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Three new species of *Callulina* (Amphibia: Anura: Brevicipitidae) highlight local endemism and conservation plight of Africa's Eastern Arc forests

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Material ascribed to the genus *Callulina* from north-east Tanzania and south-east Kenya is assessed. Three new species of *Callulina* are described from the North (***Callulina laphami* sp. nov.**) and South (***Callulina shengena* sp. nov.** and ***Callulina stanleyi* sp. nov.**) Pare Mountains in Tanzania. The species are diagnosed based on morphological, acoustic, and molecular data. A new key to the species of *Callulina* is provided. Based on an interpretation of the International Union for Conservation of Nature (IUCN) red list, we suggest that the three species will qualify as critically endangered, because of their small distributions and the ongoing threat to their habitat. We reveal the high local endemism of *Callulina* in the northern part of the Eastern Arc Mountains, with each species restricted to no more than one mountain (fragment) block. This high local endemism in *Callulina* is probably widespread across the Eastern Arc, raising further conservation concern for this group of amphibians. Based on new molecular phylogenetic data for *Callulina*, we discuss biogeographical relationships among north-east Tanzanian mountains, and evolutionary patterns in Eastern Arc breviciptids.

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ADDITIONAL KEYWORDS: Eastern Arc Mountains – Kenya – Pares – *Probreviceps* – Shimba Hills – Tanzania – tympanum loss – Usambara.

INTRODUCTION

Callulina species are highly distinctive brevicipitid frogs, with granular warty skin, truncated toe and finger tips, and which are often found climbing on vegetation (e.g. de Sá, Loader & Channing, 2004; Loader *et al.*, 2009) in the moist forests of the Eastern Arc Mountains of Tanzania and Kenya. This distinctive genus contrasts with all other breviciptids, which have rounded fingers and toes, and are generally found in leaf litter, under logs/stones, or in soil. For over 90 years *Callulina* contained a single species, *Callulina kreffti* Nieden 1911. Since Nieden's (1911) original description of *C. kreffti* from East Usambara, distribution records for the species progressively extended to other isolated forests in the Eastern Arc Mountains, including West Usambara (Philipshof), Uluguru (Barbour & Loveridge, 1928), Nguru (Emmrich, 1994), Taita Hills, and Udzungwa (Howell, 1993) (Fig. 1). Further additions to this distribution were made during the 2004 Global Amphibian

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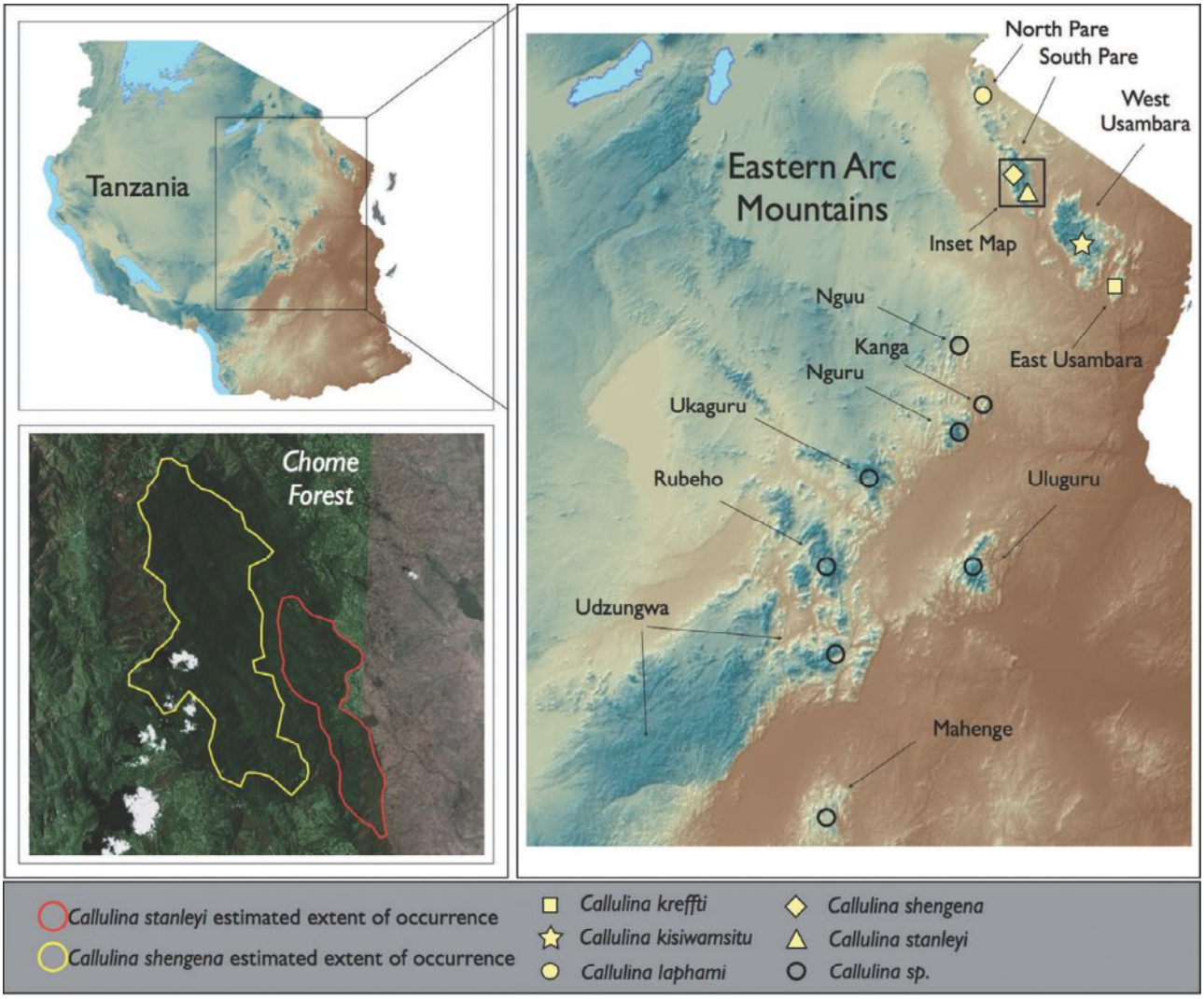


Figure 1. Map of the Eastern Arc Mountains of Tanzania showing the distribution of *Callulina*. Inset map (bottom left) showing the distribution of South Pare species ***Callulina stanleyi* sp. nov.** (elevational range of 1200–1600 m), and ***Callulina shengena* sp. nov.** (elevational range of 1920–2100 m).

Assessment, namely the montane forests of Ukaguru, Rubeho, and Nguu (IUCN, 2009). Recent taxonomic work has revealed that some of these isolated populations represent distinct species, and two have been formally described: the West Usambara *Callulina kisiwamsitu* de Sá *et al.*, 2004 and Taita Hills *Callulina dawida* Loader *et al.*, 2009. Three additional forms from a single mountain block in the Nguru are thought to be distinct new species (Menegon, Doggart & Owen, 2008), but overall the taxonomic affinity of populations of *Callulina* outside of the East Usambara (*C. kreffti*), West Usambara (*C. kisiwamsitu*), and Taita Hills (*C. dawida*) remains unclear.

Forty-seven specimens of *Callulina* of uncertain taxonomic status from north-east Tanzania (excluding material from West and East Usambara) and south-east Kenya are known in herpetological collections in the UK, Italy, and USA. Forty-six of these specimens are from the Pare Mountains of Tanzania, with a single specimen from Kwale in Kenya. These collections have accumulated sporadically over the last 50 years. Ronalda Keith (RK) collected a single specimen in November 1961 from Kwale in Kenya, from where no subsequent collections have been made. The first of these specimens to be collected from Tanzania were collected simultaneously in July 1993 by Nobert Corderio (NC) in the North Pare, and by Bill Stanley (WTS), Steve Goodman (SMG), and Charles Msuya (CM) in the South Pare. One year later, WTS collected a further six specimens at a higher altitude in the South Pare. Another collection of amphibians returned a single *Callulina* specimen from the South

Pare from Malcolm Coe's (MC) broader surveys of northern Tanzania in 1996. More recent surveys have been conducted in the Pare Mountains by the authors of this paper in 2002 and 2007–8. This consisted of a 3-day survey in the North Pare by David J. Gower, Simon P. Loader, and Wilirik Ngalason (DJG, SPL, and WN) in May 2002, followed by more substantial field surveys by Michele Menegon (MM) in the North Pare (October and November 2005) and South Pare (March 2008).

The aim of this paper is to report our taxonomic resolution of 47 specimens of *Callulina* from north-east Tanzania based on morphology (internal characters and external morphometrics), acoustic data (where collected), and mitochondrial DNA (mtDNA) sequences. Three new species are described, and a new key to the genus is provided. The biogeographic, evolutionary and conservation implications of our findings are discussed.

MATERIAL AND METHODS

Material was examined in the following institutions: The Natural History Museum, London (BMNH); Museum of Comparative Zoology, Harvard (MCZ); Field Museum, Chicago (FMNH); American Museum of Natural History (AMNH); California Academy of Sciences (CAS); and Museo Tridentino di Scienze Naturali, Trento (MTSN). The collectors' institutional affiliations are: RK (AMNH), WTS and SMG (FMNH), SPL and DJG (BMNH), WN and CM (University of Dar es Salaam), and MM (MTSN). Specimens collected from our fieldwork in the Pare Mountains were fixed in either 95% ethanol or 5% formalin, and were subsequently stored in 70% ethanol. Samples of muscle and/or liver were taken from representative individuals and preserved in 95% ethanol; these specimens are listed in Appendix.

DNA extraction, amplification, and sequencing follows the methodology of Loader *et al.* (2006, 2009). Sequences were generated for parts of the mitochondrial (mt) genes *12S*, *16S*, and cytochrome *b* (cyt*b*). GenBank accession numbers are given in Appendix. An alignment of concatenated partial *12S*, *16S*, and cyt*b* sequences was assembled, based mostly on both newly generated data and that published in Loader *et al.* (2009). Firstly, sequences were aligned in ClustalX using default parameters, then sequences were manually adjusted for obvious errors, and ambiguously aligned sites were removed. Parsimony, maximum likelihood, and Bayesian analyses were performed with PAUP*4b6 (Swofford, 1998) and MrBayes 3.1 (Huelsenbeck & Ronquist, 2001). For maximum likelihood and Bayesian analyses we used a best-fit model as determined by Modeltest 3.04 (Posada & Crandall, 1998) using the Akaike criterion. Likelihood searches were heuristic, with ten random-addition sequence replicates and tree bisection recombination branch swapping for likelihood analyses and 100 random-addition sequence replicates for maximum parsimony. MrBayes analyses were run with 2 000 000 generations, trees were sampled every 1000 generations, and the first 1000 trees were discarded. Support for clades was quantified with bootstrap proportions (Felsenstein, 1985) based on 1000 pseudoreplicates for likelihood and 10 000 for parsimony, Bayesian posterior probabilities, and Bremer support (Bremer, 1988). To test whether optimal trees significantly differ from suboptimal resolutions we employed Kishino–Hasegawa (Kishino & Hasegawa, 1989) and Templeton (Templeton, 1983) tests.

Some specimens (numbers given in species accounts below) were cleared and double-stained for bone and cartilage using a modified technique from Taylor & Van Dyke (1985). Radiographs were produced for some specimens to undertake broader checks of characters observed in cleared and stained specimens. Calls were recorded in the field using a Marantz model PMD-430 stereo cassette tape recorder and a KE66 Sennheiser directional microphone. The calls were analysed using the software package Raven (Charif, Clark & Fristrup, 2004).

Measurements were taken to the nearest 0.1 mm using digital calipers. Following Loader *et al.* (2009), the measurements taken are: ED, horizontal eye diameter; ETD, eye–tympanum distance; HL, upper arm length; HW, head width at level of jaw articulation; IN, internarial distance; IOD, interorbital distance; LF3, length of finger 3, measured from the distal edge of the basal subarticular tubercle; LT4, length of toe 4, measured from the proximal edge of the basal subarticular tubercle; NED, nostril–eye distance; NLD, nostril–lip distance; SUL, snout–urostyle length; TD, horizontal tympanum diameter; TL, tibiofibula length; TSL, length of tarsus; WDF3, width of disc of finger 3; WDTF3, width of finger 3 at level of distal subarticular tubercle. Summary statistics are provided in Table 1. Multivariate analyses were conducted using Statistica (2003). The new data were also compared with previous *Callulina* morphometric datasets (de Sá *et al.*, 2004; Loader *et al.*, 2009). Specimens examined and locality data are provided in each species account.

SYSTEMATICS

CALLULINA LAPHAMI SP. NOV.

(FIGS 1, 2, 5A, 6–8; TABLES 1, 2)

Holotype: BMNH 2002.37, an adult (gravid) female, collected in Kindoroko Forest Reserve, North Pare Mountains, Kilimanjaro Region, Tanzania

Table 1. Morphometrics of the three new species

	***Callulina laphami* sp. nov.** ($N = 21$)			***Callulina shengena* sp. nov.** ($N = 12$)			***Callulina stanleyi* sp. nov.** ($N = 11$)		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max	Mean
ED	1.6	4.5	3.8	2.6	4.9	3.8	1.6	4.6	3.1
ETD	N/A	N/A	N/A	N/A	N/A	N/A	0.7	2.8	1.4
HL	4.0	13.9	8.3	6.6	13.5	10.0	3.7	12.3	8.1
HW	4.1	16.3	9.5	6.5	16.2	10.9	5.0	13.5	8.9
IN	1.1	3.2	2.8	1.6	2.8	2.2	1.2	2.9	1.9
IOD	3.0	7.0	5.5	3.7	7.0	5.6	3.3	7.2	5.1
LF3	1.8	5.9	4.2	2.6	5.7	4.3	1.6	5.4	3.3
LT4	2.5	7.8	5.4	3.7	7.7	5.9	2.1	6.6	4.2
NED	1.4	3.4	3.1	1.8	3.2	2.6	1.4	3.1	2.2
NLD	0.7	2.0	1.2	1.1	2.0	1.6	0.8	1.8	1.2
SUL	14.2	45.4	26.1	22.1	43.2	33.4	14.0	42.5	26.4
TD	N/A	N/A	N/A	N/A	N/A	N/A	0.6	1.9	1.3
TL	4.8	15.1	9.7	8.0	15.7	11.9	4.6	14.8	9.5
TSL	4.0	11.8	7.4	5.8	12.0	8.9	3.4	10.8	6.9
WDF3	0.5	1.2	0.8	0.5	1.3	1.0	0.6	1.6	1.0
WDTF3	0.5	1.2	0.8	0.5	1.2	1.0	0.5	1.3	0.8

All measurements are given in mm. See Material and methods for a definition of the abbreviations.

(03°43′43.5″S, 37°39′16.1″E) by DJG, SPL, and WN on 10 May 2002 (see Fig. 1). This specimen has been sequenced for *12S*, *16S*, and cyt*b*. Specimen in good condition, with midventral incision into coelom, and incision around tympanic region on left and right.

Paratypes: Seventeen specimens (where determined, sex is listed): BMNH 2002.38, male, cleared and stained; BMNH 2002.39, female; BMNH 2002.40, sequenced for *12S*, *16S*, and cyt*b*; BMNH 2002.41, male; BMNH 2002.44, collected at same time and place as holotype; BMNH 2005.951, collected by NC, Kindoroko Forest Reserve, North Pare Mountains, July 1993; nine specimens collected by MM at Kindoroko Forest Reserve, North Pare Mountains, MTSN 8609, 8611, 8611 (male), 8612 (female), 8613, 8614, 8621 (female), 8622 (female), and 8632 (female); two specimens collected on 12 July 1993 by NC, Kindoroko Forest Reserve, CAS 225134 and 225135.

Referred material: Four specimens collected by MM from Minja Forest Reserve, North Pare Mountains (03°44′55.96″S, 37°38′47.09″E), October 2005: MTSN 8640, 8641, 8648 (male), and 7123.

Diagnosis: A large, stout, and robust *Callulina*. Snout–urostyle distance reaching 45.4 mm. Snout–urostyle : tibia ratio 33–37%. Tympanum absent. Toe and finger tips truncate. Dark-brown dorsally, pale-brown ventrally. Interocular band, often red, sometimes green, darkened in preservative. Subarticular tubercles of hands and feet prominent. Arms and legs show weak development of continuous glandular ridge.

Callulina laphami sp. nov. differ from *C. kreffti*, *C. kisiwamsitu*, and *Callulina stanleyi* sp. nov. in the absence of a tympanum, smoother, less granular skin, truncate finger and toe tips, and presence of bright colour in the ocular region. *Callulina laphami* sp. nov. and *C. dawida* are similar in overall appearance: both have interocular coloration, but *C. dawida* has a tympanum, albeit sometimes obscured (Loader *et al.*, 2009). *Callulina laphami* sp. nov. and *Callulina shengena* sp. nov. are most similar in external appearance, but the latter lacks prominent glands on the arms and legs. The distinctiveness of *C. laphami* sp. nov. from other *Callulina* is also supported by call, distribution, and DNA sequence data.

Description of holotype: Body robust and stout. Tips of fingers truncate (equal to width of distal subarticular tubercle), rounded edges with lateral circummarginal grooves; first finger shortest, second and fourth finger equal, third finger longest. Inner metatarsal tubercle large, rounded, and raised, separated by a middle palmar tubercle from an even larger, rounded outer metatarsal tubercle, which is raised and elongated along the margin of the hand. Smaller palmar tubercles present. Subarticular tubercles at the base



Figure 2. Dorsal, ventral, and laterial views, and ventral aspect, of hands and feet of the holotype of ***Callulina laphami* sp. nov.** BMNH 2002.37.

of each finger, large subarticular tubercles on third and fourth finger at the phalangeal joints. Third finger with two small tubercles between basal articular tubercle and subarticular tubercle. Truncate and dorsoventrally swollen toe tips without any lamellae on the ventral surface; tips of toes not expanded laterally, with circummarginal grooves; First toe same length as second. Third and fifth toes equal, fourth toe longest. Inner metatarsal tubercle large, rounded, and raised, touching a smaller, rounded, raised, outer metatarsal tubercle. Palmar tubercles present on base of foot. Subarticular tubercles at the base of each toe, large subarticular tubercles on third and fourth toe at the phalangeal joints. All tubercles on hands and feet bluish grey against a brown–grey background.

Table 2. Pairwise distances (p distance) for *12S*, *16S* and cytochrome *b* sequence data

	Mean	SD	Min.	Max.	*N*
Phrynomantis vs. *Breviceps*	0.188	0.000	0.188	0.188	2
Phrynomantis vs. *Spelaeophryne*	0.206	0.000	0.206	0.206	2
Phrynomantis vs. *Probreviceps*	0.195	0.000	0.195	0.195	2
Phrynomantis vs. *Callulina kreffti*	0.188	0.000	0.188	0.188	2
Phrynomantis vs. *Callulina kisiwamsitu*	0.189	0.001	0.188	0.189	3
Phrynomantis vs. *Callulina dawida*	0.186	0.003	0.184	0.188	3
Phrynomantis vs. ***Callulina laphami* sp. nov.**	0.196	0.000	0.196	0.196	3
Phrynomantis vs. ***Callulina stanleyi* sp. nov.**	0.189	0.000	0.189	0.189	2
Phrynomantis vs. ***Callulina shengena* sp. nov.**	0.182	0.001	0.181	0.183	3
Breviceps vs. *Spelaeophryne*	0.212	0.000	0.212	0.212	2
Breviceps vs. *Probreviceps*	0.179	0.000	0.179	0.179	2
Breviceps vs. *Callulina kreffti*	0.185	0.000	0.185	0.185	2
Breviceps vs. *Callulina kisiwamsitu*	0.184	0.001	0.183	0.185	3
Breviceps vs. *Callulina dawida*	0.183	0.001	0.183	0.184	3
Breviceps vs. ***Callulina laphami* sp. nov.**	0.188	0.000	0.188	0.188	3
Breviceps vs. ***Callulina stanleyi* sp. nov.**	0.186	0.000	0.186	0.186	2
Breviceps vs. ***Callulina shengena* sp. nov.**	0.189	0.001	0.189	0.190	3
Spelaeophryne vs. *Probreviceps*	0.176	0.000	0.176	0.176	2
Spelaeophryne vs. *Callulina kreffti*	0.180	0.000	0.180	0.180	2
Spelaeophryne vs. *Callulina kisiwamsitu*	0.196	0.000	0.196	0.196	3
Spelaeophryne vs. *Callulina dawida*	0.186	0.002	0.185	0.188	3
Spelaeophryne vs. ***Callulina laphami* sp. nov.**	0.187	0.000	0.187	0.187	3
Spelaeophryne vs. ***Callulina stanleyi* sp. nov.**	0.192	0.000	0.192	0.192	2
Spelaeophryne vs. ***Callulina shengena* sp. nov.**	0.182	0.002	0.181	0.183	3
Probreviceps vs. *Callulina kreffti*	0.148	0.000	0.148	0.148	2
Probreviceps vs. *Callulina kisiwamsitu*	0.150	0.001	0.149	0.150	3
Probreviceps vs. *Callulina dawida*	0.149	0.004	0.146	0.152	3
Probreviceps vs. ***Callulina laphami* sp. nov.**	0.143	0.000	0.143	0.143	3
Probreviceps vs. ***Callulina stanleyi* sp. nov.**	0.149	0.000	0.149	0.149	2
Probreviceps vs. ***Callulina shengena* sp. nov.**	0.143	0.002	0.141	0.144	3
Callulina kreffti vs. *Callulina kisiwamsitu*	0.068	0.001	0.068	0.069	3
Callulina kreffti vs. *Callulina dawida*	0.086	0.007	0.081	0.091	3
Callulina kreffti vs. ***Callulina laphami* sp. nov.**	0.092	0.000	0.092	0.092	3
Callulina kreffti vs. ***Callulina stanleyi* sp. nov.**	0.081	0.000	0.081	0.081	2
Callulina kreffti vs. ***Callulina shengena* sp. nov.**	0.092	0.002	0.090	0.093	3
Callulina kisiwamsitu vs. *Callulina dawida*	0.077	0.004	0.073	0.082	4
Callulina kisiwamsitu vs. ***Callulina laphami* sp. nov.**	0.095	0.002	0.094	0.096	4
Callulina kisiwamsitu vs. ***Callulina stanleyi* sp. nov.**	0.042	0.000	0.042	0.042	3
Callulina kisiwamsitu vs. ***Callulina shengena* sp. nov.**	0.099	0.002	0.097	0.102	4
Callulina dawida vs. ***Callulina laphami* sp. nov.**	0.089	0.004	0.086	0.093	4
Callulina dawida vs. ***Callulina stanleyi* sp. nov.**	0.082	0.009	0.076	0.088	3
Callulina dawida vs. ***Callulina shengena* sp. nov.**	0.092	0.006	0.086	0.098	4
***Callulina laphami* sp. nov.** vs. ***Callulina stanleyi* sp. nov.**	0.098	0.000	0.098	0.098	3
***Callulina laphami* sp. nov.** vs. ***Callulina shengena* sp. nov.**	0.080	0.001	0.079	0.080	4
***Callulina stanleyi* sp. nov.** vs. ***Callulina shengena* sp. nov.**	0.101	0.002	0.099	0.102	3
Callulina kisiwamsitu (within)	0.004	0.000	0.004	0.004	2
Callulina dawida (within)	0.015	0.000	0.015	0.015	2
***Callulina laphami* sp. nov.** (within)	0.000	0.000	0.000	0.000	2
***Callulina shengena* sp. nov.** (within)	0.003	0.000	0.003	0.003	2

Mean, standard deviation (SD), minimum (min.), maximum (max.), and number of samples (*N*).

Dorsolateral and dorsoventral aspects of arms and legs have a raised glandular area; on the arms slightly raised glandular mass from the wrist to the elbow joint on dorsal and dorsolateral surface; slightly raised glandular mass on legs from one part from knee joint to the tibiotarsal joint on the dorsal, lateral, and ventral surfaces, and on the tibia to the tibiotarsal joint to the base of the foot on the dorsal, lateral, and ventral surfaces to the foot.

Dorsum dark brown with a bright-red interocular bar that has faded to dull orange in preservative. This band connects each eyelid, marking precisely their anterior and posterior edges. Relatively smooth dorsal body surface, only slightly granular, with granular glandular masses on lateral and ventral sides, and posterior end, around the thighs and urostyle region. These glandular masses marked by slight pale-cream colour. Ventral surface pale brown. Flanks darker brown with yellowish grey mottling.

Tympanic region greyish brown. A door-like incision on surface of skin around tympanic region reveals underlying musculature and absence of any tympanum or vestigial elements. Weakly defined but darkly edged tympanic region. Loreal and canthal regions pale grey. Nostrils, snout tip, and jaw angle slightly darker grey. Snout visible from ventral view.

Measures of holotype (all measurements are given in mm): ED = 3.5; ETD = n/a; HL = 13.9; HW = 12.1; IN = 2.9; IOD = 6.7; LF3 = 5.1; LT4 = 7.7; NED = 3.1; NLD = 1.5; SUL = 45.4; TD = n/a; TL = 15.1; TSL = 11.8; WDF3 = 1.0; WDTF3 = 1.0.

Measures of male paratype (BMNH 2002.41): ED = 2.6; ETD = n/a; HL = 8.7; HW = 8.7; IN = 2.0; IOD = 4.8; LF3 = 3.8; LT4 = 4.7; NED = 2.1; NLD = 1.1; SUL = 28.6; TD = n/a; TL = 9.7; TSL = 6.8; WDF3 = 0.8; WDTF3 = 0.8.

Morphological and colour variation: In general the morphological features outlined in the holotype description are present in all paratype material. All specimens show truncate toe and finger tips. T-shaped distal phalangeal bones are clearly seen in cleared and stained specimen and radiographs. Male paratype MTSN 8648 has a slightly more raised glandular mass on the legs, although not as distinctive as in *C. shengena* sp. nov. Females (33.5–45.4 mm) are larger than males (22.8–29.0 mm), otherwise no clear sexual differences are noted externally. Some specimens have a less clear interocular band, and this has become even more faded in preservation (e.g. MTSN 8648, 8613, and 8622). Interocular band green instead of red in life in BMNH 2002.41 (faded to pale orange/brown in preservative). In some juvenile specimens (e.g. MTSN 8640 and 8612) the dorsum is reddish.

Colour in life: Dark brown dorsally fading ventrally to a cream–yellowish hue. Flanks paler than dorsum, grading into ventral colour. Distinct interocular band marking the anterior and posterior edges of the eyelid, curving slightly posteromedially, generally bright red, sometimes green (Fig. 3A).

Advertisement call: Males were heard calling only during the night, from low bushes and branches of small trees. The call is quite a fast series of *c.* 10–14 trills (Fig. 5), produced at a rate of approximately five per second, with maximum energy at 1550 Hz. Each trill consists of a group of six or seven high-energy pulses, with a pulse-group duration of about 6 ms, and an intergroup duration of about 125 ms. Compared with other *Callulina* species, call activity was less constant and less frequent, with few calls heard during our fieldwork.

Natural history: Cordeiro notes that his specimens were found 'under damp rocks of valley side of small stream in deep forest'. SL, DG, and WN found large adults during the day under rocks or logs in mature forest, and one small subadult was found at night perched on a shrub 0.3 m above the ground. MM found adults and juveniles during night searches, perched on bushes and small tree branches, from 0.3 to 1.5 m above the forest floor, both along a wet stream valley and on drier ridges.

Distribution and threat: Callulina laphami sp. nov. was collected only in the Kindoroko and Minja montane forests, at elevations between 1730 m and 2000 m (Fig. 2A). Based on current knowledge of the species' distribution and evidences on habitat preference, the estimated extent of occurrence is less than or equal to 16.5 km^2: this includes Kindoroko and Minja forest reserves. This small extent is compounded with an observed decline in area and quality of the habitat (Hall *et al.*, 2009). Currently the population density does not appear to be low, but because of the highly fragmented habitat, conservation measures for protecting the species might be important.

Etymology: The species is named for Lewis H. Lapham, who has made generous donations towards conserving Tanzania's forests. The future survival of this frog and other Eastern Arc endemics will in part depend upon such patronage.

CALLULINA SHENGENA SP. NOV.

(FIGS 1, 3, 5B, 6–8, TABLE 1, 2)

Holotype: MTSN 9285, adult (gravid) female, collected at Chome Forest Reserve, South Pare Mountains (04°17′42.907″S, 37°56′18.612″E) by MM on 12 March



Figure 3. Dorsal, ventral, and laterial views, and ventral aspect, of hands and feet of the holotype of ***Callulina shengena* sp. nov.**, MTSN 9285.

2008. This specimen has been sequenced for *12S* and *16S*. Good condition, with midventral incision into coelom, and incision around left and right tympanic region.

Paratypes: Eleven specimens: BMNH 2008.466 (formerly MTSN 9284; female; cleared and stained and sequenced for *12S*, *16S*, and cyt*b*) and BMNH 2008.465 (formerly MTSN 9287; female), collected by MM, same data as for holotype; MTSN 9288 (male), 9289 (female), and 9290 (male), collected by MM at Chome Forest Reserve, South Pare Mountains (04°17′42.907″S, 37°56′18.612″E), at about 1930 m a.s.l., between 10 and 13 March 2008; FMNH 251849 (male), 251850 (male?), 251851 (male), and 251852 (male); FMNH 255881 (female) and FMNH

255882 (female), collected by WTS at 0.7 km N and 3 km E of Mhero (4°17′S, 37°55′40″E; 2000 m a.s.l.), Chome Forest Reserve, South Pare Mountains 19–26 August 1994. FMNH 255849, 255881, and 255882, and BMNH 2008.466 have been sequenced for *12S* and *16S*.

Diagnosis: A large *Callulina*, with snout–urostyle distance reaching 43.2 mm. Snout : tibia ratio 33–38%. Tympanum absent. Toe and finger tips truncate and not expanded. Subarticular tubercles of hands and feet prominent. Coloured interocular band, usually darkened in preservative, but pinkish/reddish in life. Continuous enlarged glands on arms and legs.

Callulina shengena sp. nov. differ from *C. kreffti*, *C. kisiwamsitu*, and *C. stanleyi* sp. nov. in lacking a tympanum, smooth, less granular skin, truncate finger and toe tips, and the presence of a bright colour in the ocular region. *Callulina shengena* sp. nov. and *C. dawida* are similar in overall appearance: both have interocular coloration, but the latter has a tympanum, albeit sometimes obscured (Loader *et al.*, 2009). Furthermore, *C. laphami* sp. nov. lacks the prominent arm/leg glands of *C. shengena* sp. nov. The distinctiveness of *C. shengena* sp. nov. from all other *Callulina* is also supported by distribution and DNA sequence data.

Description of holotype: Body robust and stout. Tips of fingers truncate, only very marginally expanded laterally (equivalent to width of distal subarticular tubercle), rounded edges, with lateral circummarginal grooves; first finger shortest, second and fourth equal, third longest. Inner metatarsal tubercle large, rounded, and raised, separated by a middle palmar tubercle from an even larger, rounded, raised, outer metatarsal tubercle. Smaller tubercles present on palm. Subarticular tubercles at the base of each finger, large subarticular tubercles on third and fourth finger at the phalangeal joints. Third finger with two smaller tubercles between basal articular tubercle and subarticular tubercles. Truncate, slightly dorsoventrally expanded toe tips without any lamellae on ventral surface; tips of toes not expanded laterally, with circummarginal grooves posterior to distal phalanx. First toe same length as second, third, and fifth equal, fourth longest. Inner metatarsal tubercle large, rounded, and raised, touching a smaller, rounded, raised, outer metatarsal tubercle. Smaller palmar tubercles present on base of foot. Subarticular tubercles at the base of each toe, large subarticular tubercles on third and fourth toe at the phalangeal joints. All tubercles on hands and feet bluish grey against a brown–grey background.

Dorsal, lateral, and ventral aspects of arms and legs with large, conspicuously coloured, continuous glands; on the arms continuous from the wrist to the elbow on dorsal and dorsolateral surfaces; glands on the legs bifurcate, one part from knee joint to the tibiotarsal joint on the dorsal, lateral, and ventral surfaces, the second part continuous from tibia to the tibiotarsal joint, to the base of the foot on the dorsal, lateral, and ventral surfaces of the foot, the gland attenuating on a diagonal angle on the dorsal surface of the foot ending at the base of the fifth toe.

In life, dorsum dark brown with a pinkish/reddish interocular bar, connected to a larger continuous, roughly diamond-shaped pink/reddish area extending over the anterior part of the dorsum, paler brown on shoulders to elbows. Relatively smooth dorsal surface, only slightly granular, with larger glandular masses on lateral sides, and posterior end, around the thighs and urostyle region. Large glandular mass marked by slightly paler cream. Ventral surface paler yellow to cream–tan. Lateral sides darker brown–grey, mottled. Right-hand side of mid ventrum with irregular grey patch; left-hand side of mid ventrum shows a conspicuously marked vein, directed diagonally posteriorly and laterally spanning out from mid area. Edges of chin slightly greyish.

Tympanic region greyish brown. Door-like incision on both left and right surfaces of skin around tympanic region reveals underlying musculature, and absence of any tympanum or vestigal elements. Weakly defined but darkly edged tympanic region. Loreal and canthal regions pale grey. Nostrils, snout tip, and jaw angle slightly darker grey. Snout visible from ventral view.

Measures of holotype (all measurements are given in mm): ED = 4.4; ETD = n/a; HL = 13.5; HW = 15.4; IN = 2.7; IOD = 6.6; LF3 = 5.6; LT4 = 7.5, NED = 3.0; NLD = 2.0; SUL = 42.7; TD = n/a; TL = 15.0; TSL = 11.9; WDF3 = 1.2; WDTF3 = 1.2.

Measures of male paratype (MTSN 9288): ED = 3.5; ETD = n/a; HL = 10.0; HW = 10.5; IN = 2.2; IOD = 5.3; LF3 = 4.4; LT4 = 5.6; NED = 2.4; NLD = 1.6; SUL = 32.0; TD = n/a; TL = 11.3; TSL = 8.4; WDF3 = 1.1; WDTF3 = 1.1.

Morphological and colour variation: In general the morphological features outlined in the holotype description occur in all paratypes, including similar body proportions (Table 1). Almost all specimens show truncate toe and finger tips. Large females MTSN 9287 and FMNH 255881-82 have marginally expanded tips (subarticular width 0.92 of distal phalanx width). T-shaped distal phalanges were observed in a cleared and stained specimen (MTSN 9284). Females (39.9–43.2 mm) larger than males (22.1–32.0 mm), otherwise no clear external sexual

differences. Larger females (holotype; FMNH 255881 and 255882) have more conspicuous glands on arms and legs. Colour in FMNH 255881 and 255882 is slightly faded and much paler brown. Smaller males (FMNH 251849 and 251852) show greater variation in colour, with some dark mottling (FMNH 251851) and less well-developed glands (FMNH 251850, 251852, and MTSN 9289), whereas FMNH 251849 more closely resembles the holotype. Male paratype MTSN 9288 much darker brown on dorsum, with distinct pale-brown dorsolateral stripe from otic region to thighs; metatarsal tubercles on hands more brownish; ventral surface much darker, with midventral region cream–brownish. In some specimens a pinkish red interorbital area in life is not extended posterior to the head region.

Colour in life: Callulina shengena sp. nov. has a quite variable dorsal body and limb coloration, with specimens generally different shades of salmon to pale (flesh) pink (see Fig. 5, lower right). One specimen, however, was brown with two broad yellow dorsolateral bands (see Fig. 5, lower left). Two specimens had a bright-orange interocular band between the eyelids. The interocular band between eyelids was not present in all specimens. The flanks are always paler (grey–white) than the dorsum. The ventral surface is dark cream, and dark brown on the lateral edges. Eyes are dark brown–red (see Fig. 5).

Advertisement call: No call was heard or recorded.

Natural history: The FMMN specimens were all collected in bucket pitfalls in the forest. MM found individuals at night perched on small understory branches, as well on bushes between 1 and 2.2 m above ground. One individual was sitting among the irregularities of the bark of a tree, at over 2 m. One male and two females were found together at about 1.5 m, sitting on small tree branches.

Distribution and threat: Callulina shengena sp. nov. has only been found in montane forest dominated by the forest tree *Ocotaea usambarensis* at elevations between 1920 m and 2100 m. Based on current knowledge of the species' distribution and evidence of forest dependancy, the estimated extent of occurrence of *C. shengena* sp. nov. is less than or equal to 35 km^2, and the known area of occupancy is about 3 km^2; these are, respectively, the area including the elevational distribution (1920–2100 m) of this species in the Chome forest reserve and the area included in the polygon obtained by linking the localities where the presence of the species was recorded. The species is common in the area in which it has been found, but is confined to the montane forest zone, where persistent and large-scale logging of *O. usambarensis* occurs. Extensive areas of the forest appear to be heavily logged, and considerable habitat damage ocurrs during this process. The presence of one population at only a single, small location is compounded by an observed decline in the area and quality of the habitat (Hall *et al.*, 2009).

Etymology: The species is named after the peak in the South Pare (Shengena) from where the type series was collected. The specific epithet is considered to be a noun in apposition.

CALLULINA STANLEYI SP. NOV.
(FIGS 1, 4, 5C, 6–8, TABLE 1, 2)

Holotype: BMNH 2000.207, adult (gravid) female, collected at Chome Forest Reserve, South Pare Mountains (04°09′60″S, 37°50′48″E) by MC on 18 April 1996. Found under a rotten log in forest. This specimen has been sequenced for *12S*, *16S*, and cyt*b*. Specimen in good condition, slightly desiccated in parts, with a cross-like midventral incision into the coelom.

Paratypes: Ten specimens: BMNH 2008.467 (male; sequenced for *12S* and *16S*) and 2008.468 (male; cleared and stained), formerly MTSN 7540 and 7543, respectively; FMNH 251381–251384, collected by WTS, SMG, and CM in a pitfall trap, 7 km south of Bombo (4°20′S, 38°00′E; 1100 m a.s.l.), Chome Forest Reserve, South Pare Mountains on 20–24 July 1993; MTSN 7541 (male), 7542 (female), 7544, and 7559 (female), collected at Chome Forest Reserve, South Pare Mountains (04°19′41.382″S, 37°59′44.262″E; *c.* 1230 m a.s.l.), by MM between 13 and 15 March 2008.

Diagnosis: A medium- to large-sized robust *Callulina*. Snout–urostyle distance reaching 42.5 mm, snout: tibia ratio 0.33–0.39. Tympanum present but sometimes obscured by granular skin. Finger tips usually expanded, with the ratio between the width at first subarticular and the width at distal phalanx 0.8–1.0. Subarticular tubercles of hands and feet prominent.

Callulina stanleyi sp. nov. differs from *C. kreffti* in having a less expanded third finger (distal width at third terminal phalanx/distal width at subarticular tubercle > 0.75). *Callulina stanleyi* sp. nov. differs from *C. dawida* in having expanded finger and toe tips. *Callulina stanleyi* sp. nov. differs from *C. laphami* sp. nov. and *C. shengena* sp. nov. in the presence of a tympanum, and expanded toe and finger tips. *Callulina stanleyi* sp. nov. is morphologically very similar to *C. kisiwamsitu*, although the two species can be distinguished on the basis of DNA sequence, call, and distribution data. Additionally, the



Figure 4. Dorsal, ventral, and laterial views, and ventral aspect, of hands and feet of the holotype of ***Callulina stanleyi* sp. nov.**, BMNH 2000.207.

following morphometric characters (against SUL) differ significantly ($P < 0.05$) in the two species, based on Student's *t*-test analyses: ED, IOD, HW, ND, NLD, TD, snout length (as measured from lower lip to apex of anterior dorsal margin of snout), and outer metatarsal tubercle of the hand (as measured along its longest axis). Many of the head character differences between *C. stanleyi* sp. nov. and *C. kisiwamsitu* indicate the larger, more robust head of *C. stanleyi* sp. nov.

Description of holotype: Body robust. Tips of fingers expanded, distal width of terminal phalanx 0.85 of the width of distal subarticular tubercle. Tips of



Figure 5. Colour in life of ***Callulina laphami* sp. nov.** (top left), ***Callulina shengena* sp. nov.** (bottom left and right), and ***Callulina stanleyi* sp. nov.** (top right).

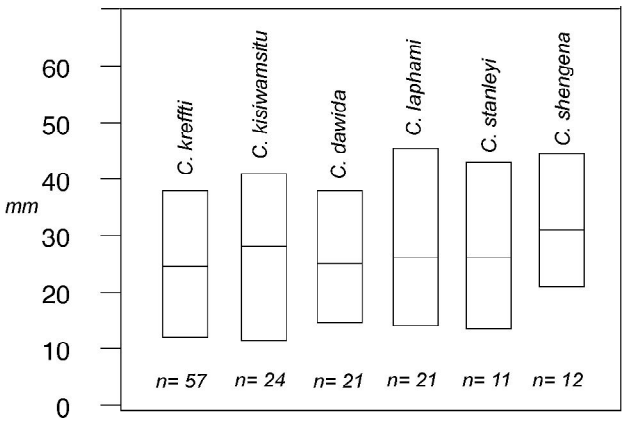


Figure 6. Snout–urostyle length in *Callulina*, showing mean, minimum, and maximum values.

fingers expanded laterally, with circummarginal grooves posterior to distal phalanx. First finger shortest, second and fourth equal, third longest. Inner metatarsal tubercle rounded and raised, separated by a middle palmar tubercle from an even larger, rounded, raised, outer metatarsal tubercle. Slightly smaller palmar tubercles present on palm. Subarticular tubercles at the base of each finger, large subarticular tubercles on third and fourth finger at the phalangeal joints. Third finger with smaller tubercles between basal articular tubercle and subarticular tubercles. Slightly dessicated but dorsoventrally expanded toe tips, with slightly folded but smooth ventral surfaces. Tips of toes slightly expanded laterally, with circummarginal grooves posterior to distal phalanx. Toe tips with smooth ventral surfaces. First toe same length as second, third and fifth equal, fourth longest. Inner metatarsal tubercle large, rounded, and raised, almost touching a similar sized, rounded, raised, outer metatarsal tubercle. Smaller palmar tubercles present on base of foot. Subarticular tubercles at the base of each toe, large subarticular tubercles on third and fourth toe at the phalangeal joints.

All tubercles on hands and feet bluish grey against brown–grey background. Dorsum brown with scattered, darker brown–black symmetric vermiculations. Skin granular, with slightly larger glandular masses on lateral sides, and posterior end around the thighs and urostyle region. Large glandular mass slightly paler brown. Ventral surface paler brown, cream–tan. Tympanic region pale brown–cream. Dark edged tympanic ridge from posterior edge of eyelid to arm insertion. Incision around tympanic region on right side. Loreal and canthal regions dark brown. Snout visible from ventral view.

Measures of holotype (all measurements are given in mm): ED = 3.0; ETD = 1.2; HL = 12.1; HW = 11.8; IN = 1.8; IOD = 7.2; LF3 = 4.7; LT4 = 6.6, NED = 2.2; NLD = 1.5; SUL = 42.5; TD = 1.3*; TL = 9.6; TSL = 10.8; WDF3 = 1.3; WDTF3 = 1.1. (*Difficult to discern, was dissected to clarify exact measurement.)

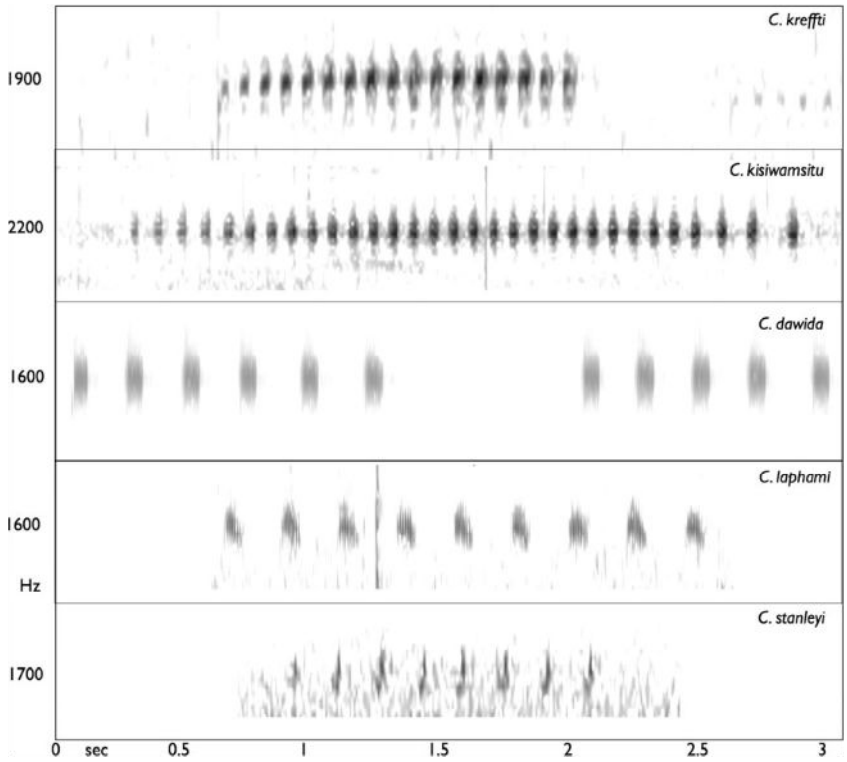


Figure 7. Sonograms of five of the six species of *Callulina*.

Measures of male paratype (MTSN 7541): ED = 4.6; ETD = 2.8; HL = 8.3; HW = 8.7; IN = 2.8; IOD = 4.4; LF3 = 3.6; LT4 = 4.7; NED = 3.1; NLD = 1.2; SUL = 24.6; TD = 1.7*; TL = 14.8; TSL = 6.9; WDF3 = 1.1; WDTF3 = 0.9. (*Difficult to discern, was dissected to clarify exact measurement.)

Morphological and colour variation: Morphological features of paratypes generally match the holotype. The proportion of TL to SUL is 0.33–0.39. The size and position of the tympana varies: TD/TL = 0.04–0.06; ETD/TL = 0.04–0.07. The ratio of the widths of the third distal phalanx to the subarticular tubercle is 0.77–1.0; most specimens are in the range 0.77–0.86. FMNH 251381 is a small (19.9 mm SUL) juvenile that is dessicated, with only marginally expanded finger tips (WDF3 = 0.6; WDTF3 = 0.6; ratio = 1), and FMNH 251384 is a small juvenile with expanded finger tips (WDF3 = 0.7; WDTF3 = 0.5; ratio = 0.71).

The juveniles FMNH 251381–251384 resemble the holotype in colour, but show different degrees of dark mottling on the dorsum, with FMNH 251382 most strongly mottled and FMNH 251383 the least mottled. The tympanum is obscured by granular skin in MTSN 7541, 7543, and 7544.

Colour in life: Generally brown, with irregular dark-brown marbling. Warts on the lateral surfaces of body clearly white. Ventral surface cream with brown marbling on edges. Eyes orange to red (Fig. 3C).

Advertisement call: Males were only calling during the night, from low bushes and branches of small trees. The call is a fast series of between seven and nine trills (Fig. 5), produced at a rate of approximately fve per second, with maximum energy at 1720 Hz. The quality of the few call recordings was not high enough to allow a detailed description of the train of pulses forming the trill. Pulse-group duration was about 25 ms, and intergroup duration was about 116 ms.

Natural history: The holotype was collected in a rotten log in the forest. The FMNH specimens were all collected in bucket pitfalls in the forest. Specimens were found on low bushes during the night, both in dense forest and along a forest road.

Distribution and threat: Callulina stanleyi sp. nov. was found only in submontane forest at elevations between 1200 and 1300 m. Based on current knowledge of the species' distribution and evidence of

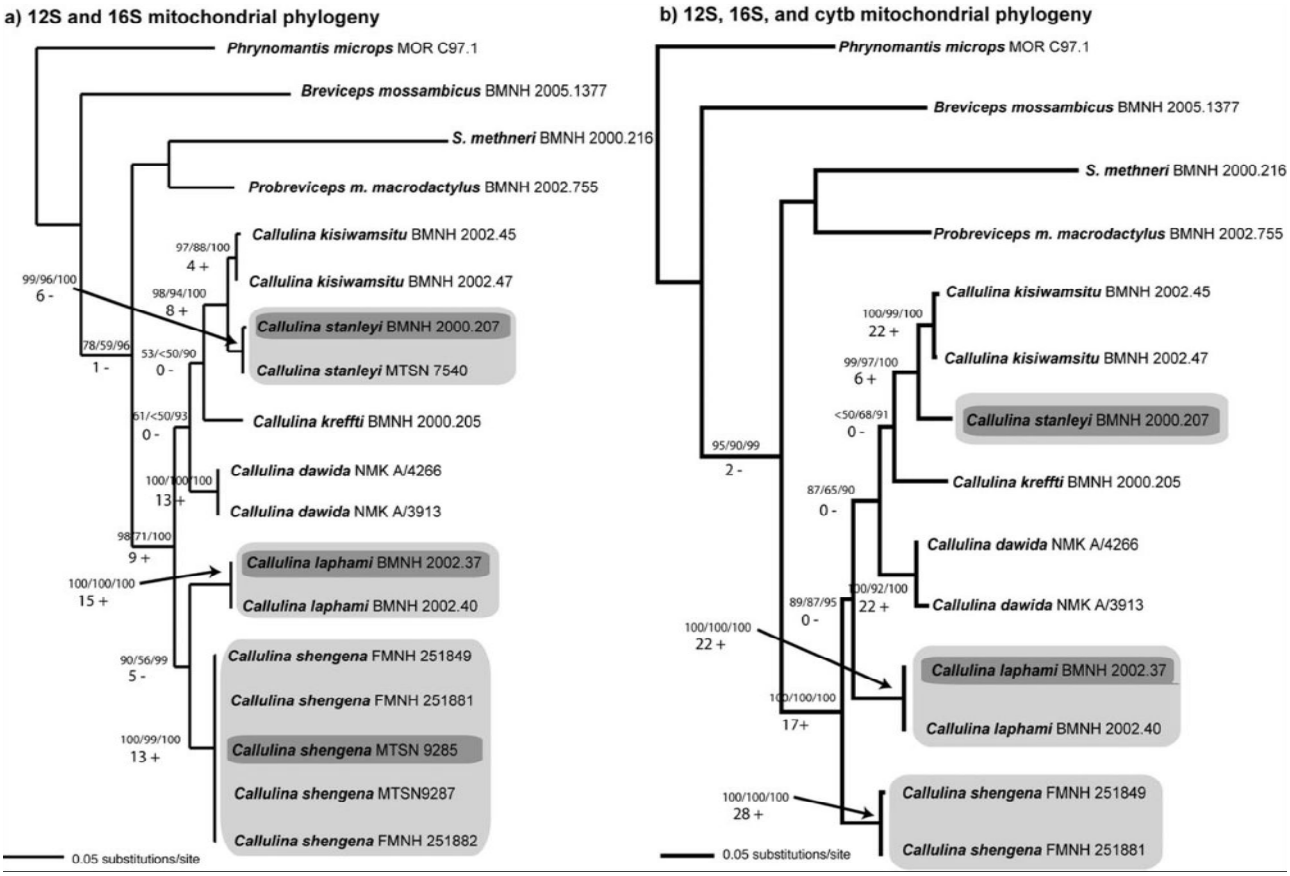


Figure 8. Maximum-likelihood phylogenies of *Callulina*, with new species marked in light-grey boxes, and with holotypes marked in dark grey. Letters above branches refer to bootstrap results (MP, ML) and Bayesian posterior probabilities. Below branches Bremer support values and Templeton test results are given, either rejected (–) or accepted (+), with 0.05 as the cut-off point.

dependency on the forest, the estimated extent of occurrence of *C. stanleyi* sp. nov. is less than or equal to 9.7 km^2: this is the area including an elevational distribution (1200–1600 m) of the submontane habitat in Chome. As with the two other newly described species, population density does not appear to be low, but *C. stanleyi* sp. nov. is confined to a small fragment of submontane forest existing along the eastern boundary of Chome forest reserve, which is bordered by extensive farmlands. The presence of one population at only a single, small location is compounded by an observed decline in the area and quality of the habitat (Hall *et al.*, 2009).

Etymology: This species is named after our colleague and good friend William T. Stanley of the Field Museum, Chicago. Bill has made numerous contributions to understanding the biological diversity of the Eastern Arc Mountains. He has also made several important field discoveries of amphibians (including those we describe from the South Pare), and we name this species in recognition of his excellent work.

INTERNAL MORPHOLOGICAL DIVERSITY IN *CALLULINA*

de Sá *et al.* (2004) and Loader *et al.* (2009) provided assessments of the internal morphology of the new *Callulina* species they described. These two studies utilized characters previously identified in Parker's (1934) review of the morphological diversity in microhylids (brevicipitids were previously classified in Microhylidae). Largen & Drewes (1989) provided additional data in their description of the new brevicipitid genus *Balebreviceps*. Largen & Drewes' (1989) and Parker's (1934) studies provided important characters for differentiating primarily among breviciptid genera, less so among species (de Sá *et al.*, 2004; Loader *et al.*, 2009). For this study we investigated the skeletal morphology of all the new species, and provide brief summaries.

Characters common to all *Callulina* species: (1) Palate with denticulate ridge in front of pharynx, and two weakly defined, broad arcuate smooth ridges anteriorly; (2) parasphenoid cultriform process broad based, but narrower, almost wing-like structure tapering medially, with the anterior end tapering only slightly; (3) mentomeckelian–posteroventral processes smaller than those of *Probreviceps*, wedge-shaped as in *Balebreviceps*, but with a more acute angle; (4) procoracoids and clavicles present, well developed, straight, slightly curved distally at the joint between coracoid and scapulae; (5) large omosternum; (6) double condylar articulation between the urostyle and the sacral vertebra; and (7) diplasiocoelous vertebrae.

Character varying among *Callulina* species: bony part of finger and toe tips expanded clearly beyond the width of the base of the terminal phalanx in *C. kreffti*, expanded marginally in *C. stanleyi* sp. nov. and *C. kisiwamsitu*, and about same width of the base of the phalanx in *C. dawida*, *C. laphami*, and *C. shengena* sp. nov.

Specimens of uncertain taxonomic affinity

Two *Callulina* specimens collected in July 1993 from Minja Forest Reserve in North Pare cannot be clearly assigned to any described species of *Callulina*. These two specimens (CAS 225136 and 225137) are both juveniles (SUL = 16.2 and 20.5 mm, respectively). One specimen (CAS 225136) is slightly dried, probably as a result of being 'found dead and floating in a small dam alongside a rivulet' (CAS catalogue) at 1500 m a.s.l., which is lower than the 1800-m a.s.l. Kindoroko locality from where the types of *C. laphami* sp. nov. were collected. The other specimen (CAS 225137), also slightly dehydrated, was found in a *Dracaena* plant in dense mist forest at 1700 m a.s.l., which is marginally lower than the Kindoroko locality. The vent in both specimens is flush with the ventral surface. Dark coloration on dorsum and dark, posteriorly-directed band connects each eyelid, although this is not continuous. Venter pale brown, dark patches around lower lip, mottled all over. Toes and feet tips rounded at distal edge, and truncated but marginally expanded. No folds or ridged surface on the generally smooth dorsum. The tympanum is indicated externally by a slightly raised, marked and dark-brown area. Dissection revealed a small tympanum in both specimens (TD = 0.5 and 0.6 mm, respectively), lying close to the eye (ETD = 0.6 and 0.9 mm, respectively). Sex could not be determined in either specimen. These specimens do not seem to be *C. laphami* sp. nov., given the presence of a tympanum, but the presence of an interocular band connecting the eyelids and truncated finger tips is notably similar to *C. laphami* sp. nov. (and to *C. shengena* sp. nov.). The presence of a tympanum aligns the species more closely with *C. kisiwamsitu* and *C. stanleyi* sp. nov., but given the isolation of the North Pare specimens relative to these species, and the very strong patterns of local endemism in this genus (Loader *et al.*, 2009), these two specimens may yet represent another new species in the Pare Mountains. More material, including tissue samples and acoustic data, is required.

A single adult specimen (AMNH 72724) collected from Kwale in Kenya awaits taxonomic clarification. The specimen was collected on 13 November 1961 by Ronaldo Keith. Field notes accompanying the specimen read 'Makadera Forest, Kwale Hills [this locality seems to have been added later in red ink, and might correspond to the Shimba Hills]. Tall virgin forest. Underbrush thick, but not impenet. . . Rains had been on heavily about three weeks. Found during day 5 above ground on leaf'. This interesting specimen, the only known example of the genus to have been found outside the Eastern Arc Mountains, most closely resembles specimens of *C. kisiwamsitu* and *C. stanleyi* sp. nov., based on the tympanum size (1.9 mm) and its distance from the eye (2.1 mm), and the expanded finger tips (1.7 mm). The ratio of the finger tip width (0.77) relative to the width at the first subarticular tubercle (1.3 mm) suggests a close relationship with the *C. kisiwamsitu* + *C. stanleyi* sp. nov. clade, which would not be unexpected given its geographic origin. Given the relatively isolated position of Kwale relative to the Eastern Arc forests and the biogeographically distinct fauna of the Shimba Hills (Waas, 1996), this population might also represent an undescribed species. Again, new fieldwork and more data are required to resolve this.

Phylogenetic analyses

To test the distinctiveness of *Callulina* species, and infer their evolutionary relationships, we constructed two alignments of sequence data for all six described species: (1) all *Callulina* individuals for which *12S* and *16S* were available; and (2) all individuals for which *12S*, *16S*, and cyt*b* were available. The two data sets comprised: (1) 18 individuals with 762 unambiguously aligned characters, of which 533 were constant, 89 variable, and 140 informative under parsimony (only one gap present, alternative coding of this gap made no difference in the resulting trees); and (2) 14 individuals with 1185 unambiguously aligned characters, of which 884 were constant, 98 variable, and 203 informative under parsimony. *Phrynomantis* was used as the out-group, as in previous analyses, following Loader *et al.* (2009).

Analysis of alignments 1 and 2 yielded single most parsimonious (MP) trees of 422 and 870 steps, respec-

KEY TO THE SPECIES OF *CALLULINA*

As Loader *et al.* (2009) outlined, *Callulina* species are distinguished from other breviciptids by their truncate to expanded toe and finger tips. Here we present a key that should identify adult specimens of all described species. It should be noted that the confident identification of species relies on an assessment of the presence or absence of a tympanum. This might require dissection, especially for small specimens, and therefore is not possible in the field. Geographical distribution (and/or morphometric/molecular analysis) can assist in distinguishing between the morphologically similar *C. kisiwamsitu* and *C. stanleyi* sp. nov. Caution is required when identifying small or poorly preserved specimens in which toe and/or finger tips might be desiccated.

1a. Tympanum present, although often obscured by granular skin.....2
1b. Tympanum absent.....5
2a. Finger tips expanded, with their width greater than the width of the distal subarticular tubercle.....3
2b. Finger tips truncated, not expanded beyond the width of the first subarticular tubercle.....*Callulina dawida*
3a. Ratio between the width of finger 3 at the level of the distal subarticle tubercle and the width of its finger tip is always more than 0.75. Distance between tympanum and eye equal to or greater than tympanum diameter...4
3b. Ratio between the width of finger 3 at the level of the distal subarticle tubercle and the width of its finger tip is less than 0.75. Distance between tympanum and eye usually less than tympanum diameter.....*Callulina kreffti*
4a. Large, robust head. Distinctive call, known only from South Pare Mountains.....*Callulina stanleyi* sp. nov.
4b. Less robust head. Distinctive call, known only from West Usambara Mountains.....*Callulina kisiwamsitu*
5a. Absence of prominent glandular masses on arms and feet. Distinctive bright red (or green) interocular band connecting the opposite anterior and posterior margins of the eyelids. North Pare Mountains.....*Callulina laphami* sp. nov.
5b. Prominent, relatively pale glandular mass on arms and feet. Less distinct and less continuous interocular band. South Pare Mountains.....*Callulina shengena* sp. nov.

tively, and the trees (not shown) are not notably different to those recovered in likelihood and Bayesian analyses. Maximum likelihood (ML) and Bayesian analyses were conducted with the GTR + I + G model. Both analyses supported the monophyly of *Callulina*, and rejected suboptimal topologies in most tests (see Fig. 8). For alignment 1, both analyses demonstrate the genetic distinctiveness of the named species, with highly supported reciprocally monophyletic groupings recovered for each, and suboptimal topologies significantly different in MP tests (apart from *C. stanleyi* sp. nov.; see Fig. 8A). The truncate finger/toe species (*C. dawida*, *C. laphami*, and *C. shengena* sp. nov.) form a paraphyletic assemblage outside a clade of all expanded finger/toe species. The sister-species grouping between *C. shengena* sp. nov. and *C. laphami* sp. nov. is weakly supported. The expanded finger/toe clade of *C. kreffti*, *C. kisiwamsitu*, and *C. stanleyi* sp. nov. is weakly supported, although the sister pair of the morphologically similar *C. kisiwamsitu* and *C. stanleyi* sp. nov. is strongly supported. Both analyses of alignment 2 recover multiple individuals of each named species (where included) together in maximally supported clades (Fig. 8B), and are significantly different from suboptimal topologies. Differing from alignment 1, *C. shengena* sp. nov. is recovered as the sister to all other species instead of grouping with *C. laphami*, although this is weakly supported. Analyses of alignment 2 (as with alignment 1) recovered a truncate-toed paraphyletic assemblage outside a clade of all expanded finger/toe species.

Constrained topologies including a monophyletic North and South Pare radiation (*C. stanleyi* sp. nov., *Callulina lapami*, and *C. shengena* sp. nov.), or a monophyletic South Pare (*C. stanleyi* sp. nov. and *C. shengena* sp. nov.) radiation, are both statistically significantly suboptimal for both alignments using MP and ML ($P \leq 0.05$). Pairwise distances are summarized in Table 2, with these values highlighting the genetic distinctiveness of all named *Callulina* species (> 4.2%, with a mean of 8.4% among all species).

DISCUSSION

Examination of all available material from north-east Tanzania and south-east Kenya has revealed patterns of high local endemism in the genus *Callulina*. From East Usambara to the Taita Hills, each fragmented Eastern Arc Mountain block is home to a distinctive species: two in the case of the South Pare. The two South Pare species are stratified by altitude, with a low submontane and high montane species: *C. stanleyi* sp. nov. and *C. shengena* sp. nov., respectively (although not sister species). The hypothesis that the three Pare *Callulina* species are monophyletic is rejected – although *C. lapami* and *C. shengena* sp. nov. are closely related, and could reflect a vicariant event between South and North Pares. Some unidentified specimens suggest that further collection in this area might reveal additional distinct species. We believe that high local endemism in *Calulina* is likely to be repeated across the rest of the Eastern Arc, with distinct species

perhaps being restricted to each of the remaining seven blocks where *Callulina* has also been recorded (Nguu, Nguru, Ukaguru, Uluguru, Rubeho, Udzungwa, and Mahenge). This will require close examination of all material, noting the possibility of multiple species per single locality. Remarkably, there appears to be at least three species in the Nguru Mountains, stratified by altitude (Menegon *et al.*, 2008).

Endemism patterns in *Callulina* contribute to a more general pattern of evolutionary diversification of amphibians, and potentially other organisms in the Eastern Arc Mountains. A recent study on minature *Arthroleptis* frogs revealed highly localized distributions of new species (Blackburn, 2009). Distinct West and East Usambara species of *Arthroleptis* were reported, and populations of the new species *Arthroleptis fichika* from South Pare and West Usambara are highly genetically divergent (16.7%). Although a distinction between South Pare and West Usambara populations was not found in a molecular phylogenetic study of double-collared sunbirds, Bowie *et al.* (2004) did distinguish between North Pare and South Pare + Usambara complexes. Increasing the number of studies of the evolutionary history of taxa in the Eastern Arc region might reveal more general patterns against those that are idiosyncratic. This will ultimately lead to a better understanding of the processes that have led to the development of this area as a global biodiversity hotspot (Myers *et al.*, 2000).

Callulina dawida, *C. shengena* sp. nov., and *C. laphami* sp. nov. are together paraphyletic, and lie outside a clade of *C. kreffti*, *C. kisiwamsitu*, and *C. stanleyi* sp. nov. (Fig. 8). The former three taxa have truncated finger and toe tips, which suggests that this is the ancestral condition for the genus. These truncated finger/toe tip taxa resemble, in some respects, leaf litter and generally the fossorial breviciptid *Probreviceps*, rather than the more arboreal *Callulina* species. Although some truncated finger/toe tip *Callulina* are known to climb (*C. laphami* and *C. shengena* sp. nov.), they are also found in forest-floor microhabitats, and their similarity to *Probreviceps* raises interesting questions. Currently, all known truncated-tip *Callulina* are found in the north-eastern part of the Eastern Arc Mountains (Usambara, Pare, and Taita Hills), areas of the Eastern Arc from which *Probreviceps* is unknown. Further work is required, especially on the *Callulina* from the southern Eastern Arc, and on ecology of brevicipitids more generally, in order to generate data that will test hypotheses explaining the distributional, phylogenetic, and ecological patterns, including ecological character displacement, radiation into empty niche space, or non-ecologically mediated historical events (e.g. extinction/historical absence of *Probreviceps* in the northern Eastern Arc).

Callulina shengena sp. nov. and *C. laphami* sp. nov. both lack tympana, but a clear call occurs in the latter species at least. Whether *C. shengena* sp. nov. calls or not is uncertain given the short field-survey period. Calling without a tympanum is not unprecedented among frogs (e.g. Jaslow *et al.*, 1988; Jaslow & Lombard, 1996). The repeated loss of the tympanum in breviciptids (apparent absence in *Breviceps* spp. and in *Probreviceps uluguruensis*), in addition to some *Callulina* species, is perhaps an adaptation to a more fossorial lifestyle, but further ecological, anatomical, acoustic, and phylogenetic data are required to fully test this.

The description of three new narrowly endemic *Callulina* species raises conservation concern for this group of brevicipitids. Typically, *Callulina* species are encountered in forest habitats, although collections in secondary forest and edge habitats are known. The generally close adherence of *Callulina* to forest means that they are threatened by ongoing forest loss. The species we describe here all have extremely narrow distributional ranges, and occur in an area that has undergone recent habitat change (Hall *et al.*, 2009). Based on International Union for Conservation of Nature (IUCN) criteria, we propose that all three new species will qualify as critically endangered. Estimated extents of occurrences are all extremely low (*C. laphami* sp. nov., 16.5 km^2; *C. shengena* sp. nov., 35 km^2; *C. stanleyi* sp. nov., 9.7 km^2), particularly when compared with previously described species (*C. dawida*, 168.2 km^2; *C. kisiwamsitu*, < 500 km^2; *C. kreffti*, > 500 km^2; IUCN, 2009). Better knowledge of the conservation biology of the three new species requires new fieldwork to clarify the extent of occurrence, generate ecological requirement and life-history information, and assess the need for and possible efficiency of any monitoring programme.

Callulina kreffti is currently considered of 'Least Concern' because of its seemingly wide distribution and tolerance to habitat modification. This rests on the assumption that several populations outside of the East Usambara are referable to *C. kreffti* (e.g. Ukaguru, Nguu, Nguru, Uluguru, Mahenge, and Udzungwa; IUCN, 2009). Based on the pattern of local endemism in *Callulina* revealed in this study, similar patterns in other amphibians (e.g. Blackburn, 2009), and other taxon groups (Stanley *et al.*, 1998; Bowie *et al.*, 2004; Fjeldså, Bowie & Kiure, 2006), this assumption must be questioned. We recommend that *Callulina* occurring outside of the Usambara, Pare, and Taita hills should, in the first instance, be considered *Callulina* sp., and not identifiable as a named species without careful consideration of morphological, molecular, and/or acoustic data.

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APPENDIX 1

Details of molecular samples used in this study. Specimen abbreviations are: MOR, M.-O. Rödel's research collection, and NMK, National Museums of Kenya, Nairobi, Kenya. Locality abbreviations are: FR. Forest Reserve.

Species	Voucher Specimen	Locality	Sequence accession numbers: 12S rRNA, 16S rRNA, and cytochrome b
Phrynomantis microps	MOR C97.1	Comoe National Park, Ivory Coast	AY531832, AY531855, FN563039
Breviceps mossambicus	BMNH 2005.1377	Sali FR, Mahenge Mts., Tanzania	AY531836, AY531859, FJ998375
Spelaeophryne methneri	BMNH 2000.216	Uluguru Mountains, Milawilila FR, Tanzania	AY531838, AY531861, FJ998376
Probreviceps m. macrodactylus	BMNH 2002.755	Nilo FR., East Usambara Mts., Tanzania	FN563040, FN563041, FN563042
Callulina kisiwamsitu	BMNH 2002.47	Ambangula FR, West Usambara Mts, Tanzania	AY531841, AY531864, FJ998379
Callulina kisiwamsitu	BMNH 2002.45	Mazumbai FR, West Usambara Mts, Tanzania	AY531840, AY531863, FJ998380
Callulina kreffti	BMNH 2000.205	Nilo FR, East Usambara Mts., Tanzania	AY531842, AY531865, FJ998381
Callulina dawida	NMK A/4266	Taita Hills, Fururu FR, Kenya	FJ998382, FJ998383, FJ998384
Callulina dawida	NMKA 3913	Taita Hills, Ngangao FR, Kenya	FJ998385, FJ998386, FJ998387
C. laphami	BMNH 2002.37 Holotype	Kindoroko FR, North Pare Mountains, Tanzania	FN563043, FN563044, FN563045
C. laphami	BMNH 2002.40	Kindoroko FR, North Pare Mountains, Tanzania	FN563046, FN563047, FN563048
C. shengena	FMNH 251849	Chome FR, South Pare Mountains, Tanzania	FN563049, FN563050, FN563051
C. shengena	FMNH 251881	Chome FR, South Pare Mountains, Tanzania	FN563052, FN563053, FN563054
C. shengena	FMNH 255882	Chome FR, South Pare Mountains, Tanzania	FN563066, FN563067, not sequenced
C. shengena	MTSN 9285 Holotype	Chome FR, South Pare Mountains, Tanzania	FN563068, FN563069, not sequenced
C. shengena	MTSN 9287	Chome FR, South Pare Mountains, Tanzania	FN563055, FN563056, not sequenced
C. stanleyi	BMNH 2000.207 Holotype	South Pare Mountains, Tanzania	FN563057, FN563058, FN563059
C. stanleyi	MTSN 7540	Chome FR, South Pare Mountains, Tanzania	FN563070, FN563071, not sequenced