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Mosaicism in somatic triploid specimens of the *Bufo viridis* complex in the Karakoram with examination of calls, morphology and taxonomic conclusions

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ABSTRACT

The discovery of triploidy in green toads from the Karakoram range and West Himalayas (Northern Pakistan) is reported. Somatic, mitotic metaphases comprising 33 conventionally and quinacrine mustard stained chromosomes are described. Meiotic metaphases and diakineses demonstrate the occurrence of different ploidy levels (diploid, triploid, tetraploid) in the male germ line of somatic triploid specimens. Flow cytometric data on DAPI stained blood samples from 54 specimens preliminarily seem to provide evidence for all-triploid populations. The mean projection areas of 30 erythrocytes per specimen were measured. The analysis of 16 morphometric characters with univariate and multivariate methods and the comparison with published data exhibited differences of the present toads to both diploid and tetraploid toads from Middle Asia. Bioacoustic analyses revealed similarity of mating calls to those of tetraploid toads. A single specimen of *Bufo (viridis) pseudoraddei* Mertens 1971, was also found to be triploid. Present populations from Karakoram represent a separate subspecies *B. pseudoraddei baturae* n. ssp. which are morphologically different from *B. p. pseudoraddei* and *B. latastii* Boulenger, 1882. A lectotype of *B. latastii* was selected. *B. siachinensis* Khan, 1997, was found to be a synonym of *B. latastii*.

KEY WORDS: *Bufo pseudoraddei baturae* n. ssp. - *Bufo viridis* complex – Triploidy – Mosaicism – Morphometry – Mating calls – Taxonomy – Karakoram.

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INTRODUCTION

Taxonomic overview and first consequences

The taxonomy of Asian green toads was reviewed by Roth (1986), Borkin & Kuzmin (1988) and Stöck & Grosse (in press). The following taxa of the *B. viridis* group (Inger, 1972) are known in the region south of the main watersheds of the Hindukush, Karakoram and Himalayas:

Bufo latastii (Boulenger, 1882) was described from Ladakh (N-India). Hemmer *et al.* (1978) considered the taxon to inhabit Middle Asia which was refuted by Pisanets & Shcherbak (1979). Their opinion was supported by Borkin *et al.* (1986b), Roth (1986) and Borkin & Kuzmin (1988). Hemmer *et al.* (1978) determined their animals without investigation of the type series and argued, they used 'topotypic material' from Kashmir and Ladakh of Gruber ("1977", publ. 1981) which would therefore represent the 'nominate subspecies' [*B. latastii latastii*]. Gruber (1981) wrote that Hemmer *et al.* (1978) "discovered that these toads belong to the subspecies *B. latastii latastii* Boulenger, 1982". One karyological study of a *Bufo* sp. from Kashmir (Duda & Opendar, 1971), which appears to involve *B. latastii* as Dubois & Martens (1977) and Roth & Ráb (1986) supposed, shows a diploid karyotype different from that of *B. viridis*. However, it is still not clear whether this karyotype really belongs to *B. latastii* or to other sympatric toad species (e.g., *B. stomaticus*, *B. himalayanus*).

The corresponding author (Stöck *et al.*, 1998) selected specimen BMNH 1947.2.21.28 (formerly 72.4.17.223) from the syntypes of *B. latastii* to be the lectotype (Fig. 1) since its dorsal colouration pattern is identical with that shown in the figure of the species description (Boulenger 1882: plate XIX). After comparisons with Gruber's and additional material (Eiselt, Schmidtler leg.) from Kashmir and Ladakh, the corresponding author found Gruber's, Eiselt's and Schmidtler's toads to be clearly different from Middle Asian taxa but similar to the *B. latastii* lectotype. These preserved *B. latastii* often show a dark brownish (in life: olive - Boulenger, 1882; Gruber U., pers. comm.) typical striped colouration and characteristic dark spots on the usually mar-

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The article is dedicated to the memory of Alessandro Morescalchi
* This publication is part of the planned dissertation thesis of the corresponding author Matthias Stöck.

Fig. 1 - Above: BMNH1947.2.21.28 (formerly 72.4.17.223), lectotype of *Bufo latastii* Boulenger, 1882. Centre: the figure of the species description by Boulenger (1882: plate XIX, Fig. 2) showing the present lectotype specimen. Bottom: Holotype of *Bufo siachinensis*, Khan, 1997, a junior synonym of *Bufo latastii*.

bled belly. Until a detailed study will be done, the ploidy level of *B. latastii* remains unknown, although the calls (Dubois & Martens, 1977) seem to provide evidence for considering the species to be polyploid (Stöck, 1998a).

Only two females from East Karakoram (Shinu, N-Pakistan), which were not analysed karyologically, formed the basis for the description of *Bufo siachinensis* Khan, 1997. The typical striped colouration (Fig. 1), the elongated parotids, the neighbourhood of the type locality to Gruber's (1981) localities (see Fig. 2), the incorrect discrimination (see Appendix) within the type description (Khan, 1997) from *B. latastii*, and finally, a morphometric analysis (see below) led the corresponding author to the conclusion that *B. siachinensis* is a junior synonym of *B. latastii*.

Bufo (viridis) pseudoraddei (Mertens, 1971) from northern Pakistan (Swat Valley) was supposed to belong to *B. latastii* (Dubois & Martens, 1977) or to be a subspecies (Hemmer *et al.*, 1978) of 'their' *B. latastii* (see above). The ploidy level was unknown. Roth (1986) announced preliminarily the form would be tetraploid.

Bufo (viridis) zugmayeri (Eiselt & Schmidtler, 1973) was described from Pishin (West Pakistan), and considered a subspecies of *B. latastii* by Hemmer *et al.* (1978). The ploidy level is unknown and the systematic position is therefore unclear (Stöck & Grosse, in press).

Origin of polyploids

After the discovery of tetraploid green toads in the northern Tien Shan (Mazik *et al.*, 1976), different authors controversially discussed the origin of polyploid forms from various localities. Karyological (Roth, 1986; Roth & Ráb, 1986), isozyme-electrophoretical (Borkin *et al.*, 1986a; Lattes A., 1997, Abstracts of the Third World Congress of Herpetology, Prague: 123) and cytometrical data (Kudryavcev *et al.*, 1988) seemed to provide arguments for their autoploidy. Conversely, Mezhzherin & Pisanets (1990, 1995a, b) found an allopolyploid origin of tetraploids with isozyme electrophoresis.

Triploidy in the Bufo viridis *complex*

Triploid individuals have been detected by Pisanets (1978) and Mezhzherin & Pisanets (1995a) in Danata (West Turkmenistan). Triploid specimens have been found in northern Kyrgyzstan (Kuzmin, 1995; Cervella *et al.*, 1997, Abstracts of the Third World Congress of Herpetology, Prague: 38; Castellano *et al.*, 1998), and south-eastern Kazakhstan (Borkin *et al.*, 1997, Abstracts of the Third World Congress, Prague: 26). Mezhzherin & Pisanets (1990) concluded the rare occurrence of triploid individuals in the Central Pamirs from isozyme data. Most of the authors supposed a hybrid origin of triploids as a result of mismatings between diploid and tetraploid toads. Preliminarily, Lattes and Cervella *et al.* (see above) indicated that triploids from Kazakhstan and Kyrgyzstan do not originate from hybridizations between diploid and tetraploids but seem to be closely related forms of the tetraploids.

Here we report the discovery of triploidy in populations of a new taxon in the Karakoram range of Pakistan.

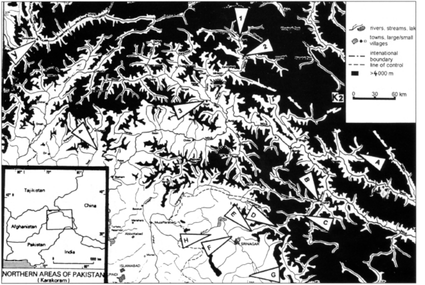

Fig. 2 - Localities. 1 to 3 - *Bufo pseudoraddei baturae*, present study. 1 - Sust, 2 - Pasu: type locality of *B. p. baturae*, 3 - Gilgit. 4 - Swat (Kulalai): *Bufo pseudoraddei pseudoraddei* Mertens, 1971. A to H: presumed *B. latastii* Boulenger, 1882: A - Shinu, Khan (1997: *B. siachinensis*), B - Kargil and near Kargil, Dubois & Martens (1977), C - Dras, Gruber (1981), D - Sonamarg and near Sonamarg, Dubois & Martens (1977), E - Naranag, Gruber (1981), F - Tangmary near Srinagar (Eiselt and Schmidtler leg.) and near Srinagar, Dubois & Martens (1977), G - Shupian, Dubois & Martens (1977), H - Gulmarg, Dubois & Martens (1977). Y: Nearest record of hitherto known polyploid toads in the map: Ishkashim, Southern Pamirs, Mezhzherin & Pisanets (1990). Map by M. Stöck after a map compiled by J. P. Mund and M. Münz (1995, unpubl.).

MATERIALS AND METHODS

We examined green toads (*Bufo viridis* complex, for definition see: Stöck & Grosse, 1997) from northern Pakistan (Karakoram Range, Western Himalayas) near Sust (36° 46' N; 74° 50' E; 2950 m a.s.l.), Pasu (36° 30' N; 74° 52' E; 2600-2800 m a.s.l.), Gilgit (35° 54' N; 74° 24' E, 1550 m a.s.l.), and Kulalai (35° 19' N; 72° 36' E; 1750 m a.s.l.) (Fig. 2).

Chromosomes

We conducted Giemsa-staining and Q-banding of metaphase plates obtained from bone marrow, intestine, and testes tissue (Schmid, 1978) in 12 specimens from Sust (2), Pasu (4), Gilgit (5) and Kulalai (1). One pair of the five toads from Gilgit reproduced in the laboratory and we also examined the chromosomes of three juvenile F_1-specimens.

DNA content

Thirty-five toads from Pasu and 19 from Gilgit were anaesthetized (MS 222) and small blood samples were taken by heart puncture (Schroer, 1996). Once specimens had recovered from anaesthesia most of them were released. The slightly heparinized blood was diffused in 70% ethanol (20° C). After transport to the laboratory, within maximally one week, samples were refrigerated for three weeks. The preparation for flow cytometry was performed according to Otto (1994). Measurements were conducted with a Partec CA II cytometer. Chicken red blood cells were used as standards.

Erythrocyte size

We used air-dried blood smears (65 specimens) and measured the projection areas of 30 erythrocytes per toad (ES) with the Cydok (Hilgers) image analyses system as described by Stöck & Grosse (1997).

Mating calls

Voices of 12 males from three localities (Pasu, Gilgit, Kulalai) were recorded with a walkman (WM 6 DC, Sony) and a microphone (ME 66 combined with module K 6, Sennheiser) during the afternoon, at dusk or at night from a distance of 0.5 to 4.0 m in the breeding ponds. Water and air temperature at the individual's calling site were taken to the nearest 0.5° C immediately after recording the call. Calls were analysed according to Stöck (1998a).

Morphometry

Fifteen body measurements of 79 living animals (females were underrepresented, see Table V) were taken to the nearest 0.1

mm with dual calipers according to Stöck (1997). Briefly, they are: snout-urostyle length (SVL), head length (HL); length of parotoid gland (PL), width of parotoid gland (PW), horizontal diameter of tympanum (HDT), vertical diameter of tympanum (VDT), horizontal diameter of eye (ED), head width (HW), internarial distance (IND), distance between nostril and anterior corner of eye (NED), length of tibia (TL), length of leg (LL), length of first toe (LFT), length of inner metatarsal tubercle (LMT), interorbital width (IOW). Mass was measured six to eight h after the toads were caught, with an accuracy of 0.5 g using a spiral dynamometer. Due to ontogenetic change, only adults were included in the morphometric analysis. The present toads were compared with 35 diploid and 106 (60 for HDT, NED, PW, IOW) tetraploid specimens from a previous study (Stöck, 1997). For statistic analyses, the programme SPSS 7.5 for Windows was used.

RESULTS

Karyotypes

All eleven specimens of the new subspecies (see below) studied from Sust, Pasu and Gilgit were found to be triploid. In addition, triploidy was also demonstrated in the three F_1 juveniles resulting from the pairing of triploid parents from Gilgit. The female from Kulalai (Swat) was also triploid.

The 33 conventionally stained chromosomes (Figs 3a, 4a) can be arranged in three groups ('triplets'), each containing 11 chromosomes (Fig. 3b). As in many other *Bufo* species (Bogart, 1972; King, 1990), the chromosomes are mostly metacentric or submetacentric. The karyotype shows a distinct demarcation in size between the larger elements (triplets 1 to 6) and the remaining five triplets. The conventionally stained chromosomes do not show differences in form and size within a triplet. The triploid karyotypes exhibit brightly quinacrine positive segments (Fig. 4b) which are situated interstitially and close to the centromere in the metacentric triplet 1, and interstitially in the smaller chromosomes of triplets 6 to 11 (Fig. 4c). Whereas chromosomes of triplet 1, 6 and 7 exhibit fluorescing segments in only one arm, the chromosomes of triplets 8 to 11 show fluorescence in both arms. In some specimens we observed that not all, but only two, chromosomes of a triplet were Q-banding positive.

Meiosis in testes tissue

In the testes tissue samples (specimens from Pasu, Sust, Gilgit) examined so far, we found triploid (mitotic) metaphases (33 chromosomes) and both diploid (22 chromosomes; Fig. 5) and tetraploid (44 chromosomes) metaphases (Fig. 6). We also observed diploid (11 bivalents) and tetraploid (22 bivalents) diakineses even in samples from the same somatic triploid male (Fig. 7). This finding preliminarily indicates that at least some adult males exhibit mosaicism because they consist of somatic triploid cells, while the testes cells are diploid, triploid and tetraploid.

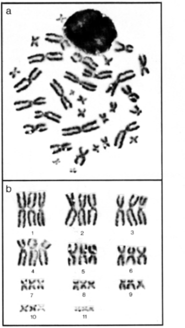

Fig. 3 - a, Conventionally Giemsa-stained mitotic metaphase of a triploid specimen from Gilgit; b, Giemsa-stained karyotype $3n = 33$.

DNA content

The mean erythrocyte DNA content in the Gilgit population was 6.13 times the content of the reference standard chicken DNA (14.34 pg if 2.34 pg/chicken nucleus: see Discussion); the mean of the Pasu population (6.44×; ≙ 15.07 pg) was about 5% higher (Table I). Mann-Whitney-U tests revealed significance ($P < 0.001$) for this difference. The mean for all triploid toads was 6.31× (≙14.77 pg) of the chicken DNA content. A blood sample from a tetraploid toad (Kashgar, China; Stöck, 1998b) was used as control and contained 7.48× (≙17.50 pg), the content of the chicken nuclei (Fig. 8). Our results confirm the triploidy of all 54 specimens examined.

Erythrocyte size (ES)

The Pasu population exhibited a distinctly higher mean of ES than the Gilgit population (Table II). The means differed significantly (t-test). The animal from Sust had a value in the lower range of the Pasu population and near the average value of the Gilgit one. We

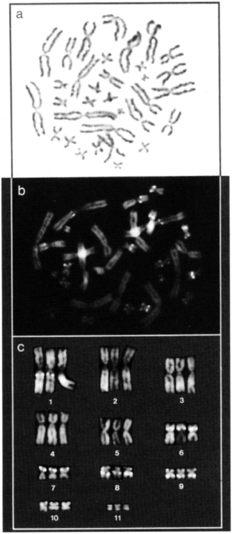

Fig. 4 - a, Giemsa-stained mitotic metaphase of a triploid specimen from Sust; b, Q-banded mitotic metaphase of a triploid specimen from Pasu; c, Q-banded triploid karyotype.

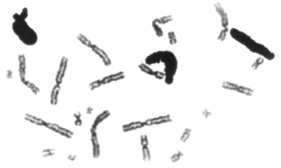

Fig. 5 - Giemsa-stained diploid metaphase containing 22 chromosomes from the testes tissue of a somatic triploid male from Gilgit (ZSM 112/1998), the same specimen as in Figure 7). Note the three black sperm nuclei.

Fig. 6 - Giemsa-stained tetraploid metaphase containing 44 chromosomes from the testes tissue in one of the somatic triploid paratypes (ZSM 101/1998) of *Bufo p. baturae* from Sust.

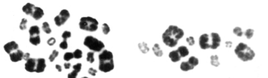

Fig. 7 - Two adjoining Giemsa-stained diakineses, one diploid containing 11 bivalents (right) and one tetraploid containing 22 bivalents (left) from the testes tissue of a somatic triploid male from Gilgit (ZSM 112/1998).

compared the ES with those of 98 Asian tetraploids and 36 diploids (Stöck & Grosse, 1997). The toads from Gilgit (and Sust) exhibited an ES in the upper range of diploid forms; toads from Pasu had an ES between those of diploids and tetraploids (Fig. 9). The animal from Kulalai had an ES value (Table II) in the lower range of the Pasu population and near the average value of the Gilgit one. We did not find any relationship between mean ES and body size (Fig. 10). A multiple LSD-test demonstrated differences of ES between the triploid, diploid and tetraploid toads at the $P < 0.001$ level (triploid/tetraploid) and at the $P < 0.025$ level (triploid/diploid).

Mating calls

Within the trills, consisting of series of pulses with a constant duration separated by constant interpulse intervals, the regular structure was reached only after about the 10th pulse (Table III, Fig. 11). First pulses were either shorter or longer and had a lower frequency (Fig. 11D). The mating call structure of toads examined from

Table I - *DNA content in two populations of* Bufo pseudoraddei baturae.

	Pasu (*n* = 35)			Gilgit (*n* = 19)		
	Ratio to chicken (= 1.00)	Ratio to mean (%)	pg	Ratio to chicken (= 1.00)	Ratio to mean (%)	pg
Mean	6.44	100.0	15.07	6.13	100.0	14.34
Max	6.88	106.8	16.09	6.48	105.7	15.16
Min	5.90	91.6	13.80	5.72	93.3	13.38
SD	0.23	-	0.53	0.24	-	0.56

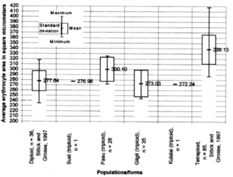

Fig. 9 - Boxplots of mean erythrocyte size of present populations from Pakistan in comparison with diploid and tetraploid toads from Middle Asia (Stöck & Grosse, 1997).

Pasu, Gilgit and Kulalai was similar in different populations. The typical relationship between call parameters and water temperature could be studied especially in the Pasu population (Figs 12, 13). Linear regressions in this population across a range of temperatures from 7° C to 27° C (Figs 12, 13) were found for interpulse interval duration: y = -2.12x + 89.23, r^2 = 0.794; for pulse duration: y = -1.67x + 63.70, r^2 = 0.95 and for the resulting pulse rate: y = 0.59x + 2.86, r^2 = 0.946.

Bufo pseudoraddei pseudoraddei

The locality (Kulalai, Swat valley), the colouration (Fig. 15H), and morphological characters of the toads from Kulalai are typical of *B. viridis pseudoraddei* Mertens, 1971. A comparison with the type series confirmed this diagnosis. The only adult female (ZSM 106/1998; Fig. 15H) which was examined karyologically was found to be triploid (see above). Mating calls of males from Kulalai (Table III, Figs 11-13) were similar to those of triploid toads from Sust, Pasu and Gilgit, and therefore different from those of diploid *B. viridis* (cf. Stöck, 1998a). With regard to the results reported above, it is very probable, that the taxon previously considered a subspecies of *B. viridis* or *B. latastii* represents a separate species, and toads from the terra typica (Swat) are consequently the nominate subspecies *B. pseudoraddei pseudoraddei*. This can be distinguished from *B. viridis* by a combination of morphological traits reported in the description of Mertens (1971). In addition, our results demonstrate that at least some specimens in the *B. p. pseudoraddei* populations are triploid. *B. p. pseudoraddei* can be distinguished by its distinctly shorter parotids from *B. latastii* (see below and Table IV). *B. p. pseudoraddei* appears to be distributed in open habitats within the zone of Himalayan dry coniferous forest with ilex oak and Himalayan moist temperate

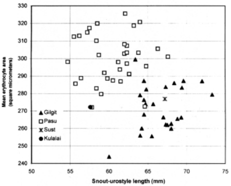

Fig. 10 - Erythrocyte size and body size of *Bufo p. baturae* (Karakoram: Sust, Pasu, Gilgit) and a single specimen of *B. p. pseudoraddei* (Western Himalayas, Kulalai).

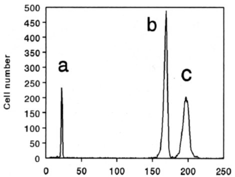

Fig. 8 - Histogram obtained by DNA flow cytometry from a mixture of DAPI stained blood samples from chicken as the standard (a), CV = 3.7%, a triploid toad from Gilgit (b), CV = 1.16%, and a tetraploid toad from Kashgar (China; c), CV = 1.98%. Total cell numer: 5830.

Table II - *Erythrocyte size (projection areas) in the populations of* B. pseudoraddei baturae *(Sust, Pasu, Gilgit) and one specimen of* Bufo p. pseudoraddei *(Kulalai).*

Parameter (μm²)	Population			
	Sust *n* = 1	Pasu *n* = 35	Gilgit *n* = 28	Kulalai *n* = 1
Mean of means in this population	276.98	300.10	273.03	272.24
Maximal mean in this population	322.77	325.57	299.54	318.11
Minimal mean in this population	224.43	272.07	243.87	216.37
Largest cell measured	322.77	373.18	361.85	318.11
Smallest cell measured	224.43	207.68	182.09	216.37
Mean standard deviation in this pop.	24.62	23.09	25.62	21.99

Table III - *Mating calls of* Bufo pseudoraddei baturae *(male 1-13) and* B. p. pseudoraddei *(male 14-15).*

Locality (number in Fig. 1)	Male	T (°C)		Pulse duration (ms)	Interpulse interval duration (ms)	Pulse rate (Hz)	Fundamental frequency (Hz)
		Air	Water				
Pasu (1)	1	17.0	7.0	56.7	76.7	7.5	1464
Pasu (1)	2	17.0	7.0	49.2	86.2	7.38	1550
Pasu (1)	3	15.5	11.5	46.2	56.1	9.77	1560
Pasu (1)	4	15.5	11.5	45.8	49.2	10.53	1378
Pasu (1)	5	14.0	15.0	37.4	62.8	9.98	1636
Pasu (1)	6	14.0	15.0	33.4	60.8	10.71	1550
Pasu (1)	7	28.0	27.0	19.2	29.4	20.58	1545
Pasu (1)	8	27.0	27.5	19.2	35.5	18.28	1550
Gilgit (3)	9	27.0	24.0	20.2	28.7	20.44	1378
Gilgit (3)	10	27.0	24.0	23.9	27.7	19.38	1380
Gilgit (3)	11	27.0	24.0	24.2	27.3	19.41	1464
Gilgit (3)	12	27.0	24.0	25.0	29.0	18.21	1553
Gilgit (3)	13	27.0	24.0	19.0	28.0	21.28	1468
				19.7	30.1	20.08	1468
Kulalai (4)	14	23.0	24.0	29.2	42.8	13.88	1552
Kulalai (4)	15	23.0	20.0	34.4	36.8	14.04	1635
				35.3	35.9	14.04	1635

forest (Roberts, 1991: maps on pl. 4-6) which are neighbouring in the upper Swat valley.

A new subspecies

The toads from Sust, Pasu and Gilgit were found to represent a new taxon:

Bufo pseudoraddei baturae n. ssp.
(proposed common name: Batura toad)

Bufo viridis: Minton (1967), Baig (1988); *Bufo viridis pseudoraddei*: Eiselt & Schmidtler (1973) in part.; *Bufo latastii pseudoraddei*: Hemmer *et al.* (1978) in part.

Etymology – The name is derived from the Batura glacier, whose mouth is situated close to the type locality. From this geographical name the genitive case was formed according to the ICZN (1985, Appendix D: IV (b), p. 197).

Holotype (Fig. 14) – ZSM 103/1998, an adult male from a plain above the right bank of the Hunza river near the mouth of the Batura glacier, opposite the mouth of the Shimshal river, north of the village of Pasu, 2700 m a.s.l., Karakoram, Pakistan, collected by M. Stöck and H. Veith, June 1997. Triploidy was determined with flow cytometry.

Paratypes (Fig. 15 in part) – ZSM 104/1998 (male), ZSM 105/1998 (a young female), ZSM 113/1998 (female), ZSM 114/1998 (male), ZMB 58769 (adult male), ZMB 58770 (adult female), same data as holotype; ZSM

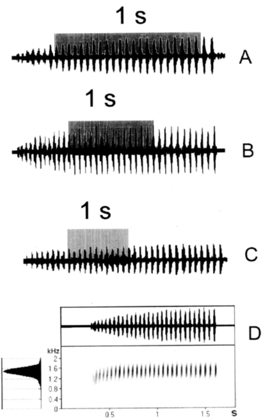

Fig. 11 - A, Oscillograms of mating calls of a triploid male *B. pseudoraddei baturae* recorded in Gilgit, water temperature 24° C. B, Male *Bufo pseudoraddei pseudoraddei* from Swat (Kulalai), water temperature 20° C. C, Male *B. pseudoraddei baturae* from Pasu, water temperature 15° C. D, Oscillogram (above), sonogram (below) and power spectrum (left) of a triploid male *B. pseudoraddei baturae* from Gilgit, water temperature 24° C.

101/1998 (male), ZSM 102/1998 (female) from Sust, from the valley slope above the settlement on the left bank of the Hunza river, 2950 m a.s.l., Karakoram, Pakistan, collected by M. Stöck, June 1996. Some specimens have had leg bones and tissue removed for chromosome and future molecular biological analyses.

Diagnosis

This is a small-sized subspecies of the *B. viridis* complex which differs from most other members of the complex by the following combination of traits: the parotids are inconspicuous and very short, their length (PL) is smaller than double their width (PW); interorbital width (IOW) is smaller than, or approximately the same size as internarial distance (IND); subarticular tubercles single on toes but often double on first, second and, in some individuals, on third finger; most specimens have very incon-

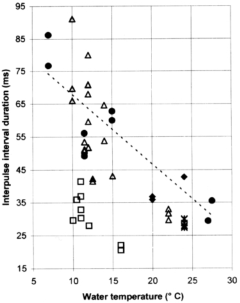

Fig. 12 - Interpulse interval duration in relationship to water temperature in males of *B. pseudoraddei baturae* from Pasu, and Gilgit and *B. pseudoraddei pseudoraddei* from Kulalai in comparison with diploid and tetraploid males from Middle Asia (Stöck, 1998a).
● Karakoram, Pasu (3n = 33); ✕ Karakoram, Gilgit (3n = 33); ◆ Swat, Kulalai (3n = 33); △ Tetraploid 4n = 44 (Stöck, 1997); □ Diploid 2n = 22 (Stöck, 1997); ---- Linear regression (Karakoram, Pasu (3n = 33)).

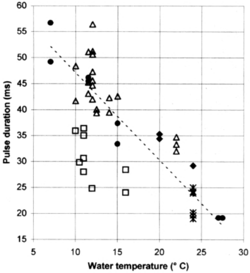

Fig. 13 - Pulse duration in relationship to water temperature in males of *B. pseudoraddei baturae* from Pasu and Gilgit, and males of *B. pseudoraddei pseudoraddei* from Kulalai in comparison with diploid and tetraploid males from Middle Asia (Stöck, 1998a). For symbols, see Figure 12.

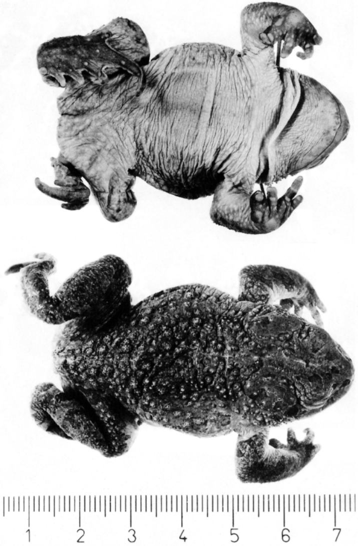

Fig. 14 - Holotype ZSM 103/1998 of *Bufo pseudoraddei baturae* n. ssp., above: ventral view; below: dorsal view. Scale is valid for both views.

spicuous tarsal folds. The somatic chromosome number in the majority (maybe in all) of the members of the populations is $3n = 33$. In life, the iris is darkly greenish-golden (Fig. 15G). The new subspecies can be distinguished from *B. latastii* Boulenger, 1882 (= *B. siachinensis*, Khan 1997; see above) whose chromosome number is apparently unknown, mainly by the distinctly shorter parotids; the difference in the ratios of PL/PW (Table IV) is highly significant between both groups of toads (Mann-Whitney-U test, $P < 0.001$). The new subspecies also differs from *B. latastii* in a combination of morphometric characters (see below), and in the colouration (see below). From the obviously allopatric *B. p. pseudoraddei* Mertens, 1971, the new subspecies differs in having a more slender habitus, a much more granular (glandular, warty) dorsal skin texture in both sexes, and a distinct sexual dimorphism in the colouration. *B. p. baturae* can be distinguished from *B.p. pseudoraddei* by a combina-

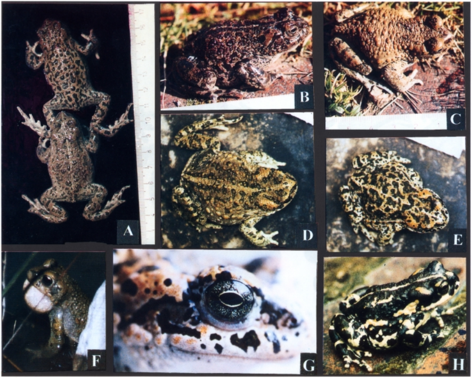

Fig. 15 - A to G: Morphological variability of *Bufo pseudoraddei baturae*. **A**, female (above, ZMB 58770) and male (below, ZMB 58769) from Pasu; **B**, female from Sust (ZSM 102/1998); **C**, male from Sust (ZSM 101/1998); **D**, male from Gilgit; **E**, female from Gilgit; **F**, calling male from Pasu; **G**, eye of a female from Gilgit; **H**, triploid female of *B. pseudoraddei pseudoraddei* (ZSM106/1998) from Kulalai, Swat valley, West Himalayas. Scales only for A-C. Photographs: M. Stöck.

Table IV - *Ratio PL/PW in adults of* B. pseudoraddei pseudoraddei, B. p. baturae *and* Bufo latastii *(different localities).*

Taxon (Localities)	*Bufo p. pseudoraddei* (Swat, Kulalai) $n = 7$	*Bufo p. baturae* (Sust, Pasu, Gilgit) $n = 78$	*Bufo latastii* (see Mat. examined) $n = 12$
Mean	1.51	1.54	2.02
Min	1.08	1.15	1.46
Max	1.82	2.05	2.71

tion of morphometric traits (see below), the frequent occurrence of double subarticular tubercles on first, second and third fingers, the absence of distinct, larger dark spots but only cloudy gray colour on the ventral side.

The new subspecies differs from the allopatric *B. viridis zugmayeri* Eiselt & Schmidtler, 1973 (unknown ploidy level) in having a distinctly smaller interorbital width (IOW), shorter and more inconspicuous parotids, and a different colouration (see below).

Measurements of the holotype (in millimeters)

SVL 58.2, HL 13.0, PL 10.2, PW 6.2, HDT 2.7, VDT 2.5, ED 6.7, HW 20.4, IND 4.5, NED 3.2, TL 22.3, LL 36.8, LFT 3.1, LMT 3.0, IOW 2.8.

Description of the holotype

An adult mature male with brown nuptial excrescences on first, second and slightly on third finger; body relatively small; head distinctly shorter than wide; snout rounded but moderately conical from dorsal view, but short and relatively blunt from lateral view; nostrils slightly directed laterally, their distance larger than the interorbital width, nostrils situated about two thirds the distance from anterior corner of eye to tip of snout; tympanum smaller than half the diameter of the eye, its anterior margin vertically under posterior corner of eye; anterior margin of the short, ellipsoid parotids close to the posterior corner of eye; parotids smooth, only with few small, warty glands; the posterior parts slightly narrower, roundish; skin on nasal and sphenethmoid region smooth; towards dorsum and posterior parts of eyelids increasingly verrucous, apex of verrucae on dorsum obviously covered with keratin, dorsal skin texture and dorsal surfaces of limbs roughened, sandpaper-like; skin of flanks from dorsum to belly smoother; belly relatively smooth, slightly rugose; subarticular tubercles double on first, second and third finger, single on toes; two palmar tubercles, inner one more prominent, its surface about half the size of outer one; third finger distinctly extending beyond fourth, tip of fourth only slightly extending beyond distal articulation of third finger; inner and outer metatarsal tubercle longish, the inner about one third longer than the outer; colouration in alcohol dorsally olive-grayish; some inconspicuous dark olive irregular spots and stripes on head and dorsal surfaces of limbs; a thin short yellowish stripe along spinal column in the interorbital region, very thin apexes of dorsal verrucae rufous; ventral side yellowish-whitish.

Colouration in life

The new subspecies exhibits a distinct sexual dimorphism in colouration: females from Pasu (Fig. 15A) and

Table V - *Morphometric parameters: mean, standard deviation, minimum, and maximum (mm) of living* Bufo pseudoraddei baturae.

Population		SVL	HL	PL	PW	HDT	VDT	ED	HW	IND	NED	TL	LL	LFT	LMT	IOW	MASS (g)
Pasu	MEAN	**62.8**	**16.6**	**12.6**	**7.3**	**3.4**	**3.3**	**7.9**	**23.6**	**4.7**	**3.9**	**23.3**	**41.0**	**4.4**	**4.1**	**3.3**	**23.7**
females	SD	4.28	0.35	1.15	1.25	0.15	0.15	0.81	1.87	0.99	0.26	0.64	0.84	0.46	0.10	0.46	3.51
$n = 3$	MIN	59.4	16.4	11.5	6.0	3.2	3.2	7.0	22.2	4.0	3.6	22.8	40.5	4.1	4.0	2.8	20.0
	MAX	67.6	17.0	13.8	8.5	3.5	3.5	8.6	25.7	5.8	4.1	24.0	42.0	4.9	4.2	3.7	27.0
Pasu	MEAN	**60.4**	**16.3**	**10.5**	**6.6**	**3.1**	**3.0**	**7.4**	**21.5**	**4.6**	**4.0**	**23.2**	**40.0**	**3.5**	**3.3**	**3.1**	**17.3**
males	SD	3.07	0.83	1.11	0.56	0.44	0.42	0.41	1.25	0.36	0.50	1.11	1.81	0.61	0.24	0.39	2.64
$n = 33$	MIN	54.7	14.9	8.4	5.5	2.3	2.1	6.7	18.9	4.0	3.1	20.9	36.4	2.7	2.8	2.4	11.5
	MAX	66.3	18.4	13.3	8.3	4.0	4.0	8.2	24.0	5.6	5.1	25.7	43.4	5.0	3.8	3.8	23.0
Gilgit	MEAN	**64.2**	**16.2**	**11.4**	**8.6**	**3.1**	**2.8**	**8.0**	**22.8**	**4.7**	**4.3**	**24.1**	**40.2**	**3.6**	**3.5**	**3.8**	**27.1**
females	SD	6.76	1.26	1.45	0.34	0.47	0.24	0.73	1.73	0.59	0.56	1.31	1.16	0.43	0.62	0.72	9.41
$n = 4$	MIN	57.4	15.2	10.0	8.2	2.4	2.5	7.3	20.8	4.2	3.7	23.0	39.0	3.2	3.1	2.7	16.0
	MAX	73.3	18.0	13.0	9.0	3.4	3.0	8.7	25.0	5.5	5.0	26.0	41.4	4.2	4.4	4.2	38.5
Gilgit	MEAN	**65.1**	**17.4**	**11.5**	**7.8**	**3.3**	**3.3**	**8.0**	**23.1**	**4.5**	**4.4**	**25.4**	**43.7**	**3.7**	**3.6**	**3.8**	**23.8**
males	SD	3.24	1.00	1.29	0.73	0.40	0.42	0.62	1.22	0.43	0.42	1.28	1.88	0.47	0.41	0.68	3.44
$n = 35$	MIN	58.5	15.5	9.4	6.0	2.3	2.4	6.8	20.0	3.6	3.2	22.7	40.5	2.7	2.6	2.4	16.0
	MAX	72.0	19.3	16.2	9.5	4.0	4.2	9.0	25.5	5.6	5.0	27.9	47.6	4.7	4.5	4.9	33.5
Sust	female	67.2	16.5	10.8	7.2	3.4	3.8	7.34	22.3	5.0	5.0	24.3	41.8	3.1	3.9	3.8	25.0
	male	56.8	15.4	9.6	5.5	2.6	3.0	7.3	20.6	4.2	4.1	22.0	42.0	3.2	2.8	2.8	16.5

Table VI - *Reclassification result of the discriminant analysis with 16 morphometric characters.*

Ploidy	Predicted group membership Number of cases (percentage)			*n*
	Diploid	Tetraploid	*Bufo p. baturae*	
Diploid	30 (88.2%)	4 (11.8%)	0	34
Tetraploid	1 (1.7%)	54 (90.0%)	5 (8.3%)	60
Bufo p. baturae	0	3 (3.8%)	76 (96.2%)	78

Table VII - *Standardized canonical discriminant coefficients of a stepwise discriminant analysis which discriminated 92.5% of diploid, tetraploid and triploid toads from different parts of Asia. Seven of 16 morphometric characters were chosen.*

Characters	Standardized canonical discriminant coefficients	
	Function 1	Function 2
LL	- 0.270	- 1.054
IOW	0.661	0.078
SVL	- 0.415	- 0.357
MASS	0.162	- 0.749
PW	- 0.187	1.185
PL	0.931	- 0.141
TL	0.122	1.048

Sust (Fig. 15B) grayish-greenish or grayish-brownish on dorsum, with irregularly formed dark green spots, mostly smaller or about the size of ED; spots partly connected, forming marbled patterns whose margins have indentations; males (Fig. 15A, C) brownish and grayish tones, distinct irregular spots are mostly limited to extremities; dorsal colouration of toads from Gilgit more diverse in both sexes, females (Fig. 15E) with dark green dorsal stripes and spots on slightly grayish-yellowish ground forming partly a continuous pale band along the spinal column. The last character was also sometimes present in males (Fig. 15D) whose brownish or greenish-brownish colouration was pale in the middle of the dorsum. In Sust, Pasu, and Gilgit populations, both sexes showed whitish bellies whose lateral parts and those between forelegs often exhibited a cloudy grayish colour. No larger dark spots on the bellies.

Morphometric comparisons

The morphometric variability of the new subspecies is presented in Table V.

Univariate statistics – The individuals from Pasu were smaller than those from Gilgit. Mann-Whitney-U tests revealed differences between these populations for all characters ($P = 0.05$) with the exception of LFT ($P = 0.198$), HDT ($P = 0.197$), and IND ($P = 0.311$). Toads from *B. p. baturae* populations were compared with diploid ($n = 36$) and tetraploid ($n = 106$) specimens from various populations of Middle Asia analysed in a previous study (Stöck, 1997). Either Mann-Whitney-U tests (ED, IOW, LFT, IND, HDT, VDT; no homogeneity of variances, Levene test) or a multiple LSD test (remaining characters) were performed. The group of the 79 specimens of *B. p. baturae* exhibited differences ($P < 0.001$) as compared with both diploid and tetraploid toads in all characters with the exception of HDT and NED (not significant as compared with diploids: $P < 0.101$ and $P < 0.23$).

Multivariate statistics – A discriminant analysis with all 16 characters reclassified 92.5% of all toads correctly into the three groups of diploid, tetraploid, and *B. p. baturae* specimens (Table VI). A stepwise discriminant analysis reclassifying the same percentage of specimens (92.5%) selected seven of 16 morphometric traits (Table VII) which had the highest values of standardized canonical discriminant coefficients. In addition, the means of the projection areas of 30 erythrocytes per toad (μm^2) were included in the analysis, as Stöck (1997) suggested. In this way the classification of 100% of all toads was possible (Fig. 16), but numbers (*n*) were lower since blood smears were not available from all animals.

A comprehensive morphometric analysis including 15 body measurements (without mass) was conducted. Four groups of toads were formed. The first two contained *B. p. baturae* from Gilgit (1) and from Pasu + Sust (2). The third group (*B. latastii*) consisted of 12 specimens of Boulenger (1982), Eiselt & Schmidtler (leg.) and Gruber (1981). The fourth group was formed with two animals from Kulalai and the type series of *B. (viridis) pseudoraddei* (Mertens, 1971). Furthermore, the holotype of *B. siachinensis* (Khan, 1997) was included in the discriminant analysis which was aimed to discriminate all four groups mutually and to classify this ungrouped case among them. Figure 17 reports the result. *Bufo p. baturae* from Gilgit and Pasu + Sust were grouped very close to one another. A reclassification showed that some animals were reclassified from Gilgit into the Pasu/Sust population and vice versa, indicating that *B. p. baturae* specimens represent a group of similar animals. In contrast, the third group (*B. latastii*) was arranged as a separate sample. The *B. siachinensis* type was classified close to *B. latastii*, supporting the decision (see above) to be arranged in the same taxon. The fourth group (*B. p. pseudoraddei*) was also separated from the two groups of *B. p. baturae*. Thus, *B. p. baturae* from the Western Karakoram range (Sust, Pasu, Gilgit) represent a separate taxon which can be distinguished morphometrically from *B. p. pseudoraddei* (Swat) and *B. latastii* (= *B. siachinensis*).

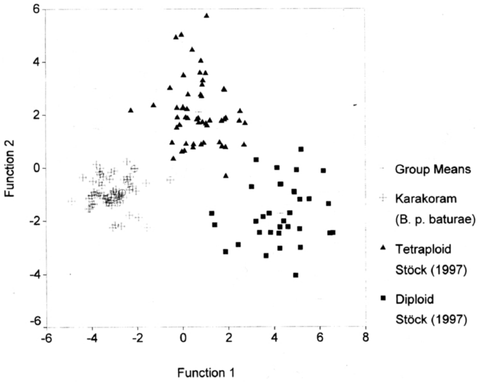

Fig. 16 - Discrimination result of an analysis with 16 morphometric characters and the mean projection area of 30 erythrocytes per toad. Data on diploid and tetraploid specimens from a previous study (Stöck, 1997).

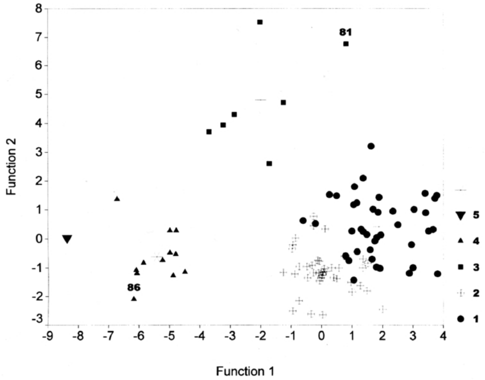

Fig. 17 - Discrimination result of an analysis with four groups of toads and one ungrouped case. Groups: 1 - *Bufo p. baturae*, Gilgit; 2 - *B. p. baturae*, Pasu and Sust; 3 - *B. p. pseudoraddei*, including the type series, holotype (SMF 65628) = No. 81, Swat, and Kulalai; 4 - *B. latastii* from different localities (see also: Material examined), including lectotype (BMNH 1947.2.21.28) = No. 86; 5 - holotype of *B. siachinensis*, junior synonym of *B. latastii*; * group means.

Distribution – Although adapted to high mountain habitats, *B. p. baturae* is, as far as we know, distributed in the Karakoram range along the main valleys of the rivers Hunza and Gilgit (and Indus?) but could not penetrate into more remote valleys with steep rocky slopes. So, the corresponding author did not find any toad under optimal breeding conditions in the Shimshal valley where rock climbing reptiles were frequently observed. In Taxkurgon (China, E- Pamirs, 3350 m a.s.l.) tetraploid toads were detected (Stöck, 1998b).

DISCUSSION

Triploidy in vertebrates

Mosaicism as well as triploidy of bisexual vertebrates are extremely rare phenomena. Mostly, triploidy is coupled with unisexuality (Dawley & Bogart, 1989), and there occur population systems in which diploid bisexual parental species coexist with all-female triploid (or even higher ploidy) hybrids (e.g., Vasil'ev *et al.*, 1989;

Bogart & Klemens, 1997) or/and specimens exhibiting mosaicism (e.g., Dawley & Goddard, 1988). Three methods of reproduction are known: parthenogenesis and gynogenesis which both produce offspring containing only maternal genes, and hybridogenesis in which the sperm is incorporated in the oocyte, but upon maturity of the offspring, one parental genome is eliminated in a meiotic or pre-meiotic event while the rest of the hybrid genome is passed on to future generations, usually in an unaltered state (Bogart & Klemens, 1997). In very scarce cases, triploids comprise males and females and are associated with individuals of other ploidy levels within the populations. The individuals either represent mosaics, as *Platemys platycephala* (Bickham *et al.*, 1993) which appear to reproduce sexually with balanced haploid sperm (Bickham *et al.*, 1993), or hybrids, as the triploids in the hybridogenetic *Rana* kl. *esculenta* complex. In this case, triploid males and females comprise up to 80% in different population systems where they occur together with diploid hybrids and/or with one of the diploid parental species (Günther, 1975, 1990: 230).

Let us examine whether *B. p. baturae* is the first somatic all-triploid bisexual reproducing vertebrate. Until now, final comments on the mode of reproduction in *B. p. baturae* remain speculation. However, all chromosome configurations observed in the testes tissue can be explained if we presume triploid hybrid spermatogonia containing two plus one sets of chromosomes (22 + 11) descending from two parental forms (as in triploid *R.* kl. *esculenta*). A hybridogenetic gametogenesis may include a premeiotic endomitotic duplication of the two different-sized parental portions of the hybrid genome and a synchronous division resulting in two unequal daughter cells containing 44 (22 + 22) and 22 (11 + 11) chromosomes. They form ('pseudo-) bivalents' (Uzzell, 1970) with 11 or 22 pairs of identical chromosomes during diakineses. Mitotic multiplication, as usual in spermatogenesis, would elucidate the finding of diploid, triploid and tetraploid metaphases. Meioses II would produce haploid (and diploid) sperm. Provided that a similar mechanism works in oogenesis, and that diploid sperm would hardly involved in fertilization (as in triploids of many water frog populations - e.g., Günther, 1990), only triploid (and diploid) F_1 can be produced. The formation of viable diploids may be interrupted by the absence of NORs in such combinations resulting from the occurrence of only two NOR-carrying chromosomes in triploid cells (preliminary data).

The phenomena observed in *B. p. baturae* require additional analyses: the investigation of inheritance patterns of the triploid F_1 from triploid parents (in prep.) will reveal whether the reproduction is of a sexual or of gynogenetic nature; the former seems more probable since many males occur and invest a lot of energy in calling and reproduction. Future studies of population genetics should reveal whether the *B. p. baturae* populations are composed purely of triploids or whether specimens of other ploidy levels coexist with a majority of triploids.

The finding of diploid (11) bivalents in the testes appears to be similar to the observations reported by Bickham *et al.* (1993) in *P. platycephala* which also appears to mask a not yet adequately examined hybridogenetic phenomenon, but in *B. p. baturae* we also found tetraploid metaphases and diakineses.

Although several polyploid species have been reported in amphibians (Green & Sessions, 1991), triploid anurans outside the *Rana* kl. *esculenta* and the *B. viridis* complex (see Introduction) have only been found as rare results of mismatings and of hybridizations between individuals of separate species and/or ploidy levels. In the genus *Bufo*, chromosome analyses of about one third of all *Bufo*-species have revealed natural polyploids (tetraploids) in only two species groups (King, 1990). A triploid specimen ($3n = 30$) similar to *B. poweri* was found by Schmid (1978) but could have been a rare spontaneous case.

Karyotypes

The conventionally stained chromosomes in the triploid karyotype are typical of representatives of the *B. viridis* complex (reviews by Roth & Ráb, 1986). A comparison of Q-banded chromosomes within the *B. viridis* complex from different regions of Eurasia (in prep.) exhibited a distribution of Q-banding positive segments in diploid forms which appears to be similar to that of triploid and tetraploid forms.

DNA-content

The best method for field preservation of blood is freezing in liquid nitrogen (Murphy *et al.*, 1997) which was not available in the Karakoram. The cytometric data obtained from ethanol fixed cells (Holtfreter & Cohen, 1990; Birstein *et al.*, 1993) may differ from those of deep-frozen samples (Murphy *et al.*, 1997). The short time of storage, the finding of distinct peaks (no bimodal curves interpreted to represent differences in the stainability of leucocytes and erythrocytes: Holtfreter & Cohen, 1990) were a demonstration of the correctness of our results. DNA content may vary among conspecific individuals, particularly among individuals from different populations and can approach 10% (ref. in Murphy *et al.*, 1997). The variation ranges (Gilgit population: 12.5%; Pasu population: 15.2%) may show slight inaccuracy due to inefficient storage of the blood samples or representing a typical variability caused by polyploidy. Nevertheless, the DNA contents measured confirm the triploidy of all specimens in addition to the karyograms. It has been demonstrated that AT-rich sequences within chromosomal DNA enhance the fluorescence intensity with DAPI and quinacrine (ref. in Schmid, 1980). Since the numbers of Q-banding positive chromosomes in triploid and in tetraploid cells are not proportional (in prep.), this might account for the fact that DAPI stained triploid nuclei exhibit more than 80% of the DNA content of the tetraploid reference cells.

The data on the absolute DNA content in chicken nuclei in the literature are various. We used the value 2.34 pg which is widely utilized in different studies (e.g. Altman & Katz, 1976; Ulrich *et al.*, 1988) and which was proved to be correct by density gradient measurements. The influence of differences in chromatin structure of phylogenetically distant animals could cause a nonproportional correlation of the DNA content, as Birstein *et al.* (1993) supposed, and might be a reason to prefer *Xenopus laevis* nuclei for measurements in amphibians.

Erythrocytes

The ES measurements confirmed the experience (ref. in Stöck & Grosse, 1997) that the cell surface is directly correlated with DNA content. This results in significant differences between the average values of erythrocyte areas in green toad populations with different ploidy levels. However, the variation range within a population is large, and the measurement of the erythrocyte area does not allow the unequivocal determination of the ploidy level of an individual. In combination with morphometric data and multivariate methods the character provides useful information for a prognosis of the classification of green toads.

Mating calls

The voices of all triploid forms from Karakoram and Western Himalayas were rather similar to those of tetraploid representatives of the *B. viridis* complex from Middle Asia (Stöck, 1998a) but differed from those of diploids (Figs 12, 13). Furthermore, the present results confirm the trends in the relationship between call variables and water temperature in the *B. viridis* complex (Lörcher & Schneider, 1973; Nevo & Schneider, 1976; Schneider & Egiasaryan, 1995; Stöck, 1998a). In the light of our study, the conservative call system within the large range of diploid green toads (Schneider & Egiasaryan, 1995; Stöck, 1998a) is supplemented by indications of an evolutionary stability of mating calls in polyploid toads (see also Stöck, 1998b). The similar calls of polyploids either only reflect their conservatism in isolated taxa or may (also) account for the origin of the present triploid forms from the tetraploids, but future studies are necessary. Calls without temperature data analysed in Kashmir and Ladakh (Dubois & Martens, 1977) in *B. latastii* have a fundamental frequency (1200-1300 Hz) only slightly lower than in our study. The pulse duration (30-60 ms) and the pulse rate (8-13 Hz) were in the range of the present results. Dubois (1998) pointed out that "significant differences in the calls of morphologically similar, and sometimes sympatric anurans have proved to indicate specific distinctness", but "the reverse is not true". Under the conditions of strong isolation in the high mountain valleys, allopatric but different taxa may have similar calls because there is no selection against this similarity.

Distribution

Our map (Fig. 2) shows the localities in the Karakoram and Western Himalayas if the material was classified with a taxonomic intention. References by Dutta (1997: 48, 53) to *B. latastii* or *B. viridis* and additional papers (e.g., Khan, 1980) only contained faunal data but failed to deal with taxonomy or morphology. Baig (1988) considered toads from some of our localities (Sust, Pasu, Gilgit) to be *B. viridis*. Bachmann *et al.* (1978) detected in a single toad from Kabul (Afghanistan) "36% more DNA than diploid *B. viridis*", and Borkin *et al.* (1997, Abstracts of the Third World Congress, Prague: 26) found toads from Pamirs to have "a DNA content 25% lower than tetraploids". Both findings may refer to the occurrence of triploid toads also in these regions. The nearest localities where polyploid green toads have been found (Ishkashim, S-Pamirs, Tadzhikistan: see Fig. 2: Y; Khorog, Tadzhikistan - Mezhzherin & Pisanets, 1990) are situated about 200 km from the Hunza valley. Although high mountain ranges probably isolate these populations from those of our study, the Indus basin (upper Hunza and Gilgit valley) and the upper tributaries of the Aral basin (Wakhan, Pyandzh valley) are extremely close to one another (Fig. 2). The lowest passes separating them are about 4000 m a.s.l. Therefore, the prerequisites to a rare exchange during evolutionary periods might be fulfilled since polyploid toads are viable in such elevations.

Taxonomy

"The application of species names to hybrids and unisexual clones is an open invitation to critics who adhere to various species concepts to expound on the utility of such designations" (Bogart & Klemens, 1997). The present triploid toads comprise males and females and appear to be bisexual reproducing mosaics. Our taxonomic and nomenclatural decision to assign the new taxon to a subspecific rank appears the best way to take the presently available data into consideration: both *B. p. pseudoraddei* and *B. p. baturae* seem to be isolated, probably allopatric, somatic triploid taxa with similar mating calls but different morphology. The rarity of triploidy in vertebrates on the one hand but the occurrence in two toad taxa, which differ by sufficient taxonomic and morphological diagnostic characters, on the other hand, provide arguments for considering these toads rather closely related. Therefore, we assigned them preliminarily to the same species but to different subspecies. In addition, both subspecies can be distinguished morphologically from *B. latastii* which appears to occur allopatrically. A revision of green toad taxonomy in India and Pakistan is very important. It should include the determination of the ploidy level(s) of *B. latastii* and *B. v. zugmayeri*, of additional specimens of *B. p. pseudoraddei* and *B. p. baturae* and should clarify the relationships of different taxa in various high mountain valleys. The occurrence of a complex of triploid toad taxa (or more complicated population systems with different ploidy levels) comprising separate species seems possible.

MATERIAL EXAMINED

Institutional abbreviations are as listed in Leviton *et al.* (1985); for localities see Figure 2.

1. *Bufo pseudoraddei baturae* n. ssp.: Gilgit, Karakoram, present paper: ZSM 111/1998, 112/1998 (see also subspecies description in the text).

2. *Bufo pseudoraddei pseudoraddei* (Mertens, 1971): Kulalai, Western Himalayas, present paper: ZSM 106/1998.

3. *Bufo (viridis) pseudoraddei pseudoraddei* Mertens, 1971: Holotype SMF 65628; paratypes SMF 65629, SMF 65630, SMF 65631, SMF 65632; Pakistan, Swat (the type locality is "Mingorah, Swat" but Mertens (1971) considered the subspecies to be a "Montanform" and the real locality in an elevation higher than 1000 m a.s.l.; Stöck could not find any green toad but only *B. stomaticus* in the surroundings of Mingorah).

4. *Bufo viridis zugmayeri* Eiselt & Schmidtler, 1973: Holotype 211/11-2, paratypes: ZSM 212/1911 (1 specimen), ZSM 211/1911/1 (1 specimen), ZSM 211/1911/3 to ZSM 211/1911/18 (16 specimens).

5. *Bufo latastii* Boulenger, 1882. Type series: BMNH 1947.2.21.28 (formerly 72.4.17.223) - lectotype (see text). Four paralectotypes of the type series: BMNH 1947.2.21.29 (formerly 72.4.17.224), BMNH 1947.2.21.30.1, BMNH 1947.2.21.31.1, BMNH 1947.2.21.31 B 1; Ladakh.

6. *Bufo siachinensis* Khan, 1997. Holotype (colour photograph and morphometric data by Khan, 1997; additional morphometric data: Khan M. S., pers. comm.). Its collection number "BMNH 1990.94" cited by Khan (1997) "has never been issued" (Clarke B., BMNH, pers. comm.); the holotype remained in the private collection of Khan (M. S., pers. comm.); Pakistan, Shinu village.

7. *Bufo latastii latastii*. Gruber (1981): ZSM 463/1976: 5 specimens, oasis near Dras, Ladakh, 3200 m a.s.l.; 464/1976: 2 juveniles, Naranag, Kashmir, 2600 m a.s.l.;

8. *Bufo latastii latastii* (unpubl.): ZFMK 36062, ZFMK 19272, India, Ladakh.

9. *Bufo latastii*: Eiselt and Schmidtler leg. (unpubl.): MTKD D 13522, 14592 to 14595: 5 specimens, Tangmary near Srinagar, 2100-2600 m a.s.l.

APPENDIX

The name *B. siachinensis* is a junior synonym of *B. latastii* Boulenger, 1882. Khan (1997: 46): "Though *latastei* [= *latastii*, lapsus calami] has a tarsal gland, longitudinal parotid and narrower interorbital space like *siachinensis* but differs from it due to its wortier [= wartier?] dorsum, presence of a distinct tarsal fold, first finger longer than second and presence of double subarticular tubercles under fingers and toes and dorsal pattern of small spots". This, the single comparison of the newly described *B. siachinensis* with *B. latastii*, contains incorrectness:

1. The lectotype of *B. latastii* (Material examined) exhibits fewer warts than the *B. siachinensis* holotype.

2. Boulenger (1882: 295) wrote about *B. latastii* only: "a tarsal fold". This character seems to be not of taxonomic value because, for instance, *B. viridis, B. raddei* (Boulenger, 1882: 283) and most of the *B. pseudoraddei baturae* specimens also exhibit an inconspicuous tarsal fold like *B. latastii*. It seems probable that *B. siachinensis* has also a very unobtrusive tarsal fold.

3. Boulenger (1882: 295) about *B. latastii*: "First finger not extending beyond second".

4. The examination of the type series of *B. latastii* revealed that some specimens have double subarticular tubercles under first and second fingers and this character appears to be very variable within this species, as already Dubois & Martens (1977) pointed out.

5. Boulenger (1882: 295) wrote: "olive above, spotted or marbled with blackish; a light vertebral stripe; beneath more or less spotted or marbled with blackish." His figure (plate XIX) of the (lecto-)type exhibits a striped dark dorsal pattern which is very similar to that of *B. siachinensis* (Fig. 1).

The material of Gruber (1981) from Dras, very similar to the lectotype of *B. latastii*, was collected only about 120 km from Shinu village, the locus typicus of *B. siachinensis*. Dubois & Martens (1977) collected *B. latastii* in Kargil, only about 90 km from Shinu. The three localities are connected by the upper tributaries of the Indus river system which provides preconditions for an easy distribution (Fig. 2).

REFERENCES

Altman P. L., Katz D. D., 1976 - Biological handbook I, Cell biology. Fed. Amer. Soc. Exp. Biology, Bethesda, Maryland.

Bachmann K., Konrad A., Oeldorf E., Hemmer H., 1978 - Genome size in the green toad (*Bufo viridis* group). Experientia, *34*: 331-332.

Baig K. J., 1988 - Anurans (Amphibia) of Northern Pakistan with special reference to their distribution. Pakistan J. Sci. Ind. Res., *31*: 651-655.

Bickham J. W., Hanks B. G., Hale D. W., Martin J. E., 1993 - Ploidy diversity and the production of balanced gametes in male twist-necked turtles (*Platemys platycephala*). Copeia, *1993*: 723-727.

Birstein V. J., Polataev A. I., Goncharov B. F., 1993 - DNA content in Eurasian sturgeon species determined by flow cytometry. Cytometry, *14*: 377-383.

Bogart J. P., 1972 - Karyotypes. *In*: W. F. Blair (ed.), Evolution in the genus *Bufo*. University of Texas Press, Austin, London, pp. 171-232.

Bogart J. P., Klemens M. W., 1997 - Hybrids and genetic interactions of mole salamanders (*Ambystoma jeffersonianum* and *A. laterale*) (Amphibia: Caudata) in New York and New England. Am. Mus. Novitates, Am. Mus. nat. Hist., New York, 3218, 78 pp.

Borkin L. Ya., Kuzmin S. L., 1988 - [Amphibians of Mongolia, species account]. *In*: Ch. Munchbajar, E. I. Vorob'eva, I. S. Darevskij, D. V. Semenov (eds), Amphibians and reptiles of Mongolian Peoples Republic. General problems. Nauka, Moskva, pp. 30-197, (in Russian).
Borkin L. Ya., Terbish Ch., Caune I. A., 1986a - Tetraploidnaya i diploidnye populyacii zhab gruppy *Bufo viridis* iz Mongolii. Doklady Akad. Nauk SSSR, *287*: 760-764, (in Russian).
Borkin L. Ya., Caune I. A., Pisanets E. M., Rozanov Y. M., 1986b - Karyotype and genome size in the *Bufo viridis*-group. *In*: Z. Rocek (ed.), Studies in herpetology. Proceed. Ord. Gen. Meet. Soc. Europ. Herpetol. Prague, pp. 137-141.
Boulenger G. A., 1882 - Catalogue of the Batrachia Salientia in the Collection of the British Museum. 2nd ed. London.
Castellano S., Giacoma C., Dujsebayeva T., Odierna G., Balletto E. 1998 - Morphological and acoustical comparisons between diploid and tetraploid green toads. Biol. J. linn. Soc., *63*: 257-281.
Dawley R. M., Bogart J. P. (eds), 1989 - Evolution and ecology of unisexual vertebrates. Mus. Bull. 466. Univ. State New York, State Educ. Dep., New York State Museum, Albany, New York.
Dawley R. M., Goddard K. A., 1988 - Diploid-triploid mosaics among unisexual hybrids of minnows *Phoxinus eos* and *Phoxinus neogaeus*. Evolution, *42*: 649-659.
Dubois A., 1998 - Lists of European species of amphibians and reptiles: will we soon be reaching "stability"?. Amphibia-Reptilia, *19*: 1-28.
Dubois A., Martens J., 1977 - Sur les crapauds du groupe de *Bufo viridis* (Amphibiens, Anoures) de l'Himalaya occidental (Cachmire et Ladakh). Bull. Soc. Zool. France, *102*: 459-465.
Duda P. L., Opendar K., 1971 - The karyotype of *Bufo* sp. from Kashmir. Chromosome Inform. Serv., *12*: 18-20.
Dutta S. K., 1997 - Amphibians of India and Sri Lanka (Checklist and bibliography). Odyssey, Bhubaneswar, Orissa (ISBN 81-86854-01-0), 342 + xxii pp.
Eiselt J., Schmidtler J. F., 1973 - Froschlurche aus dem Iran unter Berücksichtigung außeriranischer Populationsgruppen., Ann. Naturhistor. Mus. Wien, *77*: 181-243.
Green D. M., Sessions S. K. (eds), 1991 - Amphibian cytogenetics and evolution. Academic Press, inc. Harcourt Brace Javanovich, San Diego, New York, 456 pp.
Gruber U., 1981 - Notes on the Herpetofauna of Kashmir and Ladakh. Brit. J. Herpetol., *6*: 145-150.
Günther R., 1975 - Zum natürlichen Vorkommen und zur Morphologie triploider Teichfrösche "*Rana esculenta*", L., in der DDR (Anura, Ranidae). Mitt. Zool. Mus. Berlin, *51*: 145-158.
Günther R., 1990 - Die Wasserfrösche Europas. Die Neue Brehm-Bücherei, 600. A. Ziemsen, Wittenberg, 288 pp.
Hemmer H., Schmidtler J. F., Böhme W., 1978 - Zur Systematik zentralasiatischer Grünkröten (*Bufo viridis*-Komplex) (Amphibia, Salientia, Bufonidae). Abh. Mus. Tierkd. Dresden, *34*: 349-384.
Holtfreter H. B., Cohen N., 1990 - Fixation associated quantitative variations of DNA fluorescence observed in flow cytometric analysis of homopoietic cells from adult diploid frogs. Cytometry, *11*: 676-685.
ICZN, 1985 - International Code of Zoological Nomenclature. Adopted by the Assembly of the International Union of Biological Sciences. Third edition. International Trust for Zoological Nomenclature in association with British Museum Natural History London, University of California Press, Berkeley and Los Angeles, 338 pp.
Inger R. F., 1972 - *Bufo* of Eurasia. *In*: W. F. Blair (ed.), Evolution in the genus *Bufo*. University Texas Press, Austin, London, pp. 171-232.
Khan M. S., 1980 - Affinities and Zoogeography of Herpetiles of Pakistan. Biologia (Lahore), *26*: 113-171.
Khan M. S., 1997 - A New toad of the Genus *Bufo* from the foot of Siachin Glacier, Baltistan, Northeastern Pakistan. Pakistan J. Zool., *29*: 43-48.
King M., 1990 - Amphibia. Vol. 4. *In*: B. John, Y. Kayano & A. Levan (eds.), Animal cytogenetics. Chordata 2. Gebrüder Borntraeger, Berlin, Stuttgart.
Kudryavcev B. N., Ni V. V., Borkin L. Ya., Solov'eva L. V., 1988 - Soderzhanie RNK i belka v kletkakh diploidnych i tetraploidnykh zhab gruppy *Bufo viridis*. Citologia, *11*: 1324-1331, (in Russian).
Kuzmin S. L., 1995 - Die Amphibien Rußlands und angrenzender Gebiete. Neue Brehm-Bücherei. Westarp Wissenschaften, Magdeburg, 275 pp.
Leviton A. E., Gibbs jr. R. H., Heal E., Dawson C. E., 1985 - Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia *1985*: 802-832.
Lörcher K., Schneider H., 1973 - Vergleichende bio-akustische Untersuchungen an der Kreuzkröte, *Bufo calamita* (LAUR.), und der Wechselkröte, *Bufo v. viridis* (LAUR.). Z. Tierpsychol., *32*: 506-521.
Mazik E. Yu., Kadyrova B. K., Tokotosunov A. T., 1976 - Osobennosti kariotipa zelenoi zhaby (*Bufo viridis*) v Kirgizii. Zool. Zhurnal, *55*: 1740-1742, (in Russian).
Mertens R., 1971 - Die Amphibien und Reptilien West-Pakistans. 2. Nachtrag. Senckenberg. biol., *52*: 7-15.
Mezhzherin S. V., Pisanets E. M., 1990 - Geneticheskaya struktura i proischozhdenie aziatskikh poliploidnikh zhab *Bufo danatensis* Pisanets 1978. Doklady Akad. Nauk Ukrainskoi SSR, Ser. B, Geol., khim. i biolog. nauki, *8*: 71-73, (in Russian).
Mezhzherin, S. V., Pisanets E. M., 1995a - Genetic structure and origin of the tetraploid toad *Bufo danatensis* Pisanets, 1978 (Amphibia, Bufonidae) from Central Asia: Description of biochemical polymorphism and comparision of heterozygosity levels in diploid and tetraploid species. Genetica, *31*: 43-53, (in Russian).
Mezhzherin S. V., Pisanets E. M., 1995b - Genetic structure and origin of the tetraploid toad *Bufo danatensis* Pisanets, 1978 (Amphibia, Bufonidae) from Central Asia: Differentiation of geographic forms and genetic relationship between diploid and tetraploid species. Genetica, *31*: 342-352, (in Russian).
Minton, S. A., 1967 - A contribution to the herpetology of West Pakistan. Bull. Am. Mus. nat. Hist., *134*: 29-185.
Murphy R. W., Lowcock L. A., Cheryl S., Darevsky I. S., Orlov N., MacCulloch R. D., Upton D. E., 1997 - Flow cytometry in biodiversity surveys: methods, utility and constraints. Amphibia-Reptilia, *18*: 1-13.
Nevo E., Schneider H., 1976 - Mating call pattern of Green toads in Israel an its ecological correlate. J. Zool., Lond., *178*: 133-145.
Otto F. J., 1994 - High resolution analysis of nuclear DNA employing the fluorochrome DAPI. *In*: Z. Darzynkiewicz, J. P. Robinson & H. A. Crissman (eds), Methods in cell biology. Academic Press, San Diego, pp. 211-217.
Pisanets E. M., Shcherbak N. N., 1979 - Sistematika zelenykh zhab (Amphibia, Anura) fauny SSSR. Vestn. Zool., *4*: 11-16. (in Russian).
Roberts T. J., 1991 - The birds of Pakistan. Vol. 1. Oxford University Press, Karachi, 598 pp.
Roth P., 1986 - An overview of the systematics of the *Bufo viridis* group in Middle and Central Asia. *In*: Z. Rocek (ed.), Studies in herpetology. Proc. Ord. Gen. Meet. Soc. Europ. Herpetol., Prague, pp. 127-130.
Roth P., Ráb P., 1986 - Karyotype analysis of the *Bufo viridis*-group: Systematic implications. *In*: Z. Rocek (ed.), Studies in herpetology. Proc. Ord. Gen. Meet. Soc. Europ. Herpetol., Prague, pp. 131-136.
Schmid M., 1978 - Chromosome banding in Amphibia. I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla*. Chromosoma, *66*: 361-388.
Schmid M., 1980 - Chromosome banding in Amphibia IV. Differentiation of GC- and AT-rich chromosome regions in Anura. Chromosoma *77*: 83-103.
Schneider H., Egiasaryan E. M., 1995 - The mating call of the green toad, *Bufo viridis viridis* (Anura, Amphibia), in Kazakhstan. Russian J. Herpetol., *2*: 1-4.
Schroer T., 1996 - Morphologie und Ploidiegrade von Wasserfröschen aus unterschiedlichen Populationssystemen in Nor-

dost-Polen (Anura, Ranidae). Z. Feldherp., *3*: 133-150.

Stöck M., 1997 - Untersuchungen zur Morphologie und Morphometrie di- und tetraploider Grünkröten (*Bufo viridis* -Komplex) in Mittelasien (Amphibia: Anura: Bufonidae). Zool. Abh. Staatl. Mus. Tierkd. Dresden, *49*: 193-222.

Stöck M., 1998a - Mating call differences between diploid and tetraploid green toads (*Bufo viridis* complex) in Middle Asia. Amphibia-Reptilia, *19*: 29-42.

Stöck M., 1998b - Tetraploid toads (*Bufo viridis* complex) from north-western China and preliminary taxonomic conclusions for *Bufo nouettei* Mocquard, 1910. Z. Feldherp. (Bochum), *5*: 139-166.

Stöck M., Grosse W.-R., 1997 - Erythrocyte size and ploidy determination in green toads (*Bufo viridis* complex) from Middle Asia. Alytes (Paris), *15*: 72-90.

Stöck M., Grosse W.-R., in press - Der *Bufo viridis* -Komplex in Mittel- und Zentralasien: Eine Übersicht zu Verbreitung, Polyploidie, Paarungsrufen und Taxonomie. Mertensiella.

Stöck M., Grosse W.-R., Schmid M. & Steinlein C., 1998 - Ploidy level diversity in the *Bufo viridis* complex of Central Asia. Zoology, *101*, Suppl. I (1998): 76.

Ulrich W., Fritz B., Fritz J. P., 1988 - Durchflußzytophotometrische DNA-Bestimmung bei ausgewählten Amphibien- und Reptilien-Arten. Bonn. zool. Beitr., *39*: 49-58.

Uzzell T., 1970 - Meiotic mechanisms of naturally occurring unisexual vertebrates. Am. Nat., *104*: 433-445.

Vasil'ev V. P., Vasil'eva K. D., Osinov A. G., 1989 - Evolution of a diploid-triploid-tetraploid complex in fishes of the genus *Cobitis* (Pisces, Cobitidae). *In*: R. M. Dawley & J. P Bogart (eds), Evolution and ecology of unisexual vertebrates, Mus. Bull. *466*. Univ. State New York, State Educ. Dep., New York State Museum, Albany, New York, pp. 153-169.