THE MATING CALL AND MALE RELEASE CALL OF *Bufo raddei* STRAUCH, 1876 WITH SOME PHYLOGENETIC IMPLICATIONS[1]

Matthias Stöck,[2] Peter Bretschneider,[2] and Wolf-Rüdiger Grosse[2]

The mating call of *Bufo raddei* is analyzed and described for the first time. Although species-specific, important parameters (call duration, amplitude-time waveform of single pulses, absence of interpulse intervals at higher body temperatures) of the mating call show a similar structure to those of the allopatric *Bufo calamita*, mainly differing by lower pulse rates. *B. raddei* mating calls differ from those of diploid, triploid and tetraploid representatives of the parapatric Eurasian *Bufo viridis* complex which have a pulsed structure with distinct interpulse intervals. The release call of male *B. raddei* consists of a single pulse group with slight frequency modulation and appears similar to the release call of *B. calamita*, but differs from those in the *B. viridis* complex which always are subdivided in pulse groups. We suggest the occurrence of two different types of amplitude modulation as an indication for a possible divergence of two evolutionary lineages, the *B. calamita*/*B. raddei* lineage and the *B. viridis* complex lineage. Furthermore, we compare these results with other published phylogenetic data on these taxa.

Key words: *Bufo raddei*, *Bufo calamita*, *Bufo viridis* complex, Mating calls, Male release calls, Amplitude modulation, Phylogeny.

1. INTRODUCTION:
THE RECENT SYSTEMATIC POSITION
OF *Bufo raddei* AND THE NEED
FOR BIOACOUSTIC DATA

In his species description, Strauch (1876:54) considered the Mongolian toad *Bufo raddei* "a form between *Bufo calamita* and *B. viridis*, but obviously *B. raddei* is more closely related to the first of them."

Inger (1972) included *B. raddei* in his *Bufo viridis* group. The term "*Bufo viridis* complex" was used by other authors when we (Stöck and Grosse, 1997) defined it as follows: "In order to name diploid and polyploid [green toad] forms from Middle and Central Asia which are all quite closely related to *Bufo viridis* we use the term '*Bufo viridis* complex.' This excludes some species (e.g., *Bufo calamita*...) belonging to Inger's (1972) '*Bufo viridis* group,' which was defined without information about the existence of polyploid forms."

Later on, Strauch's (1876) diagnosis was supported concerning morphological similarities between *B. raddei* and *B. calamita* and by biogeogra-

phic and palaeontological evidence (summarized by Borkin, 1984), and is generally accepted to date (Borkin and Kuzmin, 1988; Kuzmin, 1999).

However, the systematic separation of *B. raddei* from the *Bufo viridis* complex was occasionally questioned (e.g., Eiselt and Schmidtler, 1973). Hemmer et al. (1978) considered the examination of living *B. raddei* necessary in order to confirm their systematic status as a species. Subsequent biochemical studies assured the rank of *Bufo raddei* as a valid species rather distant from the *Bufo viridis* complex (Maxson, 1981; Nishioka et al., 1990; Mezhzherin and Pisanets, 1995; Liu et al., 2000). Among various biochemical analyses involving toads of Inger's *Bufo viridis* group (e.g., Schenkel-Brunner and Kothbauer, 1976; see Sinsch, 1998:49), only the immunological study of Maxson (1981) included specimens of all three forms together (*B. viridis* and "*B. oblongus*" [i.e., any perhaps polyploid green toad taxon from Afghanistan, see Stöck et al. (in press)], *B. calamita*, *B. raddei*). Although Maxson's (1981) results suggested that *B. calamita* and *B. raddei* are almost equally distant from *B. viridis*, she did not directly compare the relationship between *B. calamita* and *B. raddei*.

The basic chromosome number in *B. calamita* and the *B. viridis* complex ($2n = 22$, $3n = 33$,

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$4n = 44$) is eleven; karyological studies revealed differences in the position of NORs, Q-, and C-bands (Ullerich, 1966; Bogart, 1972; Schmid, 1978; Birstein, 1981; Roth and Ráb, 1987; Sinsch, 1998; Stöck et al., 1999). The conventionally stained karyotype of *B. raddei* slightly differed from those of *B. viridis* and *B. calamita* (Borkin et al., 1986a, 1986b; Borkin and Kuzmin, 1988). Banding studies in *B. raddei* (Deng and Shang, 1984: BrdU-banding) either have not been done in *B. calamita* and the *B. viridis* complex or provided highly variable results (Shang et al., 1984) appearing not appropriate for taxonomic conclusions.

B. viridis and *B. calamita* have been used in many hybridization experiments (see Blair, 1972; Kawamura et al., 1980), but only few crosses have been performed with *B. raddei* and *B. viridis* (Kawamura et al., 1982; Nishioka et al., 1990), while none have been done with *B. raddei* and *B. calamita*. Viable offspring resulted from crossings of male *B. viridis* with female *B. calamita*, whereas reciprocal hybrids were never viable. Only a small number of viable hybrids resulted from the crossing of male *B. raddei* with female *B. viridis*. The reciprocal hybrids completed metamorphosis (Kawamura et al., 1982), however, both male and female hybrids "were completely and incompletely sterile, respectively" (Nishioka et al., 1990).

As we have shown above, phylogenetically useful studies including *B. raddei*, its presumed close relative *B. calamita* as well as toads of the *B. viridis* complex have rarely been done. Therefore, data on the phylogenetic position of *B. raddei* relative to *B. calamita* are lacking. This is the case especially concerning bioacoustics. Whereas the bioacoustics of *B. calamita* and *B. viridis* complex were examined in many papers (see references in Table 2) no data have been published on *B. raddei* (Borkin and Kuzmin, personal communication). Only the calling behavior in choruses has repeatedly been mentioned (e.g., Kuzmin, 1999:278). Recently, we examined for the first time the mating call (MCs) and the male release call (MRC, RC) of *Bufo raddei* from Mongolia and compared these calls with those of *B. calamita* as well as of diploid, triploid and tetraploid green toads from the Eurasian *B. viridis* complex.

2. MATERIAL AND METHODS

For the analyses of MCs of *Bufo raddei* we used archival recordings from two localities in Mongolia (Fig. 1) which have previously not been analyzed.

Wallschläger recorded MCs of five *B. raddei* males in the riverside landscape of the Khudshirt River (5 June 1979; 20:45 – 21:25). The temperature was not measured, but meteorological data of an avifaunistic report (Mauersberger et al., 1982) and personal information (Wallschläger) allowed the estimation that the water temperature was 8°C. Mix and Bräunlich recorded MCs of four *B. raddei* males at the Lake Khokh Nuur (5 June 1998; approx. 49°30′ N, 116°30′ E; Dornod Aimak, 560 m above sea level). The water temperature was 20°C.

A pair of *B. raddei* caught by Biedermann near the Orchon River lives in our terrarium. The RCs of the male (22.0 g body weight) were elicited by applying pressure to the sides of the animal with the fingers as described by Brown and Littlejohn (1972). To determine the effect of temperature on the structure of RCs, this male was subjected to five water baths ranging in temperature from 7 to 27°C within 5 h. RCs were recorded after the animal had adapted to the water temperature. In addition, RCs produced during interaction between animals were also recorded in two adult offspring males (20.5 and 21.0 g) descending from the Orchon pair.

For comparison with MCs of other species, we mainly used published data; for *B. calamita* (Bretschneider, 1999) and MRCs of the *B. viridis* complex (Stöck) we also present some unpublished material.

Acoustic analyses were conducted in the Institute of Zoology at the Martin-Luther-University Halle-Wittenberg. For each calling male, the call duration of at least five calls, and at least ten successive pulses and (if present) interpulse intervals from the middle section of a call were measured with the program Avisoft-SonagraphPro (ver. 2.0; Specht, Berlin). We used a sampling rate of 16 kHz (Hemming-window, FFT-length 512, frame 50%, Tchebysheff 8th order).

The call samples examined are deposited in the Animal Sound Archive at Humboldt-University Berlin, Invalidenstraße 43, D-10099 Berlin, Germany.

3. RESULTS

3.1. Mating Call

Male *B. raddei* usually call in choruses (Table 1). The MCs are short and after the onset, amplitude and frequency increase quickly. To the human ear the calls sound somewhat hard, like "ouerrrrrrr." They appear a little similar to those of *B. calamita* but can easily be distinguished from the longer, melodious-monotonous trills of representatives of the *B. viridis* complex.

Fig. 1. Localities in Mongolia: *1*) Khudshirt River; *2*) Khokh Nuur Lake; *3*) Orchon River. See *Material and Methods* for geographic details.

3.1.1. Variation of call patterns. Ten of the twelve males recorded in Khudshirt River and Lake Khokh Nuur produced single calls (second part of Fig. 2a) consisting of pulse trains separated by slightly varying intercall intervals. A second call pattern was only noticed in two males from Khokh Nuur. They produced double calls (first half of Fig. 2a, Fig. 3) consisting of two pulse trains separated only by a relatively short intercall interval. The males repeated this pattern up to five times after a slightly varying intercall interval. The same individuals sometimes changed between the two call patterns within one series of calls (Fig. 2a) which all had the

TABLE 1. Mating Call Parameters of *Bufo raddei* from Khudshirt River and Khokh Nuur Lake (Mongolia)

Locality (*n*, No.)	Water temperature, °C		Pulses per call	Pulse duration, msec	Interpulse interval, msec	Pulse rate, pulse/sec	Call duration, sec	Call interval, sec	Fundamental frequency, Hz
Khudshirt (15, 5)	8	Mean	62	10	4.52	71.27	0.986	3.06	1410
Khudshirt	8	Max	73	14	9.00	52.08	1.175	3.18	1550
Khudshirt	8	Min	53	6.7	0	100.00	0.667	2.85	1205
Khokh Nuur I (20, 5)	20	Mean	65	9.9	0	101.01	0.651	3.67	1569
Khokh Nuur	20	Max	81	11	0	90.9	0.797	5.23	1722
Khokh Nuur	20	Min	42	9	0	111.1	0.406	2.94	1378
Khokh Nuur II (20, 2)	20	Mean	65	10	0	100.0	0.562	2.25	1593
Khokh Nuur	20	Max	71	11	0	90.9	0.662	4.86	1636
Khokh Nuur	20	Min	60	9	0	111.1	0.451	0.27	1550

Designations: *n*) number of calls examined, No.) the number of specimens. Khudshirt and Khokh Nuur I: single calls which were repeated after a relatively short interval (see also Fig. 2a, second part); Khokh Nuur II: two subsequent calls separated by a very short interval were repeated after a longer interval (see also Fig. 2a, first part).
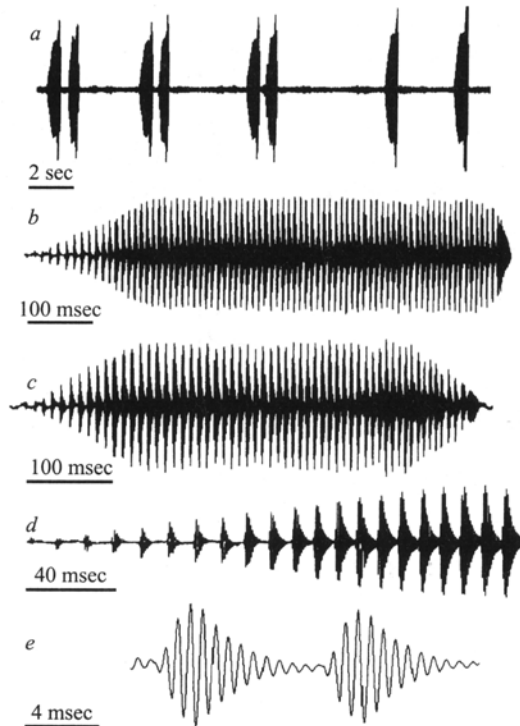
Fig. 2. Oscillograms of mating calls of *Bufo raddei*. *a*) Double and single calls produced by the same male, water temperature 20°C, Khokh Nuur, see a parts of the same call in Fig. 3; *b*) train of pulses shown in (*a*) with an expanded time base, notice the rather abrupt end; *c*) pulse train of a male from Khokh Nuur, notice the decreasing amplitude at the end, water temperature 20°C; *d*) train of pulses shown in (*a*) and (*b*) with an expanded time base to show the increasing amplitude, the distinct intervals between the first pulses decreased quickly resulting in a continuous series of pulses; *e*) two pulses of the second part of (*d*) shown with an expanded time base. Recordings: H. Mix and A. Bräunlich.

same pulse rate. Higher temperatures resulted in shorter calls (Student's *t*-test, $p < 10^{-6}$).

3.1.2. Pulse trains and fundamental frequency. The calls represented pulse trains consisting of about 40 to 80 pulses (Fig. 2*b*, *c*). Our data did not show a relationship of number of pulses per call with temperature (Student's *t*-test). Mostly, the first 12 to 15 pulses exhibited an increasing amplitude and were separated by distinct interpulse intervals which decreased continuously to zero (Fig. 2*d*). These pulses were also characterized by an increasing frequency (Fig. 3). Later on, the amplitude usually varied only slightly until the final section of the call (Fig. 2*b*, *c*). In the calls of some males from Khokh Nuur the am-

plitude was found to exhibit another short extension before the end of the call (Fig. 3). In the middle section of a call, the interpulse intervals were either very short (lower temperatures) or they were completely absent, i.e., at higher temperatures, the damped part of the pulses was cut off by the following pulse (Fig. 2*d*). For the relationship of interpulse intervals with water temperature, significance was confirmed by Student's *t*-test. Usually, the frequency increased until the middle of the call and then varied scarcely. The range of fundamental frequencies of the examined *B. raddei* MCs was found between 1205 and 1722 Hz. No correlation with water temperature was detected (Student's *t*-test); we could not examine the probable correlation with the body size since morphometric data were not available. Mostly, the calls ended rather abruptly (Fig. 2*b*), sometimes the amplitude of the final pulses decreased gradually (Fig. 2*c*).

3.1.3. Pulses. According to our data (Student's *t*-test), the number of pulses per call is not influenced by the water temperature. Pulses had a typical nearly linear onset within the first 10 to 20%, whereas their second part exhibited a damped oscillation within the remaining 90 to 80% (Fig. 2*d*, *e*). Pulse duration was not affected by water temperature (Student's *t*-test).

3.1.4. Comparisons. Table 2 shows some parameters of the *Bufo raddei* MCs in comparison to those of *B. calamita* as well as diploid, triploid and tetraploid representatives of the *Bufo viridis* complex. We did this semi-quantitatively and descriptively because our data on *B. raddei* from only two water temperatures were not suitable for an extensive comparison.

The pulse rate of *B. raddei* is the highest: their values distinctly exceed the pulse rate of *B. calamita* and the *B. viridis* complex at the same temperatures. The general range of the pulse rate of *B. raddei* only slightly overlaps beyond that of the *B. viridis* complex (Fig. 4).

B. raddei and *B. calamita* share the most obvious similarities in the amplitude-time waveform. In both species the amplitudes quickly reach their maximum within the first up to 20% of a single pulse and only gradually decrease as a damped oscillation within the remaining time (Fig. 5*b*, *c*). On the contrary, in any MC within the *B. viridis* complex amplitudes become maximal only after about 30%, keep this amplitude for another 30% and then decrease during the remaining third of the pulse (Fig. 5*a*).

Another apparent parallel in *B. raddei* and *B. calamita* is in the interpulse intervals: in pulse trains of both species these intervals are totally reduced at
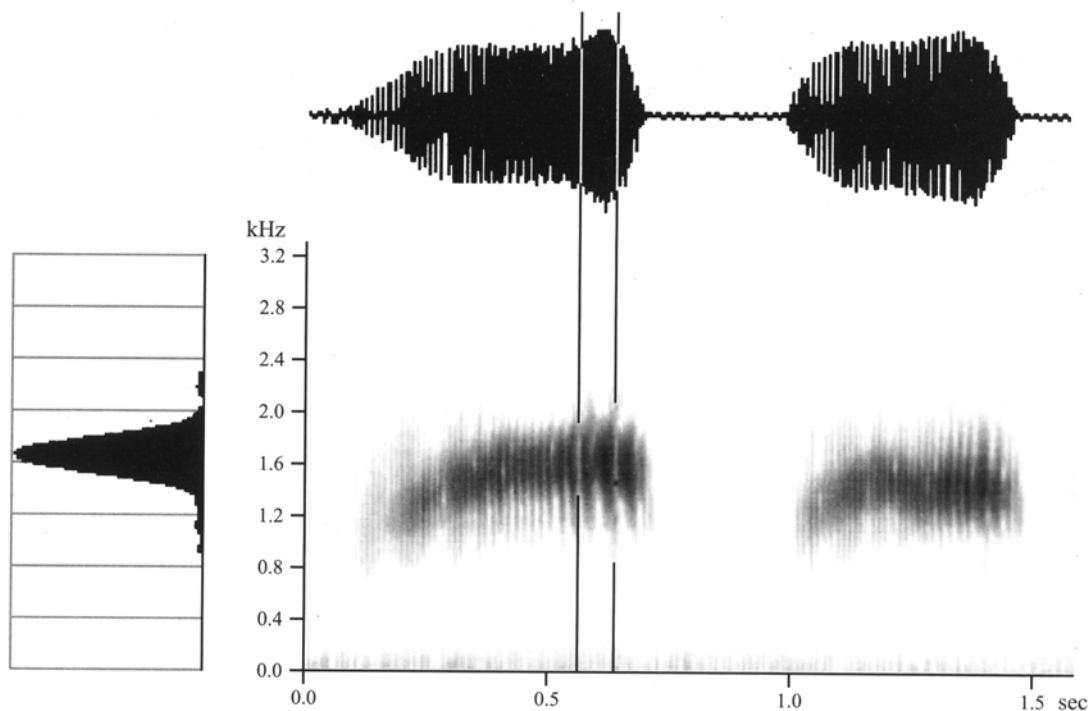
Fig. 3. Oscillogram (above), spectrogram (below), and power spectrum (left) of a double mating call of *B. raddei* from Khukh Nuur, water temperature 20°C. Recording H. Mix and A. Bräunlich.

higher water/body temperatures resulting in a continuous sequence of pulses (Fig. 5*B*, *C*) and a shortening of the call duration. On the contrary, toads of the *B. viridis* complex exhibit discrete interpulse intervals at all water/body temperatures; furthermore, increasing temperature results in a shortening of the intervals which is correlated to the reduction of the pulse duration (Fig. 5*a*).

A third common character is the call duration: *B. raddei* and *B. calamita* produce short pulse trains not exceeding 1.2 sec (Fig. 5*B*, *C*). This value corresponds to the minimal call duration in the *B. viridis* complex reaching ten times (up to 12.5 sec) the maximal values of the two other species.

The MC characters in the *B. viridis* complex, as the occurrence of distinct interpulse intervals, symmetrical amplitude-time waveforms of single pulses, lower pulse rates, and long call duration separate it from both *B. raddei* and *B. calamita*. MCs of the latter species share similar amplitude-time wave forms, high pulse rates and partly a continuous sequence of pulses within pulse trains.

The fundamental frequencies (range ~1000 – 1900 Hz) are similar in *B. raddei*, *B. calamita*, and

the taxa of the *B. viridis* complex and depend as in other toad species mainly on the body size.

3.2. Male Release Calls

3.2.1. Description. The human ear perceives those of *B. raddei* as short, nearly squealing sounds, reminding of the barking of a little dog ("oui-oui-oui"). The single note, whose frequency slightly decreases to the end, consists of a single but complex, amplitude-modulated pulse group in which the single pulses cannot be distinguished.

Figure 6 show sonograms and power spectra of MRCs. In the three specimens examined, the calls consisted of a fundamental frequency little above 1000 Hz (i.e., slightly lower than the comparable frequency of MCs) and its harmonics reaching up to 10.8 kHz (Fig. 6*a*). Usually, the fundamental frequency was also the dominant frequency. However, in some cases (Fig. 6*a*) the power of the first harmonic even surpassed the fundamental frequency.

The strength and duration of the initiating pressure stimulation generally tended to influence the duration of the MRCs which therefore exhibited a great

variation at a given temperature. However, the call duration (Fig. 7, Table 3) significantly decreased with increasing body temperature (linear regression: $y = 92.123 - 1.7651x$; $r^2 = 0.356$).

3.2.2. Comparisons. The *B. raddei* MRCs clearly differ from the MRCs which are characteristic of diploid, triploid and tetraploid representatives of the *B. viridis* complex from various regions of Eurasia. Within the *B. viridis* complex, the pulses are longer and always composed of pulse groups which are distinctly separated by intervals (Fig. 8c – h; Flindt and Hemmer, 1968; Weber, 1975, 1977; Herrmann, 1993). To the human ear, the rolling calls ("uerrrrk") of toads in the *B. viridis* complex are also easily distinguishable from the MRC of *B. raddei*.

Comparison to *B. calamita* is more difficult. The *B. raddei* MRCs appear similar to the type 1 MRCs of *B. calamita* reported by Flindt and Hemmer (1968; Sinsch, 1998:43). The type 2 (Fig. 7b) MRC of *B. calamita* differs from the comparable calls in the *Bufo viridis* complex (Fig. 8c – h) in having pulse groups nearly without interpulse intervals and a final section consisting of a longer pulse (see also: Flindt and Hemmer, 1968; Weber, 1975; Sinsch, 1998:44). This peculiarity is the most typical one of the *B. raddei* calls consisting of a long, single, amplitude and little frequency modulated pulse group. Thus, the

TABLE 2. Comparison of Mating Call Data on *B. raddei* with those on *B. calamita* and on the *B. viridis* complex

Parameter	*Bufo raddei**	*Bufo calamita***	*Bufo viridis* complex***	
Pulses rate, pulses/sec	43–111	23–67 23–59[1] 27.6–66.2[2]	6.4–45 **diploids:** **triploids:** **tetraploids:**	13–33[a], 14–36[b], 11.1–31.8[c], 15–33[d], 13.8–20.4[e], 12–45[g] 14.4–36.7[i], 9–26[k] 9–15[g], 7.5–21.3[h], 7.53–15.7[e], 6.4–24.6[f], 9–27[g], 19.4[i]
Amplitude of single pulses	maximum after 10–20% of its total duration, then damped oscillation within 90–80%	maximum after 5–10% of its total duration, then damped oscillation within 95–90%[1,3]	maximum after one third of its total duration, staying rather constant for another third then decreasing within the last third	
Pulses	form continuous series with increasing water temperature	form continuous series with increasing water temperature[1]	shortening with increasing water temperature	
Interpulse intervals, msec	0–9, vanishing with increasing water temperature	0–27[1,2], vanishing with increasing water temperature	shortening with increasing water temperature	
Call (= pulse train) duration, sec	0.4–1.175	0.28–0.9 0.3–0.9[1] 0.285–0.809[2]	1–12.6 **diploids:** **triploids:** **tetraploids:**	2–8[b,c], 3–9[e], 1–8[g], 1.26–5.4[i], 2–12.6[k] 1–8[g], 1.2–4.2[h] 3–9[e], 2–7[f], 1–8[g], 2.7–2.9[i]
Fundamental frequency, Hz	1205–1722	1200–1830[3,4] 1700[1] ~1600[5] 1312–1750[2]	1000–1900 **diploids:** **triploids:** **tetraploids:**	1140–1580[a], 1100–1550[b], 1070–1400[c], 1197–1508[d], 1270–1408[e], 1000–1300[g], 1075–1400[i] 1100–1500[g], 1378–1636[h] 1290–1479[e], 1205–1884[f], 1000–1800[g], 1378[i], 1030–1750[k]

* Data of the present paper.

** Data on *Bufo calamita*: [1] Lörcher and Schneider (1973), data from Western Germany, 10 – 20°C; [2] Bretschneider (1999), data from Central and Northern Germany, 10 – 26°C; [3] Sinsch (1998), compiled data from different European countries; [4] Arak (1983), data from Northern England, 12°C; [5] Schlyter et al. (1991), data from Southern Sweden, no temperature data.

*** Data on diploid, triploid and tetraploid green toads (we avoid here to discus their taxonomy): [a] Lörcher and Schneider (1973): diploids from Austria, 11.0 – 24.5°C; [b] Nevo and Schneider (1976): diploids from Israel, 11 – 26°C; [c] Schneider and Egiasaryan (1995): diploids from Northwestern Kazakhstan, 11.2 – 20.2°C; [d] Giacoma et al. (1997): diploids from Italy, 8 – 27°C; [e] Stöck (1998a): diploids and tetraploids from Middle Asia, 9 – 22°C; [f] Stöck (1998b): tetraploids from Northwestern China, 8 – 24°C; [g] Castellano et al. (1998): diploids, triploids and tetraploids from Southern Kazakhstan and Northern Kyrgyzstan, 10 – 28°C; [h] Stöck et al. (1999): triploids from Northern Pakistan, 7 – 27.5°C, including some unpublished data on these toads; [i] Stöck et al. (in press): original and compiled data on diploids from Central and Northern Iran from different sources and one tetraploid taxon from Eastern Iran, 11 – 30°C; [k] Castellano et al. (1999): diploids from Northwestern Italy and Sardinia, 7 – 24°C.

B. calamita MRCs with pulses being almost not separated by interpulse intervals at the beginning and a final long lasting pulse may show a related structure to both the separated pulses in the *B. viridis* complex and the single long pulse in *B. raddei*. The unpulsed structure of the *B. raddei* MRCs appears also similar to that of *B. bufo* (e.g., Flindt and Hemmer, 1968; Weber, 1975).

4. DISCUSSION

4.1. Phylogenetic Implications of MCs

Our data represent new insights from bioacoustics on the systematic relationship of the Eastpalearctic *B. raddei* and the Westpalearctic *B. calamita*. Considering that the MCs of *B. viridis* complex seem to be evolutionarily conservative characters (Stöck, 1998a, 1998b; Stöck et al., 1999), the differences found suggest a branching of the *B. raddei/B. calamita* lineage from the representatives of the *B. viridis* complex. The latter is also well defined by the simi-
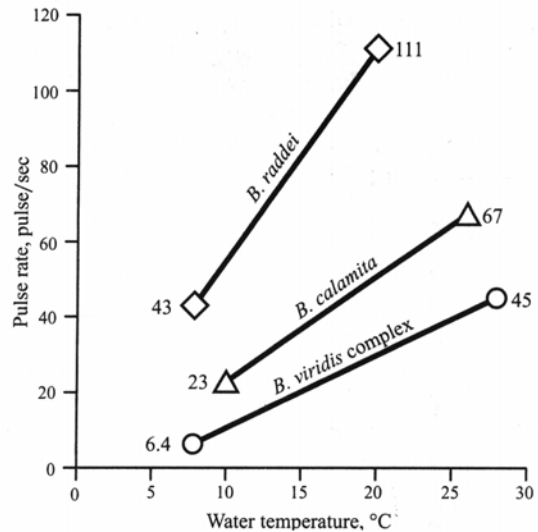
Fig. 4. Ranges with minima and maxima of the pulse rate of *Bufo raddei*, *B. calamita*, and the *B. viridis* complex depending on water temperature (see also Table 2).
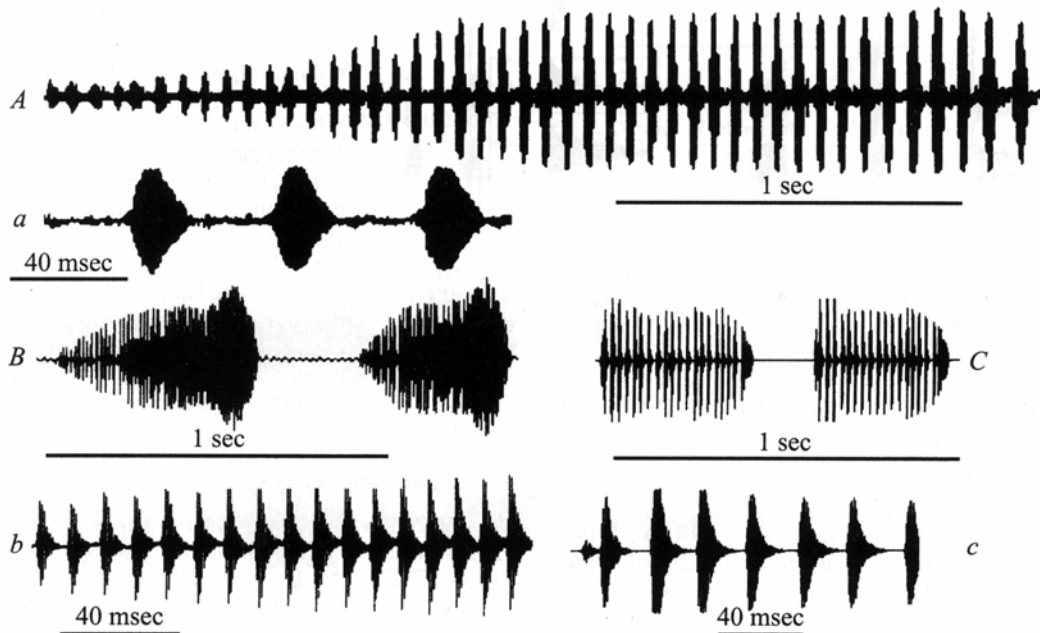
Fig. 5. Oscillograms of mating calls in the *Bufo viridis* group. *A*) *B. viridis* complex, diploid male from Fasham (Northern Iran, Elburz mountains), water temperature 10°C; *a*) detail of (*A*) with an expanded time base exhibiting the type IIIa (Martin, 1972) structure occurring in males of different ploidy in the whole Eurasian complex; *B*) *B. raddei* from Khokh Nuur (Mongolia), water temperature 20°C, two pulse trains produced by the same male; *b*) detail of B with an expanded time base exhibiting the characteristic type I (Martin, 1972) structure; *C*) *B. calamita* from Halle/Saale (Germany), water temperature 17°C; *c*) detail of (*C*) with an expanded time base also exhibiting the type I (Martin, 1972) structure. Note the nearly equal time scale in *A* – *C* (1 sec) and *a* – *c* (40 msec). Recordings: *A*, *a*) J. Martens; *B*, *b*) H. Mix and A. Bräunlich; *C*, *c*) P. Bretschneider.
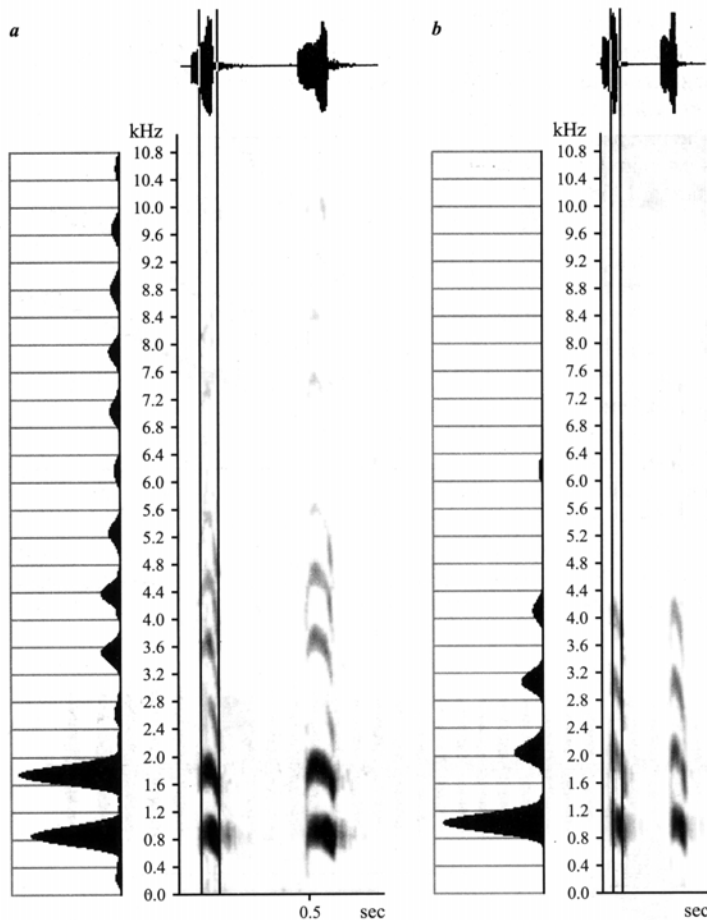
Fig. 6. Oscillograms (above) spectrograms (below), and power spectrum (left) of a release call of *B. raddei* (22.0 g body weight). Water temperature is 7 (*a*) and 22°C (*b*). Recording M. Stöck.

lar structure of their MCs and MRCs (Table 2, Fig. 8*c – h*; Stöck, in preparation).

Interestingly, Martin (1972) classified *B. calamita* calls as type I in which "the rise time is fast and is usually followed by a damped oscillation." Type I calls exhibit "passive amplitude modulation produced by vibration of the arytenoid cartilages" which is caused by an unidirectional pressure of pulmonary air which limits the call duration by the lung volume. Our results (Figs. 2*d*, *e*, 5*b*, Table 2) clearly demonstrate this type I modulation in *B. raddei*. On the contrary, Martin (1972) arranged the MCs of *B. viridis* (and this accounts for the whole complex) to his actively pulsed modulation pattern, exhibiting a "deac-

tivation of the arytenoid cartilages resulting from muscle contraction" (type IIIa).

In conclusion, we consider the resemblance of the *B. calamita* and *B. raddei* MCs to be caused by synapomorphic anatomic and functional structures which are not only phenetic similarities. Therefore, *B. raddei* appears more closely related to *B. calamita* than either is with the *B. viridis* complex. This does not contradict Borkin's (1984, 1986) theory about "Western-Eastern palearctic disjunction" of "amphiboreal lineages" which were possibly formed during the Neogene (see also Borkin, 1999). However, it may well be that the *B. calamita*/*B. raddei* lineage diverged earlier from the *B. viridis* lineage, i.e., before the hypothetically common ranges were divided in the West-Eurasian ranges of *B. calamita* as well as the *B. viridis* lineage and the Central-Eastern-Asian range of *B. raddei*. Several references (Borkin, 1984; Ratnikov, 1992, 1997; Kuzmin, 1999:276) suggested past contacts between the now allopatric *B. raddei* and *B. calamita*. The differences in their MC pulse rates, on which female preferences in anurans are often based (Gerhardt, 1994), were possibly originally an isolating barrier between *B. raddei* and *B. calamita*, as typical "of closely-related, sympatric, synchronically-breeding taxa" in which "carrier frequencies are usually similar" (Littlejohn, 1977).

4.2. Significance of MRCs

MRCs have been suggested to be "considerably more valuable a phylogenetic tool than the MC is" (Brown and Littlejohn, 1972). However, the literature exploring this hypothesis is scarce: e.g., Sullivan and Malmos (1994) discussed the phylogenetic significance of MRC compared with MC patterns in *B. alvarius*. The single long pulse of the *B. raddei* MRC and the pulses train without intervals in those of *B. calamita* contrast with the pulsed MRCs in the *B. viridis* complex, and they might also be a result of different types of modulation as in the MCs of both

species. Weber's (1979) experiments suggested differences between *B. viridis* and *B. calamita* concerning the anatomical structures involved in the RC production.

The relationship between MRC duration and body temperature corresponds to results in other *Bufo* species (e.g., Brown and Littlejohn, 1972; Matsui, 1985).

4.3. Correlation of MCs and MRCs

B. raddei exhibited the MCs with highest pulse rate and a continuous series of pulses. This species also produced the MRCs with the most continuous oscillograms seeming to consist of one pulse group. If this reflects a relationship between MC and MRC data in *B. raddei*, it requires additional analyses with MRCs from different populations. Whereas the pulse rate of the MRCs approximated that of the MC in most species of the *B. americanus* group (Brown and Littlejohn, 1972), Sullivan (1992) found no significant correlation in these call parameters in *B. americanus*. Sullivan and Wagner (1988) found MRC pulse rate, duration and frequency significantly lower than corresponding advertisement call variables in *Bufo valliceps*.

4.4. Nomenclatural Remarks

We present additional data to separate the term "*Bufo viridis* group" introduced by Inger (1972) from the term "*Bufo viridis* complex" (see *Introduction*).

Even when Blair (1972) interpreted the phylogenetic position of *B. viridis* he showed a particular position of the species within the "*B. viridis* group," and Bogart (1972) supposed a separate invasion of *B. calamita* and *B. viridis* into Eurasia. The designation "*B. viridis* group" becomes increasingly problematic because its naming species is in truth a complex of Eurasian taxa exhibiting a broad radiation which in
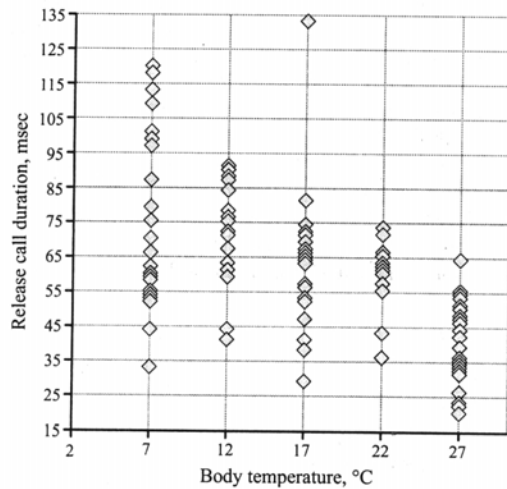
Fig. 7. Duration of release calls in relationship to body temperature in a male *B. raddei* (22.0 g body weight) from Orchon river (Mongolia).
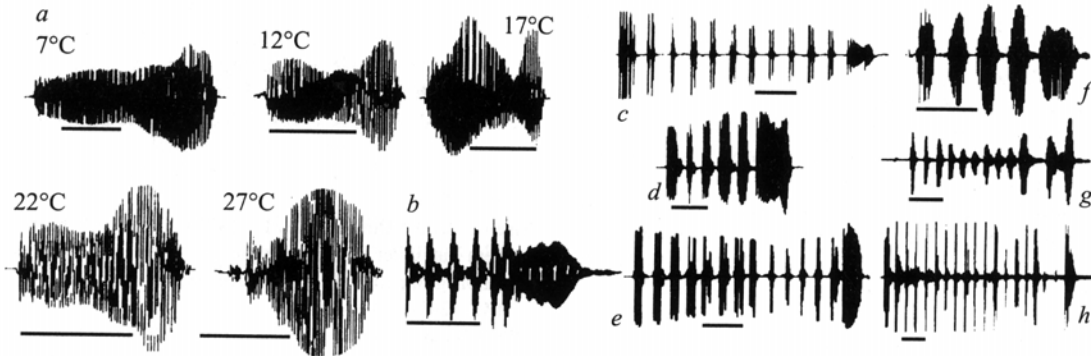
Fig. 8. Oscillograms of male release calls in the *Bufo viridis* group. *a*) *B. raddei* (22.0 g) from Orchon river (Mongolia) at different body temperatures (7 – 27°C); *b*) *B. calamita* (37.5 g) from Halle/Saale, Central Germany at 16.5°C body temperature; *c – h*) Male release calls in representatives of the *Bufo viridis* complex from different regions of Asia: *c*) tetraploid male (26.0 g) from Naryn (Kyrgyzstan, Central Tien Shan) at 9°C body temperature, *d*) tetraploid male (32.0 g) from Baghestan (Eastern Iran, Khorassan) at 25°C body temperature, *e*) tetraploid male (18.0 g) from Kashgar (Western Xinjiang, China) at 21°C body temperature, *f*) triploid male (22.5 g) from Pasu (Karakoram, Pakistan) at 22°C body temperature, *g*) diploid male (38.0 g) from Kerman (Central Iran, Kerman Province) at 22°C body temperature, *h*) diploid male (42.0 g) from 50 km east of Gonbad-e-Kavus (Northeastern Iran, Mazanderan Province) at 22°C body temperature. All bars represent 40 msec. Recordings: *a, c – h*) M. Stöck; *b*) P. Bretschneider.

Asia is strongly coupled with polyploidisation and probably with modifications of hybridogenesis (Stöck et al., 1999). Moreover, the *B. viridis* complex is well defined by bioacoustic evidence. Finally, all data also suggest the separation of *B. raddei* from the "*B. viridis* complex" where Kuzmin (1999:275) included it, but Kuzmin obviously used the term synonymously with Inger's "*B. viridis* group."

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TABLE 3. Call Duration of Male Release Calls (*n*) of *B. raddei* from Orchon River (Mongolia) at Different Body Temperatures

	Body temperature, °C				
	7	12	17	22	27
Mean, msec	77.48	72.30	62.05	60.65	37.39
Min, msec	33	41	29	36	20
Max, msec	120	91	133	73	64
SD	26.72	13.37	20.82	9.33	11.62
n	23	26	24	19	33

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