

**The tadpole of *Rhacophorus verrucosus* BOULENGER, 1893
from Vietnam
(Amphibia: Anura: Rhacophoridae)**

With 6 figures

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Abstract. The external morphology of the tadpole of *Rhacophorus verrucosus* BOULENGER, 1893, to our knowledge so far unknown, is described from southern North Vietnam (Ha Tinh Province). In addition, we give a survey of the *Rhacophorus* species known so far for Vietnam, and summarize the taxonomy, ecology and distribution of *R. verrucosus*.

Kurzfassung. Die Kaulquappe von *Rhacophorus verrucosus* BOULENGER, 1893 aus Vietnam (Amphibia: Anura: Rhacophoridae). – Es wird die äußere Morphologie der bisher unseres Wissens nach nicht bekannten Kaulquappe von *Rhacophorus verrucosus* BOULENGER, 1893 aus dem südlichen Nordvietnam (Provinz Ha Tinh) beschrieben. Zusätzlich geben wir einen Überblick über die bisher für Vietnam bekannten *Rhacophorus*-Arten sowie über Taxonomie, Ökologie und Verbreitung von *R. verrucosus*.

Key words. Amphibia, Anura, Rhacophoridae, *Rhacophorus verrucosus*, tadpole, Vietnam.

1. Introduction

The phylogenetic relationships of the Old World tree frogs of the family Rhacophoridae HOFFMAN, 1932 have been subject of intensive debate during the past decades. Due to several well-defined osteological apomorphies shared with ranid frogs, some authors proposed to place them as subfamily Rhacophorinae within the Ranidae (LAURENT 1986, DUBOIS 1992, BLUMMERS-SCHLÖSSER 1993). However, this concept was not widely accepted by most other authors, who maintained the Rhacophoridae at family status (e.g., BROWN & ALCALA 1994, RICHARDS & MOORE 1998). Most recently, VENCES & GLAW (2001) proposed a modified classification supported by molecular data. In their concept, the epifamily Ranoidea contains three families: Mantellidae, Ranidae, and Rhacophoridae. Following this approach, the Rhacophoridae are herein treated as valid family.

Within the Rhacophoridae, the genus *Rhacophorus* consists of more than 60 large and medium-sized tree frog species which are widely distributed from India to China, Japan, and the Greater Sunda Islands (GLAW et al. 1998). Concerning the Vietnamese representatives of the genus, nume-

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rous questions remain unsolved. According to INGER et al. (1999) and ORLOW & HO (2000) the following *Rhacophorus* species are currently recognised from Vietnam: *R. annamensis* SMITH, 1924, *R. appendiculatus* (GÜNTHER, 1858), *R. bathogaster* INGER, ORLOV & DAREVSKY, 1999, *R. bipunctatus* AHL, 1927, *R. calcaneus* SMITH, 1924, *R. exochopygus* INGER, ORLOV & DAREVSKY, 1999, *R. notater* SMITH, 1924, *R. reinwardtii* (SCHLEGEL, 1837), and *R. verrucosus* BOULENGER, 1893. The record of *R. binaculatus* (PETERS, 1867) from south-central Vietnam (INGER et al. 1999, ORLOW & HO 2000) appears questionable since MASTHEY & STEIOP (1998) considered the species to be restricted to the Philippines (see also the remarks in ZIEGLER & KÖHLER 2001). Records of *R. cavirostris* and *R. nigropalmatus* from northern Vietnam (TRAN et al. 1981, NGUYEN et al. 1994) were considered doubtful (INGER et al. 1999). According to INGER et al. (i.e.) the records of the latter might belong to the species *faae*. Of the *Rhacophorus* species *leporosus* (sic), *leucomystax* and *pardalis*, mentioned in NGUYEN & HO (1996) for Vietnam, the former currently is included in the genus *Theloderma* (*Rhacophorus leporosus corticalis* as *Theloderma corticalis*; *R. leporosus bicolor* as *Theloderma bicolor*), and *leucomystax* in the genus *Polypedates* (ORLOW 1997, INGER et al. 1999); the third of these species, *annamensis*, was listed as subspecies of *pardalis* by BOURRET (1942), but is now elevated to specific rank, whereas the nominal form of *pardalis* is restricted to the Philippines (see also INGER et al. 1999). Furthermore, NGUYEN & HO (1996) list the questionable *Rhacophorus* species *buergeri* and *schlegeli* for Vietnam. ÖHLER et al. (2000) described a further species of *Rhacophorus* from Vietnam: *R. duboisi*; they also list the *Rhacophorus* species *dorsoviridis* and *dugrizei* for northern Vietnam. Most recently, ZIEGLER & KÖHLER (2001) described *Rhacophorus orlovi* for southern North Vietnam.*

Rhacophorus verrucosus BOULENGER, 1893 (type locality Thao, Karin Hills, Myanmar; Ann. Mus., Genova, Ser. 2, 13: 337) was in the past regarded as subspecies of *Rhacophorus appendiculatus* (BOURRET 1942, NGUYEN & HO 1996). However, INGER et al. (1999) and ORLOW & HO (2000) regard *verrucosus* as specifically distinct from *R. appendiculatus* and from a further closely related species, *R. bisacculus* TAYLOR, 1962 from Thailand. *Rhacophorus verrucosus* is known for northeastern India, Myanmar, Cambodia and Vietnam (BOURRET 1942, ORLOW & HO 2000). In Vietnam, according to BOURRET (1942), NGUYEN & HO (1996), INGER et al. (1999), ORLOW & HO (2000), and ZIEGLER & HERRMANN (2000), the species has been collected in the northern parts, as well as in the south of the central region. INGER et al. (1999) further mention large rhacophorids of uncertain status from northern Vietnam (Tam Dao); these frogs belong into the *appendiculatus-bisacculus-verrucosus*-species complex and were later assigned to *R. appendiculatus* by ORLOW & HO (2000); a species which was known before only from India, Thailand, Malaysia, Indonesia, and from the Philippines (BOURRET 1942, INGER 1966, TAYLOR 1966, HEYER 1971, INGER & TAN 1996, DAS & DUTTA 1998). DUBOIS (1986) lists the species of the *R. appendiculatus*-group in the subgenus *Rhacophorus*, but without mentioning *verrucosus* itself.

In the framework of recent field studies in Vietnam (June to September 1997 and August to October 1998, see ZIEGLER 2000, 2002 for details) a total of 24 adult *Rhacophorus verrucosus* were collected in the surroundings of the lowland humid forest reserve Ky Anh – Ke Go in the South of Ha Tinh province, southern North Vietnam. For a thorough diagnosis of these specimens (compare also BOURRET 1942b and INGER et al. 1999), as well as for the detailed description of their morphology, pattern, colouration, bioacoustics and ecology see ZIEGLER (2000, 2002). From the tadpoles collected during field studies in the Ky Anh – Ke Go reserve, a single larva proved to be genetically identical with an adult of *R. verrucosus*. As the larval morphology of this *Rhacophorus* species is not known to our knowledge, we provide its description in the following.

2. Materials and Methods

The single tadpole was fixed in 3% formalin and later preserved in 70% ethanol. It is deposited in the Staatliche Naturhistorische Sammlungen Dresden, Museum für Tierkunde as MTKD 43475 (field number TZ '98/111).



Fig. 1: Geographical position of the Ky Anh – Ke Go reserve in the South of Ha Tinh Province, southern North Vietnam; the collecting locality of the tadpole of *Rhacophorus verrucosus*.

Frogs were fixed in 50–60% ethanol and later preserved in 70% ethanol. They are deposited in the collections of the Staatliche Naturhistorische Sammlungen Dresden, Museum für Tierkunde (MTKD) and the Zoologisches Forschungsinstitut und Museum Alexander Koenig (ZFMK), Bonn. Voucher catalogue numbers are followed by field numbers in parentheses (TZ); an asterisk marks specimens which are to be inventorized in the ZFMK collections. (1) adult males: MTKD 43470 (TZ 378), MTKD 43471 (TZ 412), MTKD 43472 (TZ 443), MTKD 43473 (TZ 418), ZFMK 74481–74483 (TZ 496–498), ZFMK 74485 (TZ 736), ZFMK 74486–74488 (TZ 741–743), ZFMK 74490 (TZ 778), ZFMK 74492 (TZ 871), ZFMK 74493 (TZ 940), ZFMK 74494 (TZ 1004), ZFMK 74495–74497 (TZ '98/83–85), TZ 738*; (2) adult females: MTKD 43474 (TZ 445), ZFMK 74484 (TZ 561), ZFMK 74489 (TZ 777), ZFMK 74491 (TZ 820), (3) cleared and stained specimen: TZ 444*. Both oocytes and contents of the gastro-intestinal tracts were removed from the preserved specimens and are all deposited separately with field numbers in the ZFMK (for details see ZIEGLER 2000, 2002). In the following description tadpole keratodont formula is set out according to DUBOIS (1995).

For molecular analyses, muscle tissue were sampled from the tail (tadpole) or thigh (frogs) of specimens using sterilized scissors and preserved in 98% pure ethanol. DNA was extracted using Qiamp tissue kits (Qiagen). We used the primers 16SA-L (5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16SB-H (5' - CCG GTC TGA ACT CAG ATC ACG T - 3') from PALUMBI et al. (1991) to amplify a fragment of the mitochondrial 16S rRNA gene. Cycling conditions were as follows: initial denaturation, 90 s at 94°C; 33 cycles: 45 s at 94°C, 45 s at 55°C, 90 s at 72°C. Products were purified using Qiaquick kits (Qiagen) and sequenced on an ABI 377 automatic sequencer. The obtained fragment (554 nucleotides) was homologous to the nucleotides 4007–4565 of the *Xenopus laevis* mitochondrial genome (ROE et al. 1985). Sequences were compared using the program Sequence Navigator (Applied Biosystems), and deposited in public databases. GenBank accession numbers are AF285226 (specimen MTKD 43470) and AF285227 (tadpole specimen MTKD 43475).



Fig. 2: Adult male of *Rhacophorus verrucosus* (MTKD 43470) from Ky Anh – Ke Go reserve (Ha Tinh Province, southern North Vietnam). – Phot. T. ZIEGLER

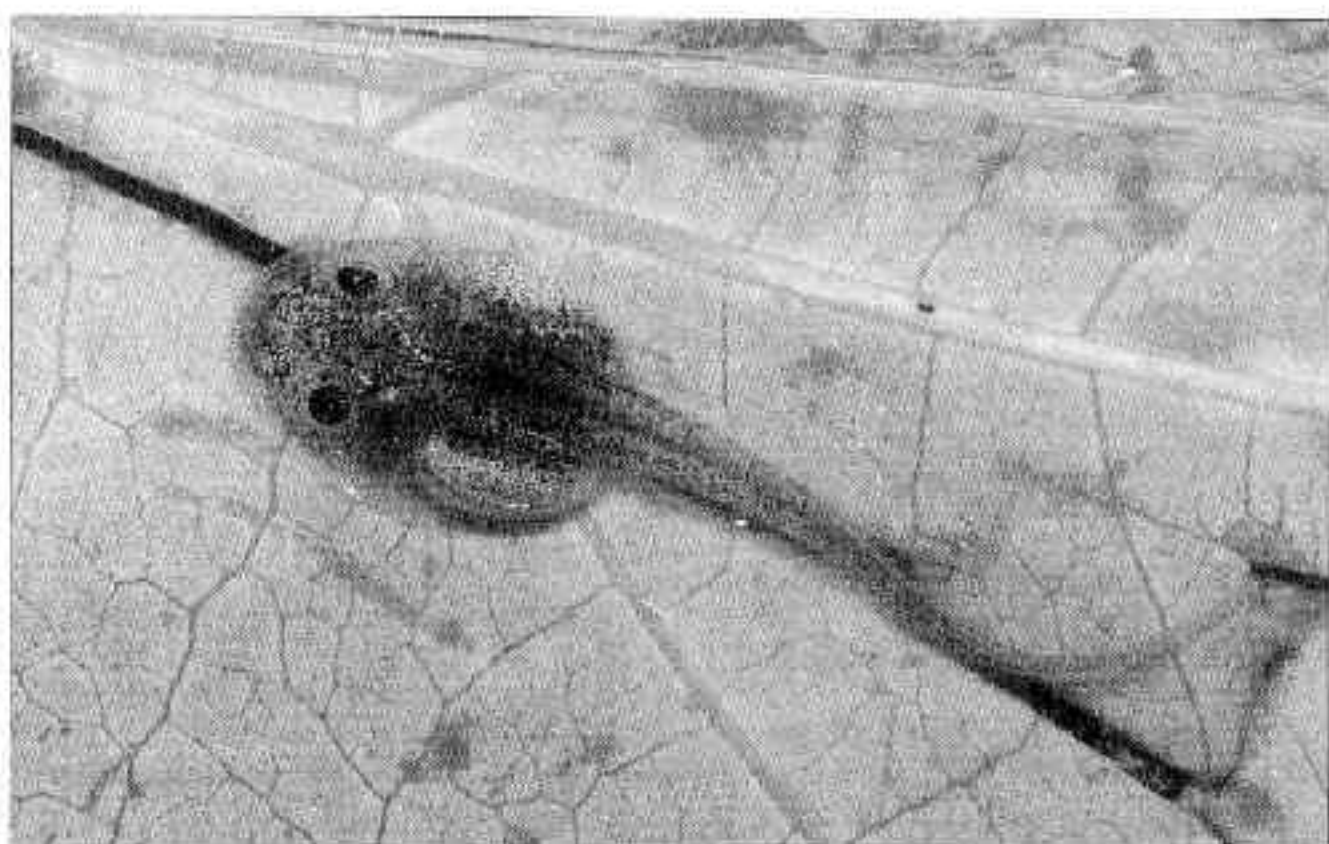


Fig. 3: Dorsal aspect of the living tadpole of *Rhacophorus verrucosus* (MTKD 43475) in GOSNER (1960) stage 30; sequences of the tail musculature proved to be 100 % identical with tissue of the syntopic male depicted in fig. 2. – Phot. T. ZIEGLER

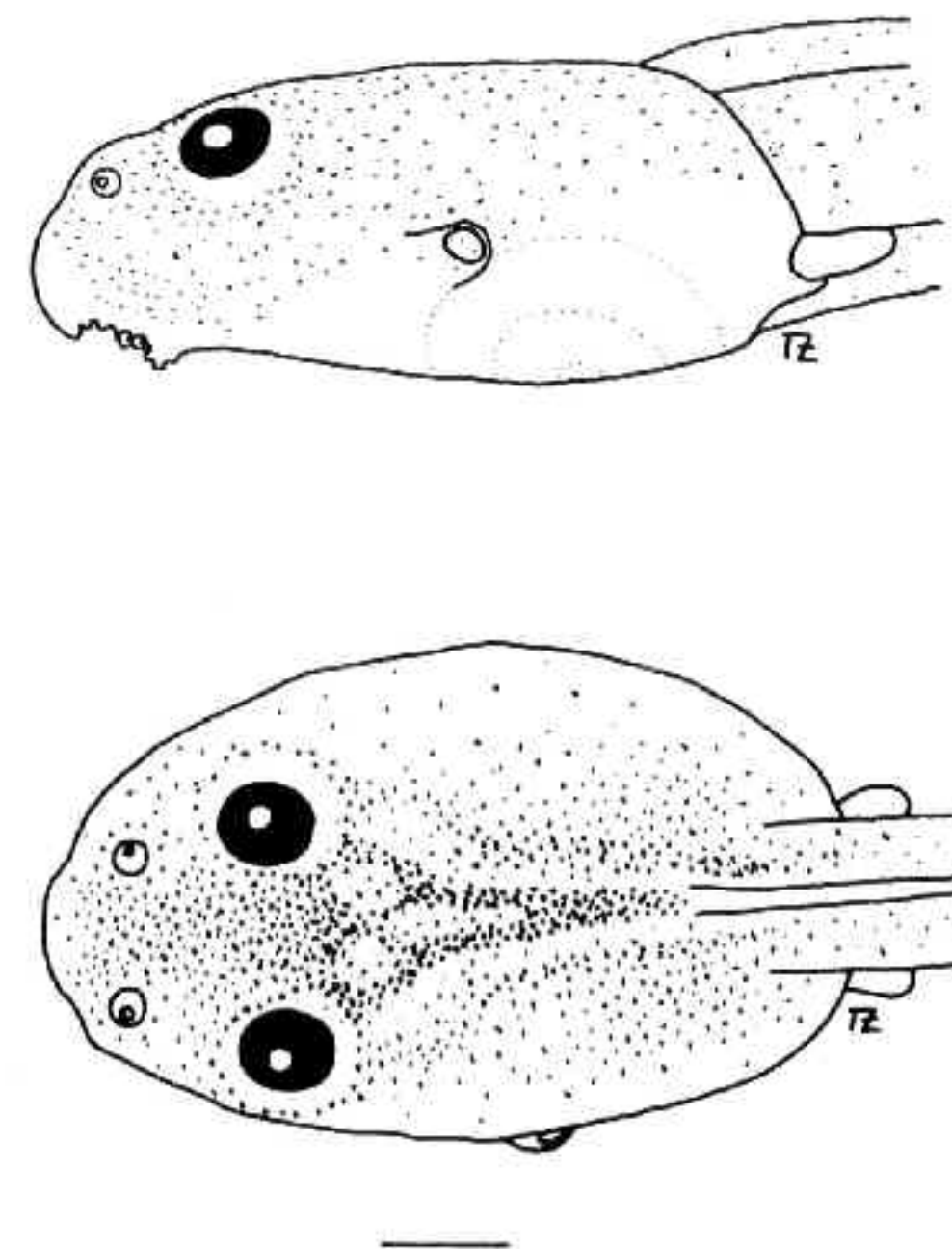


Fig. 4: Drawings of the lateral and dorsal aspect of the preserved (and, because of molecular analyses, meanwhile tailless) tadpole of *Rhacophorus verrucosus* (MTKD 43475); scale bar = 1 mm. – Orig. T. ZIEGLER

3. Results and Discussion

On 17th September 1998, during the wet season, a tadpole was caught by the senior author with a fishing-net in a muddy wayside puddle in the humid forest of the Ky Anh – Ke Go reserve in Ha Tinh Province (fig. 1), southern North Vietnam (area named "Chin Xai" by local people, meaning "nine waterfalls", vicinity of 18°04' N, 105°58' E, approximately 170 m above sea level).

Sequences of the 16S rRNA gene extracted and amplified from the tail musculature of this tadpole (MTKD 43475) proved to be 100 % identical with sequences from the syntopic adult male *Rhacophorus verrucosus* MTKD 43470 (fig. 2), but distinctly different from those of six other sympatric rhacophorid species (see below).

According to GOSNER (1960) the single tadpole of *Rhacophorus verrucosus* was in stage 30, which is defined by the length (L) / diameter (d) relationship of the developing limb bud being $L = 2 \times d$. As parts of the tail were used for molecular analyses, we merely can refer to fig. 3, for the ratio of body and tail length. Other measurements and characteristics are as follows:

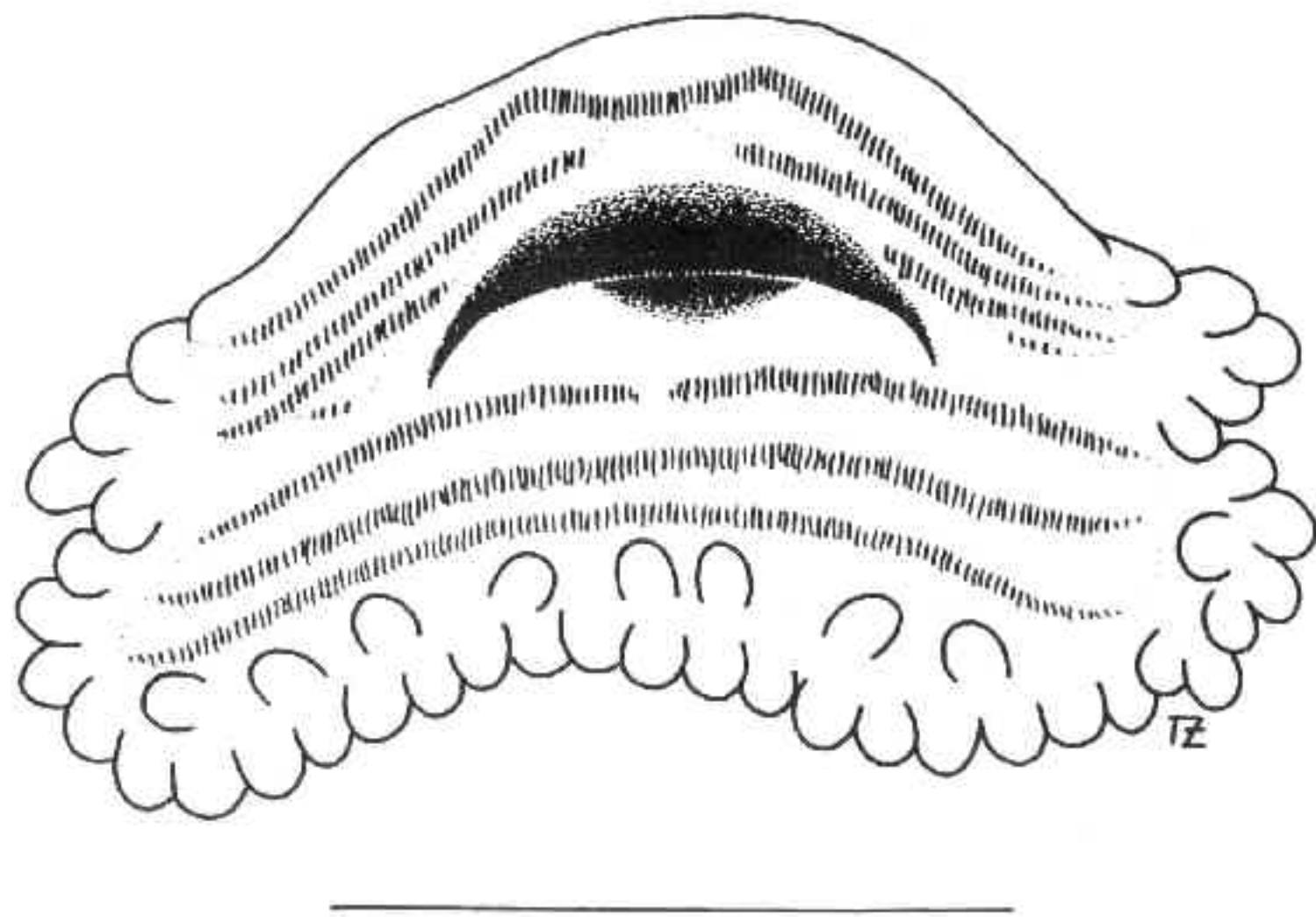


Fig. 5: Drawing of the oral disc of the tadpole of *Rhacophorus verrucosus* (MTKD 43475); scale bar = 1 mm. – Orig. T. ZIEGLER

Body length 6.0 mm; maximum body width 3.7 mm; musculature height at base of tail 1.3 mm; height of tail at base 2.0 mm; body slightly depressed, widest at midbody, wider than high; for general body shape see fig. 4.

Eyes directed dorsolaterally; dorsal interorbital distance 1.1 mm, about double of eye diameter; nostrils rounded, directed dorsolaterally, encircled by a flat rim; nostril diameter 0.2 mm; internarial distance 1.0 mm; distance between eye and nostril 0.6 mm; distance between nostril and tip of snout slightly longer than eye length.

Spiracle sinistral, its opening situated slightly below midline at a point almost two-thirds distance from tip of snout to posterior end of body; directed posterodorsally; vent tube dextral, slightly longer than limb buds; length of limb buds each 0.7 mm.

Oral disc (fig. 5) expanded laterally, in total 1.7 mm wide, ventral, subterminal; oral disc laterally and posteriorly bordered by marginal papillae; each in the lateral corners, the row of papillae is descending towards the beak; keratinized jaw sheaths about equal in length, bearing short, somewhat pointed serrations; upper jaw sheath arched with long, slender lateral processes; lower jaw sheath V-shaped; four upper and three lower keratodont rows, with only first anterior row complete and with first posterior row narrowly interrupted medially. Detailed keratodont formula thus 1:3+3/1+1:2.

For colour in life see fig. 3; colour in preservative whitish-greyish with dark grey-brown to blackish pigmentation on flanks and mainly on dorsum (see fig. 4).



Fig. 6: Habitat of *Rhacophorus verrucosus* in the Ky Anh – Ke Go reserve (August 1997), where adults and the single larva were collected. – Orig. T. ZIEGLER

In the surroundings of the Ky Anh – Ke Go reserve, at elevations of 90–350 m above sea level, numerous adults of *Rhacophorus verrucosus* were observed mainly on the border of forest clearings and on forest waysides in the surroundings of muddy puddles and ponds (where also the single tadpole was caught, see fig. 6). The species was less common in dense primary forest, swampy areas and on stream banks. Frogs sat usually in the vegetation on the ground or on branches or leaves up to 50 cm height, but sometimes also up to 1–2.5 m height above the ground. From the end of the dry season on, calling of the males was usually heard from dusk on, usually from the densely vegetated surroundings of puddles and ponds. At the beginning of the rainy season, up to 200, up to 2.5 mm large yellowish oocytes were counted in dissected females. For further ecological and bioacoustic data of *Rhacophorus verrucosus* in the Ky Anh – Ke Go reserve we refer to ZIEGLER (2000, 2002), as well as to INGER et al. (1999), ORLOW & HO (2000), and ZIEGLER & HERRMANN (2000) for general ecological data of the species in Vietnam.

As already pointed out in detail in the introduction, it is not adequately clarified how many representatives of the genus *Rhacophorus* do in fact occur in Vietnam. Currently, it can be stated according to ZIEGLER (2000, 2002) and ZIEGLER & KÖHLER (2001) that in the Ky Anh – Ke Go reserve *Rhacophorus verrucosus* occurs syntopically with the following rhacophorid species: *Chirixalus vittatus*, *Polypedates dennyi*, *Polypedates leucomystax*, *Polypedates* sp., *Rhacophorus reinwardtii*, and *Rhacophorus orlovi*. Tadpoles of *Rhacophorus orlovi* are unknown to science, and the taxonomic status of *Polypedates* sp. has still to be solved (for tadpoles see ZIEGLER 2000, 2002, and

ZIEGLER unpublished). Descriptions of the tadpoles of the remaining rhacophorids known from the area can be found in, e.g., POPE (1931), BOURRET (1942), HEYER (1973), and MANTHEY & GROSSMANN (1997). However, in general, an exact assignment of most rhacophorid larvae described so far is nearly impossible in view of numerous taxonomical problems.

The present paper exemplifies that molecular analyses can help to shed light upon the actual systematic relationships of frogs and affiliated tadpoles. DNA sequences, if deposited in public databases, provide unambiguous means to assign the larvae to adult stages, even after taxonomic re-definitions and rearrangements. They therefore provide substantial contributions to future works on larval morphology and ecology in amphibians.

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* Note added in proof:

Recently a further new *Rhacophorus* species (*R. hoanglienensis*) was described for Vietnam: ORLOV, N. I., LATHROP, A., MURPHY, R. W. & HO TITU CUC (2001): Frogs of the family Rhacophoridae (Anura: Amphibia) in the northern Hoang Lien mountains (Mount Fan Si Pan, Sa Pa district, Lao Cai province), Vietnam. – *Russ. J. Herpetol.* **8** (1): 17–44.