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Color pattern and call variation in *Kalophrynus* from south-east Asia
(Anura: Microhylidae)

Variabilität von Farbmuster und Ruf bei *Kalophrynus* aus Südostasien
(Anura: Microhylidae)

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KURZFASSUNG

Wir untersuchten Farbmuster und Morphologie von *Kalophrynus interlineatus* BLYTH, 1855 anhand von in der Literatur beschriebenen und in Museen hinterlegten Exemplaren, im besonderen den "namentragenden Typen", und beschreiben die geographische Variation des Taxons. Nach der gegenwärtigen Datenlage sollte *Kalophrynus orangensis* DUTTA, AHMED & DAS, 2000 als jüngeres subjektives Synonym von *K. interlineatus* betrachtet werden. Anzeigerufe von Tieren aus dem nördlichen Vietnam werden beschrieben und mit denen der nordthailändischen Populationen verglichen. Dabei wurden keine signifikanten Differenzen festgestellt, sodaß trotz der Unterschiede im Farbmuster die Ähnlichkeit der Paarungsrufe eine Abgrenzung in zwei Arten nicht erlaubt. Das Verbreitungsgebiet von *Kalophrynus menglienicus* (YANG & SU, 1980) schließt das nördliche Vietnam mit ein.

ABSTRACT

We compared the color pattern and external morphology of specimens of *Kalophrynus interlineatus* BLYTH, 1855 described in the literature and deposited in museums, in particular of name-bearing types, and describe the geographic variation of the taxon. According to the data available, *Kalophrynus orangensis* DUTTA, AHMED & DAS, 2000 should be considered a junior subjective synonym of *K. interlineatus*. The advertisement call of specimens from northern Vietnam is described and compared to that from a population of northern Thailand. Significant differences were not found and despite color pattern variation, similarity in the advertisement call does not allow to separate them on species level. The range area of *Kalophrynus menglienicus* (YANG & SU, 1980) was found to extend into northern Vietnam.

KEY WORDS

Amphibia: Anura: Microhylidae, *Kalophrynus interlineatus*, color pattern variation, mating call, geographical variation, systematics, taxonomy, Vietnam

INTRODUCTION

The genus *Kalophrynus* has been described by TSCHUDI (1838) concomitantly with the new species *Kalophynus pleurostigma* from Sumatra. In 1942, BOURRET recognised four species, separating *K. pleurostigma* in two subspecies following PARKER (1934). Currently 15 nominal species are known; distributed from southern China and north-eastern India to Indo-China and Indo-Malaya (FROST 1985, 2004). Borneo with eight species is a major diversity spot for this genus. From the northern part of the Indochinese subcontinent three species are recognised: *Kalophrynus orangensis* DUTTA, AHMED & DAS, 2000 (northeastern India), *Kalophrynus pleurostigma* and *Kalophrynus interlineatus* BLYTH, 1855 (southern China, Vietnam, Cambodia, Thailand) (MATSUI et al. 1996).

Kalophrynus pleurostigma has been considered a highly variable species (BOURRET 1942; INGER 1954 1966). Recent analyses of the advertisement calls showed that intraspecific variation is smaller than previously assumed (MATSUI et al. 1996) and led to the recognition of the species *pleurostigma* and *interlineatus*. New collections also allowed to define a new species (*K. minusculus*) that previously has been included into this species complex (ISKANDAR 1998). Thus, the concept of the *Kalophrynus* species has evolved.

New materials at our hand including specimens from Laos and northern Vietnam

show important variation concerning dorsal color pattern. Can this variation be considered as intraspecific or could there be sibling species subsumed under a single name? The authors present mating call analyses which do not follow the pattern of morphological variation. Name-bearing types were studied to ensure allocation of names.

MATERIALS AND METHODS

Abbreviations used. MNHN - Muséum National d'Histoire Naturelle, Paris, France; NMW – Naturhistorisches Museum, Wien, Austria; USNM - United States National Museum, Washington, USA; ZMB - Museum für Naturkunde, Humboldt-Universität, Berlin, Germany; ZSI - Zoological Survey of India,Calcutta, India. SVL - snout vent length.

Specimens studied. *Kalophrynus pleurostigma*: MNHN 1893.0061-0062, Paton (97°31'E, 17°41'N), Myanmar; MNHN 1891.0099, Sebroeang (111°53'E, 0°27'N) Borneo, Indonesia; MNHN 1974.1376, near Ho Chi Minh ("région de Saigon") (106°40' E, 10°45'N), Vietnam; USNM 37374-37375, Basilan, Philippines; NMW 22887, Mindanao (125°0'E, 8°0'N), Philippines; USNM 228778, Barrio (121°04'E, 14°57' N), Philippines; USNM 130200, Bundu Tuhan (116° 32'E, 14°32'N), Borneo, Indonesia; USNM 197671, Sungei (110°20'E, 1°02'N), Sarawak, Malaysia; *Kalophrynus interlineatus*: ZSI 9853, holotype of *Engystoma interlineatum* BLYTH, 1855, Mergui (98°36'E, 12°26' N), Myanmar; MNHN 2003.1169 (adult female; SVL 38.0 mm), Xepian National Biodiversity Conservation Area (106°12'E, 14° 34'N), Champasak Province, Laos; USNM 103422, USNM 103450, Chantaburi (102° 09'E, 12°36'N), Thailand; MNHN 1997. 5488-5490, Ben En (105°28'E, 19°35'N), Vietnam; *Kalophrynus menglienicus*: MNHN 1896.0061-0062, "Haut-Tonkin", region of Lang Son (106°30'E, 21°45'N) et Cao Bang ("Cao-Bong") (106°15'E, 22° 40'N), Vietnam.

The advertisement calls of two specimens of *Kalophrynus interlineatus* were recorded at the Ben En National Park, Thanh Hoa Province, Vietnam (19°30'-40'N, 105°21'-35'E), on 30 July 1997. The voucher specimens were deposited in the collection of the MNHN under the numbers MNHN 1997.5489-5490. Recordings were made by the junior author using a Sony TCD-D7® digital audio tape-corder with a Sony ECM-929LT® external microphone. At time of recording, air temperature was 25.0 °C and water temperature 29.5 °C. The recorded calls were analysed using Canary 1.2.4® sound analysis software from the Cornell Laboratory of Ornithology on a Power Mac G4® computer. The sampling rate used to convert the signals to digital format was 16 KHz with 16-bit precision. Frequencies above 5 KHz were filtered to discard surrounding noise. Filter bandwidths of 253.75 Hz and frame length of 256 points were used for spectrogram building. The notes are composed of groups of pulses. Notes are measured from the beginning of the first pulse to the end of the last pulse; intervals between two subsequent notes are measured from the end of the last pulse of the first note to the beginning of the first pulse of the following note; note repetition rate is the number of notes per second; pulse repetition rate is the number of pulses per second. Intervals between two subsequent notes longer than five seconds were removed from the mean because they could be due to disturbance by the recorder.

RESULTS

Dorsal color pattern in *Kalophrynus interlineatus*

The specimen collected on Boloven Highlands, southern Laos (MNHN 2003. 1169) clearly belongs to the species *K. interlineatus* as defined by MATSUI et al. (1996) in that it shows distinct dorsal lines forming a reverse V-shaped pattern on the back including a pair of internal bands and finer external lines of uniform color and smooth outlines (fig. 1a). The sides of the

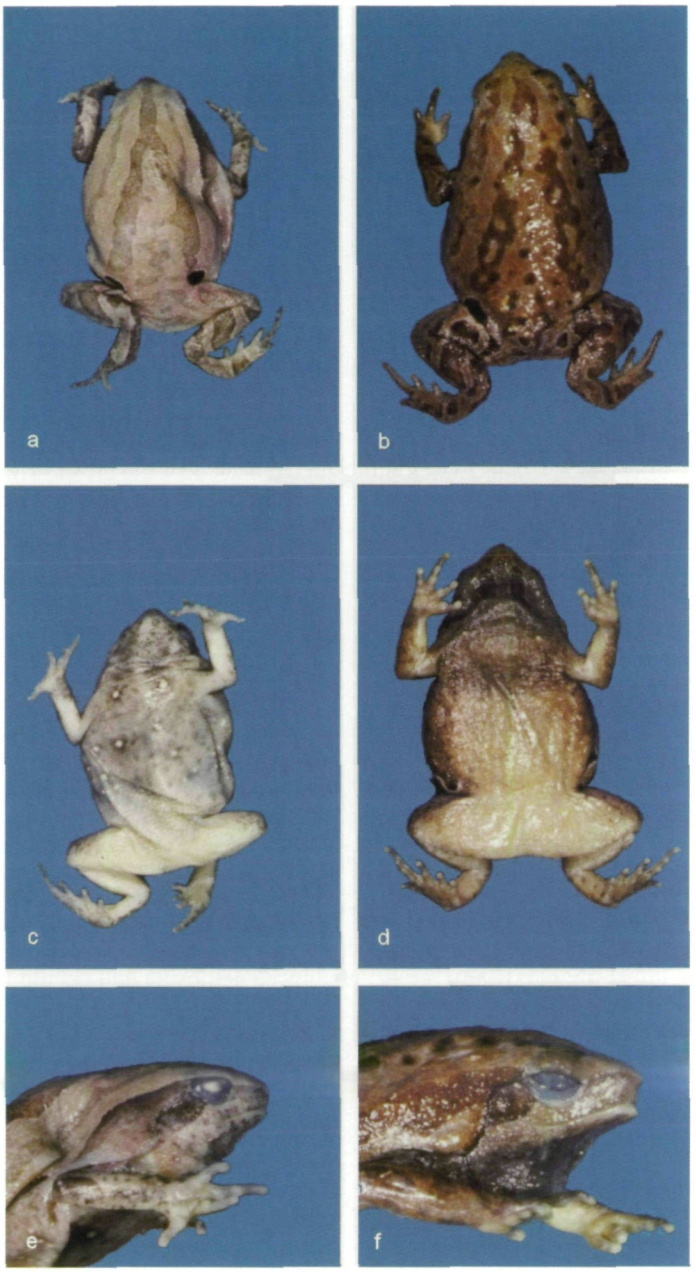

Fig. 1: *Kalophrynus interlineatus* BLYTH, 1855. 1a (dorsal view), 1c (ventral view), 1e (lateral view of head): MNHN 2003.1169, adult female from Xepian NBCA, Champasak Province, Laos. 1b (dorsal view), 1d (ventral view), 1f (lateral view of head): MNHN 1997.5489, adult male from Ben En National Park, Vietnam.

Abb. 1: *Kalophrynus interlineatus* BLYTH, 1855. 1a (Dorsalansicht), 1c (Ventralansicht), 1e (Lateralansicht des Kopfes): MNHN 2003.1169, adultes Weibchen aus Xepian NBCA, Champasak Provinz, Laos. 1b (Dorsalansicht), 1d (Ventralansicht), 1f (Lateralansicht des Kopfes): MNHN 1997.5489, adultes Männchen aus dem Ben En National Park, Vietnam.

body are distinctly darker. On the belly white macroglands are visible (fig. 1c). Specimens from northern Vietnam (MNHN 1997.5288-5290) show less clear-cut outlines in their dorsal color pattern (fig. 1b). In particular, the longitudinal dark brown bands on the back are disrupted by lighter brown spots. Lateral coloration is similar to the Boloven frog, however, there are very distinct bands on the throat in the Vietnamese specimens (fig. 1d).

When we analysed published photos and drawings of the color pattern of frogs referred to as *K. interlineatus*, we noticed that the patterns consistently mirrored the above observations. The specimens figured by BOURRET (1942: 499, fig. 181; Northern Vietnam), LARSEN et al. (1986: 33; Hong Kong, China) and FEI et al. (1991: 307; Pingxiang, Guangxi, China) resemble the North Vietnamese form showing disrupted, discontinuous longitudinal bands on the back. CHAN-ARD (2003: 97) shows a frog (mainland Thailand, without precise locality) with distinct bands on the back, quite similar to the Laos phenotype. The frogs on pages 51 and 54 in CHAN-ARD et al. (1999), presented under the name *K. pleurostigma*, come from Loei (Thailand), where MATSUI et al. (1996) reported *K. interlineatus*; they indeed show continuous lines on the back. The dorsal color pattern can be less pronounced in specimens that are all over lighter in color and the outer bands are quite distinct in specimens with darker general coloration. But none of the frogs depicted on pages 51 and 54 in CHAN-ARD et al. (1999) shows the disrupted bands present in specimens from northern Vietnam and China.

Comparison to type specimens

The holotype (ZSI 9853) of *Engystoma interlineatum* BLYTH, 1855 does not show any distinct pattern on its back which is gray-brown with small white star-like dots. There is a blackish spot in the axillary and groin regions. The limbs have indistinct brown bands and the proximal posterior part of the thigh is dark brown. The present color pattern is probably due to bleaching. The holotype of *Kalophrynus stellatus* STEJNEGER, 1908 (USNM 37355) from Basilan, Philippines, has a brown back with fine white spots wiped out by large bleached areas and two black spots on the posterior back near the groin. Limbs are of uniform color without strips. One syntype of *Calophrynus pleurostigma* var. *sinensis* PETERS, 1867 (NMW 22887) was described as coming from Hongkong, but "Mindanao, Philippinen" is written on the label. The second specimen, ZMB 5696, was said to come from Dapa, Mindanao, Philippines (BAUER et al. 1996). The first mentioned syntype, an adult female, is of small size (SVL 35.5 mm). Its dorsum is gray-brown, under the microscope, small white star-like spots can be distinguished, two dark spots are present on the back near the groin. The limbs do not show bands, but a dark zone can be distinguished on the proximal posterior part of the thigh near the vent.

The recently described species from Assam, India, *K. orangensis* DUTTA, AHMED & DAS, 2000 has distinct dark bands on the back, similar as in southern populations of *K. interlineatus*.

Kalophrynus minusculus ISKANDAR, 1998, described from West Java has previously not been distinguished from *K. pleurostigma*. It has a pair of black bands extending from between the eyes to the groin paralleled by several more black stripes on the back. It does not show a distinct spot at the sides of body near the groin. *Kalophrynus minusculus* is distinctly smaller than *K. pleurostigma* from India and the Indochinese peninsula and should be considered a distinct species.

Comparison of the type specimens resulted in the following conclusions. The names *Kalophrynus stellatus* and *Calophrynus pleurostigma* var. *sinensis* are synonyms of *Kalophrynus pleurostigma* and available for populations from the Philippines. From the morphology and color pattern of the syntypes, type locality of *Calophrynus pleurostigma* var. *sinensis* should be Philippines as stated by BAUER et al. 1996.

For *Kalophrynus* populations of Thailand and Laos two names are available: *Engystoma interlineatum* and *Kalophrynus orangensis*. The differential diagnostic characters of *K. interlineatus* mentioned by DUTTA et al. (2000) and DAS & HAAS (2003),

Table 1: Advertisement call parameters of *Kalophrynus interlineatus* BLYTH, 1855 from Vietnam. Mean ± standard deviation, range, and sample size in parentheses.

Tab. 1: Paarungsrufparameter von *Kalophrynus interlineatus* BLYTH, 1855 aus Vietnam. Mittelwert ± Standardabweichung, Streuung, und Stichprobengröße in Klammern.

Specimen Exemplar	Note length (ms) Silbenlänge (ms)	Number of pulses Anzahl Pulse	Interval between notes (s) Silben-intervall (s)	Pulse rate (pulses/s) Pulsfrequenz (Pulse/s)	Note rate (notes/s) Silbenfrequenz (Silben/s)	Fundamental frequency (Hz) Grundfrequenz (Hz)
MNHN 1997.5489	740.8 ± 77.2 645.4-1055.0 (68)	32.1 ± 3.3 28-46 (68)	1.242 ± 1.135 0.734-4.254 (67)	43.3 ± 1.0 36.5-44.9 (68)	0.56 ± 0.07 0.47-0.64 (4)	685
MNHN 1997.5490	641.3 ± 51.9 553.8-912.1 (98)	29.8 ± 2.5 25-42 (98)	1.156 ± 0.613 0.403-3.467 (94)	46.5 ± 0.6 44.6-47.8 (98)	0.59 ± 0.08 0.52-0.68 (3)	747

namely the stripe on the head not reaching the tip of the snout and the absence of an outer metatarsal tubercle can not seriously have been based on the study of the type specimen, since the holotype of *Engystoma interlineatum* has no more dorsal pattern, but it has an outer metatarsal tubercle, which is certainly indistinct, but present. This tubercle is also present, but flat, in the specimen MNHN 2003.1169 allocated to *Kalophrynus interlineatus*. As differential characters to the type-specimen are not known and as long as no further data on the taxonomic status of *Kalophrynus orangensis* are available, it should be considered a junior subjective synonym of *Kalophrynus interlineatus*.

The color pattern of the specimens from northern Vietnam and southern China cannot be allocated to any of the type specimens, but we have to be cautious as the holotype of *Engystoma interlineatum* has lost all its colors.

Advertisement call

During a single night within an observation period of two months (Ben En, Vietnam; July-August 1997), several *Kalophrynus* males were found calling in a chorus. The site was an area flooded by heavy rains fallen in the night before. The males were posted on the ground at a distance of a few decimetres from the edge of the water which was 30-40 cm deep. The following day the calling males disappeared though the area was still flooded. Calls of two males were recorded (MNHN 1997.5489, SVL 43.3 mm and MNHN 1997.5490, SVL 42.8 mm). Call parameters are given for each specimen in table 1. Data reported in the below call description are mean values from the two recorded males.

The call consisted of a single note (fig. 2) emitted at an interval of 0.403-3.467 s ($\bar{x}$ ± SD = 1.919 ± 0.863, n = 159), the note repetition rate ranged from 0.47-0.68 notes per second ($\bar{x}$ ± SD = 0.57 ± 0.07, n = 7). The notes lasted 553.8-1055.0 ms ($\bar{x}$ ± SD = 682.0 ± 80.1, n = 166) and were composed of a series of 25-46 pulses ($\bar{x}$ ± SD = 30.7 ± 3.0, n = 166), the pulse repetition rate ranged from 36.5 to 47.8 pulses per second ($\bar{x}$ ± SD = 45.2 ± 1.8, n = 166). The amplitude of the note increased quickly in its first third, then decreased more slowly until the end, as did the amplitude of the pulses. The dominant frequency equalled the fundamental frequency and spread over a frequency band of 610-890 Hz (average peak reached at 685 Hz, n = 10) for the specimen MNHN 1997.5489 and 710-930 Hz (average peak reached at 747 Hz, n = 10) for the specimen MHNH 1997.5490. Another obvious band, the third harmonic, spread over a frequency band of 1780-2030 Hz (average peak 1930 Hz) for the specimen MNHN 1997.5489 and 1990-2115 Hz (average peak 2058 Hz) for the specimen MNHN 1997.5490. Frequency modulation was absent.

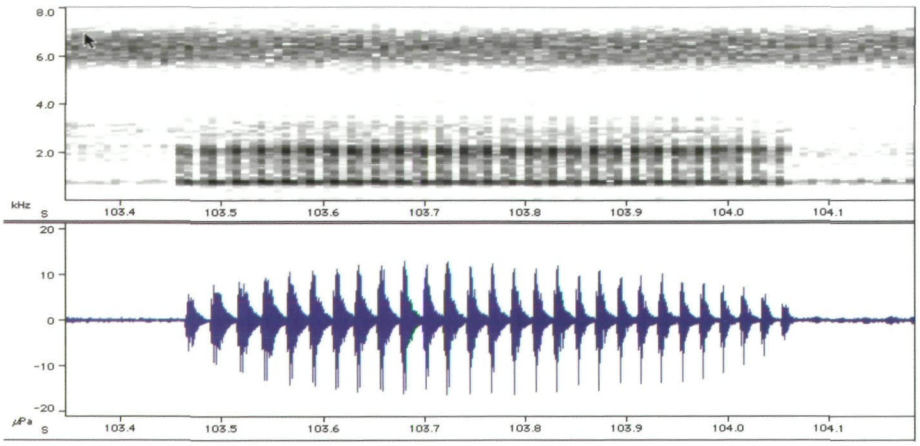

Fig. 2: Sonagram (above) and oscillogram (below) of an advertisement call of *Kalophrynus interlineatus* BLYTH, 1855 from Vietnam (MNHN 1997.5490).

Fig. 2: Sonagramm (oben) und Oszillogramm (unten) des Anzeigerufes von *Kalophrynus interlineatus* BLYTH, 1855 aus Vietnam (MNHN 1997.5490).

DISCUSSION

Morphology. Four groups of frogs can be distinguished within *Kalophrynus* as for the presence and specification of an inguinal spot. (I) A black distinct inguinal spot is always present in *K. pleurostigma*, *K. interlineatus* and *K. orangensis*. (II) In *K. subterrestris* INGER, 1966 a dark inguinal spot may or may not be present (INGER 1966: 138). (III) In some species the inguinal spot is not black. It is described as yellow spots in *K. baluensis* (KIEW 1984b) and as "one or more bluish white spots in a black inguinal area" (INGER 1966: 131) or white spots (BOULENGER 1900) in *K. heterochirus*. (IV) The inguinal spot is reported absent in *K. eok* (DAS & HAAS 2003), *K. intermedius* (INGER 1966; INGER & STUEBING 1997), *K. menglienicus* (YANG & SU 1980), *K. minusculus* (ISKANDAR 1998), *K. nubicola* (DRING 1984), *K. palmatissimus* (KIEW 1984a), *K. punctatus* (PETERS 1871; INGER 1966), and *K. robinsoni* SMITH, 1922 (BOURRET 1942). Thus the population from northern Vietnam belongs to the group with distinct black inguinal spot.

In their dorsal patterns only *K. interlineatus*, *K. minusculus* and *K. orangensis* resemble the specimens from northern Vietnam which are also distinctly larger (SVL 40.4-43.3 mm) than most of the species described, namely *K. menglienicus* (adult males SVL 19.8-23.4 mm: YANG & SU 1980), *K. minusculus* (adult males SVL 25 mm: ISKANDAR 1998), *K. intermedius* (adult males SVL 27 mm: INGER & STUEBING 1997), *K. eok* (adult males SVL 26.3 mm: DAS & HAAS 2003), *K. punctatus* (adult males SVL 22-27 mm: INGER 1966), *K. robinsoni* (adult males SVL 17 mm: BOURRET 1942), *K. nubicola* (adult males SVL 19.3-20.1 mm: DRING 1984), *K. subterrestris* (adult males SVL 23 mm: INGER 1966), *K. heterochirus* (adult males SVL 26 mm: INGER 1966).

Among the species of similar size the specimens from northern Vietnam can be distinguished by their particular dorsal pattern. Thus, *K. baluensis* has no inguinal spot nor irregular light patches on the back (INGER & STUEBING 1997), *K. baluensis* and *K. palmatissimus* have a dorsal pattern which includes a transversal bar connecting the eyes and is continued posteriorly by bands extending towards the groin or breaking up into patches (KIEW 1984a, b). Descriptions of the color pattern of *K. pleurostigma* have to be analysed carefully, as *K. pleurostigma* still may be a complex of several species. BOURRET (1942) considered *K. pleurostigma pleurostigma* to be of large size (adult males SVL 49 mm, adult females SVL 53 mm) and variable in its dorsal pat-

tern which consists of a band between the eyes continued posteriorly by a pair of branches that may be fragmented, or that pattern may be completely absent. INGER (1966) described the dorsal pattern as very variable with lines or spots forming a network and mentioned that a few specimens had no such pattern at all. The frog figured by LIM & LIM (1992) presents a uniform light colored back. Specimens depicted by ISKANDAR (1998: plate 8) show a pattern as described by BOURRET, including the transversal band connecting the eyes and bands to the groin which are fragmented by lighter spots. Only in *K. interlineatus* (BOURRET 1942; MATSUI et al. 1996) and *K. orangensis* (DUTTA et al. 2001) the dorsal pattern includes a stripe that starts on head or snout. But in both species the bands and lines that go to the groin are continuous and distinct whereas in the specimens from northern Vietnam these bands are fragmented to spots. Furthermore, the outer metatarsal tubercle is distinct in northern Vietnamese specimens while it is indistinct in *K. interlineatus* and *K. orangensis*. In conclusion the specimens from northern Vietnam show minor but consistent morphological differences that indicate a certain degree of genetic isolation.

Advertisement call. Arguing for specific recognition of *K. interlineatus*, MATSUI et al. (1996) showed that its advertisement call (MATSUI et al. 1996: 443, fig. 2) differed from that of *K. pleurostigma* (MATSUI et al. 1996: 441, fig. 1). The call characteristics described in the present paper diverge only very slightly from those of the Thai population described by MATSUI et al. (1996). Frequency bands and number of pulses per note are identical, note duration on the average is longer in the Thai population leading to a lower pulse repetition rate. The higher temperature at the time of our recording may be a sufficient explanation for this difference (ZWEIFEL 1959, 1968). Quite obviously, the advertisement call of our *Kalophrynus* from Vietnam is identical to that from Thailand, described by MATSUI et al. (1996). If advertisement call characteristics are taken for indicators of the taxonomic status, the northern Vietnamese specimens cannot be considered as a separate species (although they differ from adjacent populations in their dorsal pattern). However, in northern Thailand *K. interlineatus* breeds in small ditches and permanent pools of wetlands (MATSUI et al. 1996) while we found our northern Vietnamese *Kalophrynus* breeding in temporary pools; this might be indicative for an ecological differentiation of the populations.

Even though differences in morphology may support the decision to give the populations of *Kalophrynus* from northern Vietnam and China the status of an individual species, the mating call analysis does not corroborate this finding. The interpretation of the data available is difficult as the populations studied are allopatric to the other populations of *K. interlineatus*. Thus, character differences cannot be evaluated in the frame of the biological species concept (MAYR 2000). Mating call and color pattern cannot play any role in interbreeding between these two types of *Kalophrynus* as no contact zone between them is known.

If we analyse the color pattern phylogenetically, the presence of continuous lines in most species indicates that it is the plesiomorphic state of dorsal color pattern. The uniqueness of the disrupted dorsal pattern in the northern Vietnamese populations might be interpreted as the apomorphic state. Thus the populations from northern Vietnam and China could be defined by an apomorphy and, in consequence, considered as a distinct species in the light of the phylogenetic species concept (FROST & HILLIS 1990).

We do not wish to make further taxonomic conclusions but only draw attention to this interesting biological problem which deserves further studies. The paucity of data is mainly due to the very short breeding period and fossorial habit of these species.

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