Zootaxa 2355: 56–68 (2010)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Rediscovery of the Liberian Nimba toad, *Nimbaphrynoides liberiensis* (Xavier, 1978) (Amphibia: Anura: Bufonidae), and reassessment of its taxonomic status

LAURA SANDBERGER[1], ANNIKA HILLERS[1], JOSEPH DOUMBIA[2], NÉMA-SOUA LOUA[3], CHRISTIAN BREDE[4] & MARK-OLIVER RÖDEL[1,5]

[1]*Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity at the Humboldt University Berlin, Herpetology, Invalidenstr. 43, 10115 Berlin, Germany. E-mails: laura.sandberger@mfn-berlin.de; annika.hillers@mfn-berlin.de; mo.roedel@mfn-berlin.de*

[2]*Centre d'Étude et de Recherche en Environnement (CÉRE), Université de Conakry, BP 3817, Conakry, Guinée. E-mail: joevero76@yahoo.fr*

[3]*Société des Mines de Fer de Guinée, Cité Chemin de Fer, Immeuble Faranah, BP 2046, Kaloum, Conakry, Republic of Guinea. E-mail: nsloua@yahoo.fr*

[4]*Medizinische Klinik und Poliklinik II, Zentrum für Experimentelle Molekulare Medizin, Zinklesweg 10, 97078 Würzburg, Germany. E-mail: brede_c@medizin.uni-wuerzburg.de*

[5]*Corresponding author. E-mail: mo.roedel@mfn-berlin.de*

Abstract

We report on the search and rediscovery of the Liberian Nimba toad, *Nimbaphrynoides liberiensis*, 30 years after its original description. A small surviving population could be traced in the surroundings of the type locality Mount Alpha, Liberia. The type locality was meanwhile destroyed by open cast mining. Similar to the Guinean Nimba toad, *Nimbaphrynoides occidentalis*, the Liberian toad lives exclusively in open, savanna like habitats above 1200 m a.s.l. The presumably few surviving individuals and the small and patchy distribution classify the Liberian toads as Critically Endangered (Stuart *et al.* 2008). A morphological, acoustical and genetic comparison of Liberian and Guinean populations revealed only minor, but distinct morphological (size, colour) differences. Genetically and acoustically the two taxa were indistinguishable. We therefore propose to consider the two populations as conspecific and to consider *Nimbaphrynoides liberiensis* (Xavier, 1978) as junior synonym of *Nimbaphrynoides occidentalis* (Angel, 1943). Because of the morphological differences we propose to treat the Liberian population as a subspecies of the Guinean toad and herein introduce the new name: *Nimbaphrynoides occidentalis liberiensis*.

Key words: Conservation status, Guinea, Liberia, mining, montane grassland, *Nimbaphrynoides occidentalis*, *Nimbaphrynoides occidentalis liberiensis*, taxonomy

Introduction

In 1943 F. Angel described a new toad species from Mount Nimba as *Nectophrynoides occidentalis*. He first believed that this toad was ovo-viviparous, but soon new data on the spectacular, truly viviparous reproductive biology of this unique toad became known (e.g. Angel & Lamotte 1944, 1948; current knowledge summarized by Xavier 1986). This toad was exclusively recorded from a few km² of montane grasslands on the Guinean part of Mount Nimba (Lamotte 1959; Lamotte & Sanchez-Lamotte 1999; Hillers *et al.* 2008; very recently we detected one site on the Ivorian part of the mountain, L. Sandberger *et al.* unpubl. data). The Nimba mountain range includes parts being positioned in nowadays Guinea, Ivory Coast and Liberia (Lamotte 1998; Lamotte & Roy 2003). Due to rich deposits of iron ore the mountains were in the focus of mining prospecting activities since the 1950s (Lamotte 1983).

In the course of iron ore prospecting in the Liberian part of the Nimba range M. Coe detected Nimba toads on Liberia’s highest mountain, the Mount Alpha in 1964. These toads were subsequently described by F. Xavier as a new likewise viviparous species, *Nectophrynoides liberiensis* (Xavier 1978; Fig. 1). This

 Accepted by M. Vences: 24 Dec. 2009; published: 10 Feb. 2010

description was mainly based on morphological differences to *N. occidentalis*, i.e. *N. liberiensis* females may be as large as 35 mm, *N. occidentalis* females rarely surpass 24 mm (however: *N. occidentalis* holotype: 27.5 mm). Additional arguments for the species description were small colour differences (i.e. the presence of brown dots on the belly of most of the Liberian toads) and hybridization experiments (Xavier 1978).

More recently, both Nimba toads were placed in an own genus, *Nimbaphrynoides* by Dubois (1987) and classified as being Critically Endangered (Stuart *et al.* 2008). However, newer data on the two toads are rare (*N. occidentalis*: Lamotte & Sanchez-Lamotte 1999; Hillers *et al.* 2008) or completely missing (*N. liberiensis*). The type locality of *N. liberiensis* meanwhile was explored for iron ore (1960 to early 1990) and what formerly was Mount Alpha, now is an open cast mining pit (Fig. 2). We aimed to verify if the Liberia Nimba toad still exists and if so, to reassess its taxonomic status.

Material and methods

In order to search for Liberian Nimba toads we visited the area of the former Mount Alpha and its surroundings near Yekepa three times in 2007 (28 June, 22 July, 23–25 August) and once in 2008 (25–27 May). We investigated heavily impacted areas (e.g. old mining pit), less impacted areas (further away from the main mining site and old mining roads), open as well as forested areas, by carefully checking the vegetation and potential hiding places, between app. 900 m a.s.l up to the remaining mountain tops (1375 m a.s.l.). Searches were undertaken between 9:30 am and 4:30 pm. We employed visual encounter searches along transects (e.g. Rödel & Ernst 2004; N = 15) and standardized plot searches (N = 4) as described by Hillers *et al.* (2008). We thus investigated all potential and accessible habitats of the Liberian Nimba toad in this region. Geographic positions of study sites were recorded with a GPS receiver (Garmin 72). In total we searched for app. 63 man-hours. All investigated localities are shown in Fig. 3 and summarized in the appendix.

Toe-tips were taken for genetic comparisons and stored in 96% ethanol. Additionally, we collected a few vouchers. These were anesthetised in a chlorobutanole solution and subsequently preserved in 75% ethanol. Tissue samples and vouchers are kept in the herpetological collection of the Museum für Naturkunde, Berlin (*N. liberiensis*: ZMB 73871-73877; five females, one male, one juvenile). The type series of *N. liberiensis* was investigated in the collection of the Muséum National d'Histoire Naturelle, Paris (holotype: MNHN 1978.3088; paratypes: 1978.3089–3103; 1978.3111–3114; 1978.3116–3126; 16 females, 14 males; juveniles of the type series not investigated). These toads were compared to the *N. occidentalis* type series (holotype: MNHN 1944.149; para- and paratopotypes: 1944.151–152, 1944.154–159; nine females; three juveniles not investigated), *N. occidentalis* specimens stored in Berlin (ZMB 70660, 73878-73890; 12 females; six males) and to snout-vent length (SVL) measures taken in 2007 and 2008 in the field (*N. occidentalis*: N = 603, females: 469, males: 134; *N. liberiensis* N = 68, females: 44, males: 24). For the morphological comparison we measured SVL, head width (HW), femur length (FL), tibia length (TB) and food length incl. longest toe (FoL). With the exception of SVL, measures have been taken from museum vouchers only. All measures were taken with a dial calliper (± 0.1 mm) and are given in millimetres. For statistical comparison we carried out t-tests (all data showed a normal distribution and homogeneity in variance). All calculations were carried out with the freeware software package R.

In 2007 we succeeded in recording advertisement calls of one *N. liberiensis* male. In 2008 we recorded the calls of 11 *N. occidentalis* males. Calls were recorded with a R-09 24bit WAVE/ MP3 recorder (ERIDOL by Roland, sample rate: 44.1 kHz, record mode: wav_24bit) and a microphone (ECM-950). These calls were analysed with the programme Avisoft SAS Lab Pro 4.5 (R. Specht, Berlin, Germany).

DNA was extracted using High Pure PCR Template Preparation kits (Roche) following manufacturer's instructions. We used primers 12SA and 12SB to amplify part of the 12S gene and 16SA and 16SB for the partial 16S rRNa gene; all primers are from Palumbi (1991). The cytochrome *b* (cyt *b*) gene was amplified with the primers CBJ10933 and Cytb-C from Bossuyt & Milinkovitch (2000). Standard PCR protocols were used and PCR products were purified using High Pure PCR Product Purification kits (Roche). Purified templates were directly sequenced using an automated sequencer (ABI 3100). In total, we sequenced 16 tissue samples (nine *N. liberiensis*, seven *N. occidentalis*) for 12S (390bp), 12 (five *N. liberiensis*, seven *N. occidentalis*) for 16S (552 bp) and 10 specimens (four *N. liberiensis*, six *N. occidentalis*) for cyt *b* (608 bp; see Table 3). Sequences were validated using SEQUENCE NAVIGATOR (Applied Biosystems). We aligned them using the Clustal W option in MEGA 4.1 (Tamura *et al.* 2007). The alignment was subsequently checked by eye and refined if necessary. Uncorrected pair-wise sequence divergence was calculated using PAUP* 4beta10 (Swofford 2002).

FIGURE 1. Dorsal and ventral view of (left) the holotype of *Nimbaphrynoides liberiensis* (MNHN 1978.3088; SVL 29.2 mm) and (right) the holotype of *Nimbaphrynoides occidentalis* (MNHN 1944.149; SVL 27.5 mm).

Results

We rediscovered the Liberian Nimba toad, both north and south of the old LAMCO (Liberian-American-Swedish Minerals Company) mining pit. In total we detected 30 toads (14 adults: five females, six males, three subadults, 16 juveniles) in 2007. In 2008 we encountered a total of 22 toads, 17 females (16 being pregnant), and five males.

Most individuals were found south of former Mount Alpha and each year only one female was found north of it. At several localities we could not find any toads (Fig. 3). We observed toads only within the less impacted parts, where mining was abandoned after 10 years. They were absent from strongly impacted localities, i.e. where mining took place for more than 30 years. All places with toads had soil that was not or little compacted, i.e. holes and cracks were visible. At sites without such structures no toads could be traced. All toads were recorded in open areas at 1200 m a.s.l. and above.

FIGURE 2. Type locality of *Nimbaphrynoides liberiensis* (the meanwhile destroyed Mount Alpha, above) and a degraded habitat south of the mining pit, currently used by the Liberian Nimba toad.

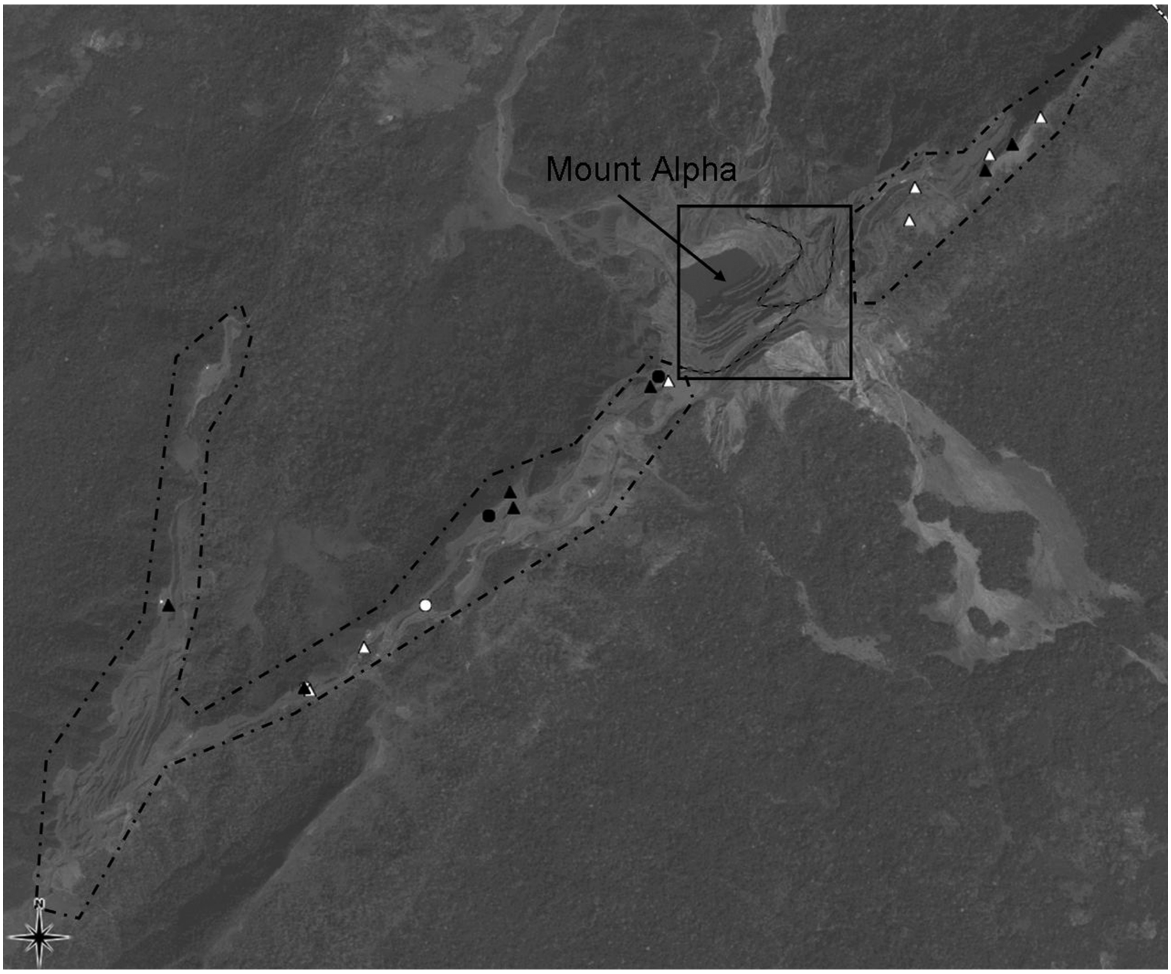

FIGURE 3. Sites investigated for Nimba toads and current distribution of *Nimbaphrynoides liberiensis*. Black dots (plots) and triangles (transects) indicate where we encountered Liberian Nimba toads. At white dots (plots) and triangles (transects) we failed to record them. The black box and the arrow indicate the water filled mining pit of the LAMCO mine, the former Mount Alpha (compare Fig. 2). Note erosion left and right of the mining pit. The area indicated by the dotted line was less intensively minded (10 years only) than Mount Alpha (1960 to early 1990). The grey line north of the mining pit indicates a further area investigated (nine man-hours) for Nimba toads without success (map adopted from Spot_Image Jannuary 2005).

For the SVL comparison we used museum vouchers and measurements taken in the field. Females were significantly larger than males in both taxa (*N. occidentalis* females: mean length 20.5 mm, N = 490; males: 18.0 mm, N = 149, $p < 0.05$; *N. liberiensis* females: 28.7 mm, N = 65; males: 22.4 mm, N = 39, $p < 0.001$; Fig. 4). *N. liberiensis* were significantly larger than *N. occidentalis* (males: $p < 0.001$; females: $p < 0.001$), respectively. Even after being corrected for size (FoL/SVL) *N. liberiensis* females (N = 19) and males (N = 13) had significantly longer feet than *N. occidentalis* (females N = 12, males N = 6; $p < 0.05$). Other morphological parameters did not differ between taxa (Table 1). To account for the possibility of geographic clinal variation we correlated all SVL field measures from 2007 and 2008 ($N_{females} = 483$, $N_{males} = 143$) with latitude. Analyses were done with respect to sex, but irrespective of the taxa. We found no geographic correlation.

The colouration of adults in both taxa was variable (Figs. 1, 5 & 6). Most individuals had white venters and brown to black backs without a distinct border between the two colours. The back may be uniform in colouration or lighter and darker browns may be irregularly mixed. Heads had always, at least on the snout and the eye-lids, lighter areas. The legs were always light brown with irregularly bordered darker stripes or dots. Juveniles had brighter coloured backs than adults. Juveniles of both taxa had a black, white bordered, lateral line, starting at the snout-tip, passing through the eyes and extending to the groin area. Their back had a golden-brownish ground colour and may carry irregular or symmetrical shaped blackish spots and figures. Their legs were yellow to brown with white stripes. With age and increasing size their backs and legs became darker, the contrast between differently coloured areas fainted. In comparison *N. liberiensis* females seemed to comprise the most uniform, *N. occidentalis,* the most contrasting individuals. None of the several hundred investigated *N. occidentalis* was found to have brown dots on the belly or throat. In contrast, some living *N. liberiensis* had small to large light brown dots on their venter (Fig. 5). These dots seemed to be generally more conspicuous in alcohol preserved material. In some of the latter specimens the venter was nearly completely brown.

TABLE 1. Mean values of morphological measures of *Nimbaphrynoides occidentalis* and *N. liberiensis* (only museum vouchers included) and t-test results between the two taxa. The sexes were compared separately. All measures had been standardized against snout-vent length prior to comparisons. Only feet (FoL) of *N. liberiensis* were significantly larger than those of *N. occidentalis.* For abbreviations compare Material & Methods; N = sample size; n.s. = not significant.

sex	variable	species	mean	N	t-value	df	p
females	HW	*occidentalis*	0.32	12	-1.8169	16.907	n.s.
		liberiensis	0.30	21			
males		*occidentalis*	0.32	6	-0.8399	5.775	n.s.
		liberiensis	0.30	15			
females	FL	*occidentalis*	0.44	12	1.4185	25.586	n.s.
		liberiensis	0.47	21			
males		*occidentalis*	0.45	6	1.3183	5.388	n.s.
		liberiensis	0.48	15			
females	TL	*occidentalis*	0.42	12	1.2579	23.932	n.s.
		liberiensis	0.44	21			
males		*occidentalis*	0.44	6	1.5919	5.291	n.s.
		liberiensis	0.48	15			
females	FoL	*occidentalis*	0.65	12	3.7655	16.996	<0.05
		liberiensis	0.73	19			
males		*occidentalis*	0.64	6	3.4065	5.789	<0.05
		liberiensis	0.73	13			

Both species start uttering advertisement calls at approximately the middle of the rainy season, i.e. July. Main calling activity is however later at the beginning or middle of September. The advertisement call was very faint, short and resembled a metallic “bing” (Fig. 7). We got recordings of this call of one *N. liberiensis* male and eleven *N. occidentalis* males, thus allowing for descriptive analyses only. The advertisement call had two distinct parts: it started with a slightly higher frequency and more energy and ended with lower energy and slightly lower frequency. The longer end part was more variable than the first one. The frequencies of the calls (total call and each part separately) of *N. liberiensis* (mean[total call] = 3568 Hz; mean[first part of call] = 3565 Hz; mean[rest of call] = 3477 Hz) were slightly higher than the frequencies of *N. occidentalis* (mean[total call] = 3141 Hz; mean[first part of call] = 3319 Hz; mean[rest of call] = 2775 Hz). The other call parameters (number of pulses, lengths of the call and call parts, proportion of the energy of the second to the first part) did not differ notably (Table 2). In *N. occidentalis* we recorded a second, relatively uniform “rasping” call, mostly heard during aggressive encounters between males as well as during mating. When uttered during antagonistic encounters this call seemed to chase an intruder away, without physical combat. This call was considerable longer than the advertisement call (0.126 sec compared to 0.018 sec). The mean frequency (3177 kHz) was similar to the advertisement call (Fig. 7). This call may start or, more often, end with the advertisement call. Schiøtz (1964) reported a third call type for *N. occidentalis*, a slow chirp with a wave-like frequency modulation. He believed that this call type could be an initial call. We so far never heard such a call.

We detected minor genetic differences between the two taxa. They were largest between the two taxa and lowest within *N. liberiensis* (Table 3). However, even the largest difference between the two taxa, found in cyt *b*, was only about 2%.

TABLE 2. Comparison of advertisement call (A in Fig. 7) parameters of one *Nimbaphrynoides liberiensis* and eleven *N. occidentalis* males. The call consists of two parts, a first part (first) which is louder and higher than the second part (rest). The latter is the more variable part of the call. For all parameters the mean and range (in parentheses) is given for the total call, the first part and the second part of the call (rest). Length of a call and length between calls is given in seconds [s]; main frequency given in hertz [Hz]. The energy of the first part of the call ($Energy_{first}$) is given as relative value to the energy of the rest of the call ($Energy_{rest}$) as $Energy_{first}$/ $Energy_{rest}$.

species		*occidentalis*	*liberiensis*
# pulses		4.9 (3–7)	6.6 (3–13)
length [s]	total call	0.018 (0.012–0.026)	0.020 (0.009–0.041)
	first	0.004 (0.003–0.005)	0.005 (0.003–0.011)
	rest	0.007 (0.015–0.022)	0.045 (0.005–0.255)
	time to next	107.012 (48.000–471.000)	104.220 (21.960–252.000)
frequency [Hz]	total call	3141 (2020–4560)	3567 (2390–5570)
	first	3319 (2190–4560)	3565 (2430–5570)
	rest	2775 (2020–4000)	3477 (2390–4500)
$Energy_{first}$/ $Energy_{rest}$		4.021 (0.555–11.730)	5.386 (0.324–22.220)

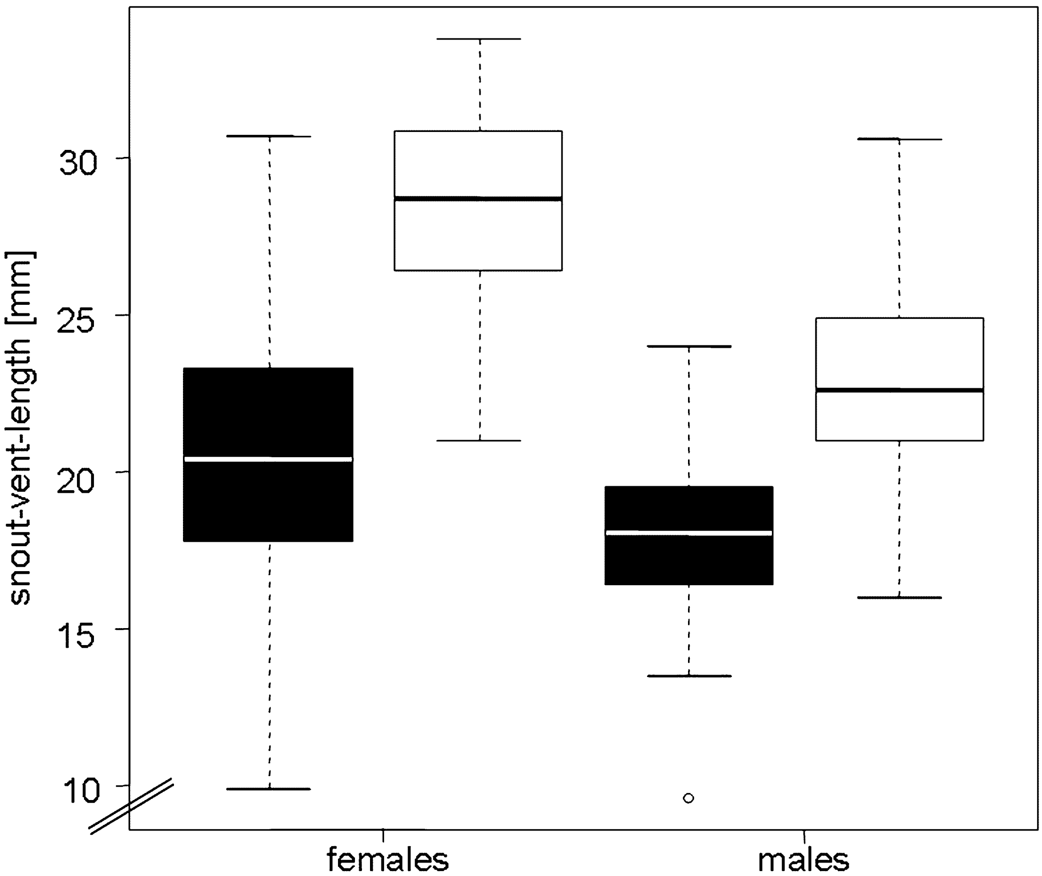

FIGURE 4. Sex dependant snout-vent-lengths comparisons of *Nimbaphrynoides occidentalis* (black) and *N. liberiensis* (white). *N. liberiensis* were significantly larger than *N. occidentalis* (see text).

TABLE 3. Genetic distances (uncorrected *p*-distance) in percent between *Nimbaphrynoides occidentalis* and *N. liberiensis* and within each taxon, for 12S, 16S and cyt*b* sequences. Given are mean, minimum (min) and maximum (max) values. Below we listed all voucher and tissue samples with their corresponding GenBank accession numbers; n.v.= no voucher collected.

Sequence comparison	12S			16S			cyt *b*		
	mean	min	max	mean	min	max	mean	min	max
Between taxa	0.002	0	0.005	0.002	0	0.004	0.020	0.016	0.023
Within *liberiensis*	0.001	0	0.005	0	0	0	0	0	0
Within *occidentalis*	0.001	0	0.003	0.002	0	0.005	0.007	0	0.013

Taxon	Voucher #	Tissue #	GB16S	GB12S	GBCyt*b*
liberiensis	ZMB 73875	MOG012	GU322838	GU322821	GU322850
	ZMB 73876	MOG013	GU322839	GU322822	GU322851
	n.v.	MTN245	GU322840	GU322823	GU322858
	n.v.	MTN246	GU322841	GU322824	GU322859
	n.v.	MTN247	GU322842	GU322825	
	n.v.	MTN248		GU322826	
	n.v.	07_204_Lib		GU322828	
	n.v.	07_205_Lib		GU322829	
	n.v.	07_215_Lib		GU322830	
occidentalis	ZMB 73881	MOG018	GU322843	GU322831	GU322852
	ZMB 73882	MOG019	GU322844	GU322832	GU322853
	ZMB 73886	MTN83	GU322849	GB322837	GU322857
	n.v.	MTN15	GU322845	GU322833	GU322854
	n.v.	MTN16	GU322846	GU322834	GU322855
	n.v.	MTN22	GU322847	GU322835	
	n.v.	MTN78	GU322848	GU322836	GU322856

Discussion

The description of *N. liberiensis* was mainly based on small morphological (size) and colour (dots on belly and throat) differences to *N. occidentalis* (Angel 1943; Xavier 1978). These differences could be confirmed herein. Additionally, F. Xavier undertook hybridization experiments between the two taxa in her Paris laboratory. She found that none of the offspring survived when fathered by a Liberian and mothered by a Guinean toad (of 15 pairings only one female gave birth, however to dead young). In contrast, 80% of the offspring fathered and mothered by *N. liberiensis* were borne alive (Xavier 1978). However, F. Xavier apparently did not try to breed a *N. occidentalis* female with a *N. liberiensis* male. The failure of getting offspring could be due to the fact that the sizes did not fit. This species has to have internal fertilization and size relation between sexes may thus be an important feature to assure successful fertilization. However, we don't believe that this is a very convincing argument (compare Fig. 5). In fact we found only very minor genetic differences in mitochondrial gene sequences, in loci usually used for DNA-barcoding in amphibians (compare Vieites *et al.* 2009). As also another important character for species delimitation in anurans, the advertisement call, did not differ, hybridization between the two taxa seems potentially possible.

Xavier (1978) gave habitat descriptions for three localities. They were described as being open, having steep slopes, loose stones or crevices and positioned near mining roads (presumably recorded here because of easier access). We could find the Liberian toad exclusively in open areas (Fig. 2), and as already stated by Xavier (1978), only above 1200 m a.s.l. and at sites with holes and cracks in the ground. The original sites

FIGURE 5. Life coloration of *Nimbaphrynoides liberiensis*; above: comparison of *Nimbaphrynoides* females, *N. occidentalis* (left) and *N. liberiensis* (right; ZMB 73875); centre: amplectant couple of *N. liberiensis* (female: ZMB 73873; male: ZMB 73874) and juvenile *N. liberiensis* (inlet left, ZMB 73877); below: two *N. liberiensis* with darker spots on throat and belly.

FIGURE 6. Female (above), male (below) and juvenile (inlet) of *Nimbaphrynoides occidentalis*.

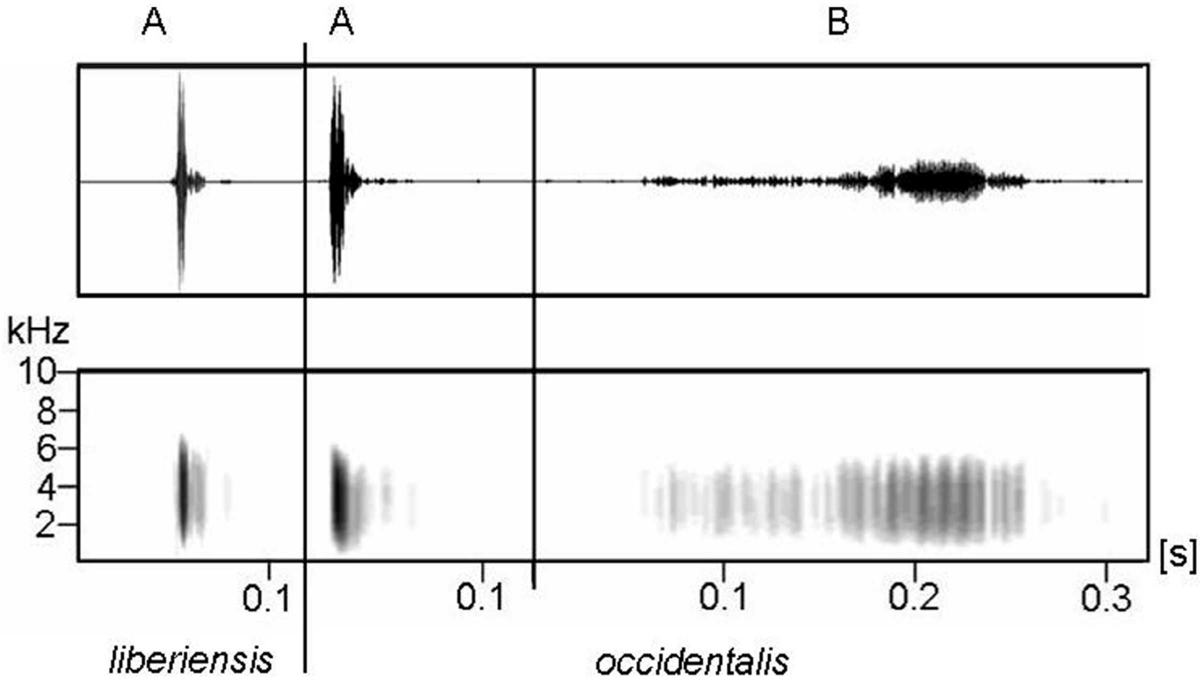

FIGURE 7. Oscillogram and waveform of the advertisement calls (A) of *Nimbaphrynoides liberiensis* and *N. occidentalis* and a presumed aggressive call of *N. occidentalis* (B, compare text).

were given as: mining plateau, Mount Alpha and mining road. Now the mining plateau and Mount Alpha are mostly reduced to altitudes below 1200 m a.s.l. (Fig. 2) and we failed to record any toads at these sites. Although many areas seem to equal the ones described by Xavier (1978), we only recorded the Liberian toad at some localities and always in comparatively low densities (see Lamotte 1959; Hillers *et al.* 2008). Most area above 1200 m a.s.l. is heavily altered, either offering only very compacted soil without hiding places or consisting of loose gravel. It is very likely that the toads need good, i.e. deep and solid hiding places, especially in order to survive the long dry season (compare Lamotte 1959). The main population now seems to be restricted to areas south of the old mining pit. North of it we only detected two females. It seems unlikely that the toads may migrate between these two sites as 1) the habitat in-between is extremely degraded and 2) lays below 1200 m a.s.l. It is a good sign that these toads were capable to survive this extreme habitat alteration. However, due to the very small and isolated habitat patches, combined with presumably very small population sizes, the Liberian Nimba toad must be still considered Critically Endangered. Its remaining habitats should be carefully monitored and maintained.

Remains the question whether the two Nimba toad taxa should be considered representing different species or not. The genetic differences between Guinean and Liberian populations are minute and far below what usually are taken to differentiate between anuran species (Rödel *et al.* 2003, 2009; Vences *et al.* 2005). In contrast, the morphological differences (size and ventral pattern) are sufficient to correctly assign at least most specimens to one or the other name. Furthermore, we are aware of the possibility that the observed genetic similarity could originate from mitochondrial introgression which is not uncommon in amphibians, as has been shown e.g. in newts (Babik *et al.* 2003) or the frog genera *Meristogenys* and *Ameerega* (Shimada *et al.* 2008; Brown & Twomey 2009). In these species nuclear genes revealed the existence of distinct differences on the species level. Unfortunately we failed to amplify nuclear genes of the Nimba toads. Both toad taxa, living exclusively in open, savanna like montane habitats, are separated by a distance of about 7.5 km, comprising a forested mountain ridge. Although it is thus unlikely that both toad taxa are in frequent genetic exchange; the observed genetic, acoustic and morphological differences altogether are too small to justify the maintenance of two different species names. However, to account for the existence of the small genetic and morphological differences, we propose to maintain a separate name for the Liberian population, although in the rank of a subspecies: *Nimbaphrynoides occidentalis liberiensis*.

Acknowledgements

We thank the "Forestry Development Authority", namely J. Woods and T. Freeman in Liberia to allow research on the Liberian part of the Nimba range. The Guinean authorities, particularly the "Ministère de l'Agriculture, de l'Elevage, de l'Environnement et des Eaux et Fôrets", represented by C.S. Kourouma, permitted work on the Guinean part of Mount Nimba. The "Centre de Gestion de l'Environnement du Nimba-Simandou", the "Projet des Nations Unies de Développement" and the three "Comités Villageois de Surveillance" allowed us to work within the World Heritage Site. The research permit in Guinea was issued by the "Ministere de l'Education Nationale et de la Recherche Scientifique/ Direction Nationale de la Recherche Scientifique et Technologique " (No. 095; MNRS/DNRST). The "Ministère du Developement Durable et de l'Environement/ Direction Nationale des forets et faune" permitted the capture and collection of the Guinean Nimba Toad (permis sceintifique de capture, No. 02/2008). We thank Arcelor-Mittal and especially M. Majumdar, for accommodation, food and the permission to search at the old LAMCO mine in Liberia. We are grateful to the "Société des Mines de Fer de Guinée" and its stuff for the financial support, and for all the other help and support, especially to J. Suter and C. Halpin. A. Ohler and A. Dubois facilitated the examination of the type material in the Paris collection. S. Soubzmaigne took the picture of the *N. liberiensis* holotype. We especially thank the "Bundesamt für Naturschutz", Bonn for issuing CITES import permits (*Nimbaphrynoides occidentalis*; E-3117/07 and E-4074/08; *Nimbaphrynoides liberiensis*; E-4509/07). The respective export permits were issued by "Le Directeur Nationale de la Protection de la Nature"(2007/ 00314) and "L'organe de Gestion CITES Guinée" (2008/ 0049) in Guinea (*Nimbaphrynoides occidentalis*) and by the "Forestry Development Authority" in Liberia (01, *Nimbaphrynoides liberiensis*). Last but not least we are much indebted to our field assistants B. Doré, K. Camara and F. Gbêmou for their excellent work and good humour.

References

Angel, F. (1943) Description d'un nouvel amphibien anoure, ovo-vivipare de la Haute-Guinée Française (Materiaux de la mission Lamotte, au Mont-Nimba) (2e note). *Bulletin du Muséum national d'Histoire naturelle*, 15, 167–169.

Angel, F. & Lamotte, M. (1944) Un crapaud vivipare d'Afrique Occidentale *Nectophrynoides occidentalis* Angel. *Annales des Sciences naturelles, Zoologie*, 6, 63–89.

Angel, F. & Lamotte, M. (1948) Nouvelles observations sur *Nectophrynoides occidentalis* Angel. Remarques sur le genre *Nectophrynoides*. *Annales des Sciences naturelles*, *Zoologie*, 11, 115–147.

Babik, W., Szymura, J.M. & Rafinski, J. (2003) Nuclear markers, mitochondrial DNA, and male secondary sexual traits variation in a newt hybrid zone (*Triturus vulgaris* x *T. montandoni*). *Molecular Ecology*, 12, 1913–1930.

Bossuyt, F. & Milinkovitch, M.C. (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences*, USA, 97, 6585–6590.

Brown, J.L. & Twomey, E. (2009) Complicated histories: three new species of poison frogs of the genus *Ameerega* (Anura: Dendrobatidae) from north-central Peru. *Zootaxa*, 2049, 1–38.

Dubois, A. (1987 '1986') Miscellanea taxinomica batrachologica (I). Alytes, 5, 7–95.

Hillers, A., Loua, N.-S. & Rödel, M.-O. (2008) Assessment of the distribution and conservation status of the viviparous toad *Nimbaphrynoides occidentalis* on Monts Nimba, Guinea. *Endangered Species Research*, 5, 13–19.

Lamotte, M. (1959) Observations écologiques sur les populations naturelles de *Nectophrynoides occidentalis* (Fam. Bufonidae). *Bulletin Biologique*, 4, 355–413.

Lamotte, M. (1983) The undermining of Mount Nimba. *Ambio*, 12, 174–179.

Lamotte, M. (1998) *Le mont Nimba. Réserve de la biosphère et site du patrimoine mondial (Guinée et Côte d'Ivoire). Initiation à la géomorphologie et à la biogéographie*. UNESCO publishing, Paris, 153 pp.

Lamotte, M. & Roy, R. (2003) Le peuplement animal du Mont Nimba (Guinée, Côte d'Ivoire, Liberia). *Mémoires du Musée national Histoire naturelle*, 190, 1–724.

Lamotte, M. & Sanchez-Lamotte, C. (1999) Adaptation aux particularités climatiques du cycle biologique d'un anoure tropical, *Nectophrynoides occidentalis* Angel, 1943 (Bufonidae). *Alytes*, 16, 111–122.

Palumbi, S.R. (1991) Nucleic Acids II: the Polymerase Chain Reaction. In: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds) *Molecular Systematics*. 2nd edition, Sinauer Associates, Sunderland, pp. 205–247.

Rödel, M.-O., Boateng, C.O., Penner, J. & Hillers, A. (2009) A new cryptic *Phrynobatrachus* species (Amphibia: Anura:

Phrynobatrachidae) from Ghana, West Africa. *Zootaxa*, 1970, 52–63.
Rödel, M.-O. & Ernst, R. (2004) Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. *Ecotropica*, 10, 1–14.
Rödel, M.-O., Kosuch, J., Veith, M. & Ernst, R. (2003) First record of the genus *Acanthixalus* Laurent, 1944 from the Upper Guinean rain forest, West Africa, with the description of a new species. *Journal of Herpetology*, 37, 43–52.
Schiøtz, A. (1964) The voices of some West African amphibians. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening*, 127, 35–83.
Shimada, T., Matsui, M., Yambun, P., Lakim, M. & Mohamed, M. (2008) Detection of two cryptic taxa in *Meristogenys amoropalamus* (Amphibia, Ranidae) through nuclear and mitochondrial DNA analyses. *Zootaxa*, 1843, 24–34.
Stuart, S., Hoffman, M., Chanson, J., Cox, N. Berridge, R. Ramani, P. & Young, B. (2008) *Threatened amphibians of the World*. Lynx Editions, Barcelona, 758 pp.
Swofford, D.L. (2002) PAUP*: *Phylogenetic Analysis Using Parsimony (and Other Methods). 4.0 Beta for UNIX or OpenVMS*. Sinauer Associates, Sunderland, Massachusetts.
Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 1596–1599.
Vences, M., Thomas, M., Bonett, R.M. & Vieites, D.R. (2005) Deciphering amphibian diversity through DNA barcoding: changes and challenges. *Philosophical Transactions of the Royal Society, B Biological Sciences*, 360, 1859–1868.
Vieites, D.R., Wollenberg, K.C., Andreone, F., Köhler, J., Glaw, F. & Vences, M. (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences*, USA, 106, 8267–8272.
Xavier, F. (1978) Une espèce nouvelle de *Nectophrynoides* (Anura, Bufonidae) des Monts Nimba, *N. liberiensis* n.sp. I – description de l'espèce. *Bulletin de la Société Zoologique de France*, 103, 431–441.
Xavier, F. (1986) La reproduction des *Nectophrynoides*. *In*: Grassé, P.-P. & Delsol, M. (Ed) *Traité de Zoologie, anatomie, systématique, biologie. Tome XIV, Batraciens, fascicule IB*. Masson, Paris, pp. 497–513.

Appendix. Study sites searched for *Nimbaphrynoides liberiensis*. Given are the area ID, the location (south or north of the LAMCO mine, former mount Alpha, compare Fig. 3), latitude, longitude, altitude, search date, the number of encountered adults (n° ad), juveniles (n° juv) and total number of toads (n° an). P1–P4 represent standardized plot searches (25 m² see Hillers *et al.* 2008 for exact description), R01–R15 represent random transects.

Area	location	latitude	longitude	altitude	date	n° ad	n° juv	n° an
P1	South	7.53468	-8.50143	1293	23/08/2007	2	0	2
P2	South	7.52675	-8.51113	1295	24/08/2007	0	1	1
P3	South	7.52168	-8.51470	1323	24/08/2007	0	0	0
P4	South	7.51698	-8.52158	1329	24/08/2007	0	0	0
R01	North	7.52168	-8.52937	1267	27/07/2007	0	1	1
R02	North	7.52723	-8.50968	1318	27/07/2007	2	10	12
R03	South	7.51698	-8.52158	1246	23/08/2007	4	4	8
R04	South	7.51698	-8.52158	1288	23/08/2007	5	0	5
R05	South	7.51928	-8.51819	1375	24/08/2007	0	0	0
R06	South	7.54545	-8.48682	1240	25/08/2007	0	0	0
R07	South	7.54936	-8.48716	1190	25/08/2007	0	0	0
R08	South	7.54639	-8.48284	1186	25/08/2007	1	0	1
R09	South	7.53442	-8.50091	1249	25/05/2008	0	0	0
R10	South	7.53408	-8.50194	1281	25/05/2008	16	0	16
R11	North	7.54944	-8.47970	1302	26/05/2008	0	0	0
R12	North	7.54785	-8.48130	1234	26/05/2008	1	0	1
R13	North	7.54731	-8.48258	1180	26/05/2008	0	0	0
R14	South	7.52808	-8.50989	1313	27/05/2008	5	0	5
R15	South	7.51685	-8.52131	1324	27/05/2008	0	0	0