

Husbandry, captive breeding, larval development and stages of the Malayan horned frog *Megophrys nasuta* (Schlegel, 1858) (Amphibia: Anura: Megophryidae)

^{1,3}MARLEN WILDENHUES, ¹ANNA RAUHAUS, ^{1,4}RIKE BACH, ¹DETLEF KARBE, ¹KARIN VAN DER STRAETEN, ^{2,5}STEFAN T. HERTWIG, AND ^{1,6}THOMAS ZIEGLER

¹Cologne Zoo, Cologne, GERMANY ²Naturhistorisches Museum der Burggemeinde Bern, Berne, SWITZERLAND

Abstract.—We report long-term experience with the successful keeping and breeding of *Megophrys nasuta* at the Cologne Zoo's Amphibian Breeding Unit and compare data with other breeding reports. In addition, we document the development and morphology of different larval stages of *M. nasuta*. Diagnostic morphological characters are provided for Gosner (1960) larval stages 18-22 and 25-46. Ovipositions were not seasonal and took place after a drier phase in the terrarium followed by intensive spraying to simulate the natural rain period. The larvae hatched about one week after egg deposition. The characteristic funnel-shaped oral disc became discernible about two weeks after egg deposition at Gosner stage 21 and degenerated at Gosner stage 42. The mean total developmental time observed for *M. nasuta* was 2.5-3.5 months. Larvae developed faster at higher temperatures and lower densities. The triangular projections at the upper eyelids, which are characteristic for advanced terrestrial stages, began to develop two or three weeks after completion of metamorphosis.

Key words. Anura, Megophryidae, *Megophrys nasuta*, husbandry, captive breeding, development, larval stages

Citation: Wildenhues M, Rauhaus A, Bach R, Karbe D, Van der Straeten K, Hartwig ST, Ziegler T. 2012. Husbandry, captive breeding, larval development and stages of the Malayan horned frog *Megophrys nasuta* (Schlegel, 1858) (Amphibia: Anura: Megophryidae). *Amphibian & Reptile Conservation* 5(3):15-28(e43).

Introduction

The Malayan horned frog, *Megophrys nasuta*, was originally described by Schlegel (1858). For some time this taxon was considered to be a subspecies of *M. monticola*, Kuhl and Van Hasselt, 1822 (e.g., Inger 1954, 1966), but is now considered to be a synonym of *M. montana*, Kuhl and Van Hasselt, 1822 (Frost 2011). The genus *Megophrys* includes the following four species besides *M. nasuta*: *M. kobayashii* Malkmus and Matsui, 1997, *M. ligayae* Taylor, 1920, *M. montana* Kuhl and Van Hasselt, 1822, and *M. stejnegeri* Taylor, 1920 (Frost 2011). The recently described *M. damrei* Mahony, 2011 and *M. takensis* Mahony, 2011 were allocated to the genus *Xenophrys* by Frost (2011), which was considered to be a junior synonym of *Megophrys* by Mahony (2011).

Megophrys nasuta is known to occur in Sumatra, Borneo, and Malaysia; records from Thailand to the Sunda Shelf may belong to other species (Frost 2011). Diagnostic characters of species are presence of a dermal rostral appendage, a triangular projection on the upper eyelid, two pairs of parallel, longitudinal, dorsolateral folds continuous between head and groin, and its large size. Females may reach a snout-vent length of 160 mm, and smaller males 105 mm (Inger 1966; Manthey and

Grossmann 1997; Malkmus et al. 2002). The head appendages and projections together with the cryptic coloration serve as phytomimesis in the leaf litter of the forest floor. *Megophrys nasuta* is regularly encountered in intact lowland and submontane rainforest up to an elevation of 1,300 m, mostly in the vicinity of forest streams. Adults are terrestrial and nocturnal and tadpoles are funnel-mouthed surface dwellers in clear forest streams (Malkmus et al. 2002; van Dijk et al. 2004).

The IUCN lists *M. nasuta* as a taxon of Least Concern because of its wide distribution range and presumed large population size. Habitat loss and fragmentation are among the major known threats to *M. nasuta* and harvesting for national and international pet trade may also threaten some populations (van Dijk et al. 2004). Because of the global amphibian crisis, including the possibility that amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) may cause extinction of local populations or species (e.g., Berger et al. 1998; Briggs et al. 2005; Mendelson et al. 2006), captive breeding programs have become crucial tools for amphibian conservation (Griffiths and Pavajeau 2008; McGregor Reid and Zippel 2008; Browne et al. 2011; Ziegler et al. 2011; Zippel et al. 2011).

Correspondence. Email: ³marlen.wildenhues@gmx.de; ⁴RikeBach@web.de; ⁵stefan.hertwig@nmbe.ch; ⁶ziegler@koelnerzoo.de (Corresponding author).

Megophrys nasuta is rarely bred in captivity (Schmidt 1976, 1977; Schmidt and Wicker 1977; Schwanz 1977; Rogner 1980; Pfeuffer 1989; Anonymus 1994; v. d. Nieuwenhuizen 2001a, b), and because of increasing threats to this and other *Megophrys* species, here we present our long-term experience with the successful husbandry of *M. nasuta* at the Cologne Zoo (see also van der Straeten et al. 2007; Ziegler et al. 2008). In addition, we present the first staging table for *M. nasuta* or for any *Megophrys* species.

Materials and methods

Collection, identification and abbreviations

When beginning our breeding program for *M. nasuta* at the Cologne Zoo, Germany, in 2005 we had access to three males and two females obtained from the pet trade. According to the trader, these frogs were from the federal states of Pahang or Perak, Malaysia. Breeding and rearing was achieved between 2006 and 2009.

For verification of species, at various times during our breeding program deceased specimens were fixed in 40-60% ethanol, preserved in 70% ethanol and subsequently deposited in the herpetological collections of the Biozentrum Grindel und Zoologisches Museum (ZMH), Universität Hamburg (ZMH A10525, A10527, A10529), of the Naturhistorisches Museum (NMBE) Bern (NMBE 1060403: adult male, 71.2 mm SVL, length of left testis 8.5 mm), and of the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK), Bonn (ZFMK 92810: adult female, 125.5 mm SVL, maximum oocyte diameter 1.0 mm). The adults were morphologically identified by characters given in Inger (1966), Manthey and Grossmann (1997), and Malkmus et al. (2002).

For molecular assignment of our specimens to populations with confirmed locality data a molecular barcoding approach was applied based on a 800 bp piece of the 16S rDNA (forward: 16SC 5' GTRGGCTAAAAGCAGC-CAC - 3', 16SA-L CGCCTGTTTATCAAAAACAT, 16SCH TCAAHTAAGGCACAGCTTA, reverse: 16SD 5' - CTCCGGTCTGAACTCAGATCACGTAG - 3', 16SB-H CCGGTCTGAACTCAGATCACGT, Vences et al. 2005; Rafe Brown, pers. comm.). Total genomic DNA was extracted from macerated muscle tissue with peq-Gold Tissue DNA Mini Kits (PEQLAB Biotechnologie GmbH) or DNeasy® Blood & Tissue Kit (Qiagen) according to the manufacturer's protocols. Cycling conditions for amplification have been published previously by Hertwig et al. (2011). Sequencing was done in both directions by Microsynth AG (Balgach, Switzerland) and Macrogen Inc. (Seoul, Korea). Sequence editing and management was done with BioEdit 7.0.5.2 (Hall, 1999, www.mbio.ncsu.edu/BioEdit/), Chromas Lite 2.01

(Technelysium Pty. Ltd., www.technelysium.com), and Geneious Pro 5.1.7 (Drummond et al., 2009) software.

The sequences were compared with samples of different populations of *M. nasuta* from the sequence database of the frogsofborneo.org project. Alignment was performed with MAFFT (Katoh et al. 2002) using the plugin of Geneious Pro with the E-INS-i algorithm and standard parameters. Genetic distances were obtained and visualized with the Geneious Pro tree builder with a neighbor-joining algorithm and the Tamura-Nei model of sequence evolution. The specimens from the breeding project were closely related to *M. nasuta* from Borneo. The lowest genetic distances of 1.2 and 1.4% respectively were found for two samples from a lowland population of this species inhabiting the Gunung Mulu National Park, Sarawak, Malaysia. This result is interpreted as indication of a possible origin of the founder animals of our breeding group from Borneo.

We photographed larval stages by placing single larvae into water filled glass vessels. Some photographs were used for ink drawings. A few freshly dead larvae at different developmental stages (Gosner stages 21, 25, 34, 39, and 44) that were first fixed in 4% formalin for some hours and subsequently preserved in 70% ethanol were used for morphological examination of character states with a Leica binocular microscope. These larvae were subsequently deposited in the collections of the Naturhistorisches Museum Bern (NMBE 1060404 [3 tadpoles]: stage 21, from 2010; stage 25, from January 2010; stage 44, from December 2009), and of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn (ZFMK 92811: stage 34, from January 2010; ZFMK 92812: stage 39, from January 2010; ZFMK 92813, 92814: stage 44, from December 2009).

Abbreviations are as follows: GH – total hardness, KH – carbonate hardness; n = number; pH – pH value; TL = total length; terminology of larval morphology followed Altig and McDiarmid (1999) and Grosjean (2005).

Captive management of adults

Megophrys nasuta were maintained at the Amphibian Breeding Unit at Cologne Zoo without public access. Adults were housed in terrariums (L145 × W60 × H56 cm) that were divided into an aquatic and terrestrial section (Fig. 1a). The back and side walls of the terrariums were covered with artificial rock like decorative substrate. The terrestrial substrate consisted of a 20 cm thick layer of leaf litter covered with about five cm of dry leaves. Measurements of the surface of the aquatic section were L72.5 × W60 cm and water depth was about 10 cm with a total volume of 40 L. The water was connected to an external filter (EHEIM professional, Type 2224) with a capacity of 700 L/h.



Figure 1. *Megophrys nasuta* enclosures in the amphibian breeding unit at the Cologne Zoo: a) terrarium of the adults, b) rearing tank for larvae at early developmental stages, c) aquaria for advanced larval stages, and d) rearing terraria for juveniles. Photos: D. Karbe.

In order to provide ready accessibility from the aquatic to the terrestrial section, as well as to provide oviposition sites, half of a cork tube was placed in the water. The terrestrial section included plants (*Asplenium nidus*) and cork tubes for shelter. Illumination was provided by fluorescent tubes (Namiba compact lights, UV replux: 36 Watt) and timer maintained photoperiod between 10 and 12 hours. Average temperatures were kept at 24-25 °C, and the humidity 80-100% through the use of a manual pump sprayer.

Captive management of larvae

Eggs were left in terrarium until hatching. The rearing tanks for larvae at early stages consisted of plastic tanks containing 13 L of water which were attached to an external filtration system (Eheim). After the hatching of the tadpoles more halves of coconut shells or cork pieces, and floating plants were added to provide hiding places (Fig. 1b). To ensure a constant water quality, part water changes were conducted every second day. Two months after hatch the tadpoles were transferred into aquariums (L54 × W65 × H30 cm), containing approximately 90 L of water, with a sand substrate and floating plants (Fig. 1c). Aquaria were connected to external filters with a

77 L filter volume which were run through 7 L pumps (Eheim).

Partial water changes were continued every second day; in addition, Catfish (*Corydoras*) were introduced to minimize the water contamination through uneaten feed. Lighting was provided by T5 fluorescent tubes (Osram FQ, 865 Lumilux daylight: 54 Watt), and water parameters were: temperature 24-27 °C (unless otherwise noted, see Table 1), pH 8.3, conductivity 320 µS, KH 2-4, and GH 6-8. Shortly before tadpoles metamorphosed, water level was reduced from 25 to 15 cm and a terrestrial section of 54 × 10 cm was established.

Captive management of metamorphs and juveniles

Metamorphs and juveniles were kept in groups of 20-30 specimens in terrariums measuring L60 × W45 × H30 cm that included a small water basin (maximum depth eight mm) and coconut husks for hiding places (Fig. 1d). For hygienic reasons, the substrate was paper tissue. Because the temperature should not exceed 23 °C, no additional illumination was used. To maintain a high humidity level, the terrarium was sprayed daily and front panels were tightly shut. Juveniles were reared to 2-4 cm and then transferred to other interested European institutions.

Nutrition

Adults were fed two or three times a week during their active periods, mostly on different invertebrates (house crickets, locusts, cockroaches), and infrequently (two times per month) on earthworms and newborn mice. Froglets were fed fruit flies (*Drosophila*) and then small house crickets (*Acheta domestica*) each day. All insects were fed a high quality herbal nutrition and dusted with minerals and vitamins (Korvimin ZVT + Reptil/Calcamineral). Tadpoles were fed on fine ornamental fish food (TetraMin). Feeding was introduced carefully when the first larvae were observed swimming at the water surface. When all tadpoles fed, food was applied 6-8 times a day, and later in the developmental progress feeding times were reduced to 2-4 times a day.

Results

Reproduction and larval development

Breeding was stimulated by providing a drier phase to the habitat, with reduced water level, during which terrarium was sprayed only as necessary for required humidity. This treatment was then followed by an artificial rain pe-

riod, with rising water level and strong daily spraying, in order to simulate a natural rainy period. After beginning the artificial rain period, males that were discernible by their smaller size, darker throats and distinct nuptial pads, started calling (Fig. 2a). The loud, metallic calls first occurred at night, but with further breeding stimulation the males also began calling during the day.

Periods of calling were interspersed with inguinal amplexus, sometimes lasting several weeks, but did not necessarily lead to oviposition. Ovipositions were not seasonal, and were observed during January, May, June, July, October, and November (Fig. 2b). The minimum interval between ovipositions was about a month, but as several females housed with the males, we could not be sure of which females spawned. During night, eggs were deposited in clutches under the cork tube in water.

The white eggs were glutinous, attached to each other, and measured about two mm in diameter (Fig. 2b). Larvae hatched about one week after egg deposition with the yolk reservoir clearly visible (Figs. 2c, 2d). Between 50 and 300 larvae hatched per oviposition. Immediately after hatching, the larvae preferred dark hiding places such as under cork pieces or halved coconut shells. About ten days after hatching, the larvae developed a brownish pigmentation; at this stage the tadpoles remained clustered in close groups on the bottom.



Figure 2. *Megophrys nasuta* at the amphibian breeding unit at the Cologne Zoo a) calling male, b) couple in amplexus during egg deposition, c) embryos, and d) hatched larvae with yolk sacs. Photos: D. Karbe, A. Heidrich, T. Ziegler.

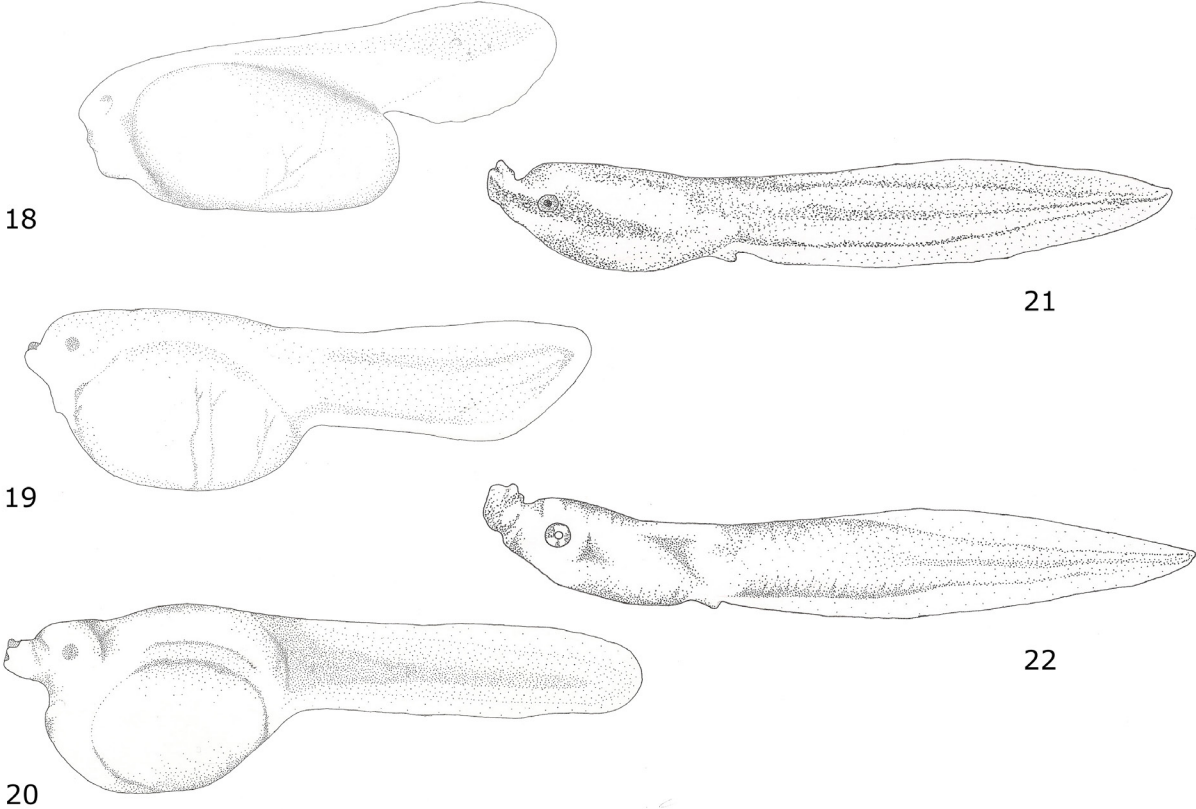


Figure 3. *Megophrys nasuta* larvae in stages 18 to 22. Drawings: R. Bach.

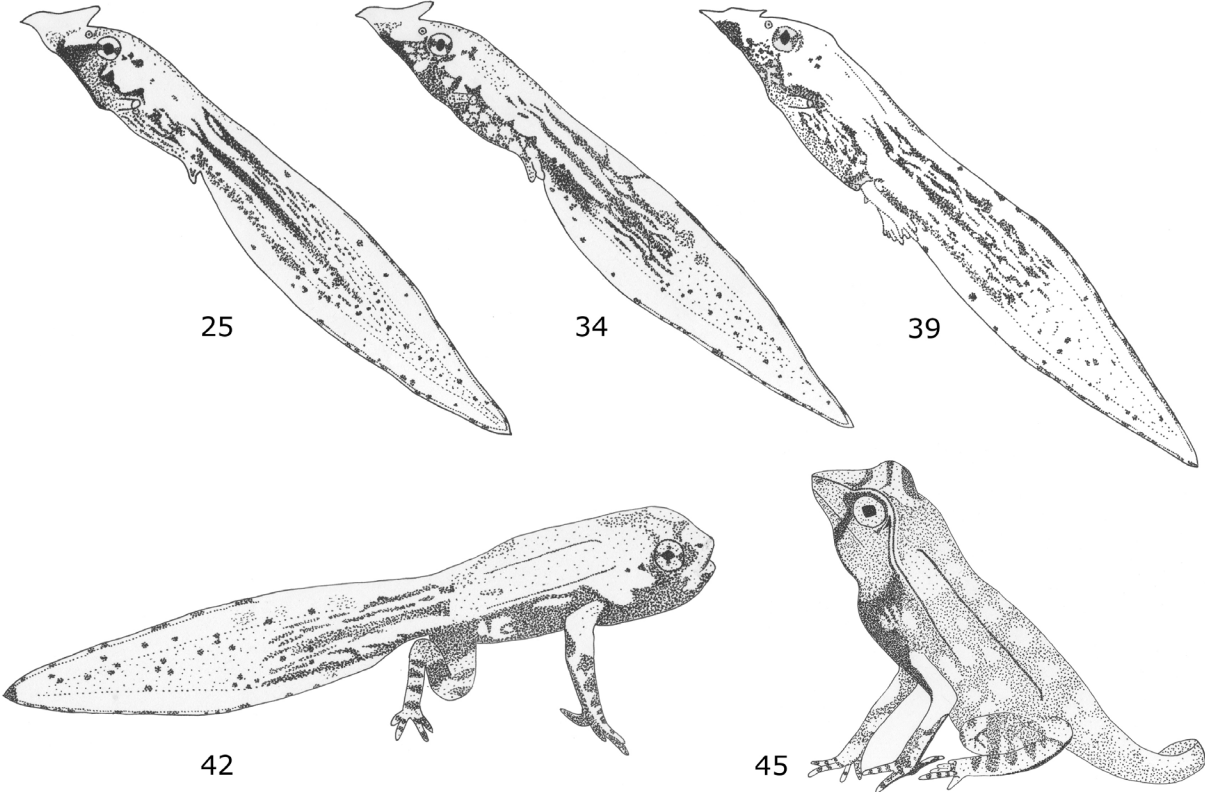


Figure 4. *Megophrys nasuta* larvae in stages 25 to 45. Drawings: M. Wildenhues.



Figure 5. *Megophrys nasuta* larvae in stages 18 to 22; blue color is caused by the blue cellular material at the aquarium ground / background while taking photographs. Photos: R. Bach, T. Ziegler, D. Karbe.



Figure 6. *Megophrys nasuta* larvae in stages 25 to 29. Photos: M. Wildenhues.

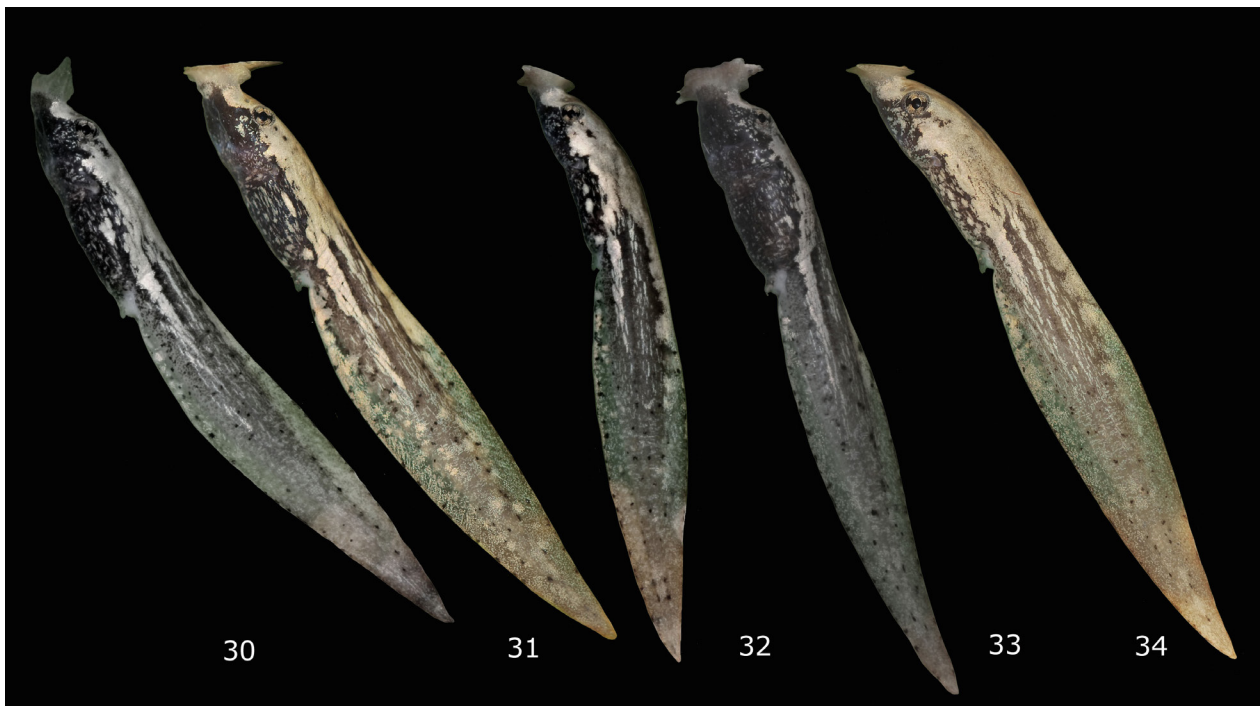


Figure 7. *Megophrys nasuta* larvae in stages 30 to 34. Photos: M. Wildenhues.

For detailed staging of the following early developmental stages see Table 1. The funnel mouth became discernible about one week after hatch. About four days later, the larvae began to move to the water surface, and after about two weeks after hatch all tadpoles were feeding. Three weeks after hatch the tadpoles had reached lengths of up to two cm. For detailed staging of the following advanced developmental stages see Table 2. After about nine weeks after hatch, some tadpoles showed a distinct ventral pattern. On average around sixty days after hatch, at Gosner stage 26 or 27, hind limbs started to develop. At this time, the largest tadpoles measured about 4.5 cm, and feeding times were reduced to two times a day because of their good nutritional condition. Shortly before metamorphosis the funnel mouth was reduced and dorsal coloration darkened.

About 2.5 months after egg deposition the first larvae moved onto the terrestrial section to metamorphose. At that time the metamorphs had body lengths of 15-18 mm. Reabsorption of the tail took two or three days, the triangular projections at the upper eyelids, which are characteristic for the advanced terrestrial stages, began to develop after about two or three weeks after completion of metamorphosis. While most of the larvae had finished their development and commenced with metamorphosis after 3.0-3.5 months, some individuals showed a distinctly slower developmental progress which took up to seven months, or longer in some cases. Larval development was both temperature and density dependent.

We generally observed a faster growth at higher water temperatures. For example, larvae that were kept at minimum temperatures of 24 °C developed dark pigmen-

tation ten days after hatch, whereas larvae kept at minimum temperatures of 22 °C developed dark pigmentation up to six days later (see Table 1). Another example from early development is the formation of the funnel mouth, which can occur 2-3 weeks after egg deposition dependent on different temperature conditions (see also Table 1). In addition, larvae kept in smaller groups (ca. 10-15 per rearing tank) grew faster compared to similar larvae in tanks with a higher density.

Morphology of developmental stages

We documented the larval development in *Megophrys nasuta* using Gosner (1960) larval stages, as reproduced in Altig and McDiarmid (1999), to describe diagnostic larval characters and stages. For developmental stages 18-22 we assessed diagnostic morphological features and age in days based on 2-6 individuals (see Table 1 and Figs. 3 and 5). For morphological description of developmental stages 25-46 (see Table 2 and Figs. 4, 6-9), we increased the number of larvae up to 12 individuals and measured length instead of age in days.

Compared to standard developmental tables, proposed for most other anuran species (e.g., Pan and Liang 1990), the funnel-shaped oral disc of tadpoles, typical for other megophryid genera (such as *Brachytarsophrys*, or *Xenophrys*), served as an additional character for staging. We have not presented a detailed morphological larval description in an advanced stage because several papers have already described these. General larval views including short descriptions were provided (e.g.,

Table 1. Developmental stages of *Megophrys nasuta* bred at the Cologne Zoo from stage 18-22, including age and diagnostic features ($n = 2-8$). Some of the larvae were reared under lower water temperatures than previously described (minimum value ca. 22 °C) which explains the somewhat slower development compared with tadpole growth described in results; stage diagnostic characters according to Gosner (1960) are in italics. ¹Could not be observed in our sample.

Stage number	Age (days)	Diagnostic features
18	11 ($n = 2$)	<i>muscular response to water movement</i> ; eye region begins to develop
19	16 ($n = 8$)	<i>heart beat visible</i> ; eye pigmentation distinctly discernible; oral region begins to stretch upwards; developing dark pigmentation on body dorsum and tail; <i>yolk reservoir reduced and blood vessels discernible</i>
20	- ($n = 5$)	<i>(development and circulation of external gills¹)</i> ; elongated oral region; last stage with distinctly visible yolk reservoir; tail longer than body
21	~21 ($n = 7$)	<i>cornea transparent</i> ; funnel mouth discernible; dark body and tail musculature with transparent and distinctly developed fin
22	60 ($n = 7$)	<i>fin circulation begins</i> ; dark dorsal pigmentation brightens

by Nodzenski et al. 1989, including the description of the visceral organization; Manthey and Grossmann 1997; and Malkmus et al. 2002); more detailed larval drawings (including lateral and oral disc) were provided by Schmidt (1976). The most detailed descriptions are in Inger (1966: under the name *M. monticola nasuta*), Inger (1985), who described internal buccopharyngeal morphology including scanning electron microscopy, and Leong and Chou (1999) (see also Das and Haas 2005).

Discussion

During keeping and breeding of *Megophrys nasuta* at Cologne Zoo we found drier conditions followed by phases of intense water spraying (rain simulation) to be important triggers for subsequent reproductive behavior and reproduction. Similar observations have been made by other authors (see Table 3b). In contrast to Pfeuffer (1989), who only noticed mating during increased temperatures in spring, we did not recognize seasonal related breeding behavior. Pfeuffer (1989) also observed egg depositions only during the daytime, whereas ovipositions at Cologne Zoo only took place during dusk and night (see also Schmidt 1976, 1977, Table 3b). In addition, we realized that housing several males with females stimulated mating, probably because of male-male competition.

We observed a wide variation in developmental time of *M. nasuta*. Whereas the first tadpole finished metamorphosis about 2.5 months after egg deposition, others did not metamorphose for seven months. We cannot know whether this wide variation also takes place under natural conditions or whether this is due to the artificial environment. Dependent on the species and the rearing conditions captive bred individuals, even in the first generation, may not be physiologically equivalent to wild individuals (Ron Altig, in litt.).

Nevertheless, mean developmental times at water temperatures of 24-26 °C were 2.5-3.5 months. We reared *M. nasuta* larvae under different water temperatures and observed development was faster at higher water temperatures. Schmidt (1977) also observed faster growth at higher temperatures of larvae kept at 22-28 °C compared with larvae reared at 19-20 °C. Development under natural conditions may also take longer than in our study because water temperatures of 18-21 °C were found in the habitat of *M. nasuta* (Malkmus 1995).

Lower density of larvae in the rearing tanks also appeared to increase developmental rate (see also Schmidt 1976, 1977) perhaps because of better accommodation and optimum nutrient availability in smaller groups. Thus, differences in temperature, population density, and greater nutrient supply appear to be the causes of different body sizes and development stages of tadpoles, of the same age. In general, larvae that developed faster led to comparatively smaller metamorphs and juveniles (e.g., 10 mm after 2.5 months developmental time versus 15-17 mm after 3.5 months). The effects of possible differences in metabolism or a different genetic background on development rates cannot be excluded. Further studies regarding the rearing of *M. nasuta* tadpoles might help to better understand factors that influence their development.

Appropriate staging of the larval period is fundamental to various life history studies of amphibians (e.g., Shimizu and Ota 2003). While trying to morphologically describe the larval stages of *M. nasuta*, we found differences compared with methodology applied by Gosner (1960). While Gosner stages 26-34 are characterized by development of hind limbs, such approach is difficult in *M. nasuta* because hind limbs of larvae are white during early development (as is likewise the case in other anurans). Although differentiation of these stages is possible to diagnose in life with a microscope or a hand loupe,

Husbandry and development of *Megophrys nasuta*

Table 2. Developmental stages of *Megophrys nasuta* bred at the Cologne Zoo from stage 25-46 including total lengths (TL) and diagnostic features ($n = 1-12$); stage diagnostic characters according to Gosner (1960) are in italics.

Stage number	TL (in mm)	Diagnostic features
25	22.07-30.94 ($n = 6$)	<i>spiracle opening sinistral</i> ; pigmentation complete; funnel mouth complete
27	24.90-31.75 ($n = 10$)	hind limb buds visible; <i>length of hind limbs $> 0.5 \times$ basal width</i>
28	27.54-32.08 ($n = 12$)	<i>length of hind limbs $>$ basal width</i> ; length of hind limbs $<$ length of vent tube
29	28.31-31.30 ($n = 7$)	<i>length of hind limbs $> 1.5 \times$ basal width</i>
30	30.78-34.85 ($n = 8$)	<i>length of hind limbs $= 2 \times$ basal width</i> ; length of hind limbs = length of vent tube
31	33.35-34.85 ($n = 2$)	<i>foot paddle-shaped</i>
32	32.11 ($n = 1$)	<i>indentation between 4th and 5th toe</i>
33	30.37-34.08 ($n = 3$)	<i>indentation between 3rd and 4th toe</i>
34	31.39-34.10 ($n = 5$)	<i>indentation between 2nd and 3rd toe</i>
35	33.33-35.00 ($n = 3$)	<i>indentation of all toes</i> ; hind limb $>$ vent tube
36	33.30-36.54 ($n = 4$)	<i>toes 3-5 separated</i>
37	34.78-37.93 ($n = 2$)	<i>all toes separated</i> ; pigmentation of hind limbs darkens
38	33.51-35.80 ($n = 6$)	<i>metatarsal tubercle visible</i>
39	32.56-35.62 ($n = 2$)	<i>subarticular patches slightly visible</i>
40	33.37-35.70 ($n = 2$)	fore limb bumps visible; hind limbs with distinct pattern; <i>last stage with vent tube</i>
41	31.63-32.40 ($n = 2$)	funnel mouth atrophy; <i>vent tube gone</i>
42	29.80-34.90 ($n = 3$)	funnel mouth degenerated; <i>fore limbs emerged</i> ; spiracle opening disappeared; mouth beneath nostril
43	31.04 ($n = 1$)	snout pointed; eyeballs starting to protrude; <i>mouth between nostril and eye</i>
44	24.05-35.73 ($n = 3$)	terrestrial life modus; tail atrophy; eyeballs further pointed; longitudinal ridges on back; <i>mouth beneath eye</i>
45	15.50-18.20 ($n = 3$)	tail mostly reduced; <i>mouth posterior to eye</i>
46	–	change of pigmentation (cream, fawn); lappet of snout and eyeballs visible; ridges on back and head become more distinct; <i>tail completely resorbed</i>

such attempt is difficult based only on photographs. This is the reason why we could not provide photographic evidence at stage 26.

In contrast, the development of the funnel mouth and length of the hind limb bud compared to the vent tube serve as additional characters in early larval stages of *M. nasuta*. The atrophy of the funnel mouth, the eye development, and the longitudinal ridges serve as diagnostic features of the species' advanced stages. Compared with Gosner (1960), we could also observe that the development of the forelimb bumps and of mouth shape in relation to position of the nostril and eye developed formerly in *M. nasuta*. Further studies on the egg development of *M. nasuta* and descriptions of stages 23, 24, and 26 are required to complete our preliminary development table.

Outlook

In general, the megophryid *M. nasuta* is relatively easy to keep, presupposed that sufficient land and water space, appropriate climatic conditions, and sufficient substrate and hiding places are provided. Breeding is possible, when drier phases followed by subsequent intensive spraying, as important triggers for reproductive activities, are provided. During the rearing of larvae, tanks

must be clean, group sizes should not be too large, and a continuous, multiple feeding per day (in particular) during early larval development should be provided. In addition, sufficient filtration and proper water exchange must be guaranteed. The rearing of the metamorphs and juveniles is time consuming but feasible.

M. nasuta is a large and attractive anuran with interesting ecological adaptations such as camouflage and somatolysis (figure dissolution) and thus is quite suitable for public zoo exhibits. This species occurs in high numbers in the international pet trade, and while few captive breeding successes have been reported, we would like to encourage other zoos and amphibian keeping facilities to keep and breed this species. Breeding activities under captive conditions, such as in zoos, especially with focus on amphibians, might considerably help to reduce the number of wild caught *M. nasuta* by providing this demand with captive bred individuals.

However, there are less understood and more endangered megophryids than *M. nasuta*, such as some of the *Megophrys* congeners, for which this overview paper might be a useful guide in future conservation breeding programs. For such conservation breeding purposes, the parental generation should at least have proper locality information or should be genetically screened, because there is still some taxonomic uncertainty among

Table 3a. Basic husbandry parameters based on the papers by Schmidt (1976, 1977), and Pfeuffer (1989) in comparison with our own results.

	Schmidt (1976, 1977)	Pfeuffer (1989)	Wildenhues et al. (2012)
adult husbandry			
terrarium size (cm)	120 × 70 × 100	85 × 60 × 50	145 × 60 × 56
land (cm)	30 × 50 (foam material)	42.5 × 60 (foam & synthetic rubber)	72.5 × 60 (leaf litter)
water depth (cm)	8	8	10
equipment	cork tubes, <i>Scindapsus</i> , <i>Philodendron</i>	cork tube caves, roots, flat stones, twine	cork tubes, <i>Asplenium nidus</i>
illumination	–	fluorescent tubes (20 Watt)	fluorescent tubes (54 Watt)
temperature	not exceeding 25 °C (preferred temperature up to 22 °C)	ca. 22-25 °C	24-25 °C
heating	–	slight floor heating	–
nutrition	crickets, earthworms, newborn mice	everything they could swallow	crickets, earthworms, newborn mice
larval husbandry			
water parameters	temperature 24 °C, GH 12.5, KH 9.5, pH 7.8	temperature 24-26 °C	temperature 24-27 °C, GH 6-8, KH 2-4, pH 8.3, conductivity 320 µS
juvenile husbandry			
terrarium size (cm)	100 × 40 × 30 (<i>n</i> = 102 froglets) 19 × 19 × 8.5 (<i>n</i> = 12 froglets)	–	60 × 45 × 30 (<i>n</i> = 20-30 froglets) –
equipment	synthetic foam, cork pieces	–	paper tissues, coconut husks
nutrition	small crickets, house crickets, wax and flour moth larvae	small earthworms, slugs	fruit flies, later on small house crickets

**Figure 8.** *Megophrys nasuta* larvae in stages 35 to 40. Photos: M. Wildenhues.

Husbandry and development of *Megophrys nasuta*

Table 3b. Breeding data based on the papers by Schmidt (1976, 1977), Pfeuffer (1989), and Anonymous (1994) compared with our own results; ¹when eggs were removed from the water part of the terrarium and fungus was eliminated; ²when eggs remained in the water part of the terrarium; ³before the development of the funnel mouth, larvae proved to be sensitive towards low temperatures (fatalities occurred at 18-20 °C); ⁴after egg deposition.

	Schmidt (1976, 1977)	Pfeuffer (1989)	Anonymous (1994)	Wildenhues et al. (2012)
calls	from middle of December onwards, at dusk	throughout the whole year, most common during spring, at that time also during daytime	–	after beginning of rain period, first at night, later also during daytime
oviposition months, and time	December, July and August, at night	March, 10:00-18:00	August, during artificial rain period	January, May, June, July, October, and November, at night
egg number	1,474-2,033	1,500-2,000	~ 300	–
hatching⁴	6 days	~ 4 days	one week	~ one week
hatching success	6-26% ¹ or 72-88% ²	~ 90%	–	–
first feeding⁴	~ 25 days	–	–	~ 20 days
developmental time (from egg deposition onwards)	first metamorphosis took place after 3 months ³	first metamorphosis took place after 4 months	–	first metamorphosis took place after 2.5 months
froglet size after metamorphosis (cm)	1.0-1.6	1 or 2	–	1.0-1.7



Figure 9. *Megophrys nasuta* larvae in stages 41 to 46. Photos: M. Wildenhues.

megophryids and species descriptions pending. A good example is the only recently described, endemic *M. kobayashii*, IUCN status near threatened and is only known from a geographically very limited range (Borneo's Mount Kinabalu, the Crocker Range, and Mount Trus Madi, in Sabah, Malaysia, at 1,300-1,600 m elevation; Frost 2011).

Acknowledgments.—The senior author would like to thank Anna Gawor (Cologne) for her kind support. Many thanks also to Professor Dr. Alexander Haas (University Hamburg) for initiating molecular comparisons with specimens collected by his working group. Professor Dr. Ronald Altig (Mississippi State University), Dr. Robert Browne (Sartenaja, Belize), and an anonymous reviewer kindly helped to improve a previous version of the manuscript. Thanks also to Lieselotte Schulz (Cologne Zoo) for her support in record keeping and to Astrid Heidrich, Perth, for providing a photograph taken at Cologne Zoo.

Literature cited

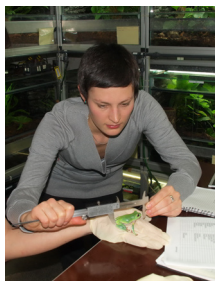
- ALTIG R, McDIARMID RW. 1999. *Tadpoles: The biology of anuran larvae*. The University of Chicago Press, Chicago, London.
- ANONYMUS 1994. Erfolgreiche Nachzucht des Zipfel-frosches. *DATZ* 47(6):346.
- BERGER L, SPEARE R, DASZAK P, GREEN DE, CUNNINGHAM AA, GOGGIN CL, SLOCOMBE R, RAGAN MA, HYATT AD, McDONALD KR, HINES HB, LIPS KR, MARANTELLI G, PARKES H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America* 95(15):9031-9036.
- BRIGGS CJ, VREDENBURG VT, KNAPP RA, RACHOWICZ LJ. 2005. Investigating the population-level effects of chytridiomycosis: An emerging infectious disease of amphibians. *Ecology* 86(12):3149-3159.
- BROWNE RK, WOLFRAM K, GARCIA G, BAGATUROV MF, PEREBOOM ZJJM. 2011. Zoo-based amphibian research and conservation breeding programs. *Amphibian and Reptile Conservation* 5(3):1-14.
- DAS I, HAAS A. 2005. Sources of larval identities for amphibians from Borneo. *Herpetological Review* 36(4):375-382.
- DRUMMOND AJ, ASHTON B, CHEUNG M, HELED J, KEARSE M, MOIR R, STONES-HAVAS S, THIERER T, WILSON A. 2009. Geneious v4.7. [Online]. Available: www.geneious.com [Accessed: 23 July 2009].
- FROST DR. 2011. Amphibian Species of the World: An Online Reference. Version 5.5 (31 January 2011). [Online: Electronic Database. American Museum of Natural History, New York, New York]. Available: <http://research.amnh.org/vz/herpetology/amphibia/> [Accessed: 18-01-2012].
- GOSNER KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190.
- GRIFFITHS RA, PAVAJEAU L. 2008. Captive breeding, reintroduction, and the conservation of amphibians. *Conservation Biology* 22(4):852-861.
- GROSJEAN S. 2005. The choice of external morphological characters and developmental stages for tadpole-based anuran taxonomy: A case study in *Rana* (*Sylvirana*) *nigrovittata* (Blyth, 1855) (Amphibia, Anura, Ranidae). *Contributions to Zoology* 74(1/2):1-22.
- HALL TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.
- HERTWIG ST, DAS I, SCHWEIZER M, BROWN R, HAAS A. 2011. Phylogenetic relationships of the *Rhacophorus everetti*-group and implications for the evolution of reproductive modes in *Philautus* (Amphibia: Anura: Rhacophoridae). *Zoologica Scripta* 41(1):9-46.
- INGER RF. 1954. Systematics and Zoogeography of Philippine Amphibia. *Fieldiana Zoology* 33:183-531. Natural History Museum, Chicago, Illinois. 531 p.
- INGER RF. 1966. *The Systematics and Zoogeography of the Amphibia of Borneo*. Fieldiana Zoology 52. Field Museum of Natural History, Chicago, Illinois. 402 p.
- KATO H, MISAWA K, KUMA K, MIYATA T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14):3059-3066.
- LEONG TM, CHOU LM. 1999. Larval diversity and development in the Singapore Anura (Amphibia). *The Raffles Bulletin of Zoology* 47(1):81-137.
- MAHONY S. 2011. Two new species of *Megophrys* Kuhl & van Hasselt (Amphibia: Megophryidae), from western Thailand and southern Cambodia. *Zootaxa* 2734:23-39.
- MALKMUS R. 1995. Die Krötenfrösche der Gattung *Megophrys* KUHL & VAN HASSELT, 1822 am Mount Kinabalu, Nord - Borneo. *Herpetofauna* (Weinstadt) 17(94):6-10.
- MALKMUS R, MANTHEY U, VOGEL G, HOFFMANN P, KOSUCH J. 2002. *Amphibians and Reptiles of Mount Kinabalu (North Borneo)*. Ruggell (A.R.G. Gantner Verlag K.G.). 424 p.
- MANTHEY U, GROSSMANN W. 1997. *Amphibien und Reptilien Südasiens*. Natur- und Tier Verlag, Münster. 512 p.
- MCGREGOR REID G, ZIPPEL KC. 2008. Can zoos and aquariums ensure the survival of amphibians in the 21st century? *International Zoo Yearbook* 42:1-6.
- MENDELSON JR III, LIPS KR, GAGLIARDO RW, RABB GB, COLLINS JP, DIFFENDORFER JE, DASZAK P, IBANEZ R, ZIPPEL KC, LAWSON DP, WRIGHT KM, STUART SN, GASCON SC, DE SILVA HR, BURROWES PA, JOGLAR RL, LA MARCA E, LÖTTERS S, DU PREEZ L, WELDON C, HYATT A,

- RODRIGUES-MAHECHA JV, HUNT S, ROBERTSON H, LOCK B, RAXWORTHY CJ, FROST DR, LACY RC, ALFORD RA, CAMPBELL JA, PARRA-OLEA G, BOLANOS F, DOMINGO JJC, HALLIDAY T, MURPHY JB, WAKE MH, COLOMA LA, KUZMIN S, PRICE MS, HOWELL KM, LAU M, PETHIYAGODA R, BOONE M, LANNOO MJ, BLAUSTEIN AR, DOBSON A, GRIFFITHS RA, CRUMP ML, WAKE DB, BRODIE JR ED. 2006. Biodiversity - Confronting amphibian declines and extinctions. *Science* 313(5783):48.
- NODZENSKI E, WASSERSUG RJ, INGER RF. 1989. Developmental differences in visceral morphology of megophryine pelobatid tadpoles in relation to their body form and mode of life. *Biological Journal of the Linnean Society* 38(4):369-388.
- PAN J, LIANG D. 1990. Studies of the early embryonic development of *Rana rugulosa* Wiegmann. *Asiatic Herpetological Research* 3:85-100.
- PFEUFFER FJ. 1989. Der Zipfelfrosch *Megophrys monticola nasuta*. *DATZ* 42(3):150-151.
- ROGNER M. 1980. Viel Freude mit Zipfelkrötenfröschen, *Megophrys nasuta*. *Aquarium Aqua Terra* 14(134):425-427.
- SCHLEGEL H. 1858. *Handleiding tot de Beoefening der Dierkunde*. Volume 2. Breda: Koninklijke Militaire Akademie. 57 p.
- SCHMIDT AA. 1976. Erst-Nachzucht des Zipfelfrosches *Megophrys nasuta* (Amphibia, Salientia, Pelobatidae). *Salamandra* 12(2):55-68.
- SCHMIDT AA. 1977. Weitere Beobachtungen bei der Nachzucht des Zipfelfrosches (*Megophrys nasuta*). *Salamandra* 13(1):43.
- SCHMIDT AA, WICKER R. 1977. Weitere Beobachtungen bei der Nachzucht des Zipfelkrötenfrosches *Megophrys nasuta*. *Salamandra* 13(1):43-48.
- SCHWANZ S. 1979. Der Zipfelkrötenfrosch. *Aquarien (Magazine)* 13(12):610-612.
- SHIMIZU S, OTA H. 2003. Normal development of *Microhylya ornata*: The first description of the complete embryonic and larval stages for the microhylid frogs (Amphibia: Anura). *Current Herpetology* 22(2):73-90.
- V. D. NIEUWENHUIZEN A. 2001a. *Megophrys monticola nasuta* (deel 1). *Aquariumwereld* 54(11):254-259.
- V. D. NIEUWENHUIZEN A. 2001b. *Megophrys monticola nasuta* (slot). *Aquariumwereld* 54(12):285-289.
- VAN DER STRAETEN K, KARBE D, OLBORT S, ZIEGLER T. 2007. Erste Nachzucht des Zipfelkrötenfrosches, *Megophrys nasuta*, im Aquarium des Kölner Zoos. *Terraria* Nr. 6 2(4):45-51.
- VAN DIJK PP, ISKANDAR D, INGER R. 2004. *Megophrys nasuta*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. [Online]. Available: www.iucnredlist.org. [Accessed: 20 January 2012].
- VENCES M, THOMAS M, BONETT RM, VIEITES DR. 2005. Deciphering amphibian diversity through DNA barcoding: Chances and challenges. *Philosophical Transactions of the Royal Society B* 360:1859-1868.
- ZIEGLER T, DANG TT, NGUYEN TQ. 2011. Breeding, natural history and diversity research: *Ex situ* and *in situ* Asian amphibian projects of the Cologne Zoo and the Institute of Ecology and Biological Resources. In: *Biology and Conservation of Tropical Asian Amphibians*. Editors, I Das, A Haas, AA Tuen. Proceedings of the Conference "Biology of the amphibians in the Sunda region, South-east Asia," Sarawak, Malaysia, 28-30 Septemebr 2009 – Institute of Biodiversity and Environmental Conservation, Universiti Malaysia Sarawak, Kota Samarahan. 137-146.
- ZIEGLER T, VAN DER STRAETEN K, KARBE D. 2008. *Der Zipfelkrötenfrosch Megophrys nasuta*. Natur und Tier-Verlag, Münster. 63 p.
- ZIPPEL K, JOHNSON K, GAGLIARDO R, GIBSON R, MCFADDEN R, BROWNE R, MARTINEZ C, TOWNSEND E. 2011. The amphibian ark: A global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6(3):340-352.

Received: 03 February 2012

Accepted: 20 February 2012

Published: 24 March 2012



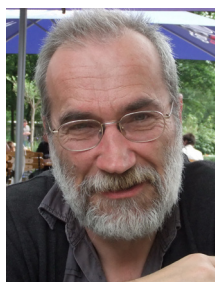
MARLEN WILDENHUES is working for the Rhine-Bergish District as a local species conservation appointee since 2011. Her interest for amphibians started with two practical courses on larval morphology and development at the Cologne Zoo in 2009, resulting so far in two scientific publications. She completed her Master Thesis in 2010 at the Faculty of Mathematics and Natural Sciences of the Rhineland Friedrich-Wilhelms-University Bonn in collaboration with the Cologne Zoo and the Institute of Ecology and Biological Resources in Hanoi Vietnam, focusing on the larval morphology and development of Vietnamese tree frogs.



ANNA RAUHAUS started her career at the Aquarium / Terrarium Department of the Cologne Zoo in May 2011. She finished her apprenticeship as zoo keeper in the year 2010. Her focus of expertise is herpetology and behavioral training.



RIKE BACH, diploma biologist from Bonn, conducted her thesis in the ichthyology section of the Zoologisches Forschungsmuseum Alexander Koenig, Bonn. During her education at the Rheinische Friedrich-Wilhelms-Universität in Bonn she attended to a research project in the Cologne Zoo where she documented the early developmental stages of *Megophrys*. Her previous research was focused on the evolutionary biology of aquatic vertebrates in Southeast Asia.



DETLEF KARBE has been an employee of the Cologne Zoo since 1974 where he has worked for 20 years as gardener in the Aquarium / Terrarium Department. He is now a zoo keeper with his main focus of work on the construction of amphibian facilities and husbandry and breeding of anurans and salamanders. He has been involved in the successful breeding of *Megophrys nasuta* at the Cologne Zoo (and its respective record keeping), and in the breeding of threatened amphibian species such as *Atelopus flavescens* and *Tylototriton shanjing*.



KARIN VAN DER STRAETEN has been an employee of the Cologne Zoo since 1970 and is head keeper in the Terrarium Department. She is a zoo keeper focusing on amphibians and reptiles and during her career she has successfully bred more than ten species of amphibians.



STEFAN T. HERTWIG is the Head Curator of the Department of Vertebrate Animals at the Naturhistorisches Museum der Burgergemeinde Bern, Switzerland, and lecturer at the University of Bern. He studied biology and finished his Ph.D. at the Institute of Systematic Zoology, Friedrich Schiller University Jena, Germany. His scientific interests focus on biodiversity, phylogeny, and evolution of the frogs of Southeast Asia.



THOMAS ZIEGLER has been the Curator of the Aquarium / Terrarium Department at the Cologne Zoo since 2003 and is the coordinator of the Zoo's Biodiversity and Nature Conservation Projects in Vietnam. He completed his Ph.D. in the year 2000 at the Mathematical Scientific Faculty of the Rhineland Friedrich Wilhelms University Bonn focusing on the amphibian and reptile community of a lowland forest reserve in Vietnam. Since 1994, he has published 246 papers and books, mainly dealing with herpetodiversity. Since 2008, Thomas has been a member of the IUCN/SSC Amphibian Specialist Group within the Mainland Southeast Asia Region. His main research interests include diversity, systematics, and zoo biology of Southeast Asia's herpetofauna, in particular amphibians, monitor lizards, snakes, and crocodiles. Since February 2009, he has been Associate Professor at the Zoological Institute (Biocentre) of Cologne University.