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A new dwarf forest toad (Amphibia: Bufonidae: *Nectophrynoides*) from the Ukaguru Mountains, Tanzania

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Abstract

A new species of *Nectophrynoides* from the Ukaguru Mountains, Eastern Arc Mountains Tanzania, is described. The new species is diagnosed by the presence of large prominent spines with keratinized tips, positioned on the dorsum, head and limbs. In addition, the combination of the following characters - presence of large tympanum and annulum, foot longer than tibia, the lack of parotoid glands and advertisement call features, allow this species to be distinguished from all other species in the genus**.** The new species adds to our understanding of the amphibian fauna of the Ukaguru Mountains, relatively undersampled and poorly understood.

Key words: *Nectophrynoides*, Ukaguru Mountains, Eastern Arc, Biodiversity hotspot, Tanzania

Introduction

The genus *Nectophrynoides* Noble, 1926 is strictly confined to the sub-montane and montane moist forests of the Eastern Arc Mountains and the Southern Highlands of Tanzania. The genus was established by Noble (1926) to accommodate the dwarf toad *Nectophryne tornieri,* described by Roux in 1905 and subsequently the toad *Pseudophryne vivipara* described by himself in 1906. The new genus was separated from other bufonid genera based on presence of an omosternum, T-shaped but not flattened or spatulated phalanges, and by the 'viviparous' (ovoviviparous as in Duellman and Trueb, 1986) mode of reproduction. Since the description of *N. viviparus* (Noble, 1926) another ten species have been described: *N. cryptus* Perret, 1971; *N. minutus* Perret, 1972; *N. wendyae* Clarke, 1988; *N. asperginis* Poynton *et al.* 1998, *N. vestergaardi, N. pseudotornieri, N. frontierei, N. laevis, N. poyntoni* Menegon *et al.* 2004 and finally *N. laticeps* Channing *et al.* 2005. Despite the relatively rapid increase in the number of *Nectophrynoides* species described over the last 20 years, diagnosis of *Nectophrynoides* Noble 1926, and therefore its content still remains unsatisfactorily resolved (Clarke, 2001; Menegon *et al.* 2004). This in spite of significant contributions made by Grandison (1978) and Wake (1980) to understand the morphology and reproductive biology of non-*Bufo* African bufonids (Clarke, 1988).

Although there remain some difficulties in assigning new species to the not clearly defined genus *Nectophrynoides* (*sensu* Dubois, 1987 (1986)), here we describe a species of *Nectophrynoides* found in the Ukaguru Mountains during an amphibian and reptile survey in January 2004. The new species is compared with all other currently recognised species, using mainly morphology and call characteristics. Further work on the genus will be needed to clarify the phylogenetic relationships of these species, and its corresponding classification.

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Material and methods

Specimens were collected from Mamiwa-Kisara Forest Reserve during a single survey conducted by Michele Menegon and Wilirk Ngalason in January 2003. One site was sampled intensively, mainly by visual encounter surveys (Corn and Bury, 1990) and by means of pitfall traps with drift fences (Corn, 1994). Specimens belonging to the species herein described were captured exclusively during visual night searches. Voucher specimens were fixed in 70% ethanol. Tissues for DNA analysis were fixed in 96% ethanol. The type series is deposited in the collections of Museo Tridentino di Scienze Naturali (MTSN), Trento, Italy and the Natural History Museum, London. Specimens were measured using callipers; all measurements are given in millimetres, to the nearest 0.1 mm. The following morphometric measures were taken: HL = head length (from the base of the lower jaw to the snout); HW = head width (at jaw articulation); ND = nostril distance; FL = forearm length; SUL = snout-urostyle length; TL = tibia length, (taken with tibia completely folded); ML = metatarsum length (taken with metatarsum forming a square angle with tibia); FOL = foot length.

Calls were recorded using a Sharp MT 877 mini-disc recorder and a Sony TCM directional microphone and analysed using the software Raven 1.3 (Charif et al., 2004).
Comparative material examined is listed in the appendix.

Systematic Account

***Nectophrynoides paulae* sp. nov.**
(Figs. 2-4)

Holotype. An adult male in the Museo Tridentino di Scienze Naturali, Trento, MTSN 5630 collected 25 January 2004 in Mamiwa-Kisara Forest Reserve by Michele Menegon and Wilirk Ngalason.

Type locality. Mamiwa-Kisara North Forest Reserve (Fig. 1), at 1800 m above sea level, Ukaguru Mountains, Kilosa District, Morogoro Region, Tanzania (UTM coordinates: 37M 0270973 / 9295414).

Paratypes. MTSN 5621 (male), BM 2005.1589 (formerly MTSN 2622) (male), BM 2005.1590 (formerly MTSN 5623) (male), MTSN 2624 (male), MTSN 5625 (male), MTSN 2626, MTSN 5627 (male), MTSN 2628 (female containing about 20 yolking eggs), MTSN 2629 (male). All specimens were collected at the same locality, with the same collection data as the holotype.

Etymology. The species is named in honour of Paola Magagnato, a dedicated natural sciences teacher. Paola conveyed her love and enthusiasm of nature to her family, friends and students; she tragically passed away in August 2004.

Diagnosis. A small-sized *Nectophrynoides* with snout-urostyle (SUL) ranging from 15.1 to 23.7 mm, characterised by prominent, large conical shaped spines covering head, eyelids, dorsum and hindlimbs. These spines are conical in shape ending with a conspicuous clear keratinized horny tip. A set of six spines, often larger than the others, situated on head and shoulders are arranged symmetrically (Fig. 1). Spines often are concentrated also on the anterior part of the head, dorsum, sacral region and limbs. Tips of fingers and toes are rounded, not or slightly expanded, never truncated. The foot length is greater than or equal to length of tibia (Table 1). Tympanum and tympanic annulus are present and clearly visible. Parotoid glands are absent. Hands are not webbed, while fingers are only webbed at their base. The two distal phalanges of the fifth toe are free of main webbing, the three distal phalanges of fourth toe are free of main webbing on both sides. The upper lips often show dark markings on a paler ground colour (Figs 1 and 2). These features distinguish *N. paulae* from all others known species.

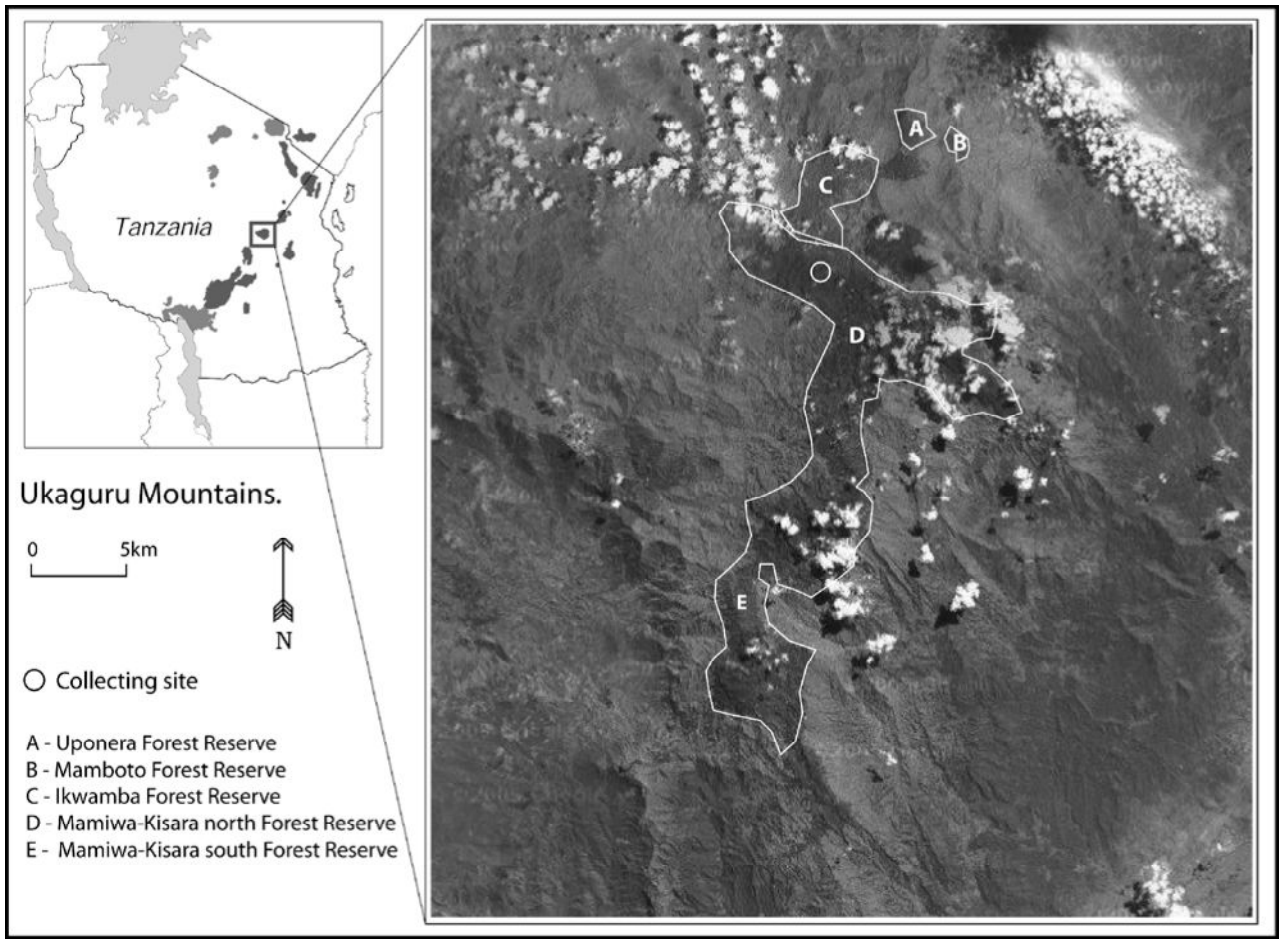


FIGURE 1. Location of the Ukaguru Mountains, forest reserves demarcation and collecting site.



FIGURE 2. *Nectophrynoides paulae*, male paratype MTSN5629 in life.

TABLE 1. Morphometric characters of *Nectophrynoides paulae* new species. For abbreviations see Material and Methods.

CODE	Sex	SUL	HL	HW	ND	FL	TL	ML	FOL
MTSN 5621	male	21.1	6.9	6.9	1.9	6.4	9.4	6.0	9.7
BM2005.1589	male	17.1	6.4	6.0	1.7	5.5	8.5	5.4	8.5
BM2005.1590	male	23.6	7.9	8.1	2.0	6.8	10.3	6.4	10.7
MTSN 5624	male	21.7	7.3	7.3	1.9	6.3	10.1	6.2	10.1
MTSN 5625	male	19.9	6.8	6.6	1.8	6.1	9.0	5.9	9.6
MTSN 5626	male	23.7	7.6	7.4	2.0	7.0	9.9	6.7	11.0
MTSN 5627	male	16.6	5.7	5.6	1.6	5.1	7.5	4.9	7.5
MTSN 5628	female	16.9	6.0	5.9	1.7	5.2	8.1	5.2	8.8
MTSN 5629	male	15.1	5.6	5.4	1.5	4.7	7.3	4.7	7.3
MTSN 5630	male	25.3	8.2	8.1	2.0	7.3	10.7	7.1	12.0

Comparison with all other described species of Nectophrynoides

Nectophrynoides paulae can be distinguished from:

N. tornieri by having the finger tips rounded, just slightly expanded and without truncated ends (as in *N. tornieri*), by the presence of several enlarged spines on dorsum, eyelids and limbs, the spines are symmetrical arranged on head and shoulders. Features of advertisement call consisting of a sequence of similar pulse groups, each of them composed of 3 to 5 pulses (see Figure 5. and cf. Menegon *et al.*, 2004, fig. 5 page 111, for call characteristics of *N. tornieri*).

N. asperginis, *N. laevis, N. pseudotornieri, N. frontierei* and *N. wendyae* by having a fully formed tympanum with evident annulus and by the presence of enlarged spines on dorsum and hindlimbs.

N. cryptus , *N. vestergaardi*, *N. poyntoni* and *N. laticeps* by the presence of enlarged spines on skin and the absence of parotoid glands.

N. minutus by having the feet longer than or equal to tibia (foot much shorter than tibia in *N. minutus*), and by the presence of enlarged spines on dorsum and hindlimbs.

N. viviparus by the absence of parotoid glands and by the absence of massive glands on the limbs, by the presence of enlarged spines on dorsum and hindlimbs and by its smaller size.

Description of the holotype. MTSN 5630, adult male. Distance from tip of snout to urostyle 25.3 mm, width of head at jaw articulation 8.1 mm, length of tibia 10.7 mm, length of foot 12.0 mm. Tympanum and tympanic annulus easily discernible. Parotoid glands absent. Snout short, nostrils much closer to the snout tip than eyes, prominent and visible ventrally, canthus rostralis concave. Eye pupil horizontal. No web on hands. Two phalanges of fifth toe free of main webbing, third and fourth toe free of main webbing on both sides, Tips of fingers and toes rounded, only slightly expanded but never truncated. The body skin is completely covered by blunt spines with several glandular humps surmounted by elogated clear spines on the whole body. Spines are enlarged on hind limbs, on the anterior part of the head, on the sacrum area and on the shoulder area. Some spines on head and shoulder area are symmetrically arranged. The tibia/foot ratio is 0.89 (Table 1).

Colour pattern in life: the dorsal surface of the body and limbs is uniform brown, a broad cream vertebral strip begins between the middle of the eyes running almost reaching the urostyle, positioned anteriorly. A light, subtriangular maxillary patch is present, dark edged along the canthus rostralis and toward the tympanum, small darker patches are present in the lower maxillary patch. There are a few sparse condensations of melanophores on limbs, giving a mottled impression. Venter and gular regions are paler with condensations of melanophores regularly scattered.

Variation in the paratypes series. Little variation is observed in the further eight specimens collected. In preserved specimens dorsal ground colour varies from uniform dark brown to light grey with a variable pat-

tern. Ventral colour pattern is relatively constant and similar to the holotype. Maxillary brown blotches on the side of the head are always present (cf. fig. 2;3), but tend to disappear in dark specimens (e. g. BM 2005.1589 and MTSN 5625 have a white stripe from eye to the lip). A pale mid dorsal vertebral line is often present, sometimes interrupted in some specimens. The tibia/foot ratio in the type series varies between 0.89 to 1.0 - mean 0.96 ± 0.04 (Table 1).



FIGURE 3. *Nectophrynoides paulae*, male paratype MTSN5624 in life.

Sexual dimorphism. With just one female and nine males collected only a very limited assessment of variation between the sexes was possible. Based on the external morphometric characters examined there appear to be no differences between males and females in body proportions. Females of *Nectophrynoides* species do tend to attain a larger body size (unpublished obs.) but given the small sample size here we are unable to evaluate this for *N. paulae*. Both sexes have prominent spines, one character which diagnoses this species.

Advertisement call. The male high frequency advertisement call is monophasic (Fig. 4). It consists of a sequence of similar pulse groups, each of them composed of 3 to 5 pulses. The group duration is about 0.6 sec. The pulse duration is about 27 msec (average 27.4 ± 2.1) with an inter-pulse duration of about 120 msec (average 121.4 ± 27). Each pulse has a very stable dominant frequency of about 3.1 kHz, with a third harmonic emphasized at about 9.4 kHz. Males were calling from leaves at about 0.5 – 1.5 m above forest floor after dusk.

Habitat and life history notes. The type series was collected in a wet valley with montane forest, dominated by the tree fern *Cyathea manniana,* at about 1800 m, (cf. Fig 5). All specimens were found on vegetation up to 1.30 m from soil during rainy evenings along a stream and close to standing water. In terms of microhabitat selection *N. paulae* was most often captured on small tree shrubs or bushes with males calling from exposed sites. The syntopic *N. laticeps* (Channing *et al.*, 2005) has never been found on bushes or trees but only on the forest floor within leaf litter. Perhaps these differences reflect some sort of niche separation. Further investigation of the ecology of these species will be necessary.

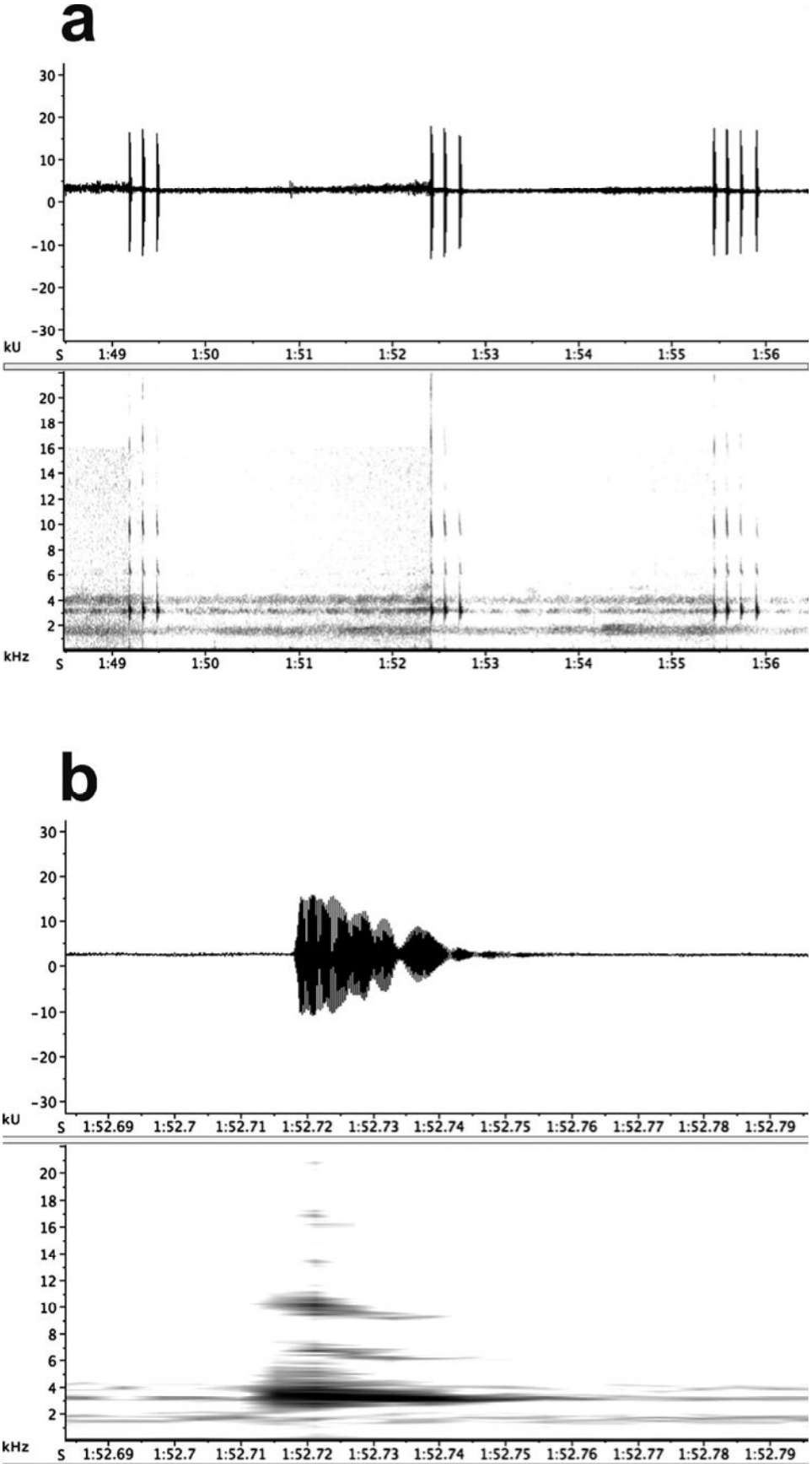


FIGURE 4. *Nectophrynoides paulae* advertisement call (MTSN 5626). (a) Three pulse groups with 3, 3, and 4 pulses each; (b), detail of one of the pulses. Legends is in minutes:seconds.

The presence of a small number of large developing eggs (about 20) in the dissected female suggests that the species is ovoviviparous.

The analysis of gastrointestinal tracts dissected from nine specimens yielded preliminary information on food habits (Table 2). Several small-sized nematodes, possibly commensals or parasitic, were found both in stomach and intestine and they were not considered as preys. Overall, 29 prey items were recognised, ants and coleopterans representing the bulk of the species diet, with spiders and mites also being present.

TABLE 2. Gut contents of *Nectophrynoides paulae* new species (sample size n = 9) obtained by dissection.

Taxa	Number of specimens	Number of stomachs
Aracnida		
Acarina	3	2 (22%)
Araneidae	2	2 (22%)
Insecta		
Hymenoptera		
Formicidae	13	6 (67%)
Coleoptera	11	6 (67%)
Nematoda	14	6 (67%)

Conservation status. We considered the conservation status of *N. paulae* according to the IUCN guidelines (IUCN, 2001). Furthermore, details outlined in this paper on the habitat of the new species, biology and its distribution have been forwarded to the ongoing Global Amphibian Assessment (Stuart *et al.* 2004). Based on current knowledge of the species distribution and habitat preference, the maximal area of occupancy for *N. paulae* must be equal or less than 172 km^2, an area that comprises the maximal extent of the forest reserves of the Ukaguru Mountains (comprising the contiguous Mamiwa-Kisara North and South Forest Reserves, Ikwamba Forest Reserve, Mamboto Forest Reserve and Uponera Forest Reserve- see Fig. 3; see also Burgess, et al. 2007 for forest area estimates). As a preliminary assessment for *N. paulae*, according to red list categories based on the criterion of a geographic range less than 5,000 km^2, the species qualifies as being Endangered. Further studies will be necessary to confirm its presence throughout the Ukaguru Mountains. If studies show that the species is in fact confined to just part of the Mamiwa-Kisara FR, equalling less than 100km^2 area of occupancy, then the conservation status will need to be re-evaluated. Furthermore, if the area undergoes any change such as accelerated habitat destruction, or evidence is presented showing the species population is small, or declining, then the status will need to be elevated to the Critically Endangered category. It is worrying to note that this species was not recorded during previous surveys in the area and in nearby forest patches, perhaps a result of its low population density. Monitoring of the amphibian fauna of the Ukaguru Mountains now seems be a priority, also given the presence of other endemic species and genera (Channing & Stanley 2003; Channing et al., 2005; Loader et al., 2006).

Discussion

The biological richness of the Eastern Arc Mountains forest habitats has been well-documented, and impressively high concentrations of endemic species of plants and animals are known from this relatively small area of tropical rainforest (as summarised in Poynton *et al.* 2007; Burgess *et al.*, 2007). For amphibians, until fairly recently this diversity appeared to be unevenly distributed, with high species diversity found in just a few mountain blocks, principally East Usambara, Uluguru, and Udzungwa. However, with only a few mountains sufficiently explored to allow any quantitative assessment of diversity (Poynton *et al.*, 2007), sampling ine-

qualities among mountains along the arc chain could explain the skewed diversity, Sampling differences between mountains have yet to be fully tested as an explanation of differences, and evidence from other groups seems to suggest that some mountain blocks might hold disproportionate amounts of species diversity (Burgess *et al.*, 2007).



FIGURE 5. Tree ferns (*Cyathaea manniana*) at the type locality of *Nectophrynoides paulae.*

Recent amphibian surveys have focused on the more poorly explored areas of the Eastern Arc, and based on preliminary findings these studies seem to indicate impressive levels of endemism and diversity (M. Menegon & S.P. Loader unpubl. data). In the case of the Ukagurus, despite only a few, short surveys (Akker & Highstead, 1992; Menegon *et al.*, 2004), some highly significant discoveries have been reported. One endemic amphibian genus, the toad *Churamiti* has been described (Channing & Stanley, 2003). This is the only known endemic amphibian genus restricted to a single mountain block in the Eastern Arc, and furthermore comprises one of only 14 newly collected amphibian species worldwide that have been described as new genera over the last ~30 years (Glaw *et al.,* 2006). A further two endemic species have been described, the forest toad *Nectophrynoides laticeps* Channing *et al.*, 2005 and the semi-fossorial brevicipitid *Probreviceps durirostris* Loader *et al.*, 2006. From collections held in the museum in London, UK and Trento, Italy we are also aware of other undescribed amphibian species, similarly restricted to high elevation forest habitats in the Ukagurus (M. Menegon and S.P. Loader, unpubl. data).

The high level of endemism in amphibians seems to be repeated throughout the Eastern Arc chain, with many undescribed species awaiting description. With improved techniques in species identification (e.g. molecular data) allowing cryptic species to be detected, and better sampling throughout the Eastern Arc range each mountain block within the Eastern Arc will likely become home to its own endemic fauna. With species descriptions expected to increase, the Eastern Arc chains importance as a repository for amphibian diversity will rise to an almost unprecedented level for the continent of Africa, comparable to Cameroon (LeBreton, 1999). Conservation of the Eastern Arc Mountains is therefore a priority if we are to successfully protect a significant proportion of African amphibian species.

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Appendix. List of comparative specimens examined.

Abbreviations of institution and field tags are as follow: MTSN= Museo Tridentino di Scienze Naturali; BMNH = British Museum of Natural History, London; KMH = Kim Howell field tags; MCZA = Museum of Comparative Zoology, Harvard Usa; RO = field tags of the Zoological Museum of University of Copenhagen; UDSM = University of Dar es Salaam ; WTS = William Stanley field tags.

Nectophrynoides asperginis Poynton, Howell, Clarke & Lovett, 1998
UDSM 7798, UDSM 2285, UDSM 2296, UDSM 2305 from Kihansi River Gorge, Udzungwa Mountains.

Nectophrynoides cryptus Perret, 1971
BMNH 1992-273 Bondwa peak, Uluguru Mountains. MCZ A-12470, MCZ A-12471, MCZ A-84830 from Nyingwa, Uluguru Mountains.

Nectophrynoides minutus Perret, 1972
KMH 21465, KMH 21468, KMH 21471, KMH 21472, KMH 21474, KMH 21479, from Uluguru Mountains, KMH 25059, KMH 25495 from Ukwiva, Rubeho Mountains, KMH 25523 from Mangalisa, Rubeho Mountains.

Nectophrynoides tornieri (Roux, 1906)
5429, MTSN 5430, MTSN 5431, MTSN 5432, MTSN 5433, MTSN 5434, MTSN 5435 from Udzungwa Scarp Forest Reserve. KMH 21457, KMH 21493, KMH 25045, KMH 25046 from Ruvu Forest Reserve, Uluguru Mountains.

Nectophrynoides wendyae Clarke, 1988
MTSN 5642, MTSN 5644, MTSN 5647

Nectophrynoides poyntoni Menegon, Salvidio & Loader, 2004
MTSN 5075, MTSN 5076, MTSN 5080

Nectophrynoides viviparus (Tornier, 1905)
MTSN 5339, MTSN 5340, MTSN 5341, MTSN 5341, MTSN 5255, MTSN 5256, from Kiolela Forest, Udzungwa Mountains. MTSN 5248, MTSN 5249, MTSN 5250, MTSN 5251, MTSN 5254 from Udzungwa Scarp Forest Reserve, Udzungwa Mountains.

Nectophrynoides laticeps Channing, Menegon, Salvidio & Akker, 2005
MTSN 5635, MTSN 5637, MTSN 5641 from Mamiwa-Kisara Forest Reserve, Ukaguru Mountains. WTS 4209, WTS 4213 from Ukaguru Mountains

Nectophrynoides pseudotornieri Menegon, Salvidio & Loader, 2004
RO 2020, RO 2157, RO 2143