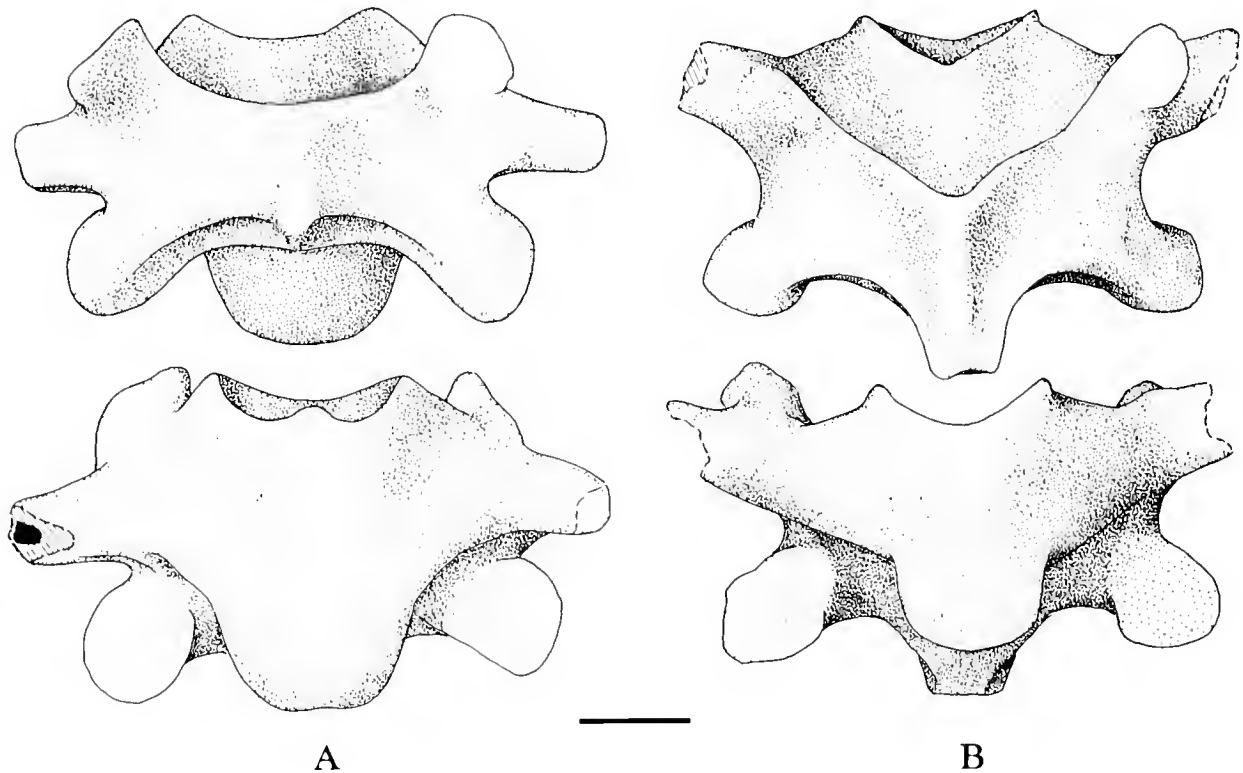


FIG. 6. Ranidae indet. Prae sacral vertebrae in dorsal (above) and ventral (below) views. A- V5 (ZIL/EL/17). B- V2 (ZIL/EL125). Bar equals 1 mm.

the crista in most individuals. This is why more precise assignment is difficult.

Material: Praesacral vertebrae V2 - ZIL/EL/8 (Fig. 5C) and ZIL/EL/125 (Fig. 6B); V3 - ZIL/EL/9; V5 - ZIL/EL/17 (Fig. 6A); V6 - ZIL/EL/70 (Fig. 5B).

Description: The centra are procoelous, dorsoventrally compressed. In V2, the diapophyses are distinctly inclined anteriorly and slightly also ventrally; they are oval in cross-section due to moderate dorsoventral compression. ZIL/EL/125 is similar in its preserved characters but differs in having the postzygapophyses more robust, and the posterior convexity of the centrum more prominent (see Fig. 6B). Besides that, the anterior-posterior distance between the prae- and postzygapophyses is greater than in ZIL/EL/8 because the former processes extend anteriorly beyond the level of the centrum. The neural arches of ZIL/EL/125 (they are not preserved in

ZIL/EL/8) produce distinct proc. spinosus which is, as usually in V2 of ranids, directed posteriorly. Its dorsal surface is flat, only anteriorly there is a narrow and low ridge paralleled by a depression on either side. V3 is represented by ZIL/EL/9 which is preserved only as fragment lacking the centrum, but its diapophysis with some rugosity in the middle of its length, as well as an extent of its neural canal and shape of its praezygapophysis, suggest its relations to the ranids. ZIL/EL/17 is V5; it has its proc. spinosus directed dorsally (again, as usual in ranids). Perhaps it might be associated with ZIL/EL/70 (see below), judging by the shape of the centrum in ventral view (also in this specimen the posterior convexity is divided by a slot, though visible only in posterior aspect). Peculiar feature of this specimen is the ventral edge of its anterior concavity which runs out anteriorly in the mid-line (see Fig. 6A). It is difficult to say whether this is of some taxonomic

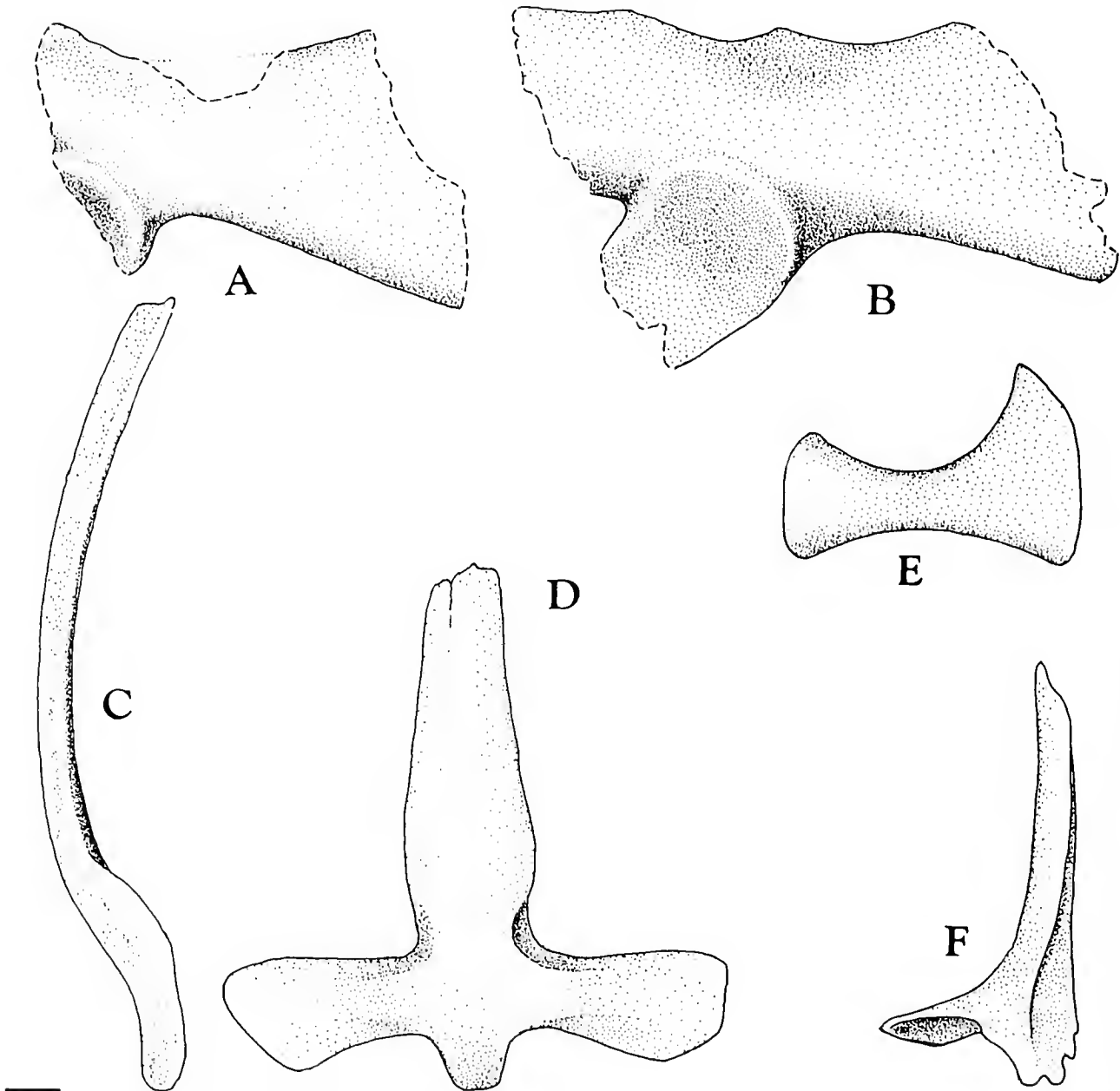


FIG. 7. A- cf. *Bufo* sp., left scapula in lateral view (ZIL/EL/140). B- *Bufo* sp., left scapula in lateral view (ZIL/EL/7). C- Anura indet., left prearticular in dorsal view (ZIL/EL/12). D- Anura indet., parasphenoid (ZIL/EL/185). E-Ranidae indet., right coracoid in ventral view (ZIL/EL/54). F-Anura indet., right pterygoid (ZIL/EL/141). Abbreviations: c. gl. - cavitas glenoidalis; i. gl. - intumescentiaglenoidalis; m.a. - margo anterior; p. a. pars acromialis; p. gl. - proc. glenoidalis. Bar equals 1 mm.

importance. V6 is represented by ZIL/EL/70. Its diapophyses are rounded in cross-section, and are of the same diameter both proximally and distally. They are horizontal, not inclined dorsally. The posterior convexity is clearly divided vertically by a slot which is better

developed than in ZIL/EL/17. Signs of such slots may be observed in corresponding vertebrae of some individuals of the contemporary Ranidae (e.g., *Rana esculenta*). All the described praesacral vertebrae have in common a distinct indentation along the posterior edge



of the neural arches; this is interrupted only in the mid-line where a distinct ridge runs down from the proc. spinosus.

All the above features suggest that vertebrae of the Ranidae should be concerned. Bufonids are excluded mainly because of the morphology of their proc. spinosus and because their neural canal is less spacious.

Material: Sacral vertebra ZIL/EL/10 (Fig. 5A).

Description: The centrum is dorsoventrally compressed, bicondylar posteriorly, both condyli being separated by a comparatively wide notch. The anterior side of the centrum is not preserved but comparison with contemporary ranids suggests that there could be a mineralized intervertebral disc. The diapophyses are inclined dorsally, and are distinctly compressed dorsoventrally. The articulation surface of the praezygapophyses is, in correspondence with the inclination of the diapophyses, facing dorsomedially.

Anura indet.

Material: Parasphenoid, ZIL/EL/185 (Fig. 7D).

Description: The shape and proportions of the bone may be seen from Fig. 7D. Among the characters that should be mentioned are the proc. posterior which is well developed, narrow proximal parts (compared with the distal sections) of the lateral processes and of the pars medialis, and distinct indentations on the transition between the pars medialis and lateral processes caused by a low ridge on either side; similar ridge continues on both sides from the lateral edge of the proc. posterior onto the surface of the proc. lateralis where it disappears.

Material: Left praearticular, ZIL/EL/12 (Fig. 7C).

Description: The proc. coronoideus is well developed, nearly vertical in position.

The sulcus pro cart. Meckeli is, especially in the posterior moiety of the bone, only moderately developed. These characters do not allow precise assignment.

Material: Right pterygoid, ZIL/EL/141 (Fig. 7F).

Description: The inner surface (margo orbitalis) of the ramus maxillaris does not bear any crista and the ramus itself is almost straight. The distal section of the ramus posterior is broken off, so the real proportions of the bone are difficult to reconstruct.

Material: Ilium, ZIL/EL/3 (Fig. 3D).

Description: Although this ilium is preserved only as a small section, important diagnostic characters are preserved. The crista ossis ilii is well developed, and may be distinguished both in medial and lateral view. In contrast to ranids, the torus superior is not developed, and the anterior margin of the pars descendens is directed posteroventrally instead of ventrally or even anteroventrally (see Bohme 1977, fig. 9).

Material: Praesacral vertebra (most probably V5 or V6), ZIL/EL/11.

Description: Only the centrum and bases of the left transverse process incl. adjacent praezygapophysis are preserved. However, one can conclude after the declination of the proximal part of the transverse process that V5 or V6 should be concerned. The centrum is dorsoventrally compressed and procoelous, though its posterior side is also slightly concave. As its surface does not display spongy structure (indicating a crack) it can be admitted that there could be a free intervertebral disc that in living animal adhered the posterior end of the centrum. The ventral surface of the centrum is almost at the same level as the proximal section of the transverse processes, and the centrum itself is short antero-posteriorly. The praezygapophysis is comparatively small and located far laterally (its distance from the lateral edge of the proximal concavity of the centrum is about half the diameter of

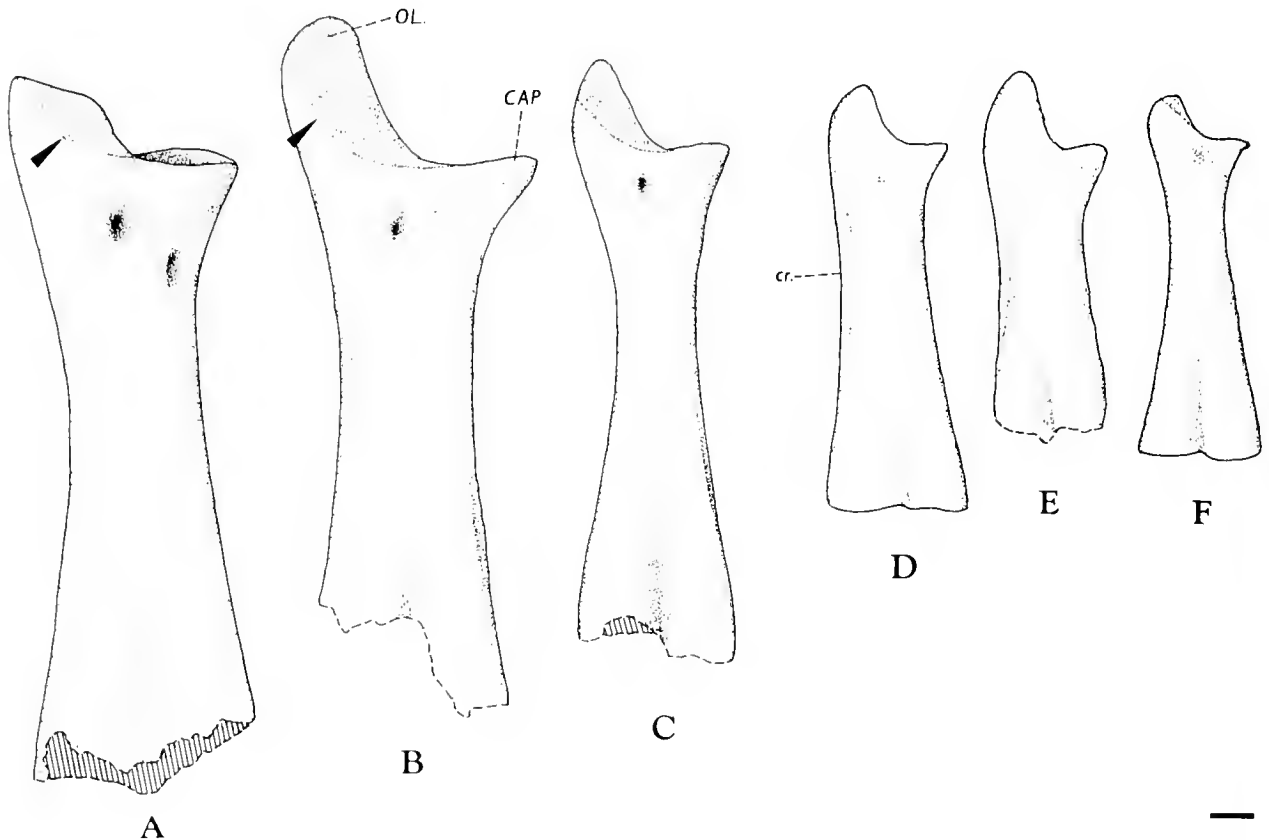


Fig. 8. Radioulnae in medial view. A- *Bufo* sp. (ZIL/EL/62). B-*Bufo* sp. (ZIL/EL/106). C- *Bufo* sp. (ZIL/EL/120). D- Anura indet. (ZIL/EL/36). E- Anura indet. (ZIL/EL/46). F- Anura indet. (ZIL/EL/72). D-F reversed for comparison. Arrows indicate border between periost and that part of the bone without periostal surface. Abbreviations: CAP. - capitulum radioulnae; OL. - olecranon; cr. - crista on the ulnar margin. Line equals 1 mm.

this concavity). The neural arches are comparatively thin, and the neural canal was obviously quite spacious.

Material: Urostyle, ZIL/EL/88.

Description: This element fits morphologically into the variation range of contemporary Ranidae. Both in Ranidae and Bufonidae this range is rather broad which precludes precise assignment of the specimen.

Material: Radioulnae ZIL/EL/36 (Fig. 8D), ZIL/EL/46 (Fig. 8E), ZIL/EL/72 (Fig. 8F), ZIL/EL/73, ZIL/EL/114, ZIL/EL/152.

Description: These radioulnae are medium to small sized (compared with those identified as *Bufo*). A conspicuous

character is that most of them are laterally compressed in their columnar section. This results in that their anterior and posterior margins run out in a distinct ridge. The smallest specimen (ZIL/EL/72), however, has its margins rounded. These radioulnae might be ascribed to the Ranidae, however, lack of diagnostic characters of these elements casts some doubts on this assignment.

#### *Accompanying Vertebrate Fauna in the Sample*

From Kudaro I Cave, Tsepkin (1980) gave a list of accompanying fishes, Darevsky (1980) mentioned one lizard genus (*Lacerta* sp.), Burchak-Abramovich (1980) gave a list of birds, Gadzhiev (1980) bats, Gromov & Fokanov (1980)

rodents, and Vereshchagin & Baryshnikov (1980) large mammals. In the sample that was placed at my disposal there were, besides frogs, also two different forms of birds, and following mammals (determination by Dr. Ivan Horacek, Department of Zoology, Charles University, Prague): *Talpa* cf. *caeca*, *Prometheomys schaposchnikovi*, *Arvicola* cf. *terrestris*, *Microtus* cf. *gud*, *Microtus* ("Pitymys") cf. *majori*, and cf. *Lupus*.

### Conclusions

Determination of the material revealed that its substantial part belongs to the genus *Bufo* and to the family Ranidae. Minor part (represented by fragmentary or less numerous elements) could be determined only as Anura indet. Precise determination was impossible because of supposed individual and developmental variation. Nevertheless, results of this determination show that generic composition of the Holocene anuran fauna in this region was basically the same as contemporary one. The genus *Bufo* in the corresponding altitudes of Caucasus is nowadays represented by *Bufo verrucosissimus*, and *B. viridis*, genus *Rana* by *R. macrocnemis*, and possibly also by *R. ridibunda* (Bannikov et al., 1977; Kuznetsov, 1974; Tuniyev, 1990). Besides, there occurs sporadically also *Pelodytes caucasicus* in South Ossetia, however, presence of this genus in the fossil material could not be confirmed.

### Acknowledgments

I am grateful to Professor I. S. Darevsky (Zoological Institute, St. Petersburg) for the loan of the fossil material for study, and to Dr. I. Horacek (Department of Zoology, Charles University, Prague) for the determination of accompanying micromammalian fauna. Thanks are due also to Dr. B. Sanchiz (Museum of Natural History, Madrid) for his valuable suggestions.

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## Karyotype, C-Band and Ag-Nors Study of Three Stink Frogs

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**Abstract.**—The karyotypes, C-bands and Ag-NORs of *Rana kuangwuensis*, *R. andersonii* and *R. margaratae* were analyzed. Intra- and interspecific chromosome variations, including centromeric type and C-banding patterns, were detected. It was assumed that the Guizhou Plateau was the distributional center of the original place of the group.

**Key Words:** Amphibia, Ranidae, *Rana kuangwuensis*, *Rana andersonii*, *Rana margaratae*, China, karyotype, C-band, Ag-NORs.

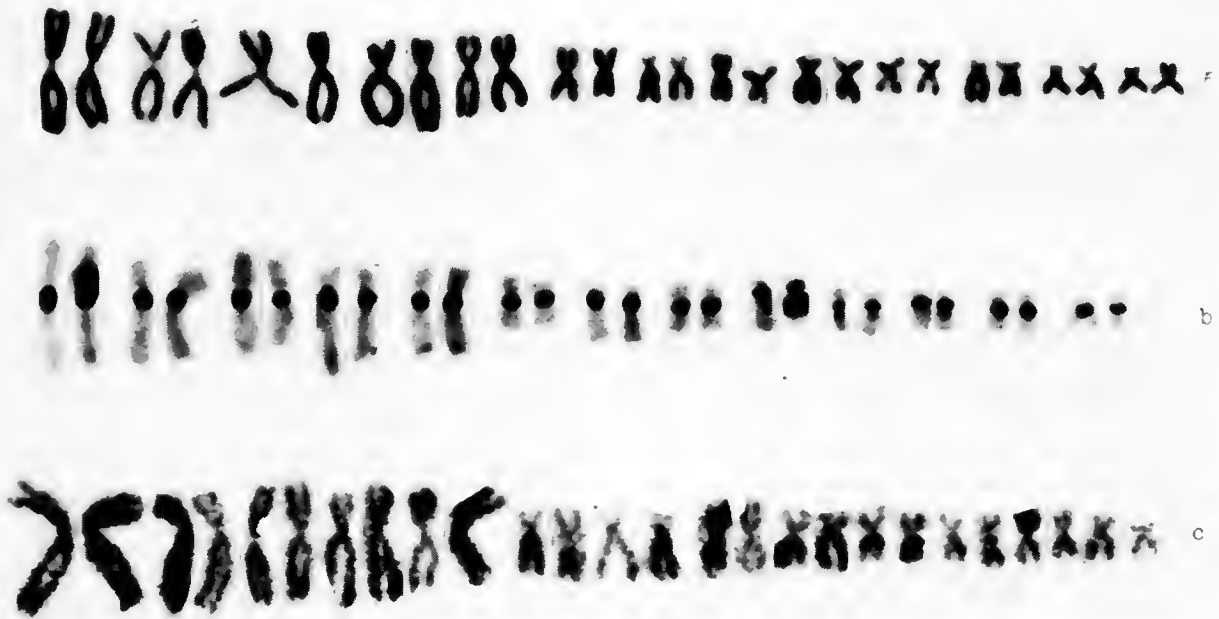


FIG. 1. a: Karyotype of *Rana andersonii*. b: showing C-bands. c: showing Ag-NORs.

### Introduction

The group of stink frogs which have a special stink smell from the skin consists of nine species, i.e. *Rana andersonii*, *R. anlungensis*, *R. grahami*, *R. kuangwuensis*, *R. lungshengensis*, *R. margaratae*, *R. schmackeri*, *R. tiannanensis* and *R. wuchuanensis*. They are considered to be phylogenetically close because of similar morphological characters in adults and tadpoles. Among them, the karyotype and C-bands of *R. grahami* from Kunming, Yunnan has been studied by Li (1982). In addition, the karyotype, C-bands and Ag-

NORs of *R. margaratae* from Emei Mountain, Sichuan have been analyzed by Wang (1983) and Wu (1990). In the present paper, the karyotypes, C-bands and Ag-NORs of *R. kuangwuensis*, *R. andersonii* and *R. margaratae* were analyzed.

### Methods

Two females and one male *R. andersonii* were captured at Qianxi, Guizhou Province, (27°20' N, 106°16' E). One female and three male *R. kuangwuensis* were captured at Nanjiang, Sichuan Province (32°30' N, 106°40' E) and one female and

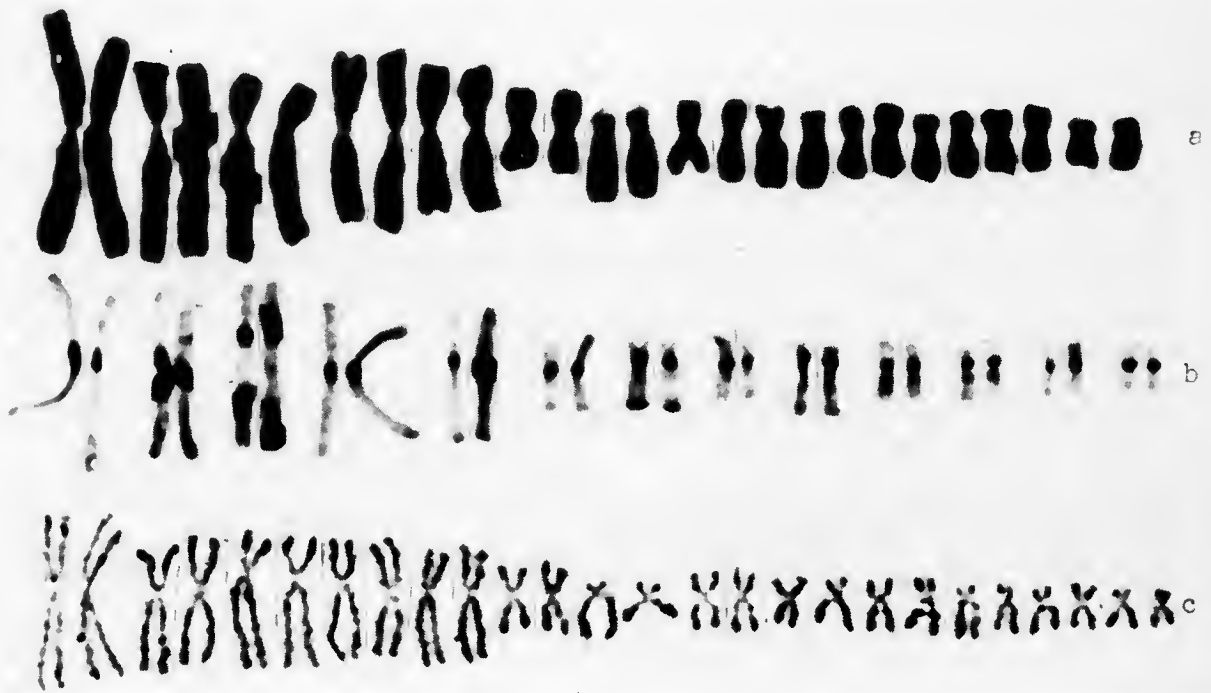


FIG. 2. a: Karyotype of *Rana kuangwuensis*. b: showing C-bands. c: showing Ag-NORs.

one male *R. margaratae* from Zunyi (27°40' N, 106°50' E) were captured. Karyotypes, C-bands and Ag-NORs preparations were made after Wei et al. (1990).

### Results

Figures 1, 2, and 3 depict the karyotypes, C-bands and Ag-NORs of *R. andersonii*, *R. kuangwuensis* and *R. margaratae*. For the measurement of the karyotypes see table 1. The diploid number of the species are all the same,  $2n=26$ , comprising two groups.

The large chromosome group includes chromosome Nos. 1-5, with a relative length (R.L.) larger than 9%. With regard to the arm ratio (A.R.), chromosome Nos. 1 and 5 are metacentric in all three species. No. 2 is metacentric in *R. margaratae* and *R. andersonii*, but submetacentric in *R. kuangwuensis*. No.3 is submetacentric in *R. margaratae* and *R. kuangwuensis*, but metacentric in *R. andersonii*. No.4 is submetacentric in *R. andersonii* and *R. margaratae*, but metacentric in *R. kuangwuensis*.

The small chromosome group comprises chromosome Nos. 6-13, with a R.L. less than 7%. Nos.6, 8, 10, 12 and 13 are metacentric, No.7 is submetacentric in all the three species. Nos. 9 and 11 are submetacentric in *R. kuangwuensis* and *R. andersonii* but metacentric in *R. margaratae*. Secondary constrictions are observed in the long arms of No. 10 of *R. margaratae* (only one homologous) and *R. andersonii* but not observed in *R. kuangwuensis*.

Treatment of the chromosome of the three species according to the C-banding method shows that each species has a centromeric C-band on each chromosome. For interstitial C-band, it is quite different among the species. There is only an interstitial C-band in 10q in *R. andersonii*. And there is an interstitial C-band in 2p (stained weakly), 3q and 4q (only one homologous) in *R. margaratae*. But there are much more interstitial C-bands in *R. kuangwuensis* than in the other two species. *R. margaratae* and *R. andersonii* have not any telomeric C-band. But *R. kuangwuensis* has some telomeric C-bands.



FIG. 3. a: Karyotype of *Rana margaratae*. b: showing C-bands. c: showing Ag-NORs.

Specific staining of the NORs with silver (Ag) confirms that the regions of NORs in all the three species are the same, in the long arms of chromosome No.10. But the relation between the NORs and the constrictions is quite different. The regions of the one pair of NORs in *R. andersonii* correspond to the regions of the secondary constrictions. *R. margaratae* has only one NORs in the chromosome where the secondary constriction locates, and no NORs is observed in the other homologous which has no secondary constriction. While for *R. kuangwuensis*, no secondary constriction is observed in the regions of the NORs.

### Discussion

Comparing the karyotypes between the three species, we detect some interspecific variations. The secondary constriction is not detected in *R. kuangwuensis*. The chromosomes consist of 8 metacentric and 5 submetacentric pairs in *R. kuangwuensis* and *R. margaratae*, while 9 metacentric and 4 submetacentric in *R. andersonii*.

C-banding patterns of all 13 pairs of *R.*

*margaratae* are similar to those of *R. andersonii*, except for the variant bands on chromosomes 2, 3, 4, and 10. While C-banding pattern of *R. kuangwuensis* is quite different from those of the other two species, for *R. kuangwuensis* has more interstitial C-bands and telomeric C-bands as well. And the relation between the NORs and the secondary constrictions is quite different among the three species.

This study also indicates that intraspecific chromosome variations exist in *R. margaratae* from different distributional areas. We observe 8 metacentric and 5 submetacentric pairs in the present study, as opposed to 12 metacentric and 1 submetacentric pairs from Emei Mountain, Sichuan (Wang et al., 1983) and 9 metacentric and 4 submetacentric pairs also from Emei Mountain (Wu 1990).

The C-banding pattern of chromosomes 1-13 of *R. margaratae* from Zunyi is compared with those from Emei Mountain. There is a centromeric C-band in each chromosome and a terminal C-band at each chromosome terminus, and an interstitial C-band in the long arm of No.3 from Emei

TABLE 1. Karyotypic data for *Rana margaratae*, *R. andersonii*, and *R. kuangwuensis*.

Chromosome Nos.	<i>R. margaratae</i>	<i>R. andersonii</i>	<i>R. kuangwuensis</i>
1	R. L. 14.64±0.83	R. L. 14.30±0.91	R. L. 15.70±0.91
	A. R. 1.26±0.31	A. R. 1.26±0.33	A. R. 1.46±0.26
2	R. L. 12.38±0.84	R. L. 11.90±0.70	R. L. 13.11±0.32
	A. R. 1.48±0.38	A. R. 1.43±0.35	A. R. 1.54±0.41
3	R. L. 11.73±0.86	R. L. 11.20±0.68	R. L. 11.93±0.94
	A. R. 1.72±0.49	A. R. 1.57±0.28	A. R. 2.01±0.39
4	R. L. 11.39±0.77	R. L. 10.21±0.49	R. L. 11.42±0.92
	A. R. 1.76±0.39	A. R. 1.71±0.41	A. R. 1.29±0.39
5	R. L. 10.20±0.84	R. L. 9.58±0.34	R. L. 10.25±0.84
	A. R. 1.39±0.45	A. R. 1.33±0.20	A. R. 1.41±0.37
6	R. L. 6.52±0.45	R. L. 6.82±0.48	R. L. 6.59±0.65
	A. R. 1.21±0.35	A. R. 1.32±0.29	A. R. 1.20±0.16
7	R. L. 5.65±0.37	R. L. 6.09±0.61	R. L. 5.92±0.41
	A. R. 2.53±0.37	A. R. 2.14±0.30	A. R. 2.54±0.39
8	R. L. 5.44±0.48	R. L. 6.01±0.60	R. L. 5.40±0.35
	A. R. 1.39±0.38	A. R. 1.38±0.46	A. R. 1.24±0.37
9	R. L. 5.27±0.42	R. L. 5.73±0.57	R. L. 5.13±0.27
	A. R. 1.92±0.34	A. R. 2.06±0.39	A. R. 2.01±0.36
10	R. L. 4.73±0.43	R. L. 5.30±0.53	R. L. 4.87±0.32
	A. R. 1.35±0.26	A. R. 1.27±0.25	A. R. 1.55±0.43
11	R. L. 4.39±0.38	R. L. 4.92±0.32	R. L. 4.34±0.36
	A. R. 1.32±0.36	A. R. 1.57±0.42	A. R. 1.92±0.37
12	R. L. 4.28±0.56	R. L. 4.56±0.41	R. L. 4.21±0.32
	A. R. 1.27±0.29	A. R. 1.53±0.37	A. R. 1.45±0.37
13	R. L. 3.98±0.34	R. L. 4.12±0.39	R. L. 3.55±0.40
	A. R. 1.47±0.39	A. R. 1.66±0.33	A. R. 1.66±0.35

Mountain (Wang et al., 1983). Besides those above, there is an interstitial C-band in the acro long arm of No.7, and even heterogeneity observed in No.9. There is an interstitial C-band in the middle of the long arms of both homologues of No.9 in female, while only one homologous of No.9 is observed having an interstitial C-band, the other homologue has not an interstitial C-band in the middle of the long arm, but has an interstitial C-band near the terminus of the long arm (Wu 1990). We also observed indeed C-band heterogeneity of chromosome No.9 from Emei Mountain. Yet in our present study, we do not detect C-band heterogeneity of chromosome No.9 from Zunyi. And we detect other interstitial C-band (2p, 4q) but no telomeric C-band has been observed.

In the early stage of the karyotypic evolution, a karyotype had generally more metacentric chromosomes. With the

development, the karyotype differentiated in the direction of having more submetacentric or telocentric chromosomes (Li, 1985). Generally speaking, karyotypes with more telomeric and less interstitial C-band are more original. Between the two distributional areas of *R. margaratae*, the specimens from Emei Mountain has 12 (Wang 1983) or 10 (Wu, 1990) metacentric, and has more telomeric and less interstitial C-bands, and that from Zunyi has 8 metacentric and has less telomeric and more interstitial C-bands. On the view of point above, *R. margaratae* from Emei Mountain is more original than that from Guizhou.

The stink frog group is composed of 9 species. The distributions of them are as follows:

*R. andersonii*: upper Burma to Yunnan, Guizhou, Guangxi, Hainan



*R. anlungensis*: Guizhou (Anlung County)

*R. grahami*: Sichuan, Guizhou, Yunnan

*R. kuangwuensis*: Sichuan (Nanjiang County)

*R. lungshengensis*: Guizhou, Guangxi, Hunan

*R. margaratae*: Gansu, Sichuan, Guizhou

*R. schmackeri*: Henan, Gansu, Sichuan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang, Jiangxi, Hubei, Guangdong

*R. tiannanensis*: Yunnan, Hainan

*R. wuchuanensis*: Guizhou (Wuchuan, Libo)

From the description above, it could be found that the distributional areas of some species are very limited, only one or two counties. So they are very rare and precious wildlife. And it could also be found that the stink frog group is distributed mainly in the south of China, and most of them (7 species) are found on the Guizhou Plateau. So, the Guizhou Plateau might be the distributional center of the group.

There were another two species of stink frogs. Their karyotypes and C-banding patterns were published. They are *R. grahami* and *R. schmackeri*. Both the species have 10 metacentric and 3 submetacentric pairs in their karyotypes, and both species have one telomeric C-band, but the former has 5 and the latter has 4 interstitial C-bands.

Among the 5 species published their karyotypes and C-banding patterns, *R. margaratae* from Emei Mountain has most metacentric pairs and most telomeric C-bands. Although it does not have less interstitial C-bands, it could still be considered as the most original in the viewpoint of cytogenetics. Considering that *R. margaratae* from Guizhou is more evolved than that from Emei Mountain, it might be as-

sumed that the stink frog group originated in Emei Mountain and its adjacent plateau.

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The Variegated Toad Agama in Djungar Gate (Eastern Kazakstan) with Notes on Certain Systematic Problems of *Phrynocephalus versicolor* Str. (Reptilia: Agamidae)

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**Abstract.**—The distribution of *Phrynocephalus versicolor* in Djungar (=Junggar) Gate (Eastern Kazakstan) was investigated. The characteristics of these lizards are: absence of red axillary spots and the presence of red-orange subcaudal coloration. The taxonomic status of this population and of subspecies of *P. versicolor* is discussed. The variegated toad agama is presumed to be a "composed" species.

**Key Words:** Reptilia, Sauria, Agamidae, *Phrynocephalus versicolor*, Kazakstan, China, Djungar Gate, systematics, distribution.

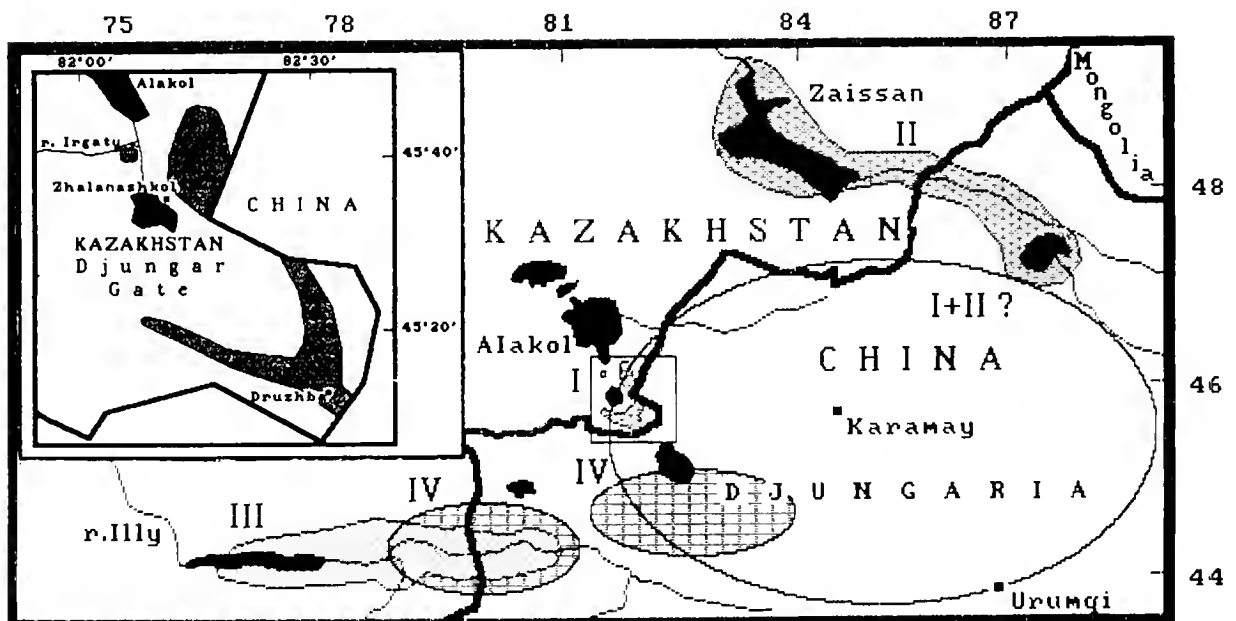


FIG. 1. The distribution of *Phrynocephalus versicolor hispida* (I), *P. guttatus salenskyi* (II), *P. g. alpherakii* (III), and *P. v. doriai* (IV) in Eastern Kazakhstan and Chinese Djungaria.

### Introduction

There are few reliable literature citations concerning the distribution of the variegated toad agama, *P. versicolor* Str. from the eastern part of the Balkhash-Alkol Depression. Only Paraskiv (1956) reported finding the toad agama "near Lake Zhalanashkol". He also indicated that the distribution of this lizard is a natural continuation of the the Djungar Gate in the Alakol Depression, "somewhere along the northern Lake Alakol shore" (Paraskiv,

1956). According to Kubykin (1975), one specimen of this lizard was captured by him on Sredniy Island (Lake Alakol). However, this specimen is not mentioned in the collection list of the Institute of Zoology of the Kazakh Academy of Sciences (Brushko and Kubykin, 1988). Semenov (1986) and Semenov et al. (1987) referred to toad agamas collected "from Alakol Hollow" in the Zoological Museum of Moscow State University. All other references to the distribution of this lizard cite the above references.



FIG. 2. Habitat of *Phrynocephalus versicolor* in Djungar Gate near Druzhba Railway Station: crushed-stony and gravel semidesert covered with boyalych (*Salsola orboscula*).

### Methods

We studied the distribution of the variegated toad agama in June 1991 during investigations of the Djungar Gate territory (Fig. 1).

### Results and Discussion

The Djungar Gate is a relatively narrow pass between the Balkhash-Alakol Depression and Chinese Djungaria. This pass is oriented from the northwest to the southeast and rising in elevation towards the southeast. The pass enters China near the Druzhba ("Friendship") railway station along the Lankol Valley. The Djungar Gate Valley is formed by the broad alluvial plain and gentle foothills of Maily Ridge and the Djavlau Mountains to the northeast and the more abrupt upthrust of the Djungar Alatau to the southwest. The surface of the valley alluvial plains varied with the degree of slope and ranged from boulders, rubble,

gravels and fine gravels on a loess base with the finer sorted materials deposited farther from the mountains slopes. Southeast of Lake Zhalanashkol the valley floor is a broad alkaline plain (20-25 km.) with subsurface water.

The dominate plant, *Salsola orboscula*, (common Russian name = boyalych, also known in the United States as Russian thistle) is found on the lower slopes and alluvial plains of the valley. It is more widely distributed on the northeastern slopes but it is sometimes replaced by saxaul (*Haloxylon sp.*). Wormwood (*Artemisia sp.*) is dominate among the grasses and nearly the only plant on flats without shrubs.

From the Lankol Valley the toad agamas are distributed along both the northeastern and southwestern slopes above the Djungar Gate. Along the southwestern alluvial plain the toad agama is distributed 15-20 km

from the Druzhba station to the northwest. The lizard occurs along the foothills of the Maily Ridge and the Djavlau Mountains for 55-60 km where the northern limit of its distribution coincides with the border of the boyalych dominate, gently sloping alluvial plain composed of rock rubble and gravels. Further north, on the steeper slopes of the alluvial plain composed of larger rock rubble and dominated by wormwood, the sunwatcher (*P. helioscopus*) is found. It is possible that *P. versicolor* occurs much further to the northeast into the Alakol Depression along the foothills, however field work in this area is difficult because of the presence of military installations along the border. A small isolated area with conditions which would make good habitat for this species is found along the railway tracks between the Zhalanashkol Station and the 19th Station near the mouth of the Irgaty River.

Toad agamas are found under single bushes in small groups composed of 1-2 males and 2-5 females of different ages. In addition, groups of up to 10 subadults were observed. The density of the lizards is variable, with higher densities in gravelly areas with boyalych (Fig. 2) as well as in areas of colonies of the great gerbil (*Rhombomys opimus*) that have excavated through the darker colored gravels and rock rubble and where the lighter loes makes up the predominant coloration of the surface.

It is interesting to note that the lizards inhabiting the gravel plains in the Djungar Gate have retained the sand burrowing behavior, involving rapid lateral movements of the body, observed in populations inhabiting sandy areas.

Pregnant *P. versicolor* as well as females of other *Phrynocephalus* species assume the "copulation avoidance" posture when pursued by males (Polynova, 1982; 1989; Rogovin, 1991; and our observations of *P. strauchi* in the Fergan Valley). To assume the "copulation avoidance" posture the female turns onto her back as the male approaches and maintains this position while he is nearby.

Currently *P. versicolor* is considered to be a polymorphic species and it is interesting to determine the subspecific position of the form inhabiting the Djungar Gate.

For a long time it was assumed that in eastern Kazakhstan this toad agama was found in three isolated populations: the Zaissan Depression, Alakol Depression and the Ili River Depression (Paraskiv, 1956; Bannikov et al., 1977). Peters (1984) considered the Zaissan Depression and Ili River Depression lizards to be two separate species: *P. salenskyi* Bedr. and *P. alpheraki* Bedr. Three years later a new subspecies, *P. versicolor paraskiwi* (Semonov et al., 1987), was described from the Ili River Depression. These authors speculated that the two Chinese Djungar Depression subspecies, *P. v. hispida* Bedr. and *P. v. doriai* Bedr. were conspecifics. However, because of a shortage of material, they did not determine the taxonomic status of the Alakol Depression variegated toad agama. Soon after the most significant attempt to analyze the intraspecific variation of *P. versicolor* was undertaken (Semenov and Shenbrot, 1989).

Semenov and Shenbrot (1989) examined 675 specimens: 580 from Mongolia and Tuva, 65 from the Ili River Depression, 19 from Chinese Kuldja (now Yining, Xinjiang, China), 11 from the Alakol Depression, but no specimens from the remainder of the range of this species in China. The authors, using discriminant analysis techniques, felt their material was adequate to discuss all known subspecies of the variegated toad agama.

Semenov and Shenbrot (1989) indicated that *P. v. paraskiwi* was detached from the main group. Also, *P. v. doriai* from Kuldja and *P. v. kulagini* from western Mongolia were resurrected. The Alakol variegated toad agama was singularly attributed to *P. v. doriai*. These authors were unable to distinguish the lizards from Mongolian Djungaria from "typical" *P. v. hispida*, however they did not indicate which *P. v. hispida* they considered

TABLE 1. Differences between Djungar subspecies of *Phrynocephalus versicolor* (after Bedriaga, 1909).

Characters	<i>P. v. hispida</i>	<i>P. v. doriai</i>
Body length (L.)	122 mm	133 mm
Tail length (L. cd.)	Medium (male), short (female)	Long
Supraocular scales	Slightly smaller than surrounding	Distinctly smaller
Head scales	Large	Small
No. of scales across top of head	21-26 (23-24)	25-29 (rarely 23)
No. of scales along top of head	10-13	12-15
Thigh scales	Smooth	Smooth or keeled
Dorsal coloration	Gray, olive, light brown, gray-brown	Dark gray, grey-brown, dirty red
Dorsal bands	Can be distinguished on shoulders and hind part of dorsum	Distinct to absent
Ventral coloration	White, throat and chest slightly pigmented	Commonly dark
Axillary spots	Tracks of yellowish or pink-yellow spots may be present	?

typical. They rejected their original view on the close relationship of the Djungar forms. *Phrynocephalus v. hispida* was recognized as identical to the nominative form.

Discriminant analysis also has shown that the presence of red axillary spots are useful characteristics for separating the closely related pairs of subspecies, *P. v. versicolor* - *P. v. kulagini* and *P. v. paraskiwi* - *P. v. doriai*. The remaining characteristics were found not to be useful for this purpose. This was already noted in earlier research (Nikolsky, 1915; Leroy, 1940; Terentjev and Chernov, 1949).

However, a number of questions remain unanswered. Has it been demonstrated that axillary spots are absent in *P. v. doriai* and in the Alakol toad agama? Is it appropriate to include in the nominative subspecies, characterized by the presence of axillary spots, the form *P. v. hispida* in which the axillary spots may be present or absent (Bedriaga, 1909)? If so, then what are the reasons for separating into distinct taxa *P. v. kulagini*, which lacks axillary spots and *P. v. paraskiwi* which has the axillary spots? The latter form should be excluded from further discussion since it has been shown (Golubev, 1989) that it was erroneously described and should be attributed to *P. guttatus alpherakii*.

Discriminant analysis did not clarify the relationship between *P. v. doriai* and *P. v. hispida*.

It is clear (Table 1) that such characteristics as the relative size of the head and supraocular scales can not be used unless they are standardized. Dorsal and ventral coloration vary widely and are effected in life by such physiological considerations as body temperature and ambient light and in preserved specimens by the manner of preservation. Body length to tail length, when expressed in ratios (Bedriaga, 1909) and repeated measurements of type specimens from the Zoological Institute of the Russian Academy of Sciences, St. Petersburg (ZIN) did not confirm the differences noted by Bedriaga (*P. v. hispida* ZIN 6637 females: 0.78-0.81; males 0.66-0.68; *P. v. doriai* ZIN 5549, 8160 females 0.71-0.74; males 0.64-0.72). The ratios could be confirmed by using liner dimension L. and L. cd. but this was not done. Two other characteristics, number of scales along and across the top of the head are known to vary widely among populations. Only presence or absence of axillary spots remains as a useful character for separating subspecies. However, we have no information concerning this character in *P. v. doriai*. Bedriaga (1909) used material that had been in preservative for more than

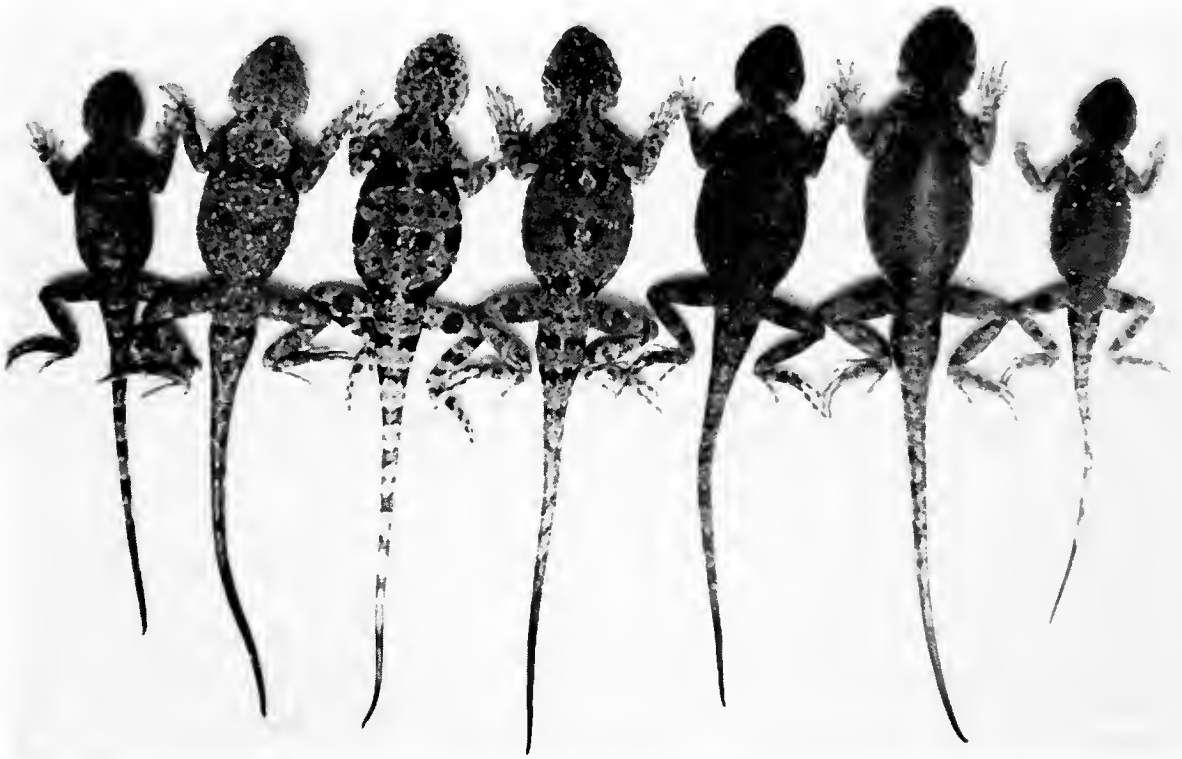


FIG. 3. Dorsal view of *Phrynocephalus versicolor* from Djungar Gate (Djungar Railway Station).

10 years and these spots might have disappeared during this time. Also there are no detailed data on the distribution of this character in lizards from eastern Djungaria. It is known only that such spots are present in the Mongolian part of the range of the toad agama (Semenov and Shenbrot, 1989). However, the nearly isolated Mongolian Djungaria (Barun-Khuray Depression) differs from Chinese Djungaria in several geographic parameters such as altitude.

The type specimens of *P. v. hispida* are dated 1879 (ZIN 6637 and 6638) and 1880 (ZIN 6639). Nikolay M. Przewalsky's Third Central Asian Expedition (First Tibetan Expedition) took place at this time (Dubrovin, 1980). Przewalsky left the city of Zaissan on March 21 (April 4 by the modern calendar) and reached Ulungur Lake (Ulungur Hu) on March 31 (April 12 by the modern calendar). He followed the Urungu River (Ulungur He) and its tributary the Bulugun to the Barun-Khuray Depression and crossed it from north to south. He then crossed the Baytik Shan

Ridge and the plains of south eastern Djungaria. On May 18 (May 30 by the modern calendar) he reached Barkul and did not return to Djungaria during this expedition (Przewalsky, 1883). In Przewalsky's journal the toad agamas (*Phrynocephalus* sp.) are casually mentioned for the middle and lower reaches of the river. Thus, it is possible to draw two conclusions: (1) the *P. v. hispida* type specimens may have been collected in different parts of Djungaria and thus include different forms, (2) the date of collection for the ZIN 6639 sample is incorrect.

There is an overlap between both subspecies of toad agamas from the Djungar Gate region in body proportions (females 0.6-1.06; males 0.64-0.75), number of scales across (19-29; not counting the supraocular scales) and along (8-14) the top of the head. The scales on the thigh are smooth and dorsal and ventral coloration are highly variable (Figs. 3, 4, 5). In the material we collected, a slight shift in these character's values toward

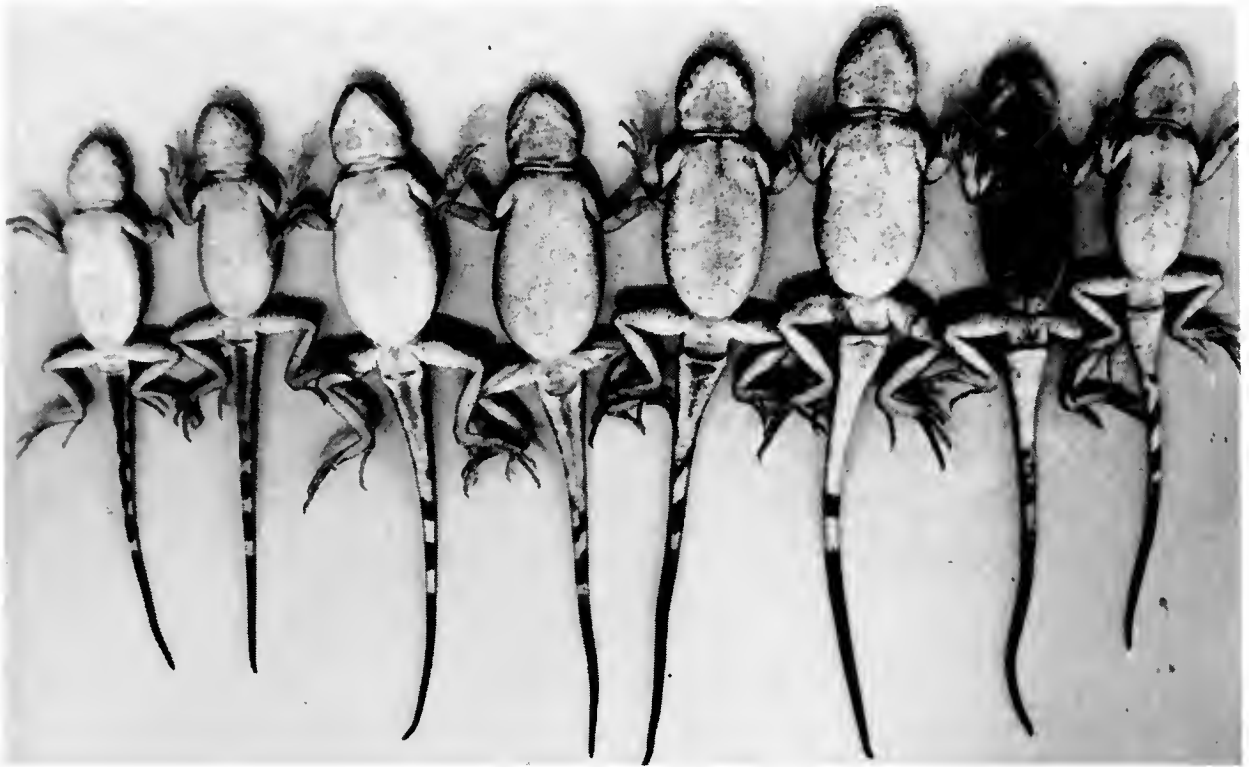


FIG. 4. Ventral view of *Phrynocephalus versicolor* from Djungar Gate (Djungar Railway Station).

*hispidus* can be noted. It is important to note that the subcaudal surface in living lizards of both sexes is a red-orange color, that with age loses its lustre and disappears (Fig. 4). We also discovered remnants of the red-orange coloration in one of 13 specimens from "Alakol Depression" (Zoological Museum of Moscow State University, MSU R7779). Recently we examined specimens from China with the same subcaudal coloration in the California Academy of Sciences collected in the central (northeast of the city of Karamay) and southeastern (east from the city of Urumqi) Djungaria. In all specimens examined there are dark transverse bars on the ventral tail surface and this agrees with Bedriaga (1909) but not with Semenov (1986).

From the above data, it follows that variegated toad agamas inhabiting the Alakol Depression and Djungar Gate to southern Djungaria (>500 km) differ from all other forms of *P. versicolor* in the absence of axillary spots and brightly colored subcaudal surface. Does this

indicate the existence of a new subspecies? There are also red tailed toad agamas in northern Djungaria and the Zaisan Depression variously described as *P. guttatus*, *P. salenskyi* and *P. versicolor*. In the Zaisan Depression this lizard is mostly sand-dwelling and similar in habits to *P. guttatus* and *P. frontalis* (Golubev, 1989), while in northern Djungaria and in some places in the Zaisan Depression they are found in more stabilized soils. From the Alakol Depression and the Djungar Gate, where this species is found, there are over 300 km of continuous habitat without noticeable barriers into central Djungaria where *P. versicolor* is found. This may represent a cline with a gradual transition from one form to the other.

In September 1991 repeated copulations between a male *P. salenskyi* (Zaisan Depression) and a female *P. versicolor* (Djungar Gate) were observed in the Kiev Zoo terrarium. If precopulation barriers exist, they apparently can be broken in terrarium conditions. Both Przewalsky (1883) and Potanin (1948), when traveling



along the lower reaches of the Urungu River and the southern shore of Ulungur Lake (type locality of *P. salenskyi*), noted the variable coloration of toad agamas. We discovered fragments of a light longitudinal caudal stripe (a characteristic of *P. g. salenskyi*) in some specimens of *P. versicolor* from Djungar Gate (Fig. 5). Red axillary spots are present not only in the Alashan variegated toad agama (species type locality) but in lizards inhabiting the area south of Beishan Ridge in the Gashun Goby. However, here *P. versicolor* are connected by coloration and pattern transitions with *P. axillaris* Blanf.

Thus, the question of the taxonomic status of the variegated toad agamas from Djungaria and Alakol Depression should again be considered open as does the question of the position of *P. v. doriai*. The Kuldja and western Djungarian populations are separated by the Tianshan Mountains. There are reasons to believe that the Kuldja *P. v. doriai* is actually an ecological race of the Ili *P. g. alpherakii* while the Ebinurian *P. v. doriai* is assignable to the *acutirostris* group (which also might be no more than one of the color variants of the *axillaris-guttatus* complex).

In summary, it appears that only two subspecies of the variegated toad agama can be recognized. *P. v. kulagini* inhabits southern Tuva and northwestern Mongolia and forms a narrow zone of intergradation with the nominative subspecies *P. v. versicolor*. However, there are doubts that the axillary red spots constitute a characteristic which allows one to delineate populations specifically on the level of geographaic race, i.e. subspecies. It is possible that *P. versicolor* consists of isolated, genetically differentiated color morphs associated with stabilized soils. Taxonomic separation of these variants should occur only after a detailed study of the entire group. Bedriaga (1909) recognized five subspecies and considered *P. versicolor* to be a species composed of many highly variable populations. Bedriaga expressed concern that his taxonomic arrangement of these subspecies did not represent a natural assemblage.



FIG. 5. A specimen of *Phrynocephalus versicolor* from Djungar Gate (Djungar Railway Station) with light longitudinal caudal stripe.

Further he stated "[only when we are more familiar with the fauna of Central Asia, will it be clear whether I have exaggerated distinguishing characters]". Leroy (1940) proposed that the species *P. versicolor* be abolished. Leroy's point of view may be closest to the truth.

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## Allozyme Variation and Genetic Relationships within the *Phrynocephalus guttatus* Species Group (Sauria: Agamidae) in the Former USSR.

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**Abstract.** -An electrophoretic analysis of several populations of *Phrynocephalus guttatus* s. lato. shows that there are two groups with a remarkable level of genetic differentiation. There is an eastern Palearctic *P. versicolor* from southern Mongolia, and a western Palearctic *P. guttatus* s. str. which includes: *g. guttatus*, *g. kushackewitschii*, *g. alpherakii*, *g. salenskyi*, *g. moltschanovii*, *guttatus* ssp. from northern Turkmenia and *versicolor hispida* from Djungar Gate. There are no objective criteria for subspecific separation by biochemical genetic markers.

**Key words:** Reptilia, Sauria, Agamidae, *Phrynocephalus*, electrophoresis, systematics.

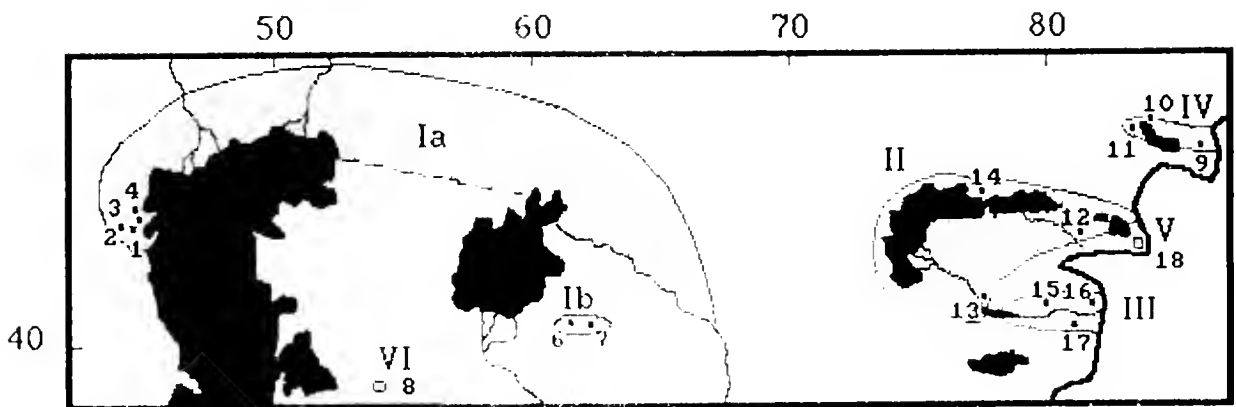


FIG. 1. Distribution of the *Phrynocephalus guttatus* species group in the former USSR. Ia- *P. g. guttatus*; Ib- *P. g. moltschanovii*; II- *P. g. kushackewitschii*; III- *P. g. alpherakii*; IV- *P. g. salenskyi*; V- *P. versicolor hispida*; VI-*P. guttatus* ssp. The numbering of the populations is as given in Table I.

### Introduction

The agamid genus *Phrynocephalus* includes some polytypic species groups. One of the most complicated species complexes is *Phrynocephalus guttatus* s. lato. Representatives of this species group are widely distributed in Middle and Central Asia from the northern Caucasus to China. The systematics of this species group is highly controversial and needs revision.

There are some alternative viewpoints on the status and systematic relationships of its representatives. The classical viewpoint of Terentjev and Chernov (1949) recognized only two species: *P. versicolor* and *P. guttatus* (*P. g. guttatus* and *P. g. kushackewitschii*). A new concept was

developed during the last decade by Semenov and Shenbrot (Semenov and Shenbrot, 1982; Shenbrot and Semenov, 1987; Semenov et al., 1987). According to this concept, the *guttatus*-group consists of four species: *P. guttatus* (Gmel), *P. moltschanovi* Nik., *P. melanurus* Eichw. (= *P. salenskyi* Bedr.) and *P. versicolor* Str. The last form includes the nominal subspecies (China: Alashan to Djungaria), *P. v. kulagini* (Tuva, Russia; western Mongolia) and the western Palearctic subspecies, *P. v. paraskii* Semenov, Brushko, Kubykin et Shenbrot. Golubev (1989) lowered the status of "*salenskyi*" to subspecific level, united *P. v. paraskii* with *P. guttatus alpherakii* Bedr. and included "*moltschanovi*" only as a color variation of *P. g. guttatus*.

TABLE 1. Localities, sample sizes and taxa of *Phrynocephalus guttatus* S. lato. populations collected and investigated in this study.

N	Taxa	Locality	No.
1	<i>P. g. guttatus</i>	NORTHERN TRANSCAUCASUS REGION	
		Daghestan: Tersky Sands near Chervlenny Buruny	2
		Russia: Stavropol Dist., Tersky Sands: Roshchino	3
		Chencheno-Ingushety: near Starogladkovsky	3
		N. Daghestan: sands on the right bank of Kuma River	1
2	<i>P. g. moltschanovi</i>	N. Kysylkum in Karakalpakia: Beltau Mount.	4
		N. Kysylkum in Karakalpakia: Kostruba Well	5
3	<i>P. guttatus ssp.</i>	N. Turkmenia: Kazakhlyshor near Kumsebshen Well	3
4	<i>P. g. salenskyi</i>	E. KAZAKHSTAN: Zaissan Depression:	
		Left bank fo Black Irtysh near Karatal	22
		Irtysh Sands near Chingildy	18
		Left bank of Bukhtarma Reservior: Kuludjunsy Sands	18
5	<i>P. g. kushackewitschii</i>	E. KAZAKHSTAN: Taldy-Kurghan Dist. (Alakol Basin): Near Andreevka (left bank of Chyndjaly River	23
6	<i>P. g. kushackewitschii</i>	E. KAZAKHSTAN: Taldy Kurghan Dist. (Balkhash Basin):	
		NW bank of Kapchagay Reservior	10
		SW of Balkhash: Ortadyressin	2
7	<i>P. g. alpherakii</i> (+ <i>P. v. paraskii</i> )	E. KAZAKHSTAN: Taldy-Kurghan Dist. (right bank of Illi );	
		Panfilov Region: 25 km. from Aidarly	2
		Kerbulak Region: Ayakkalkan	7
		Alma-Ata Dist.: near Chundja (left bank of Illi)	2
8	<i>P. versicolor hispida</i>	E. Kazakhstan: Djungar Gate	7
9	<i>P. v. versicolor</i>	Mongolia: South Gobi Aimag: Dalanzadagad	5
10	<i>P. strauchi</i>	Fergan Valley: left bank of Kajrakkum Reservior near Kyly	20
11	<i>P. helioscopus saidalievi</i>	S. part of Fergan Valley near Kim	7

A high level of morphological variation on the one hand, and caryological conservatism on the other, doesn't allow one to decide problems of systematic status and specific identity of representatives of *P. guttatus* s. lato. Therefore, in order to decide controversial systematic problems of this agamid group, we used biochemical genetic markers.

### Methods

Electrophoretic analysis was carried out on geographic forms of *P. guttatus* s. lato from different geographic regions (Fig. 1, Table 1). The geographic form from northern Turkmenia was excluded from *P. g. guttatus* on the basis of the red spots on the arm pits, a very rare characteristic in *P. guttatus*. We also studied two well differentiated species, *P. strauchi* Nik. and *P. helioscopus saidalievi* Sattorov, both from the Fergan Valley, as an external

control for genetic differentiation.

Each adult specimen was processed in the laboratory for blood and muscle samples and immediately studied by standard vertical acrylamide electrophoresis. Homogenates obtained from muscle, crushed in distilled water with 5 per cent sucrose, were processed for the following enzymes and proteins (Table 2).

Isozymes were numbered in order of decreasing mobility from the most anodal one. Allozymes were designated numerically according to their mobility, relative to the most frequent allele (100), faster mobility (>100), slower mobility (<100). The genetic divergence between populations and divergence time were estimated with indices of standard genetic distances by formulas proposed by Nei (1975).

TABLE 2. Enzymes studied and electrophoretic conditions employed.

Enzyme or protein	Locus abbrev.	EC no.	Tissue	Buffer
Aspartate aminotransferase	s-Aat	2.6.1.1	Muscle	TEB
Glycerol-3-phosphate dehydrogenase	G-3-pdh	1.1.1.18	Muscle	TEB
Isocitrate dehydrogenase	s-Idh	1.1.1.42	Muscle	TEB
Lactate dehydrogenase	Ldh-A	1.1.1.27	Muscle	TEB
Lactate dehydrogenase	Ldh-B	1.1.1.27	Muscle	TEB
Malate dehydrogenase	s-Mdh	1.1.1.37	Muscle	TEB
Malic enzyme	s-Me	1.1.1.40	Muscle	TEB
Superoxide dismutase	s-Spd	1.15.1.1	Muscle	TEB
6-phosphogluconate dehydrogenase	6-pgdh	1.1.1.44	Muscle	TEB
Phosphoglucomutase	Pgm	2.7.5.1	Muscle	TG
Esterase	Es-D	3.1.1.1	Muscle	TEB
Esterase	Es-2	3.1.1.1	Muscle	TG
Esterase	Es-3	3.1.1.1	Hemolizate	TG
Hemoglobin	Hb	-	Hemolizate	TG
Albumin	Alb	-	Muscle	TG
Structural muscle proteins	Pt-1, 2, 3	-	Muscle	TG

Note: TEB- Tris-EDTA-NA<sub>2</sub> boric acid Ph 8.5 (Peacock et al., 1965). TG- Tris-glycin, disc-electrophoresis (Davis, 1964)

## Results

*Allozyme variation.*—Four of 18 loci analyzed (Es-3, Pt-2, Pt-3, IdhS) were monomorphic and fixed for the same allele in all populations and species considered. Fourteen loci were polymorphic within or between population and their allelic frequencies are given in Table 3. Expected genotypes distributions were in equilibrium according to Hardy-Weinberg formula in the investigated populations at all loci observed. Exceptions were obtained only in "*salenskyi*" and "*kushackewitschii*" at the Ldh-B locus (Table 4). In our opinion, absence of heterozygote genotypes can be explained by introgression of Ldh-B 90 from "*salenskyi*" to "*kushackewitschii*" or vice versa.

Levels of genetic variation are given in Table 3 (Mean proportion of heterozygosity observed (H obs.) and expected (H exp.)). H obs. ranged from 0 in "*salenskyi*" to 0.05 in "*kushackewitschii*" with a mean of 0.04. This meaning of heterozygosity is near the level usual for Reptilia (Nevo, 1984).

*Genetic divergence.*—Only two loci

(Me-S, Mdh-S) display fixation or predominance of alternative alleles between *P. versicolor* (Mongolia) and the *P. guttatus* group. All representatives of Western palearctic *P. guttatus* have common gene pools of the loci considered. *Phrynocephalus v. hispida* is an exception and has alternative allelic fixation at Es-2 (Table 3). Genetic distances among *P. guttatus* forms are rather low and range from 0 to 0.05 i.e. on interspecific level of differentiation.

There are four loci which display alternative fixation between *P. strauchi* and *P. guttatus*. It shows a clear intraspecific level of genetic divergence. The largest genetic distance is between *P. h. saidalievi* and *P. guttatus* species group (D=0.832). Differences between these species include 8 loci which display alternative allelic fixation (Pgm, Mdh-S; Aat-S, Me-S, Alb; Pt. 1; Ldh-B, Es-2).

From the allelic frequencies at 18 loci tested, we calculated Nei's genetic distance and constructed a matrix of genetic distances (Table. 5). A UPGMA phenogram was calculated on the basis of this matrix. This reflects the relationships

TABLE 3. Allelic frequencies.

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11
s-Aat	90											0.97
	95					0.12						0.03
	100	1.00	1.00	1.00	0.98	0.88	1.00	1.00	1.00	1.00	0.80	
	105				0.02						0.20	
G-3-pdh	95		0.25									
	100	1.00	0.75									
Ldh-A	90		0.25		0.08	0.92	0.81	0.39		0.67		
	100	1.00	0.75	1.00	0.92	0.08	0.19	0.61	1.00		1.00	1.00
Ldh-B	90	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00		
	100						0.03				1.00	1.00
s-Mdh	-100									0.93		
	80				0.02					0.17		
	90											1.00
	100	1.00	1.00	1.00	0.98	1.00	1.00	1.00	1.00		1.00	
s-Me	-100									0.50		
	90										1.00	
	91											1.00
	98									0.40		
	100	1.00	1.00	1.00	0.20	0.83	1.00	1.00	1.00	0.10		
	102				0.53	0.13						
	105				0.14	0.04						
	108				0.11							
Pgm	95									0.17		
	100	1.00	1.00	1.00	0.97	1.00	1.00	1.00		0.83	1.00	
	102				0.03							1.00
	105											
6-pgdh	80				0.03	0.04				0.08		
	85									0.32		
	88				0.01			0.08				
	90	1.00	1.00	1.00	0.96	0.96	1.00	0.88	1.00	0.60		
	98											0.03
	100											0.94
	103											0.03
	108										0.05	
	112										0.87	
s-Sod	100	1.00	1.00	1.00	1.00	1.00	0.91	1.00	1.00	1.00	1.00	1.00
	110						0.09					
Es-D	95				0.10							
	98							0.23				
	100	0.95	1.00	1.00	0.86	1.00	1.00	0.77	1.00	1.00	1.00	1.00
	105	0.05			0.04							
Es-2	94									0.17		
	96		0.12	0.25	0.50	0.04	0.05	0.55			0.57	
	98	1.00	0.88	0.75	0.50	0.92	0.95	0.35		0.50	0.37	
	100					0.04				0.33	0.07	
	103											1.00
	105							0.05	0.21			
	110							0.05	0.70			
	115								0.09			
Hb	99											1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
	102										1.00	
Alb	98											1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
Pt-1	a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	b											1.00
H obs. %		0.6	3.0	2.2	6.3	4.9	3.4	6.9	4.0	6.6	5.6	1.0
H exp. %		0.6	3.2	2.1	8.0	5.0	4.3	7.8	3.8	13.7	5.8	1.0

Under the electrophoretic condition used, the following loci are monomorphic: s-Idh, Es-3, Pt-2, Pt-3. See table 1 for population numbers.

between the *P. guttatus* s. lato representatives and the other two species that we studied (Fig. 2).

### Discussion

Two distinctive gene pools,

differentiated from one another only by two diagnostic loci, were found between representatives of *P. guttatus* s. lato and *P. versicolor* (southern Mongolia). The genetic differentiation corresponds to the division of eastern Palearctic (*P. versicolor* from southern Mongolia) and Western

TABLE 4. Distribution of genotypes at the Ldh-A locus in populations of different geographic forms of toad agamas of the *Phrynocephalus guttatus* group.

Form	Locality	Genotypes distribution				
<i>salensky</i>	Zaissan	O	42	0	6	
		E	36.8	10.5	0.75	$\chi^2 = 13.44^{**}$ d. f. = 1
<i>kushackevitschi</i>	Andreevka	O	2	0	10	
		E	0.31	3.2	8.5	$\chi^2 = 4.05^*$ d. f. = 1
<i>kushackevitschi</i>	Kapchagay	O	1			
		E	0.6			
<i>moltschanovi</i>	Beltau	O	7			
		E	6.75			

Note: O- observed distribution; E- expected distribution; \* $p < 0.05$ ; \*\* $p < 0.001$

TABLE 5. Matrix of genetic distances (D, Nei, 1975) among the taxa *P. guttatus* s. lato, *P. strauchi*, and *P. helioscopus saidalievi*.

	1	2	3	4	5	6	7	8	9	10	11
1	x	0.004	0.003	0.043	0.053	0.038	0.033	0.046	0.151	0.257	0.809
2		x	0.002	0.041	0.028	0.012	0.016	0.042	0.128	0.281	0.820
3			x	0.032	0.055	0.041	0.019	0.035	0.146	0.269	0.795
4				x	0.078	0.078	0.040	0.049	0.132	0.234	0.878
5					x	0.003	0.043	0.099	0.117	0.344	0.920
6						x	0.032	0.084	0.120	0.326	0.896
7							x	0.043	0.133	0.280	0.845
8								x	0.165	0.303	0.784
9									x	0.388	0.749
10										x	0.659
11											x

Palaearctic forms (*P. guttatus*) which diverged around 500,000 years ago (Late Pleistocene).

*P. guttatus* consists of conspecific forms, diverse morphologically, but conservative on the molecular level. In this species, the more differentiated form is *P. v. hispida*. This is supported by the fixation of Es-2 (94) which is absolutely absent in *P. v. versicolor*.

The level of genetic differentiation of *P. guttatus* s. lato from *P. strauchi* and *P. helioscopus saidalievi* is higher and corresponds to good species which diverged about 1,500,000-2,000,000 years ago, i.e. in Middle or Early Pleistocene.

On the basis of the data we obtained, our main conclusion is that *P. guttatus* s. lato. consists of two groups with a remarkable

level of genetic differentiation. There is an eastern Palaearctic *P. versicolor* from southern Mongolia, and a western Palaearctic *P. guttatus* s. str.\* which includes: *g. guttatus*, *g. kushackewitschii*, *g. alpherakii*, *g. salenskyi*, *g. moltschanovii*, *guttatus ssp.* from northern Turkmenia and *versicolor hispida* from Djungar Gate. There are no objective criteria for subspecific separation by biochemical genetic markers.

\*This abbreviation, which as generally known means "sensu stricto", was erroneously deciphered as "s. Strauch" (Mezhzherin and Golubev, 1992). This somewhat distorted the intended meaning.

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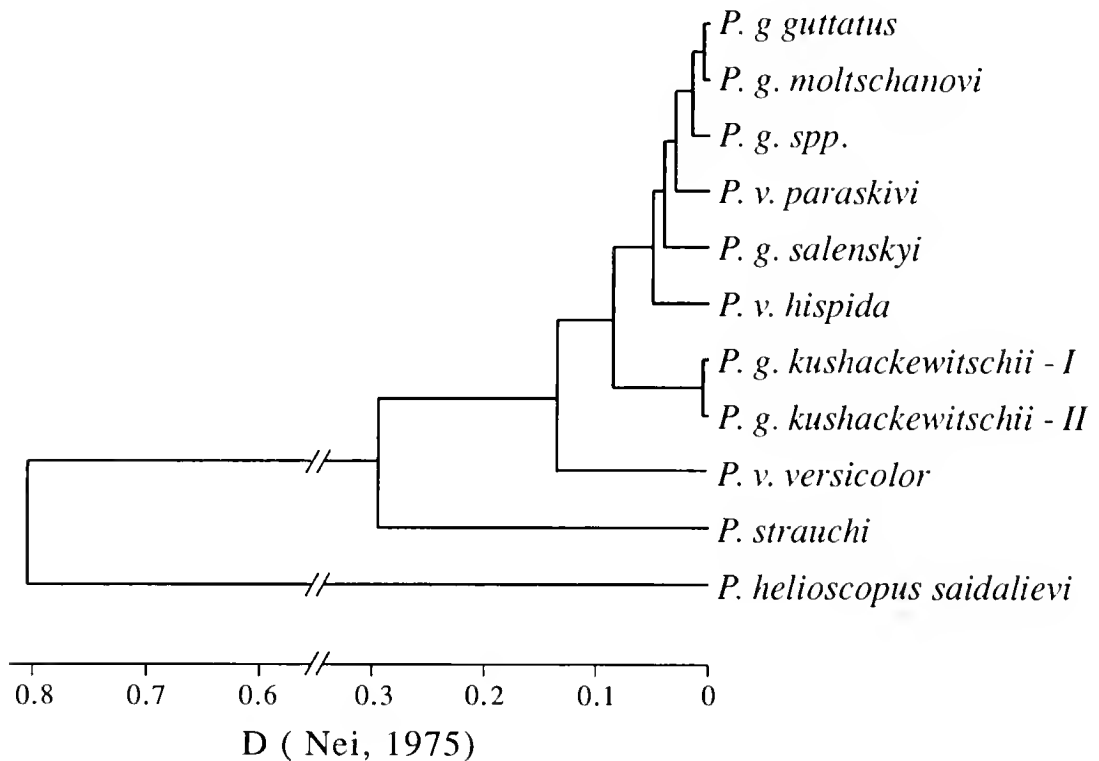


FIG. 2. UPGMA Phenogram of relationships among *Phrynocephalus guttatus* s. lato.

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## Geographic Variation and Diversity in Three Species of *Phrynocephalus* in the Tengger Desert, Western China

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**Abstract.** -Univariate and multivariate statistical analysis of 5 meristic characters, 4 metric characters and 8 ratio characters recorded for 9 samples of lizards were used to assess non-geographic variation and geographic variation in the southeast area of the Tengger Desert. A cluster analysis indicated that there were three major groups which represented *Phrynocephalus versicolor*, *P. przewalskii*, and *P. frontalis*, based on morphological characters, respectively. The cluster analysis and canonical analysis showed that among samples, phenetic similarity was not always predicted by geographic proximity. Dispersal and divergence of these species of *Phrynocephalus* with the relationships of paleogeography and paleoclimatology are discussed. It is evident that the Yellow River fails to cause geographic isolation.

**Key words:** Reptilia, Sauria, Agamidae, *Phrynocephalus*, China, biogeography.

### Introduction

Strauch (1876) described four new species of *Phrynocephalus* from the Altan Desert (including the Tengger Desert) and the Mu Us Desert (Ordos). They were *P. przewalskii*, *P. versicolor*, *P. affinis*, and *P. frontalis*. He pointed out that *P. affinis* was very similar to *P. przewalskii*. Pope (1935), with out any discussion, considered *P. affinis* to be synonymous with *P. przewalskii*. Leroy (1939), who studied the geographic variation and distribution of *P. przewalskii*, *P. frontalis*, and *P. vlangalii*, failed to recognize *P. frontalis* and *P. versicolor*, which were quite different on morphological characters. Therefore, his distribution of *P. frontalis* included *P. versicolor*. Zhao (1979) deduced that the distribution of *Phrynocephalus* in China almost reached the shore of the Bohai Sea and in the Altan Desert (including the Tengger Desert) no distribution for *P. frontalis* was mentioned.

In this paper we discuss (1) the geographic variations and distributions of *P. przewalskii*, *P. frontalis*, and *P. versicolor*, and (2) the dispersal and divergence tracts of the three species in the Tengger Desert.

### Materials and Methods

**Study Areas.**—The Tengger Desert is

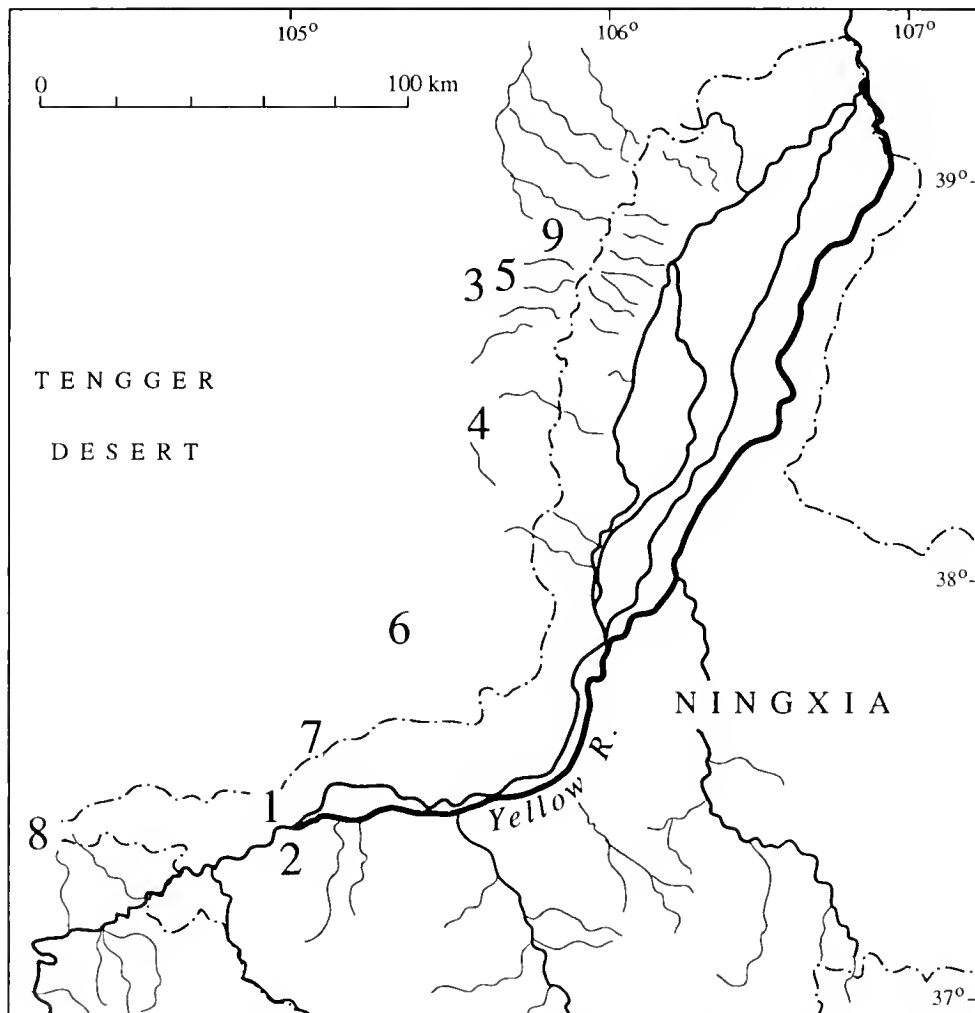
situated in western Nei Mongol Autonomous Region, China. The east edge reaches the Helan Mountains, and the southern edge, the Yellow River. In the west and north, the study area connects with the Badain Jaran Desert and the Ulan Behou Desert, respectively. The climate in these areas is arid-continental. The topography is moving sand dunes and gobi. In this area the vegetation is scarce. *Ammopiptanthus sp.*, *Potaninia sp.*, *Teraena sp.*, and *Caragana sp.* are present. Populations of *P. przewalskii*, *P. frontalis*, and *P. versicolor* were sampled from nine localities in the southeast area of the Tengger Desert from the south and north shore of the Yellow River, through southeast Tengger Desert to Hala Woo Valley of the Helan Mountains, and in the west at Xial Hong Shan (Fig. 1 and Table 1).

**Methods.**—Five meristic characters, four metric characters and eight ratio characters were chosen for study. These characters are shown in Table 2.

**Statistical analysis.**—For analysis of geographic variation, lizards were grouped into nine samples (Fig. 1 and Table 1) within which gene flow was assumed to occur freely. Sexual variation in metric characters was examined in sample 1 (representing *P. przewalskii*), and samples 2 and 3 (representing *P. frontalis*). We

TABLE 1. Locality data for *Phrynocephalus* from the Tengger Desert, western China used in this study.

Population	Locality	Males	Females
1	Shapotou North shore of the Yellow River (37°30'N 104°58'E)	26	14
2	Shapolton South shore of the Yellow River	31	12
3	7.2 km SW of Alxa Zuogi (38°50'N 105°32'E)	21	19
4	2.4 km north of Mujingzi (38°20'N 105°42'E)	9	15
5	1.9 km SW of Alxa Zouqi (38°50'N 105°32'E)	10	9
6	1.7 km SW of Luanjing (37°58'N 105°32'E)	6	4
7	47.3 km SW of Luanjing	5	2
8	9.3 km east of Xiao Hong Shan (37°31'N 104°27'E)	5	1
9	Hala Woo Valley Helan Mountains (38°51'N 105°34'E)	5	3

FIG. 1. Locations in the Tengger Desert for the nine samples of *Phrynocephalus* used in the study of geographic variation (see Table 1 for exact localities).

determined that individuals of the three species are reproductively mature at an SVL of 45 mm or larger.

The ANOVA test was used to test for

differences between the sexes in samples 1, 2, and 3. Standard univariate statistics were calculated for these three samples and to examine geographic variation in single meristic characters for each sample. For all

TABLE 2. Characters used in analysis of variation in three species of *Phrynocephalus*.

## A. Meristic Characters

1. NSPL	No. of supralabials
2. NIFL	No. of infralabials
3. NSDT	No. of subdigital lamellae on longest toe
4. NSNT	Number of scales between nostrils
5. NDVT	No. of ventral dark bands on tail

## B. Metric Characters

6. SVL	Snout-vent length
7. TL	Tail Length
8. LFL	Foreleg length (including fingers)
9. LHL	Hindleg length (including longest toe)

## C. Ratio characters

10. SVL/TL
11. SVL/LFL
12. SVL/LHh
13. TL/LFL
14. TL/LHL
15. LFL/LHL
16. HL/HW\*
17. LNE/LN\*\*

\* Head length (rostral up to and including parietales)/Head width (front of ears).

\*\* Length from nostril to eye/Length between nostrils.

samples, meristic characters were calculated and standard values from the matrix of intersample phenotypic distances were clustered with the unweighted furthest-neighbor method using arithmetic average. To overcome some disadvantages of the clustering, the multivariate analysis of variance (MANOVA) and canonical analysis were used to provide weighted combinations of characters to analyze variations of the nine samples. A set of canonical was calculated and the mean values of each sample were plotted on the first two axes. Additionally, the relative contribution of each character in Table 4 to each of these two axes was calculated, which collectively accounted for over 86% of the total variation. All calculations were performed on an IBM-PC/XT computer at the Chengdu Library, Academia Sinica by means of FORTRAN Program II.

TABLE 3. Sexual variation in three samples of *Phrynocephalus* (sample number as in Fig. 1 and Table 1. Levels of significance are indicated. (\*= $p<0.05$ ; \*\*= $p<0.01$ )

Character	n	M (SD)	n	M (SD)	ANOVA
Males			Females		
Population 1					
SVL	26	61.51 (1.42)	14	58.65 (2.88)	4.11**
TL	26	86.20 (3.50)	14	75.72 (3.54)	1.02
LFL	26	31.35 (1.10)	14	29.47 (1.17)	1.13
LHL	26	51.10 (2.16)	14	47.17 (1.82)	1.41
Population 2					
SVL	31	51.51 (1.45)	12	52.08 (1.64)	1.28
TL	31	72.55 (2.67)	12	65.39 (2.00)	1.78
LFL	31	26.09 (1.42)	12	25.36 (1.01)	1.98
LHL	31	43.05 (2.83)	12	40.16 (1.69)	2.80**
Population 3					
SVL	21	58.07 (1.59)	19	54.97 (2.37)	2.22*
TL	21	77.85 (3.23)	19	67.86 (3.59)	1.24
LFL	21	29.51 (1.28)	19	27.25 (0.69)	3.44**
LHL	21	47.68 (1.79)	19	42.52 (0.73)	6.01**

## Results

*Sexual variation (non-geographic variation).*—Results for intersex comparisons (adults only) in three samples are summarized in Table 3. In ANOVA the sexes from sample 1 differed significantly in SVL. In sample 2, the metric character LHL showed significant sexual dimorphism. In sample 3, three metric characters, SVL, LFL, and LHL showed significant sexual dimorphism.

*Multivariate analysis.*—Fig. 3 presents a distance phenogram, based on 5 meristic characters, clustering 9 OTUs which correspond to the group sample localities (Fig. 1 and Table 1) used in this study.

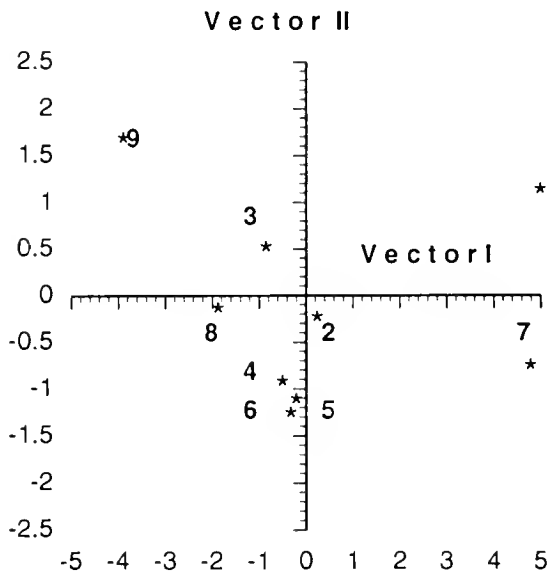


FIG. 2. Projections on the first two canonical vectors of centroids representing the nine samples of *Phrynocephalus*. The number of centroid corresponds to the localities in Fig. 1.

The cophenetic correlation of the phenogram with the distance matrix was 0.912. The first major dichotomy separates sample 9 from all others, and the second major dichotomy groups samples 1 and 7. The third major dichotomy groups samples 2, 3, 4, 5, 6, and 8. Most of the clusters group samples that do not have any geographic affinities or relationships. For example, samples 3 and 9 in fairly close geographic proximity (about 10 km separate each other) were separated by different dichotomies in the phenogram, conversely, samples 2, 8, and 3 clustered with one another, despite being separated by over 150 km.

*Univariate analysis (geographic variation).*—Standard statistics are presented for two meristic characters. Fig. 4 and Fig. 5 depicts geographic variation in NSPL and NIFL counts for nine samples (males only). The mean NSPL counts of samples 1 and 7 were 17.50 and 16.00, respectively. For samples 2, 3, 4, 5, 6, and 8, the mean NSPL counts were between 13.00 and 14.90, and for sample 9 was 15.00 (Fig. 4). Fig. 5 shows NIFL counts for all nine samples. Samples 1 and 7 show higher NIFL counts, 17.10 and

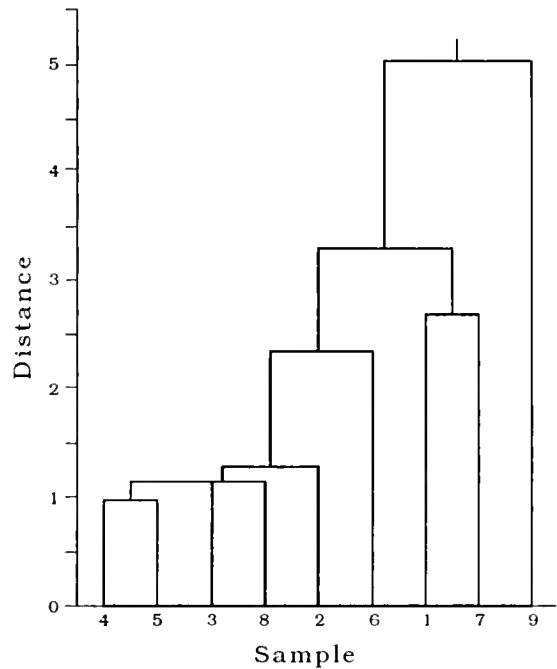


FIG. 3. Distance phenogram resulting from the cluster analysis with five meristic characters of nine samples (each considered an OTU) of *Phrynocephalus*. The cophenetic correlation coefficient was 0.912.

16.00 respectively, than those of samples 2 (15.00), 3 (14.80), 4 (14.10), 5 (14.00), 6 (13.20), and 8 (14.00), as well as 9 (14.00).

In the MANOVA, the overall tests of the hypothesis of no effect due to geography were rejected ( $P \leq 0.001$ ) for Wilks' Criterion, and Roy's Maximum Root Criterion. The first two canonical vectors extracted from the variance-covariance matrix accounted for 77.58 % and 8.67 % of the total variation. The samples are plotted along these two vectors in Fig. 2 and Table 4 summarizes the percent influence of each character to each of the two vectors.

Three major groups were discernible in Fig. 2, the most strongly differentiated of which separated primarily along vector I and to some extent along vector II. The second group in clustering (samples 1 and 7) was separated along vector I, which also separated by high NSPL and NIFL counts in Figs. 4 and 5, and form a distinct phenetic cluster in Fig. 3. The first group

TABLE 4. Variable coefficients for canonical variates I and II and estimated % influence of each vector for nine samples of *Phrynocephalus*.

Character	Vector I (77.58%)		Vector II (8.67%)	
	Variable coefficients	% influence	Variable coefficients	% influence
NSPL	0.332	8.040	0.715	4.430
NIFL	0.355	9.780	0.715	4.430
NSDT	0.871	58.862	-0.450	1.763
NSNT	0.035	0.097	-0.245	0.011
NDVT	-0.093	0.007	-0.036	0.001
SVL/TL	-0.008	0.000	-0.004	0.000
SVL/LFL	0.026	0.001	-0.043	0.000
SVL/LHL	-0.004	0.000	0.005	0.000
LNE/LN	0.032	0.001	-0.010	0.000
LFL/LHL	-0.001	0.000	0.001	0.000
HL/HW	0.003	0.000	0.009	0.000
TL/LFL	0.007	0.000	-0.003	0.000
TL/LHL	0.023	0.000	0.031	0.000

TABLE 5. Characters for three species of population-groups of *Phrynocephalus*.

Character	<i>P. frontalis</i> Range (Mean)	<i>P. przewalskii</i> Range (Mean)	<i>P. versicolor</i> Range (Mean)
SVL	45.8-57.2 (51.5)	50.7-71.4 (61.6)	46.1-55.6 (49.5)
TL	58.4-82.1 (72.4)	76.1-101.5 (86.3)	53.9-69.4 (60.8)
LFL	23.3-29.5 (26.1)	27.4-35.9 (31.4)	22.7-26.9 (24.4)
LHL	37.1-48.7 (43.1)	43.7-59.6 (51.1)	36.5-43.4 (39.1)
NSNT	2-4 (3.2)	2-4 (2.9)	2-4 (2.0)
NSPL	11-17 (14.9)	15-20 (17.6)	13-17 (15.0)
NIFL	12-17 (15.1)	14-20 (17.1)	12-16 (14.0)
NDVT	2-5 (2.8)	1-4 (2.2)	0-3 (2.0)
NSDT	24-30 (26.4)	27-34 (29.9)	21-26 (22.0)
Color on ventral surface of tail tip	dark	dark	dark
Color on armpits	grey-white	grey-white	grey-white
Color on back of body	2-5 pairs of black spots	irregular spots longitudinal line	2-4 transverse black- reddish bands
No. of teeth on maxillary	11	11	10
No. of teeth on dentary	11	12	11
Ishium	Cartilage	Cartilage	Posterior 1/2 is cartilage
Meckel's cartilage	Covered by splenial	Covered by splenial	Native not covered by splenial

in clustering (sample 9) was separated along vector II and vector I. The third group, represented by samples 2, 3, 4, 5, 6, and 8 in clustering, was plotted along the centroid in Fig. 2.

Characters having the greatest influence

to vector I and vector II are NSDT, NSPL, and NIFL, respectively.

Only males were used in univariate and multivariate analysis for geographic variation.

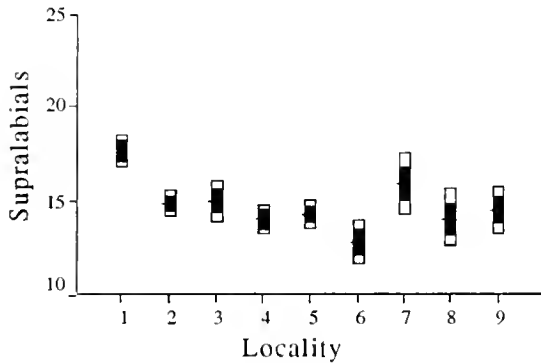


FIG. 4. Diagram depicting geographic variation among nine samples of *Phrynocephalus* in supralabial number. Verticle line represents sample mean; open and closed bars represent range and one standard deviation, respectively.

### Discussion

According to the morphological characters, populations of all nine samples can be divided into three major groups as shown in Fig. 2, 3, and Table 5. The first group (sample 9), a second group (samples 1 and 7), and a third group (samples 2, 3, 4, 5, 6, and 8). These are very similar to the descriptions by Strauch (1876) of *P. versicolor*, *P. przewalskii* and *P. frontalis*, respectively.

It is apparent that *P. frontalis* is distributed in the Tengger Desert. This was not mentioned by authors who had studied the distribution of *P. frontalis* before.

Strauch (1876) had pointed out that the color patterns of *Phrynocephalus* were very variable. In our study, we found that the color on the armpits of the group of *P. frontalis* and *P. versicolor* in the Tengger Desert was grayish-white. This was different from *P. frontalis* in the Mu Us Desert which has reddish on the armpits, and *P. versicolor* with reddish-blue on the armpits at Anxi and Wuwui in Gansu Province. In 142 specimens of *P. frontalis* in the Tengger Desert, only 4 specimens had reddish on the armpits. Therefore, we suggest that the color on armpits could not be a character for taxonomy in the genus *Phrynocephalus*.

In the group of *P. frontalis*, sample 2 is

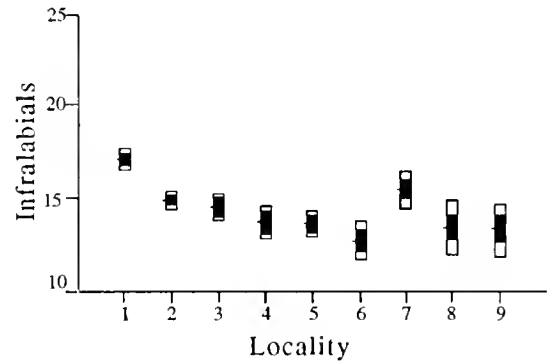


FIG. 5. Diagram depicting geographic variation among nine samples of *Phrynocephalus* in infralabial number. Verticle line represents sample mean; open and closed bars represent range and one standard deviation, respectively.

located on the south shore of the Yellow River, but samples 3, 4, 5, 6, and 8 are located on the north side of the Yellow River. They are very similar in morphological characters and they belong to the same species, *P. frontalis*. It is evident that the Yellow River does not function to geographically isolate *P. frontalis*. According to information on paleogeography and paleoclimatology, in Late Tertiary the Tengger Desert began to form. The area was very dry and cool. The Yellow River began to form in the Pleistocene with the uplift of the Qinghai-Xizang Plateau (Li, 1984). This means that the Tengger Desert was forming earlier than the Yellow River. Additionally, the Yellow River in this area had shifted its route several times from west to east during the past. We suggest the *Phrynocephalus* might have invaded and dispersed into the Tengger Desert before the formation of the Yellow River and that the river fails to be a geographic barrier for these lizards. Although the group of *P. przewalskii* located on the north side of the Yellow River is the nearest neighbor of the group of *P. frontalis*, they are quite different from each other on morphological characters. It is believed that this is the result of interspecific isolation.

According to the study of morphological and skeletal characters of *P. versicolor*, *P. przewalskii* and *P. frontalis*, *P. versicolor* was quite different from the other two

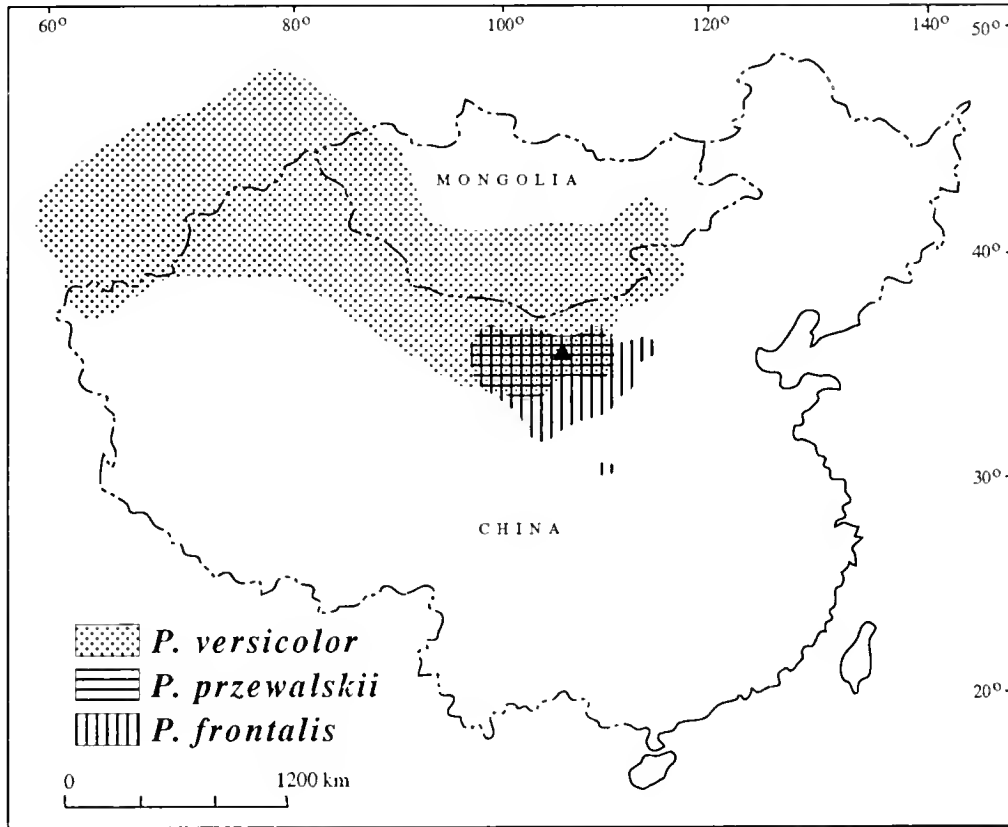


FIG. 6. Geographic distribution of *P. versicolor*, *P. przewalskii*, and *P. frontalis*.

(Table 5; Wang, 1987), and showed the characters replacement. For example, the pro-half part of the ischiopubis in *P. versicolor* is ossified and the post-half part still is cartilage. The ischiopubis in *P. przewalskii* and *P. frontalis*, conversely, is all cartilage. Additionally, the Meckle's cartilage is not covered by the splenial in *P. versicolor* but it is covered by the splenial in *P. przewalskii* and *P. frontalis*. Electrophoresis also showed that *P. versicolor* is different from *P. przewalskii* and *P. frontalis*, the latter two species being similar (R. Macey pers. comm.). Therefore we suggest that *P. versicolor* was probably derived earlier from the ancestral stock than *P. przewalskii* and *P. frontalis*.

The distribution of *P. versicolor* is from central Asia through western China to the central parts of Mongolia (Boblov, 1986) and Nei Mongol (Zhao, 1978; 1975). *Phrynocephalus przewalskii* occurs from

Zhang Ye, Gansu Province through the Tengger Desert to the west edge of the Helan Mountains. *Phrynocephalus frontalis* is found from Zhang Ye (Yao, 1983) through the Tengger desert to the Mu Us Desert (Ordos) (Schmidt, 1927) and forward to the south in the east part of Gansu Province. There is also an isolated population at Xun Yang in Shaanxi Province (Song, 1987) (Fig. 6). *Phrynocephalus* is distributed to the east to about 112°E but does not reach the shore of the Bohai Sea as Zhao (1979) stated.

In the Early Tertiary, there was an arid-subtropical continental climate belt from central Asia through the southern edge of the depression areas in the Tian Shan Mountains and the north edge of the Tarim Depression, the north part of the Qinghai-Xizang "initial plateau", the Qaidam Depression, depression areas in the Qilian-Qinling Mountains, and the south edge of depression areas in the Helan-Liupan

Mountains to the central part of China (Li, 1984). In some areas, desert had been forming and the ancestral stock of the Agamidae might have followed this belt to invade the areas of the south side of the depression areas in the Helan-Liupan Mountains from central Asia through western China. As evidence, some fossils of Agamidae were discovered from the Eocene of western and central China (Li, 1984). We suggest that *P. versicolor* might have derived from the ancestral stock during that time. After the Late Tertiary and Early Quaternary, with the uplift of the Qinghai-Xizang Plateau, this arid-continental climate belt shifted forward to northern regions, including the Tengger Desert (Li, 1984). The ancestral stock of *Phrynocephalus* and *P. versicolor* might have followed the shift of this belt again to disperse into northern regions, including the Tengger Desert and *P. przewalskii* and *P. frontalis* derived from ancestral stock in the Tengger Desert during that time. Then *P. frontalis* dispersed further east to the Mu Us Desert (Ordos) and south part of the Qinling Mountains. According to the distribution of *P. frontalis*, it is obvious that it had a wider range in the past.

*Phrynocephalus przewalskii* and *P. frontalis* show sexual dimorphism. According to observations in the breeding season on mating behavior, males run after females quickly for mating (Song et al. 1987). We deduced that the individual males with long legs would have more successful opportunities for mating with females than males with shorter legs. We suggest that the sexual dimorphism between males and females was the result of sexual selection.

### Acknowledgments

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## Sympatric Amphibians of the Yew-box Grove, Caucasian State Biosphere Reserve, Sochi, Russia

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**Abstract.**-The Yew-box Grove of the Caucasian State Biosphere Reserve is home to seven species of amphibian. These species occur in a wide range of aquatic environments. The species composition, physical characteristics and history of each aquatic site was evaluated. The reproductive biology and food habits of each species was studied. These amphibians divide their niches on daily activity, seasonal activity, breeding site, microhabitat and food habits. The highest amphibian diversity and species overlap occurs in the most stable aquatic environments.

**Key Words:** Amphibia, Russia, Caucasus, ecology.

### Introduction

It is important when studying the influence of environmental factors on life history characteristics to distinguish those factors that are significant and those that are part of the "neutral background" (Monchadsky, 1958). The study of environmental influences is accomplished on sympatric species, usually closely related species (Orr and Maple, 1978; Ananjeva, 1981) at the population level (Pianka, 1973; Schoener, 1974; Schoener, 1977; Lyapkov and Severtsev, 1981). This is the study of the ecological niche (Pianka, et al., 1979).

In the former Soviet Union one of the areas of highest amphibian diversity is found in the western Caucasus. The Yew-box Grove of the Caucasian State Biosphere Reserve is inhabited by eight amphibian species: *Triturus vulgaris lantzi*, *T. cristatus karelini*, *T. vittatus ophryticus*, *Pelodytes caucasicus*, *Bufo verrucosissimus*, *Hyla arborea schelkownikowi*, *Rana ridibunda*, and *R. macrocnemis*.

### Methods

Field studies were conducted from 1980-1982 in the Yew-box Grove (approximate area 302 ha) in the Caucasian State Biosphere Reserve and on adjacent land. Transect routes and study sites were selected on the basis of local forest topography (Gulisashvili, et al., 1975). Observations on these study sites along the

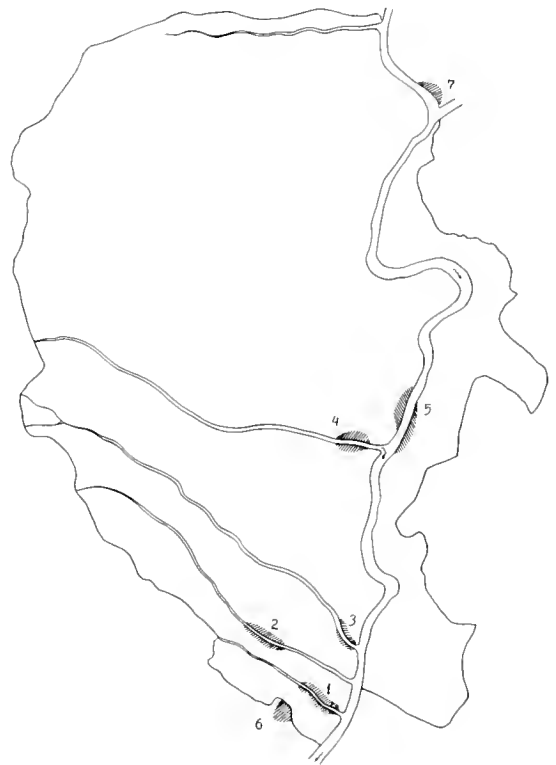


FIG. 1. Study sites of sympatric amphibians in the Yew-box Grove, Caucasian State Biosphere Reserve. 1- Spring 118; 2- Opolznevaya Ravine; 3- Labirintovaya Ravine; 4- Glubokaya Ravine; 5- Khosta River; 6- Samshit Pond; 7- Pond on Malaya Khosta River.

transects were made throughout the year (Fig. 1). The intensity of calls was recorded in spring (2-3 times per week), summer (1-2 times per month) and fall (2-3 times per week). Over 200 individuals were recorded.

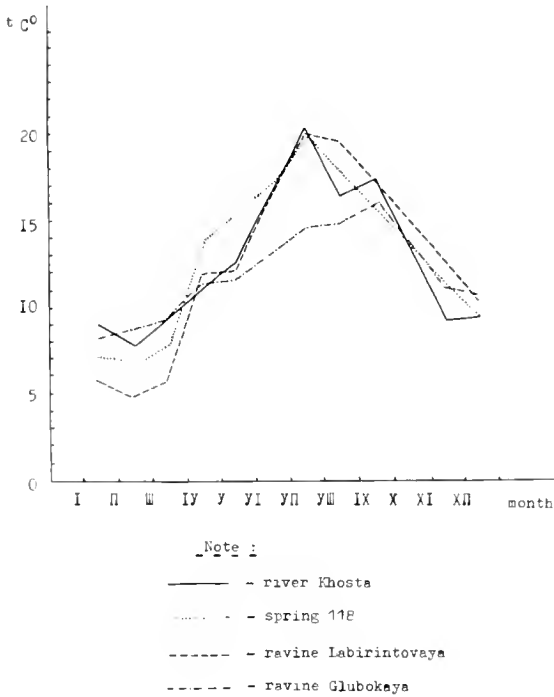


FIG. 2. Mean monthly temperatures of perennial bodies of water in the Yew-box Grove (1980-1982).

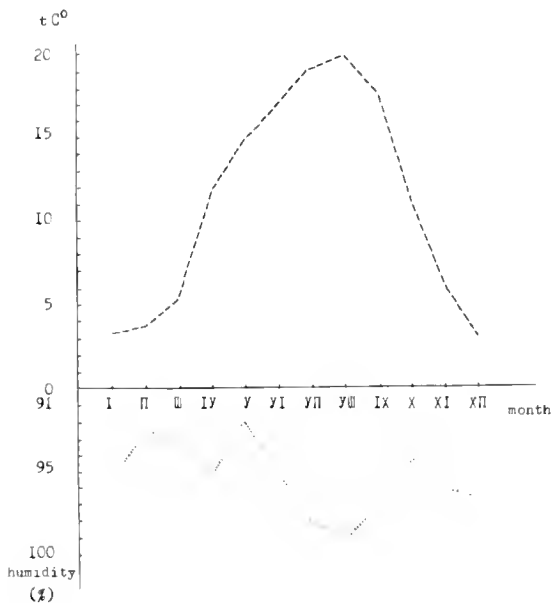


FIG. 3. Climatram of the Yew-box Grove (1982).

The location, weather conditions, air and body temperature, and behavior of each specimen was recorded. During 1982, detailed microclimate records were made in

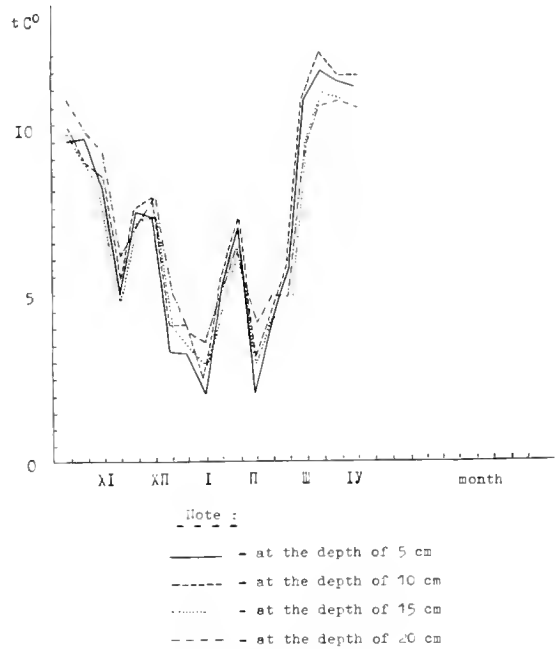


FIG. 4. Winter soil temperature in the Yew-Box Grove (1982-1983).

the Yew-box Grove (Fig. 2). Air temperature and humidity above the surface were recorded weekly by a thermograph (M-16AN) and a hydrograph (M-16AN) placed in a meteorological kiosk (Fig. 3). Soil temperatures were recorded at 5, 10, 15 and 20 cm depths (Fig. 4).

Water samples were taken periodically for chemical analysis by established methods (Anonymous, 1978). Ambient light was measured with a light meter and converted into percent relative illumination. Climagrams were made (Formozov, 1934).

Species composition of each biotope was calculated using conventional methods (Kashkarov, 1927; Dinesman and Kaletskaya, 1952). Food habits were studied using non-lethal methods (Verzhutsky and Zhuravlev, 1977).

## Results

### Description of Study Sites

*Study Site 1. Spring 118.* This site contains a small perennial stream running

TABLE 1. Some hydrochemical indices of the study sites.

Sample site No. of samples	pH Mean	ions, total mg/l	total hardness mg-equiv/l	nitrogen			mineral
				ammonium	nitrites	nitrates	phosphorous mg/l
Glubokaya 15	7.36-8.30 8.02	196.0-292.1 223.7	2.42-3.60 2.78	0.00-0.04 0.02	0.00-0.026 0.009	0.16-0.37 0.24	0.003-0.008 0.003
Labirintovaya 10	7.30-8.45 7.87	182.8-380.1 868.7	2.28-3.67 3.08	0.00-0.60 0.19	0.00-0.020 0.004	0.03-0.55 0.18	0.003-0.029 0.009
Opolznevaya 10	8.00-8.27 8.17	254.3-350.0 301.3	3.04-3.71 3.42	0.01-0.11 0.05	0.00-0.034 0.020	0.08-1.41 0.45	0.003-0.009 0.006
Spring 118 10	7.54-8.50 8.12	347.1-472.9 413.4	3.91-5.61 4.85	0.01-0.45 0.12	0.001-0.07 0.019	0.97-2.67 1.78	0.013-0.164 0.089
Khosta River 10	7.70-8.50 8.12	208.2-315.9 252.3	2.35-3.39 2.98	0.00-2.32 0.22	0.00-0.016 0.006	0.02-1.44 0.37	0.003-0.022 0.008

through a sub-tropical, mixed broad-leaved forest (*Fagus orientalis*, *Taxus baccata*, *Carpinus betulus*) with an evergreen understory (*Buxus colchicus*, *Ilex colchicus*, *Laurocerasus officinalis*) and lianas (*Hedera colchica*, *Smilax excelssior*). Relative illumination is 1-2%. The stream flows over a bed composed of clay and sandstone. The stream flow derives from runoff and a sub-surface aquifer. The water chemistry of the spring water was hydrocarbonic-calcic with moderate mineralization, and moderately hard (Table 1). Hydrogen ion concentration is neutral to slightly basic, pH ranges from 6.89-8.50. The concentration of nitrogen and nitrates (0.97-2.67 mg/l) is higher than in other waterways. This is a result of the subterranean flow. Ammonium concentration is normally low and increases during flash floods (up to 0.45 mg/l). Nitrites are also found in low concentrations except during flash floods (up to 0.08 mg/l). Phosphorus concentration is considerably higher than in other waterways (up to 0.16 mg/l). *Rana macrocnemis*, *R. ridibunda*, *Bufo verrucosissimus*, *Hyla arborea schelkownikowi*, *Pelodytes caucasicus*, and *Triturus vittatus ophryticus* are found at this study site and the latter two species breed there (Fig. 5, Table 2).

*Study Site 2. Opolznevaya Ravine.* A small intermittent waterway flows through an eroded ravine through carbonic rock and

clay. The vegetational community is analogous to Study Site 1, but box yews and beeches are dominate. Relative illumination is 2%. Stream flow is derived from runoff and aquifer. The water chemistry of the spring water was hydrocarbonic-calcic with moderate mineralization, and soft. Hydrogen ion concentration is neutral to slightly basic, pH ranges from 8.0-8.27. No amphibians were observed at this study site.

*Study Site 3. Labirintovaya Ravine.* A small intermittent, vernal-autumnal stream flows through an eroded, karst gorge with steep walls in a box yew forest. Relative illumination is 2%. Stream flow is derived from runoff and subsurface flow. During low waters periods the stream falls into a number of stagnant pools. The water chemistry of the spring water was hydrocarbonic-calcic with moderate mineralization, and soft. Hydrogen ion concentration is neutral to slightly basic, pH ranges from 7.3-8.45. Water content of nitrogen compounds is low but increases during flash floods. *R. ridibunda* is found here at this study site and *P. caucasicus* reproduces here.

*Study Site 4. Glubokaya Ravine.* This site is a small pond in a limestone gorge with steep walls and surrounded by a box yew forest. Relative illumination is 2-3%. The pond is fed by a small, relatively constant spring flowing from Karst.

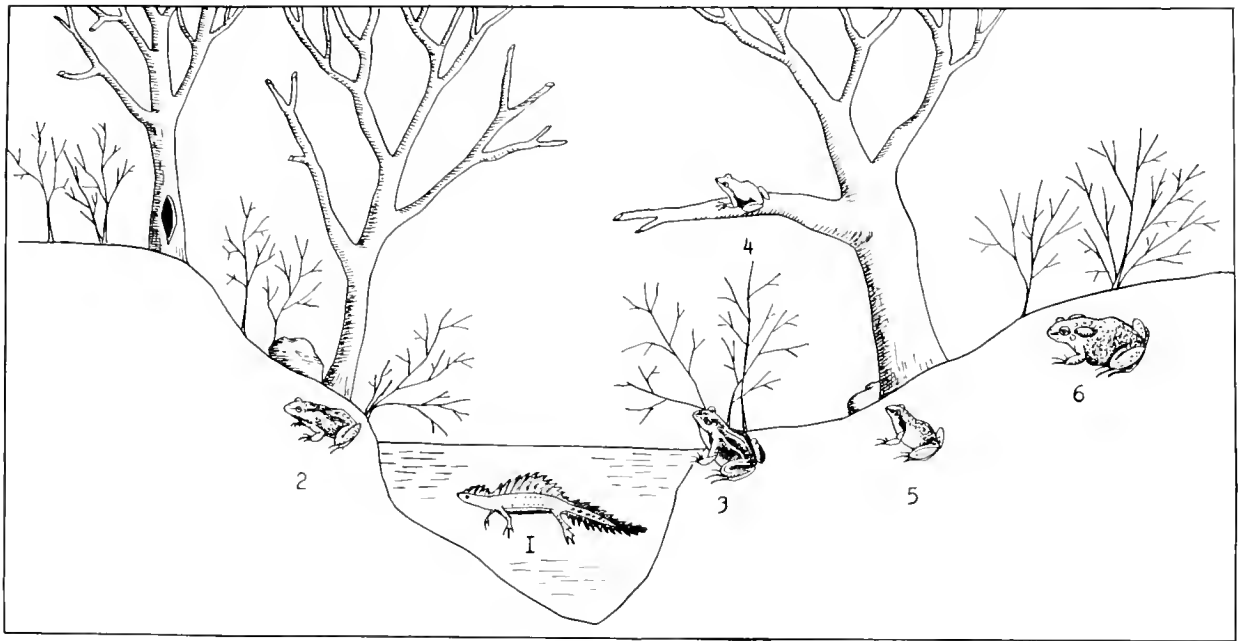


FIG. 5. Distribution of sympatric amphibians at Spring 118 in the Yew Box Grove. 1- *Triturus vittatus ophryticus*; 2- *Pelodytes caucasicus*; 3- *Rana ridibunda*; 4- *Hyla arborea schelkownikowi*; 5- *Rana macrocnemis*; 6- *Bufo verrucosissimus*.

Spring temperature ranges from 11-16° C. The water chemistry of the spring water was hydrocarbonic-calcic with moderate mineralization, and moderately hard (2.42 - 3.6 mg-eq/l). Ammonium concentration is low (maximum = 0.04 mg/l) and in winter drops to 0. Nitrates are highest during summer low water period (up to 0.37 mg/l). Phosphorus concentration is very low (>0.01 mg/l). *Bufo verrucosissimus*, *R. macrocnemis*, *P. caucasicus*, *R. ridibunda* and *T. v. ophryticus* are found living at this study site and the last three species breed here.

**Study Site 5. Khosta River.** This site is a small mountain stream 21 km long that drains a watershed of approximately 96 km<sup>2</sup>. The stream flows through a canyon formed in Cretaceous limestone at an average of 5 m<sup>3</sup>/sec. Water flow is derived from runoff and springs arising in karst rock formations. Vegetation is a broad-leaved, subtropical Colchis type forest. Relative illumination is as high as 100%. The water chemistry of this stream is hydrocarbonic-calcic with moderate mineralization and basic pH (7.7-8.5). Ammonium concentration is not high and

varies from 0 to 0.07 mg/l, but during floods it can reach 2.32 mg/l. This stream is subject to occasional flooding. Concentration of nitrates reaches a maximum during floods of 1.44 mg/l. Dissolved oxygen is 10-15mg/l and carbonic acid is 10mg/l.

The Nizhe-Khostinsky Spring, flowing out of a karst formation, contributes 1-1.5 m<sup>3</sup>/sec of flow at 11-13° C to the Khosta River. In low water periods the Khosta River above the spring nearly dries up and its temperature ranges from 0.6 (the river freezes) - 26° C. During low waters on the Khosta River the Nizhe-Khostinsky Spring provides a relatively stable flow and temperature regime. This spring serves as a barrier for dispersal of amphibians at this study site. *Rana ridibunda* and *Hyla arborea schelkownikowi* live and reproduce above the spring. *Pelodytes caucasicus* and *Bufo verrucosissimus* live and breed below the spring.

**Study Site 6. Samshit Pond.** This site is a small pond with flowing water in a stand of hornbeam (*Carpinus betulus*) in the broad-leaved forest. Relative

TABLE 2. Diversity of amphibian species at the study sites.

Species	Study Sites						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No.7
<i>Triturus vulgaris</i>						X	X
<i>Triturus vittatus</i>	X			X		X	X
<i>Bufo verrucosissimus</i>	O			O	X		O
<i>Pelodytes caucasicus</i>	X		X	X	X		
<i>Hyla arborea</i>	O					O	X
<i>Rana ridibunda</i>	O		O	X	X	O	
<i>Rana macrocnemis</i>	O	O		O	O	X	X

NOTE: X- reproduce, O- inhabit  
illumination is 100%. The pond flow is feed by run off and flow from the aquifer. The water chemistry of this pond is hydrocarbonic-calcic with moderate mineralization, moderately hard and alkaline. *Hyla arborea schelkownikowi*, *Triturus vulgaris lantzi*, *Triturus vittatus ophryticus*, *Rana ridibunda*, and *R. macrocnemis*, are found living at this study site and the last three species breed here.

*Study Site 7. Pond on the Malaya Khosta River.* This site is a small stagnant pond located in the flood plain forest. The pond is filled by runoff and flow from the aquifer. Relative illumination is 50%. *Bufo verrucosissimus*, *T. vulgaris lantzi*, *T. v. ophryticus*, *H. a. schelkownikowi* and *R. macrocnemis*, are found living at this study site and the last two species breed here.

### Species Accounts

*Triturus cristatus karelini.* This newt is an extremely rare and declining species along the Caucasian Black Sea coast. It was observed only once, at Study Site 1, in the box yew forest.

*Triturus vulgaris lantzi.* This species exclusively inhabits stagnant ponds and ponds with flowing water, in well illuminated stands of hornbeam in broad-leaved forests, and adjacent areas. These newts are found in the ponds beginning in early March. Reproductive activity begins when water temperature reaches 10° C (usually from mid-March to early April.

Females lay their eggs in shallow, thoroughly warmed waters at a depth of 5 cm and remain in the pond until the end of June. The newts over-winter in forest leaf litter and underground (Table 3).

*Triturus vittatus ophryticus.* This species is found in both well illuminated broad-leaved flood plain forests and thick yew-box groves. It appears in bodies of water from the end of November through January. Reproduction occurs from January until the middle of April at water temperatures of 7-9° C. Females lay their eggs at depths of 5-10 cm. The adults stay in the water until the end of May. The newts over-winter in forest leaf litter.

*Bufo verrucosissimus.* This toad is found throughout the Yew-box Grove with the exception of the steeper parts of the Khosta Canyon. Reproduction takes place in well illuminated running water in the Khosta River from February until May at water temperatures from 9.5-16° C. Eggs are deposited at a depth of 20-70 cm in strings through vegetation and other underwater objects. These toad over-winter in forest leaf litter beginning in December.

*Pelodytes caucasicus.* This species inhabits back-water vegetation communities with flowing water. Reproduction lasts from the end of May until the end of October at water temperatures of 13-16° C. Females lay their eggs at a depth of 10-20 cm. These anurans over-winter in forest leaf litter.

TABLE 3. Ecological characteristics of sympatric species of the Yew-box Grove amphibians

DISTRIBUTION:	SPECIES:						
	<i>Triturus vulgaris</i>	<i>Triturus vittatus</i>	<i>Bufo verrucosissimus</i>	<i>Pelodytes caucasicus</i>	<i>Hyla arborea</i>	<i>Rana ridibunda</i>	<i>Rana macrocremisi</i>
at summer biotopes:							
flood plain forest	x	x	x	x	x	x	x
box tree forest		x	x	x		x	x
hornbeam forest		x	x	x	x	x	x
ash and oak forest	x		x	x			x
yew and cherry laurel forest			x		x		x
agrocenosis			x			x	x
at spawning site:							
river			x	x		x	x
pools (rain)							
lotic streams		x		x		x	
semi-lotic streams		x		x		x	
ponds	x	x		x	x	x	
at hibernation sites:							
stagnant, semi-lotic body of water	x	x	x	x	x	x	x
soil and litter							
Breeding periods:							
January		x					
February		x	x			x	x
March	x	x	x			x	x
April	x	x	x			x	x
May			x	x	x		
June-October							
Depth of egg-laying from water surface:							
0-5 cm	x						x
5-10 cm		x					
10-20 cm				x		x	
20-50 cm			x				
over 50 cm			x				
Preferable water temp. for breeding:							
4-5 degrees Celcius							x
5-7 degrees Celcius							x
7-9 degrees Celcius		x				x	
9-18 degrees Celcius	x		x	x		x	

TABLE 4. Comparison of development periods of *Triturus vittatus ophryticus* and *Pelodytes caucasicus*.

SPECIES:	MONTHS:											
	XII	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
<i>Triturus vittatus</i> (ad.)	x	x	x	x	x	x						
<i>Triturus vittatus</i> (larvae)				x	x	x	x	x	x			
<i>Pelodytes caucasicus</i> (ad.)						x	x	x	x	x	x	
<i>Pelodytes caucasicus</i> (larvae)	x	x	x	x	x	x	x	x	x	x	x	x

\* Note: Shaded area represents the periods of mutual number limitation

*Hyla arborea schelkownikowi*. This species is found in open, well illuminated ecotones throughout the grove. Reproduction take place from March until October in warm ( $>11^{\circ}$  C), stagnant waters. Eggs are laid at a depth of 10-12 cm. These frogs over-winter in the forest leaf litter.

*Rana ridibunda*. This frog is numerous in Khostinsky Canyon in open, well illuminated areas in the water-box yew forest ecotone. Reproduction last from January until March at water temperatures from  $5-9^{\circ}$  C. Frogs over-winter at the bottom of stagnant bodies of water.

*Rana macrocnemis*. This frog is found in low numbers in all areas of the grove except in the rocks. Reproduction takes place from February to March in warm ( $4-9^{\circ}$  C), shallow water. Eggs are laid at 0-5 cm depth. During cool winters this frog over-winters in the forest leaf litter and during warm winter it remains abroad.

### Discussion

During the summer all of the species of amphibians in the Caucasian Biosphere Reserve are broadly sympatric. However, during reproduction and winter retreat there is habitat segregation (Table 3). The highest level of overlap occurs in the summer in the flood plain forest where all of the above are found. The lowest level of overlap occurs in the box yew-cherry laurel

stands with only *B. verrucosissimus*, and *R. macrocnemis* present. The box yew-cherry laurel stands are the most ancient forest type preserved in the Yew-box Grove. This ancient forest is dominated by box yew trees ranging from 500 to 2000 years old and has been virtually unchanged in appearance during that time. It is interesting that this ancient forest is inhabited by the indigenous Caucasian species *B. verrucosissimus*, and *R. macrocnemis*.

The hornbeam tree is a pioneering species that invades disturbed areas such as those that have burned or been logged. It also grows in barren areas. The diversity of amphibian species in the hornbeam forest is caused by a number of factors: 1) secondary character of hornbeam forest; 2) relatively higher illumination (compared to the box yew forest; 3) presence of suitable water conditions for reproduction. When the hornbeam trees are young and the habitat is still open, such species as *R. ridibunda*, *T. v. lantzi*, and *H. a. schelkownikowi* are found. Later, when the trees become mature and the forest more closed and less illuminated, *B. verrucosissimus*, *R. macrocnemis* and *P. caucasicus* become established. This environment supports the highest level of sympatry of amphibian species in the Yew-box Grove. In box yew stands *T. v. lantzi* and *H. a. schelkownikowi*, the most illumination tolerant species, are not represented. These two species appear in



TABLE 5. Daily activity of amphibians of the Yew-box Grove.

SPECIES:	TIME OF ACTIVITY:																										
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1	2	3			
<i>Triturus vittatus</i>		x	x	x	x		x	x	x	x	x	x	x	x													
<i>Triturus vulgaris</i>		x	x	x	x	x	x	x	x	x	x	x	x	x													
<i>Pelodytes caucasicus</i>	x																			x	x	x	x	x	x		
<i>Bufo verrucosissimus</i>																		x	x	x	x	x					
<i>Hyla arborea</i>				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
<i>Rana ridibunda</i>		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x									
<i>Rana macrocnemis</i>	x	x																									

the ecotone adjoining the southwest boundary of the grove (Table 3).

It is interesting to note that *B. verrucosissimus* and *R. macrocnemis* have very specific breeding requirements in terms of the aquatic environment required, but they occur over a large area during the terrestrial stages of their life history. They can be termed stenotopic or very restricted in terms of the reproductive requirements (*B. verrucosissimus* lays its eggs in rivers and *R. macrocnemis* in small pools) and eurytopic in terms of their general distribution (Dazho, 1975). These two species are autochthones or indigenous to the broken country Caucasian region and reproduce in rapid mountain stream and ephemeral pools. The majority of lakes in this region are of recent origin and formed by glaciation, karst or from landslides. As this range of aquatic environments became available at the end of the last glacial period, amphibians successfully colonized those environments which met their reproductive requirements (Monchadsky, 1958).

*Bufo verrucosissimus* lays its eggs in strings and wraps them around aquatic vegetation and other anchored objects in the water. This allows the toad to lay its eggs

in fast flowing mountains streams which are generally unsuitable for other species. It lays its eggs at depths of 20 to 70 cm thus providing some protection from flash floods though many eggs perish in such floods. *Pelodytes caucasicus* and *R. ridibunda* are found sympatric with *B. verrucosissimus*. *Rana ridibunda* lays its eggs in the shallow, slow-moving sections of the river or in pools formed by floods. Reproduction in *R. ridibunda* is limited to the short period of winter low water and lasts from the end of January to the end of March.

*Pelodytes caucasicus* is isolated from other breeding anurans temporally. It attaches its eggs to thin roots (2-10 cm) beginning in mid-June, when other species have finished breeding. Typically breeding sites are in the backwaters of streams, under vegetation canopies where temperatures are moderate. In small streams *R. ridibunda* and *T. v. ophryticus* are sympatric with *P. caucasicus*.

At Glubokaya Ravine, Study Site 4, there is a high level of sympatry among the amphibian species. There is significant temporal segregation. Adult *T. v. ophryticus* remain in the water from the end of November to the end of May. Larval

TABLE 6. Faeces composition of sympatric amphibian species of the Yew-box Grove

	SPECIES:					
	<i>Triturus cristatus</i>	<i>Triturus vittatus</i>	<i>Bufo verrucosissimus</i>	<i>Pelodytes caucasicus</i>	<i>Rana ridibunda</i>	<i>Rana macrocnemis</i>
<b>COLEOPTERA:</b>						
Curculionidae			x	x	x	x
Carabidae		x	x	x	x	x
Cerambycidae			x		x	
Chrysomelidae			x	x	x	
Coccinellidae					x	x
Scarabaeidae			x			
Lucanidae			x			
Silphidae			x			
Elateridae			x			
non det.	x		x			
<b>DIPTERA:</b>						
Tipulidae			x			
Muscidae					x	
non det.	x					x
<b>HYMENOPTERA:</b>						
Apidae				x		
Vespidae			x	x	x	
Formicidae			x			
<b>HEMIPTERA:</b>						
Pentatomidae			x	x	x	
Pyrrhocoridae			x	x		
<b>ANNELIDES:</b>						
Hirudinea		x				
Oligochaeta		x				
<b>ARTROPODA:</b>						
Lepidoptera			x			
Scorpiones			x			
Amphipoda		x				
Isopoda			x	x	x	
<b>MOLLUSCA:</b>						
<i>Pisidium</i>	x					
<i>Oxychilus</i>						x

development occurs from March until August (Table 4). *Pelodytes caucasicus* can be heard calling from May until the middle of October.

The adults of *T. v. ophryticus* and *P. caucasicus* prey upon each others larval stages. When post metamorphic *T. v. ophryticus* begin leaving the water in late

July they are prey upon by adult *P. caucasicus*. In December when adult newts enter the water to breed they capture the smaller sizes of *P. caucasicus* tadpoles. During these periods of intense competition and predation both species adopt several strategies for preying upon the other for avoiding predation (Smith 1981).

TABLE 7. Size limits of feeding objects of sympatric amphibians in the Yew-box Grove

SPECIES:	SIZE OF FEEDING OBJECTS ( in mm)								
	up to 3	3-3. 5	3. 5-4	4-4. 5	4. 5-5	5-5. 5	5. 5-14. 5	14. 5-19	19-36
<i>Triturus vulgaris</i>					x	x			
<i>Triturus vittatus</i>							x	x	
<i>Bufo verrucosissimus</i>		x	x	x	x	x	x	x	x
<i>Pelodytes caucasicus</i>	x	x	x	x	x	x	x		
<i>Rana macrocnemis</i>				x	x	x	x	x	
<i>Rana ridibunda</i>						x	x	x	

The highest population densities of *T. v. ophryticus*, *T. v. lantzi* and *T. c. karelini* occur in small forest lakes. Much lower population densities are found in mountain streams. Recently formed lakes are apparently the most suitable habitat for these species of newts. As lakes mature, sediments accumulate and they become less suitable habitat and populations decline and are preserved at low levels in nearby streams.

The highest level of sympatry occurs in lakes during breeding season (Table 3). There is, however, very little competition because of temporal and microhabitat segregation for egg deposition and deposition sites.

Lakes and deep pits (Study Site 7) are breeding sites for *H. a. schelkownikowi* where it is spatially segregated from other amphibians but overlaps temporally with *P. caucasicus* (Table 3) and activity patterns (Table 5).

In the Yew-box Grove *P. caucasicus* and *H. a. schelkownikowi* are allopatric at breeding sites. On the Caucasian Black Sea Coast they are sympatric at breeding sites. The interrelationships of these populations in the zones of sympatry have not been studied.

On the Caucasian Black Sea Coast winters are mild with abundant precipitation. Most amphibians remain active and abroad through the winter. Though, on the occasional cold days they become torpid. The exceptions are adult *P. caucasicus* and *H. a. schelkownikowi*.

During some cold winters when night temperatures fall to -10 to -12° C all amphibian species enter hibernation.

*Bufo verrucosissimus*, *P. caucasicus*, *H. a. schelkownikowi*, and *T. v. lantzi* pass hibernation hidden in the soil and leaf litter. *Rana macrocnemis* hibernates in the soil, leaf litter and in the water. Winter soil temperatures at 20 cm depth ranges from 3.5 - 7.2° C. In the spring the water warms more quickly than the soil and this may explain why the those amphibians hibernating in the water breed earlier than those hibernating in the soil.

The mechanism of niche isolation of symbiotopic species includes layering, or the formation of adaptive groups (Dinesman 1948a) and differences in daily and seasonal activity. For example, at Study Site 1, *T. v. ophryticus* occupies the deepest aquatic level, *R. ridibunda* and *P. caucasicus* are found in the lower intermediate levels, *R. macrocnemis* and *B. verrucosissimus* occupy the higher intermediate levels and *H. a. schelkownikowi* is found at the shallowest level and onto land (Fig. 4). Some of the pattern of species distribution can be explained by varying tolerance of desiccation among the different species (Dinesman, 1948b). Those species that are found at the same level are active at different times of the day (Table 5).

The amphibians of the Yew-box Grove can be divided into three groups based on their food habits: 1) feeding on hydrobionts (newts), 2) feeding on arboreal invertebrates (tree frogs), 3) feeding on

terrestrial invertebrates (all other species). *Rana ridibunda* feeds on both aquatic and terrestrial invertebrates (Table 6, 7).

In a given area species diversity is dependent upon niche separation (Pianka, 1981). The seven amphibian species studied here are characterized by biotopic (including breeding site choice, hibernation sites and summer activity ranges), seasonal activity period, daily activity period and food habits isolation. In the Yew-box Grove the highest level of species diversity is achieved in the most stable aquatic environments. Water temperature during the breeding season, level of illumination and water chemistry appear to be an important characteristics.

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## Notes on a Collection of Squamate Reptiles from Eastern Mindanao, Philippine Islands Part 1: Lacertilia

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**Abstract.** -In 1982, I spent six months collecting the herpetofauna of several areas in eastern Mindanao, Philippine Islands. I present species accounts for all lizards collected at these sites, and draw conclusions about the biodiversity of second-growth and primary forest habitats. I found second-growth habitats to be depauperate compared to primary forest habitats. Species which may depend on primary forest habitat and also species apparently restricted to such habitats are detailed. The importance of primary forest reserves, selective logging, and a mosaic of successional habitats within primary forest reserves is discussed.

**Key words:** Reptilia, Squamata, Lacertilia, Philippines, taxonomy, ecology, biodiversity

### Introduction

Little is known of the ecology, distribution, and life history of Philippine lizards. Taylor (1922a, b, c; 1923; 1925) investigated the systematics and zoogeography of the Philippine herpetofauna and provided some ecological information. Brown and Alcala (1961, 1964, 1978, 1980) undertook ecological and systematic studies on some islands, Alcala (1986) recently reviewed the herpetofauna, and Auffenberg (1988) has detailed the ecology of the Gray's monitor, *Varanus olivaceus*. However, many islands remain poorly known. This and a subsequent paper provide information on the natural history of squamate reptiles collected in eastern Mindanao, Philippine Islands, during a collecting trip to the area made in 1982. I also comment on squamate assemblages occurring in primary and second-growth dipterocarp forest in an effort to pinpoint species of special concern should primary forest continue to be lost to logging and agricultural practices.

**Site Description Vegetation.**—The vegetation of the Philippines was described by Brown (1919) and Dickerson (1928) and a detailed study of southeast Asian rain forests was published by Richards (1952). Trees of the family Dipterocarpaceae dominate the rain forests of the Philippines. For the purposes of my study, primary dipterocarp forest is defined as dipterocarp forest that has apparently never been

logged. Early second-growth dipterocarp forest is defined as dipterocarp forest selectively logged one or two years previously. It has abundant ground cover of rattan, herbaceous vines, shrubs, and lianas. A few small (<2 m) trees were present, and the ground was littered with fallen logs. Selective logging was practiced at my study sites, and some small dipterocarps were left standing, though not enough to cast appreciable shade. Late second-growth dipterocarp forest is defined as forest selectively logged three or more years ago. It usually had a closed canopy composed of young fast-growing trees (mostly *Trema* species) about 10-15 m tall. Small dipterocarps were present, and the herbaceous understory was extremely dense. The vegetation of my study areas is discussed in more detail in Smith (1985).

**Site 1.**—This site was located in the coastal mountains (Diwata Range) of east-central Mindanao, 55 km south, 20 km west of Bislig Bay and 10 km southeast of Mt. Agtuaganon (Fig. 1). Areas from 500-800m elevation were sampled. Vegetation of this area was early second-growth and primary dipterocarp forest. Slopes were generally very steep (Fig. 2, 3, 4). This site was sampled from April 6 to August 15, 1982.

**Site 2.**—This site was also in the Diwata Range, 33 km south and 7 km west of Bislig Bay (Fig. 1). Vegetation at this site consisted entirely of late second-growth

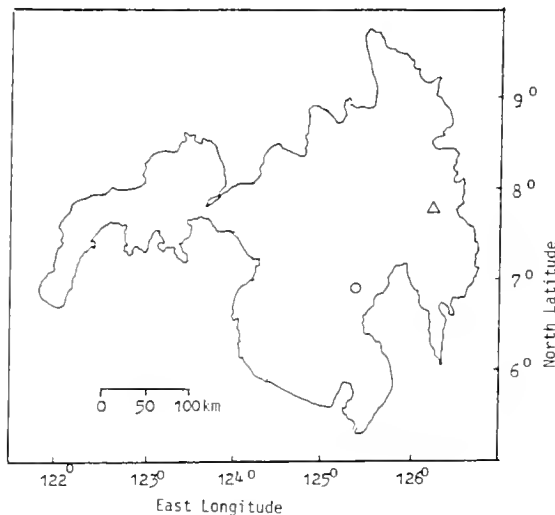


FIGURE 1. Map of Mindanao, Philippine Islands, showing locations of collecting sites 1 and 2 (triangle) and site 3 (circle).

dipterocarp forest. Slopes were moderately inclined to steep. The site was 400 m in elevation, and was sampled from August 21 to September 4, 1982.

*Site 3.*—I also visited Mount Talomo, a 2693 m peak located 30 km west of Davao City in the Mount Apo Range of southern Mindanao (Fig. 1). I sampled areas around the Philippine Eagle Captive Breeding Project (PECBP) field station (about 1000 m elevation) from September 8-13, 1982. Workers at the field station made incidental collections at this site from April to September. According to residents, logging in this area was discontinued sometime in the mid- to late-1960's. The area was selectively logged and appears to be more similar to typical pristine forest than many other logged areas I visited. Human disturbance is considerable on the slopes of Mount Talomo. Farms extend up the slopes from Davao Gulf to about 900 m elevation. Coconuts, bananas, pineapples, coffee, and various fruits and vegetables are grown. Areas sampled range from 600-1050 m. Slopes are gently to steeply inclined.

*Climate.*—Rainfall was heavy at all sites. At sites 1 and 2, there is no marked dry season. The wetter season usually

occurs from November to March, with the heaviest rains in December and January (Census Office of the Philippine Islands 1920; Dickerson, 1928; Willmott et al., 1981). Annual rainfall in Surigao (the nearest weather station to sites 1 and 2) is 3647 mm, with 2360 mm falling from November to March, and 1191 mm of this amount in December and January alone (Willmott et al., 1981). Surigao is in the lowlands, and rainfall at my collecting sites may have been higher. Site 3 also does not have a marked wet or dry season (Census Office of the Philippine Islands, 1920; Dickerson, 1928). Annual rainfall in Davao (at the base of the Mount Apo range) is 1942 mm (Willmott et al., 1981). This figure undoubtedly increases with elevation. During some times of the year, the PECBP station may be shrouded in clouds for weeks at a time. Temperature was relatively constant at all sites. At site 1 it usually ranged from 20-25 C under the canopy, and from 20-30 C in the open. The minimum temperature recorded was 18 C, the maximum was 34 C. Temperatures at site 2 were similar. Temperature readings were not available from site 3, but it was slightly cooler because of the higher elevation. Humidity at all sites varied from 79-100%. Occasionally typhoons strike Mindanao, but they generally lack the severity of those hitting more northerly islands (Census Office of the Philippine Islands, 1920, Dickerson, 1928). In March of 1982 a typhoon struck Bislig Bay near sites 1 and 2. The only effect at the study sites was moderate, steady rain for several days.

## Methods

Specimens were collected using drift fences (Gibbons and Semlitsch, 1981) and by hand. Drift fences were 0.5 m high and 18 m long and constructed as detailed in Gibbons and Semlitsch (1981). Pit-cans and funnel traps were placed at either end of the fence. Pit-cans seemed effective at capturing all terrestrial lizards except *Varanus* spp. Funnel traps were primarily useful in capturing snakes. Drift fences appeared to adequately sample reptiles moving on the soil surface. To sample



FIGURE 2. Primary forest at the edge of a road cut at site 1. The ridge top is about 900m elevation.

arboreal fauna, I examined epiphytic ferns and trees felled during logging. These efforts were largely unsuccessful and arboreal species are under-represented in the collection. Data on macrohabitat (primary, early, or late second-growth dipterocarp forest), microhabitat (fossorial, terrestrial, or arboreal), elevation, date, and time of day were recorded for each specimen collected. Standard measurements including snout-vent (SVL), tail (TL), and total length (TTL) were taken in the field on freshly killed specimens. General weather conditions were noted daily. Specimens were dissected in the laboratory to determine sex, stomach contents, and reproductive condition of females. Food contents were identified usually to family for the arthropod prey of these lizards. Standard scale counts were also taken, but no deviation from

previously published data was noted, and scale count data are not reported herein.

### Species Accounts

#### *Family Gekkonidae*

*Cyrtodactylus agusanensis*:—Specimens of this species were collected in all habitats at sites 1 and 2. This species' morphology suggests nocturnal habits (vertically elliptical pupils), but half the specimens were captured in drift fences during the day. Specimens captured at night were taken on bushes and logs, 1-3 m above ground. My observations do not agree with Alcalá (1986), who states that this species is found in swamps and along rivers. My specimens were all taken far from such habitats. I also did not find this species to be particularly rare, as did Alcalá

(1986). Females taken June 14 and 23 had one large egg (15.7-18.2 mm long) in each oviduct. Small males were captured April 2 and 9 and a small female July 31. One juvenile was captured at site 2 on August 26. Stomach contents included insects of several families and a shed skin, probably of *C. agusanensis*.

Specimens examined: LSUMZ 41601-41609, 41640.

#### Family Agamidae

From their morphology, all agamids collected on the study sites appear to be arboreal, although most specimens, except *Draco* species, were caught on or near the ground.

*Calotes cristatellus*.—A female was collected at about 550 m in early second-growth forest at site 1. A male specimen lacks additional data. This species is rare, transient in the habitats sampled, or mostly arboreal and hence under-represented in the collection. It has morphology indicative of a highly arboreal lifestyle. The female, collected on July 1, had one large egg (38.4 and 35.2 mm) in each oviduct. Stomach contents included lepidopteran larvae, unidentified insects, and a snail.

Specimens examined: LSUMZ 41737, 41738.

*Draco mindanensis*.—Taylor (1922a) collected only two specimens of this species. They were taken at 1100 ft. at the base of Malindang Mountain, northwestern Mindanao. Inger (1983) examined taxonomic characters in nine specimens but gave no ecological data for them. My specimens were taken at 650 m at site 1 in primary forest. The species may be confined to primary forest. *Draco mindanensis* is diurnal and arboreal. A female contained two oviducal eggs 17.5 and 18.4 mm long. The date of capture of this specimen is unknown. Stomach contents consisted of several families of insects. This species is apparently not an ant-feeding specialist like its congener, *D. volans* (see below). *D. mindanensis* is

reported from Catagan and Malindang Mountain in northwestern Mindanao (Taylor, 1922a) and the Diuata Mountains in the province of Davao del Norte, east-central Mindanao (this study).

Specimens examined: LSUMZ 41678-41680.

*Draco volans*.—This species is very common in early second-growth forest and is probably the most conspicuous lizard species in this or any other habitat. Individuals are commonly seen running along branches, displaying, and gliding. They are diurnal and exclusively arboreal. This species was never seen in primary forest. A female containing two eggs (14.7 and 13.9 mm long) was collected on July 20. Taylor (1922a) stated, and my data confirm, that this species feeds exclusively on ants.

Specimens examined: LSUMZ 41741-41748.

*Gonyocephalus semperi*.—Although arboreal by morphology, five specimens were captured on the ground in drift fences. One was caught by hand 1 m above the ground on a large tree. This species is diurnal, as is its' congener *G. godeffroyi* in the Solomon Islands (McCoy 1980). Four were captured in primary forest, two in late second-growth forest. Due to the species' arboreal habits, it is highly likely that *G. semperi* does not occur in highly disturbed areas largely lacking trees. McCoy (1980) found that *G. godeffroyi* also avoids open areas in the Solomons. One *G. semperi* exhibited aggression and grunted when handled. The single female captured June 16 contained three developing eggs (5.8, 6.6, and 7.3 mm in length). Stomachs examined contained the remains of insects of the families Chilopoda, Coleoptera, Hymenoptera, Orthoptera, and larval Lepidoptera.

Specimens examined: LSUMZ 41730-41735.

*Hydrosaurus pustulosus*.—This is a juvenile specimen collected by a native on





Figure 3. Early second-growth forest at site 1. This site had been selectively logged two or three years prior to this photograph. Trees in the middle background were deliberately left standing during the selective logging procedure.

June 19. Its' stomach was empty. It is said by Alcalá (1986) to be omnivorous, which generally agrees with observations of captive specimens at the Dallas Zoo. Auffenberg (1988) states that adult *H. pustulosus* are entirely folivorous in the wild. Captive specimens usually lay 6-8 eggs measuring roughly 50 mm in length about once a year (Mitchell 1985). This species is said to be common in the Philippines near unpolluted mountain streams (Alcalá 1986). It has also been observed around coastal fishing villages, utilizing as vertical perches the stilts or piers that support homes over water (L. A. Mitchell, personal observation).

Specimen examined: LSUMZ 41739.

#### *Family Scincidae*

In the Philippines, skinks far exceed the other lizard families in number of species, abundance, and probably in the variety of niches they occupy. They are often the most abundant lizards in all habitats sampled, with the exception of early second-growth forest, where *Draco volans* is more abundant. The leaf litter herpetofauna is dominated by species of the family Scincidae.

*Brachymeles gracilis hilong*:— Specimens were captured only in late second-growth forest (site 2, 400 m) and at Mount Talomo (site 3). Brown and Alcalá (1980) stated that this fossorial species is found under leaves, duff, rotting logs, and



Figure 4. Small permanent stream in primary forest at site 1. Small streams such as this one were common in areas of primary forest at all sites.

in loose soil, usually only in primary forest from 50-1000 m elevation. This species is apparently rare or absent in early second-growth forest. Stomach contents indicate that this species is a generalized insectivore, however, part of a skink tail (*Mabuya* or *Sphenomorphus* species) was found in one stomach.

Specimens examined: LSUMZ 41719-41725.

*Brachymeles schadenbergi orientalis*.— This fossorial species was often caught in drift fences after long, steady rains. It is found under logs and in leaf litter and loose soil in primary and second-growth forests at elevations from 50-1000 m (Brown and Alcalá, 1980). A female collected July 5 had three developing embryos 18.1, 15.9,

and 15.0 mm in her oviduct. Brown and Alcalá (1980) stated that this subspecies is ovoviviparous and usually has 2 or 3 young. This species is also a generalized insectivore. In addition, a lizard tail (probably *Brachymeles* species) was found in one specimen's stomach.

Specimens examined: LSUMZ 41726-41729.

*Lamprolepis smaragdina philippinica*.— This was a very common arboreal species in cultivated areas and villages, and a single specimen was taken in a coconut plantation on Mount Talomo between 700 and 800 m elevation. In contrast to Brown and Alcalá (1980) and Alcalá (1986), I never observed this species in primary or second-growth forests. My study sites did not encompass

agricultural areas or villages.

Specimen examined: LSUMZ 41648.

*Lipinia semperi*.—Taylor (1922a) noted that this species is commonly found in old tree stumps and hollow trees. Alcalá (1986) states that it is arboreal and rare. My specimen was taken in daylight from an epiphytic fern 4 m above ground in primary forest at 800 m elevation.

Specimen examined: LSUMZ 41740.

*Mabuya multicarinata multicarinata*.—This is a very active and abundant skink that favors open areas. It is diurnal and terrestrial. All but two specimens were caught in early second-growth forest. One specimen was taken in late second-growth forest. An additional specimen was observed in primary forest, but this was within 100 m of a logging road and it may have been transient. This species seems to be a lizard of open areas, and it may have originally occupied natural treefall gaps in the forest. With extensive clearing of areas for logging and cultivation, *M. m. multicarinata* has probably increased substantially in numbers. Of eleven females collected throughout this study, only two failed to contain well-developed eggs. The rest had 2 (6 specimens) or 3 (3 specimens) large oviducal eggs 11.0–15.5 mm long. Juveniles 29–41 mm SVL were captured on June 24 and August 1, 4, 12, and 26. Another juvenile was taken from the stomach of a snake (*Cyclocorus nuchalis taylori*) that was collected July 1. Stomachs examined contained insects of many taxa, and I consider this species a generalized insectivore.

Specimens examined: LSUMZ 41610–41639.

### *Sphenomorphus* Species

Lizards of the genus *Sphenomorphus* dominate the leaf litter herpetofauna of the primary forest in the areas I investigated, and they are sometimes conspicuous in secondary growth as well. *Sphenomorphus* exceeds all other genera at

my sites in number of species and individuals. Its' species are mostly diurnal and terrestrial.

*Sphenomorphus acutus*.—Brown and Alcalá (1980) state that this species is strictly arboreal, which may account for the paucity of specimens collected. However, three specimens were collected in drift fences during daylight, so they are at least occasionally found on the ground. This agrees with Alcalá's (1986) observations. Stomach contents included arachnids and orthopterans.

Specimens examined: LSUMZ 41713–41715.

*Sphenomorphus coxi coxi*.—This was the most common ground-dwelling lizard in the primary forest and is the most conspicuous member of the leaf-litter herpetofauna in this habitat. It is fairly common in second-growth forest as well, but it is not seen in the open as often as *Mabuya multicarinata multicarinata*. Although unquantified, I believe that these observations represent a real difference, and that *M. m. multicarinata* replaces *S. c. coxi* as the dominant skink in second-growth habitats. Gravid females were collected April 27 (1 egg, 5.2 mm long), July 3 (2 eggs, 8.5 and 9.2 mm), and August 5 (2 eggs, 14.7 and 15.2 mm). One juvenile was collected May 21 and two on May 24. Four others were collected August 9, 13 (2), and 28. These juveniles measured 34–45 mm SVL. One small specimen identified as a male measured 45 mm SVL. These data suggest two hatching seasons during the six months of this study, one in May and the other in August. Apparently, reproductive maturity is reached at approximately 45 mm SVL. Stomachs examined contained insects of many taxa. I consider this species to be a generalized insectivore.

Specimens examined: LSUMZ 41649–41677.

*Sphenomorphus decipiens*.—Although Alcalá (1986) states that this species is rare, I found it to be a fairly common diurnal

member of the leaf litter herpetofauna in primary forest. It was never found in second-growth forest. A juvenile (25 mm SVL) was collected June 23. Stomachs contained larval lepidopterans and other insects. Specimens examined: LSUMZ 41704-41711.

*Sphenomorphus fasciatus*.—Brown and Alcalá (1980) noted that this species was a common terrestrial skink. During my study, I collected only one specimen at site 1. Since my capture techniques seemed especially efficient in sampling the terrestrial herpetofauna, I conclude that *S. fasciatus* was rare at my collecting sites. The stomach of this specimen contained insects of several taxa.

Specimen examined: LSUMZ 41716.

*Sphenomorphus steerei*.—This species is common in primary forest, and is occasionally found in second-growth forest. In my study, it was more common above 650 m elevation, although Brown and Alcalá (1980) stated that it occurs from sea level to 2000 m. Stomachs contained insects of several different taxa.

Specimens examined: LSUMZ 41697-41703, 41712.

*Sphenomorphus variegatus*.—This species is a common diurnal leaf-litter lizard of the primary forest. It was never found in early second-growth forest, but two specimens were taken in late second-growth forest. *S. variegatus* appeared to be completely absent from highly disturbed areas. Females taken April 11 and May 24 each contained two eggs (11.0 and 10.3 mm; and 6.5 and 6.1 mm, respectively). Juveniles (SVL 24-39 mm) were captured May 23, June 29, August 9, and August 26. Stomachs contained insects of many different taxa.

Specimens examined: LSUMZ 41681-41696.

*Tropidophorus partelloi*.—This species was found to be active during the day and night. Specimens collected during the day

were either captured in drift fence traps or found under cover. At night, individuals were found actively foraging. This species is apparently secretive during daylight. Specimens frequently grunted during capture and attempted to bite. All specimens were caught in primary forest except one caught in late second-growth forest. This species is apparently absent from highly disturbed areas. Gravid females were caught March 16 (5 eggs, 3.5-6.3 mm) and June 14 (5 eggs, 12.3-13.9 mm).

Specimens examined: LSUMZ 41641-41647.

## Discussion

The continuing destruction of tropical rain forest worldwide makes it imperative that species of special concern are identified in these habitats. In the section below, I will attempt to pinpoint species which could be adversely affected by continuing deforestation, where my data are adequate to do so. I make special reference to the lizards of the forest floor, since I feel that these lizards were the most accurately sampled species with the techniques that I used. These species are all skinks: *Mabuya multicarinata multicarinata*, *Sphenomorphus coxi coxi*, *S. decipiens*, *S. steerei*, *S. variegatus*, and *Tropidophorus partelloi*. *Sphenomorphus fasciatus* is not considered, since only one specimen of this species was collected.

### Primary Forest

This was the richest habitat sampled, containing all six of the skinks mentioned above. Of these six, five seem to be regular inhabitants of the primary forest. In addition, there were two common arboreal lizards: The gecko *Cyrtodactylus agusanensis* and the agamid *Gonyocephalus semperi*. The most commonly collected terrestrial lizards listed in decreasing order of abundance were: *Sphenomorphus coxi coxi*, *S. variegatus*, *S. steerei*, *S. decipiens*, and *Tropidophorus partelloi*. Species which were sparsely collected in primary forest include the

arboreal lizards *Calotes cristatellus*, *Draco mindanensis*, *Lipinia semperi*, and *Sphenomorphus acutus*, and the terrestrial lizard *Mabuya multicarinata multicarinata*. I do not consider *M. m. multicarinata* to be a regular inhabitant of primary forest.

#### Early Second-Growth Forest

This was the most depauperate habitat sampled. Two arboreal lizards were common: *Cyrtodactylus agusanensis* and *Draco volans*, the latter species being the most common lizard observed in any habitat. The common terrestrial lizards were: *Sphenomorphus coxi coxi* and the extremely abundant *Mabuya multicarinata multicarinata*. This habitat contained only three of the six terrestrial skinks of interest. The skink *Sphenomorphus steerei* was only represented by two individuals collected in this habitat, and I consider it to be rare in early second-growth habitats. *M. m. multicarinata* was virtually absent in primary forest, but it was found to be twice as common in second-growth habitats as the next most common lizard, *S. c. coxi*. I spent approximately two man-months apiece working in primary forest and early second-growth forest at site 1. I have assumed that the time spent in these two habitat types was equal and have calculated the Brillouin diversity index (Krebs 1989) for each habitat using the absolute number of specimens collected in each habitat type of the six terrestrial skinks of interest noted above. Indices of 0.610 and 0.309 are calculated for primary forest and early second-growth forest habitat types, respectively. Although not an exact measurement, this rough estimate of diversity points out the major difference in biodiversity between these two habitat types as regards terrestrial skinks.

#### Late Second-Growth Forest

I spent very little time in this habitat type, yet the species collected here provide valuable insights into possible lizard population successional patterns. The arboreal agamid *Gonyocephalus semperi*, absent in early second-growth forest, was found in late second-growth forest,

whereas *Draco volans*, common in early second-growth forest, was absent from late second-growth forest. The terrestrial skinks *Sphenomorphus variegatus* and *Tropidophorus partelloi*, both absent from early second-growth forest and common in primary forest, were collected in late second-growth forest.

#### Species of Special Concern

It is clear that less complex habitats will support a less complex community of plants and animals, by definition. Although my data are sparse, it is obvious that the biodiversity of terrestrial skinks (and other lizards) is far different after logging operations alter the forest structure. The clearing of primary forest creates habitat for open habitat specialists such as *Mabuya multicarinata multicarinata* and *Draco volans*, while decreasing habitat for the species which seem to be confined to the primary forest. Especially notable is the lack of *Sphenomorphus variegatus*, which is common in primary forest but absent in early second-growth forest. Other species which may be adversely affected by logging activities include the skinks *Tropidophorus partelloi*, *Sphenomorphus steerei*, and *S. decipiens*; and the agamids *Draco mindanensis* and *Gonyocephalus semperi*. Except for *D. mindanensis*, all these species were collected in late second-growth and primary forest. *D. mindanensis* was only found in primary forest. *Sphenomorphus coxi coxi* was the only lizard commonly found in all habitats, although it was less frequently seen in the open where it was syntopic with *Mabuya multicarinata multicarinata*.

#### Conclusions

The collection of primary forest species in areas of late second-growth forest points towards the possibility of a sustained harvest of the primary forest for lumber. However, there is absolutely no data on the periodicity of such a harvest, nor any precise information on changes in biodiversity through a successional series. It is imperative that primary forest be conserved as a refuge for certain species

which may only occur there, such as *Draco mindanensis*. Possibly, a system of reserves with rotating areas of selective logging may help to conserve Philippine lizard species. However, at the present time research towards such an end is entirely lacking, and the conversion of large parts of the Philippines towards a much impoverished lizard fauna continues unabated.

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## Notes on a Collection of Squamate Reptiles from Eastern Mindanao, Philippine Islands Part 2: Serpentes

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**Abstract.** -A systematic collection of the herpetofauna occurring in the Diuata Range of eastern Mindanao, Philippine Islands, with incidental collections from the Mount Apo Range, was made from April-September 1982. This paper completes taxonomic and ecological notes that were taken on the squamates of this region. Although conclusions about the effect of habitat alteration on snake populations are necessarily tentative due to sampling difficulties, some comments on apparent and potential shifts in population size and habitat use of the more common snake species are made.

**Key words:** Reptilia, Squamata, Serpentes, Philippines, taxonomy, ecology

### Introduction

Except for reviews by Taylor (1922) and Leviton (1959) and biogeographic works by Brown and Alcalá (1970), Leviton (1963a, 1970) and Wüster and Thorpe (1989, 1990, 1991a), very little is known about the ecology and distribution of Philippine snakes. This paper describes ecological and distributional data taken on the snakes of an area in eastern Mindanao, Philippine Islands, that I visited from April-September 1982. An earlier paper on the lizards of this area describes the sites and methodologies I used during this study. Scallation data taken on snakes included dorsal scale rows at ten ventral scutes posterior to the head, mid-body, and ten ventral scutes anterior to the anal plate; ventral scute counts; and subcaudal scale counts. This data is given only when it adds to data previously published.

### Species Accounts

#### *Family Colubridae*

*Ahaetulla prasina preocularis*.—Two specimens were taken, one lacking specific data that was probably collected at or above the upper elevational limit of 800 m given by Leviton (1967). This species has a morphology typical of arboreal snakes, but is frequently taken on the ground, where it may descend to forage on *Mabuya* skins (Leviton 1967). If *Mabuya* is a favored food item, then it is highly likely that *A.*

*prasina* favors open areas, as does *Mabuya*. A female was taken on April 6 on a road in early second-growth habitat near site 1. Five oviducal eggs (22.8-31.1 mm in length) were found in this specimen.

Specimens examined: LSUMZ 41804-41805.

*Boiga cynodon*.—This snake is arboreal by morphology, but two specimens were captured in drift fences and one found dead on a road at site 1. They were all collected or killed at night, and were found in both early second-growth and primary forest habitats. Prior studies give no elevational information (Alcalá, 1986; Leviton, 1968a; Smith, 1943; Taylor, 1922); the present specimens were found at 450-650 m. This species is aggressive and has very large palatine teeth which are quite effective in defense. Taylor (1922) stated that this species is rare in the Philippines, but it was the most commonly captured *Boiga* species at my study sites. As Leviton (1968a) noted, *B. cynodon* has an arboreal morphology and diet (birds and bird eggs), but all specimens were captured on the ground.

Specimens examined: LSUMZ 41814-41816.

*Boiga dendrophila latifasciata*.—This specimen was taken on a road at night at about 500 m elevation in early second-growth forest. Previous reports indicate



that this species is a common inhabitant of lowland swampy areas (Taylor, 1922; 1965; Tweedie, 1983), but the present specimen was found in hilly country far from such habitat. The stomach of this specimen contained bird feathers. Previous studies found this species to also eat bats and lizards (Alcala, 1986; Leviton, 1968a; Taylor, 1922).

Specimen examined: LSUMZ 41812.

*Calamaria gervaisi*.—This burrowing species was common in primary forest, but was rarely found in early second-growth forest. One specimen was collected 20 cm underground when digging a pit-can hole. Four adults were taken in a single pit-can during four days in April. The significance of this aggregation is unknown. A female collected April 10 contained one egg 3.4 mm in length. Segmented worms were found in the stomachs of two specimens.

Specimens examined: LSUMZ 41769-41778.

*Cyclocorus nuchalis taylori*.—One specimen was found in leaf litter in the primary forest, the other was caught in a drift fence in early second-growth forest. One specimen had eaten a juvenile *Mabuya multicarinata*. Leviton (1965) found specimens of the skink genera *Mabuya* and *Sphenomorphus* in stomachs of the *C. nuchalis* he examined.

Specimens examined: LSUMZ 41796-41797.

*Dendrelaphis caudolineatus terrificus*.—Leviton (1968b) gave an elevational range of 0-35 m for this species, but I collected specimens of this snake from 100-1000 m. Also, I did not note any particular association with water, as did Alcala (1986). Although thought to be primarily arboreal (Leviton, 1968b), all the specimens I collected were taken on the ground. One specimen had eaten a terrestrial skink, *Sphenomorphus coxi*. A specimen examined by Leviton (1968b) also contained a terrestrial skink, *Mabuya* species. One specimen that I collected was

taken in grassland in an extremely large ca. 100 square km clear-cut area. This species is arboreal in its morphology, but these data indicate common use of the terrestrial microhabitat.

Specimens examined: LSUMZ 41798-41800.

*Dryphiops philippina*.—This specimen was collected by a native near Mount Talomo and is the first specimen of this species collected on Mindanao. The ventral scute count of this specimen is 172, increasing the known variation of 177-188 reported by Leviton (1964a). Previous specimens were taken near sea level (Leviton, 1964a), but this specimen may have been collected as high as ca. 1000 m. This species was previously known only from Luzon, Negros, and Sibuyan (Leviton 1964a). Subsequent investigations should discover it on other large islands in the Philippine archipelago.

Specimen examined: LSUMZ 41790.

*Elaphe erythrura erythrura*.—This species is a common ground-dwelling diurnal inhabitant of all forest situations. It feeds on lizards, birds, and mammals (Alcala, 1986; Leviton, 1977; Taylor, 1922). My specimens commonly had mammals or mammal remains in their stomachs, and I consider this species to be a typical, heavy-bodied, mammal-eating constrictor that opportunistically takes other prey. It is also a common food item of the Philippine Eagle (*Pithecophaga jefferyi*) and the Philippine Serpent-Eagle (*Spilornis holospilus*). Alcala (1986) reports an altitudinal range to 500 m, but I took specimens up to 1065 m. I collected young snakes August 1 and September 8 (two snakes). These specimens measured 465, 383, and 409 SVL, respectively. The two largest of this group were identified as young males. Leviton (1977) stated that year-old young probably measure 400 mm SVL.

Specimens examined: LSUMZ 41807-41811.

*Oligodon maculatus*.—This is only the fifth known specimen of this species. Taylor (1922) took two specimens beneath sod and trash piles at Bunawan, Agusan, in the Agusan Valley of Mindanao. A third specimen was taken in northern Surigao Province (Taylor, 1925). A fourth was collected on Mount Todaya in the Mount Apo Range (Leviton, 1962). My specimen was taken during daylight in the forest floor litter on Mount Talomo, Mount Apo Range, at about 1000 m elevation. Alcalá (1986) reports two specimens from 400 and 850 m elevation, but gives no further information. Including my data, an altitudinal range of 400-1000 m is indicated. This species is known only from eastern Mindanao. Leviton (1962) stated that this species has 17 dorsal scale rows throughout; my specimen reduces to 15 in the posterior third of the body. Leviton also noted that the loreal may be present or absent; in my specimen it is absent. This specimen also differs from the ones analyzed by Leviton in having one instead of two preoculars. My specimen also has fewer scutes than those examined by Leviton, increasing the known variability in ventral scute counts to 156-164. The specimen I collected is male. The stomach was empty.

Specimen examined: LSUMZ 41806.

*Oxyrhabdium modestum*.—The specimen taken was a gravid female with eight eggs measuring 18.9-26.1 mm in length. This specimen was collected on a road at about 400 m elevation in late second-growth forest. There is no further data to add to that given by Leviton (1964b).

Specimen examined: LSUMZ 41803.

*Psammodynastes pulverulentus*.—This is a common and aggressive rear-fanged colubrid of the forest floor litter. It is diurnal and was taken in all habitat types. Juveniles 190 and 197 mm SVL were collected May 3 and May 23 at site 1. I found insects, a lizard tail, and a snake (*Calamaria gervaisi*) in the stomachs of the specimens I examined. This species is primarily known as a lizard feeder, but

frogs and snakes are also taken (Greene, 1989; Leviton, 1983). Greene (1989) did not report insects as a food item in the specimens he examined.

Specimens examined: LSUMZ 41779-41789.

*Rhabdophis auriculata auriculata*.—This species is a common diurnal member of the leaf-litter herpetofauna in all habitat types investigated. I did not find it to be associated with water, as reported by Alcalá (1986). These are small inoffensive snakes which usually attempt to conceal themselves rather than flee or bite when captured. I collected gravid females June 17 (three eggs, 9.9-10.5 mm in length) and 30 (one egg, 9.0 mm), and July 8 (three eggs, 5.3-7.6 mm) and 23 (two eggs, 9.0-9.2 mm). Juveniles 135-235 mm SVL were collected April 7, 11, and 12, May 18 and 23, June 14, and July 8. The gradually increasing SVL of these specimens indicate that they could be the members of a single cohort. Leviton (1970) indicated two hatching seasons in the Mount Apo Range, during June-July and October-November. My observations within the Diuata Range indicate that the hatching season here could occur as early as March, but data are scanty. Specimens had eaten frogs and frog eggs, as also reported by Leviton (1970).

Specimens examined: LSUMZ 41749-41768.

*Stegonotus muelleri*.—This is the eighth known specimen of this species, and only the second taken on Mindanao (Leviton 1959). Virtually no ecological data are available. This specimen was found dead on a logging road in early second-growth forest at about 590 m elevation. Leviton (1959) found three adult *Rana limnocharis* in the stomach of a large adult; the present specimen's stomach was empty. The specimen I collected is male. Its ventral scute count is higher than that given for males by Leviton (1959), thereby increasing the range of this measurement to 217-236.

Specimen examined: LSUMZ 41802.

*Tropidonophis dendrophiops dendrophiops*.—Specimens were collected during the day in all habitats, but always near swift-flowing streams. The altitudinal range given by Alcalá (1986) extends to 700 m; one individual I collected was taken at 900 m. Juveniles were collected April 18 (161 mm SVL), May 21 (242 mm SVL), and June 24 (308 mm SVL) at site 1. These could be members of a single cohort born in March or April. One specimen was collected in a pit-can eating a frog. Taylor (1922) also reported frogs as common food items of this species. Alcalá (1986) refers to this species as *Natrix dendrophiops*. Malnate and Underwood (1988) have recently assigned this species to the genus *Tropidonophis*.

Specimens examined: LSUMZ 41791-41795.

#### Family Elapidae

*Maticora intestinalis philippina*.—One specimen was taken in a pit-can at site 2 in late second-growth forest at 400 m elevation. This snake is thought to be rare in the Philippines (Alcalá, 1986; Taylor, 1922). Leviton (1963b) gives no ecological information, and Taylor (1922) describes an apparent anti-predator display during which specimens exhibit aimless thrashing motions, similar to displays described for other New and Old World coral snakes (Greene, 1973). Despite Alcalá's (1986) statement that this species is found in arboreal ferns as well as under rotten logs, I consider this species to be a typical semi-fossorial coral snake. This agrees with Taylor's (1922) observations. There are no data on food habits of this species; my specimen's stomach was empty.

Specimen examined: LSUMZ 41817.

*Naja samarensis*.—This is an alert diurnal species. It is quite common around habitations and early second-growth habitats. Contrary to Alcalá (1986) and Taylor (1922), this species was never

found in primary forest, and residents told me that they never saw individuals of this species in the forest. It is likely that the conversion of much of the Philippines into altered habitat has resulted in an increased abundance of this highly venomous snake. I saw or collected this species from sea level to 1000 m elevation. Despite its highly toxic venom (Minton 1967), the local residents believed that this snake brought luck, and individuals found under and around houses were invariably left alive. I actively sought out reports of envenomation resulting from bites of *N. samarensis*, but received no such reports, and was told by locals that bites of this species were seldom problematical. This is in contrast to conclusions reached by Reyes and Lamana (1955). This is a spitting cobra, and there is at least one report of an accurate strike in the eyes resulting in a great deal of pain but no serious after-effects (Van Wallach, personal communication). I handled many specimens, but never saw one exude any appreciable quantity of venom. This species is known to eat frogs, snakes (*Calamaria gervaisi*), and rodents (Gressitt, 1937; Leviton, 1964c; Taylor, 1922). A specimen I examined contained a small *Bufo marinus* (SVL 95mm), and Van Wallach reports (personal communication) only *B. marinus* in the stomachs of *N. samarensis* he collected in rice paddies on Mindanao near the city of Surigao. *N. samarensis* itself is eaten by the Philippine Eagle (*Pithecophaga jefferyi*) and the Philippine Serpent-Eagle (*Spilornis holospilus*). Two juveniles were taken with an adult male from a hole in the ground at 1000 m on Mount Talomo. These specimens were brought to me by a local resident. It was not possible to ascertain whether there was any significance to this aggregation, but other cobras are known to guard both eggs and young (Campbell and Quinn, 1975; Tryon, 1979; Tweedie, 1983). Wüster and Thorpe (1991b) have recently elevated *Naja naja samarensis* to full species status, and I use this new species designation in this paper.

Specimens examined: LSUMZ 41819-41824.

## Discussion

Unlike lizards, snakes are rarely taken in such numbers that it is possible to gauge their relative abundance in any given habitat. Therefore, ecological conclusions based on studies such as this one are few and tentative. Most of these conclusions have already been reached in individual species accounts.

The pace of destruction of primary forest in the Philippines has created a great deal of second-growth habitats of various types. At least one snake, *Naja samarensis*, has probably benefitted from this trend. Taylor (1922) observed this species in primary forest, but neither I nor any other biologists that I worked with, nor any local residents, ever told me of seeing this species in the primary forest. I consider it a common snake of second-growth and agricultural areas. Other species, such as *Elaphe erythrura erythrura* and *Rhabdophis auriculata auriculata* appear to be common in all habitats, although they have not undergone an apparent shift in habitat preference similar to *N. samarensis*. The only other very abundant snake, *Calamaria gervaisi*, may be adversely affected by deforestation, in that it was common in primary forest but not in second-growth habitats. Logging causes both soil compaction from the movement of heavy equipment and soil drying from increased insolation. It seems likely that these changes would adversely affect a burrowing animal such as *C. gervaisi*.

Snake ecology suffers from the typical problems inherent to studies of higher level predators. Since they are frequently at the top of the food chain, snakes never seem to be very abundant. In addition, they are secretive by nature, making collection and observation difficult. If we are to understand the effect of habitat loss on snake populations, it will be necessary to use novel approaches which are uniquely designed to overcome these specific problems.

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## First Records for *Ophisaurus harti* and *Python molurus bivittatus* from Jiangxi Province, China

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**Abstract.**—*Ophisaurus harti* and *Python molurus bivittatus* are reported for the first time from Jiangxi Province China. The measurements, characteristics, and distributions of these two species and subspecies are described in detail.

**Key Words:** Reptilia, Lacertilia, Anguillidae, *Ophisaurus harti*, Serpentes, Boidae, *Python molurus bivittatus*, China, distribution

### *Ophisaurus harti* Boulenger (Fig. 1)

On October 22, 1980 a specimen of *O. harti* was caught by Weitao Ji and Songlin Cheng of the Wuyi Shan Natural Reserve at an altitude of 900 meters on Mt. Wuyi, Yanshan County, Jiangxi Province, China. The specimen is kept in the Department of Biology, Jiangxi Medical College, Nanchang.

#### *Measurements of specimen, in mm.*

Specimen Number	600
Sex	male
Head width	21
Snout to ear	28
Body width	19
Total length	279
<u>Tail* length</u>	166

\*Regenerated

**Characteristics.**—Body cylindrical and no vestiges of limbs externally. Head with large symmetrical shields; two shields in a line between the nasal and the azygous prefrontal; ear-opening minute, smaller than the nostril. Dorsal scales keeled, in 16 longitudinal rows and 99 transverse series (counted in the length of the lateral fold); ventrals smooth, in 10 longitudinal series. The tail is long and fragile, and regenerates quickly. The regenerated tail is shorter than the original one, and the regenerated scales are smaller than the original ones. Brown above, with 21 transverse blue marking; under parts whitish.

**Distribution.**—Vietnam; China: Sichuan, Yunnan, Guizhou, Anhui, Jiangsu, Zhejiang, Jiangxi (Mt. Wuyi in



FIG. 1. *Ophisaurus harti*.

Jiangsu, Zhejiang, Jiangxi (Mt. Wuyi in Yanshan County), Hunan, Fujian, Taiwan, Guangxi.

### *Python molurus bivittatus* Schlegel (Fig. 2)

A piece of skin of *P. molurus bivittatus* was collected in 1965 from the people of Mt. Daji in Quannan County by the author, and one living specimen was caught in a mountain stream in the countryside of the city of Longnan County by a fisherman with a fishing net. The specimen was purchased by Chunhuo Teng of Nanchang People's Park in 1979.

#### *Measurements of specimen, in mm.*

Specimen Number	1001
Sex	unknown
Head width	55
Body width	120
Total length	2936
Tail length	34



FIG. 2. *Python molurus bivittatus*.

*Characteristics*.—Size large, with vestiges of hind limbs externally. Head distinct from neck, with large symmetrical shields; rostral with a deep pit on either side; two internasals; two pairs of prefrontals, the anterior pair is longer than the posterior one; frontal a little larger than the supraocular, divided longitudinally; parietal, loreal and temporal regions covered with irregular scales; supralabials 13, the first two deeply pitted, 6th and 7th separated from the eye by suboculars. Scales smooth, in 50 rows on neck, 75 rows on midbody and 38 rows before the vent; anal entire. Tail rather short. Ventrals and subcaudals were not counted. Light yellowish above, with a dorsal series of large, more or less subquadrangular dark gray, black-edged spots; flanks with smaller, rounded or irregularly-shaped spots of the same color. A lance-shaped mark on the top of the head and the neck; yellowish below, with a border of dark spots on the outermost row of the scales; tail below marbled with yellow and black.

*Distribution*.—Asia, Indo-Australian Region; China: Yunnan, Jiangxi (Dajishan in Quannan County, Longnan County), Fujian, Guangdong, Hainan, Guangxi.

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## Karyotype Information on some Toad Agamas of the *Phrynocephalus guttatus* Species Group (Sauria, Agamidae) of the former USSR.

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**Abstract.** -Karyotypes of several toad agamas of the *Phrynocephalus guttatus* species group (*sensu lato*) were investigated in specimens from a variety of localities of the former USSR. Differences in the diakinetetic stage of meiosis have been observed, permitting distinctions among three groups of species. The forms *guttatus*, *moltschanovi*, *kushackewitschii*, and *alpherakii* comprise Group I; *P. guttatus salenskyi* represents the second group; and *P. versicolor hispida* represents Group III.

**Key words:** Reptilia, Sauria, Agamidae, *Phrynocephalus guttatus*, Kazakhstan, Middle Asia, Precaucasus, karyology.

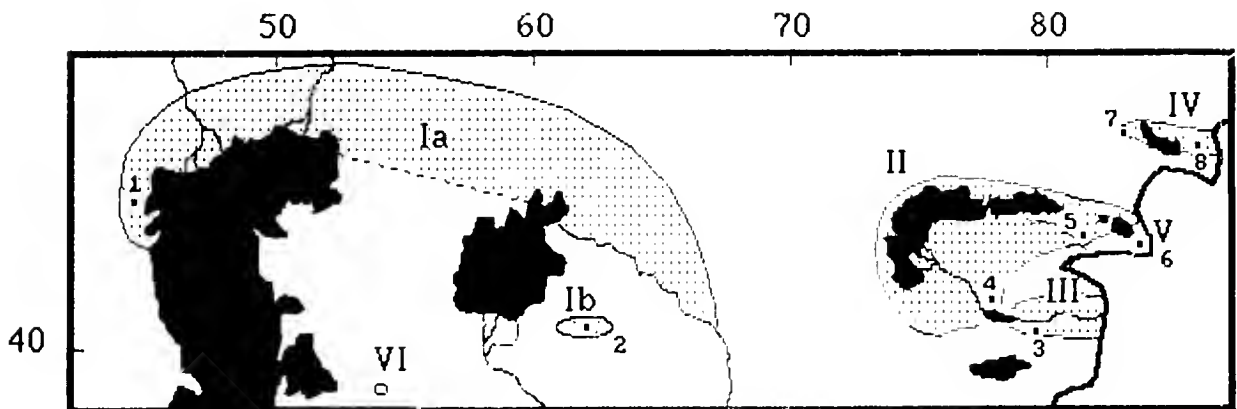


FIG. 1. Scheme of distribution of forms of *P. guttatus* species group of the former USSR fauna: Ia- *P. g. guttatus*; Ib- *P. g. moltschanovi*; II- *P. g. kushackewitschii*; III- *P. g. alpherakii*; IV- *P. g. salenskyi*; V- *P. versicolor hispida*; VI- *P. guttatus* spp. (the numbering of populations is in accordance with the data in table I).

### Introduction

The first and only extensive karyological investigation of the agamid lizard genus *Phrynocephalus* Kaup is the work of Sokolovsky (Sokolovsky, 1974; 1977). Karyotype characteristics permitted the recognition of five groups. The "*guttatus*" group included two species, *P. guttatus* (Gmel.) and *P. versicolor* Str. These species have a diploid number of 46, all chromosomes are telocentric. The karyotypes could be divided into 12 pairs of macrochromosomes and 11 pairs of microchromosomes. Approximately 50% of the metaphase plates in *P. guttatus* contained satellite chromosomes on the first pair of chromosomes, but these were never observed in the *P. versicolor* karyotype.

The specimens examined came from Daghestan (*P. guttatus*) and Central Gobi, Mongolia (*P. versicolor*) and were believed to represent the nominative forms of both species.

The systematics of the *P. guttatus* group based on external morphological characteristics is extremely complicated and remains unclear. At various times the forms *alpherakii* Bedr., *moltschanovi* Nik., *kushackewitschii* Bedr., *salenskyi* Bedr., etc. have either been included in *P. guttatus*, *sensu stricto* or treated as related species. The forms *bogdanowi* Bedr., *hispida* Bedr., and *paraskiwi* Semenov et al. have been assigned to *P. versicolor*. (Bedriaga, 1909; Nikolsky, 1915; Terentjev and Chernov, 1949; Peters,

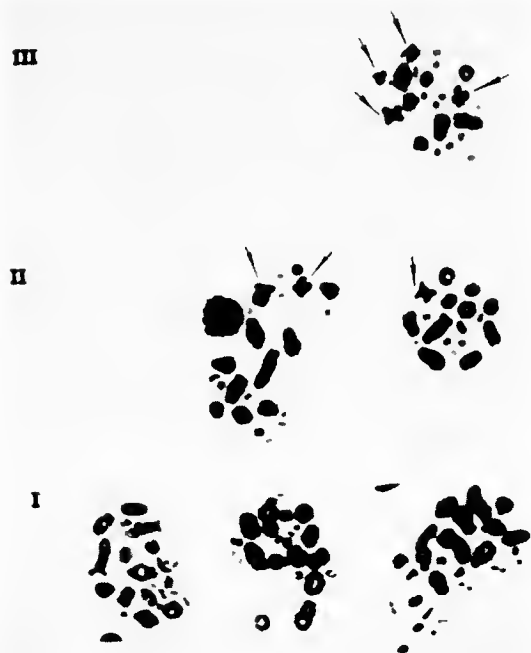


FIG. 2 The karyogramme of *Phrynocephalus guttatus salenskyi*.

1984; Semenov et al. 1987). Golubev (1989) suggested that *P. guttatus* and *P. versicolor* from Kazakhstan are conspecific. Karyotype details of the forms listed above have never before been examined. The purpose of this study is to determine whether karyotype information will aid in our understanding of the systematics and evolution of *Phrynocephalus*.

### Methods

Between 1989-1991 we collected specimens of nearly all listed forms of both species of *Phrynocephalus* inhabiting the territory of the former USSR with the exception of *P. v. bogdanowi* from the extreme south of Tuva (Central Asia) and *P. guttatus* ssp. from Turkmenistan (Fig. 1 and Table 1). Chromosome samples were prepared from cellular suspension of bone marrow, blood, and testis. We used a smear method and a method known as "digging out" in conformity with procedures described by Ford and Hamerton (1956) and McGregor and Varley (1986) as partially modified by Manilo (1986). Chromosomal staining was

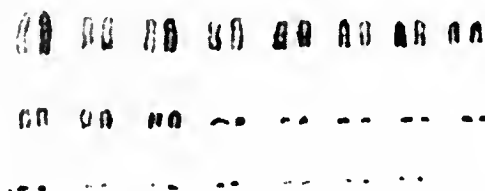


FIG 3. Diakinetik stage of meiosis of five forms of *P. guttatus* species group. s. lato: I- all elements are ring- or stick-shaped; (*guttatus*, *moltschanovi*, *kushackewitschii*, *alpherakii*); II- one or two elements are cross-shaped (*salenskyi*); III- two and more (up to four) elements are cross-shaped (*hispidi*).

performed by Giemsa stain (2% solution) in 0.01 M sodium-phosphate buffer (pH 6.8) for 20-30 minutes. After washing in distilled water, the preparations were passed through alcohols and xylols (orthoxytol) and subsequently embedded in Canadian Balsam. In excess of 30 metaphase plates from each form were investigated using a Biolam 1-212 microscope.

Metaphase plates of spermatogonial division, spermatocyte I (diakinesis) and spermatocyte II (metaphase II) bivalents were investigated in testis preparations. Chromosome morphology is described according to the classification proposed by Levan et al. (1964).

### Results and Discussion

Our data support the findings of Sokolovsky (1974, 1977). The diploid number is uniformly 46 and the Fundamental Number (NF) is 46. In several forms (*guttatus*, *moltschanovi*, *kushackewitschii*) we noted satellite chromosomes on several plates; whereas in other forms (*alpherakii*, *salenskyi*) we saw no evidence of satellites. The revelation of this structure largely depends on the degree of spiralization. It is possible that satellites will be found in the latter forms with further investigation and more extensive material.

TABLE 1. Localities, sample sizes, and taxa of *Phrynocephalus guttatus* s. lato populations collected and investigated in this study (numbering of populations is given in accordance with the data shown in Fig. 1.

No	Taxa	Locality	Sample size, sex
1	<i>P. g. guttatus</i>	N. Transcaucasus: N. Daghestan: sands on right bank of Kuma River	2 male
2	<i>P. g. moltschanovi</i>	N. Kysylkum in Karakalpakia: Kostruba Well	2 male
3	<i>P. g. alpherakii</i>	E. Kazakhstan: Alma Ata District: Near Karakuldek	3 male
4	<i>P. g. kushackewitschii</i>	E. Kazakhstan: Taldy-Kurghan District: NW bank of Kapchagay Reservior	3 male; 2 female
5	<i>P. g. kushackewitschii</i>	Near Andreevka (left bank of Chyndjaly River)	9 male
6	<i>P. versicolor hispida</i>	E. Kazakhstan: Djungar Gate	4 male
7	<i>P. g. salenskyi</i>	E. Kazakhstan: Zaissan Depression: Left bank of Bukhtarma Reservior: Kkuludjunsky Sands	3 male
8	<i>P. g. salenskyi</i>	Left bank of Black Irtys near Karatal	3 male

Sokolovsky described all chromosomes as telocentric. We cannot confirm this with confidence. Second arms are clearly visible on metaphasic plates with premetaphasic (elongated) chromosomes on several pairs of large elements. Such chromosomes could be acro- or even subtelocentric. The karyotype of *salenskyi* is an example (Fig. 2). This characteristic is not peculiar to any one form or group of forms of the *guttatus* group and cannot be used to distinguish a subordinate group.

We also observed distinct peculiarities of chromosome morphology in meiosis in the diakinetic stage. The chromosome bivalents of the various taxa differ in the number of ring-shaped and cross-shaped bivalents. Based on this difference in pairing, it is possible that the taxa of the toad agamas of the "*guttatus*" group might be grouped in the following way:

I: *guttatus*\*, *moltschanovi*, *kushackewitschii*, *alpherakii* all diakinetic bivalents are ring or stick-shaped.

II: *salenskyi* one or two of the elements are cross-shaped (Fig. 3), the remainder as in Group I.

III: *hispida* from two to four cross-shaped elements (Fig. 3), the remainder as in Group I.

This grouping by cross-shaped elements in diakinesis is a continuum. In this system, *P. v. hispida* is closer to *P. g. salenskyi* from the Zaissan Depression [sometimes attributed to *P. versicolor* (Paraskiv, 1953; Bannikov et al., 1977)] than to other forms of *P. guttatus sensu stricto*. However, it may be important that the toad agama from the Zaissan Depression occupies an intermediate position between Groups I and III. It is interesting to note the absence of chromosomal differences in the I-st. group, while its members, as mentioned above, are attributed by a number of authors to different species.

The data may be interpreted to suggest uniformity in the species of the *guttatus* group from Kazakhstan, Middle Asia, and the Precaucasus (Golubev, 1989) as well as a close relationship between *salenskyi* from Zaissan Depression and *hispida* from Djungar Gate and northern Djungaria (Golubev, 1992).

\* On several testis preparations of the nominative form we observed a picture similar to that of the preparations in Group II.

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## A Karyosystematic Study of the Plate Tailed Geckos of the Genus *Teratoscincus* (Sauria, Gekkonidae)

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**Abstract.**—Karyotypes of two subspecies of *Teratoscincus scincus* are described (*T. s. scincus* and *T. s. rustamowi*). Both have  $2n=36$ , and 46 arms in the karyotype (N. F. =46). A minor difference in centromere position in two of the smaller chromosome pairs was noted, and may characterize the respective subspecies sampled, or may reflect intra-population variation or even error in preparation. The karyotypes differ from an earlier published description for *T. scincus*, in which de Smet (1981) reported  $2n=34$ , N. F. =42. Whether this represents intraspecific variation, error in preparation or interpretation, or a suggestion that the name *T. scincus* is being applied to more than a single species will only be resolved with further systematic study of this gekkonid lizard.

**Key words:** Reptilia, Sauria, Gekkonidae, *Teratoscincus scincus*, Kazakhstan, Tadjikistan, karyology.

TABLE 1. Karyotypic data for *Teratoscincus scincus*. Legends: M- macrochromosome, m- microchromosome, v- metacentric, sT- subtelocentric, a (A)- acrocentric, NF- basic number.

Species, subspecies	Chromosomal formula	2n	NF	Author
<i>T. scincus</i>	4sT+4v+26A	34	42	de Smet
<i>T. s. scincus</i>	24M(6sT+18A)+12m(4v+8a)	36	46	our data
<i>T. s. rustamowi</i>	24M(6sT+18A)+12m(4v+8a)	36	46	our data

### Introduction

The Central Asian Gekkonid genus *Teratoscincus* is comprised of four recognized species (Szczerbak and Golubev, 1986). Karyotype data are available only for the species *Teratoscincus scincus* (de Smet, 1981). *T. scincus* is presently divided into three subspecies: *T. s. scincus*; *T. s. rustamowi*; and *T. s. keyserlingii*. This paper provides karyotypic descriptions of two races of *T. scincus*.

### Methods

A total of seven females and four males representing four populations from Turkmenistan (20 km north of Bami station; 50 km north of Bakhardok; 45 km north of Ashkabad; and the vicinity of Gyaurs) were studied. Also, a Kazakhstan population (the Chimkent Region, Syutkent Settlement) was sampled, as were three additional males of *T. s. rustamowi* from Tadjikistan (Leninabad Region, in the vicinity of Yakkatarak Settlement).

Chromosomal samples were prepared from cellular suspensions of bone marrow, blood and testis by the smear method and by using the method of "digging out" as described in Ford and Hamerton (1956) and McGregor and Varley (1986), as modified in part by Manilo (1986).

Cellular mitotic activity was increased by injections of phytohemagglutinine solution (0.02 ml/g body mass) and chorionic gonadotrophin (50 units/g body mass).

Chromosome preparations were stained with Giemsa (2% solution) in a 0.01 M sodium-phosphate buffer (pH 6.8) for 20-30 minutes. After washing in distilled water, the preparations were passed through alcohols and xylols (ortho-xylol) and subsequently embedded in Canadian balsam.

An NU-2 microscope with a 100x10 magnification was used for microscopy and photomicrography. Chromosomes are described using the centromeric position to define morphology following the



FIG. 1. *Teratoscincus scincus scincus*. a- mitotic metaphase of a dividing cell of bone marrow; b- bivalents of diakinesis; c, d- karyotype of female and male, respectively; e- idiogram of the karyotype.

classification suggested by Levan et al. (1964).

*Karyotype descriptions*

*Teratoscincus scincus scincus* (Schlegel, 1858).

Type locality: The Ili River in "Turkestan"

The diploid chromosome consists of 36 chromosomes. The karyotype is provisionally divisible into 24 macrochromosomes (M) and 12 microchromosomes (m), but there is no sharp demarcation between M and m. Chromosomes decrease in size gradually from largest (pair 1) to smallest (pair 18). Chromosome pairs 4, 7, and 9 appear subtelocentric; pairs 14 and 15 are metacentric; and the remaining pairs are



FIG. 2. *Teratoscincus scincus rustamowi*. a- mitotic metaphase of a dividing blood cell; b, c- male karyotype; d- idiogram of the karyotype.

acrocentric. The chromosomal formula could be stated:  $2n=24 M (6 sT+18A) + 12m (4v+8a) = 36$ . The "fundamental number" (N. F.) is 46. Sex chromosomes are not evident. Male and female karyotypes do not appear to differ in chromosome number or morphology. (Fig. 1).

In male meiosis, the number of bivalents at diakinesis is 18. The bivalents which correspond to macrochromosomes have a ring-like shape; the smaller bivalents (microchromosomes) have a rod-like shape (Fig. 1b).

*Teratoscincus scincus rustamowi* (Szczerbak, 1979)

Type locality: Fergan Valley in the sands in the vicinity of Cokand and Kairakkum.

As in *T. s. scincus* the diploid number is

36 with a somewhat arbitrary division between 24 macrochromosomes and 12 microchromosomes; no obvious sex chromosome heteromorphism; and chromosome pairs 4, 7, and 9 are subtelocentric. One possible difference noted between the subspecies, however, is that pairs 13 and 15 (instead of 14 and 15) appear to be metacentric. Meiotic material was not studied (Fig. 2).

*Comparative analysis of karyotypes of the genus*

The karyotype of *T. scincus* was first described by de Smet (1981). He reported  $2n=34$  with the following formula:  $4sT+4V+26A$ . The total number of arms (Fundamental Number, or N. F.) was 42. Subsequently Manilo and Pisanets (1984) obtained a different result ( $2n=36$ ). This stimulated us to re-examine the group in a more detailed manner (Table 1). Our conclusion is that two of the three subspecies have very similar karyotypes (the karyotype of *T. s. keyserlingii* remains unknown). The present studies with relatively small sample sizes, do not permit us to determine whether the difference we describe between the two subspecies represents individual variation or a real difference.

Because de Smet did not indicate the locality for his specimens, we cannot determine whether his description of  $2n=34$  and our description of  $2n=36$  represent a difference in diploid number among populations; variation within populations; or problems in preparation and description. Clearly this group of geckos should be studied more extensively.

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Resting Metabolic Rate in Three Age-groups of *Alligator sinensis*

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**Abstract.**-The resting metabolic rate in three age-groups of *Alligator sinensis* is influenced the temperature, season, body weight and other factors. Among these factors, the effect of body weight, temperature, season, which are greater than that of others. The relationship between the body weight and the resting metabolism is not consistent with the "third-quarter" surface area law. In the equation  $M=aW^b$ , the value of "a" ranges from 0.009 to 0.028, and the value of "b" ranges from 0.522 to 0.591, so that b is approximately equal to 2/3 in empirical equation.

**Key Words:** Reptilia, Crocodylia, Alligatoridae, *Alligator sinensis*, China, metabolism.

## Introduction

*Alligator sinensis* is a kind of special and precious reptile. It is classed as a first grade protected form of wildlife in China. Much research on the adaptability of *Alligator sinensis* have been done in the fields of morphology, distribution, reproduction and so on (Chen, 1985; Chen and Wang, 1984). Zhang (1986; 1989) reported information about infant *Alligator sinensis* whose weights ranged from 35 to 50 grams. Because energy metabolism is a very important criterion for the adaptability of *Alligator sinensis*, the purpose of our work was to explore the regularity of daily gain in total energy from diet, with relation to digestibility and energy allocation and to explore the resting metabolic rate of *Alligator sinensis*. This paper deals with the resting metabolic rate of three age groups in *Alligator sinensis*.

## Materials and Methods

The *Alligator sinensis* that we used were provided by the Shanghai Zoo. The total number of tested animals was 13. Five of them were born in 1980, and their average body weight was  $2.84 \pm 0.053$  (M $\pm$ SD) kg at the beginning of the experiment in 1986 and  $3.126 \pm 0.053$  kg at the end of 1987. Four of them were born in 1981, and their average body weight was  $1.54 \pm 0.59$  kg at the beginning of the experiment in 1986 and  $2.139 \pm 0.857$  kg at the end of the experiment in 1987. Four of them were born 1982, and their average body weight

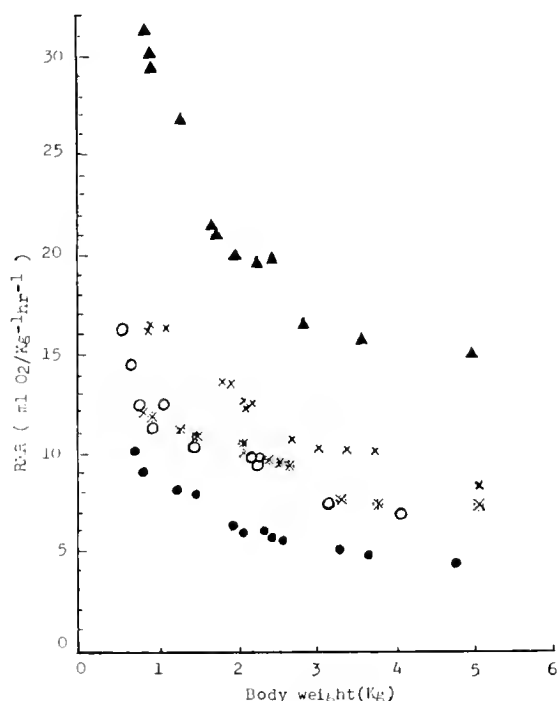


FIG. 1. Relationship between RMR and body weight in *Alligator sinensis*. Open circle- May (20°C); solid triangle- July (28°C); x- September (24°C); \*- October (20°C); solid circle- January (12°C).

was  $0.900 \pm 0.284$  kg at the beginning of the experiment and  $1.231 \pm 0.536$  kg at the end of the experiment in 1987.

The experiment began in May 1986 and ended in January 1987. These animals were reared in the three ponds according their age differences. These ponds were simple artificial environments and the size of each pond was 3x4 square meters.



Table 1. The linear regression equations and the approximate surface area equations of *Alligator sinensis*.

Months	Ta=C	Linear regression equations	Approximate surface area equations
May	20°	$\log Y = \log -1.911 + 0.568 \log X$	$M = 0.012W^{0.568}$
May	25°	$\log Y = \log -1.789 + 0.549 \log X$	$M = 0.016W^{0.549}$
July	25°	$\log Y = \log -1.584 + 0.579 \log X$	$M = 0.026W^{0.579}$
July	28°	$\log Y = \log -1.549 + 0.571 \log X$	$M = 0.028W^{0.571}$
Sep.	24°	$\log Y = \log -1.750 + 0.542 \log X$	$M = 0.018W^{0.542}$
Sep.	25°	$\log Y = \log -1.730 + 0.550 \log X$	$M = 0.019W^{0.550}$
Oct.	20°	$\log Y = \log -1.882 + 0.591 \log X$	$M = 0.013W^{0.591}$
Oct.	25°	$\log Y = \log -1.774 + 0.525 \log X$	$M = 0.017W^{0.525}$
Jan.	12°	$\log Y = \log -2.050 + 0.544 \log X$	$M = 0.009W^{0.544}$

TABLE 2. T-test of regression coefficient.

Months	May		July		September		October		January
T=C	20°	25°	25°	28°	24°	25°	20°	25°	12°
t-value	1201	1203	1072	637	302	1819	1043	1272	4599

Note: all t values are over  $t_{0.001df_6}=5.96$  and  $t_{0.001df_5}=6.86$ .

In this paper the measure of the resting metabolic rate is in  $\text{ml O}_2/\text{kg}^{-1}\text{hr}^{-1}$  or  $\text{ml O}_2/W^{0.56}\text{hr}^{-1}$ . The closed-system respirator meter of Wang et al. (1980) was used to measure the oxygen consumption of *Alligator sinensis* under two different temperatures. The first, 25°C, is the contrast temperature that comes from the adaptive temperature of *Alligator mississippiensis* reported by Coulson and Coulson (1986). The other is the seasonal temperature that is derived from the average temperature of each month in the last five years in Shanghai (Table 1). The ingestive food behavior in the animals was fasted to avoid its effect on metabolism during the measuring of oxygen consumption.

## Results and Discussions

### *The relationship between the body weight and the resting metabolic rate (RMR)*

The relationship between the body weight and the resting metabolic rate of *Alligator sinensis* is summarized in Fig. 1. The resting metabolic rate to unit weight declines with the raise of individual weight in each month or under each temperature. That is, there is a negative correlation between the body weight and the weight-specific resting metabolic rate of *Alligator sinensis* that corresponds with the surface area law. The further analysis of the correlation between the weight and the

TABLE 3. A comparison of RMR in three age groups of *Alligator sinensis* from 1986-1987.

Age groups	RMR	May 1986		July 1986		Sept. 1986		Oct. 1986		Jan. 1987
		20°C	25°C	25°C	28°C	24°C	25°C	20°C	25°C	12°C
	$\text{ml O}_2/\text{kg}^{-1}\text{h}^{-1}$									
	Mean	8.52	10.28	16.19	17.54	10.51	11.10	8.80	10.31	5.44
	S. E.	0.77	1.07	1.15	1.24	0.71	0.79	0.62	0.75	0.36
A group	%	100	100	100	100	100	100	100	100	100
Born 1980	$\text{ml O}_2/\text{W}^{0.56}\text{h}^{-1}$									
	Mean	13.26	16.39	27.98	28.87	17.44	18.40	14.12	16.84	8.76
	S. E.	0.39	0.46	1.85	0.66	0.32	0.37	0.34	0.91	0.09
	%	100	100	100	100	100	100	100	100	100
	$\text{ml O}_2/\text{kg}^{-1}\text{h}^{-1}$									
	Mean	10.98	13.89	21.08	21.85	13.09	13.84	9.87	12.19	6.49
	S. E.	0.64	0.72	2.35	2.76	1.33	1.45	0.79	1.51	0.66
B group	%	128.9	135.1	130.2	124.6	124.5	124.7	100.8	118.2	119.3
Born 1981	$\text{ml O}_2/\text{W}^{0.56}\text{h}^{-1}$									
	Mean	12.61	16.55	26.41	27.23	17.73	18.72	13.53	16.55	8.63
	S. E.	0.53	0.35	0.42	0.52	0.13	0.23	0.22	0.62	0.67
	%	95.1	101.0	94.5	94.3	101.7	101.7	95.8	98.3	98.5
	$\text{ml O}_2/\text{kg}^{-1}\text{h}^{-1}$									
	Mean	13.87	18.62	25.08	27.42	15.18	16.52	11.61	14.52	8.47
	S. E.	1.16	1.42	1.84	2.33	0.76	1.46	0.73	1.14	0.79
C group	%	162.8	181.1	154.9	156.3	144.4	148.8	131.9	140.8	155.7
Born 1982	$\text{ml O}_2/\text{W}^{0.56}\text{h}^{-1}$									
	Mean	12.28	15.96	26.79	29.19	17.00	18.31	12.87	15.88	8.87
	S. E.	0.50	0.49	1.44	0.80	0.61	0.04	0.76	0.14	0.20
	%	92.6	97.4	95.8	101.1	97.5	99.5	91.2	94.3	101.3

resting metabolic rate of *Alligator sinensis* begin by converting or "transforming" observed values to their logarithms and the linear regression equation and the approximate surface area equation (Table 1) which are formed on the base of their logarithms according to the methods of Avery (1979). In equation  $M=aW^b$  from Table 1, the value "a" ranges from 0.009 to 0.028, the value "b" ranges from 0.522 to 0.591.

The significance of coefficients on the linear regression equations in Table 1 are also examined through the T-test ( $T=b/sb$ ) and the results are shown in the Table 2. All values of "t" are larger than  $t_{0.001df5}=6.86$  and  $t_{0.001df5}=5.96$  (Table 2), so the values of p are less than 0.001. We may be to deduce that the body weight has a great effect on the metabolic rate, and has a similar effect in other crocodylians (Coulson and Hernandez, 1983).

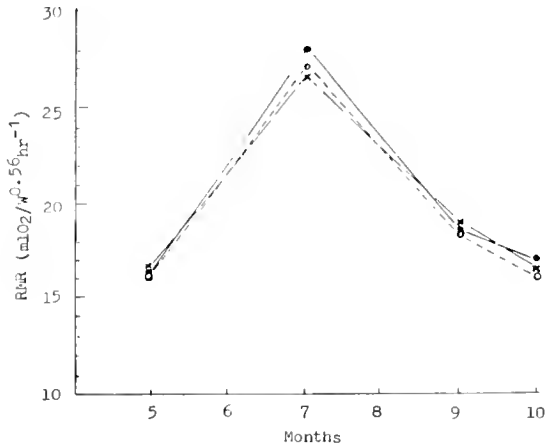


FIG. 2. Seasonal influence on the RMR of *Alligator sinensis* maintained at 25°C. Solid circle-1980 age group; x- 1981 age group; open circle-1982 age group.

#### Effects of temperature and season

Table 3 shows that the resting metabolic rate of *Alligator sinensis* affected by the temperature and season. In the cool season or under the low temperature, the resting metabolic rate is at a lower level, and vice versa. This is similar to that of the other reptiles (Coulson and Coulson, 1986; Huggins et al., 1971; Wang and Lu, 1986; Wang and Xu, 1987; Wang et al., 1983, 1988). This metabolic character is due to the result of acclimatization of seasonal temperature rhythm in evolution of animals.

We further analyze the relationship between RMR (resting metabolic rate) and temperature as well as season. We used 0.56 power of body weight to adjust all values of observing, so that the effect of body weight in RMR is eliminated. The results are summarized in Table 3. The values of  $\text{ml O}_2/\text{W}^{0.56}\text{hr}^{-1}$  from Table 3 show the temperature and season effect on RMR. The levels of RMR are higher under high temperatures than low, and it is a similar state that there is a higher level of RMR during hot seasons than during cool seasons.

From Fig. 2, it is further shown that the RMR changes with seasons under the same temperature of 25°C. In July, the RMR is

the highest level; in September, the RMR becomes lower and it continues to fall in October. This suggests that the energy consumption is relevant to the seasonal change which also corresponds with the rules of the energy consumption and requirements of *Alligator sinensis*. Among the growth months of *Alligator sinensis* as in July, there is a high water temperature, and there is intensive metabolism and rapid growth in *Alligator sinensis*. The data below support this statement. The group born in 1980 ingested daily  $172.02 \pm 77.65$  ( $M \pm SD$ ) g fresh fish in June,  $221.35 \pm 35.58$  g fresh fish in July,  $54.73 \pm 41.14$  g fresh fish in September. The daily body weight growth of the group was also rapid, such as 10.3 g in June, 44.6 g in July and 15.8 g in September. In May, *Alligator sinensis* had just awaked from hibernation, when the RMR was lower. In October, *Alligator sinensis* will stop the feeding when the RMR declined to a low level, and there were some changes in their physiological attributes for the coming hibernation stage. It is indicated that *Alligator sinensis* has a series of adaptive strategies for the seasonal changes.

#### The relationship between age and RMR

The relationships between age and RMR in three age groups are shown in Table 3. There are two kinds of data on the RMR. The first RMR in Table 3 is influenced by body weight, and does not eliminate the effect of body weight. It is expressed in  $\text{ml O}_2/\text{kg}^{-1}\text{hr}^{-1}$ . The second RMR eliminates the effect of body weight by expressing RMR in  $\text{ml O}_2/\text{W}^{0.56}\text{hr}^{-1}$ . A comparison on the level of both kinds of RMR in three age groups is based on 100 in RMR of age groups in 1980. The results of comparison suggest that the first kind of RMR falls as the age of groups increases. The second kind of RMR slightly falls in younger groups, except in a few months.

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## Effects of Chinese Snake Venoms on Blood Coagulation, Purified Coagulation Factors and Synthetic Chromogenic Substrates

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**Abstract.** -We examined the action of venoms from common Chinese *Crotalidae* and *Elapidae* snakes on blood coagulation mechanisms. Procoagulant effects were observed with venoms from *Agkistrodon acutus*, *Trimeresurus stejnegeri*, *Ophiophagus hannah* and *Bungarus fasciatus*, the latter two only in the presence of  $Ca^{2+}$ . After treatment with a serine protease inhibitor (phenylmethanesulfonyl fluoride, PMSF), *Agkistrodon acutus* venom lost its ability to clot purified fibrinogen but retained its capacity to clot human plasma in the absence of  $Ca^{2+}$ . An anticoagulant action was obtained with venoms from *Trimeresurus mucrosquamatus*, *Agkistrodon halys* and *Naja naja atra*. This action was abolished after treatment with a specific inhibitor of  $PLA_2$  activity (*p*-bromophenacyl bromide, BrPBr), revealing a procoagulant action with high concentrations of treated venoms in the cases of *Trimeresurus mucrosquamatus* and *Agkistrodon halys*. The effects of these venoms on hemostasis have been further characterized by measuring their phospholipase  $A_2$  activity, their ability to hydrolyze synthetic chromogenic substrates and to activate purified blood coagulation factors (prothrombin, factor X, protein C and plasminogen). These venoms showed an amidolytic activity which was mainly due to serine proteases (90 to 95% of inhibition with PMSF). Combining the observations obtained with human plasma and purified blood coagulation factors, we concluded that: i) six of the eight tested Chinese venoms (*i.e.*: *Ophiophagus hannah*, *Bungarus fasciatus*, *Agkistrodon acutus*, *Trimeresurus mucrosquamatus*, *Trimeresurus stejnegeri* and *Naja naja atra*) contain components which activate factor X in a  $Ca^{2+}$ -dependent manner; ii) three venoms (*Agkistrodon acutus*, *Agkistrodon halys* and *Trimeresurus stejnegeri*) contain prothrombin activators; iii) *Ophiophagus hannah* venom has a weak protein C activating activity; and iv) *Trimeresurus stejnegeri* venom possesses plasminogen activating activity. In addition, several of these venoms have previously been shown to contain thrombin-like and fibrinogenolytic enzymes, anticoagulant phospholipases  $A_2$  ( $PLA_2$ s) and/or non enzymatic anticoagulant components.

**Key Words:** Snake venoms, blood coagulation, purified blood coagulation factors, chromogenic substrates.

### Introduction

Snake venoms are known to be a rich source of hydrolytic enzymes, mainly proteases and phospholipases  $A_2$  and of non-enzymatic proteins, which induce disorders of blood coagulation, hemorrhage and shock (Pirkle and Markland, 1988; Ouyang and Teng, 1972; Teng and Seegers, 1981). Many proteases acting on different steps of the blood coagulation cascade have been purified from snake venoms. They cleave blood coagulation factors, either in a specific or in a non-specific manner, and cause acceleration or retardation of blood coagulation (Pirkle and Markland, 1988). Some of these proteases, such as thrombin-like enzymes (Stocker and Meier, 1988) or protein C activators (Kisiel et al., 1987), are serine proteases which may be rapidly and

irreversibly inactivated by alkylation with PMSF. Other procoagulant or anticoagulant proteases, like factor X or prothrombin activators from *Bothrops atrox* venom (Hofmann and Bon, 1987a; 1987b) or from *Echis carinatus* venom (Morita and Iwanaga, 1978) are insensitive to PMSF and have been postulated to be metalloenzymes.  $PLA_2$ s have also been recognized for their anticoagulant activity, which has been attributed to their ability to antagonize the procoagulant action of negatively charged phospholipids (Ouyang et al., 1978).

In order to better understand the pathophysiological action of snake venoms on haemostasis, and to examine the potential use of their procoagulant or anticoagulant components as pharmacological tools, we examined the

effects of various snake venoms on blood coagulation mechanisms *in vitro*, using human plasma, purified blood coagulation factors (fibrinogen, prothrombin, factor X, protein C and plasminogen), and synthetic chromogenic substrates. We examined in detail venoms from the eight most common venomous snakes in China; four belonging to the *Elapidae* family (*Ophiophagus hannah*, *Naja naja atra*, *Bungarus fasciatus* and *Bungarus multicinctus*) and the other four to the *Crotalidae* family (*Trimeresurus mucrosquamatus*, *Trimeresurus stejnegeri*, *Agkistrodon halys* and *Agkistrodon acutus*).

### Methods

Venoms were supplied by the Kunming Institute of Zoology (Academia Sinica, China). The venoms were collected from snakes living in the southern provinces of China and stored desiccated. They were dissolved in 50 mM Tris-HCl buffer, pH 7.8, at a concentration of 1 mg·ml<sup>-1</sup> and were used immediately.

Bovine factor X, human prothrombin and human Glu-plasminogen were obtained from Sigma (St. Louis, MO, USA). Human protein C was obtained from Diagnostica Stago (Asnières, France). Human fibrinogen (grade L) from Kabi Vitrum (Stockholm, Sweden) was treated with diisopropylfluorophosphate according to the instructions of the manufacturer, in order to irreversibly inactivate traces of thrombin or other blood coagulation factors. Platelet-poor human plasma was the supernatant of human blood mixed with 1/10 volume of 3.8% sodium citrate and centrifuged at 3000 rpm for 15 min. Pools of normal citrated plasma obtained from 5-10 healthy donors were stored at -20°C.

Chromogenic substrates H-D-Phe-Pip-Arg-pNA (S-2238), H-D-Val-Leu-Lys-pNA (S-2251), H-D-Val-Leu-Arg-pNA (S-2266), H-D-Pro-Phe-Arg-pNA (S-2302) and Bz-Ile-Glu-Gly-Arg-pNA (S-2222) were obtained from Kabi Vitrum (Stockholm, Sweden) and the chromogenic substrate H-D-Lys(Cho)-Pro-Arg-pNA (CBS65-25) was from Diagnostica Stago

(Asnières, France). Phenylmethanesulfonyl fluoride (PMSF) and *p*-bromophenacyl bromide (BrPBr) were purchased from Sigma (St. Louis, MO, USA). All other reagents were of the highest purity available.

### Chemical modifications

Inactivation of serine proteases by PMSF was performed in 50 mM Tris-HCl, pH 7.8. Venom samples (2 mg·ml<sup>-1</sup>) were incubated at 37°C for two hours with 5 mM PMSF (stock solution: 0.1 M in dimethylsulfoxid). Inactivation of PLA<sub>2</sub>s was carried out in the same buffer by incubating the venom (1 mg·ml<sup>-1</sup>) at 37°C for one hour with 2 mM BrPBr (stock solution: 0.1 M in acetone). Treated venom samples were then dialyzed for 4 to 8 hours against large volumes of the same buffer.

### Chromogenic assays

Amidolytic activity was measured with a Kontron spectrophotometer in 1 cm path-length plastic cuvettes. Assays were performed in 500 µl of 50 mM Tris-HCl, pH 7.8, containing the appropriate chromogenic substrate (0.2 mM). The reactions were initiated by addition of the sample to be tested (5 mg·ml<sup>-1</sup> to 100 mg·ml<sup>-1</sup>, final concentrations) and the formation of *p*-nitroanilide was monitored at 405 nm. The amount of substrate hydrolyzed was calculated using a molar extinction coefficient of 10,000 M<sup>-1</sup>·cm<sup>-1</sup> for free *p*-nitroanilide.

### Determination of PLA<sub>2</sub> activity

PLA<sub>2</sub> activity was determined by the titrimetric method described by Desnuelle et al. (1955), according to the procedure of Radvanyi and Bon (1982).

### Effects of the venoms on blood coagulation

Citrated platelet-poor human plasma (200 µl) was incubated at 37°C for 1 min, then a 20 µl aliquot of diluted venom samples was added and clotting time was recorded. In some cases, 5 µl of CaCl<sub>2</sub>

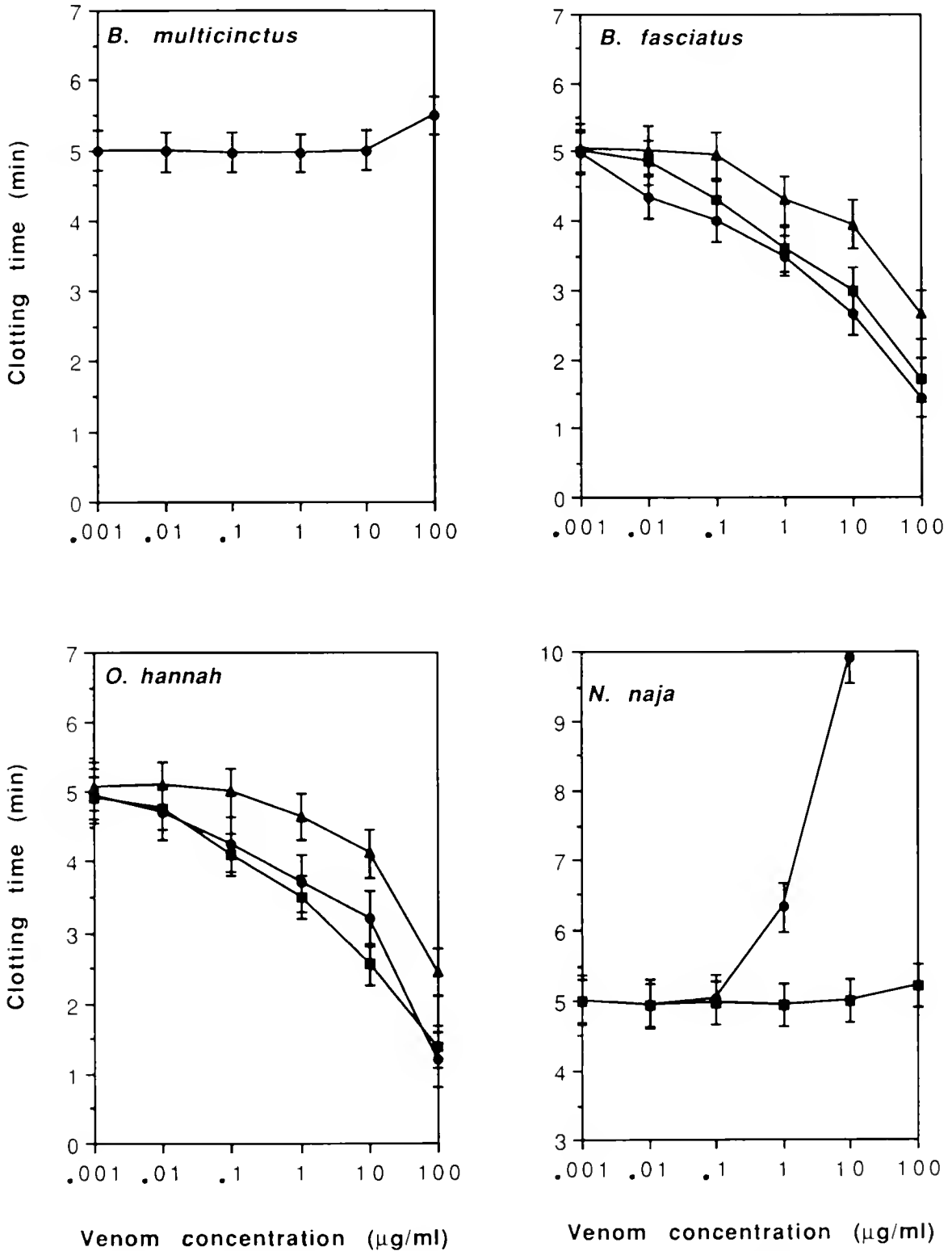


FIGURE 1. Effects of *Elapidae* snake venoms on blood coagulation. Citrated platelet-poor human plasma (200 ml) was incubated at 37°C for 1 min; dilutions of the sample to be tested (20 ml) were then added simultaneously with 5 ml of 0.45 M CaCl<sub>2</sub> (10 mM final concentration); native venom (●), PMSF-treated venom (▲) or *p*-bromophenacyl bromide-treated venom (■). Values are the mean of triplicates.

TABLE 1. Phospholipase A<sub>2</sub> activity of the venoms from the common Chinese venomous snakes ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ ).

	native venom	treated venoms
<i>Agkistrodon acutus</i>	37±2	<1.0
<i>Agkistrodon halys</i>	220±9	<1.0
<i>Trimeresurus mucrosquamatus</i>	48±3	<1.0
<i>Trimeresurus stejnegeri</i>	50±6	<1.0
<i>Ophiophagus hannah</i>	95±8	<1.0
<i>Bungarus fasciatus</i>	160±11	<1.0
<i>Bungarus multicinctus</i>	180±12	<1.0
<i>Naja naja</i>	35±4	<1.0

The phospholipase A<sub>2</sub> activity of the venoms were determined as described by Radvanyi and Bon (1982) using egg lecithin solubilized by sodium cholate. Venoms were treated by *p*-bromophenacyl bromide as indicated in the method then dialyzed to remove the excess of reagent. Each value is the mean of three determinations ± standard deviation.

(10 mM final concentration) were added simultaneously with the venom samples. Thrombin-like activity was determined by measuring the clotting time of purified human fibrinogen (0.5%) in 50 mM Tris-HCl, pH 7.8, containing 0.1 M NaCl. Fibrinogen (200 ml) was incubated for 2 min at 37°C before addition of 20 ml of diluted venom samples.

#### *Activation of blood coagulation factors by snake venoms*

Activation of prothrombin and factor X was performed as described by Hofmann and Bon (1987a; 1987b). Briefly, purified human prothrombin (50 mg·ml<sup>-1</sup>) was incubated at 37°C in 50 mM Tris-HCl, pH 7.8, containing 0.1 M NaCl and different concentrations of the samples to be tested. Aliquots (50 ml) were removed at various times and their amidolytic activity was tested in 500 ml of the same buffer containing S-2238 (0.2 mM). Purified bovine factor X (25 mg·ml<sup>-1</sup>) was incubated at 37°C in 50 mM Tris-HCl, pH 7.8, containing 0.1 M NaCl, 10 mM CaCl<sub>2</sub> and different concentrations of the samples to be tested. Aliquots (50 ml) were

removed at various times and their amidolytic activity was immediately assayed in 500 ml of the same buffer containing S-2222 (0.2 mM).

Protein C activation was assayed according to the method of Orthner et al. (1988), with minor modifications. Human protein C (5 mg·ml<sup>-1</sup>) was incubated at 37°C in 50 mM Tris-HCl, pH 7.8, containing 1 mg·ml<sup>-1</sup> polyethylene glycol, 5 mM EDTA and dilutions of the samples to be tested. At various times, aliquots (50 ml) were taken to measure the amidolytic activity of activated protein C, in 500 ml of the same buffer containing CBS65-25 (0.3 mM).

#### *Plasminogen activation assay*

Human Glu-plasminogen (100 mg·ml<sup>-1</sup>) was incubated at 37°C in 200 ml of 50 mM Tris-HCl, pH 7.8, containing 0.1 M NaCl, 0.01% Tween-80, and different concentrations of the samples to be tested. Aliquots (50 ml) were taken at various times and assayed for plasmin activity. They were introduced into a plastic cuvette containing 450 ml of the same buffer



TABLE 2. Amidolytic activity ( $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$ ) of venoms from Chinese snakes.

Venom		Substrate					
		S-2238	S-2251	S-2222	S-2302	S-2266	CB S65-25
<i>A. acutus</i>	Native	230	<1	20	110	50	550
	Treated	<1	<1	<1	<1	<1	<1
<i>A. halys</i>	Native	30	50	70	360	290	260
	Treated	<1	<1	<1	10	40	30
<i>T. mucrosquamatus</i>	Native	2200	110	20	1500	2900	1600
	Treated	40	<1	<1	130	60	70
<i>T. stejnegeri</i>	Native	800	120	30	2400	950	1400
	Treated	10	<1	<1	240	10	10
<i>O. hannah</i>	Native	10	<1	10	60	50	70
	Treated	<1	<1	<1	<1	<1	<1
<i>B. fasciatus</i>	Native	<1	<1	<1	<1	<1	<1
<i>B. multicinctus</i>	Native	<1	<1	<1	<1	<1	<1
<i>N. naja atra</i>	Native	<1	<1	<1	<1	<1	<1

The amidolytic activities of each venom were determined as described in Methods, with the indicated substrates. Venoms were treated by PMSF as indicated in Methods, then dialyzed to remove the excess of reagent. Indicated values are the means of three determinations (standard errors were less than 10%).

supplemented with S-2251 (0.3 mM) and the formation of *p*-nitroanilide was monitored at 405 nm.

## Results

### *Procoagulant and anticoagulant properties of the venoms*

The procoagulant and anticoagulant actions of the venoms from the eight most common venomous snakes in China (*Ophiophagus hannah*, *Naja naja atra*, *Bungarus fasciatus*, *Bungarus multicinctus*, *Trimeresurus stejnegeri*, *Trimeresurus mucrosquamatus*, *Agkistrodon halys* and *Agkistrodon acutus*) were examined with human plasma in the presence and in the absence of calcium ions. Each venom was tested in its native form, after treatment with a specific and irreversible inhibitor of serine proteases (PMSF), or after treatment with a specific and irreversible inhibitor of PLA<sub>2</sub>s (BrPBr) (Volwerk et al., 1974).

As indicated in Figure 1, *Bungarus multicinctus* venom did not modify blood coagulation *in vitro*. Venoms from *Bungarus fasciatus* and from *Ophiophagus hannah* showed a procoagulant action, dependent on the presence of Ca<sup>2+</sup>. They were unable to clot purified fibrinogen (result not shown), indicating the absence of thrombin-like enzymes. Their procoagulant effect might therefore result either from an ability to convert prothrombin into thrombin in a calcium-dependent manner, or more probably, from a direct or indirect activation of factor X into factor Xa. The fact that a treatment of these venoms with PMSF significantly but not completely reduced their procoagulant action (Figure 1) suggests that this effect is due, at least in part, to serine protease(s).

The venom from *Naja naja atra* was characterized by a pronounced anticoagulant action (Figure 1). It did not prevent clotting of purified human

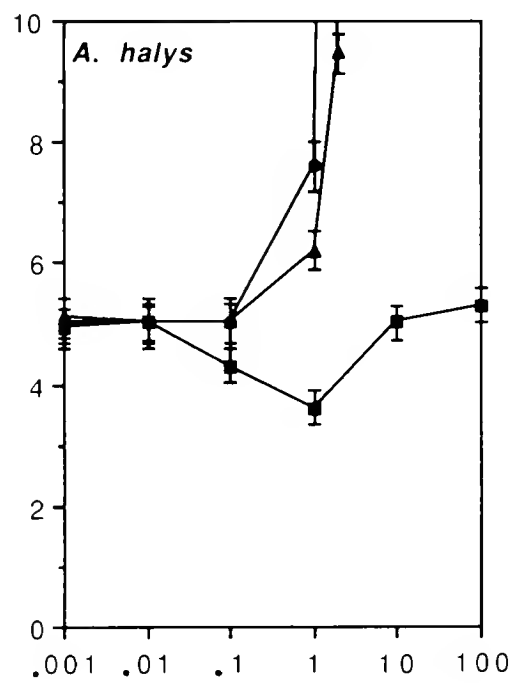
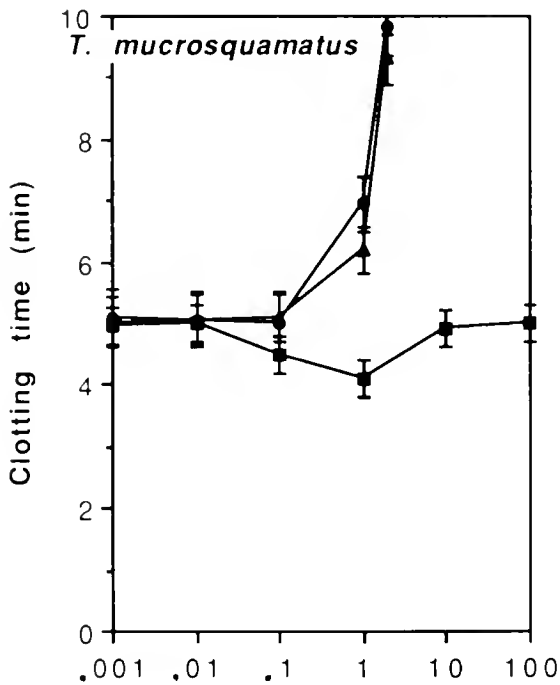
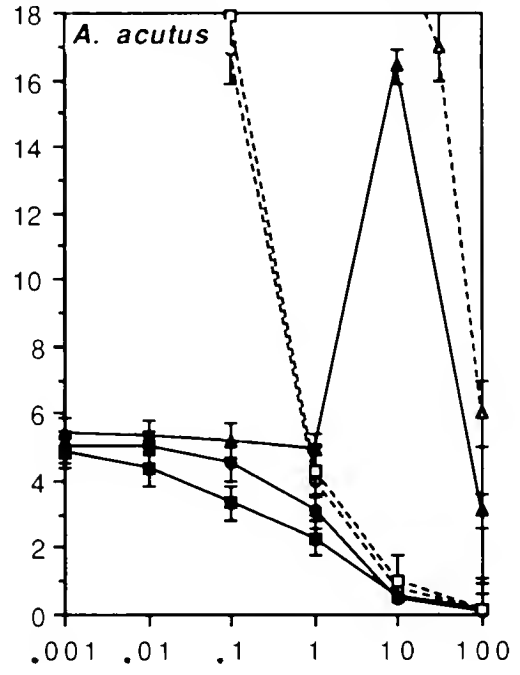
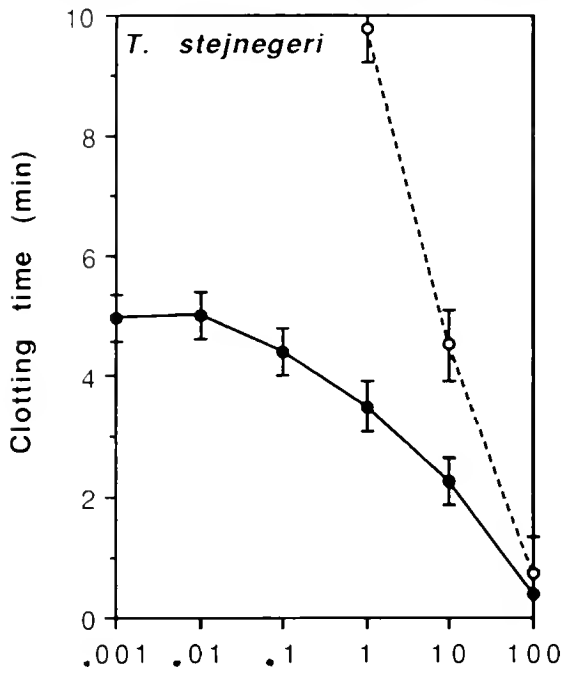
fibrinogen in the presence of thrombin (result not shown), indicating that the anticoagulant action is not due to fibrinogenolysis. The anticoagulant effect of *Naja naja atra* venom was completely and irreversibly prevented by treatment with BrPBr (Figure 1) which inhibited PLA<sub>2</sub> activity of the venom (Table 1). This activity is therefore due to one or several anticoagulant PLA<sub>2</sub>s, as reported in the case of many other snake venoms.

The effects of venoms from Chinese *Crotalidae* snakes on blood coagulation mechanisms (Figure 2) appeared much more complex than those observed with *Elapidae* venoms (Figure 1). The venoms from *Agkistrodon acutus* and from *Trimeresurus stejnegeri* were characterized by a procoagulant action, observed both in the presence and in the absence of Ca<sup>2+</sup> (Figure 2). These venoms also clotted purified fibrinogen in a calcium-independent manner (result not shown), indicating that they contain potent thrombin-like enzymes, in agreement with the observations reported by Ouyang *et al.* (1971) and by Liu and Xiong (1990). Treatment of *Agkistrodon acutus* venom with PMSF completely abolished its ability to clot purified fibrinogen (result not shown) and strongly reduced its procoagulant action (Figure 2), as expected since this effect is mainly due to thrombin-like serine proteases. However, PMSF-treated *Agkistrodon acutus* venom showed a complex action, an anticoagulant activity at 10 mg·ml<sup>-1</sup>, and a clotting activity at higher concentrations (Figure 2), suggesting that it contains other procoagulant components, in addition to thrombin-like enzymes. The anticoagulant effect of PMSF-treated *Agkistrodon acutus* venom is consistent with the presence of an anticoagulant protein of 26 kD, which is devoid of enzymatic activity and which prevents the formation of thrombin by binding to the prothrombin activation complex (Teng and Seegers, 1981). In addition to this non-enzymatic component, *Agkistrodon acutus* venom might contain anticoagulant PLA<sub>2</sub>s, since treatment with BrPBr (Table 1) significantly increased its procoagulant effect (Figure 2).

*Agkistrodon halys* and *Trimeresurus mucrosquamatus* venoms were characterized by an anticoagulant action which was completely abolished after treatment with BrPBr, but not with PMSF (Figure 2). This indicates that they contain potent anticoagulant PLA<sub>2</sub>s, as previously reported for *Trimeresurus mucrosquamatus* (Ouyang *et al.*, 1978) and for *Agkistrodon halys* (Chen *et al.*, 1987). In fact, treatment of *Agkistrodon halys* and *Trimeresurus mucrosquamatus* venoms with BrPBr suppressed the anticoagulant activity of these venoms and revealed a weak procoagulant activity (Figure 2), indicating the presence of procoagulant components. Further, this procoagulant action was observed only in the presence of Ca<sup>2+</sup>, suggesting that the procoagulant components are able to convert prothrombin into thrombin or to activate factor X. High concentrations (100 mg·ml<sup>-1</sup>) of *Agkistrodon halys* venom were able to clot purified fibrinogen (result not shown). This may be explained by the presence of a thrombin-like enzyme, which has been purified (Guan *et al.*, 1988) but the level of this enzyme in the venom is low and its activity is weak.

#### *Action of the venoms on purified blood coagulation factors*

We determined the amidolytic activity of the venoms on a number of chromogenic substrates, classically used for assaying blood coagulation factors: thrombin (S-2238), factor Xa (S-2222), activated protein C (CBS65-25), kallikrein (S-2302 and S-2266) and plasmin (S-2251). Assays were performed with native, PMSF-treated and BrPBr-treated venoms (Table 2). The venoms from *Elapidae* snakes did not present detectable activities, with the exception of the venom from *Ophiophagus hannah* which hydrolysed several substrates (Table 2). The venoms from *Crotalidae* snakes exhibited significant activities towards most chromogenic substrates, but with important species differences: the venoms from *Trimeresurus* snakes presented much higher activities than those of *Agkistrodon* snakes and the venom from *Agkistrodon*



Venom concentration (µg/ml)

Venom concentration (µg/ml)

Figure 2. Effects of *Crotalidae* snake venoms on blood coagulation. Citrated platelet-poor human plasma (200 ml) was incubated at 37°C for 1 min then the sample to be tested (20 ml) was added simultaneously with calcium (5 ml of 0.45 M CaCl<sub>2</sub>; 10 mM final concentration; closed symbols) or without calcium (open symbols and dashed lines). Native venom (●; ○); PMSF-treated venom (▲; △) or *p*-bromophenacyl bromide-treated venom (■; □). The values are the mean of triplicates, standard errors being 10% of the values.

TABLE 3: Activation of factor X, prothrombin, protein C and plasminogen by the venoms from common Chinese snakes.

	Activation activity (Arb. Unit)			
	Factor X	Prothrombin	Protein C	Plasminogen
<i>A. acutus</i>	0.67	1.3	0	0
PMSF treated	0.59	1.0	ND	ND
<i>A. halys</i>	0	3.0	0	0
PMSF treated	ND	2.9	ND	ND
<i>T. mucrosquamatus</i>	0.66	0	0	0
PMSF treated	0.58	ND	ND	ND
<i>T. stejnegeri</i>	0.27	0.16	0	0.42
PMSF treated	ND	ND	ND	0
<i>Ophiophagus hannah</i>	9.7	0	0.44	0
PMSF treated	4.2	ND	ND	ND
<i>B. fasciatus</i>	13.3	0	0	0
PMSF treated	5.8	ND	ND	ND
<i>B. multicinctus</i>	0	0	0	0
PMSF treated	ND	ND	ND	ND
<i>N. naja</i>	0.22	0	0	0
PMSF treated	ND	ND	ND	ND

The activation of the indicated blood coagulation factors was determined as described in Materials and Methods by measuring the amidolytic activity of the factors after activation. Each value is the mean of at least three independent determination, standard errors being less than  $\pm 10\%$ . ND means not determined. The results were expressed as the  $\Delta$  O.D./min at 405 nm acquired in analysed solution divide incubation time and divide venom concentration in activation solution.

*acutus* was 10 times more active on substrate S-2238 than that from *Agkistrodon halys*; in contrast substrate S-2251 was hydrolysed by the venom of *Agkistrodon halys* but not by the venom of *Agkistrodon acutus*. These results are in agreement with the general concept that the proteolytic activities of the venoms from *Elapidae* snakes are much lower than those of *Crotalidae* snakes.

The procoagulant action of the various venom-purified bovine factor X, human prothrombin, protein C and plasminogen, and measuring their amidolytic activity after activation (Table 3). Except for *Agkistrodon halys* and *Bungarus fasciatus*, all venoms were able to activate factor X, those from *Ophiophagus hannah* and *Bungarus fasciatus* being far more active than the others. Furthermore the ability of *Bungarus fasciatus* venom to activate factor X was markedly reduced after treatment with PMSF (Table 3), suggesting that the venom components

responsible for this activity may be serine proteases. It should however be noticed that neither *Ophiophagus hannah* nor *Bungarus fasciatus* venoms showed any activity on prothrombin or plasminogen, emphasizing their rather specific action on factor X.

On the other hand, three venoms (*Agkistrodon acutus*, *Agkistrodon halys* and *Trimeresurus stejnegeri*) among the eight which have been tested, possessed componentss was further examined in detail, using which converted prothrombin into thrombin. In all cases, this activity was insensitive to PMSF, suggesting that it is not due to serine proteases. Table 3 also shows that *Ophiophagus hannah* venom was able to activate protein C with a low activity compared to that observed in the venom of *Agkistrodon contortrix contortrix* (Kisiel et al., 1987). Interestingly, the venom from *Trimeresurus stejnegeri* was characterized by the capacity to activate plasminogen *in vitro* (Table 3). This action

appeared to be due to (a) serine protease(s), since it was completely abolished by PMSF.

We observed no correlation between the amidolytic activity of snake venoms, measured with the chromogenic thrombin substrate (S-2238) and their thrombin-like activity as determined by their ability to clot fibrinogen (result not shown). Similarly, there was no correlation between activation of prothrombin (Table 3) and the amidolytic activity measured with factor Xa substrate S-2222 (Table 2). These results emphasize the differences which exist between the substrate specificity of human coagulation factors and snake venom activators, and the existence in snake venoms of proteases which are able to hydrolyse chromogenic substrates without possessing the capacity to activate the corresponding blood coagulation factors.

### Discussion

In the present study, we found that, except for *Bungarus multicinctus*, the venoms from common Chinese venomous snakes (*Ophiophagus hannah*, *Naja naja atra*, *Bungarus fasciatus*, *Trimeresurus stejnegeri*, *Trimeresurus mucrosquamatus*, *Agkistrodon acutus* and *Agkistrodon halys*) possessed procoagulant and/or anticoagulant activities. An *in vitro* analysis of these venoms indicated that their action on blood coagulation results from the combined effects of several procoagulant and anticoagulant components. In particular, comparing the effects of native venom with those of venom in which PLA<sub>2</sub> activity has been blocked revealed the presence of anticoagulant PLA<sub>2</sub>s in *Agkistrodon halys* venom, similar to that described by Chen et al. (1987), which masked the effect of procoagulant component(s). We also showed that the procoagulant action of *Agkistrodon acutus* venoms does not result only from the previously described thrombin-like enzyme (Ouyang et al., 1971), but also from at least two other components, a PMSF-insensitive prothrombin activator and a calcium-dependent factor X activator. This illustrates the complexity of action of the

venoms on blood coagulation mechanisms.

We demonstrated the presence of prothrombin activators in *Agkistrodon acutus*, *Agkistrodon halys* and *Trimeresurus stejnegeri* venoms, as well as of factor X activators in *Agkistrodon acutus*, *Trimeresurus mucrosquamatus*, *Trimeresurus stejnegeri*, *Ophiophagus hannah*, *Bungarus fasciatus* and *Naja naja atra* venoms. These studies further indicated that prothrombin and factor X activators from Crotalidae (*Agkistrodon acutus*, *Agkistrodon halys*, *Trimeresurus mucrosquamatus* and *Trimeresurus stejnegeri*) venoms are PMSF-insensitive, and Ca<sup>2+</sup>-dependent in the case of factor X activators. These activators might be similar to those of *Bothrops atrox* and *Vipera russelli* venoms (Kisiel et al., 1976; Hoffman and Bon, 1987a; 1987b). Interestingly, factor X activators found in Elapidae venoms (*Ophiophagus hannah* and *Bungarus fasciatus*) were inactivated after treatment with PMSF, suggesting that they might be serine proteases.

We also showed the existence of a protein C activator in the venom of *Ophiophagus hannah*, although its activity was low compared to that found in the venom of *Agkistrodon contortrix contortrix* (Kisiel et al., 1987; Orthner et al., 1988).

These studies also revealed the first evidence of the existence of a plasminogen activator. *Trimeresurus stejnegeri* contains a PMSF-sensitive plasminogen activator. Its biochemical structure and mechanism of action are currently under investigation.

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## Herpetogeographical Map of Turkmenistan

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**Abstract.** -The herpetological map presented in this paper shows the distribution and abundance of the reptiles of Turkmenistan. The country is divided into 17 complexes and the 84 species and subspecies found in Turkmenistan are listed as occurring in mountains, plains, or both.

**Key words:** Reptilia, Turkmenistan, biogeography, distribution.

### Introduction

In the mid-1960's biogeography entered a new state of development with the practice of ecosystems mapping (Chyel'tsov-Bebutov, 1963, 1964, 1970, 1976). We do not here discuss the principles of the preparation and classification of geographical maps which depict animal population areas. We can only note that they make-up the series of sections included in many integrated regional atlases. Special surveys (Chel'tsov and Chibisova, 1976; Chel'tsov-Bebutov et al., 1972) have dealt with them as well. Nevertheless, the above mentioned maps were prepared for birds and mammals. Until now there have been no geographical maps (as geographical science visualized these) which present the quantitative proportions of reptiles in the total animal kingdom of any region (Chel'tsov-Bebutov and Chibisova, 1976).

The three authors of this article (Ataev, Rustamov, and Shammakov, 1989) created a color version of the Herpetogeographical Map of Turkmenistan in 1989. It was presented in 1989 at the All-Union Seminar dealing with the animal kingdom registration and cadastre (in Ufa), the Zoological Section of the Moscow Naturalists Society (in Moscow) and the VII-th All-Union Herpetological Conference (in Kiev). This article presents a black and white version of the map, giving no consideration to color qualitative background, on the scale 1:2,000,000, to be included into the Turkmen SSR Geographical Atlas (Fig. 1).

Field data, gathered throughout the whole Turkmenistan during 1960-1985 (Schammakov, 1981; Ataev, 1985), served as the main sources for this map. Other data were obtained from literature (Rustamov, 1966, 1981; Ataev, 1975; Rustamov and Schammakov, 1982; Ataev, Rustamov and Schammakov, 1985; Rustamov and Shcherbak, 1986; Makeev et al., 1988). Topographic maps on the scale of 1:1,000,000 and 1:500,000 were used as the cartographic basis.

The taxonomic generalization level of the topological contours shown on the map were dependent on both its scale and an analysis of data gathered by Ataev and Schammakov, unfortunately, apart, not in assemblage, with the zoogeographical survey of the country by Rustamov. A further point: the whole complex of a habitat and the animal population, which it supports, was taken as a unit undergone to zoogeographical mapping (Chel'tsov-Bebutov, 1963, 1964, 1976). We tried to single out the larger habitats at a level of an ecosystem (landscape or land system, according to Christian, 1975), not of the land unit, which is in close correlation with both the chosen scale and the content of the rest of the maps belonging to the Nature Division of the Atlas. The map scale provoked the necessity to single out such complexes of the reptiles population territorial aggregation, which should be grouped into a definite unity with regard to both common conditions of the habitats (the integral components of which are those aggregations) and the dominant species prevailing in number. A total of 17 complexes as such were revealed. Thus,

the map was build up on consideration of the habitats of reptiles and their species composition and density. Any territorial differentiation not proved by distinctions in the reptile population was not, as a rule, taken into account.

The reptile fauna of the Turkmenistan (Table 1) includes 78 species (84 subspecies) which belong to 2 orders and 14 families. The fauna consists of 3 species (3 subspecies) of tortoises, 47 (51) lizards, and 28 (30) snakes. The information on the reptiles species and population quantities distributed through every complex is placed in a special table that is not given in the atlas, as well as the Table 1, because of the lack of space. One needs this table because the map contours contain no concrete figures on the general density and species number of reptiles. The reptile populations are characterized only according to their appropriate abundance levels. This is quite enough for examining the general content of the map. Nevertheless, we provide herpetologists using this map with more concrete figures (Table 2). Reptile distributions, their abundance, and correlation are dependent upon habitats diversity as well as the fauna richness and specific ecologico-geographical peculiarities (Rustamov, 1966, 1981; Ataev, 1975; Rustamov and Schammakov, 1982). This, in turn, forms the physiognomy of the 17 territorial herpetological complexes.

To optimize the reptiles population characteristics, the map legend was made up of 2 parts: the table (placed at the Supplement) and the text. In addition, the insets give information on the fauna composition and contain the out-scale signs characterizing the loci of the habitats. The tables series are arranged according to the principle that permitted us to depict the territorial structure of herpetological complexes, although the map scale and content give no possibility to illustrate the morphological specification of the habitats occupied by these complexes. For example, the table-legend horizontal columns present the main groups of the territorial herpetological complexes revealed

on the basis of common ecosystems availability within the compared habitats. Those (groups) are: plain-desert (4 habitats), flood-plain valley (7), piedmont semi-desert (3), and mountain-arid (3). The vertical columns present the territorial units obtained as a result of geographical regionalization that, in our case, merely ground the boundaries of the herpetocomplexes. Such units of the regionalization scheme (zoogeographical regions) within Turkmenistan include: 1 area, 1 sub-area, 3 provinces, 4 districts, 6 regions and 10 sections (Rustamov and Scherbak, 1986).

The text of the legend gives the reptile population characteristics for every habitat gone into either complex. In front of the latter's name there is a circle under the correspondent number, the color map has qualitative background representing the complex. The latter's name is followed by the species number and the animals total density index (individuals per ha). The text of the legend is reduced in this article as the abundance indices are brought out in the special table (see Table 2).

Further reptile population characteristics for every complex are presented with species numeration of a fixed sequence: first species which use large areas are listed, then the stenotypic ones, which are confined to individual, smaller habitats within a contour. For example, clay surface, solonchaks, construction sites, etc., which are evidently differentiated due to their decreased sizes. The species names are arranged one after another according to decreasing population number within the habitat, of which a brief description is given immediately prior to the species enumeration (see the text of the legend). The dominant species are followed by (1), the codominant ones by (2), and the minor species by (3). The dominant species are defined by us as those whose number is over 10 per hectare, codominant species from two to nine per hectare, and minor species only one per hectare.

Thus, the map shows the herpetological territorial complexes differentiated



according to their species composition, total abundance and dominance levels (with regard to the species number) as well as principle features of the territory's morphology and its ecosystems structure, including the pattern of soils and vegetation cover.

Mapping had proved to be the most effective means to manifest and analyze the

reptiles population richness throughout the country. The present map can serve as the data source to evaluate the actual situation with the Turkmenistan reptile resources, or to elaborate the practical measures on resource use and conservation. The map can be a help to anybody who will create new, more detailed, large-scaled herpetological maps of either Turkmenistan or any other country.

TABLE 1. Reptiles of Turkmenistan. Su- USSR Red Data Book; T- Turkman SSR Red Data Book.

	Mountains	Plains	Mountains & Plains
Order Testudines			
<i>Emys orbicularis</i> (Linnaeus, 1758)	-	-	+
<i>Mauremys caspica</i> (Gmelin, 1774)	-	-	+
<i>Agrionemys horsfieldi</i> (Gray, 1844)	-	-	+
Order Squamata Suborder Sauria			
<i>Phrynocephalus helioscopus helioscopus</i> Pallas, 1771	-	+	-
<i>P. interscapularis</i> Lichtenstein, 1858	-	+	-
<i>P. maculatus</i> Anderson, 1872 (Su, T)	-	+	-
<i>P. mystaceus mystaceus</i> Pallas, 1776	-	+	-
<i>P. raddei raddei</i> Boettger, 1888	-	+	-
<i>P. r. boettgeri</i> Bedriaga, 1905	-	+	-
<i>P. reticulatus reticulatus</i> Eichwald, 1831	-	+	-
<i>P. r. bannikovi</i> Darevsky, Rustamov et Schammakov, 1976	-	-	+
<i>P. rossikowi rossikowi</i> Nikolsky, 1899(Su, T)	-	+	-
<i>P. r. schammakowi</i> Szczerbak et Golubev 1979, (Su, T)	-	+	-
<i>Stellio caucasius caucasius</i>	+	-	-
<i>Stellio c. triannulatus</i> Ananjeva et Ataev, 1984	-	-	+
<i>S. chernovi</i> (Ananjeva, Peters et Rzepakovsky, 1981)	+	-	-
<i>S. erythrogaster</i> Nikolsky, 1896	+	-	-
<i>S. lehmanni</i> Strauch, 1896	+	-	-
<i>Trapelus sanguinolentus aralensis</i> (Lichtenstein, 1823)	-	-	+
<i>Pseudopus apodus apodus</i> Pallas, 1775	+	-	-
<i>Alsophylax laevis</i> Nikolsky, 1907 (Su, T)	-	+	-
<i>A. loricatus szczerbaki</i> Golubev et Sattorov, 1979 (Su, T)	-	+	-
<i>A. pipiens</i> (Pallas, 1814) (T)	-	+	-
<i>Bunopus tuberculatus</i> Blanford, 1874 (Su, T)	+	-	-
<i>Crossobamon eversmanni</i> (Wiegmann, 1834)	-	+	-
<i>Cyrtopodion caspius caspius</i> Eichwald, 1831	-	-	+
<i>C. fedtschenkoi</i> (Strauch, 1887)	+	-	-
<i>C. longipes microlepis</i> Lantz, 1918 (Su, T)	+	-	-
<i>C. russowi</i> (Strauch, 1887)	-	-	-
<i>C. spinicauda</i> (Strauch, 1887) (Su, T)	+	-	-
<i>C. turcmenicus</i> (Szczerbak, 1978) (Su, T)	+	-	-
<i>Eublepharis turcmenicus</i> Darevsky, 1977 (Su, T)	+	-	-
<i>Teratoscincus scincus scincus</i> Schlegel, 1858	-	+	-

<i>Eremias arguta uzbekistanica</i> Chernov, 1934 (T)	-	+	-
<i>E. grammica</i> (Lichtenstein, 1823)	-	+	-
<i>E. intermedia</i> (Strauch, 1876)	-	+	-
<i>E. lineolata</i> (Nikolsky, 1896)	-	+	-
<i>E. nigrocellata</i> Nikolsky, 1896 (T)	-	+	-
<i>E. persica</i> Blanford, 1874	-	-	+
<i>E. regeli</i> Bedriaga, 1905 (T)	-	-	+
<i>E. scripta scripta</i> Strauch, 1867	-	+	-
<i>E. strauchi kopetdaghica</i> Szczerbak, 1971	+	-	-
<i>E. velox velox</i> Pallas, 1771	-	-	+
<i>Lacerta raddei raddei</i> Boettger, 1892 (T)	+	-	-
<i>L. strigata</i> Eichwald, 1831	-	-	+
<i>Mesalina guttulata wotsonana</i> Stoliczka, 1872	-	+	-
<i>Ablepharus deserti</i> Strauch, 1868	-	+	-
<i>A. pannonicus</i> (Lichtenstein, 1823)	-	-	+
<i>Chalcides ocellatus ocellatus</i> Forskal, 1775 (Su, T)	+	-	-
<i>Eumeces schneideri princeps</i> Eichwald, 1839	-	-	+
<i>E. taeniolatus taeniolatus</i> Blyth, 1854	-	-	+
<i>Mabuya aurata septemtaeniata</i> Reuss, 1834	-	-	+
<i>Ophiomorus chernovi</i> Anderson et Leviton 1966 (Su, T)	+	-	-
<i>Varanus griseus caspius</i> Eichwald, 1831 (Su, T)	-	-	+

## Suborder Serpentes

<i>Eryx elegans</i> (Gray, 1849) (Su, T)	+	-	-
<i>E. miliaris miliaris</i> Pallas, 1773	-	+	-
<i>E. tataricus speciosus</i> Tsarevsky, 1915 (T)	+	-	-
<i>Boiga trigonatum melanocephalia</i> Annandale, 1904 (Su, T)	-	-	+
<i>Coluber caspius</i> Gmelin, 1789 (T)	-	-	+
<i>C. karelini karelini</i> Brandt, 1838	-	+	-
<i>C. najadum najadum</i> Eichwald, 1831 (T)	+	-	-
<i>C. ravigieri</i> Menetries, 1832	-	-	+
<i>C. rhodorhachis rhodorhachis</i> (Jan, 1865)	-	-	+
<i>C. r. ladacensis</i> (Anderson, 1871)	-	-	+
<i>Eirenis medus</i> (Gernov, 1949)	+	-	-
<i>Elaphe dione</i> (Pallas, 1773)	-	+	-
<i>E. quatuorlineata sauromates</i> Pallas, 1814 (T)	-	+	-
<i>Lycodon striatus bicolor</i> Nikolsky, 1903 (Su, T)	+	-	-
<i>Lythorhynchus ridgewayi</i> Boulenger, 1887 (Su, T)	-	-	+
<i>Natrix natrix persa</i> Pallas, 1814	-	+	-
<i>N. tessellata</i> (Laurenti, 1768)	-	-	+
<i>Oligodon taeniolatus</i> (Jordan, 1853) (Su, T)	+	-	-
<i>Psammophis lineolatum</i> (Brandt, 1838)	-	+	-
<i>P. schokari schokari</i> Forskal, 1775	+	-	-
<i>Pseudocyclophis persicus persicus</i> Anderson, 1872	+	-	-
<i>Ptyas mucosus nigricans</i> Chernov, 1949 (Su, T)	-	-	+
<i>Spalerosophis diadema schiraziana</i> Jan, 1865	-	-	+
<i>Telescopus rhynopoma</i> (Blanford, 1874) (Su, T)	+	-	-
<i>Agkistrodon halys caraganus</i> Eichwald, 1831 (T)	-	+	-
<i>A. h. caucasicus</i> Nikolsky, 1916 (T)	+	-	-
<i>Naja oxiana</i> (Eichwald, 1831) (Su, T)	-	-	+
<i>Typhlops vermicularis</i> Merrem, 1820	+	-	-
<i>Echis multisquamatus</i> Cherlin, 1981	-	-	+
<i>Vipera lebetina turanica</i> Chernov, 1940	-	-	+

TABLE 2. Abundance and proportions of ecologico-systematic groups within the territorial complexes of Turkmenistan. 1\*- species number; 2\*- individuals per hectare.

Systematic groups and abundance Complexes	Testudines			Sauria			Serpentes			Total	
	1*	2*	%	1	2	%	1	2	%	1	2
1. South-Ustjurt	1	3.7	2.4	17	141.4	92.8	9	7.3	4.8	27	151.5
2. Caspian	1	3.6	3.3	16	95.0	84.8	13	13.2	11.8	30	111.8
3. Karakum	1	3.7	2.2	23	155.0	89.5	10	14.4	8.3	34	173.1
4. Sundukli	1	3.8	3.2	17	106.9	90.6	7	7.3	6.2	25	118.0
5. Sarykamysh	1	3.7	3.1	14	104.1	88.0	7	10.5	8.9	22	120.3
6. Uzboi	2	7.8	6.9	16	92.7	81.9	7	8.0	11.2	26	107.5
7. Atrek-Sumbar	3	7.9	8.4	12	65.5	69.6	10	20.5	22.0	25	93.9
8. Tedzhen-Haushan	1	3.4	3.2	16	99.6	84.6	12	14.4	12.2	29	117.4
9. Murgab	1	3.5	2.2	16	153.0	88.3	12	14.5	9.5	29	171.0
10. Amu-Darya	1	3.3	2.0	19	172.7	90.5	13	14.2	7.5	33	190.2
11. Kopetdag piedmont-anthropogenic	1	3.9	7.4	6	35.5	72.6	8	10.5	20.0	15	49.9
12. Kopetdag piedmont	1	3.6	2.3	19	148.2	93.1	10	7.3	4.6	30	159.1
13. Kugitang piedmont	1	3.8	3.8	16	86.7	88.8	8	7.3	7.4	25	97.8
14. Badghyz-Karabil	1	11.5	27.2	18	27.4	59.2	12	3.8	13.6	31	42.7
15. Balkhan	1	1.2	4.4	6	21.2	89.0	4	1.5	6.6	11	23.9
16. Kopetdag mountain	3	9.1	11.3	16	58.6	81.5	18	5.2	7.2	37	71.9
17. Kugitang mountain	1	1.3	4.8	9	22.6	83.4	10	3.2	11.8	20	27.1

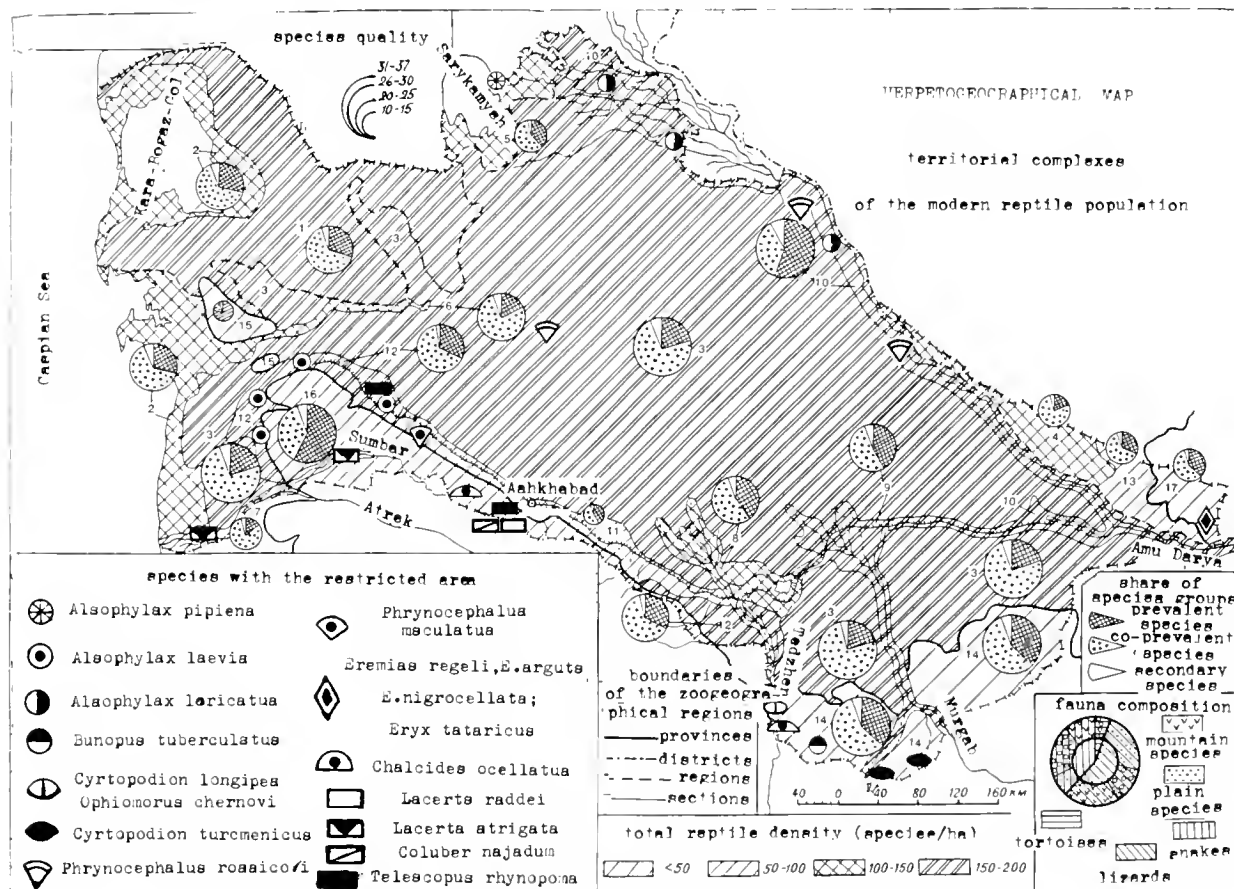


FIG. 1. Herpetological map of Turkmenistan. The species composition of various habitats within each of the 17 complexes is listed below. We define dominant species (1) as those that number over 10 per hectare, co-dominant species (2) as those that number from 2-9 per hectare, and minor species (3) as those that number one or less per hectare.

### 1. South-Ustjurt Complex

Various types of northwestern Turkmenistan deserts—*Cyrtopodion caspius* (1), *Eremias intermedia* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Eryx miliaris* (2), *Psammophis lineolatus* (2), *Coluber karelini* (3), *Spalerosophis diadema* (3). Clay, crushed-stone and solonchak habitats—*Phrynocephalus helioscopus* (2). Sandy and clay—*Varanus griseus* (3), *Naja oxiana* (3), *Boiga trigonatum* (3), *Agkistrodon halys* (3). Clay—*Cyrtopodion russowi* (2). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (2), *Eremias grammica* (2), *Eremias scripta* (2), *Phrynocephalus mystaceus* (2).

### 2. Caspian Complex

Various types of eastern Caspian deserts—*Cyrtopodion caspius* (2), *Eryx miliaris* (2), *Coluber karelini* (3), *Eremias intermedia* (2), *Eremias velox* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Echis multisquamatus* (2), *Psammophis lineolatus* (2), *Echis multisquamatus* (2). Sandy, clay and solonchak habitats—*Eremias lineolata* (2). Clay, crushed stone and solonchak—*Phrynocephalus helioscopus* (2). Sandy, clay and construction sites—*Eumeces schneideri* (2), *Coluber rhodorhachis* (3), *Varanus griseus* (3), *Naja oxiana* (3), *Boiga trigonatum* (3). Sandy and clay—*Agkistrodon halys* (3). Sandy and crushed stone—*Phrynocephalus reticulatus* (1). Sandy and on construction sites—*Coluber ravergieri* (3). Clay and on construction sites—*Mabuya aurata* (2). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (3), *Eremias grammica* (3), *Eremias scripta* (3), *Phrynocephalus mystaceus* (3). Clay—*Elaphe quatuorlineata* (3). Solonchak—*Lythorhynchus ridgewayi* (3). By water bodies—*Natrix tessellata* (3).

### 3. Karakum Complex

Various types of Karakum deserts—*Cyrtopodion russowi* (2), *Cyrtopodion caspius* (2), *Agrionemys horsfieldi* (2), *Eryx miliaris* (2), *Coluber karelini* (3), *Eremias grammica* (2), *Trapelus sanguinolentus* (2), *Psammophis lineolatus* (2), *Eremias velox* (2), *Echis multisquamatus* (2), *Echis multisquamatus* (2), *Spalerosophis diadema* (3). Sandy, clay and solonchak habitats—*Phrynocephalus raddei* (2), *Eremias grammica* (2). Clay, crushed stone and solonchak—*Phrynocephalus helioscopus* (2). Sandy, clay and on construction sites—*Eumeces schneideri* (2), *Coluber rhodorhachis* (3), *Varanus griseus* (3), *Naja oxiana* (3), *Boiga trigonatum* (3). Sandy, less common crushed stone—*Phrynocephalus reticulatus* (1). Sandy, clay, less common crushed stone—*Mesalina guttulata* (2). Sandy and on construction sites—*Coluber ravergieri* (3). Clay and crushed stone—*Lythorhynchus ridgewayi* (3). Clay and on construction sites—*Mabuya aurata* (2). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (2), *Eremias grammica* (2), *Eremias scripta* (2), *Phrynocephalus mystaceus* (2), *Vipera lebetina* (2).

### 4. Sundukli Complex

Various types of Sundukli massif deserts—*Cyrtopodion caspius* (1), *Eremias intermedia* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Eryx miliaris* (2), *Psammophis lineolatus* (2), *Coluber karelini* (3), *Spalerosophis diadema* (3). Sandy, clay and solonchak habitats—*Phrynocephalus raddei* (2), *Eremias lineolata* (2), *Eremias velox* (2), *Cyrtopodion russowi* (2), *Echis multisquamatus* (2). Clay, crushed-stone and solonchak—*Phrynocephalus helioscopus* (2). Sandy and clay—*Varanus griseus* (3), *Naja oxiana* (3), *Boiga trigonatum* (3). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (2), *Eremias grammica* (2), *Eremias scripta* (2), *Phrynocephalus mystaceus* (2). Crushed stone—*Cyrtopodion fedtschenkoi* (2).

### 5. Sarykamysh Complex

Various habitats of the Sarykamysh Depression—*Coluber karelini* (3), *Eremias velox* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Cyrtopodion caspius* (2), *Psammophis lineolatus* (2), *Eryx miliaris* (2), *Spalerosophis diadema* (3), *Varanus griseus* (3). Sandy, clay and solonchak habitats—*Eremias grammica* (2). Sandy, clay, solonchak and on construction sites—*Cyrtopodion russowi* (2). Clay, crushed-stone and solonchak—*Phrynocephalus helioscopus* (2). Sandy, clay and solonchak—*Eremias lineolata* (2). Sandy, clay and on irrigated lands—*Agkistrodon halys* (3). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (2), *Eremias grammica* (2), *Phrynocephalus mystaceus*

(2). Solonchak habitats, along collectors and canals, in settlements—*Elaphe dione* (3). On irrigated lands and water bodies—*Natrix tessellata* (2).

#### 6. Uzboi Complex

Various habitats of Western Uzboi Valley—*Coluber karelini* (3), *Cyrtopodion caspius* (2), *Agrionemys horsfieldi* (2), *Trapelus sanguinolentus* (2), *Eremias velox* (2), *Echis multisquamatus* (2), *Eryx miliaris* (2), *Psammophis lineolatus* (2), *Spalerosophis diadema* (3), *Coluber rhodorhachis* (3), *Varanus griseus* (3), *Naja oxiana* (3). Sandy, clay, solonchak and on construction sites—*Cyrtopodion russowi* (2). Sandy, clay, solonchak and crushed-stone—*Eremias intermedia* (2). Flood-plain, clay and on construction sites—*Mabuya aurata* (2). Clay, crushed-stone and solonchak—*Phrynocephalus helioscopus* (2). Sandy, clay and solonchak—*Phrynocephalus raddei* (2), *Eremias lineolata* (2). Flood plain—*Emys orbicularis* (2). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (2), *Eremias scripta* (2), *Eremias grammica* (2), *Phrynocephalus mystaceus* (2).

#### 7. Atrek-Sumbar Complex

Various habitats of Atrek and Lower Sumbar valleys—*Trapelus sanguinolentus* (2), *Eremias velox* (2), *Agrionemys horsfieldi* (2), *Echis multisquamatus* (2), *Eumeces schneideri* (2), *Cyrtopodion caspius* (2), *Coluber karelini* (3), *Elaphe dione* (3), *Varanus griseus* (3), *Psammophis lineolatus* (2), *Spalerosophis diadema* (3), *Boiga trigonatum* (3). Flood plains and irrigated lands—*Natrix natrix* (2), *Natrix tessellata* (2), *Ablepharus pannonicus* (2), *Coluber caspius* (3). Sandy, clay, crushed stone, and solonchak habitats—*Eremias intermedia* (2), *Eryx miliaris* (2). Clay, crushed-stone and solonchak—*Phrynocephalus raddei* (2). Clay, crushed-stone and solonchak—*Phrynocephalus helioscopus* (2). By water bodies—*Emys orbicularis* (2), *Mauremys caspica* (2). Sandy—*Teratoscincus scincus* (1), *Crossobamon evermanni* (2).

#### 8. Tedzhen-Hauzkhan Complex

Various habitats of Tedzhen Valley and Hauzkhan Massif—*Eremias velox* (2), *Natrix tessellata* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Echis multisquamatus* (2), *Eumeces schneideri* (2), *Mabuya aurata* (2), *Coluber karelini* (3), *Coluber rhodorhachis* (3), *Naja oxiana* (3), *Varanus griseus* (3), *Coluber ravergieri* (3), *Cyrtopodion caspius* (2), *Psammophis lineolatus* (2), *Boiga trigonatum* (3), *Vipera lebetina* (2), *Eumeces taeniolatus* (3), *Spalerosophis diadema* (3), *Eryx miliaris* (2), *Eremias intermedia* (2). Sandy, clay and solonchak habitats—*Phrynocephalus raddei* (2), *Eremias lineolata* (2). Clay, crushed-stone and solonchak—*Phrynocephalus helioscopus* (2). Sandy and clay—*Mesalina guttulata* (3). Sandy—*Phrynocephalus interscapularis* (1), *Phrynocephalus mystaceus* (2), *Eremias grammica* (2). Clay—*Lytorhynchus ridgewayi* (3).

#### 9. Murgab Complex

Various habitats of the Murgab Valley—*Eremias velox* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Cyrtopodion caspius* (2), *Echis multisquamatus* (2), *Eumeces schneideri* (2), *Psammophis lineolatus* (2), *Vipera lebetina* (2), *Mabuya aurata* (2), *Coluber karelini* (3), *Coluber rhodorhachis* (3), *Varanus griseus* (3), *Naja oxiana* (3), *Spalerosophis diadema* (3), *Ptyas mucosus* (3), *Boiga trigonatum* (3). On flood-plains and irrigated lands—*Ablepharus deserti* (1), *Ablepharus pannonicus* (2), *Natrix tessellata* (2), *Eumeces taeniolatus* (3). Sandy, clay, crushed-stone and solonchak habitats—*Eremias intermedia* (2), *Eryx miliaris* (2). Sandy, clay, solonchak habitats and on construction sites—*Cyrtopodion russowi* (2). Sandy, clay and solonchak habitats—*Phrynocephalus raddei* (2), *Eremias lineolata* (2). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon evermanni* (2). Clay—*Lytorhynchus ridgewayi* (3).

#### 10. Amu Darya Complex

Various habitats of the Amu Darya Valley—*Ablepharus deserti* (1), *Eremias velox* (2), *Natrix tessellata* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Cyrtopodion caspius* (2), *Echis multisquamatus* (2), *Psammophis lineolatus* (2), *Vipera lebetina* (2), *Eumeces schneideri* (2), *Mabuya aurata* (2), *Coluber karelini* (3), *Spalerosophis diadema* (3), *Varanus griseus* (3), *Naja oxiana* (3), *Coluber ravergieri* (3), *Elaphe dione* (3), *Boiga trigonatum* (3), *Agkistrodon halys* (3), *Eumeces taeniolatus* (3). Sandy, clay, crushed-stone and solonchak habitats—*Eryx miliaris* (2), *Eremias grammica* (2). Clay, crushed stone and solonchak—

*Phrynocephalus helioscopus* (2). Sandy, clay and crushed stone—*Phrynocephalus raddei*(2), *Eremias lineolata*(2). Sandy and crushed stone—*Phrynocephalus reticulatus*(1). Clay and crushed stone—*Lythorhynchus ridgewayi* (3). Construction sites—*Cyrtopodion fedtschenkoi* (2).

#### 11. Kopetdag Piedmont Anthropogenic Complex

Various habitats of Kopetdag piedmont oases—*Mabuya aurata* (1), *Cyrtopodion caspius* (1), *Eremias velox* (2), *Natrix tessellata* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Coluber rhodorhachis* (3), *Coluber ravergieri*,(3), *Naja oxiana* (3). In flood-plains of shallow rivers and on construction sites—*Eryx miliaris* (2), *Echis multisquamatus* (2), *Spalerosophis diadema* (3). On flood plains—*Eremias lineolata* (2), *Lythorhynchus ridgewayi* (3).

#### 12. Kopetdag Piedmont Complex

Various habitats of the Kopetdag piedmont plain—*Cyrtopodion caspius* (1), *Phrynocephalus raddei* (2), *Phrynocephalus helioscopus* (2), *Eremias intermedia* (2), *Eremias lineolata* (2), *Eremias velox* (2), *Cyrtopodion russowi* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Eryx miliaris* (2), *Echis multisquamatus* (2), *Psammophis lineolatum* (2), *Coluber karelini* (3), *Spalerosophis diadema* (3), *Varanus griseus* (3), *Naja oxiana* (3). Clay and crushed-stone habitats—*Mabuya aurata* (1), *Lythorhynchus ridgewayi* (3). Sandy and clay—*Mesalina guttulata* (2), *Boiga trigonatum* (3). Clay—*Pseudocyclophis persicus* (3), *Eirenis medus* (3). Sandy—*Phrynocephalus interscapularis* (1), *Teratoscincus scincus* (1), *Crossobamon eversmanni* (2), *Eremias grammica* (2), *Eremias scripta* (2), *Phrynocephalus mystaceus* (2).

#### 13. Kugitang Piedmont Complex

Various habitats of Kugitang piedmont plain—*Cyrtopodion caspius* (1), *Phrynocephalus raddei* (2), *Eremias intermedia* (2), *Phrynocephalus helioscopus* (2), *Eremias lineolata* (2), *Eremias velox* (2), *Trapelus sanguinolentus* (2), *Agrionemys horsfieldi* (2), *Eryx miliaris* (2), *Echis multisquamatus* (2), *Psammophis lineolatum* (2), *Coluber karelini* (3), *Spalerosophis diadema* (3). Clay and crushed-stone habitats—*Cyrtopodion fedtschenkoi* (2), *Lythorhynchus ridgewayi* (3). Sandy and clay—*Varanus griseus* (3), *Naja oxiana* (3), *Boiga trigonatum* (3). Sandy—*Phrynocephalus interscapularis* (1), *Phrynocephalus mystaceus* (2) *Eremias grammica* (2), *Eremias scripta* (2).

#### 14. Badghyz-Karabil Complex

Various habitats of the Badghyz and Karabil hills—*Agrionemys horsfieldi* (1), *Mabuya aurata* (2), *Cyrtopodion caspius* (2), *Trapelus sanguinolentus* (2), *Eremias velox* (2), *Ablepharus pannonicus* (2), *Stellio erythrogaster* (2), *Eumeces taeniolatus* (2), *Eumeces schneideri* (2), *Pseudophus apodus* (3), *Vipera lebetina* (3), *Naja oxiana* (3), *Psammophis lineolatum* (3), *Varanus griseus* (3), *Spalerosophis diadema* (3), *Coluber ravergieri* (3), *Coluber rhodorhachis* (3). On slopes covered by stones and mud-streams—*Stellio caucasicus* (2), *Typhlops vermicularis* (3). On bare rocks-outcrops—*Lycodon striatus* (3). On stone surfaces—*Pseudocyclophis persicus* (3). On slopes covered by loess and stones—*Psammophis schokari* (3). Food-plains and mud-streams—*Eremias persica* (2). Mud-streams—*Oligodon taeniolatus* (2). Flood plains—*Natrix tessellata* (3).

#### 15. Balkhan Complex

Various habitats of the Great and Small Balkhan Mountains— *Cyrtopodion caspius* (1), *Stellio caucasicus* (2), *Trapelus sanguinolentus* (2), *Eremias velox* (2), *Agrionemys horsfieldi* (2), *Coluber rhodorhachis* (3), *Naja oxiana* (3), *Ablepharus pannonicus* (3). Piedmonts and inter-ridge hills—*Varanus griseus* (3). Mud streams, undulated surfaces, piedmonts and inter-ridge hills—*Psammophis lineolatum* (3). Inter-ridge hills, piedmonts and construction sites—*Spalerosophis diadema* (3).

#### 16. Kopetdag Mountain Complex

Various habitats of the Kopetdag Mountains—*Stellio caucasicus* (2), *Ablepharus pannonicus* (2), *Agrionemys horsfieldi* (2), *Coluber rhodorhachis* (3), *Vipera lebetina* (3), *Naja oxiana* (3), *Cyrtopodion caspius* (1), *Trapelus sanguinolentus* (2), *Coluber ravergieri* (3), *Pseudopus apodus* (3), *Mabuya aurata* (1), *Eremias strauchi* (2), *Eremias velox* (2), *Typhlops vermicularis* (2), *Eumeces schneideri* (2), *Eumeces*

COMPLEXES OF THE MODERN REPTILE POPULATION										
ARID MEDITERRANEAN-ASIATIC SUB-AREA										
zoogeographical regions  main groups of the territorial herpetological complexes	provinces	TURAN PLAIN-DESERT						TRANSITORY IRAN-AFGHAN		MOUNTAIN-ASIA
	districts	K A R A K U M				KYZYLKUM		IRAN-AFGHAN UPLAND		CENTRAL ASIA MOUNTAIN
	regions	Ustjurt by-Caspian	K a r a k u m			Central-Kyzylkum		North-Iran-Afghan upland		Gissar-Alaj
	sections	Ustjurt crushed stone	Karakum sand	Karakum takyr	Atrek tugai	Amu Darya tugai	Kyzylkum sand	Kyzylkum crushed stone takyr	Badghyz-Karabil-piedmont	Khora-san-Kope t dag mountain
PLAIN DESERT	1	2	3				4			
FLOOD-PLAIN VALLEY	5	6		7	8					
			11		9					
					10					
PIEDMONT SEMI-DESERT			12				13	14		
MOUNTAIN ARID		15							16	17

FIG. 2. Supplement to herpetological map of Turkmenistan.

*taeniolatus* (2), *Cyrtopodion spinicauda* (3), *Pseudocyclophis persicus* (3). Steppe-like, stone and inter-ridge hills—*Coluber caspius* (3). Stone inter-ridge hills and piedmonts—*Lycodon striatus* (3), *Psammodon schokari* (3). Steppe-like inter-ridge hills and piedmonts—*Eirenis medus* (3). Inter-ridge hills, piedmonts, and on construction sites—*Psammodon lineolatus* (3), *Spalerosophis diadema* (3). Stone and inter-ridge hills—*Oligodon taeniolatus* (3), *Eublepharis turkmenicus* (3). Piedmonts—*Varanus griseus* (3). Along shallow rivers and other water bodies—*Natrix tessellata* (2). Juniper stands—*Eryx elegans* (3), *Agkistrodon halys* (3).

17. Kugitang Mountain Complex

Various habitats of the Kugitang Mountains—*Cyrtopodion fedtschenkoi* (1), *Agrionemys horsfieldi* (2), *Vipera lebetina* (3), *Coluber ravergieri* (3), *Stellio lehmanni* (2), *Eremias velox* (2), *Naja oxiana* (3), *Ablepharus pannonicus* (2), *Coluber rhodorhachis* (3), *Eumeces schneideri* (2). Inter-ridge hills, piedmonts, and on construction sites—*Trapelus sanguinolentus* (2). Piedmonts, stone habitats, and on construction sites—*Cyrtopodion caspius* (2). Stone habitats and juniper stands—*Stellio chernovi* (3). Piedmonts, stone and inter-ridge hills—*Spalerosophis diadema* (3). Inter-ridge hills and stone surfaces—*Pseudopus apodus* (3), *Typhlops vermicularis* (3). Stone surfaces and piedmonts—*Psammodon lineolatus* (3). Piedmonts—*Lytorhynchus ridgewayi* (3). Along shallow rivers and other water bodies—*Natrix tessellata* (2).

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## A Karyosystematic Study of the Genus *Bombina* from China (Amphibia: Discoglossidae)

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**Abstract.** -Chromosome number, morphology and positions of Ag-NORs were determined for four Chinese species of *Bombina*. Chromosome numbers are: *B. orientalis* (2n=24, NF=48), *B. maxima* (2n=28, NF=56), *B. microdeladigitora* (2n=28, NF=56). The Ag-NORs of *B. orientalis* are located on the long arm of the 7th chromosome pair, where as those of the latter three are on the short arm of the 11th pair. The subdivision of *Bombina* into two subgenera is supported by the karyology. A close relation between *Bombina* and *Discoglossus* is suggested.

**Key words:** Anura, Discoglossidae, *Bombina*; karyotypes, Ag-NORs, China.

TABLE 1. Species, localities, and number of individuals used in this karyological study.

Species	Locality	No. of Individuals	
<i>B. (G.) fortinuptialis</i>	Jinxiu, Guangxi	5 males	1 female
<i>B. (G.) maxima</i>	Dayao, Yunnan	11 males	5 females
<i>B. (G.) microdeladigitora</i>	Jingdon, Yunnan	5 males	2 females
<i>B. (B.) orientalis</i>	Qindao, Shandong	3 males	1 female

### Introduction

There are five genera in the family Discoglossidae. The systematics of this family have long been under discussion. The systematic position of the genus *Bombina* within this family is the most problematical. A variety of studies dealing with this genus have been presented during the past years (summarized by Lang, 1988; 1989a; 1989b). However, the systematics of *Bombina* is still quite confusing. The relationships within *Bombina* have not been fully worked out.

Only six species belong to the genus *Bombina*. All are distributed in Eurasia. Karyological data are known for *B. bombina* (2n=24, NF=48), *B. variegata* (2n=24, NF=48) [Morescalchi, 1973], *B. orientalis* (2n=24, NF=48) [Jiang et al., 1984], *B. maxima* (2n=28, NF=56) [Zhao, 1986, no photographs presented]. No chromosome banding data are available for *Bombina*.

Careful morphological analysis and banding of the chromosomes may yield useful information not only on the

phylogeny of the genus itself, but also on the relationships between *Bombina* and other genera of the family Discoglossidae. The purpose of this study is to analyze the karyotypes and Ag-NORs of four species in the genus from China (Liu and Hu, 1961). The results, when compared with known karyological data of *B. bombina*, *B. variegata* and other genera of Discoglossidae, should be helpful in understanding the taxonomy and phylogeny of *Bombina*.

### Materials and Methods

The specimens used in this investigation are listed in Table 1. The toads were collected by the authors at time of the year when both sexes are active for mating. The specimens were kept at room temperature (15-20°C) until the time of investigation.

To block mitosis at metaphase, we used a freshly prepared colchicine solution of 0.05%, and injected intraperitoneally 1/20ml of this solution per gram of body weight. The animals were sacrificed 20-24 hrs later, the spleen and small intestine were

TABLE 2. Observational results of the diploid chromosome number of four species of *Bombina* from China.

Species	# of cells observed	Number of diploid chromosomes							
		22	23	24	25	26	27	28	29
<i>B. (G.) fortinuptialis</i>	113					1	12	99	1
percentage (%)						0.88	10.6	87.6	0.88
<i>B. (G.) maxima</i>	198				3	3	14	176	2
percentage (%)					1.5	1.5	7	88.9	1
<i>B. (G.) microdeladigitora</i>	238				2	4	22	209	1
percentage (%)					0.8	1.7	9.2	87.8	0.4
<i>B. (G.) orientalis</i>	116	2	8	104	0	2			
percentage (%)		1.7	6.9	89.5		1.7			

removed, and the intestine was opened with a pair of fine scissors to expose the inner epithelial surface. The exposed epithelial surface was washed with a 0.64% NaCl solution for several minutes in order to remove all mucous and debris. The tissue was cut into small pieces and placed in a Petri-dish.

We added 8-10 ml of 0.4% KCl into the dish, suspended vigorously with a Pasteur pipette. The hypotonic treatment lasted 30-40 minutes. It was centrifuged at 1000 rpm for five minutes, and then the hypotonic solution was removed. The tissue was fixed with 8-10ml of freshly prepared solution of 3:1(v/v) absolute methanol-glacial acetic acid for three times, with a total time of 60 minutes. The samples will keep indefinitely in the fixative if stored at 1-4°C.

We prepared slides by transferring 3-4 pieces of the fixed tissue onto a dry, warm (about 50°C) slide. We then added 5-10 drops of 60% acetic acid and siphoned the solution up and down until the solution evaporated completely. Slides were stained in 10% Giemsa (pH 6.8) for 10 minutes. Staining of nucleolus organizer regions (NORs) followed the methods of Howell et al. (1980).

A total of 675 mitotic chromosome spreads were observed. Ten selected

metaphase plates for each species were photographed, enlarged, and measured. The chromosome nomenclature used is that suggested by Levan et al. (1964). For the convenience of comparison, the chromosomes are defined as being large (A group), medium (B group) and small (C group) according to their relative lengths. Large chromosomes have a value of 100 units or more, small chromosomes have a value of 40 or fewer units. Chromosomes whose length falls between 40-99 units are considered to be medium.

## Results

The observed diploid chromosome numbers are presented in Table 2. Measurements of metaphase chromosomes of four Chinese species of *Bombina* are shown in Table 3.

It is obvious that, the karyotypes of *maxima*, *microdeladigitora* and *fortinuptialis* are equal to each other. They have  $2n=28$ ,  $NF=56$ , composed of 6 pairs of large homologous, one pair of medium-small chromosomes and seven pairs of small homologous; all the chromosomes are metacentric (m), except for 6th, 7th, and 9th pair, which are submetacentric (sm). A weak secondary constriction is observed on the short arm of 11th pair. It appears in about 10% of the cases. The Ag-NORs are observed on short arm of 11th pair and

TABLE 3. Measurements of metaphase chromosomes of four Chinese species of *Bombina* Mean  $\pm$  SE

<i>B. fortinuptialis</i>					<i>B. maxima</i>				
Group	No.	Relative length	ratio	type	Group	No.	Relative length	ratio	type
A (1-6)	1	160.0 $\pm$ 4.22	1.18 $\pm$ 0.06	m	A (1-6)	1	165.2 $\pm$ 5.30	1.23 $\pm$ 0.10	m
	2	147.0 $\pm$ 3.72	1.36 $\pm$ 0.03	m		2	144.4 $\pm$ 4.03	1.43 $\pm$ 0.07	m
	3	129.9 $\pm$ 5.36	1.48 $\pm$ 0.05	m		3	127.9 $\pm$ 6.21	1.47 $\pm$ 0.07	m
	4	119.8 $\pm$ 2.10	1.47 $\pm$ 0.06	m		4	122.8 $\pm$ 4.15	1.58 $\pm$ 0.11	m
	5	109.9 $\pm$ 5.20	1.21 $\pm$ 0.08	m		5	108.1 $\pm$ 6.52	1.38 $\pm$ 0.11	m
B (7)	6	107.3 $\pm$ 3.94	1.86 $\pm$ 0.13	sm	B (7)	6	105.8 $\pm$ 4.30	1.71 $\pm$ 0.06	sm
	7	40.5 $\pm$ 1.05	2.51 $\pm$ 0.11	sm		7	40.9 $\pm$ 3.25	2.69 $\pm$ 0.23	sm
	8	33.5 $\pm$ 0.97	1.28 $\pm$ 0.02	m		8	33.4 $\pm$ 2.51	1.56 $\pm$ 0.09	m
	9	28.4 $\pm$ 0.69	2.19 $\pm$ 0.09	sm		9	30.1 $\pm$ 1.75	2.33 $\pm$ 0.12	sm
C (8-14)	10	27.4 $\pm$ 1.27	1.50 $\pm$ 0.03	m	C (8-12)	10	28.1 $\pm$ 1.02	1.37 $\pm$ 0.06	m
	11	26.4 $\pm$ 0.78	1.48 $\pm$ 0.05	m		11	26.7 $\pm$ 1.33	1.42 $\pm$ 0.08	m
	12	25.4 $\pm$ 0.76	1.62 $\pm$ 0.08	m		12	24.5 $\pm$ 1.06	1.55 $\pm$ 0.03	m
	13	23.1 $\pm$ 0.41	1.44 $\pm$ 0.07	m		13	22.8 $\pm$ 1.10	1.44 $\pm$ 0.02	m
	14	21.4 $\pm$ 0.71	1.22 $\pm$ 0.04	m		14	20.3 $\pm$ 1.04	1.30 $\pm$ 0.03	m

<i>B. microdeladigitora</i>					<i>B. orientalis</i>				
Group	No.	Relative length	ratio	type	Group	No.	Relative length	ratio	type
A (1-6)	1	160.4 $\pm$ 6.24	1.16 $\pm$ 0.03	m	A (1-6)	1	152.3 $\pm$ 4.89	1.14 $\pm$ 0.02	m
	2	142.8 $\pm$ 5.87	1.45 $\pm$ 0.10	m		2	133.8 $\pm$ 5.01	1.27 $\pm$ 0.03	m
	3	130.1 $\pm$ 5.56	1.54 $\pm$ 0.08	m		3	130.0 $\pm$ 4.74	1.37 $\pm$ 0.03	m
	4	120.5 $\pm$ 3.78	1.52 $\pm$ 0.07	m		4	125.7 $\pm$ 7.51	1.34 $\pm$ 0.08	m
	5	111.3 $\pm$ 5.65	1.23 $\pm$ 0.04	m		5	113.0 $\pm$ 2.30	1.22 $\pm$ 0.11	m
B (7)	6	104.6 $\pm$ 4.19	1.90 $\pm$ 0.13	sm	B (7)	6	108.4 $\pm$ 3.10	1.71 $\pm$ 0.07	sm
	7	41.8 $\pm$ 2.96	2.56 $\pm$ 0.16	sm		7	86.0 $\pm$ 2.05	1.43 $\pm$ 0.04	m
	8	34.7 $\pm$ 1.01	1.39 $\pm$ 0.07	m		8	38.4 $\pm$ 3.25	1.07 $\pm$ 0.03	m
	9	32.3 $\pm$ 1.06	2.26 $\pm$ 0.09	sm		9	34.7 $\pm$ 2.15	1.33 $\pm$ 0.04	m
C (8-14)	10	28.0 $\pm$ 1.18	1.39 $\pm$ 0.04	m	C (8-12)	10	28.7 $\pm$ 1.76	1.42 $\pm$ 0.06	m
	11	27.8 $\pm$ 1.47	1.48 $\pm$ 0.05	m		11	26.1 $\pm$ 1.75	1.15 $\pm$ 0.13	m
	12	24.1 $\pm$ 1.12	1.46 $\pm$ 0.06	m		12	24.1 $\pm$ 1.04	1.53 $\pm$ 0.05	m
	13	22.1 $\pm$ 2.04	1.44 $\pm$ 0.08	m					
	14	20.8 $\pm$ 2.36	1.21 $\pm$ 0.11	m					

relative length= (chromosome length/total of haploid chromosome length) x 1000

ratio= long arm/short arm

coincide with the position of secondary constriction. *B. orientalis* has  $2n=24$ ,  $NF\leq 48$ , consisting of 6 pairs of large homologues, one pair of medium-large chromosomes and 5 pairs of smaller homologues. All the chromosomes are m, except for the 6th pair, which is sm. A

clear secondary constriction is found on the long arm of 6th pair, and a weak one is observed on the short arm of the 8th pair, the latter appears in a case of 5%. Ag-NORs were only observed on the long arm of 7th pair. No heteromorphic chromosomes were found. The karyotypes

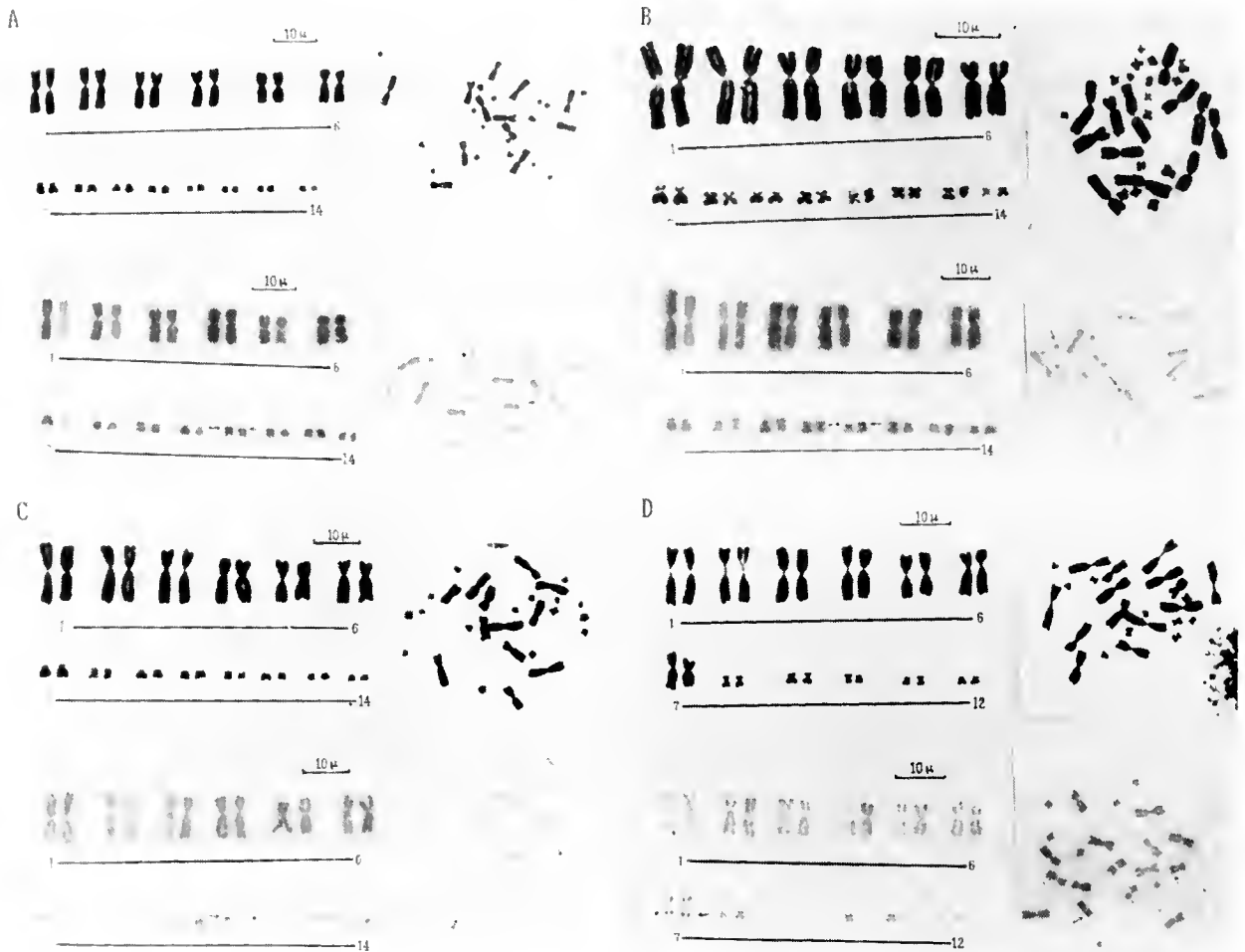


FIG. 1. Karyotypes and Ag NO<sub>3</sub> stained karyotypes of *Bombina* from China. Arrows show Ag-NORs. A: *B. fortinuuptialis*; B: *B. maxima*; C: *B. microdeladigitora*; D: *B. orientalis*.

are presented in Fig. 1.

### Discussion

Now, karyotypes are known for all the recognized species of *Bombina*. We compare the karyotypes and Ag-NORs of them in Table 4.

All the chromosomes of *Bombina* have median or submedian centromeres. In the discoglossids, *Alytes* are rich in acrocentrics, and with some microchromosomes ( $2n=38$ ,  $NF=64-72$ ), *Discoglossus* have  $2n=28$ ,  $NF=54$ , with one pair of telocentrics (Morescalchi, 1973). So, from the karyological point of view, *Bombina* is the most highly differentiated.

Within *Bombina*, two different kinds of karyotypes exist. The differences between the two are mainly as follows: 1). The morphology of 7th pair are quite different. The 7th pair of *maxima*, *microdeladigitora* and *fortinuuptialis* are medium-small and s, where as those of *bombina*, *variegata* and *orientalis* are medium-large, m, with a clear secondary constriction on the long arm. 2). The number of smaller homologues is different. The former three have 7 pairs of small homologues, but the latter three have only 5 small homologues. Tian and Hu (1985) suggested a subdivision of *Bombina* into two subgenera, the subgenus *Bombina* containing the Palearctic *bombina*, *variegata* and *orientalis*, and the Oriental *Glandula* containing *maxima*, *microdeladigitora* and *fortinuuptialis*. Our

TABLE 4. Comparison of karyotypes and Ag-NORs in *Bombina*.

Species	2n	NF	Chromosome formula	Secondary centromere	Locality of A-NORs	Data
<i>B. (G.) fortinuptialis</i>	28	56	22m+6sm	No. 11	No. 11	Present study
<i>B. (G.) maxima</i>	28	56	22m+6sm	No. 11	No. 11	Present study
<i>B. (G.) microdeladigitora</i>	28	56	22m+6sm	No. 11	No. 11	Present study
<i>B. (G.) orientalis</i>	24	48	22m+2sm	Nos. 7,8	No. 7	Present study
<i>B. (G.) bombina</i>	24	48	unknown	Nos. 7,8	unknown	Morescalchi 1973
<i>B. (G.) variegata</i>	24	48	unknown	Nos. 7,8	unknown	Morescalchi 1973

karyological evidence support this subdivision.

In *B. maxima*, *microdeladigitora* and *fortinuptialis*, the karyotypes are practically equal to each other which indicates that these three species may have diverged recently. In the group consisting of *B. bombina*, *variegata* and *orientalis*, *bombina* and *variegata* are equal to one another (Morescalchi, 1973), but *orientalis* has some differences from them. The 8th and 12th pairs are m in *orientalis*, but st in *bombina* and *variegata*. Thus the two European species are more closely related, which is congruent with immunological evidence of Maxon (1979) and Maxson and Szymura (1984).

Morescalchi et al. (1977) could not resolve relationships within Discoglossidae because the karyotypes of *Discoglossus*, *Alytes* and *Bombina* are so different from each other. Our investigation indicated that *Discoglossus* and the *Glandula*-group of *Bombina* have the same diploid chromosome number (Lanza et al., 1975; 1976). We suggest that those two genera may be related. The secondary constriction is the only "marker" currently available for analysis in most anuran karyosystematics studies. However, in the present study, we found that the secondary constriction is abrupt and the position of it is quite variable. The Ag-NORs are stable and clear and may be a more useful tool than the

place of the secondary constriction in some cases. The mechanisms of karyotype evolution of *Bombina* may take place through unequal translocation. This is still an open question. A further chromosome banding study is necessary.

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## A Study on the Purification and Pharmacological Properties of Two Neurotoxins from the Venom of the King Cobra (*Ophiophagus hannah*)

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**Abstract-** Using Sephadex G-50, CM-Sephadex C-25 and CM-cellulose 52 columns, two neurotoxins of *Ophiophagus hannah* were purified to be homogeneous on acidic PAGE which contain 53 and 73 amino acid residues respectively. The two neurotoxins were used as a substitute for morphine. Using a morphine withdrawal jumping model, the results demonstrated that the effects of the two neurotoxins when administered by injecting are very significant ( $P < 0.01$ ), and when administered orally are significant ( $P < 0.05$ ).

**Key words:** King Cobra, *Ophiophagus hannah*, neurotoxins, morphine addiction, naloxone jumping model.

### Introduction

The King Cobra (*Ophiophagus hannah*) is the largest poisonous snake in the world (Tu, 1977). It is extensively distributed in southern China, India, Thailand and other Asian countries. Joubert (1973) reported on the purification and sequence determination of two toxins from the venom of King Cobras grown in Thailand. Shun (1981) reported on the purification of four postsynaptically acting toxins from the venom of the King Cobra in Guangxi, China. Our research showed that among four toxins, there is a postsynaptically acting toxin containing only 63 amino acid residues. We determined the complete amino acid sequence of neurotoxin, which contain 73 amino acid residues. This neurotoxin's sequence is analogous to that of the neurotoxins determined by Joubert (1973), but its C-terminal four amino acid residues are very similar to that of bungartoxin in hydrophobicity (Lin and Wang, 1984). Now, the sequence of the neurotoxin containing 53 amino acid residues is in the process of being determined.

Xiong and Wang (1987) reported the clinical observation results of using the neurotoxin from cobra venom to achieve better analgesic effect on morphine addicted patients. The possibility to use the neurotoxins from snake venom as a substitute for morphine was also first reported by Xiong (1990). The mechanism has been discussed in the other papers. In

this paper, the research concentrated on the purifying of two neurotoxins from the venom of King Cobras from Guangxi, China and using them as a substitute for morphine during tests on the mice-jumping model.

### Methods

Venom of King Cobras was purchased from Guangxi Province. Male and female mice were provided by the feed lot in our institute. Naloxone and morphine were purchased from Qinghai Medical Factory. CM-Sephadex C-25, Sephadex G-50, and CM-Cellulose 52 were provided by our pharmacy. Other chemicals of A. R. grade were produced in China.

1. The purification of two neurotoxins from the venom of King Cobras: The dry venom powder was dissolved in the buffer solution (pH 5.8, 0.05 M HAC-NaAc buffer), then the solution was centrifuged to discard the insoluble material. The supernatant was loaded on a Sephadex G-50 column (25 x 200 cm). The same buffer was used to elute the column. The fractions which contained only low molecular weight components were collected (The process was directed by acidic PAGE).

The collected material was desalted and concentrated. It was then loaded on a CM-Sephadex C-25 column (4x80 cm). The column was eluted first by the equilibrium buffer, then was eluted by the buffer

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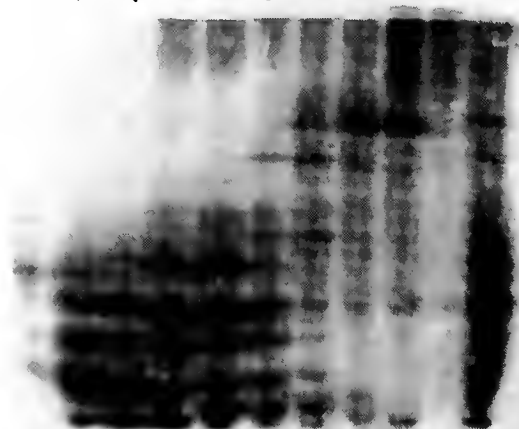


FIG. 1. The Acidic PAGE of the different fractions after separation with a Sephadex G-50 column (2.5 x 200 cm). 1: Crude venom; 2-11: Different fraction after separation with a Sephadex G-50 column. Fractions 7-11 were collected together for the further isolation.

containing NaCl gradient (0.04) and finally was eluted by the buffer containing NaCl gradient (0.4~0.8). The two neurotoxins were eluted during the first gradient process.

The neurotoxins from CM- Sephadex C-25 still showed two other minor bands on acidic PAGE. So each of them was further purified on the CM- Cellulose 52 Column. The equilibrium buffer is 0.05M, pH 5.8 HAc-NaAc buffer. The NaCl gradient is 0~0.5M in the same buffer.

2. Determination of purities: Acidic PAGE was used. The separating gel was 15 %, and the spacial gel was 2.5 %. The gel was stained by R- 250 dissolved in 375 ml ethanol, 125 ml water and 5 ml methanol.

3. Determination of amino acid composition: The samples were analyzed with a Hitachi Model 835-50 High Speed Amino Acid Analyzer.

4. Determination of amino acid sequence of the neurotoxin: The method was in accordance with the method of Lin and Wang (1984).

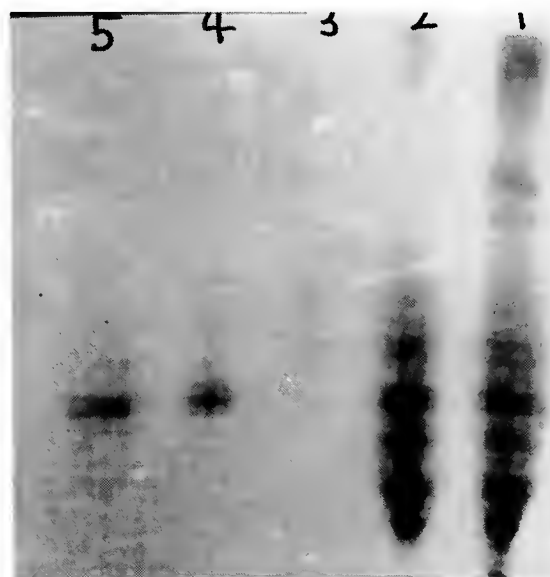


FIG. 2. The acidic PAGE of the neurotoxins from CM-Sephadex C-25 CM-Cellulose columns. 1. Crude venom; 2. The fraction containing neurotoxins from Sephadex G-50; 3. Neurotoxin 1; 4. Neurotoxin 2 from CM Sephadex C-25; 5. Neurotoxin 2 further purified on CM cellulose 52.

5. Determination of LD 50: Mice were used as the material. The method was according to Gu (1965).

6. Morphine withdrawal jumping model: Mice were divided into random groups, with each group consisting of 10 mice, half male and half female, with body weights of  $20 \pm 2$ g. The groups were treated by injecting S. V. with morphine, for four days, three times at a dose of 10 mg/kg, three times at a dose of 20 mg/kg and 12 times at a dose of 30 mg/kg. After morphine was given the last time, the neurotoxins were administered either orally or by injecting. The control group was treated with physiological saline of 0.2 ml per mouse. Three hours later, naloxone was administered S. V. to different groups at a dose of 15 ml/kg. Then the jumping numbers of the mice during a 30 minute period were recorded. The data were analyzed with statistical methods.

## Results

The lyophilized venom powder was dissolved in the buffer (0.05M, pH 5.8.



TABLE 1. The amino acid compositions of neurotoxins 1 and 2.

Amino Acid	Neurotoxin 1		Neurotoxin 2	
	Min. Residue	Residue Number	Min. Residue	Residue Number
	Numbers	100 Residues	Numbers	Number
Lys	6	6.81	4	7.55
His	0	0	1	1.89
Arg	4	5.26	1	1.89
Asp	8	10.53	6	11.33
Thr	9	11.84	2	3.77
Ser	3	3.95	5	9.43
Glu	5	6.58	2	3.77
Pro	7	9.21	4	7.55
Gly	4	5.26	4	7.55
Ala	4	5.26	3	5.66
Val	6	7.89	2	3.77
Met	1	1.32	0	0
Ile	3	3.95	3	5.66
Leu	1	1.32	3	5.66
Try	2	2.63	0	0
Phe	2	2.63	4	7.55
1/2 Cys	8	10.53	7	13.21
Try	3	3.95	2	3.77
Total Residue		76		53

HAc-Ac buffer), loaded on the Sephadex G-50 column, then eluted with the same buffer. The fractions containing neurotoxins were put together, m desalted, concentrated, then loaded on the CM-sephadex C-25 column, which was eluted first with equilibrium buffer, then with different NaCl gradient. After the CM-Sephadex C-25 column, the two neurotoxins were further purified on a CM-Cellulose column.

The crude venom was isolated first on a Sephadex G-50 column, then CM-Sephadex C-25, and finally, a CM-Cellulose column. Figure 1 and 2 shows the acidic PAGE of different fractions.

The amino acid sequence of neurotoxin 1 was determined by the Immobilized Phase Edman Method as: 1 Thr • Lys • Cys • Tyr • Val • Thr • Pro • Asp • Val • Lys • Ser • Glu • Thr • Cys • Pro • Ala • Gly • Gin • Asp • Leu • Cys • Tyr • Thr • Glu • Thr • Trp • Cys • Val • Ala • Trp • Cys • Thr • Val • Arg • Gal • Lys • Arg • Val • Ser • Leu • Thr • Cys • Aal • Ala • Ile • Cys • Pro • Ile • Val • Pro • Pro • Lys • Val • Ser • Ile • Lys • Cys • Cys • Ser • Thr • Asp • Aal • Cys •

Gly • Pro • Phe • Pro • Thr • Trp • Pro • Asn • Val • Arg

The toxicity of neurotoxins 1 and 2 was determined by the following method: The mice, with body weight of 18-20 g, were divided at random into groups of 5 mice. The different doses of the neurotoxins were given to the groups S. V. The death numbers of mice within 24 hours were recorded and the LD 50 was determined by modified a Ginsberg method. For neurotoxin 1 the LD 50=0.21±0.013. For neurotoxin 2 the LD 50=0.24±0.009

For the morphine withdrawal jumping model, the effects of purified neurotoxins on mice jumping numbers and the results of statistical analysis are shown in Table 2. The results demonstrated that the two neurotoxins display very significant effects (P<0.01) as a substitute for morphine, by injecting S. V. and significant effects (P<0.05) when administered orally.

## Discussion

The origination and evolutionary relationships of neurotoxins and

TABLE 2. The effects of the two neurotoxins on mice jumping test.

Groups	Administered S. V.			Administered Orally		
	Dose	No. of jumps	Statistics	Dose	No. of jumps	Statistics
Saline	0.1 ml	44.4		0.1 ml	44.4	
Neurotoxin 1	0.1µg/20 g	11.3	p<0.01	1.0 µg/20g	18.2	p<0.05
Neurotoxin 2	0.1 µg/20 g	19.3	p<0.01	1.0 µg/20 g	16.8	p<0.05

phospholipase A2 from snake venom are still disputed problems. Some experts thought that the original molecule is the postsynaptical toxins containing only 60-61 amino acid residues. By increasing the cycles in the molecules, the short-chain neurotoxins evolved into long-chain neurotoxin containing 7374 amino acid residues, then evolved into phospholipase A2. On the other hand, some experts thought Phospholipase A2 was the original molecule which diverged into neurotoxins (including postsynaptical and presynaptical toxin), proteinase inhibitor and a new neurotoxin (dendrotoxin). In King Cobra venom, both short and long chain neurotoxins were isolated. Especially, a postsynaptical toxin containing 53 amino acid residues was purified. So this neurotoxin is very important to understand the evolutionary relationship of the neurotoxin. Now, we are focused on the determination of its sequences and its conformational properties in the solution by means of 2D- NMR method (mainly by different correlated spectrum and NOSY spectrum).

By using the withdrawal jumping model, two neurotoxins display significant effects on substituting for morphine. The clinical observation in Kunming also demonstrated remarkable effects. These facts suggest that these two neurotoxins from the King Cobra venom may be a better medicine to be used as a substitute for morphine. Now, experiments have

been accomplished to understand its mechanism.

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## The Ecology of the Caucasian Salamander (*Mertensiella caucasica* Waga) in a Local Population

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**Abstract.** -The different aspects of ecology of *Mertensiella caucasica* Waga, 1876 were investigated in a local population from Borjomi Canyon (central Georgia) for five years (1985-1990). The aspects of the species' life cycle were more precisely determined. The main fecundity is about 16.9 eggs per female. There are about 2 years in a period from egg deposition (June to first half of July) to the end of metamorphosis in nature. Animals have spent most of the time in shelters after metamorphosis. They appear on the ground surface at night during the breeding period. Commonly the adults don't retreat to a great distance from population localities. Localities are situated in comparatively small plots (100-300m) along the streams. Estimation of adult animal number showed that the population consists of 1189 specimens (1989). Annual adult survival is higher than known values of most amphibians (approaches 0.77). Larval survival is 0.27-0.32 in the second year of life. The characteristics of demography (especially, low renewal rates) and spatial restriction in localities depends mostly on subtle constitution of the species (which is a result of allometric growth specifics). The small recent geographical range of *M. caucasica* is explained as a result of morphological and ecological peculiarities. General morphological constitution limits adaptive possibilities of any particular representative of the European salamander tribe. This is an explanation of quite high ecological similarity of *M. caucasica* and *Chioglossa lusitanica*.

**Key Words:** Amphibia, Caudata, Salamandridae, *Mertensiella caucasica*, Caucasus Mountains, Georgia, population ecology.

### Introduction

Natural populations are the single way of species existence. Autoecological research doesn't allow a complete understanding of the life of a species in nature. That is why there must be information of life cycles, geographical range, population size, number dynamics, etc. On the other hand it is hard to explain ecological aspects of the species existence without any information of their habitat preferences, feeding habits, breeding sites, etc.

By analyzing connections between species population ecology and autecology, as well as morphology and geographical distribution, the most complete notion can be formed. Investigations on some amphibian species biology have allowed scientists to elaborate complex works connected with different aspects of their life history. A wonderful example is Bell's works on the Smooth Newt (Bell and Lawton, 1975; Bell, 1977).

There aren't many data of regular

stationary investigations about the ecology of the rare or narrow-ranged species. Long term research of such species enlarges the knowledge of the biology of wide taxonomic groups. Moreover, these investigations may be useful to find out ways of rare species preservation.

A local population of the endemic salamander, (*Mertensiella caucasica*), from the western Caucasus of Georgia has been investigated for a five year period (1985-1990). This work gives additional information about the life history of this species.

The geographical distribution of the Caucasian Salamander was mainly established in the beginning of this century. Information was summarized by Nikolsky (1913). Later investigations commonly took place in earlier reported localities or in adjacent areas. Some new localities for salamanders were found by Bakradze and Tartarashvili (pers. comm.). The real geographic range of *M. caucasica* was established. The Caucasian Salamander is

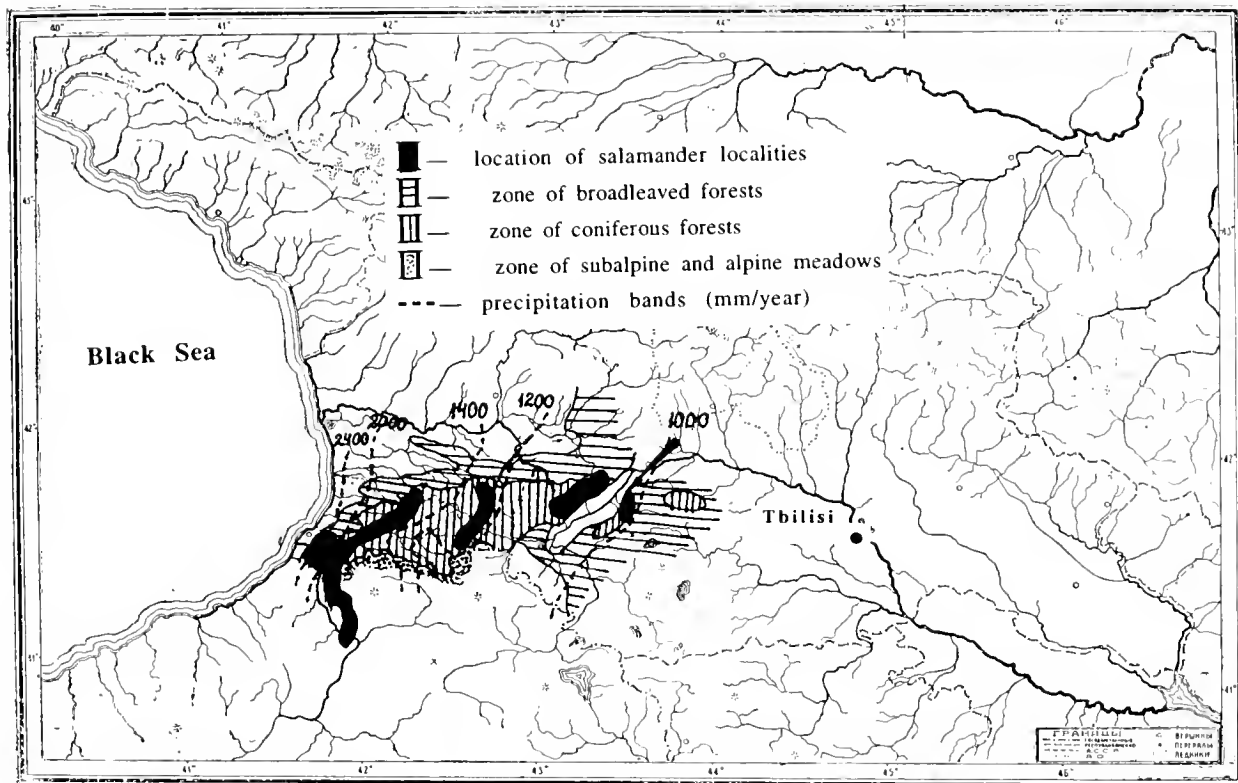


FIG. 1. Distribution of *Mertensiella caucasica*.

distributed in external spurs of the Trialetian Mountain Range. Probably it is the result of historical changes in the Kura River bed (Fig. 1). Populations are mainly distributed in the forest belt, but in some places they can be found close to subalpine meadows. Humidity in the species' locations reaches 1000 mm or more per year (another narrow-ranged representative of the salamander tribe, *Chioglossa lusitanica*, has similar requirements of humidity).

In the most dry part of the range of *M. caucasica*, the eastern one, salamanders live only in coniferous forest. When humidity reaches 1200 mm/year in the middle area, they can also be found in subalpine meadows. Salamanders are distributed in deciduous forest only close to the Black Sea coast, where humidity is very high (2000-2400 mm/year; Fig. 1). The high dependence of the animal on humidity does not itself limit the species distribution, but determines sensitivity of specimens to other environmental factors. It is very interesting that the rheophilous species *Ranodon*

*sibiricus*, more restricted to water habitats than *M. caucasica*, is geographical limited by coniferous forests like *M. caucasica* in eastern localities (Paraskiv, 1953). Local populations, distributed along tributaries of the Chorokh and Kura rivers (in upper flow), are formed by salamanders within its area. Width of streams in salamander plots is not more than 1-1.5 m in spring and because of stepped disposition of streams, they run slowly in some places. There are many slowly draining pools about 20-30 cm in depth with a lot of shelters. The bottoms of streams and pools are covered with stones, and there is a lot of non-decayed organic matter. Stepped disposition of streams is formed by stoned conglomerations and fallen logs. Apparently, mountain ranges between stream canyons don't allow wide salamander migration and local populations are comparatively isolated. There is no evidence that direct migrations of animals occurs during their life cycle. Individuals are found a maximum of 200-300 m distance from streams.

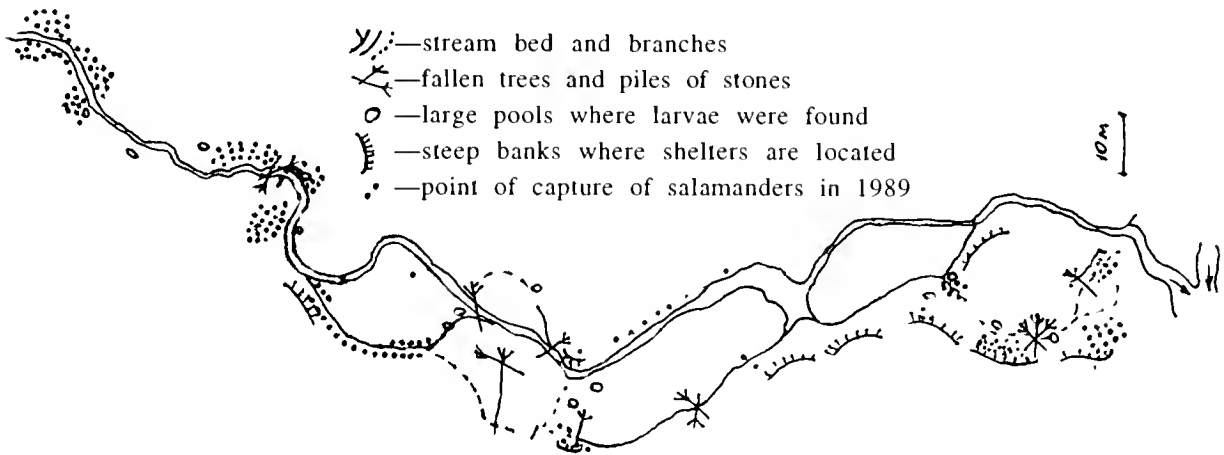


FIG. 2. Schematic diagram of the study site for *Mertensiella caucasica*.

### The Study Area

The studied population inhabits coniferous forest ecosystems along the second range tributary of the Kura River in Borjomi Canyon (eastern part of the species' range) (Fig. 1). The plant association is formed by *Taxus baccate*, *Picea orientalis* and deciduous spots. The size of the inhabited location is a bit more than 200 m, and it is situated between 1000 and 1300 m altitude, about 2 km from the stream mouth. Slopes are precipitous, built by corrosion of underground tree roots or relatively gradual, partially covered by pteridium, *Matteuccia struthiopteris*, from the adjoining stream banks. There are some stone conglomerations and fallen trees in the study area, shown on the map (Fig. 2). Air temperature is close to stream water temperature (13-15°C in summer) in shelters formed by stones and logs. Dynamics of air temperature in Borjomi Canyon in May to July, 1989 is shown in Fig. 3. Quiet pools and shelters are relative rare, slopes are steeper and stream flow is faster at upper and lower localities. Density of salamanders here falls rapidly as well as away from the stream banks in these places.

### Methods

The main quantitative data were obtained during excursions with a lantern after sunset along the study area. The location

of each adult animal was mapped, substrate type and distance from stream bank (more or less than 50 cm) was recorded. Adult animals were marked individually by toe-clipping. Combinations from clipped digits in hind-limbs (not more than 2 in 1 foot) responds to individual number of animals from 1 to 99. Zero-1 clipped digit in the front leg mean number of hundred. Marks of salamanders recaptured in the next year were renewed. Data of capture-recapture were statistically counted as in Kaughley (1977). Substrates of animals caught were subdivided in 6 types: shallow water; sand and pebbles above water shore; wet stones; wet ground; moss or lichens; dry ground and stones. These types were ranked according to their humidity. Basic investigations were conducted on 8-10 and 21-23 June, 1986, 24-28 June, and 5-7. August, 1987, 3-5 and 21-24 July, 1988, 16 June-12 July, 1989, 2-9 July, 1990.

We had 337 contacts with males and 202 with females (including specimens found two or more time). Recording of larvae was conducted during night excursions.

We caught females from nature in the reproductive period and obtained eggs using a hormonal stimulation method (Gontcharov et al., 1989) to study some ecological and morphological features of early development. Eggs were incubated in Weiss bowls in dechlorinated water at a temperature of 14°C as well as in aquaria at

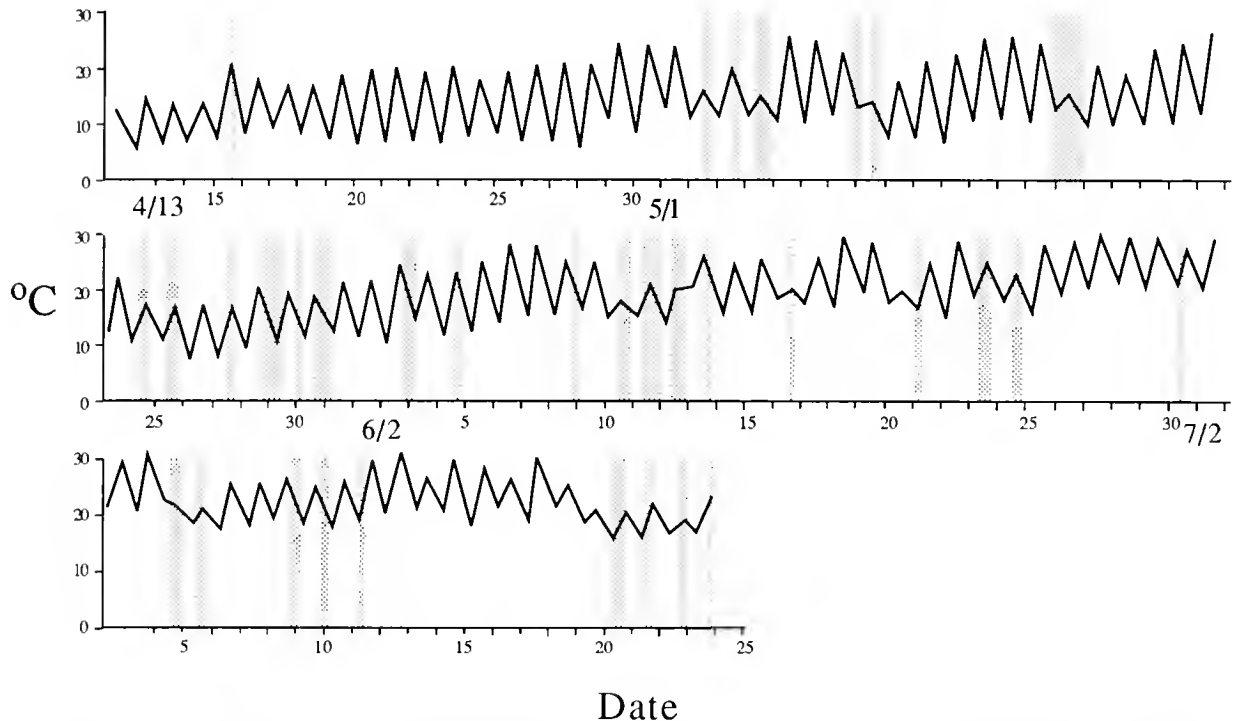


FIG. 3. Air temperature at the *Mertensiella caucasica* study site during the period of reproductive activity. Stippled bars represent periods of rainfall.

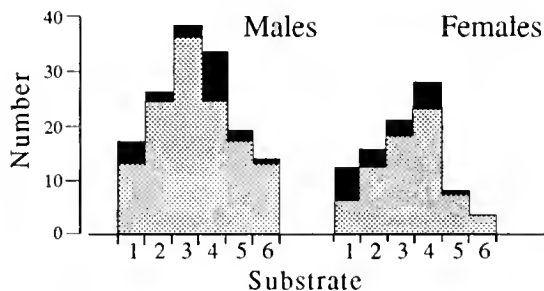


FIG. 4. Location of salamanders by substrate type. 1- shallow water; 2- wet sand and pebbles; 3- wet stones; 4- moist ground; 5- moss or lichens; 6- dry stones. Solid bars represent males. Stippled bars represent females.

a temperature varying from 5-22°C. Before completion of metamorphosis, larvae were kept in 20 liter aquaria, where water was changed every third day. Food consisted of crustaceans (*Daphnia*, *Cyclops*), *Tubifex* and Chironomid larvae.

Morphological studies were conducted on larvae, juvenile and adult animals with a binocular magnifies and calipers. Their snout-vent length (L), head length (Lc), and tail length (Lcd) were measured with a

precision of 0.1 mm. The coloration patterns of some animals was also recorded.

## Results

### *The niche of larvae and adult specimens.*

Salamanders don't have an even distribution within the study site. Preference to every substrate depends on the amount of moisture of each particular substrate. Frequency of captures decreases with distance from water or potential shelters. The number of animals captured out of shelters depends on time and season. Most adult specimens were recorded close to the stream (less than 50 cm from the water shore):  $60 \pm 4\%$  of males,  $62 \pm 5\%$  of females. In contrast to data on the ecologically similar species, *Chioglossa lusitanica* (Arntzen, 1981), there is no difference between male and female attachment to water in *M. caucasica*. Animals commonly may be found on wet sand or stones at the water shore, and they avoid dry soil and stones. The distribution of substrate type of captured animals for the

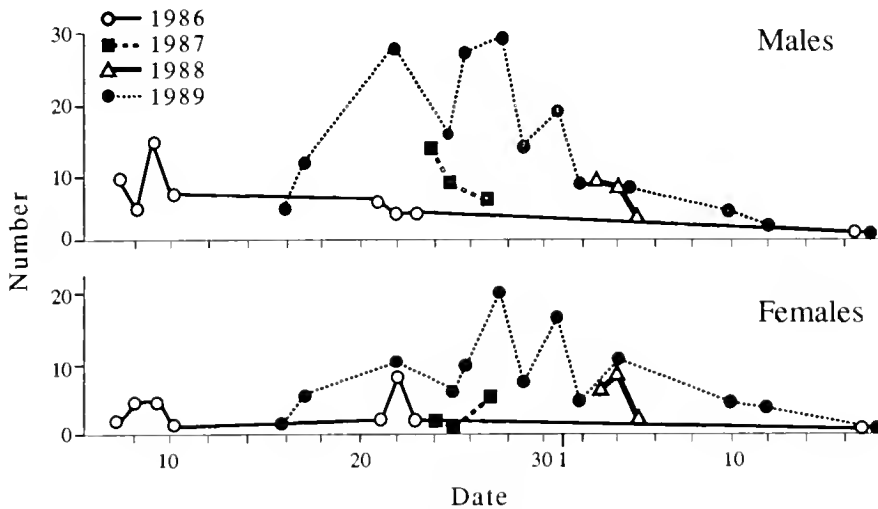


FIG. 5. The seasonal dynamics of salamander occurrence.

period from 1988-1989 is presented in Fig. 4. It is obvious that females avoid open rocky plots, fallen leaves or moss cover. According to our observations, adult salamanders spend only a small part of their life on the surface of the ground. Even during the active period, only a small part of the population leaves their shelters at night. Apparently, salamanders spent the rest of the time in shelters, where they live and feed. Seasonal dynamics and diurnal activity are reported on a number of captures during excursions in a 4 year period (Fig. 5). The number of animals found above ground decreased in July. The decrease in capture was especially sharp in the 5-15 July period. Even after rains, individuals could hardly be found in the second half of July (Fig. 5). Earlier seasonal activity of males is noted for other flowing-water (Arntzen, 1981) and standing-water (Golubev, 1981; Beneski et al., 1986) tailed amphibians. According to phenological data the large number of salamanders found in June is connected with their reproductive activity.

The beginning of the reproductive period is determined by air and water temperature. The active season begins when minimal night temperature is about 15°C (Fig. 3). After mating and egg deposition, activity decreases. Apparently, the decreasing number of specimens found in July does not depend on regular migrations. Observations of adjacent parts of the stream

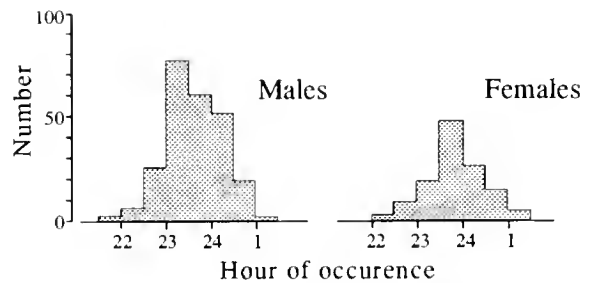


FIG. 6. Number of salamanders observed by hour of day.

did not have any result in late July and August. The decrease in animal numbers either depends on the dispersion of individuals in the forest (or along stream banks, as proposed by Arntzen, 1981, for *C. lusitanica*), or more probably, they are in shelters most of the time because of greater abundance of their food, such as Gammaridae and Lumbricidae, there.

The main nocturnal period of activity of *M. caucasica* is between 2200 and 0100. No active salamander has been found before 2130, and active animals have been rather rare before 2230. The number of active animals decreased after 0130-0200. The peak of activity was observed at about 2300 (Fig. 6).

Other nocturnal tailed amphibians also have a short active period and the time of activity is species specific (Semlitch and Peachmann, 1985). In the summer,

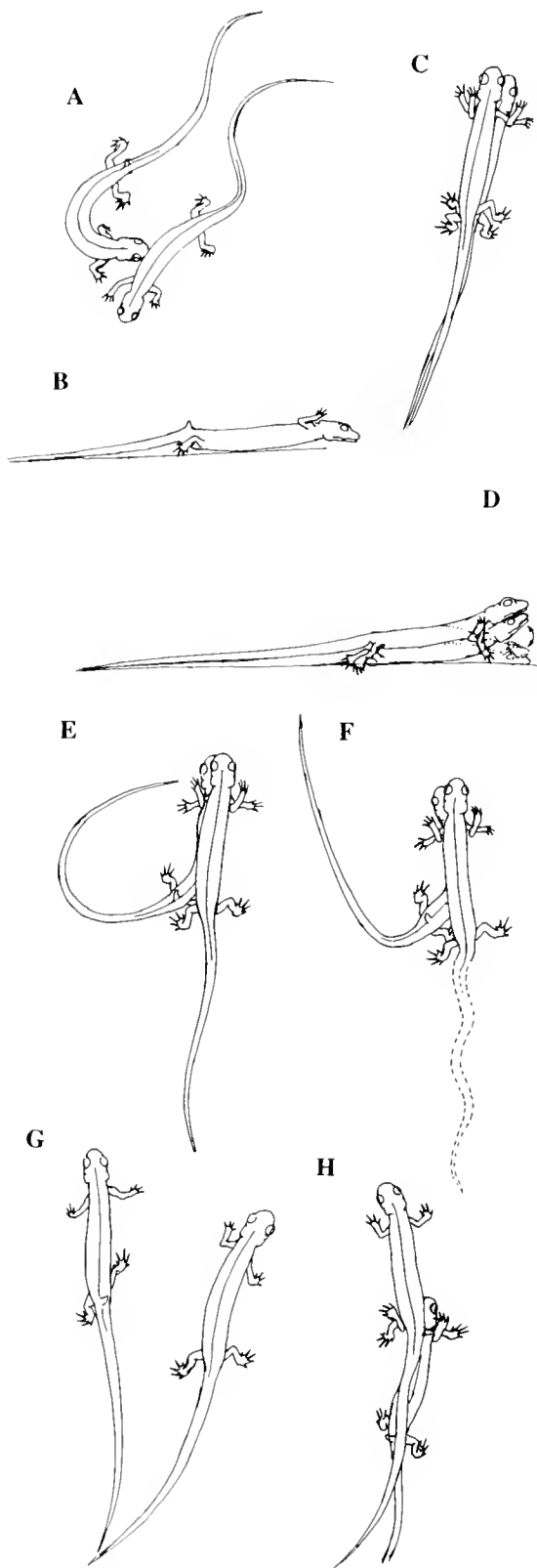


FIG. 7. Consecutive stages of courtship and amplexus in *Mertensiella caucasica*.

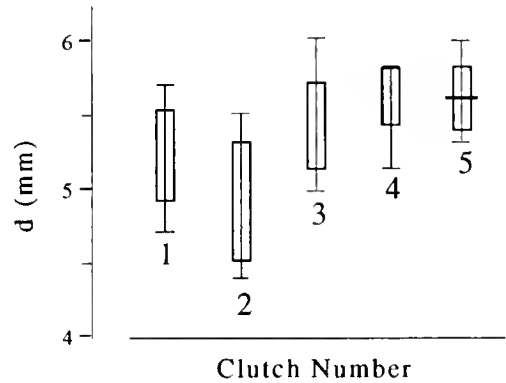


FIG. 8. Inter and intra-clutch variability in fertilized eggs of *Mertensiella caucasica*.

salamander larvae can be found in some parts of the stream bed in small pools with slowly drying water. Outside of the shelters, they are mainly in shallow water with a depth of less than 5 cm. Generally, the number of larvae doesn't exceed 10 individuals (maximum 14) in a pool. Comparatively large premetamorphosed larvae can be found in the stream. They move actively along the stream. Like adults, larvae spend most of the time in shelters. The first individuals can be seen in open water in late May. Larvae leave their shelters at twilight, when absolute sunlight is about 10 Lk. There are no nitrates in the water composition in salamander breeding and developing sites. The pH reaches 7.8-8.3 and the hardness of the water is 0.6-2.8 mg/equivalent l. Juveniles rarely leave their shelters during the period from the end of metamorphosis until first breeding.

The diet of larvae, juveniles, and adults reflects their biotopical preferences and does not show specialization in any invertebrate group (Kuzmin, 1992). Both terrestrial and aquatic organisms are found in the diet of adult salamanders (Ekvtimishvili, 1948; Kuzmin, 1992). Only terrestrial organisms are found in the diet of juveniles (Kuzmin, 1992).

#### *Space used by salamanders.*

The requirements of salamanders to environmental characters limit the space they use even within a local habitat. Thus, salamander distribution isn't homogeneous



in a given locality. Obviously, animals prefer places with plenty of shelters. The mean number of salamanders captured during night excursions per 10 m along the stream bank in 1986, 1987, and 1989 was  $9.2 \pm 1.86$  males and  $5.26 \pm 1.13$  females. According to the "mean crowding" index of Lloyd (1967) ( $m = \bar{m} + \frac{\sigma^2}{\bar{m}} - 1$ ) the mean value is respectively  $21.84$  and  $12.78$ . The highest density was observed in places where there were logs and wooden blocks, combined with stone conglomerations, and a lot of small pools and shelters under tree roots. The total number of adults recorded for the 1986-1990 period, including recaptures was 455. The place where each specimen was found in 1989 is noted in Fig. 2. This data could give a notion of real space distribution of animals. Note that larvae can be found outside of the local population habitat significantly more often than adults. This is connected with the fact that some of the larvae leave the shelters and continue development in the lower parts of the stream (see below). Apparently, this process does not disturb normal metamorphosis and juvenile animals return to the population locality.

### Life Cycle.

The salamander breeding period in investigated habitats occurs from the second half of June until early July. Most of the females found in July are ready for egg deposition. The large oocytes can be observed through the transparent ventral skin. There are well distinguished mating corns at the adult male shoulders. Amplexing animals were found on the ground close to shelters. Cyren (1911) and Obst and Rotter (1962) described normal sexual behavior of salamanders in water in natural and laboratory conditions. We observed normal sexual behavior twice: on 28 June, 1988 and 4 July, 1990. In the first case it took place about 2 m from the stream bank in a conglomeration of tree roots. In the second case it happened close to water, at the entrance of a rock chink. We don't exclude the possibility of normal copulation in water. For example, mating of *C. lusitanica* may take place both in streams and on the shore. The consequent

states of courtship and amplexus are shown in Fig. 7, b-f. According to our observations, sexual behavior of *M. caucasica* is similar to that of *Salamandra salamandra* (Joly, 1966). The corn on the dorsal side of male tails has no special role in courtship and amplexus. We have also observed an attempt of copulation (Fig. 7h) on 28 June, 1989 and 2 males in an amplexus pose on 8 June, 1986. Apparently, we have observed, in the latter case, rival combats, described for *S. salamandra* by Kästle (1986), but in this work the behavioral display is not the same.

Copulated females have a slightly opened cloaca. There are more than three days between copulation and egg deposition. Each female deposits from 11 to 24 eggs ( $N=9$ ,  $M=16.9$ ,  $\sigma=3.9$ ). Inter- and intra-clutch variability of fertilized eggs sizes is shown in Fig. 8. Darevsky and Polozhikhina (1966) found that the sizes of 90 eggs found in nature ranged from 5.0-5.6 mm. Females deposit separate eggs, sticking them to the substrate in shaded places. Activity of animals gradually decreases after the completion of the reproductive period.

Egg development takes 45 days until hatching in aquaria, where the average temperature is  $14.8^\circ\text{C}$ . When the temperature changes from  $6^\circ$  to  $26^\circ$  ( $M=16.5$ ,  $\sigma=4.7$ ) development is extended to about 48-51 days. We can expect similar developmental rates in nature, when the temperature of the water is about  $14-15^\circ$  in July to August. The hatching of most part of the generation takes place not earlier than late August. Larvae found in June can be divided into 3 groups on the basis of snout-vent length: the I group-  $L=14.6-19.5$  mm ( $M=106-180$  mg); the II group-  $L=23.7-27.5$  mm ( $M=330-664$  mg); the III group-  $L=29.0-35.4$  mm ( $M=632-1400$  mg). By virtue of larval size distribution, Freytag (1954) as well as Koroljov (1986) concluded a 3 year period of larval development in the Caucasian Salamander. Kuzmin (1992) established no annual ring in the I and II larvae group hip bones and only one annual ring in the III group larvae

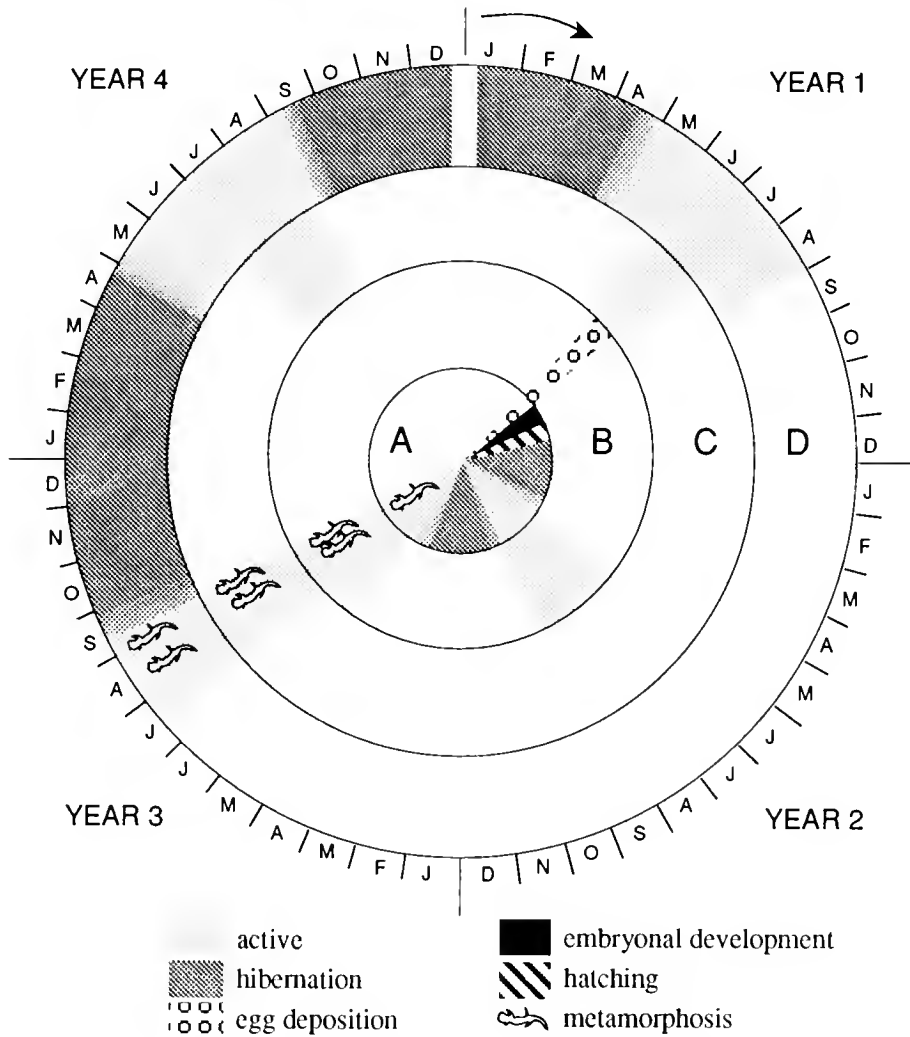


FIG. 9. The reproductive cycle of *Mertensiella caucasica*. A- underground parts of the stream bed; B- open water; C- surface and temporary shelters on the ground; D- constant shelters on the ground.

hip bones. On the basis of this information Kuzmin (1992) supposed that larvae of I and II size groups had most probably hatched in the given year. Nevertheless, analysis of time of reproduction and embryonic development during the year opposes Kuzmin's opinion.

Apparently, salamander larvae remain in shelters after hatching and go out when water temperature increases at least to 13°C (at the beginning of the next summer). Lack of annual ring on hip bones can be explained by incomplete development of hind legs just after the hatch. Larvae growth is delayed by autumn temperature decreasing. Thus, animals of the I and II size groups have a previous year hatch and

represent a single generation.

Size differences within a generation are formed by prolonged breeding time (not only between breeding locations [Mertens, 1968], but within populations, too) and/or by variation of individual growth rates. Larvae have developed during the warm period of the second year. After hibernation, they have a metamorphosis in July-August of the 3rd year. Their development from fertilized egg to completed metamorphosis takes about two years in nature.

Salamanders have a concealed life during the period after metamorphosis and before maturity. According to Kuzmin (1992), 3-

5 annual rings can be observed in hip bones of adults. Therefore, salamanders can first breed in the 3rd year after metamorphosis. The total life cycle of the Caucasian Salamander from egg to egg is about 4 years (Fig. 9).

### Growth and Development

Embryogenesis of *M. caucasica* is similar to other large-size egg amphibian development (Fig. 10 a-c). Analyzing experimental observations, hatching takes place when total length is 17-20 mm ( $L=10.5-11.5$  mm). When hatching starts, a larvae has well developed external gills and a tail fin. Sometimes the rudiments of 3 toes can be distinguished on the hind legs. Pigmentation is formed by a couple of faded pigmented stripes. There are rare individual melanophores on the surface of the stripes. A line of small circular non-pigmented patches lays along each stripe (Fig. 10 d). The gut is filled with yolk. The total length of the smallest larva caught in nature was at least 25 mm (commonly,  $L=15$  mm, minimum= $14.6$  mm). Larvae found in nature already have no yolk in the gut. Their external gills are smaller than those of animals that have not yet hatched. There are 4-5 toes on the hind legs and more pigment cells (Fig. 10 e).

In laboratory conditions, when temperature is  $14.8^{\circ}\text{C}$ , yolk disappears from the frontal part of the gut 16 days after hatching at a length of  $13.8114$  mm. The first larvae with a snout-vent length of  $15-16$  mm can be found in streams in early June, but most of them appear in July. The small larvae, which have over wintered, appear in stream pools in small, probably sibling groups. The largest group (8 larvae) was found on July 5, 1988. Individual sizes in that group provides some information about intra-clutch larval size variation: when  $L=17.35\pm 0.48$ ,  $\text{min}=16.0$ ,  $\text{max}=19.2$ , coefficient of variation approaches  $7.8$ ,  $L_{\text{total}}=29.25\pm 0.60$ ,  $\text{min}=27$ ,  $\text{max}=31.3$ ,  $\text{CV}=5.8\%$ . Summer growth of first hibernated animals (in 1985) is shown in the histograms of June and August larval size distribution (Fig. 11). Mean total

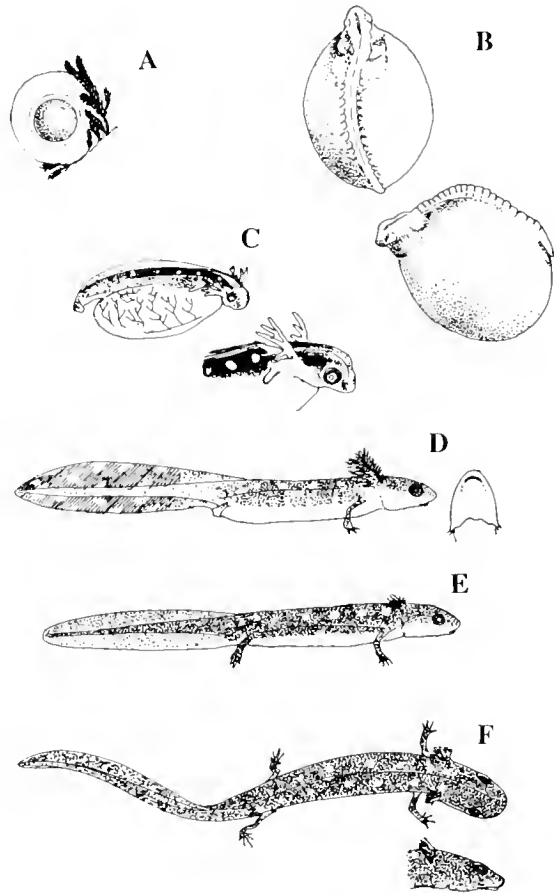


FIG. 10. Embryo and larval development of *Mertensiella caucasica*.

length increases  $6.27$  mm for 70 days and the specific growth approaches  $0.24\%/day$ . At the same time homogeneity of generation increases:  $\text{CV}=23\%$  in June and becomes  $9.9\%$  in August. This process is probably caused by more rapid growth and/or comparatively high mortality of the small larvae.

Morphological changes, connected with metamorphosis (i.e. yellow coloration of unpigmented spots, decreasing of gill size, reduction of tail fin- Fig. 10 f) began in animals with at least  $30$  mm snout-vent length. They approach that size in the 3rd year of larval development. Comparing sizes of the 2nd and 3rd year larvae, the specific body growth rate is  $0.12\%/day$  in the period between August and June of the next year. Commonly, metamorphosis takes place at a snout-vent length of  $30-35$

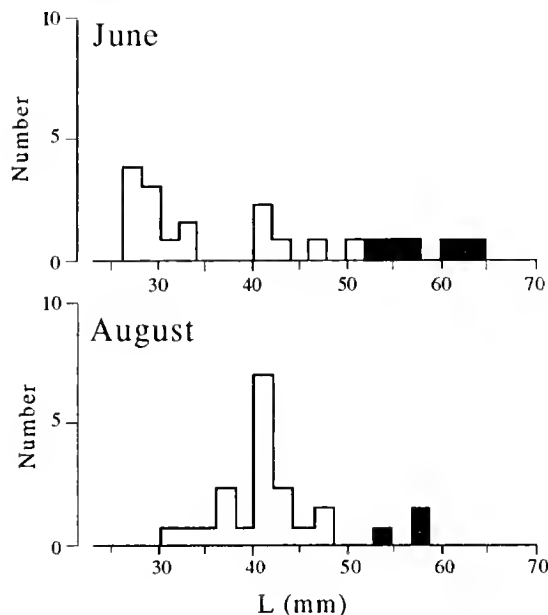


FIG. 11. Size distribution of *Mertensiella caucasica* larvae at the study site in 1985.

mm. Maximal larval size approaches 35.4 mm (L total=70.3 mm) in the population studied. Snout-vent length of metamorphosed animals varies within the limits of 32.9-42.4 mm (body mass, M=694-1592 mg, N=11). At the same time 2 larvae, with L=43.8 and 44.6 mm, are in a collection from the surroundings of Batumi (Kuzmin, pers. comm.). Animals of all three size groups can be found in streams even in May (Korolyov, 1986).

Peculiarities of natural growth of *M. caucasica* are similar to *C. lusitanica*. Although this species passes metamorphosis at smaller sizes (i.e. L=24-25 mm) their linear growth for two summer months approaches 0.29%/day and 0.10%/day for the rest of the year (Arntzen, 1981) and it is very similar to the analogous index of *M. caucasica*. The slow growth of salamander larvae is mainly the result of low water temperature in streams. The specific total length growth rate of a single animal (from 27.9 to 56.7 mm) was 0.54%/day, and total length increased from 37.0 to 59.2 mm was 0.42%/day under laboratory conditions, at 23-25°.

The snout-vent length of adults varies insignificantly. Data on animals measured

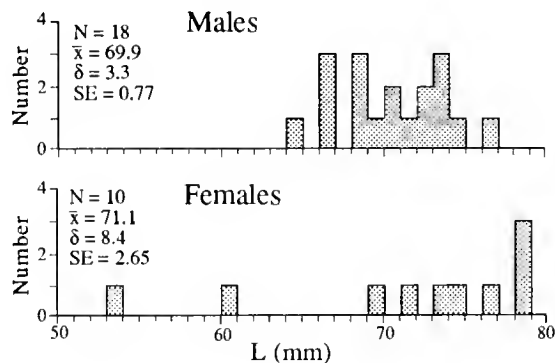


FIG. 12. Size distribution of adult *Mertensiella caucasica* in the study area. L- snout-vent length.

in the studied population are shown in Fig. 12. There aren't considerable intersexual differences in sizes and general body proportions, but apparently females can begin breeding at a smaller body size. Other authors (Cyren, 1911; Knoblauch, 1905; Nesterov, 1911) reported that mean L in males approached 68.9 mm (N=7) and females, 63.5 mm (N=11). The animal body length of an outlying population (Goderdzi Mountain Pass) varies between 68-77 mm in males and 56-73 mm in females. However, our population does not show any specifics in adult animal size distribution.

We will briefly discuss morphological changes in the period from the beginning of active feeding to the end of metamorphosis. When larvae begin to feed, melanophores gradually disperse from the lateral sides, filling the ventral surface of the larval body. Even the size II larval group have only a narrow non-pigmented stripe remaining on the ventral side. All lower surface is filled by pigmented cells and non-pigmented patches remain only on the lateral sides of the size III larval group. These patches are used as a substrate of xanthophores and iridiophors, forming yellow spots later on (Tarkhnishvili and Tartarashvili, 1987). The intensity of basic coloration is correlated with the size of the animal that has already started metamorphosis. The animals with a large size at the beginning of metamorphosis have a dark-brown (not as dark as in spotted salamander) coloration with bright and comparatively large yellow spots. Smaller size larvae do not have such

an intense basic coloration and spot pattern is more or less reduced (spots are smaller and/or poorly expressed). The intensity of salamander pigmentation, like other tailed amphibians, may vary depending on the light intensity at the larval location (Fernandez and Collins, 1988).

Ground coloration of adults varies from reddish-brown (similar to *Chioglossa lusitanica* or some *M. luschani* subspecies (Winter et al., 1987) to dark brown. The spotted pattern may be expressed in a different degree to full reduction (especially in light colored specimens) (Fig. 13). Poorly pigmented animals with comparatively reduced spots (described by Tartarashvili and Bakradze (1989) as the subspecies *M. c. djanashvilii*- Fig. 13 a) predominate in some populations from the surroundings of Batumi, at the Black Sea coast. Nevertheless, dark colored animals, with well developed spots (Fig. 13 e, f) predominate in the population from the subalpic zone (Mountain Pass Goderdzi, in Bakradze's collection). Salamanders with an intermediate intensity of coloration are more abundant in our studied population, but there are some specimens with less or more reduced spot pattern. There is also a female, colored as the form described by Tartarashvili and Bakradze. Probable, the specific coloration of adults is connected with the character of larval development, which depends on the special climatic conditions of each habitat. That is why many light-colored animals occur in the warm sea coast habitat and dark colored ones are found at high altitudes. Populations from Borjomi Canyon are in an intermediate place. Of course, we don't exclude the possibility of inheritable fixing of one or another coloration type in different populations.

The Caucasian salamander is included with the Luschan Salamander, *M. luschani*, in the same genus because of the tail corn, the secondary sexual character of males (Özeti, 1967). This character appears in males with a length of at least 130 mm and it seems to be of no functional importance as some investigators have proposed, for example Cyren, (1911).

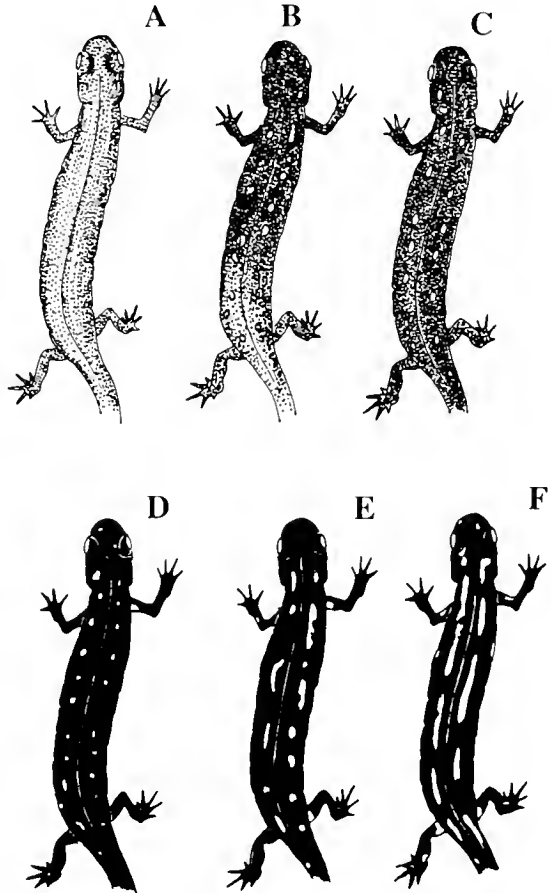


FIG. 13. Color variation in *Mertensiella caucasica*.

The main changes of general body proportion occur during ontogenetic development. First, relative tail length increases after hatch. Mertens (1968) reported this for larvae with a total length of more than 45 mm. Based on our data, comparatively rapid tail growth begins at the earliest stages of development and extends to the adult stage. On the other hand, comparative length of head decreases (Fig. 14). The changes of general proportions have different intensity in different developmental stages. Allometrical dependence of head and body length on the total length of the I and II size group larvae is described by equations:

$$Lcd=0.35L^{1.26}$$

$$Lv=1.71L^{0.26}$$

The coefficients of allometric equations for the III size group larvae is different:

$$Lcd=0.08L^{1.71}$$

$$Lc=1.5L^{0.52}$$

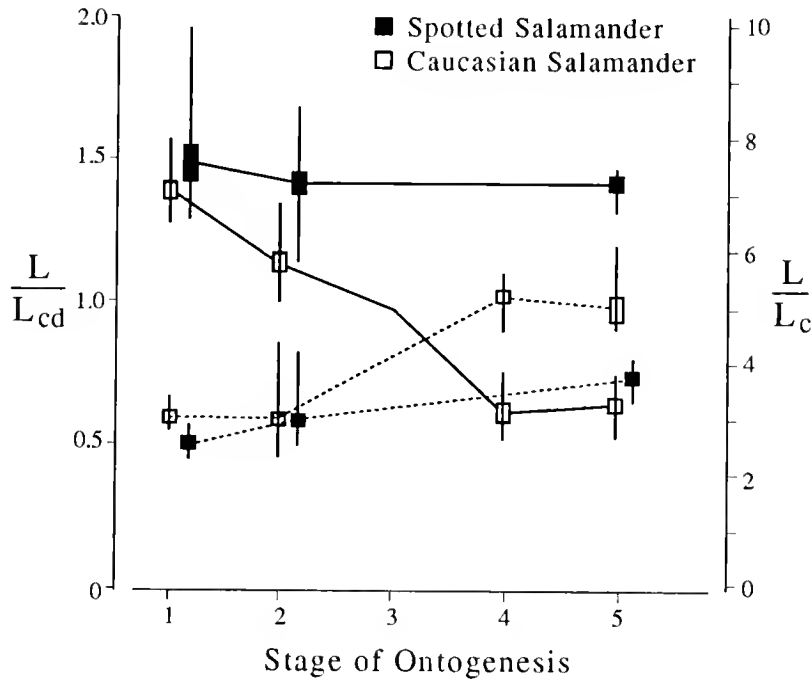


Fig. 14. Changes in body length proportions in *Mertensiella caucasica* (open square) and *Salamandra salamandra* (solid square) during ontogenesis. 1- larvae with total length less than 35 mm; 2- larvae with total length greater than 35 mm; 3- yearlings; 4- juveniles; 5- adults. solid line-  $L/L_{cd}$ ; broken line-  $L/L_c$ .

The coefficients of static allometrical equations for recently metamorphosed animals are:

$$L_{cd}=0.23L^{1.42}$$

$$L_c=1.04L^{0.62}$$

Hence, the most rapid comparative increasing of tail length is during late larval development. The comparative decreasing of head is the most rapid in the I and II size groups, but later this process isn't so clear. Nevertheless, it takes part in metamorphosis.

Plenty of eco-morphological features separate *M. caucasica* from the other representatives of European salamander tribe, depended on subtilization (Özeti, 1967). The latter is a base determined ecological similarity between *M. caucasica* and *C. lusitanica* (Borja-Sanchiz and Mlinarsky, 1979). Perhaps this is a reason of similar breeding ways of these species differing from other European salamanders. The changes of general proportion of *M. caucasica* and *S. salamandra*, which are shown in Fig. 14, allow a comparison of these species. The general trends of body

proportion changes are common in the two species (as in most Tetrapoda): the comparative length of head ( $L/L_c$ ) decreases and that of tail ( $L/L_{cd}$ ) increases. But in these tendencies, both are quite rapid in *M. caucasica*. In *S. salamandra* the changes are gradual and moreover, tail length growth is poorly distinguished (Fig. 14).

#### Population Number Dynamics and Regulation.

The analysis of our 1986-1990 capture-recapture data allow us to study population number, number dynamics and demographic peculiarities. We obtained quite full information in 1989. In that year the captured animal number, in relation to real population number, was comparatively high. The general picture of results is shown in Table 1. We had 532 contacts with animals. We met an animal twice in the same night only on 7 occasions. They were on the surface, essentially not moving. In all cases animals were captured again in the same plot, not more than an hour later. The 50 animals of 68 recaptured

TABLE 1. Mark-recapture results for *Mertensiella caucasica* in 1989.

Date	i	n <sub>i</sub>	R <sub>i</sub>	Males											
6-16	1	5	5	1											
6-17	2	11	11	0	II										
6-22	3	27	26	0	1	III									
6-25	4	15	7	0	1	1	IV								
6-26	5	26	29	0	2	5	0	V	x <sub>ij</sub>						
6-28	6	28	28	2	2	1	2	2	VI						
6-29	7	13	13	1	0	1	0	0	0	VII					
7-1	8	18	18	0	1	2	0	1	1	0	VIII				
7-2	9	8	8	0	0	2	0	1	1	0	0	IX			
7-4	10	7	7	1	1	1	0	0	0	0	0	1	X		
7-10	11	4	4	0	0	0	0	1	0	0	0	0	1	XI	
7-12	12	2	2	0	0	1	0	1	0	0	0	0	0	0	
r <sub>i</sub>				4	8	14	2	6	2	0	0	1	1	0	
N <sub>i</sub>								579	619	349	681			104	44
SE <sub>i</sub>								151	376	135	474			97	25
P <sub>i,i+1</sub>								1	1	1	0.22			0.45	
									(P <sub>6,9</sub> )						
A <sub>i,i+1</sub>								70							
							(A <sub>3,5</sub> )								

Date	i	n <sub>i</sub>	R <sub>i</sub>	Females											
6-16	1			I											
6-17	2			0	II										
6-22	3			0	0	III									
6-25	4			0	1	0	IV								
6-26	5			0	0	0	0	V	x <sub>ij</sub>						
6-28	6			1	1	0	1	0	VI						
6-29	7			0	0	0	0	1	0	VII					
7-1	8			0	1	0	0	2	0	0	VIII				
7-2	9			0	0	0	0	0	1	0	0	IX			
7-4	10			1	0	0	0	1	2	0	0	0	X		
7-10	11			0	0	0	0	0	0	1	0	0	0	XI	
7-12	12			0	0	0	0	2	0	0	0	0	0	0	
r <sub>i</sub>				2	3	0	1	6	3	1	0	0	0	0	
N <sub>i</sub>								78	340	497					
SE <sub>i</sub>								66	200	476			97	25	
P <sub>i,i+1</sub>								1	1						
A <sub>i,i+1</sub>								70	265	157					
							(A <sub>3,5</sub> ) (A <sub>4,6</sub> )								

Note: i, j- the number of census; n<sub>i</sub>- the number of specimens examined in i-th census; R<sub>i</sub>- the number of recaptured and escaped specimens; x<sub>4, III</sub>- the number of animals recaptured in the 4-th census that had been marked during the 3-rd census; r<sub>i</sub>- the total number of recaptured specimens that had been marked in the i-th census; N<sub>i</sub>- the estimate of population size in the moment i, using the Jolly-Seber method; SE<sub>i</sub>- standard error (Jolly-Seber); P<sub>i, j</sub>- the probability of the individual remaining in the active part of the population between the i-th and j-th census; A<sub>i, j</sub>- the number of specimens supplemented the active part of the population between the i-th and j-th census.

in a year of marking were caught in the same or adjacent plot (the place of 28 captured animals was not recorded). On the basis of these data, we conclude that these salamanders have a low moving

ability and a short period of nocturnal activity. Only an approximate representation of activity dynamics can be given by captured animal number, though in some cases, data of captured animals are

used as an index of number (Bozhanski and Semenov, 1982). We used the Jolly-Seber method (see Caughley, 1977) to estimate the real number of the local population and its dynamics during the breeding period in 1989. The total number of breeding animals was 1187 and the percentage of males was  $58 \pm 0.001$ . The greatest number of active animals was in the end of June. Females appeared a bit later than males.

Unfortunately, the data of 1986-1988 don't allow us to correctly estimate the number of salamanders in those years. It varied from 10-20 to 460 individuals, when the errors exceeded mean values, i.e. significantly lower than real quantity. The highest value (460) was recorded when the Schumacher method was used on 1990 data. The values recorded for the 1986-1988 data didn't exceed 200. Thus, the annual number value significantly increases when the research period is prolonged and captured specimen number increases. That is because only a small part of the population left their shelters, even in the highest activity period in late June to early July.

The estimate of the *C. lusitanica* population (Arntzen, 1981) is different because of the permanent migration of part of the population. The number of two local populations of this species is 1236 and 1324 respectively. This is similar to our information about *M. caucasica*, moreover, the study sites have a size similar to ours. Usually, the number of animals in the widely distributed in Europe *S. salamandra* populations can exceed some thousand specimens (Klewen, 1986). Their populations are spread over several hectares, and animals can be found far from the breeding sites.

The capture of salamanders marked in previous years gives some information about mortality rates of adults. Ninety eight males and 45 females were marked in June, 1989. There were 38.5% (OM=7.8%) recaptured males of the total of 39 found in July 1989, and 28.9% (OM=7.3%) recaptured females of the 38 total found. The 31.8% (OM=5.7%) of

males and 17% (OM=5.9%) of females captured in July 1990 were marked in June 1989. Hence, survival rates of the period from July, 1989 to July, 1990 is  $P_m = 31.8/38.5 = 0.83$  for males and  $P_m = 17/28.9 = 0.59$  for females. The part of all marked adults was  $33.8 \pm 9.3\%$  in July, 1989 and  $26.2 \pm 4.2\%$  in July, 1990. Hence, the annual survival approached 0.77. It should be noted that only 8 individuals ( $9.0 \pm 2.0\%$ ) of all 89 marked in 1986-1988 were found again in 1989. This is quite a high number because 89 individuals aren't more that 10% of the adult population.

Unfortunately, there is a lack of information about renewal rates of tailed amphibian populations. Ignoring age structure, the annual mean survival of *Ambystoma maculatum* approaches 0.72 for males and 0.60 for females (the mean male number is 641 and capturing of males is a bit more often (Husting, 1965). These data are quite similar to ours. On the other hand, annual survival of the Smooth Newt is only 0.45 for males and 0.55 for females in England (Bell, 1977). There are considerable low survival rates of *Notophtalmus viridescens* and some Anura when higher mortality of males is observed (Ischenko, 1989). Klewen's (1986) quantitative data for *S. salamandra* show that annual survival varies between 0.55-0.81 (mean 0.66 in four years of investigation. A higher female survival was recorded for the genus *Desmognathus* (Husting, 1965).

The Caucasian Salamander has a comparatively low population renewal rate, when mortality is low. Perhaps, this kind of population dynamics is typical for populations with low total number and high male survival. Organ (see Husting, 1965) mentioned that a higher male survival was a result of significant energy expenses of females during breeding.

We can only indirectly estimate salamander mortality before mating. On the basis of adult female number and mean fecundity, we estimate that there were 8000-9000 eggs deposited in the study site.



TABLE 2. Data on mark-recapture of *Mertensiella caucasica* from 1986 to 1988.

Sex	Data	1986							1987			1988							
		6-7	6-8	6-9	6-10	6-21	6-22	6-23	6-24	6-25	6-27	7-3	7-4	7-5	7-22				
f	n <sub>1</sub>	9	5	14	7	6	4	4	49	13	8	6	27	6	7	2	1	16	95
	R <sub>1</sub>	9	2	8	7	5	2	3	36	10	8	4	23	5	4	1	1	11	70
	m <sub>1</sub>	0	1	2	0	0	1	1	5	0	0	0	0	0	1	0	0	1	6
m	n <sub>1</sub>	2	5	5	2	2	8	2	28	2	0	5	7	6	8	1	0	15	48
	R <sub>1</sub>	2	4	4	1	1	4	2	18	0	0	0	0	0	1	1	1	3	20
	m <sub>1</sub>	0	1	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0	3

Note: n<sub>i</sub>- number of individuals examined in the i-th census; R<sub>i</sub>- number of recaptured and escaped individuals; m<sub>i</sub>- total number of individuals captured in the i-th census that were marked in the same year (but on another day).

Nevertheless, significantly few larvae could be found in the stream when salamander density was the highest.

Korolyov (1986) found only 90 larvae in 1984 in the whole area of the stream described here. Bozhansky and Semenov (1982) counted 1-33 larvae in every 700 m of flow in August. We found 116 larvae in the study site in June, 1985, of which 91 had already gone through hibernation. In the beginning of July, 1990, 74 larvae of the I and II size groups (hibernated once) were counted in the same plot. The number of second year larvae is quite constant in different years. Although it was considerably lower than the total number of eggs deposited, it did not have an influence on the real larval mortality rates in the first year. More probably, larvae were carried by flow along the stream and were distributed more uniformly than adults and eggs.

On the base of the 2nd and 3rd year larvae proportion at the site, we were able to judge larval mortality from egg deposition to the second year. The twice hibernated were 27.8% (1985) to 31.9% (1990) of the total number of once hibernated specimens. Apparently these values express the real survival rates of a year (within twelve months).

The stable larval density and ratio of the second and third year animals at the locality is a result of the stability of the adult salamander population number, and moreover, conversely to stagnant water amphibians, quite constant developmental

conditions. The later is a reason of Caucasian Salamander number dynamics peculiarities. The basic reasons of natural salamander mortality are not completely clear. Perhaps, egg and larval mortality caused by ponds drying up is not as important for *M. caucasica* as it is for many amphibians (for example, *Ambystoma maculatum*, Albers et al., 1987). There are practically no predatory insect larvae dangerous to salamander larvae in the stream. Young trout (*Salmo trutta labrax*), which are possible predators, are also very rare. Perhaps the main reasons of larval mortality are over wintering and larval diversion into the stream flow. Grass Snakes (*Natrix natrix*) could of course cause great damage during metamorphosis. There were from one to five just metamorphosed specimens in the stomachs of the five Grass Snakes captured in the salamander locality. We did not find any adult salamanders in snake stomachs. It is unlikely the low vulnerability of adults is connected with autotomy ability or coloration. The juvenile animals do not have the same coloration as adults and they are able to autotomise also (Golubev, 1981). The reason is rather adult animal size and antipredator behavior of this species described by Brodie et al. (1984).

**Discussion**

According to the view of adaptionists, the peculiarities of the morphology of the Caucasian Salamander mainly are a result of general body constitution. The vulnerability of this species results in the high requirements to environmental

condition, especially temperature and humidity. The comparative large body surface reduces homeostatic ability. Another main feature of *M. caucasica* as well as of *C. lusitanica* delimiting these species from all other European salamanders is breeding by egg deposition, when fecundity is comparatively low.

The biogeographical and ecological characteristics of *M. caucasica* can be explained by consequences of its morphological type. On the basis of paleontological date, *Mertensiella* aff. *caucasica* was distributed sympatrically with the Spotted Salamander in an area extending to central Europe in the Pliocene. The reduction of the range of *Mertensiella* was the result of the last glacial periods (Borja-Sanchiz and Mlinarsky, 1979). Nevertheless, the present range of *S. salamandra* is quite wide (Thorn, 1968), while the range of *M. caucasica*, like other representatives of the tribe, *M. luschani* and *C. lusitanica*, is comparatively narrow and in areas with mild climate. It is unlikely that the Spotted Salamander can affect the geographic range of these species. Although members of the genus *Mertensiella* are allopatric to *S. salamandra*, *C. lusitanica* has a wide sympatric zone with this species (Bas Lopez, 1984). The absence of *S. salamandra* in the Caucasus, including the Great Caucasus, obviously depends on historical reasons. The geographic range of subtle species is limited most of all by climatic factors. Wolterstorff et al. (1936) mentioned that the range of *M. caucasica* has not changed considerably since the Eocene. Perhaps the low homeostatic ability of adults limits their migratory possibilities.

As we already noted, the captures of animals far from their population locality were very rare. The salamanders don't penetrate the comparatively distant mountain systems like the Great Caucasus. They also don't occur in comparatively dry localities along the Trialeti Mountain Range in the East (Fig. 1) where there is no relief limit. This is one of the reasons for the restricted salamander distribution. On the basis of different research (Obst and Rotter,

1962; Tartarashvili, pers. comm.) we conclude that the area of salamander localities of high altitude and on the Black Sea coast isn't larger than ours. *Chioglossa lusitanica* localities have a similar distribution (Arntzen, 1981). On the other hand, a small population area might depend on attachment to the breeding sites (stream plots suitable for egg deposition and larval development). Spotted salamander populations are always distributed in significantly wider areas (Klewen, 1985).

As a result of the small area of the localities and the lack of breeding sites, the population number is limited at a comparatively low level, about 1000 individuals, when the sex ratio is close to equal. This amount is enough to maintain the populations demographically and genetically (Lande and Barrowclough, 1989). The potential population growth rate is also limited by comparatively low fecundity. Nevertheless, the breeding sites are used rather efficiently. Temporary ponds vulnerable to periodic natural disturbances aren't used for egg deposition. Thus, the population renewal possibilities of *M. caucasica* are different from stagnant-water amphibians. A significant part of the latter species are not able to breed efficiently because many of the breeding ponds within localities are destroyed during egg and larval development. Apparently the egg deposition of *M. caucasica* is done only in places suitable for further development. This type of reproduction is correlated with the high stability of population number though the resilience to habitat transformation is low.

This described model of population dynamics practically excludes the number of outbursts caused by climatic perturbations which could stimulate considerable migrations. Since, settling of investigated species is determined by the low tolerance of adults, the small area of population localities and breeding sites, and the low fecundity. Hence the main reason for the narrow geographical range of *M. caucasica* are its morphological features and the stable type of population cycle.

In analyzing morphological reasons of Caucasian Salamander ecological specificities in comparison to related species, we can not accept as a main character only body proportions. Ecological particularities of all the European salamander tribe mainly depend on comparatively large egg formation (and probably skin structure). These characters limit European salamanders to breed only in flowing water in comparatively wet places.

Although the European salamander adaptive type allows comparatively wide interspecies variability in some features, for example coloration patterns and degree of subtilisation, the general morphological constitution restricts adaptive ability of particular representatives of the group. In a sense *M. caucasica* is a morpho-ecological equivalent of *C. lusitanica*. These species have similar life cycle, population spatial structure and number dynamics, climatic and biotopic preferences, etc. The central adaptive possibility and the feature determined place in the group can be distinguished among a lot of morphological characteristics. There are some other features which separate these species and reveal the independent origin of both of them. This is, for example, tail corn in males. Nevertheless, this structure does not take place among main ecological features of species and reflects only the complexity of the phylogenetical ways.

Color patterns have rarely been used in phylogenetical speculations, but this characteristic is a favorable object of adaptationists. The presence of light-colored specimens in some *M. caucasica* populations, their predominance in other populations of this species and in *M. lusitani* and, finally, fully reduced of spotted specimens in *C. lusitanica* are not connected with variability of the plant cover in localities and do not affect their ecological preferences. We can't speculate about the adaptive meaning of coloration in this case. Coloration is closely related to the climate type of localities. Apparently, we could consider this characteristic as a fixed non-adaptive reaction to temperature and humidity changes.

In conclusion, we would like to give an opinion on an interesting detail connected with the distribution of *M. caucasica*. Three anuran species, the Colchic Toad (*Bufo verrucosissimus*), the Caucasian Parsley frog (*Pelodytes caucasicus*), and the Asia Minor Frog (*Rana macrocnemis*) live sympatrically with the Caucasian Salamander. *Rana macrocnemis* is distributed all over the Caucasus. *Bufo verrucosissimus* and *Pelodytes caucasicus* like *M. caucasica* do not penetrate the eastern part of the Trialeti Mountains because of lack of humidity. However, they are distributed in some locations in the Great Caucasus. The northern parts of the Trialetian and Adjaro-Imeretian mountains are more that 50 km from the southern parts of the Great Caucasus in Central Georgia. There are no suitable localities for forest amphibians between these mountain systems. The comparatively small transitional zone could have been crossed many times by *B. verrucosissimus* and *P. caucasicus* after the Great Caucasus system was formed. If we take into consideration the comparatively high fecundity (about 500 eggs per year for *P. caucasicus* and 10,000 eggs per year for *B. verrucosissimus*) and the temporal variability of the breeding sites, a few climatically favorable seasons could cause a great increase in population number and as a final result, a massive migration. According to the peculiarities of *M. caucasica* population dynamics, we can not expect any similar process. Hence, the lack of *M. caucasica* in the Great Caucasus has historical rather than autecological reasons.

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## GUIDELINES FOR MANUSCRIPT PREPARATION AND SUBMISSION

### Summary

Manuscripts must:

- 1) be written in English.
- 2) be of letter quality (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 "The three snakes examined were female."

**Passive voice:**

"Lizards were observed to be extremely common on the site." and "Three snakes were examined and were found to be female."

**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.

*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.

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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.

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- 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).

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- 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.**
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- 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.

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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 1987, Vol. 6 numbers 1-4 and 1988, Vol. 6 numbers 1-2.

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.

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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.

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*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.

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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.

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