Captive management and reproductive biology of Orlov's Treefrog, *Rhacophorus orlovi* Ziegler & Köhler, 2001 (*Amphibia: Anura: Rhacophoridae*), including larval description, colour pattern variation and advertisement call


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Abstract

In this paper, the successful keeping and breeding of Orlov’s Treefrog, *Rhacophorus orlovi* Ziegler & Köhler, 2001, is described for the first time. Breeding took place at low temperatures of 19° to 24° C, based on two couples from northern Vietnam. The species turned out to have a noticeably rapid development. Slightly more than two weeks after foam nest building, larvae developed hindlimbs, and
after about four weeks, all larvae were metamorphosed, with a snout-vent length of about 10.5 mm. In addition, we provide for the first time the morphological description of the larva of *R. orlovi*, and we describe so far unknown subadult and adult colour patterns and the advertisement call of this species from Vietnam.

**Keywords:** *Rhacophorus orlovi*; Captive management; Reproduction; Larval description; Colour variation; Advertisement call

**Introduction**

*Rhacophorus orlovi* has been described a decade ago by Ziegler and Köhler (2001) from the lowland forests of Ha Tinh Province in Vietnam. It was named after the Russian herpetologist Nikolai Orlov from the Zoological Institute of the Russian Academy of Sciences, St. Petersburg. Orlov’s Treefrog (Fig. 1) is characterized amongst others by its medium size in combination with its typical pattern consisting of a reddish-brown dorsum with dark brown markings, dark interorbital bar, dark brown loreal region (mostly with irregular yellow blotches), and yellow or bluish turquoise spotting on flanks and posterior side of thighs. It is further characterized by its distinct canthus rostralis and supratympanic fold, lack of dermal flaps or ridges on limbs and around venter, tibiotarsal articulation reaching between eye and nostril when hind limb is adpressed forward, webbing between outer fingers reaching between distal subarticular tubercle and disc, and fully webbed feet (Ziegler & Köhler, 2001; Orlov, Nguyen, & Ho, 2008).

![Adult *Rhacophorus orlovi* from Phong Nha - Ke Bang, Quang Binh Province, Vietnam.](image-url)
Rhacophorus orlovi meanwhile has been recorded from diverse provinces in northern and central Vietnam (from North to South: Lao Cai, Ninh Binh, Nghe An, Ha Tinh, Quang Binh, Quang Tri, Thua Thien - Hue, Kon Tum, and Gia Lai). According to Stuart (2005), Rhacophorus binaculatus reported by Inger, Orlov, & Darevsky (1999) from Vietnam in reality represents R. orlovi. Besides its occurrence in Vietnam, R. orlovi also has been reported from Laos and northeastern and eastern Thailand (Ziegler & Köhler, 2001; Ziegler, Herrmann, & Köhler, 2002; Nguyen et al., 2009; Frost, 2011). Most probably, Orlov’s Treefrog has an even wider distribution range than current records suggest. The species inhabits surroundings of streams in lowland primary forests up to 850 m a.s.l. R. orlovi also can be found in semi-deciduous forests, karst forests and in associated limestone caves and karst crevices (e.g., IUCN Red List of Threatened Species, 2011; own observations).

However, knowledge about the natural history of the species is rather limited, and information on its reproduction, larval stages and development is still lacking, as it is also the case for numerous other rhacophorids from Vietnam. For example, larval descriptions are only available for less than one third of the 18 Rhacophorus species currently recognized from the country (AmphibiaWeb, 2011; Nguyen et al., 2009; Rowley, Le, Dao, Stuart, & Hoang, 2010), and only two of these larval descriptions are based on species records from the country itself, i.e., R. annamensis (Hendrix et al., 2007) and R. maximus (Wildenhues et al., 2010).

The importance of detailed morphological larval descriptions was for a long time underestimated and more attention has been given to it only recently (e.g., Chou & Lin, 1997; Haas, 2003; Hendrix et al., 2007; Gawor, Hendrix, Vences, Böhme, & Ziegler, 2009; Haas, Wolter, Hertwig, & Das, 2009; Wildenhues et al., 2010). In times of the global amphibian crisis, also ex situ captive-breeding programs have proven to be valuable tools both in species conservation and natural history as well as larval development research (Gagliardo et al., 2008; Ziegler et al., 2011). The importance of zoos and aquariums for amphibian conservation breeding was also highlighted by McGregor Reid and Zippel (2008). Breeding of endangered species can help to build up reserve populations or to provide specimens for reintroduction into nature. By keeping and breeding endangered or barely known species important insights into the reproductive biology can also be achieved. The morphological descriptions of the larval stages help to identify early stages of amphibians in the field as prerequisite for subsequent ecological research and adequate conservation measures.

In this article we report for the first time about the successful keeping and breeding of Rhacophorus orlovi from Vietnam in the Leningrad Zoo, St. Petersburg, Russia. Furthermore, based on specimens from Vietnam, we provide the morphological description of the larva of R. orlovi, describe so far unknown colour pattern variation and for the first time the advertisement call of the species.

Captive management

The breeding stock was built up by two wild-caught pairs of Rhacophorus orlovi from Vietnam. Adult specimens derived from Ha Tinh Province in the North of the country (1.2 from Rao An mountain forest; 1.0 from Khe Bun) and were provided by Dr. Sergey Ryabov
via Dr. Nikolai L. Orlov in June 2010 to the Department of Insectarium and Amphibians of the Leningrad Zoo, St. Petersburg, Russia. Due to limited space resources adult couples initially could only be kept in a small glass terrarium with the measurements of ca. 20 × 40 × 30 cm (length × width × height), but already here reproduction took place during the first two months of keeping. The enclosure was equipped with branches and living plants (Philodendron sp., Dracaena sp.) for hiding and climbing. The terrarium substrate consisted of watery coco-substrate (see Fig. 2). Light was provided by the ceiling illumination only. Air and water temperatures (identical with the room temperature) measured 22° to 24° C during day time and 19° to 21° C at night time. No additional source for heating was used. Ambient humidity was around 90% due to the presence of a water basin and daily misting (at least 1-2 times per day). Frogs were fed twice a week. The food for the adults mainly consisted of crickets (Acheta domesticus), but occasionally also Blatta lateralis cockroaches were provided. Crickets and cockroaches were dusted with vitamin-calcium supplement before feeding. Water was at least changed one day after feeding, usually twice a week.

Tadpoles were kept in a plastic tank which measured 39 × 28 × 22 cm. The tank was filled with about 6 litres of water and was covered with a perforated lid. An aquarium pump was installed for oxygenation (see Fig. 2). The tadpole tank was only equipped with a piece of a flower pot or brick which should serve as shelter, but neither was used by the tadpoles. Larvae were fed with aquarium fish flakes 2-3 times per day. Debris and food left overs were removed every day with a medical syringe and water was replaced up to 0.5 litre per day.

Froglets were kept in groups of 15-17 specimens in small glass terraria with a screen top measuring 23 × 21 × 31 cm. Keeping conditions corresponded to these described for the adults. In addition, light was provided by daylight bulbs and as plants Sansevieria trifasciata were placed into the terrarium as hiding places. Froglets were fed on winged Drosophila and pinhead crickets (Acheta domesticus) every second day. Drosophila and crickets were dusted with vitamin-calcium supplement before feeding. Water was sprayed at least once a day and the water of the water bowl was changed every second day. After three months they were rehoused into a larger tank, measuring 30 × 30 × 50 cm and densely planted with Phyllodendron erubescens and Sansevieria trifasciata. At this time they were fed only 2-3 times per week.

Reproduction

The first foam nest was discovered on 6 August 2010, approximately 1.5 months after the receipt of the adult specimens. Previously, mating behaviour including amplexus and calling was recorded several times. The eggs of the first foam nest proved to be completely infertile. Seventeen days later, on 23 August 2010, the second foam nest was discovered. This foam nest measured 14 × 9 cm and amounted for about 55, mostly fertilized eggs. The fertilization rate of the eggs in this foam nest was about 90%. The fertilization rate of the eggs in the third foam nest, which was deposited about one month later, at the night from 23 to 24 September, amounted for hardly 30%, which means that only 12 larvae developed. Altogether, eleven foam nests were built in a seven months period, from August 2010 to March 2011. The next reproduction phase began in June 2011, when new nests could be
observed. All nests were built on the terrarium back wall next to the side wall on which the water bowl was attached (see Fig. 2). However, 50% of these foam nests carried only infertile eggs. All nests were built overnight. Nest building was observed only once on 12 December 2010, at about 9 p.m. after lights were turned off (see Fig. 2). During 15 minutes of observation the couple was sitting at the edge of the water reservoir. Meanwhile, the male moved its hind legs from time to time, foaming the skin secretion and constructing the nest. The next morning the nest was located at the same place, but filled with eggs inside the foam mass and attached to the terrarium surface.
Fig. 3 Developmental stages of *Rhacophorus orlovi*: A) Tadpole ZISP 10529 in Gosner stage 40, B) Freshly metamorphosed individual in Gosner stage 44, C) One month old froglet, and D) Two months old juvenile.

Four to five days after the second foam nest (with first fertile eggs) was discovered, the larvae dropped into the water. Approximately 16 days after deposition of the foam nest, larvae developed hind limbs at Gosner (1960) stage 31. Already after about four weeks, all larvae had completed metamorphosis. All froglets had left the water and entered the tank walls within only a few days. Freshly metamorphosed froglets had a snout-vent length of about 10.5 mm at Gosner stage 44. The colouration at this stage was dark grey to silvery grey dorsally with a greenish part at the top of the head and a darker loreal region. The cross bars at the hind limbs were already visible (Fig. 3). Approximately one month after metamorphosis first signs of the adult colour pattern were already discernible, with some variation of the ground colour (Fig. 3). At the age of about two months, *R. orlovi* offspring already displayed the typical adult colour pattern (Fig. 3). Six months after foam nest building the snout-vent length of juveniles measured about 26 mm. We also noticed that young frogs started with calling activities at the age of 5.5 to 6 months. At that time their sizes were slightly beyond half of the adult size. But no reproductive behaviour such as amplexus could be observed. 12 months after foam nest building, juveniles had reached snout-vent lengths of 38-44 mm, however, with no traces of sexual size dimorphism. At this age, regular calling of males all day round, especially after misting, was noted. Concerning
the sex ratio of the offspring, about 30% of the first two fertile nests could be identified as males due to calling activities.

Most of the offspring is still kept at the amphibian facility of Leningrad Zoo. Some younger froglets were provided to other zoos but shortly died after arrival. Probably the species is sensitive to transfer and changes of environment, at least as froglets. During the maintenance of the young frogs at Leningrad Zoo only few of them were lost. Until June 2011 the offspring still did not reach maximum adult sizes, but had fully developed adult colouration.

**Larval description**

The first description of the larval stage of *Rhacophorus orlovi* is based on a freshly dead tadpole in Gosner (1960) stage 40 from the second foam nest (9 September 2010). The larva subsequently was deposited in the Zoological Institute, St. Petersburg, Russian Academy of Sciences, Russia (collection number: ZISP 10529). For assured allocation of this larva to the species *R. orlovi*, it was matched to an adult specimen (Fig. 5) by the method of DNA barcoding. The adult *R. orlovi* was a male (41.6 mm snout-vent length) from Phong Nha - Ke Bang, Quang Binh Province, bordering Ha Tinh Province, from where the species originally was described. The male originated from nearby the type locality of this species and is deposited at the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (collection number: ZFMK 92291). It morphologically matched with the species descriptions provided by Ziegler and Köhler (2001) and Orlov et al. (2008). The method of DNA barcoding is based on the comparison of partial mitochondrial 16S rRNA gene. The method is considered as a reliable identification tool in amphibian taxonomy (e.g., Vences, Thomas, van der Meijden, Chiari, & Vieites, 2005; Bwong, Chira, Schick, Veith, & Lötters, 2009).

Molecular data were obtained from the tadpole by taking a tissue sample from the tail musculature, and from the adult specimen by taking a tissue sample from the thigh musculature. DNA was extracted using the peqGold Tissue DNA Mini Kit (PEQLAB Biotechnologie GmbH). The primers 16sar-L (light chain; 5’- CGC CTG TTT ATC AAA AAC AT - 3’) and 16sbr - H (heavy chain; 5’- CCG GTC TGA ACT CAG ATC AC - T - 3’) of Palumbi et al. (1991) were used to amplify a section of the mitochondrial 16S ribosomal RNA gene (575 bp). PCR cycling procedure followed Schmitz, Ineich, & Chirio (2005). PCR products were purified using QIAquick purification kits (Qiagen). Sequences (including complimentary strands for ensuring the accuracy of the sequences) were obtained using an automatic sequencer (ABI). Sequences were examined and aligned manually using the original chromatograph data in the program BioEdit (Hall, 1999). The same program was used to calculate the direct pairwise similarities for the resulting sequences. An unambiguous assignment of the larva ZISP 10529 to the species *R. orlovi* was guaranteed by its negligible 16S sequence divergence (0.35%, corresponding to only two base pairs difference) to the non-syntopic adult specimen ZFMK 92291.

The terminology of tadpole morphology and respective abbreviations (partly modified) followed Altig and McDiarmid (1999), Grosjean (2005), and Altig, McDiarmid, Nichols, & Ustach (accessed 2010). The labial tooth row formula (LTRF) was determined according to
Table 1. Measurements (in mm) of the tadpole of *Rhacophorus orlovi* (ZISP 10529) from Ha Tinh Province, Vietnam in Gosner stage 40.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Measurements (in mm)</th>
</tr>
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<tbody>
<tr>
<td>body height (midpoint of body)</td>
<td>BH 3.51</td>
</tr>
<tr>
<td>body length</td>
<td>BL 8.3</td>
</tr>
<tr>
<td>body width (midpoint of body)</td>
<td>BW 4.8</td>
</tr>
<tr>
<td>maximum diameter of eye (horizontal)</td>
<td>ED 1.31</td>
</tr>
<tr>
<td>interpupilar distance (from midpoint of eyes)</td>
<td>IP 3.03</td>
</tr>
<tr>
<td>internarial distance</td>
<td>IND 1.11</td>
</tr>
<tr>
<td>narro-pupilar distance</td>
<td>NPD 1.5</td>
</tr>
<tr>
<td>rostro-narial distance (from tip of snout)</td>
<td>RND 1.02</td>
</tr>
<tr>
<td>snout-pupil distance</td>
<td>SP 2.49</td>
</tr>
<tr>
<td>oral disk width</td>
<td>ODW 1.55</td>
</tr>
<tr>
<td>number of keratodonts (per 0.5 mm of the A3 keratodont row)</td>
<td>NK –</td>
</tr>
<tr>
<td>number of papillae around mouth</td>
<td>NP ~66</td>
</tr>
<tr>
<td>snout-spiracle distance (to opening of spiracle)</td>
<td>SS 3.81</td>
</tr>
<tr>
<td>total length (from beginning of ventral tube)</td>
<td>TL 24.45</td>
</tr>
<tr>
<td>tail length (from beginning of ventral tube)</td>
<td>TAL 16.94</td>
</tr>
<tr>
<td>height of tail musculature at base</td>
<td>TMH 2.17</td>
</tr>
<tr>
<td>width of tail musculature at base</td>
<td>TMW 1.62</td>
</tr>
<tr>
<td>height of upper tail fin (midpoint of tail)</td>
<td>UF 0.79</td>
</tr>
<tr>
<td>height of lower tail fin (midpoint of tail)</td>
<td>LF 0.78</td>
</tr>
<tr>
<td>total tail height (midpoint of tail)</td>
<td>MTH 3.1</td>
</tr>
<tr>
<td>keratodont row formula</td>
<td>KRF 1:3+3/1+1:2</td>
</tr>
<tr>
<td>labial tooth row formula</td>
<td>LTRF (2-4)/3(1)</td>
</tr>
</tbody>
</table>

Altig and McDiarmid (1999). Larval stages were determined according to Gosner (1960). The ecological assignment of larval types followed Orton (1953) as extended in Altig and McDiarmid (1999). Colouration in life was described from photographs. Drawings of the dorsal view, lateral view and oral disc of the preserved specimen (fixation and preservation was done in 70% ethanol) were prepared by using a stereo microscope. All measurements are approximate values and were taken in millimetres (mm) with a digital calliper gauge. For detailed measurements see Table 1.

Tadpoles of *Rhacophorus orlovi* are ecologically exotrophic, lentic: bentic larvae of Orton’s type IV.

Colouration in life (ZISP 10529, see Fig. 3): Body is densely covered with dark grey pigments dorsally, which are less vivid at the anterior body. The pigmentation fades to brown-reddish before changing to transparent laterally. Venter is completely transparent, except for a few brownish pigments at the anterior margins, with the white intestinal coil and the reddish glimmering internal gills very well visible. The tail musculature is light grey to uncoloured and scattered with grey to brown pigments. The tail fins are transparent,
whereas the pigmentation of the tail musculature spreads slightly at the upper tail fin being strongest at the first third. The hind limbs are light grey to uncoloured and pigmented dark greyish dorsally and laterally. The iris of the eye is marbled brown with light blotches at the iris periphery and a black pupil.

Colouration in preservative (ZISP 10529): Colouration and pattern of pigmentation are the same as in life with pigments being dark grey to brown throughout. The intestinal coil is visible from lateral and ventral view. One type of pigment is shaped like merged ice crystals and the other type is filamentary-shaped and scattered densely on dorsum and less densely at the tail and the anterior body.

Dorsal view (ZISP 10529, see Fig. 4): Body is oval-shaped, elongated with weak lateral constrictions (body width 0.58 of body length) and with a tapered snout. Eyes are of
remarkable size (maximum diameter of eye 0.16 of body length), dorso-laterally positioned and directed at the first third of the body (snout-pupilar distance is 0.30 of body length). The interpupilar distance is 0.63 of the body width. Nares are round, antero-laterally positioned and laterally directed (rostro-narial distance is 0.68 of naro-pupilar distance). The internarial distance is 0.37 of the interpupilar distance. Spiracle is not visible in dorsal view. A lateral line organ is not distinct. At the anterior snout the bulge of the oral disc is distinctly visible in dorsal view. The tail musculature is of moderate size (width of tail musculature at base is 0.34 of body width).

Lateral view (ZISP 10529, see Fig. 4): Body is depressed (body height is 0.73 of body width). Spiracle is sinistral and ventro-laterally positioned at mid body (distance of snout tip to opening of spiracle is 0.46 of body length). The spiracle opening is rather small, rounded and directed postero-laterally. Spiracle is attached to the body wall. A lateral line organ is not distinct. The marginal papillae of the upper and the lower lip, as well as the upper lip itself, are sticking out anterior-ventrally. The tail is of remarkable size (tail length is 2.04 of body length). The tail musculature is moderately developed (height of tail musculature at base is 0.62 of body height and 0.70 of maximum tail height) and is more or less equal in height until the second third of the tail before gradually tapering to the tail tip. Myotomes of the tail musculature are only distinct at the last quarter of the tail. The upper fin originates at the end of the body and is gradually elevating from the base to the first third of the tail. The lower tail fin is equal in height to the upper tail fin at the mid point of the tail (lower fin is 0.99 of the upper fin). The tip of tail is rounded and slightly sloped. Vent tube is positioned ventro-medially with its body visible in lateral view. The opening is large and rounded, dextral and postero-laterally directed. The inner wall is adnated to the lower fin.

Oral disc (ZISP 10529, see Fig. 4): Oral disc is positioned anterior-ventrally and laterally emarginated (oral disc width is 0.32 of body width). Papillae are absent at the emargination. The marginal papillae of the anterior labium have a large dorsal gap with a fleshy labium. The marginal papillae of the posterior labium are arranged in one row laterally and partly in two rows ventrally. A ventral gap and submarginal papillae are absent. All papillae are of moderate size, rounded and coloured in white to transparent. The oral disc shows approximately 66 papillae in total and the LTRF is 5(2-4)/3(1). Due to the advanced Gosner stage of the tadpole some labial tooth rows are not complete anymore and the number of keratodonts per 0.5 mm could not be determined. Keratodonts of row P1 are remarkably larger than others. At the lateral end of keratodont row P2 two teeth are separated on a bulge or a submarginal papilla, respectively. The jaw sheaths are dark brown to black and serrated except for the lateral endings of the upper jaw sheath. The serration of the upper beak is more regular and tooth like than at the lower beak. The upper jaw sheath is curved and with a short lateral process. The lower jaw sheath is V-shaped.

**Colour variation**

Ziegler and Köhler (2001) already pointed to some degree of colour variation in the paratype series of *Rhacophorus orlovi*, mainly referring to irregular yellow spots, which may be present or absent at the loreal region and on the flanks, independent of the sex. Here
we can report that such yellow spots also may be present on the back, as is discernable from an adult *R. orlovi* photographed in Phong Nha - Ke Bang, Quang Binh Province, central Vietnam (see Fig. 5). Based on the adult female ZFMK 86412 (40.71 mm snout-vent length, with developed oocytes) likewise from Phong Nha - Ke Bang, we herein describe another so far unknown dorsal colour variation of *R. orlovi*: instead of a greyish-brown dorsal ground colouration, this individual, which has been collected at the beginning of the raining season, had the margins of head and back for large parts yellowish-green coloured (Fig. 5). Such a variable yellowish-green back pattern, that also can include green back spots, also was observed in captive held specimens (Fig. 2). Whether such colour pattern is only present in females, still has to be clarified. However, the in part green colouration of the dorsum at least can also occur in subadults (Fig. 2).

**Advertisement call**

Recordings of the advertisement call of *Rhacophorus orlovi* were obtained on 11 July 2006 with a digital camera (Pentax Optio W10) in Phong Nha - Ke Bang National Park, Quang Binh Province, central Vietnam. At that time a freshly collected adult male was
kept for one night in a fabric bag at the station. After morphological identification based on the diagnosis given in Ziegler and Köhler (2001), the adult male specimen was released into the karst forest of Phong Nha - Ke Bang. The recordings were later analyzed by Raven Pro 1.3 software with the following parameters: call duration (ms), intercall interval (call/s), call repetition rate (call/s), number of notes per call (notes/call), and dominant frequency (Hz). Unfortunately, temperature data which were taken during call recordings were subsequently lost and thus are not available. *R. orlovi* has a call of single unpulsed notes (see Fig. 6, Table 2) with an average duration of 9.5 ± 2.14 ms (n = 52) and a call repetition rate of 1.36 ± 0.72 calls/s (n = 2). Intervals of the calls range between 109 and 4,645 ms (average 893.24 ± 854.60 ms; n = 50). The call has energy in a frequency modulated from 959.2 to 3648.7 Hz; dominant frequency ranges from 1599 to 3075 Hz (average: 2753.31 ± 287.33 Hz; n = 52).
Table 2. Parameters of the advertisement call of *Rhacophorus orlovi* from Phong Nha - Ke Bang National Park, Quang Binh Province, Vietnam.

<table>
<thead>
<tr>
<th></th>
<th>record 1</th>
<th>record 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calls</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>Notes/call</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Call (note) duration (ms)</td>
<td>11.19 (7-15)</td>
<td>8.49 (4-10)</td>
</tr>
<tr>
<td>Pulses/note</td>
<td>31.46 (15-44)</td>
<td>22.51 (11-27)</td>
</tr>
<tr>
<td>Pulses repetition rate (pulses/ms)</td>
<td>2.71 (2-3)</td>
<td>2.54 (2.33-2.89)</td>
</tr>
<tr>
<td>Dominant frequency (Hz)</td>
<td>2848.95 (1599-3075)</td>
<td>2690.23 (2460-2952)</td>
</tr>
</tbody>
</table>

Discussion

Facing our husbandry experiences, the fast developmental time from egg to froglet in approximately one month at 19° to 24°C is most striking. Development usually takes three to four months in other rhacophorid species, such as in *Rhacophorus annamensis* (Rybal’tovskiy, 1999), *Kurixalus verrucosus*, *R. dennyi*, *R. feae*, and *R. maximus* (own observations), and also in most species of *Theloderma* (Bagaturov, 2011). Moreover, unlike other rhacophorid species, froglets of *R. orlovi* apparently do not need UV-light for growing. Both, the apparent low need for UV radiation and the short development time might be adaptations due to their hidden lifestyle in limestone caves and crevices, the undergrowth of densely covered forests with low light intensity, and the high desiccation risk of ephemeral crevice puddles where the tadpoles grow in. Interestingly, males seem to reach sexual maturity at an earlier stage than females. Based on our husbandry experience and several cases of froglet transfers with subsequent deaths we believe the species at least in young stages to be sensitive to transfer. The species also seems to suffer from high temperatures (> 27°C), especially in combination with insufficient air circulation. Consequently, low to average temperatures of 19° to 24°C in combination with clean keeping conditions and sufficient air circulation seem to be crucial for the successful husbandry of *R. orlovi*. We also recommend a mild hibernating period for breeding success in this species.

Our morphological tadpole description of *Rhacophorus orlovi* largely agrees with the general larval descriptions given by Bourret (1942) and Inger (1966) for rhacophorids: e.g., eyes positioned laterally or dorsally; sinistral spiracle; dextral vent (which does not reach the edge of the ventral fin); dorsal tail fin does not reach beyond base of tail; long papillae on the upper lip absent; within the general keratodont formula 4-7/3-4. Compared with other rhacophorid tadpoles at Gosner stages 40/41 the larva of *R. orlovi* is quite small with a total length of 24.45 mm versus for example 34.37-41.69 mm in *R. annamensis* (Hendrix et al., 2007) and 42.8 mm in *R. maximus* (Wildenhues et al., 2010). The low number of four keratodont rows at the upper labium is also a striking difference. Other *Rhacophorus* species from Vietnam, with available larval descriptions, all possess 5-8 keratodont rows at the upper labium (Hendrix, 2007; Hendrix et al., 2007; Wildenhues et al., 2010; Wildenhues, 2010). Hendrix et al. (2007) mentioned that some tadpoles of the genus *Rhacophorus* possess an uninterrupted row of marginal papillae on the lower labium whereas others show a short medial gap. The larva of *R. orlovi* belongs to the first group.
Concerning the colour variation in *Rhacophorus orlovi* it can be stated that many anuran species exhibit striking colour or dorsal pattern polymorphisms (Hoffman & Blouin, 2000). The presence and absence of yellow spots shown in *R. orlovi* are also known from other anuran species (e.g., Cei, 1959). Variation in colouration and colour patterns in anurans can have different backgrounds. The most important factors that affect movement of the pigments, and hence the colour change in frogs are temperature, light and humidity (Hoffman & Blouin, 2000). However, much further research is required here, concerning geographical variation, individual variation within populations and finally whether the here described green dorsal pattern is due to sexual dimorphism or not.

Facing the advertisement call of *Rhacophorus orlovi*, there is a similarity of the call structure between the latter species and *Polypedates leucomystax*, as was described by Trépanier, Lathrop, & Murphy (1999). Both species have a call with a single note. The call of *R. orlovi* is distinguished from that of *P. leucomystax* by an unpulsed note versus 2-7 pulses per note in the latter species. Moreover, the range of the dominant frequency from 1599 to 3075 Hz in the call of *R. orlovi* is wider than that of *P. leucomystax* (from 2250 to e550 Hz). The call of *R. orlovi* differs from that of *R. dennysi*, another rhacophorid which has a call consisting of three notes (Ziegler, 2002), by having a single unpulsed note. In addition, the call of *R. orlovi* has a shorter duration (9.5 ± 2.14 ms) and a higher call repetition rate (1.36 ± 0.72 calls/s) compared to the call of *R. dennysi* with an average duration of 155.5 ± 4.8 ms and a call repetition rate of 40 calls/min., which is equivalent to 0.67 calls/s (Ziegler, 2002). Moreover, the call of *R. orlovi* has a wider frequency range (959.2-3648.7 Hz) and a much higher dominant frequency (2753.31 ± 287.33 Hz) compared to *R. dennysi*’s call that has a frequency range of 1300-1800 Hz and a dominant frequency at 1600 Hz. Compared to *R. kio*’s call, described by Ziegler (2002) under the name of *R. reinwardtii*, the call of *R. orlovi* differs not only in structure but also in energy. *R. orlovi* has only one type of call consisting of a single note whereas *R. kio* has two call types: (1) call comprises two unpulsed-notes and (2) call consists of about 10 non-pulsed single notes. The call repetition rate of about 1.36 call/s of *R. orlovi* is slightly slower than that of *R. kio* with 2 calls/s for type 1 and 3.5 calls/s for type 2. Also the frequency range (959.2 to 3648.7 Hz) in the call of *R. orlovi* is broader than in the call of *R. kio* (900 to 1000 Hz in type 1; 600 to 2000 Hz in type 2). Moreover, the average dominant frequency of 2753.31 Hz of *R. orlovi*’s call is much higher than that of the *R. kio*’s call (890 Hz for type 1; 1000 Hz for type 2).

**Outlook**

The rapid development, the multiple building of nests per year with an average number of eggs, and the relatively easy keeping conditions make *Rhacophorus orlovi* an attractive candidate for zoo exhibits. Although *R. orlovi* is currently evaluated as being not endangered, it is a species that might face habitat loss. The breeding success and the new data we could collect both on the keeping and on the natural history of the species will be especially helpful when an ex situ captive breeding program is recommended for the species.

Both in situ and ex situ conservation engagement for amphibians and research is required to contribute towards a better understanding of the natural history and the successful keeping
and breeding in particular of still poorly known amphibian species. The implementation of the breeding of a species and the setting up of captive breeding programs might be one step towards the important role of zoos and aquariums as institutions being involved in species conservation action and as representing modern arks (e.g., McGregor Reid & Zippel, 2008; Ziegler, 2010; Gawor, Straeten, Karbe, Manthey, & Ziegler, 2011; Ziegler et al., 2011).

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References


