Tetraploid toads (*Bufo viridis* complex) from north-western China and preliminary taxonomic conclusions for *Bufo nouettei* MOCQUARD, 1910*

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Tetraploide Kröten (*Bufo viridis* complex) aus Nordwest-China und vorläufige taxonomische Schlußfolgerungen für *Bufo nouettei* MOCQUARD, 1910

Einer der Syntypen von *Bufo nouettei* MOCQUARD, 1910 aus Kashgar (China) repräsentiert ein tetraploides Taxon. Er wurde als Lectotypus (MNHN 19090087) von *B. nouettei* MOCQUARD, 1910 ausgewählt und beschrieben. Die übrigen Paralectotypen gehören zu *B. raddei*. Von lebend untersuchten Vergleichstieren der Terra typica des Lectotypus (Kashgar) werden Fotografien mitotischer Metaphasen mit 44 Giemsa-gefärbten Chromosomen und eine durchflußzytometrische Messung gezeigt. Die Paarungsrufe der Kröten aus Kashgar sind ähnlich denen anderer tetraploider Kröten. 22 topotypische Adulti, die dem Lectotypus morphometrisch ähnelten, zeigten eine heterogene Färbung, die von einfarbig Kakaobraun bis Kakaobraun mit kleiner olivgrüner Fleckung reichte. Ein Kreuzungsexperiment mit einem Männchen aus dem West-Tien Shan ergab deformierte Kaulquappen und eine starke Zunahme der Mortalität, die eine Separation von *B. nouettei* von den mittelasiatischen tetraploiden Taxa anzeigt. Aus dem Ost-Tien Shan und dem Ost-Pamir werden Metaphasen und Paarungsrufdaten anderer tetraploider Grünkröten vorgestellt.

Schlagwörter: Amphibia, Anura, Bufonidae, *Bufo viridis* Komplex, *B. nouettei* Lectotypus, *B. raddei*, Chromosomen, DNA, Paarungsrufe, Morphologie, Morphometrie, Verbreitung, Systematik.

Summary

One of the syntypes of *Bufo nouettei* MOCQUARD, 1910 from Kashgar (China) represents a tetraploid taxon. It is selected and designated as the lectotype (MNHN 19090087) of *B. nouettei* MOCQUARD, 1910. The remaining paralectotypes belong to *B. raddei*. Photographs of mitotic metaphases with 44 Giemsa-stained chromosomes and a flow cytometric measurement of living reference specimens from the terra typica of the lectotype (Kashgar) are shown. The mating calls of toads from Kashgar are similar to those of other tetraploid toads. 22 topotypic adults being morphometrically similar to the lectotype exhibited a heterogeneous coloration which varies from uniformly cacao-brown to cacao-brown with small olive-green spots. A crossing experiment involving a tetraploid male from W-Tien Shan resulted in deformed tadpoles and strong increase of mortality indicating a separation of *B. nouettei* from the Middle Asian taxa. Mating call data and metaphases of other tetraploid green toads from the East Tien Shan and the East Pamirs are presented.

Key words: Amphibia, Anura, Bufonidae, *Bufo viridis* complex, *B. nouettei* lectotype, *B. raddei*, chromosomes, DNA, mating calls, morphology, morphometry, distribution, systematics.

* This publication is part of the planned dissertation thesis of the author.

1 Introduction

Bufo nouettei was described by MOCQUARD (1910) from northern China. The specimens from the type series were collected at three localities (Fig. 1): one specimen from Kashgar (= Kashi Shi, Xinjiang, 39° 29′ N, 75° 58′ E, ZHAO & ADLER 1993), and the other ones from the west and north slope of the Nan-Shan: five specimens from Cha-Tcheou, Kan-Sou (= Dunhuang Xian, Gansu Sheng, 40° 10′ N, 94° 50′ E, ZHAO & ADLER 1993), and one specimen from Sou-Tcheou, Kan-Sou (= Jiuquan Xian, Gansu, 39° 46′ N, 98° 34′ E, ZHAO & ADLER 1993). MOCQUARD (1910) unequivocally wrote that seven specimens were captured, and he listed how many individuals originated from each locality, but he did not note the collection numbers. Today, e i g h t specimens (MNHN 19090081-87 and 19090038) are designated to be syntypes of *B. nouettei*.

Obviously, the specimen with the disconnected number MNHN 19090038 arrived at the collection at the same time as the remaining types and it is not understandable that MOCQUARD only mentioned seven specimens (OHLER in litt.). Interestingly, POPE & BORING (1940/41) also wrote »MOCQUARD based *nouettei* on eight specimens«.

MOCQUARD (1910) supposed that his new species would be closely related to *B. raddei* STRAUCH, 1876. MOCQUARD (1910) distinguished *B. nouettei* from *B. raddei* particularly by the following characters: its more slender habitus, a more elongated and depressed, gutter-like hollowed mouth, its longer parotids, a more developed webbing of the digits, a smaller outer metatarsal tubercle, and finally its coloration.

The coloration is described by MOCQUARD (1910): the dorsal part uniformly brown blackish, or with some black dorsal spots, the ventral view backwards mouselike grey, becoming more light (distinct?) up to the forepart, and shading into a rose under the throat.

POPE (POPE & BORING (1940/41) examined the (»eight«) types of »MOCQUARD's *nouettei* in Paris«, and »found nothing to separate them from *raddei*«. This determination is also cited by LIU (1950). Although *B. nouettei* has been repeatedly synonymised until today with *B. raddei* (e.g., LIU & HU 1961, ZHAO & ADLER 1993), in fact, systematical work examining the type series has been rarely done. Only MERTENS (1971) examined the syntypes (how many?) and previously presumed that *B. nouettei* were a subspecies of *B. raddei*. PETERS (1971) supposed a taxonomic heterogeneity within the type series when he mentioned in a footnote that one of seven of MOCQUARD's specimens came from Kashgar and would be probably a *B. viridis* whereas the others represent, according to POPE & BORING (1940/41), *B. raddei*. EISELT & SCHMIDTLER (1973), examining two topotypic females from Kashgar, presumed that *B. nouettei* could be a subspecies of *B. viridis*. HEMMER et al. (1978) arranged *B. nouettei* into »their« *B. latastii* cf. *oblongus* (which was considered a taxonomic misinterpretation by some authors: e.g., PISANETS & SHCHERBAK 1979, BORKIN & KUZMIN 1988), but HEMMER et al. (1978) did not exclude a re-evaluation of *B. nouettei* in future. BORKIN & KUZMIN (1988) cited EISELT & SCHMIDTLER (1973) as well as HEMMER et al. (1978) and mentioned that the ploidy level of *B. nouettei* is unknown.

Since cytogenetic investigations in north-western China (WHU & ZHAO 1987) only revealed tetraploid toads from western Xinjiang, STÖCK (1995, 1997a) previously supposed that the taxon could be also tetraploid.

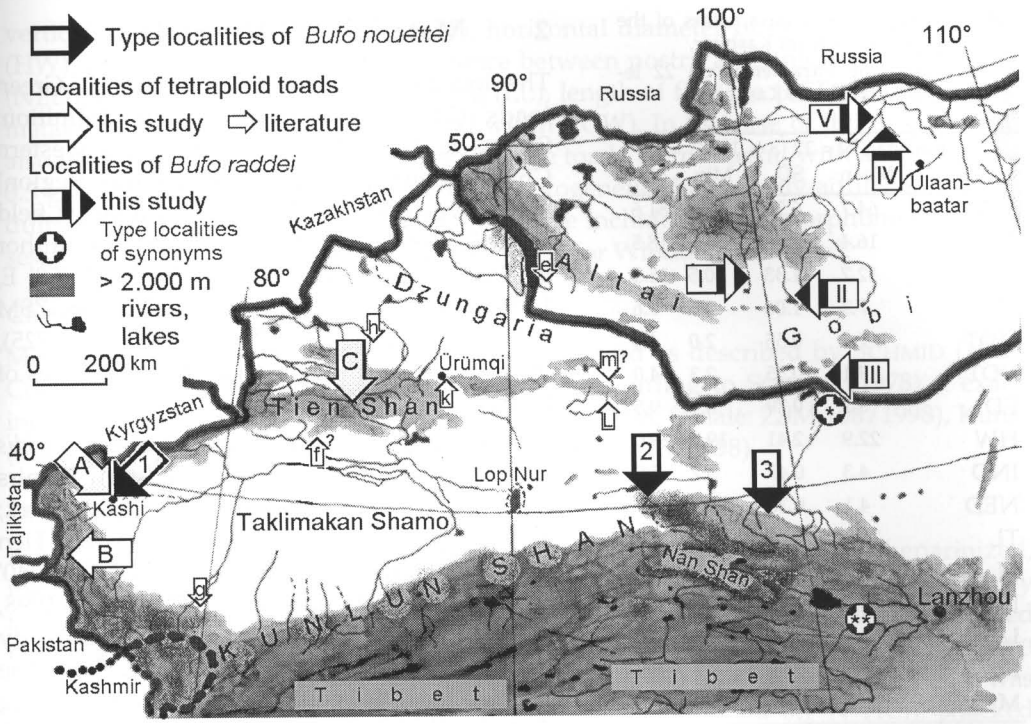


Fig. 1: Localities in Mongolia and China (Xinjiang Uygur Autonomous Region). [Fundorte in der Mongolei und China (Xinjiang, Autonome Uygurische Region).] 1–3: Localities of syntypes of *B. nouettei* MOCQUARD, 1910. [Fundorte der Syntypen von *B. nouettei* MOCQUARD, 1910]. 1: Kashgar (= Kashi Shi, Xinjiang), MNHN 190987, now Lectotype of *B. nouettei*, see text. 2: Cha-Tcheou (Kan-Sou = Dunhuang Xian, Gansu Sheng), MNHN 19090081 to 19090085. MNHN 19090038. 3: Sou-Tcheou (Kan-Sou = Jiuquan Xian, Gansu), MNHN 19090086 (MNHN-catalogue: »Marais de Hé ti man, Ngun Si tchen, Kan Sou«). A–C: Localities of tetraploid green toads investigated by the author in the field. [Fundorte tetraploider Grünkröten, die vom Autor im Freiland untersucht wurden.] A: Kashgar (including: ZSM 107/1998, ZSM 108/1998, MTKD D 40725, MTKD D 40724). B: Taxkurgan (including: ZSM 110/1998). C: Kunas (including: ZSM 109/1998, MTKD D 40726). e–m: Localities of tetraploid green toads or presumed tetraploids from literature. [Fundorte tetraploider Grünkröten oder vermuteter Tetraploider aus der Literatur.] e: Bulgan Gol (Mongolia), tetraploid toads, MEYER (1991), MTKD D 40627 to 40632. See also many records of tetraploids in this region in the environs of about 50 km in the map of BORKIN & KUZMIN (1988: 60). [Siehe auch zahlreiche Nachweise von Tetraploiden im Umkreis von ca. 50 km in der Karte von BORKIN & KUZMIN (1988).] f?: Koutchar (= Kuqa Xuan, Xinjiang), '*B. viridis*', MOCQUARD (1910), MNHN 190980, 190977. Presumed tetraploids (only morphologically recorded). [Vermutete Tetraploide (nur morphologisch festgestellt).] g: Khotan (WHU & ZHAO 1987), tetraploid karyotype. h: Wusu (WHU & ZHAO 1987), tetraploid karyotype. k: Ürümqi (WHU & ZHAO 1987), tetraploid karyotype. L: Hami (WHU & ZHAO 1987), tetraploid karyotype. m?: Hami prefecture, 12 km NE Koumenzi, 2 090 m a.s.l. (ZHAO & ADLER 1993), presumed tetraploid specimen (only morphologically recorded). [Vermutetes tetraploides Exemplar (nur morphologisch festgestellt).] I–V: Mongolian localities of *B. raddei* investigated in the present study. [Mongolische Fundorte von *B. raddei*, die in vorliegender Arbeit untersucht wurden.] I: Baydrag Gol river, W Bömbögör, 46° 10' N; 99° 20' E, MTKD D 40623 to 40626 (leg. D. HEIDECKE, 01.06.88) and bridge over Baydrag Gol river, 150 km W Ulanbataar, MTKD D 40612 to 40622 (leg. A. STUBBE, July 1989); II: Ich-Bogd, Orog-Nuur, Bayanchongor-Aimak, MTKD D 17324 to 17327 (leg. M. DORN, 14.08.1979); III: 150 km SW Dalandzadgad, ZMB 47853 (leg. N. SCHNEEWEISS); IV: Baruunharaa, ZMB 51060 (leg. OBST); V: Orchon river, MTKD D 3234 (leg. F. J. OBST) and living specimen (leg. M. BIEDERMANN) in captivity.

The present study was mainly aimed to collect data on living green toads from north-western China, to compare them with toads from other parts of Asia and to clarify the systematic rank of *B. nouettei*.

Tab. 1: Morphometric parameters of the 22 living specimens from Kashgar.
Morphometrische Parameter von 22 lebenden Exemplaren aus Kashgar.

	Kashgar, N = 22 (3 ♀, 19 ♂)			
(mm)	Mean	SD	Min	Max
SVL	64.7	6.72	52.0	81.6
HL	16.4	1.46	14.0	18.5
PL	12.7	1.33	10.5	15.5
PW	7.3	1.06	5.6	9.6
HDT	2.8	0.55	2.0	4.0
VDT	3.1	0.55	2.3	4.0
ED	8.2	0.79	6.9	9.5
HW	22.9	2.41	19.9	29.0
IND	4.3	0.47	3.2	5.0
NED	4.1	0.36	3.5	4.9
TL	23.9	2.76	17.0	28.9
LL	40.7	3.43	37.0	48.0
LFT	3.6	0.56	2.8	5.1
LiMT	3.8	0.52	2.8	4.6
IOW	4.5	0.61	3.7	5.9
Mass (g)	22.8	6.97	14.0	40.5

2 Material and Methods

The localities are shown in Figure 1. Green toads (*Bufo viridis* complex; for definition: STÖCK & GROSSE 1997) from north-western China (Xinjiang Uygur Autonomous Region) were investigated in June 1996. Intensive field investigations were carried out by the author in Kashgar (= Kashi Shi, 39° 29' N, 75°58' E, 1 350 m a.s.l.; including: ZSM 107/1998, ZSM 108/1998, MTKD D 40724, MTKD D 40725), the most western of the three type localities of *B. nouettei*.

Furthermore, some field data on the *B. viridis* complex were collected in the eastern Pamirs (Taxkurgan: 37° 47' N, 75° 14' E, 3350 m a.s.l.; including: ZSM 110/1998) and the eastern Tien Shan (E-Narat-Shan, Kunas: 43° 14' N, 84° 40' E, 2 145 m a.s.l.; including: ZSM 109/1998, MTKD D 40726). These records have already been published (STÖCK et al. 1998). Since the last two localities could be visited only during short journeys, the sample size is very small. In Taxkurgan, only one female was caught.

Into the morphological comparisons I included 22 living animals from Kashgar (females were underrepresented, see Tab. 1), 34 diploid green toads from Middle Asia (STÖCK 1997a), 106 (59 for HDT, NED, PW, IOW and for multivariate comparisons) tetraploid specimens from Middle Asia (STÖCK 1997a) and six tetraploid toads from Bulgan Gol (Mongolia, Hovd Aimak, MTKD D 40627–40632; MEYER 1991).

Furthermore, I examined the eight specimens designated as syntypes of *B. nouettei* (MNHN 19090081 to MNHN 19090087, MNHN 19090038), five additional preserved specimens (MNHN 19090074 to 190977, 19090080; MOCQUARD 1910: 153 – »*B. viridis*«) from Koutchar = Kuqa Xian, Xinjiang, 41° 43' N, 82° 54' E, ZHAO & ADLER (1993).

In addition, I included 24 specimens of *B. raddei* from different localities, most of them from Mongolia: 15 specimens from Baydrag Gol (MTKD D 40612 to 40626), four specimens from Ich-Bogd (Orog-Nuur, Bayanchongor-Aimak, MTKD D 17324 to 17327), two specimens from Orchon river (MTKD D 3234 and one in captivity), one specimen from Suuncharaa (ZMB 51060), one specimen 150 km SW from Dalandzadgad (ZMB 47853). Two *B. raddei* were from Russia: Blagoreshensk (MTKD D 18273) and Kissakewitch (ZMB 9868).

Morphometry

15 body measurements were taken to the nearest 0.1 mm with dial callipers according to STÖCK (1997a): 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 (LiMT), interorbital width (IOW). In the case of living specimens, also mass was measured 6 to 8 hours after the toads were caught, with an accuracy of 0.5 g using a spiral dynamometer. Due to ontogenetic change only adults and semiadults (secondary sex differences in males) were included in the morphometric analyses. For statistic analyses, the program SPSS 7.5 for Windows was used.

Chromosomes

Metaphase plates from bone marrow were prepared as described by SCHMID (1978). Conventional Giemsa-staining was performed according to SCHMID (1978). I examined four animals: from Kashgar (male: ZSM 107/1998, female: ZSM 108/1998), Kunas (male: ZSM 109/1998) and Taxkurgan (female: ZSM 110/1998).

DNA content

A blood sample of one toad from Kashgar (MTKD D 40724) was slightly heparinized, injected quickly into 70 % ethanol (room temperature), and diffused in the solution by repeated pumping through the needle. Preparation for flow cytometry was performed in the Department of Human Genetics, Würzburg, following a protocol by OTTO (1994): 1.5 ml ethanol fixed cells were centrifuged (1400 min^{-1}; 8 min), the pellet was resuspended in 1 ml Pepsin solution (50 mg Pepsin + 10 ml 0.1 N HCl), shacked 15 min at room temperature and stained for 2–3 hours with 4.5 ml DAPI solution (5.9 g tri-Sodiumcitrate × 2 H_2O + 0.2 mg DAPI in 100 ml H_2O). Measurements were conducted with Partec CA II cytometer. Chicken red blood cells were used as standards.

Erythrocyte size

I used air dried blood smears and measured the projection areas of 30 erythrocytes per toad (22 specimens) with the image analyses system Cydok (HILGERS) as described by STÖCK & GROSSE (1997).

Mating calls

Voices of eleven males from Kashgar, of three males from Kunas and of two males from Taxkurgan were recorded with a walkman (WM 6 DC; Sony) and a stereo microphone (ME 66 with module K 6; Sennheiser) during dusk or night from a distance of 1.0 to 4.0 m in the breeding ponds. Air and water 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 (1998). Call duration and call interval duration were determined only if more than one call was recorded without visible disturbing of the male by other males or by the operator.

Crossing experiment

A female tetraploid toad from Kashgar (MTKD D 40725) was crossed with a tetraploid male from Chatkal (Western Tien-Shan, 41° 35′ N, 70° 07′ E, Uzbekistan, 80 km E Tashkent, Chatkal Nature Reserve, 5 km E Burchmulla, alt. 900 m; MTKD D 40727).

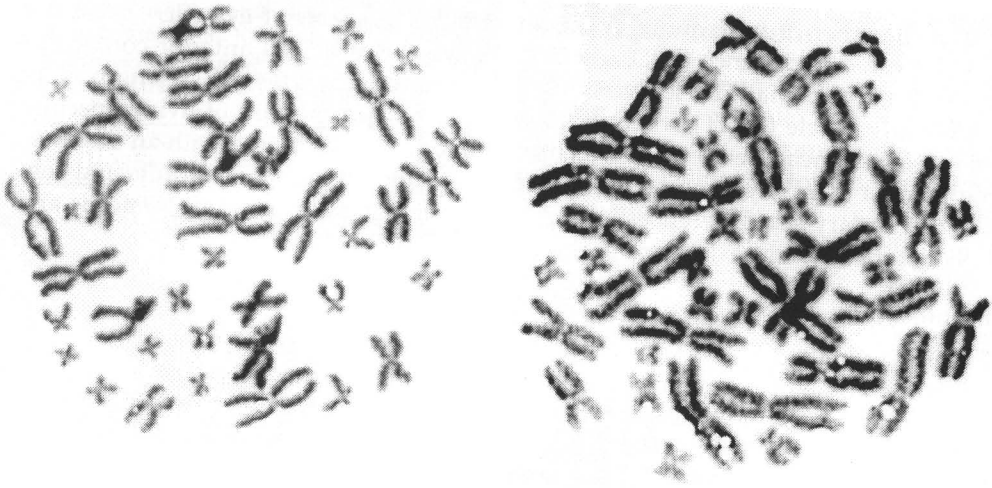


Fig. 2/3: Conventionally Giemsa-stained metaphases of a ♂ from Kashgar (ZSM 107/1998) (left) and a ♀ from Taxkurgan (ZSM 110/1998) (right).
Konventionell Giemsa-gefärbte Metaphase eines ♂ aus Kashgar (ZSM 107/1998) (links) und eines ♀ aus Taxkurgan (ZSM 110/1998) (rechts).

Both specimens still live in captivity. As a control, the same female from Kashgar was isolated from the first male and was allowed to reproduce with a tetraploid male (MTKD D 40724) from the Kashgar population. In both experiments the males started calling in the terrarium indicating their fitness for reproduction. After hibernation in a terrarium, the female was stimulated with an intramuscular injection of the hormone Predalon® (Human Choriongonadotropin, ORGANON, Germany) according to HERRMANN (1988). Clutch strings were allowed to develop in separate similar aquariums ($20 \times 30 \times 15$ cm^3). The first week of the development of the F_1 embryos and tadpoles was documented using tables of staged series by FOX (1986) and GROSSE (1997). After this, from each pairing 30 randomly chosen tadpoles (stage III_{10}, = stage 25) were fed with Tetramin® until metamorphosis, if they survived. The ploidy level of one tadpole from each F_1 was determined using intestine tissue and the method of MACGREGOR & VARLEY (1983).

3 Results

Karyotype

All four specimens studied from the three different localities were found to be tetraploid. The photographs of the metaphases show 44 conventionally stained chromosomes (Fig. 2/3). A detailed cytogenetic study including toads from present localities is in preparation. As in many other *Bufo* species (BOGART 1972, KING 1990), the chromosomes are largely metacentric or submetacentric. The metaphases show a distinct demarcation in size between the larger elements (quartets 1 to 6) and the remaining five quartets. The conventionally stained chromosomes do not show differences in form and size within a quartet.

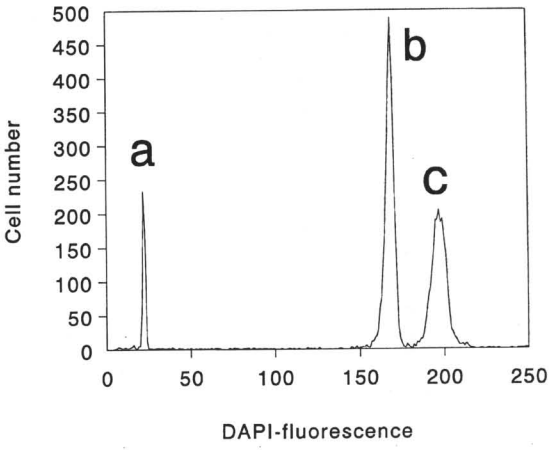


Fig. 4: Histogram obtained by DNA flow cytometry from a mixture of DAPI stained blood samples with chicken as the standard (a), CV = 3.7 %, a triploid green toad from Gilgit, Pakistan (STÖCK et al. 1998) (b), CV = 1.16 %, and a tetraploid green toad from Kashgar (China) (c), CV = 1.98 %. Total cell number 5 830.

Histogramm, das mittels DNA-Durchfluß-zytometrie aus einer Mischung DAPI-gefärbter Blutproben mit Huhn als Standard (a), CV = 3.7 %, einer triploiden Grünkröte aus Gilgit, Pakistan (STÖCK et al. 1998) (b), CV = 1.16 %, und einer tetraploiden Grünkröte aus Kashgar (China) (c), CV = 1.98 % gewonnen wurde. Gesamtzellzahl 5 830.

DNA content

The relative mean DNA content in the Kashgar specimen was 7.48× the content of the reference standard chicken DNA (Fig. 4.). I calculated an absolute DNA content of 17.5 pg per tetraploid nucleus since I used the value 2.34 pg/chicken nucleus.

Tab. 2: Erythrocyte size (projection areas) in the population Kashgar.
Erythrozytengröße (Projektionsflächen) in der Population Kashgar.

Parameter (µm²)	Kashgar N = 22
Mean of means in the population	333.21
Maximal mean in the population	359.24
Minimal mean in the population	323.75
Largest cell measured	428.39
Smallest cell measured	264.91
Mean standard deviation in the pop.	26.30

Erythrocyte size

The parameters are shown in Table 2. 22 toads from Kashgar exhibited an average erythrocyte area of 333.21 µm². Since the mean erythrocyte size was larger than 323 µm² in all toads examined (including the karyotyped specimens), I concluded that all specimens investigated from Kashgar were tetraploid according to STÖCK & GROSSE (1997).

Mating calls

Within the monotonous 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 (Fig. 5). Typically, first pulses were either shorter or longer and had a lower frequency.

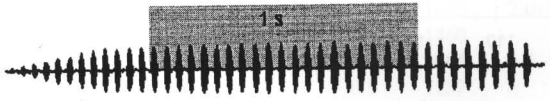


Fig. 5: Oscillogram of a mating call of a tetraploid ♂ recorded in Kashgar, water temperature 24 °C.
Oszillogramm eines Paarungsrufes eines tetraploiden ♂, aufgenommen in Kashgar, Wassertemperatur 24 °C.

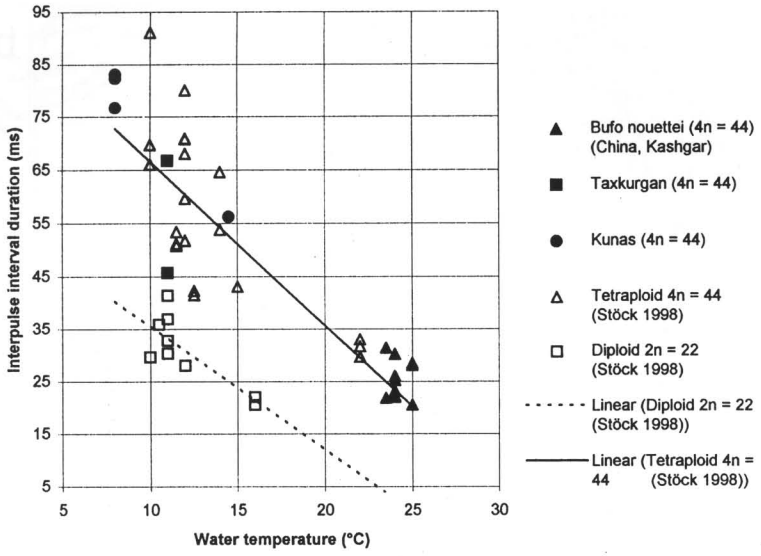


Fig. 6: Interpulse interval duration in relationship to water temperature in ♂ from Kashgar, Kunas and Taxkurgan in comparison with diploid and tetraploid ♂from Middle Asia (STÖCK 1998).
Interpuls-Intervall-Dauer in Beziehung zur Wassertemperatur bei ♂ aus Kashgar, Kunas und Taxkurgan im Vergleich mit diploiden und tetraploiden ♂ aus Mittelasien (STÖCK 1998).

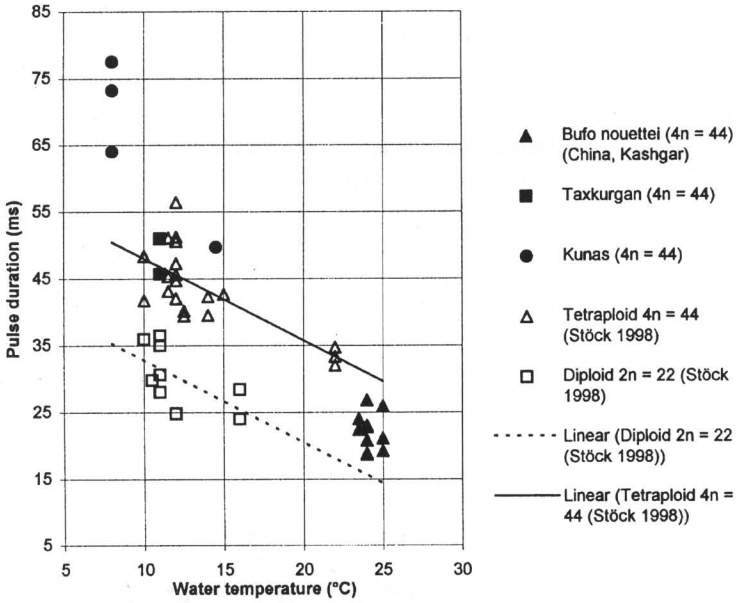


Fig. 7: Pulse duration in relationship to water temperature in ♂ from Kashgar, Kunas and Taxkurgan in comparison with diploid and tetraploid ♂ from Middle Asia (STÖCK 1998).
Pulsdauer in Beziehung zur Wassertemperatur bei ♂ aus Kashgar, Kunas und Taxkurgan im Vergleich mit diploiden und tetraploiden ♂ aus Mittelasien (STÖCK 1998).

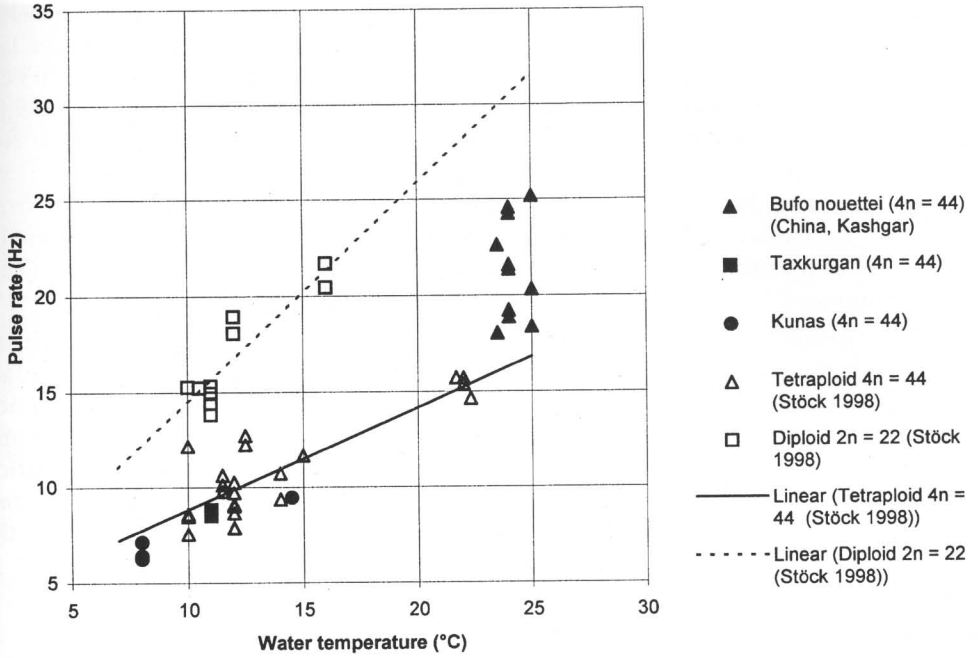


Fig. 8: Pulse rate in relationship to water temperature in ♂ from Kashgar, Kunas and Taxkurgan in comparison with diploid and tetraploid ♂ from Middle Asia (STÖCK 1998).
Pulsrate in Beziehung zur Wassertemperatur bei ♂ aus Kashgar, Kunas und Taxkurgan im Vergleich mit diploiden und tetraploiden ♂ aus Mittelasien (STÖCK 1998).

Tab. 3: Mating call data on tetraploid toads from Kashgar, Taxkurgan and Kunas.
Paarungsrufdaten der tetraploiden Kröten aus Kashgar, Taxkurgan und Kunas.

Locality (letter in fig. 1)	♂	Air T. (°C)	Water T. (°C)	Inter-pulse interval duration (ms)	Pulse duration (ms)	Pulse rate (s^{-1})	Fundamental frequency (Hz)	Call duration (s) [N of calls measured]	Intercall interval (s) [N of intervals measured]
Kashgar (A)	1	23	24	26	20.8	21.37	1636	2.0–5.1 [2]	4.1–8.3 [2]
Kashgar (A)	2	23	24	23.2	23	21.6	1884	2.4–2.8 [4]	3.3–3.9 [3]
Kashgar (A)	3	22	24	22.3	18.9	24.27	1722	2.1–2.3 [3]	2.9–3.7 [2]
Kashgar (A)	4	22	24	22	18.7	24.57	1640	1.6–1.8 [2]	5.3 [1]
Kashgar (A)	5	22	23.5	21.8	22.4	22.62	1464	2.5–3.0 [5]	4.2–5.9 [4]
Kashgar (A)	6	22	23.5	31.4	24	18.05	1808	-	-
Kashgar (A)	7	23	24	30.2	22.8	18.87	1550	-	-
Kashgar (A)	8	23	24	25.2	26.8	19.23	1722	-	-
Kashgar (A)	9	24	25	28.1	21.1	20.32	1550	-	-
Kashgar (A)	10	24	25	28.5	25.9	25.19	1635	2.0–2.7 [6]	3.2–5.1[6]
Kashgar (A)	11	24	25	20.5	19.2	18.38	1460	-	-
Taxkurgan (B)	12	15.5	11	66.7	51.0	8.5	1205	6.3–6.7 [3]	4.8–8.4 [2]
Taxkurgan (B)	13	15.5	11	68.0	45.7	8.8	1237	-	-
Kunas (C)	14	9.5	14.5	56.2	49.7	9.44	1464	-	-
Kunas (C)	15	6	8	82.3	77.5	6.26	1450	6.3–7.0 [3]	5.2–7.2 [3]
Kunas (C)	16	6	8	83.1	73.2	6.4	1330	4.9–5.6 [2]	4.3–5.3 [2]
Kunas (C)	17	6	8	76.7	64.0	7.12	1464	-	-

The toads from Kashgar were found in large muddy basins near a water channel in the southern environs of Kashgar Lake. Recordings were made in the middle or late phase of the breeding period (tadpoles and juveniles from the same year, about 1.5 cm SVL, were observed). Within the small range of water temperatures observed (23.5 °C to 25 °C), toads from Kashgar exhibited a pulse duration ranging from 18.7 ms to 26.8 ms and an interpulse interval duration ranging from 20.5 ms to 28.1 ms which resulted in pulse rates between 18.05 s^{-1} and 24.6 s^{-1} (Tab. 3, Fig. 6–8). Independent from occasionally noticed influences by interactions with other males, the call duration (1.6 s to 5.1 s) as well as the intercall interval duration (2.9 s to 8.3 s) varied strongly (Tab. 3). The fundamental frequencies were measured between 1 460 Hz and 1 884 Hz. *

Males from Kunas called at distinctly lower temperatures than toads from Kashgar. During dusk, the quickly falling temperature in small ponds flooded by a mountain stream allowed the record of calls from 14.5 °C to 8 °C. Although the phenomenon was observed in different individuals it is possible to conclude according to literature data (e.g., STÖCK 1998) that this cooling resulted in a prolongation of pulse duration (in these cases e. g., 49.7 ms to maximally 77.5 ms), a prolongation of interpulse interval duration (56.2 ms to maximally 83.1 ms) and consequently in a reduction of the pulse rate (9.44 s^{-1} to minimally 6.24 s^{-1}). Fundamental frequencies were found in Kunas toads between 1330 Hz and 1464 Hz.

The two males from Taxkurgan called at 11 °C water temperature in the flat water of ponds near a small irrigation channel. Whereas the pulse duration reached from 45.7 ms to 51.0 ms, the interpulse interval duration varied between 66.7 and 68 ms. This resulted in a pulse rate of about 8.5 s^{-1}.

Morphological characters

Living specimens: The topotypic toads from Kashgar exhibited a slightly-reddish dark-brown (= cacao-brownish) dorsal colouring. This character was the main obvious attribute shown by all males and females in this population (see below for juveniles). The second very striking character was the occurrence of uniformly brown females (Fig. 9). Usually, small dark olive or greenish spots (smaller or about the size of ED; brownish or blackish in preserved specimens) were typical of the females as well as of some males (Fig 10, 11). These spots were always isolated, and rather rounded and never formed a marbled pattern. Some specimens displayed stripes coloured as the patches across the dorsal parts of the hind legs. The males mostly tended to be invariably brownish and distinct patches were mostly restricted to extremities (Fig. 25). Both sexes of toads from Kashgar had white or whitish bellies (Fig. 14) which exhibited often a greyish cloudy colour in some areas. The colour of the iris in all examined specimens from Kashgar tended to be darkly yellow-golden (Fig. 9, 10).

Fig. 9–13 (right): 9: Uniformly cacao-brownish ♀ from Kashgar (ZSM 108/1998). 10: Tetraploid ♂ from Kashgar (ZSM 107/1998). 11: Tetraploid ♀ from Kashgar. 12: Foot of a tetraploid ♀ from Kashgar (ZSM 108/1998), note the outer metatarsal tubercle (arrow). 13: Tetraploid ♀ from Taxkurgan (ZSM 110/1998).
9: Einheitlich kakao-braunes ♀ aus Kashgar (ZSM 108/1998). 10: Tetraploides ♂ aus Kashgar (ZSM 107/1998). 11: Tetraploides ♀ aus Kashgar. 12: Fuß eines tetraploiden ♀ aus Kashgar (ZSM 108/1998), beachte den äußeren Metatarsaltubercel (Pfeil). 13: Tetraploides ♀ aus Taxkurgan (ZSM 110/1998).


9


10


11


12
1
2
3
4


13

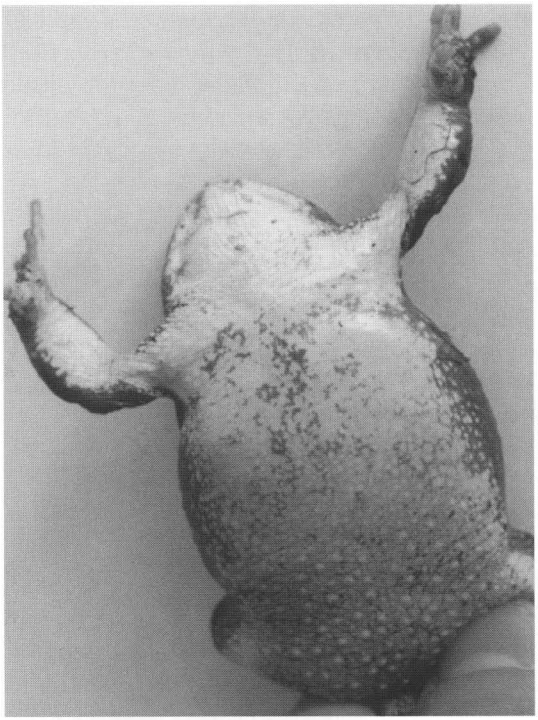


Fig. 14: Whitish belly with greyish cloudy coloration in some areas in a ♂ from Kashgar.
♂ aus Kashgar mit weißlichem Bauch in einigen Bereichen und gräulich wolkiger Färbung

From the dorsal view, the snout was rounded and short and the head did not look conic but typical circularly (»*B. viridis* like«) rounded. The subarticular tubercles were usually single but often double under first and second finger. Most of the toads had extremely inconspicuous tarsal folds. The outer metatarsal tubercle was always well developed, longish and longer than about 2 mm (Fig. 12). The dorsal coloration of the toads from Kunas, where only males were observed, was uniformly brownish or greenish/greyish-brownish. The bellies were greyish or yellowish with grey cloudy spots.

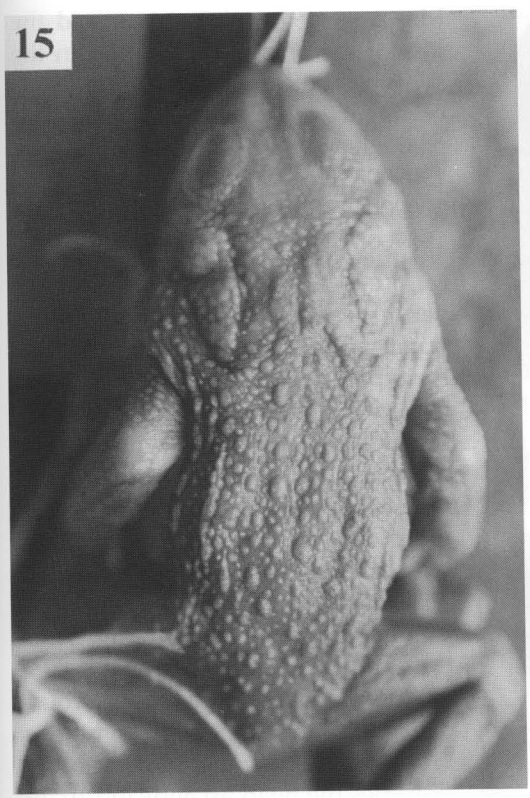
The males from Taxkurgan could be only observed in the light of an electric torch and seemed to resemble those from Kunas. The female caught (Fig. 13) displayed a marbled olive pattern of spots on a dark greyish ground. The belly was greyish with grey cloudy spots.

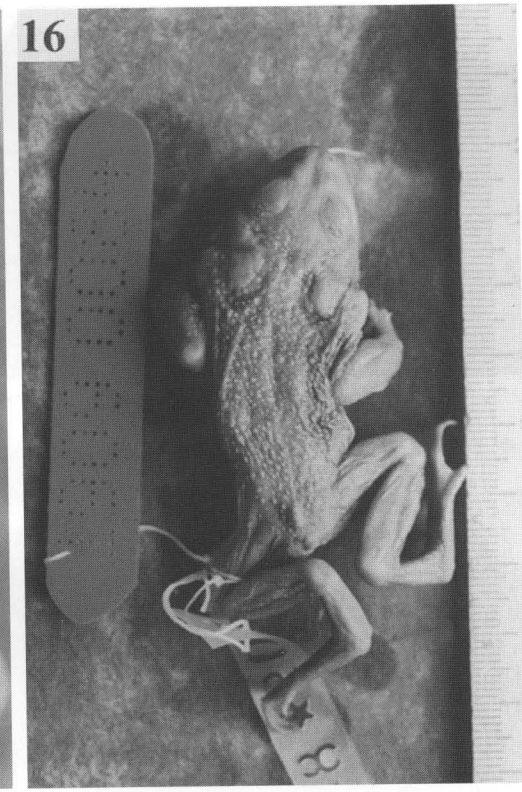
Syntypes of *Bufo nouettei* (Fig.: 15–18, 19–21): An analysis of the morphology of the syntypes included the following difficulties:

1. Most of the specimens are doubtless not adult (see also MOCQUARD 1910). Consequently, I refused to include MNHN 19090086 (SVL only about 38 mm) into the morphometric analyses and I also decided to exclude the juvenile MNHN 19090038 (SVL only 17 mm) from all analyses (see also 2.).

2. Some of the individuals are little destroyed, especially the trunk of MNHN 19090084 is strongly deformed (see multivariate statistics), and the very small MNHN 19090038 has been shrivelled substantially in the alcohol.

3. The coloration became nearly uniformly brownish because of the long time of the preservation.





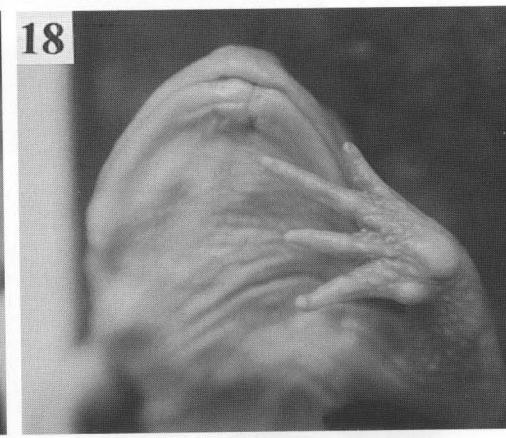


Fig. 15–18: 15: MNHN 19090083, dorsal view, *B. raddei*. 16: MNHN 19090084, *B. raddei*, dorsal view, note the deformed trunk because of preservation. 17: MNHN 19090083, *B. raddei*, foot, see the small and round outer metatarsal tubercle. 18: MNHN 19090081, *B. raddei*, detail of the hand, note the short fourth finger.
15: MNHN 19090083, Dorsalansicht, *B. raddei*. 16: MNHN 19090084, *B. raddei*, Dorsalansicht, beachte den durch die Präparation deformierten Rumpf. 17: MNHN 19090083, *B. raddei*, siehe den kleinen und runden äußeren Metatarsaltubercel. 18: MNHN 19090081, *B. raddei*, Detail der Hand, beachte den kurzen vierten Finger.

Fig. 19–21: MNHN 19090087, Lectotype of *B. nouettei* MOCQUARD, 1910.
MNHN 19090087, Lectotype von *B. nouettei* MOCQUARD, 1910.

MERTENS (1971) already noticed the loss of the original coloration in the syntypes but remarked the dark tips of fingers in some specimens. This character was still visible in MNHN 19090083 to 19090086, very bad in 19090082, but not in 19090081, and 19090087.

MERTENS (1971) also remarked the very weakly developed or absent outer metatarsal tubercles of the syntypes. I can confirm this statement for all syntypes except MNHN 19090087 (Fig. 19–21) whose outer metatarsal tubercles are clearly bigger than those of all other specimens. Unfortunately, the character was not measured but was clearly observed in representative photographs. In addition, the outer metatarsal tubercle is in MNHN 19090087 a little elongated and not as roundish as in the remaining syntypes. From the lateral view, in most of the syntypes the head ascends gradually (premaxilla to frontoparietal region), whereas in MNHN 19090087 the snout looks blunt. MNHN 19090087 has another obvious peculiarity: from the dorsal view its snout looks short and more or less circularly rounded whereas snouts appear more conic (»acute«) and prolonged in the remaining syntypes. Finally, the very long fourth toe is a special character of MNHN 19090087.

Morphometric analyses

The parameters are shown in Table 1. Univariate statistics: Toads from Kashgar were compared with diploid (N = 34) and tetraploid (N = 106 or 59, see Material and methods) specimens used in my previous study (STÖCK 1997a). Since the Levene-Test revealed no homogeneity of

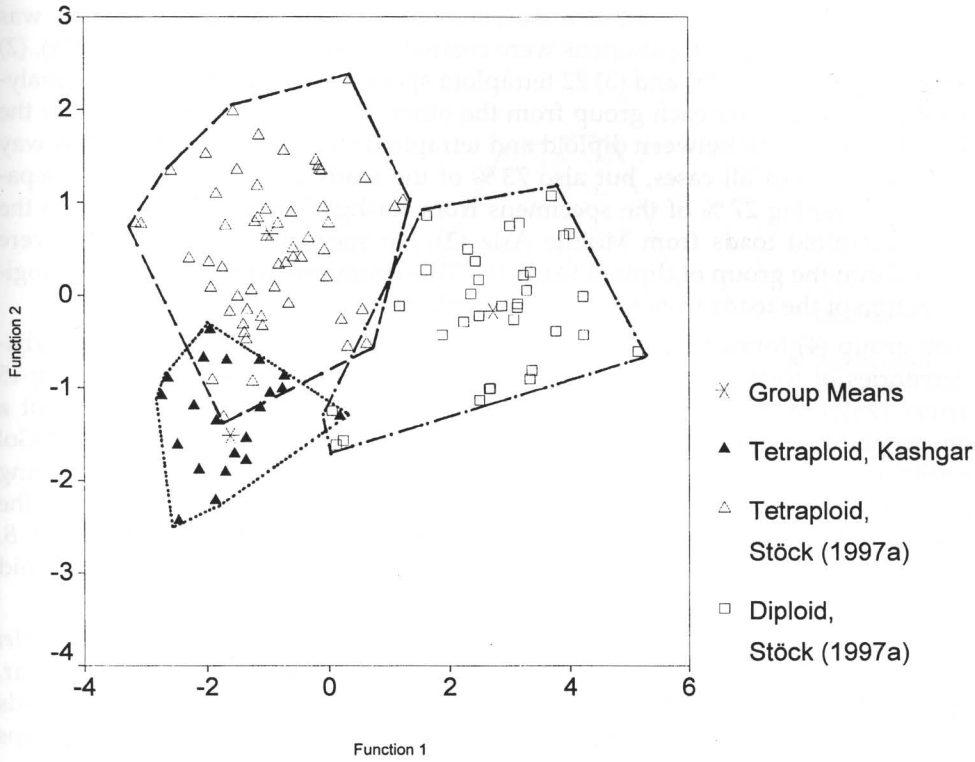


Fig. 22: Result of a discriminant analysis with 16 morphometric characters.
Ergebnis einer Diskriminanzanalyse mit 16 morphometrischen Parametern.

variances for the groups of diploid, tetraploid and tetraploid toads from Kashgar for NED, LL and PW, Mann-Whitney-U-tests for two independent samples were conducted. For the remaining characters a multiple LSD-test was performed.

The group of the 22 individuals from Kashgar differed significantly ($P < 0.001$) from the group of diploid toads in all characters. Toads from Kashgar did not show significant differences from the group of Middle Asian tetraploid toads in SVL, ED, LiMT, IND, HDT and VDT whereas the remaining characters differed at least at the $P < 0.05$ level. Although the group of the Middle Asian tetraploid toads represents a large morphometric variability within a broad geographic region between Turkmenistan and Kyrgyzstan, the toads from Kashgar are different in nine of the 16 characters.

Mann-Whitney-U-tests revealed differences at least at the $P < 0.05$ level between the 22 tetraploid toads from Kashgar and the 24 *B. raddei* specimens in all characters except NED, IND, PW, and PL.

Tab. 4: Result of a discriminant analysis with 16 morphometric characters.
Ergebnis einer Diskriminanzanalyse mit 16 morphometrischen Merkmalen.

Group	Total N (%)	Predicted group membership Diploid	Tetraploid	Kashgar
(1) Diploid (Stöck 1997a)	34 (100 %)	31 (91 %)	2 (6 %)	1 (3 %)
(2) Tetraploid (Stöck 1997a)	59 (100 %)	1 (2 %)	55 (93 %)	3 (5 %)
(3) Tetraploid, Kashgar	22 (100 %)	0 (0 %)	6 (27 %)	16 (73 %)

Multivariate statistics: First, a discriminant analysis with 16 characters was conducted. Three groups of specimens were created: (1) 34 diploids (STÖCK 1997a), (2) 59 tetraploids (STÖCK 1997a) and (3) 22 tetraploid specimens from Kashgar. The analysis was aimed to separate each group from the other. Table 4 and Figure 22 show the result. A discrimination between diploid and tetraploid toads was possible in this way in more than 90 % of all cases, but also 73 % of the toads from Kashgar were separated. The remaining 27 % of the specimens from Kashgar were reclassified into the group of tetraploid toads from Middle Asia (2). No specimens from Kashgar were reclassified into the group of diploid toads (1). This result indicates some morphological distinction of the toads from Kashgar from other tetraploids.

A fourth group (4) formed by 24 *B. raddei* from different parts of Mongolia and adjacent territories of Russia was included into a second discriminant analysis using 15 characters (without mass because of preserved specimens). In order to represent a greater variability of Asian tetraploid toads six tetraploid specimens from Bulgan Gol (Mongolia) were added to the group (2). The outcome is shown in Table 5. Concerning the Middle Asian diploids, the Middle Asian and Mongolian tetraploids and the Kashgar tetraploids the result is similar to the first analysis. In addition, the 24 *B. raddei* specimens were separated completely from both diploid (1) and tetraploid toads (2, 3)!

In the third discriminant analysis, six toads from the type series of *B. nouettei* (Kashgar, and Cha-Tcheou) and two supposed tetraploids from Koutchar. (MOCQUARD 1910 considered them to be *B. viridis* but in Xinjiang only tetraploid toads of the *B. viridis* complex have been found, see discussion) were added to the groups

Tab. 5: Result of a discriminant analysis with 15 morphometric characters.
Ergebnis einer Diskriminanzanalyse mit 15 morphometrischen Merkmalen.

	Total	Predicted group membership			
Group	N (%)	Diploid	Tetraploid	Kashgar	*B. raddei*
(1) Diploid (STÖCK 1997a)	34 (100 %)	30 (88 %)	2 (6 %)	2 (6 %)	0 (0 %)
(2) Tetraploid (STÖCK 1997a) +Bulgan Gol	65 (100 %)	2 (3 %)	59 (91 %)	4 (6 %)	0 (0 %)
(3) Tetraploid, Kashgar	22 (100 %)	0 (0 %)	8 (36 %)	14 (64 %)	0 (0 %)
(4) *Bufo raddei*	24 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	24 (100 %)

Tab. 6: Result of a discriminant analysis with 15 morphometric characters.
Ergebnis einer Diskriminanzanalyse mit 15 morphometrischen Merkmalen.

	Total	Predicted group membership			
Group	N (%)	Diploid	Tetraploid	Kashgar	*B. raddei*
(1) Diploid (STÖCK 1997a)	34 (100 %)	30 (88 %)	2 (6 %)	2 (6 %)	0 (0 %)
(2) Tetraploid (STÖCK 1997a) + Bulgan Gol	65 (100 %)	2 (3 %)	59 (91 %)	4 (6 %)	0 (0 %)
(3) Tetraploid, Kashgar	22 (100 %)	0 (0 %)	5 (23 %)	17 (77 %)	0 (0 %)
(4) *Bufo raddei*	24 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	24 (100 %)
Ungrouped cases: 6 specimens from the type series of *Bufo nouettei* + 2 supposed tetraploids: MNHN 190977, 190980	8 (100 %)	0 (0 %)	2 (25 %) (MNHN 190984, 190977)	2 (25 %) (MNHN 190980, 190987)	4 (50 %) (MNHN 190981, 190982, 190983, 190985)

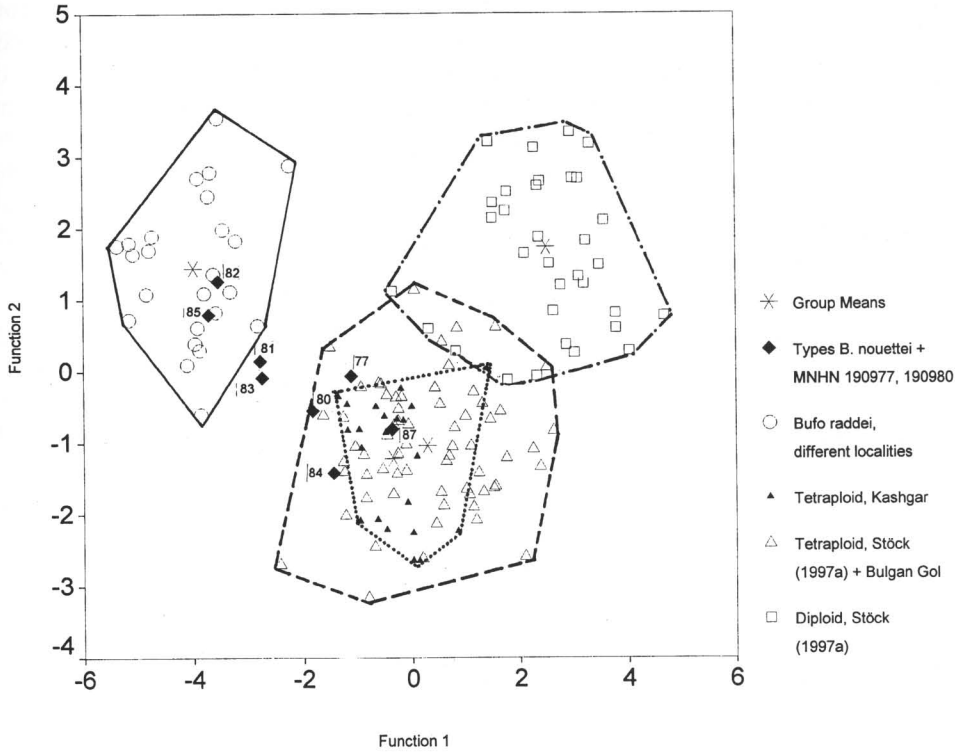


Fig. 23: Result of a discriminant analysis with 15 morphometric characters. Syntypes classified to resemble *B. raddei*: 81 = MNHN 19090081, 82 = MNHN 19090082, 83 = MNHN 19090083, 85 = MNHN 19090085. Presumed tetraploids (MOCQUARD (1910): '*B. viridis*'): 77 = MNHN 19090077, 80 = MNHN 190980 and lectotype 87 = MNHN 19090087 classified to resemble tetraploid toads. Exception 84 = MNHN 190900084, possibly »false« classified since the trunk is deformed (see Fig. 16).
Ergebnis einer Diskriminanzanalyse mit 15 morphometrischen Parametern. Syntypen, die als *B. raddei* ähnlich klassifiziert wurden: 81 = MNHN 19090081, 82 = MNHN 19090082, 83 = MNHN 19090083, 85 = MNHN 19090085. Vermutete Tetraploide (MOCQUARD (1910): '*B. viridis*'): 77 = MNHN 19090077, 80 = MNHN 190980 und Lectotypus 87 = MNHN 19090087, die als tetraploiden Kröten ähnlich klassifiziert wurden. Ausnahme 84 = MNHN 190900084, vielleicht »falsch« klassifiziert, da sein Rumpf deformiert ist (siehe Fig. 16).

from the second analysis as »ungrouped cases«. This third analysis was aimed to discriminate all four groups mutually and to classify the six ungrouped syntypes and the two supposed tetraploid specimens among them.

The results are presented in Figure 23 and Table 6. The ungrouped specimens do not form a homogenous group but were classified to resemble different morphometrically defined toad taxa. Analysing these animals individually (Fig. 23, Tab. 6), interestingly, the specimen MNHN 19090087 which was classified to resemble those of Kashgar tetraploids is in fact the only type specimen collected in the environs of Kashgar. The separate morphological position of MNHN 19090087 within the type series which has been described above was now supported by the result of the multivariate morphometric analysis. The two supposed tetraploids from Koutchar (MNHN 19090080, 19090077) were also classified to resemble the tetraploids.

Those toads from the *B. nouettei* type series which were assigned to the *B. raddei* group (MNHN 19090081, 19090082, 19090083, 19090085) were collected in Cha-Tcheou, more than 1900 km to the east from Kashgar. As shown above, all these toads (except the Kashgar specimen) also exhibit some common qualitative characters: rather conic snout from dorsal, gradually ascending head from lateral, very small outer metatarsal tubercles, short fourth fingers, and often dark tips of fingers and toes. These characters correspond to those in the 24 *B. raddei* of the present study as well as to the diagnostic descriptions of this species by BOULENGER (1882: 294), LIU (1950), or BORKIN & KUZMIN (1988: 94, 97). So, I conclude that these syntypes represent indeed *B. raddei* as it was supposed by some of the previous researchers (see introduction).

Within the third discriminant analysis, the classification of MNHN 19090084 (Fig. 16) seems to be an artefact. Although this toad was classified as belonging to the group of tetraploid toads, I consider it a *B. raddei* specimen. Its morphometric data might have been not adequate because this specimen was destroyed (see above). Since MNHN 19090084 exhibits some qualitative characters corresponding to the remaining syntypes (conic snout, small outer metatarsal tubercles, dark tips of fingers and toes, short fourth fingers) this conclusion seems to be correct. Furthermore, zoogeographical implications (see discussion) support the determination as *B. raddei.*

Crossing experiment

Many of the tetraploid F_1-hybrids resulting from the crossing of the tetraploid female (MTKD D 40725) with the tetraploid male from Western Tien Shan (MTKD D 40727) exhibited after three days (stage III_2) externally visible disturbances in their development (Tab. 7). Among those individuals which reached stage III_2 some had substantial morphological anomalies and died. In stage III_6 to III_7 the number of deformed tadpoles, which were unable to normal swimming, was high. Furthermore, a strong increase of mortality was noticed (>75 %). During the subsequent days, I observed also distinct retardments in the development of many individuals compared with others and with the control group (Fig. 24). Only two of the 30 randomly chosen hybrid tadpoles completed metamorphosis but died some days after. Despite the reali-

Tab. 7: First week of the development of embryos and tadpoles under identical conditions (22 °C, artificially ventilated aquariums). * visible deformations in few embryos; ** visible deformations in many embryos; *** many deformations, strong increase of mortality, > 75 %.
Erste Woche der Entwicklung der Embryos und Kaulquappen unter identischen Bedingungen (22 °C, künstlich belüftete Aquarien). * sichtbare Mißbildungen bei wenigen Embryos; ** sichtbare Mißbildungen in zahlreichen Embryos; *** zahlreiche Mißbildungen, starkes Anwachsen der Mortalität, > 75 %.

Control Kashgar (♀) x Kashgar (♂)		Time after deposition of the clutch	Crossing Kashgar (♀) x Chatkal (♂)	
stage (FOX 1986)	stage (GROSSE 1997)	days (d), hours (h)	stage (FOX 1986)	stage (GROSSE 1997)
II_{12}	14, 15	1 d 23 h	II_{12}	14, 15
III_1	17	2 d 9 h	II_{13}	15
III_1 to III_2	18	2 d 20 h	III_1	17
III_2	18	3 d 11 h	III_1 to III_2*	18*
III_5 to III_6	19, 20	4 d 11 h	III_4* to III_5*	19*
III_6 to III_8	20 to 21	5 d 9 h	III_6**	20**
III_8 to III_9	23, 24	6 d 11 h	III_6 to III_7***	21***

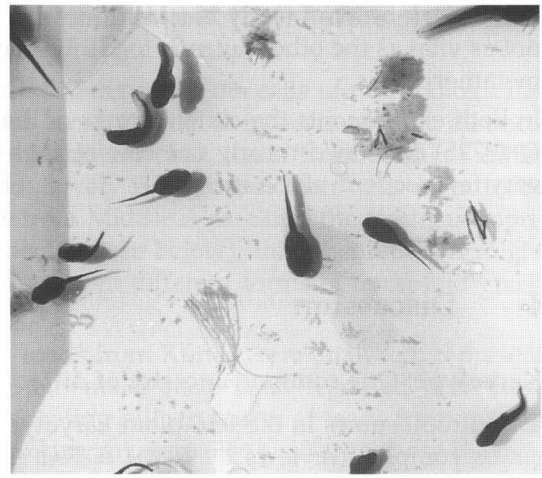


Fig. 24: F_1 tadpoles resulting from the crossing experiment of a tetraploid ♀ from Kashgar (MTKD D 40725) and a tetraploid ♂ from Western Tien Shan (MTKD D 40727). Note the deformed tadpoles, which were unfit for normal swimming, and the distinct retardments in the development of many individuals compared with others.

F_1-Kaulquappen, die aus dem Kreuzungsexperiment eines tetraploiden ♀ aus Kashgar (MTKD D 40725) und einem tetraploiden ♂ aus dem West-Tien Shan (MTKD D 40727) hervorgingen. Beachte die deformierten Kaulquappen, die unfähig zu normalem Schwimmen waren, und die deutliche Verzögerungen in der Entwicklung vieler Individuen im Vergleich mit anderen aufwiesen.

sation of only one experiment the described result might have revealed a postmating reproductive isolation caused by genetic barriers between the Kashgar tetraploid taxon and the Chatkal tetraploid.

In contrast, the control mating of the same female and a male (MTKD D 40724) from her native population resulted in a certainly normal development. 26 of the 30 randomly chosen tadpoles completed metamorphosis. The specimen tested was tetraploid. Some juveniles still live in the terrarium and had after one year (no cool hibernation) a SVL of about 35 mm (e.g., MTKD D 40728).

The metamorphosed toads resulting from the control pairing of Kashgar toads demonstrated the high variability in the coloration caused by the combination of different genes from only one polyploid pair. Although the cacao-brownish colour was typical of all toadlets, some juveniles were uniformly brownish without any spots, others showed different size and intensity of olive spots in their coloration. Interestingly, among the 26 metamorphosed toads two melanistic specimens were observed. They

Fig. 25: Pair of tetraploid toads from Kashgar depositing clutch strings. ♀ (MTKD D 40725), ♂ (MTKD D 40724).

Paar tetraploider Kröten aus Kashgar bei der Laichablage. ♀ (MTKD D 40725), ♂ (MTKD D 40724).

had a dark brown, nearly blackish dorsal colour without spots and were apparently not as vital as the other juvenile toads. Both melanistic animals died a few weeks after metamorphosis.

In both experiments the clutch strings of the tetraploid female from Kashgar (MTKD D 40725) contained mostly one row of eggs (Fig. 25). Eggs formed two rows only in very few sections of the string.

4 Discussion

Karyotype: One common ancestor of different tetraploid toads?

The chromosomes in the tetraploid karyotypes are typical of representatives of the *B. viridis* complex (reviews by ROTH & RÁB 1986). Only elaborate staining techniques (chromosome banding, in prep.) will reveal more detailed cytogenetic data. Preliminary results (in prep.) provide evidence that tetraploids from Kashgar are related with some other tetraploid populations from Middle Asia, and therefore, appear to originate from a common ancestor.

DNA-content

The data on the absolute DNA content of chicken nuclei are various. I used the value 2.34 pg which is widely utilised in different studies (e. g. ALTMAN & KATZ 1976, ULRICH et al. 1988) and which was proved to be correct by density gradient measurements.

Erythrocyte size: Typical of tetraploids

The toads from Kashgar exhibited a mean erythrocyte size which is near the average value of the 98 tetraploids (338 μm^2) from various regions of Middle Asia reported by STÖCK & GROSSE (1997).

Mating calls: Similar calls do not mean the same taxon

The sample size of calls of tetraploid toads at temperatures near 25 °C is still rather low, also in the literature. CASTELLANO et al. (1998) detected a pulse rate of about 25 s^{-1} at 25 °C (body temperature) in tetraploids form southern Kazakhstan and northern Kyrgyzstan. STÖCK (1998) found a pulse rate of about 16 s^{-1} at 22 °C (water temperature) in toads from Tashkent (Fig. 8).

Mating calls in green toads are considered to be evolutively conservative characters within diploids (SCHNEIDER & EGIASARYAN 1995) and within tetraploids as well (STÖCK 1998). The distinct differences between mating calls of diploids and tetraploids (STÖCK 1997b) probably appeared (suddenly?) due to polyploidisation (STÖCK 1998). The small temperature range in which present measurements of calls in toads from Kashgar were made, and the small amount of data do not allow to decide finally if the calls differ from those of other tetraploid populations. The data provide arguments to consider the calls in the range of variability of tetraploids. This would support the

hypothesis of the conservatism of the mating calls in toads of the same ploidy level. Further investigations are necessary to clarify this question.

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«. In the present case, toads from Kashgar differ morphologically and appear to be reproductively isolated but their calls seem to be similar to those of some other tetraploids from Middle Asia. Since the Kashgar population probably belongs to an allopatric isolate (see below), this phenomenon might occur in spite of high mountains barriers between different forms of tetraploid green toads if the calls are evolutively stable.

The water temperatures at which calls in toads from Kunas were analysed, represent the lowest range at which calling tetraploid males have been recorded until now (CASTELLANO et al. 1998, STÖCK 1998). The mating call structures of toads from eastern Tien Shan (Kunas) and eastern Pamirs (Taxkurgan) were also similar to those in some other tetraploid populations of Middle Asia (STÖCK 1998). These results provide additional arguments for the relative stability of this character during evolution.

Crossing experiment: Toads from Kashgar appear to be reproductively isolated

In the *B. viridis* complex, artificial experimental crossings have been performed quite often (ORLOVA & UTESHEV 1986, PISANETS 1992) but the F_1 ploidy level was not analysed. Among the crossings by PISANETS (1992) a probably tetraploid female from Khorog (Tadzhikistan) was crossed with a tetraploid male from Danata (Turkmenistan). The offspring exhibited a high rate of metamorphosis. STÖCK (1995, unpubl.) crossed a tetraploid female from Issyk-Kul Lake (Kyrgyzstan) with a tetraploid male from Danata (Turkmenistan) which resulted in normally developed tetraploid F_1. One male of this F_1 reproduced at the age of three years with a tetraploid female from Nuratau (Uzbekistan) and this tetraploid F_2 developed normally (STÖCK unpubl.). In contrast, the present crossing of the tetraploid male from Chatkal with the tetraploid female from Kashgar did not provide viable offspring. I consider this a first indication of a genetic barrier. DUBOIS (1998) wrote »the use of hybridisation data in systematics also requires some methodological precautions (...) a n e g a t i v e result (i. e. failure of hybridisation), if obtained in fully rigorous experimental conditions (i.e. with several experimental and control crosses) involving different individuals from different populations), clearly means that different species are involved«. While the negative result is obvious in the present case, the number of experiments is too low and therefore the finding is only a preliminary evidence which requires additional confirmations.

Morphology: The tetraploids from Kashgar differ from Middle Asian diploids, tetraploids and *B. raddei*

In some other diploid and tetraploid populations of the *B. viridis* complex, examined by the author from throughout Middle and Central Asia as well as from different collections, uniformly brownish females like in the taxon from Kashgar (e. g., Fig. 9: ZSM 108/1998, in this case female sex was confirmed by a dissection during chromosome preparation) are not found. Furthermore, the coloration pattern of toads from Kashgar generally appears to be unique among the known tetraploid toads.

ROTH (1986) mentioned a high morphometric variability of green toad populations from Eurasia. Unfortunately, these data have never been published (ROTH in litt.) and were mostly without ploidy determination. STÖCK (1995, 1997a) partly confirmed the high variability among populations but he also remarked that morphometric differences between toads of different ploidy levels appear to exceed the variation within groups of the same ploidy level. The sample of the tetraploids which were compared with those from Kashgar included toads of known ploidy from various populations in Middle and Central Asia. Univariate and multivariate analyses of morphometric traits along with the analysis of some non-metric characters clearly demonstrate the morphological separation of the Kashgar population from the other known tetraploid toads. The variability of the polymorphic (BORKIN & KUZMIN 1988) *B. raddei* seems to be also at least partly represented by the used material from different geographic origin, mainly from Mongolia (and adjacent parts of Russia). In addition to the specimens from the type series, material from north-eastern Tibet (Nan Shan region), a zone of taxonomic controversies (see below), was not analysed.

Zoogeography: Only tetraploid green toads and no *B. raddei* occur in Western Xinjiang

Although for instance ZHAO & ADLER (1993), CHANGYUAN et al. (1993) and ZHAO (1995) listed (diploid) *B. viridis* for northern China, in truth no cytogenetic study (WHU & ZHAO 1987, present paper, see also Fig. 1) has revealed any diploid *B. viridis* individual in this region and it seems doubtful that diploid *B. viridis* occur in Xinjiang. The nearest sites of diploid green toads (*B. viridis*) have been recorded in the north of the northern Tien Shan and one doubtful (see: DUJSEBAYEVA et al. 1997) record has been reported from Alakol Lake in East-Kazakhstan by GOLUBEV (1990). From the central and southern Tien Shan, the Western and Central Pamirs only (triploid? and) tetraploid toads (e. g. MEZHZERIN & PISANETS 1991) have been recorded with more or less exact methods of ploidy determination. Triploid populations have been discovered southern the Karakoram watershed (STÖCK et al. 1998).

The western Tarim Basin with the Kashgar oasis (type locality of MNHN 19090087) is effectively isolated by systems of high mountain ranges, most of them higher than 4000 m a.s.l.. These are in the west the mountainous desert of the Pamirs, in the south Tibet and the Kunlun Shan and in the north the dry south-slopes of the Tien Shan. The east is covered by the drainless Taklamakan sand desert, the most climatically extreme desert of Asia (WALTER & BRECKLE 1986). Moreover, ZUGMAYER (1909) supposed that the long distances [in the formerly poorly cultivated landscape] between the oasises in »East-Turkestan« could favour the development of local races of green toads.

The ranges of (tetraploid) representatives of the *B. viridis* complex and of *B. raddei* in north-western China have been insufficiently investigated yet. PETERS (1971) reviewed the knowledge about distribution of *B. viridis* (probably tetraploid toads) and *B. raddei* in Mongolia and China in detail and he supposed that the border between the ranges of both species is situated between 90° and 94° E. BORKIN & KUZMIN (1988) presented a detailed map of *B. raddei* records in Mongolia and cited LIU & HU (1961) which showed the occurrence of *B. raddei* in the environs of Altyn-Tagh (= Altun Shan)

(about 90° E) in Xinjiang (BORKIN & KUZMIN 1988: 93). ZHAO & ADLER (1993) as well as KUZMIN (1995) only listed »N China« for *B. raddei*. ZHAO (1995) published an actualised table similar to that by ZHAO & ADLER (1993) which only shows a »+« for the Xinjiang Province. Future investigations may reveal some localities of sympatry between tetraploid green toads and *B. raddei* whose ranges hitherto appear to be parapatric. The records by WANG & SHI (1958) would be an example of sympatry (comp.: WHU & ZHAO 1987), but the determination of toads from Ürümqi as *B. raddei* was probably an error. Furthermore, sympatric records can be expected in western Mongolia, in the Altai region (PETERS 1971, BORKIN & KUZMIN 1988, MEYER 1991) and between the environs of the Karlik Shan peak (4925 m), with the most eastern records of tetraploid toads (Fig. 1: m, L?) and the north-west and north parts of the Qilian Shan (Gansu province), where *B. raddei* probably occurs. The definite determination of the western border of the *B. raddei* range in China also requires a final solution of several taxonomic problems occurring in the region between the North-east of Tibet and Inner Mongolia. BORKIN & MATSUI (1986) specified the type locality of *B. brevipes* ZAREVSKY, »1925« (1926) which was found to be a synonym of *B. raddei* (see also ADLER & ZHAO 1993) to the Ji-us-shan, some east of Lake Kuku-nor (= Qinghai Hu, Fig. 1: **). This locality is about 400 km south-west from Sou-Tcheou (Fig. 1: 3), the most eastern locality of MOCQUARD'S *B. nouettei* syntypes (= *B. raddei*). The type locality of *Bufo koslovi* ZAREVSKY, »1924« (1925), another synonym of *B. raddei* (BORKIN & KUZMIN 1988, ADLER & ZHAO 1993), near Edsin-gol (= Ruo) river, Nei Mongol (= Inner Mongolia) (Fig. 1: *), lies about the same distance to the north-west from Sou-Tcheou (Fig. 1: 3). For the polytypic *B. raddei*, whose geographic variability in the whole range has not been examined (BORKIN & KUZMIN 1988: 87), further taxonomic investigations with a higher number of specimens are necessary, especially in these regions.

Despite the insufficient clarification of the western boundary of the *B. raddei* range in Xinjiang, it can be assumed with a high probability that PETERS' (1971) opinion is still quite valid. The most western occurrence of *B. raddei* is surely more than 800 km east of Kashgar. Older references to *B. raddei* from Pakistan and Afghanistan (reviewed by PETERS 1971) can be also resolved now as taxonomic mistakes and no (valid) record of *B. raddei* is known western 87° E.

The present record of tetraploids in Kunas provides additional arguments for a rather continuous distribution of tetraploid toads from Eastern Tien Shan to the Dzungarian Gobi in Mongolia. The record from Taxkurgan is situated in a very remote region from which there are only extremely scarce (if any) data on green toads. Future studies should clarify the relationship between toads from Taxkurgan and toads in Gorno-Badakhshan (Central Pamirs) and/or to those in the southern Tarim Basin which is connected with the Taxkurgan region via the Yarkant river tributaries.

Taxonomy: The lectotype of *Bufo nouettei*

When MOCQUARD (1910) described his new species *B. nouettei*, *B. raddei* STRAUCH, 1876 had been known already 34 years and MOCQUARD's intention was to distinguish the new taxon from *B. raddei* (see introduction). Although MOCQUARD (see partly above) mainly seemed to describe *B. raddei*, some of the characters given in the species description (uniformly brown blackish above, blunt snout) appear only to refer to the

Tab. 8: Morphometric data of six specimens from the type series of *B. nouettei* MOCQUARD, 1910. MNHN 19090081 to 85 (now: paralectotypes) represent *B. raddei*. MNHN 19090087 is the designated lectotype of *B. nouettei*.

Morphometrische Daten von sechs Exemplaren der Typusserie von *B. nouettei* MOCQUARD, 1910. MNHN 19090081 bis 85 (jetzt: Paralectotypen) stellen *B. raddei* dar. MNHN 19090087 ist der ausgewiesene Lectotypus von *B. nouettei*.

Specimen (mm)	MNHN 19090081	MNHN 19090082	MNHN 19090083	MNHN 19090084	MNHN 19090085	MNHN 19090087 Lectotype
SVL	54.3	56.6	52.1	57.6?	48.0	52.6
HL	16.6	16.2	14.6	16.7	13.9	14.5
PL	11.9	13.7	11.0	11.0	12.2	11.7
PW	11.5	9.0	5.0	6.5	7.0	6.7
HDT	3.1	3.2	3.1	3.3	3.2	2.7
VDT	4.0	4.1	3.5	3.4	3.5	3.0
ED	6.9	7.3	7.3	7.3	6.8	8.0
HW	20.3	21.3	18.7	19.2	18.5	20.0
IND	3.9	4.0	4.0	3.6	3.6	4.0
NED	3.7	4.3	3.9	4.0	3.7	4.2
TL	22.0	20.9	20.7	21.6	18.5	20.9
LL	36.5	34.0	34.0	38.0	30.5	39.7
LFT	3.4	3.3	3.0	3.8	3.0	3.7
LiMT	3.0	3.4	3.0	3.5	3.2	3.7
IOW	4.9	4.1	3.7	4.0	3.8	4.4

taxon (i. e. the only specimen: MNHN 19090087) from Kashgar which is also the first specimen mentioned in the species description. *B. raddei* does not occur in Kashgar. Furthermore, the morphology of the MNHN 19090087 exhibits characters of tetraploids from the same locality. Therefore, it can be concluded that this individual is a tetraploid toad representing the same taxon as those 22 tetraploid animals investigated in the present study. For this taxon the name *B. nouettei* MOCQUARD, 1910 is available. This decision agrees with MAYR & ASHLOCK (1991) who emphasised: »a type specimen is nothing more and nothing less than a specimen (or taxon) which tells us to which taxon a given name should be attached«. The remaining syntypes represent *B. raddei*. Since the status of *B. nouettei* has been doubtful and questionable many decades and no previous reviser restricted the name to one of the components of the type series, a lectotype should be selected (ICZN 1985: Article 74, Mayr & Ashlock 1991).

Designation of a lectotype of *Bufo nouettei* MOCQUARD, 1910 (Fig. 19–21, for morphometric data see Tab. 8): Among the syntypes described by MOCQUARD, MNHN 19090087 is selected to serve as the type. MNHN 19090087: collected in October 1906 (according to the catalogue of the MHNH) by Dr. LOUIS VAILLANT; type locality: the environs (the catalogue of the MHNH: »env. de Kachgar«) of Kashgar (= Kashi Shi, Xinjiang, China); dorsal colour (after 81 years in alcohol) uniformly dark brownish; the remains of dark stripes dorsally across femur and tibia; belly whitish-yellowish, some grey cloudy-marbled tones laterally of the throat and mouth; parotids inconspicuous, the posterior parts weakly rounded, not acute; snout short, rounded but not conic from the dorsal view, blunt from lateral view; tips of fingers and toes whitish; subarticular tubercles under first finger double but apparently weakly shrivelled by

preservation; fourth finger considerably extending the first articulation of the third finger; outer metatarsal tubercle nearly as big as the inner; fourth toe long, about half of its length without webbing; karyotype (according to topotypic specimens analysed in the present study) highly probable tetraploid.

The description of the lectotype has restricted the type locality (ICZN 1985: Article 74) to the environs of Kashgar. The remaining specimens from MOCQUARD's type series (now: paralectotypes, ICZN 1985: Article 74) originated form Cha-Tcheou and Sou-Tcheou, a region where taxonomic investigations on the polytypic *B. raddei* are necessary. Since other names, e.g. *B. brevipes* or *B. koslovi*, could possibly serve as name bearers if the discovery of a (species or) subspecies in the region north-east of Tibet would result from such investigations, the restriction of the name *B. nouettei* to tetraploid toads from the environs of Kashgar is also recommended. Finally, the problem with the seven (MOCQUARD 1910) or eight syntypes (MNHN catalogue) does not disturb the nomenclature any longer since the name is now restricted to the unequivocally identified specimen from Kashgar.

The qualification of the tetraploid toads from Kashgar as a separate taxon based on its morphology, its geographical isolation and references to a reproductive isolation. In any case, these results found with various techniques strongly support that a speciation process is observable in the Kashgar taxon (= *B. nouettei*). However, a later review of Asian green toad systematics may reveal that »*nouettei*« is only a subspecies within the obviously polytypic tetraploid toads whose name most likely will be *Bufo oblongus* NIKOLSKY, 1896 (STÖCK in prep.).

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6 Abbreviations used in the text

MNHN - Muséum National d'Histoire Naturelle Paris.
MTKD D - Staatliches Museum für Tierkunde Dresden.
ZMB - Museum für Naturkunde der Humboldt-Universität zu Berlin.
ZSM - Zoologische Staatssammlung München.

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