



## A new brevicipitid species (Brevicipitidae: Callulina) from the fragmented forests of the Taita Hills, Kenya

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### Abstract

A new species *Callulina dawida* is described from the Taita Hills, Kenya. It is distinguished from other members of the genus on the basis of the degree of digital expansion. The species further differs from other members of the genus based on molecular sequence comparisons and on its call. The morphological variation in the new species is described, including a comparison of internal and external characters and sexual dimorphism with other species of *Callulina*. The conservation status of the species, on the basis of its restricted distribution and land use changes in the area, is considered to be of high concern. An updated key of the species of *Callulina* is provided.

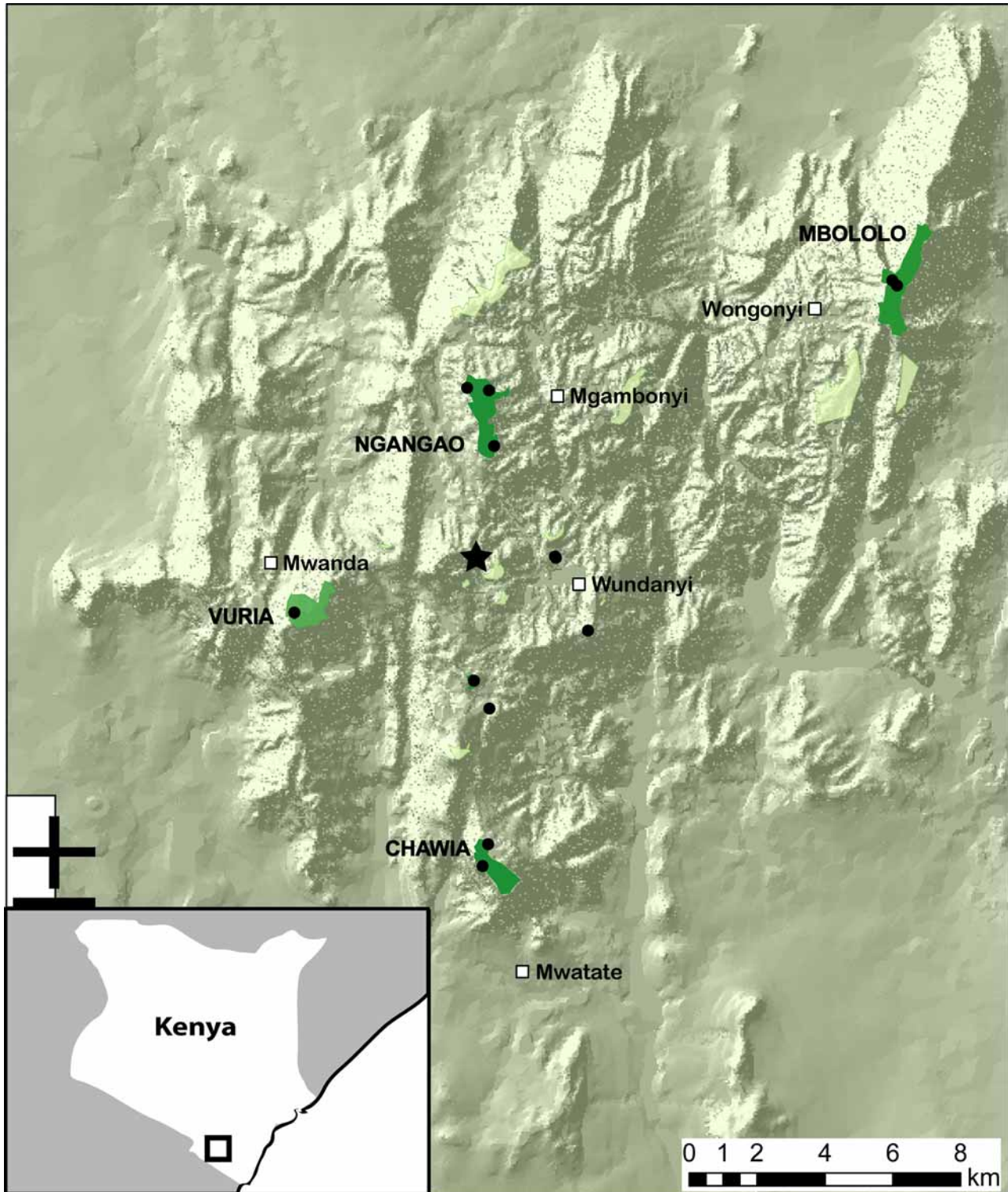
**Key words:** *Callulina*, Brevicipitidae, Taita Hills, Eastern Arc Mountains, Afromontane

### Introduction

The brevicipitid genus *Callulina* is found throughout the Eastern Arc Mountains (EAM), from its northern most extent in the Taita Hills of Kenya (Howell, 1993) down to the Udzungwa Mountains (Frontier, 2001) in southern Tanzania. Described species are generally associated with forest habitats. Species of *Callulina* are easily distinguished from other brevicipitines by the degree of expansion of the finger and toe tips (Parker, 1934; de Sá *et al.* 2004). Whereas all other brevicipitines (*Breviceps*, *Probreviceps*, *Balebreviceps*, and *Spelaeophryne*) have round finger and toe tips and are predominantly fossorial, species of *Callulina* show expanded finger and toe tips that are likely to be an adaptation to their more arboreal lifestyle. *Callulina* was long considered to be a monotypic genus, with *C. kreffti* distributed throughout the EAM (Schjøtz, 1981). This notion was recently challenged with the description of populations from the West Usambaras as *C. kisiwamsitu* (de Sá *et al.* 2004). The recognition of two distinct species in geographically adjacent areas of the Usambaras highlighted an uncertainty about the taxonomic status of other mountain populations distributed throughout the EAM (de Sá *et al.* 2004). Populations of *Callulina* that are isolated and geographically widely separated in the Eastern Arc Mountains, deserve detailed investigation and ongoing work on *Callulina* indicates that many new species await description (Menegon *et al.* 2008).

In this paper we describe a new species of *Callulina* from the Taita Hills, Kenya and provide details on internal and external morphological variation, and the advertisement call of the species. We focus on a complex of morphological characters that Parker (1934) identified as being taxonomically informative for distinguishing between brevicipitid genera and species. More recently, the morphological characters that

Parker (1934) identified have been used by de Sá *et al.* (2004) and Largen and Drewes (1989) for their respective descriptions of *C. kisiwamsitu* and *Balebreviceps hillmani*. Although not a comprehensive survey of the internal morphology, these characters are useful at discriminating between species and genera (Parker, 1934; Largen and Drewes, 1989). Using molecular evidence we also investigate the phylogenetic relationships of these species, adding to previous analyses (Loader *et al.* 2004; 2006).



**FIGURE 1.** Map of the Taita Hills. White squares show localities of (black lowercase) named towns, filled polygons are principle remaining indigenous (black uppercase) named forests, and filled circles collection localities for specimens of *Callulina dawida*, holotype collection locality is shown with a star.

## Materials and methods

Specimens were collected from remnants of indigenous forest patches in the Taita Hills, Kenya (Fig 1). Specimens were fixed in 5% formalin and subsequently stored in 70% ethanol; sample tissues of muscle and liver were removed and preserved in 96% ethanol. One *Callulina* specimen was cleared and double-stained for bone and cartilage based on the method of Taylor and Van Dyke (1985). Specimens examined are in the following institutions: Natural History Museum, London, UK (BM), Museum of Comparative Zoology, Harvard University, Boston, USA (MCZ), National Museums of Kenya, Nairobi, Kenya (NMK), California Academy of Sciences, San Francisco, USA (CAS), Museum für Naturkunde Berlin, Germany (ZMB), Natural History Museum of Geneva, Switzerland (MHNG), and US National Museum of Natural History (Smithsonian Institution, Washington, DC, USA) (USNM). Further details are provided in Appendix 1, in addition to specimens listed by de Sá *et al.* (2004).

Standard measurements were taken to the nearest 0.1 mm using digital calipers. Measurements are: SUL (snout-urostyle length), TL (tibiofibula length), ED (horizontal eye diameter), TD (horizontal tympanum diameter), ETD (eye—tympanum distance), IN (internarial distance), NED (nostril-eye distance), HW (head width at level of jaw articulation), LF3 (length of Finger three measured from the distal edge of the basal subarticular tubercle), LT4 (length of Toe four measured from the proximal edge of the basal subarticular tubercle), TSL (length of tarsus), HL (humerus length), NLD (nostril—lip distance), WDF3 (width of disc of Finger three), WDTF3 (width of Finger three at level of distal subarticle tubercle), and IOD (interorbital distance).

Calls were recorded using a stereo cassette tape recorder (Marantz PMD-430) with a directional microphone. Calls were analysed using Raven (Charif, *et al.* 2004). DNA Sequences for all *Callulina* species were obtained to investigate genetic variation among species. Tissue samples were taken from specimens listed in Appendix 1. Genomic DNA was extracted from liver or thigh muscle preserved in aqueous 96% ethanol, purified and sequenced following Loader *et al.* 2006. Primers used in amplification and sequencing were 12Sa and 12Sb for the 12S rRNA gene (Kocher *et al.* 1989), and 16Sa and 16Sb for the 16S rRNA gene (Palumbi 1996), CB2F frog and CB3R frog (Palumbi 1996) for the Cytochrome b gene. GenBank accession numbers are given in Appendix 2.

## Systematics

### *Callulina dawida* sp. nov.

(Figures 2–5)

**Holotype.**NMK A/4267 (Field No. JM01239), adult male, collected by J. Mwandoe & T. Mwakio, on 16 December 2004, in Kiangungu forest patch near Iyale forest, 37M 426787, UTM 9623928.

**Paratypes.**NMK A 3913 (MW03197) collected by G. J. Measey on 7 May 2002, in Ngangao forest), A 3534/3 (NK091), A 3534/2 (NK090), and A 3534/1 (NK089) collected by R. Odhiambo on 10 February 1998, in Ngangao forest, A 1499/1 collected by H. J. Beentje and J. Lorubun on 10 May 1985 in Ngangao forest, A 3703 (CHA2) collected by P. K. Malonza, V. Muchai and R. Mwakondi on December 1999, in Chawia forest, A 4266 (JM01235) collected by B. A. Bwong and G. J. Measey on 16 December 2004, in Fururu forest, A 4268/2 (JM01632) and A 4268/1 (JM01628) collected by G. J. Measey and J. Mwandoe 28 December 2004 in Ngangao forest, A 3535 (NK592) collected by R. Odhiambo on 22 March 1998, in Chawia forest.

All the following specimens were collected by P. M. Odongo: BMNH 2005.1574 (formerly NMK A 3647/4- FNK998) and BMNH 2005.1575 (formerly NMK A 3647/3 – FNK1206) collected between 4–8 June 1998 in Kenya, Taita-Taveta district, Taita Hills, Ndiwenyi forest, A 3613/2 (FNK906) collected on 29 May 1998, in Fururu forest, A 3647/5 (FNK956) and A3647/6 (FNK 1205) collected on 4 June 1998 in Ndiwenyi forest,

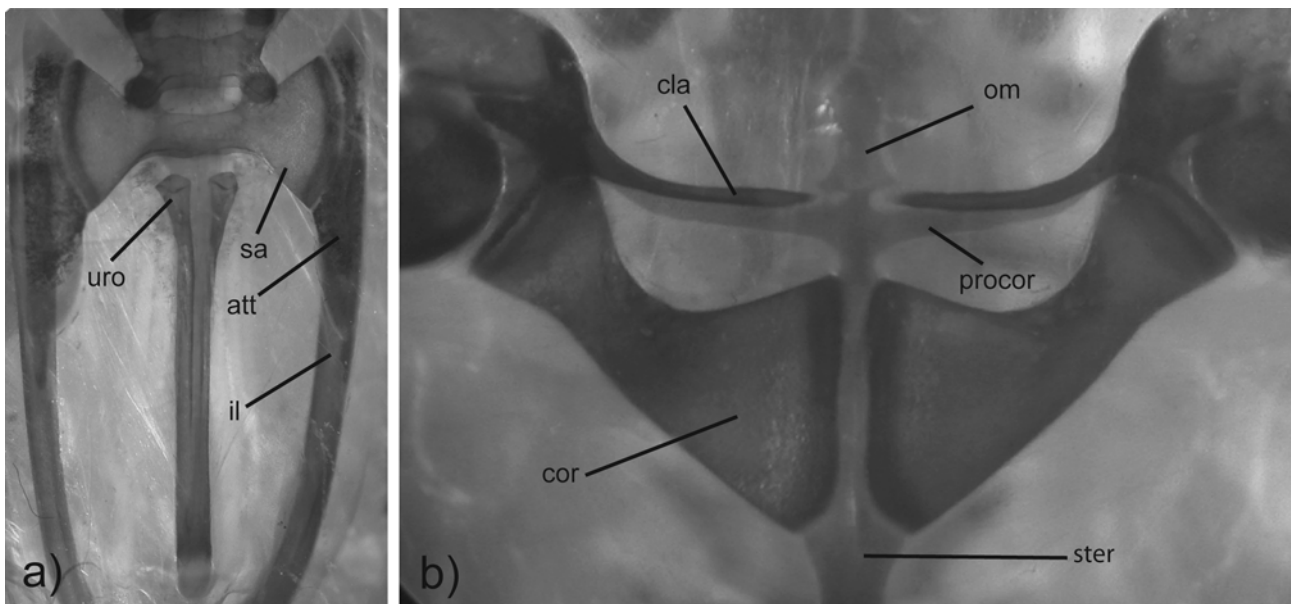
A 3613/1 (FNK817) collected on 21 May 1998, in Fururu forest, A3647/1 (FNK106) and A 3647/2 (FNK 1207) collected on 8 June 1998 in Ndiwenyi forest, A 3619 (FNK826) collected on 25 May 1998, in Ndiwenyi forest, and A 3617(FNK957) collected on 1 June 1998, in Fururu forest.



**FIGURE 2.** Dorsal, ventral and lateral views of the holotype of *Callulina dawida* (NMK A/4267).

**Diagnosis.** The new species is assigned to the genus *Callulina* based on the following characteristics: Truncated or expanded terminal phalanges (simple in *Spelaeophyrne*, *Probreviceps*, *Breviceps*, and *Balebreviceps*); single posterior denticulated row in the palate of *Callulina* (two denticulated rows in

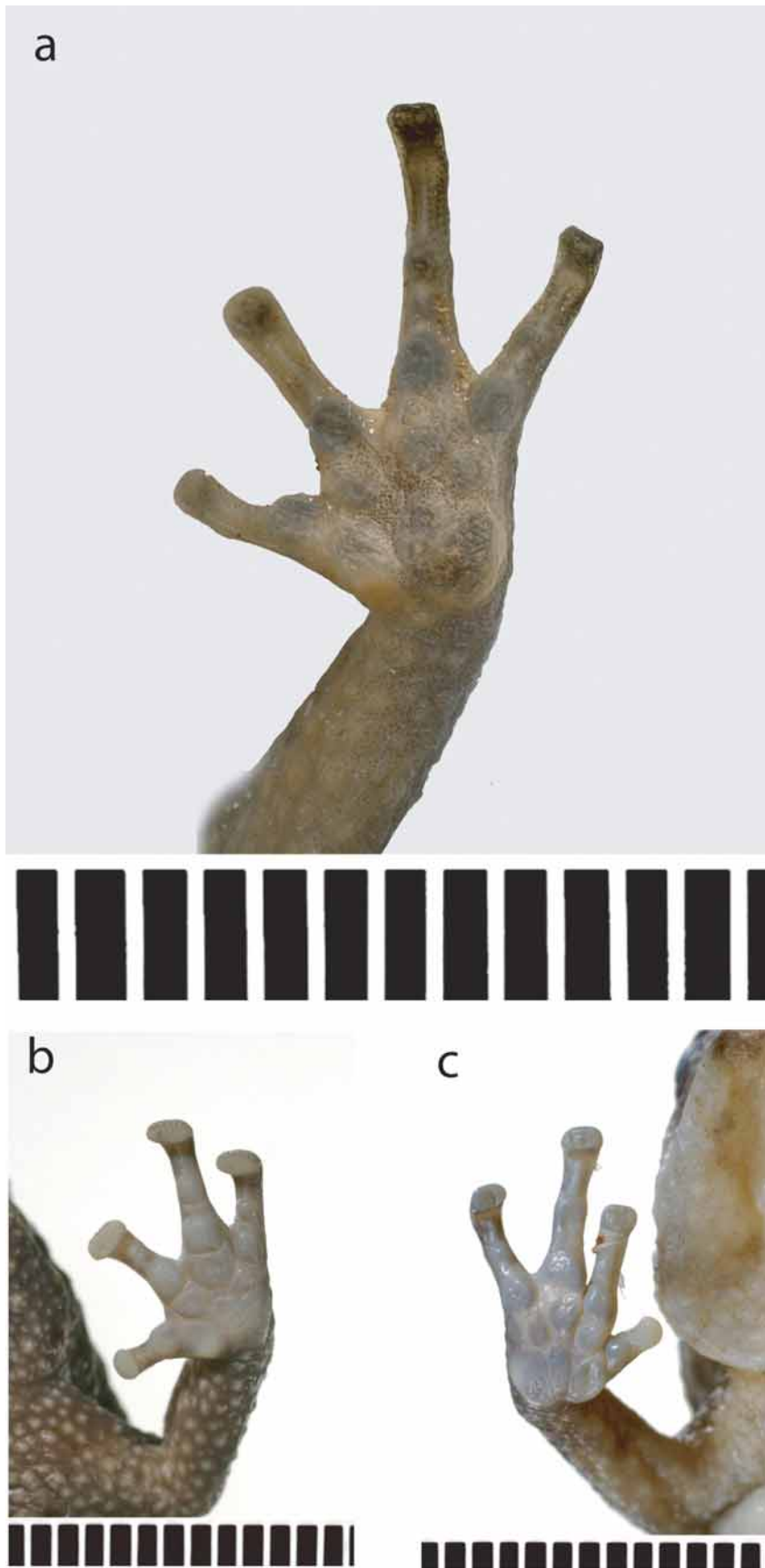
*Probreviceps*, and a glandular mass in *Breviceps*); choanae present (absent in *Balebreviceps*); moderately sized wedge shaped lobes on the mentomeckelian elements, posteroventrally directed (in *Probreviceps*, *Balebreviceps*, and *Breviceps* variously reduced and enlarged, Largen and Drewes, 1989); cultriform process of the parasphenoid with broad based but narrow alary processes, tapering laterally (cultriform process of the parasphenoids in known brevicipitids show wide diversity, Largen and Drewes, 1989); nasals almost meet at midline (broadly separated in *Breviceps* and *Balebreviceps*); clavicle well-developed and straight though slightly curved anteriorly at the point of contact of the coracoid and scapulae (clavicle straight in *Breviceps*, *Probreviceps*, and *Spelaeophryne*) (see Figure 3a); omosternum large (rudimentary or small in *Breviceps*, *Probreviceps*, and moderate in *Balebreviceps*, Figure 3a); tympanum present and usually well-differentiated (absent in *Balebreviceps* and *Probreviceps uluguruensis*); double condylar articulation between the urostyle and the sacral vertebra (fused in *Balebreviceps*, *Breviceps*, and *Probreviceps*, Figure 3b).



**FIGURE 3.** a) Urostyle elements of *Callulina dawida* (A3535), Abbreviations, uro= Urostyle, sa= Sacrum, att= ilial-urostyle muscle attachment, il= ilium. b) Pectoral elements of *Callulina dawida* (A3535). Abbreviations, omo= Omosternum, clav= Clavicle, cor= Coracoid, procor= Procorocoid, ster= Sternum.

*Callulina dawida* is a slightly smaller, robust shaped *Callulina* that is morphologically distinct from the two other species in this genus (*C. krefftii* and *C. kisiwamsitu*). The new species is distinguished by the following characters: truncate finger tips, rounded at edges, and only slightly expanded (if at all) beyond the width of the subarticular tubercle (finger tips expanded beyond the width of subarticular tubercle in *C. kisiwamsitu* and *C. krefftii*; see Figure 4); terminal phalanges Y-shaped but not expanded beyond the width of the base of phalange. *Callulina kisiwamsitu* and *C. krefftii* have terminal phalanges expanded distally beyond the width of the base of the phalange, *C. krefftii* has T-shaped terminal phalanges and some individuals exhibit an intermediate ‘T’ and ‘Y’ shape (e.g. CAS162505).

**Remarks on diagnosis.** Tubercles on the hands and feet were treated as diagnostic for species in *Callulina*; de Sá *et al.* (2004) stated that in *C. kisiwamsitu* “there is no contact between inner and outer metatarsal”, however an examination of additional specimens indicates that this character is more variable than previously considered. Because inner, mid and outer tubercles seem to be quite variable in their size and position, and definition they cannot consistently discriminate between these species. Given that tubercles can also be desiccated, they can be difficult to discern precisely. We suggest that this character needs to be further examined, among and within populations of *Callulina* to determine its systematic utility in the genus. The new species shows the inner and outer tubercles separated on the hand by a mid palmar tubercle (Figure 4) that is also present in other *Callulina* species.



**FIGURE 4.** Ventral close up of hands (1mm scale) (a) *Callulina dawida* (NMK A/4267) (b) *C. krefftii* (2000.196) (c) *C. kisiwamsitu* (BM 2002.45).



**FIGURE 5.** *Callulina dawida* in life.

**Description of the Holotype.** Body stout, head wider than longer. Snout truncate in lateral view (Figure 2), snout tip extending anteriorly beyond the jaws. Snout tip flattened not pointed at apex, with slightly rounded edges. Lower jaw has small warts, concentrated on chin and lateral sides near to tympanum, less dense on upper jaw. Tongue rounded. Canthus rostralis rounded, loreal region sloping slightly concavely. Nostrils rounded, directed laterally, nearer to the tip of snout than the eye. Interorbital space flat, larger than width of eyelid. Pupil horizontal. Tympanum distinct and ovoid, transversely smaller than longitudinally. Tympanum defined by smooth, light coloured skin with warts around edge of the disc. Tympanic ridge is prominent, marked by a darker brown colouration, starts at posterior corner of eye to the arm insertion. Warts on dorsal surface of head and eyelids small, rounded. Dorsal and ventral surfaces of body granular, with rounded, shallow warts; lateral warts slightly larger. Forelimbs slender, covered with small warts that are larger and more densely concentrated at the arm joint than on ventral surfaces. Hind limbs stout and also covered with small warts, which are larger and concentrated at the leg joint. Digits of hands moderately long, relatively slender. Tip of digits truncate, slightly rounded at the edges, but not expanded beyond the width of the first subarticular tubercle. First, second, and fourth fingers of similar length, first being the shortest, third finger longest. Toes are truncate with slightly rounded edges, first smallest in length, followed by second, third, and fifth, with fourth toe being the longest. An evident fold of skin defines the dorsal junction between the penultimate and ultimate phalanges in hands and feet, marked as a narrow white coloured band. No webbing present on either hands or feet. Subarticular tubercles are distinct and rounded. Palmar tubercles are distinct and well-separated, inner and outer metatarsal tubercles distinct, not contacting each other and separated by a medial tubercle, inner one larger. Vent is ventro-posteriorly positioned.

**Measurements.** SUL = 29.6; TL = 10.4; ED = 3.0; TD = 1.6; ETD = 1.4; ND = 1.9; NED = 2.5; JW = 8.9; LF3 = 3.8; LT4 = 5.2, TSL = 6.8; HL = 8.9; NLD = 1.4; WDF3 = 0.9; WDTF3 = 0.9; IOD = 4.7.

**Variation.** The tympanum is usually distinct in *Callulina dawida*. However, in a small number of

specimens (NMK A3534/3, A3534/1, A 3534/2, A 4268/1), the tympanum poorly demarcated. In this case, the tympanum was distinguished either by smoother skin or by dissection of the tympanic region to verify the presence of the tympanum. Variation in the size, shape and presence/absence of the tympanum is a character often utilized in brevicipitine systematics (e.g. Parker, 1931; de Sá *et al.* 2004; Loader *et al.* 2006), though caution is needed because the granular skin of brevicipitids can obscure its presence.

The width of the distal phalange in *Callulina dawida* is less expanded than other *Callulina* species currently recognized, with the truncate end being almost always equal to the width at the first subarticular tubercle. However, in some specimens the finger-tips are slightly more expanded than the width of the tubercle (BMNH 2005.1574, A4268, and NMK A3647/1), but never reaching the degree of expansion found in either *C. krefftii* or *C. kisiwamsitu*. The size and condition of hand and foot tubercles vary slightly between specimens, with some being slightly desiccated, such as specimen NMK A3534/3 where tubercles are less clear.

**Sexual dimorphism.** There is some size morphological difference between the sexes, mean SVL males = 27.1 (n = 9: 21.2—31.3) whereas mean SVL females = 29.1) (n = 9: 24.1—38.3). Three specimens were immature and not sexed or analysed. Females are larger than males, though not significantly (t-test, p = 0.5962). The position and size of the tympanum between male and females differ. Males have a significantly larger tympanum, e.g. tympanum-snout urostyle (t-test = > 0.001); and the position of the eye to the tympanum (t-test = > 0.001) is also greater in males. Besides these differences, we did not identify any other significant differences in body proportions. Males often appear lighter or more brightly coloured than females.

**Colouration.** In life, the dorsal colour is quite variable, ranging from shades of light yellow through orange, brown to dark brown. In darker individuals, the flanks, hind- and fore quarters are usually lighter, sometimes with the tubercles having white tips (Figure 3). The ventral surface of body is pale and occasionally spotted. In preservative, the dorsal surface is dark tan brown with lighter patches towards the lateral sides of mid-body and head. A distinct, broad, and posteriorly directed light brown interorbital band emanates from both eyelids, meeting at centre of cranium. Almost continuous with the interorbital patterning, a light brown dorsolateral stripe continues from the eyelid along the sides to the hind limb insertion. The brown dorsolateral stripe darkens slightly posteriorly. Limbs are lighter in colour to dark brown dorsum, with their ventral side a light brown beige colour. The underside of the chin has distinctive brown markings. Dark brown colouration encroaches on the middle of throat region, resembling two triangles with the apex of each point meeting at the centre of the throat. The patterning varied between specimens, perhaps influenced by preservation, with some specimens showing a strong interorbital band and dorso-lateral stripe, and others with less clear markings.

**Advertisement Call.** We made a detailed temporal analysis of 1.5 minutes of calling from a single male recorded on 15 September 2008 in Wundanyi forest. This period comprised 10 calls, each call being made up of a mean of 7 "chirps" (range 5 to 9; std dev 1.15) with a mean duration of 0.06 seconds (range 0.05 to 0.07; std dev 0.007), and a mean interval of 0.17 seconds between chirps (range 0.14 to 0.28; std dev 0.03), three such chirps are shown in Fig. 6. The interval between calls had a mean of 7.21 seconds (range 0.75 to 15.11, std dev 4.82). Peak dominant frequency of *Callulina dawida* is at 1.6 KHz, with a notable harmonic at 3.2 KHz. The call and harmonic fall below 3.5 KHz (Figure 6), whereas *C. kisiwamsitu* is always below 2 KHz, and peak dominant frequency in *C. krefftii* is always above 2 KHz but below 3 KHz (de Sá *et al.*, 2004). The call can best be described as a fast repeated "brrr brrr brrr...".

**Etymology.** The specific name *dawida* is a noun in apposition. It is derived from Dawida, the language spoken by the inhabitants of Taita Hills where this species is found. The local Ki-dawida name king'ombe refers to the similarity in the movement of the animal to that of a cow (Ng'ombe = Ki-swahili for Cow). The common name Taita Warty Frog is given to reflect the restricted distribution of this species to Dawida and Mbololo blocks of Taita Hills, Kenya.

**Morphometrics Analysis.** We conducted a morphometric analysis on 101 *Callulina* specimens: *Callulina krefftii* (57 individuals), *C. kisiwamsitu* (23 individuals), and *C. dawida* (21 individuals). Using an ANOVA analysis, we tested three sets (each species) for all measures including ratios using snout-urostyle

length, to see if there were any statistically significant differences. Statistical significance was shown in the following characters: snout-urostyle length ratios, distal phalange width ( $p = \geq 0.001$ ), infraorbital distance ( $p = \geq 0.001$ ), and jaw width ( $p = \geq 0.001$ ) (Table 1). The degree of digital expansion is identified as a key diagnostic feature for discriminating *Callulina* species. Furthermore, the robust shape of the head is shown to be statistically different in the new species from other species, as indicated by the significant larger jaw width (Table 1). The infraorbital distance is also shown to be significantly different between species. The size of the tympanum is largest in the new species, although not statistically significant. All other morphological characters examined were not shown to be statistically significant, reflecting the generally similar shape and morphology of all species of *Callulina*.

**TABLE 1.** ANOVA analysis on select morphological characters in *Callulina*. See text for abbreviations. Group 1—*C. krefftii*, Group 2—*C. kiswamsitu*, and Group 3—*C. dawida*.

SUL/JAW WIDTH						
Group	N	Mean	Min	Max	Variance	St. dev
1	57	0.264	0.228	0.359	0.000490	0.022144
2	23	0.252	0.223	0.278	0.000228	0.015089
3	21	0.322	0.274	0.392	0.000928	0.030458
ANOVA	Current effect (group): F (2, 40)= 76.223, p= 0.00000					

SUL/DISTAL PHLANGE WIDTH						
Group	N	Mean	Min	Max	Variance	St. dev
1	57	0.0454	0.0340	0.0580	0.000036	0.006038
2	23	0.0345	0.0314	0.0415	0.000005	0.002340
3	21	0.0338	0.0283	0.0388	0.000009	0.002924
ANOVA	Current effect (group): F (2, 40)= 76.923, p= 0.00000					

SUL/INTERORBITAL						
Group	N	Mean	Min	Max	Variance	St. dev
1	57	0.1988	0.1604	0.2463	0.000306	0.017496
2	23	0.1819	0.1643	0.2037	0.000088	0.009377
3	21	0.1780	0.1587	0.2157	0.000207	0.014374
ANOVA	Current effect (group): F (2, 38)= 25.508, p= 0.00000					

**Phylogenetic Analyses.** An alignment of sequences was gathered to test two specific hypotheses, 1) that the new species forms a clade with *Callulina* species and not other brevicipitids, and 2) *Callulina dawida* was sister group to a clade including *Callulina krefftii* and *Callulina kisiwamisitu*. To test these hypotheses an alignment of eleven DNA-sequences were assembled. This resulted in a matrix of 1129 unambiguously aligned characters, of which 725 were constant, 110 variable, and 294 parsimony informative. *Hoplophryne* and *Phrynomantis*, two microhylids, were used as outgroups. An exhaustive search option using parsimony yielded one best tree (863 steps; Fig. 7a). Maximum Likelihood analyses were also conducted (using a heuristic search option using 10 random addition sequence replicates and TBR swapping method under a GTR + G model as suggested by Modeltest 3.04, Posada and Crandall, 1998). The tree resembles the parsimony tree (Fig. 7b), only differing in the position of *Spelaeophryne* – shown to have an ambiguous phylogenetic position in previous analyses (Loader *et al.* 2004). Support for clades was measured with bootstrap proportions (Felsenstein, 1985, 10,000 pseudoreplicates). In all analyses, *Callulina* species form a well-supported clade – as demonstrated by high bootstrap values. Within this grouping, the geographically

close Usambara species *C. krefftii* and *C. kisiwamsitu* form a clade – albeit weakly supported – with *C. dawida* a sister group to this clade. Overall the analyses demonstrate the genetic distinctiveness of the three *Callulina* species.

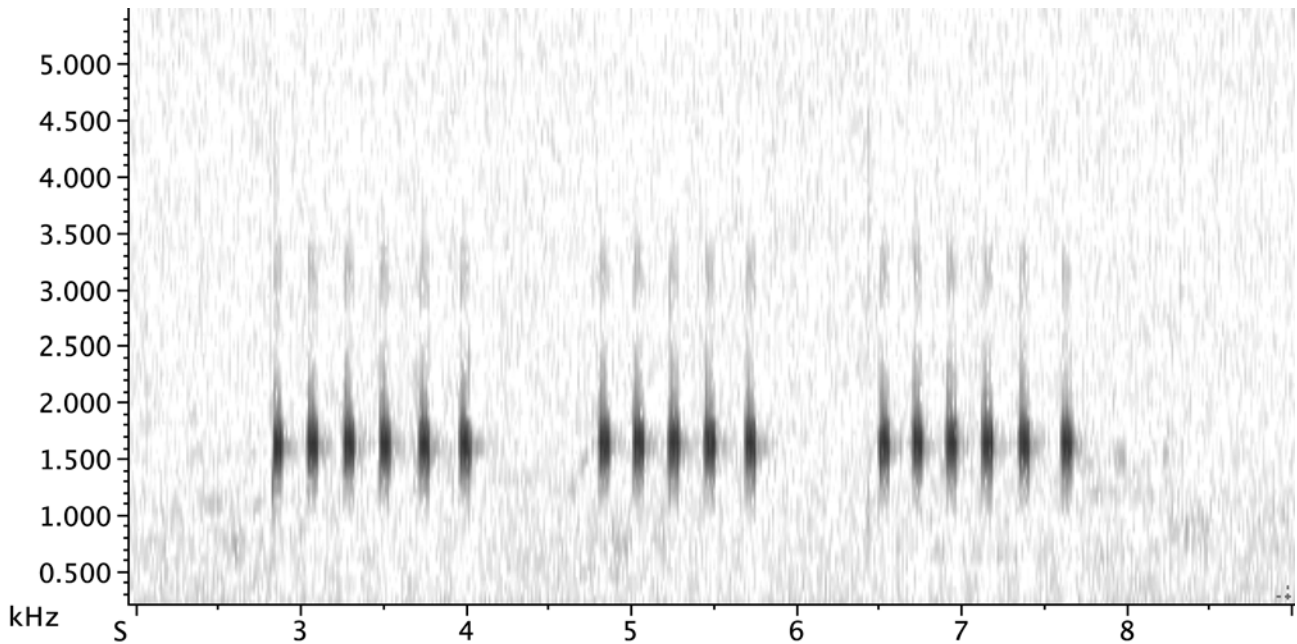


FIGURE 6. Sonogram of *Callulina dawida*.

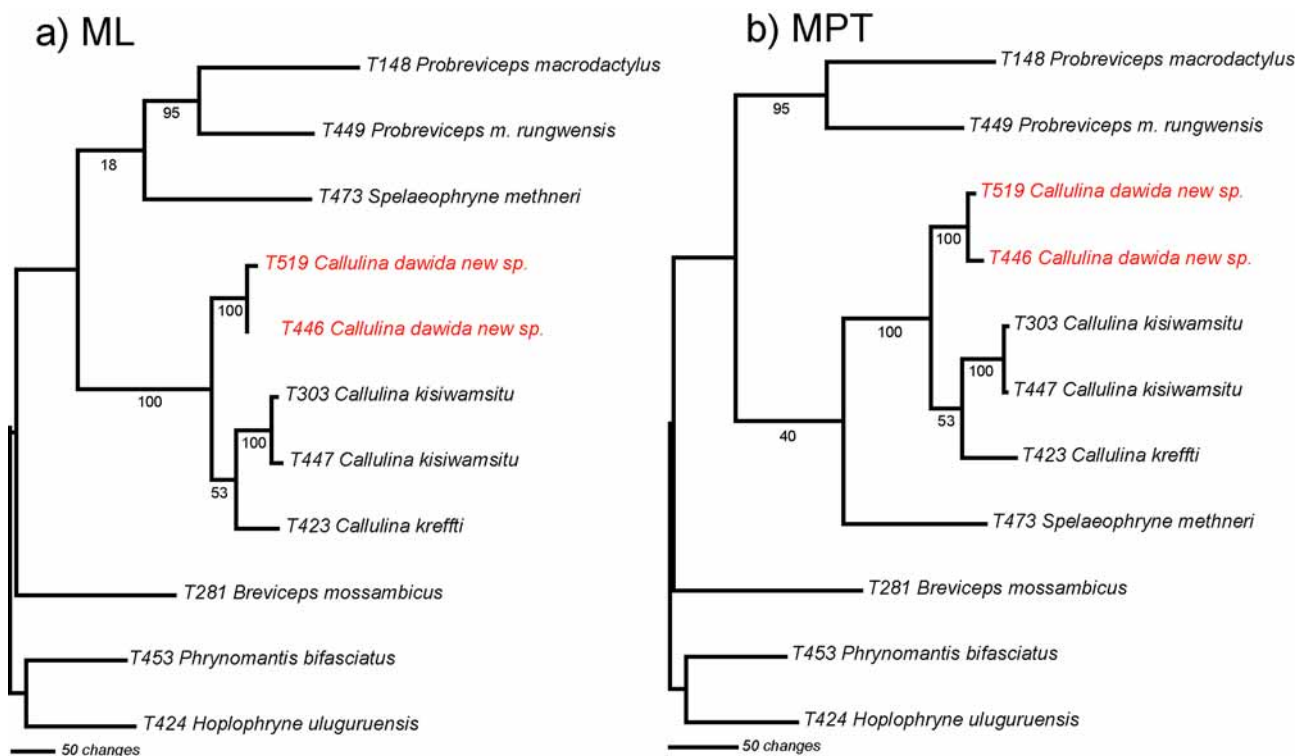


FIGURE 7. a) Maximum likelihood tree showing branch lengths. ( $-ln$  likelihood = 5576.01891). Nucleotide frequencies A= 0.3306 C= 0.2645 G= 0.1413 T= 0.2636, Nst=6, Rmat= 1.0000 2.2193 1.0000 1.0000 and 4.7793, Rates=gamma, Shape= 0.7896, Pinvar= 0.3855. Numbers below branches refer to bootstrap support. b) Maximum Parsimony exhaustive tree showing branch lengths. Numbers below branches refer to bootstrap support.

**Natural History.** During the day this species can be found buried in deep and wet leaf litter or associated

with decomposing logs. At night, individuals are encountered moving through the leaf litter, where males have been seen to call. A minority of specimens were found low down on branches in trees.

**Distribution and Conservation Status** The new species is presently only known from remnant forest patches in the Taita Hills, Kenya. Specimens were recorded from Mbololo and Dawida blocks (Mbololo, Ngangao, Chawia, Fururu, Vuria, Ndiwenyi, Mwachora and Boma-Wundanyi forests; Figure 2). Based on surveys conducted in the Taita Hills, we calculated the spatial distribution of *Callulina dawida*. The estimated extent of occurrence of *C. dawida* is equal to 168.2 km<sup>2</sup> and the estimated area of occupancy is about 4.3 km<sup>2</sup>. These are respectively the area included in the polygon (minimum convex hull polygon) obtained by linking the localities where presence of the species was recorded and the area of eight forest fragments in the Taita Hills where *C. dawida* is known to occur. Based on these spatial estimates we applied the criteria set out by the IUCN for assessing the conservation status of species. The area of occupancy, consisting of eight severely fragmented locations, was estimated to be less than 10 km<sup>2</sup>. In addition, some of the forest fragments in which this species occurs are under severe pressure from an increasing local population who utilise forest products (both cutting sticks and collecting dead wood). Therefore, according to the IUCN criteria, the species conservation status is critically endangered (CR B1a, b (ii, iii)). Positive steps are being made to conserve the indigenous forest of the Taita Hills. Several eucalyptus and pine plantations in the area have been earmarked for conversion back to indigenous forest, and it is hoped that these measures will increase the suitable habitats available for many of the endemic species of the Taita Hills.

### Key to the species of *Callulina*

*Callulina* species are distinguished primarily in the field by the truncate to expanded toe- and fingertips. Parker's (1934) initial distinction of breviciptid genera is based on the double condylar articulation between the urostyle and the sacral vertebra; a synapomorphy of *Callulina* and *Spelaeophryne*. *Spelaeophryne* and *Callulina* were distinguished by Parker (1934) on the basis of toe tip morphology.

- 1a. Fingers are expanded, the finger-tip width is greater than the width of the finger at the distal subarticular tubercle..2
- 1b. Fingers truncated, the finger-tip is not expanded beyond the width of the finger at the distal subarticular tubercle.....  
..... *Callulina dawida*
- 2a. The ratio between the widths of Finger 3 at the level of the distal subarticle tubercle relative to the width of its toe tip is always more than  $\frac{3}{4}$ . The distance between the tympanum and the posterior corner of the eye is equal to or greater than the tympanum diameter ..... *Callulina kisiwamsitu*
- 2b. The ratio between the widths of Finger 3 at the level of the distal subarticle tubercle relative to the width of its toe tip is less than  $\frac{3}{4}$ . The distance between the tympanum and the posterior corner of the eye is usually less than the tympanum diameter ..... *Callulina krefftii*

### Discussion

In the field, species of *Callulina* are easily distinguished from other breviciptids on the basis of the expansion of their toe and finger tips. *Callulina krefftii* and *C. kisiwamsitu* are often found perched on shrubs, banana plants, and trees (Channing and Howell, 2005). The ability to climb is presumably attained by the finger and toe expansion in *Callulina*, coupled with their ability to grasp with their weakly opposable hand shape (Figure 4). The new species amends the characterization of the genus. *Callulina dawida* has a truncate finger and toe shape, although still distinctly different from the rounded toes and fingers of all other breviciptids. Quantitative data is still required, but preliminary evidence suggests this species occupies an ecological niche most closely resembling that of a leaf litter, fossorial breviciptid species rather than its arboreal conspecifics. Given the basal position of the new species on the molecular tree, *C. dawida* could be an interesting transition form from fossoriality in breviciptids to arboreality in the derived *Callulina* species. Ecological and

phylogenetic studies will be required to understand the evolutionary history of *Callulina* species to investigate this more thoroughly.

Largen and Drewes (1991) showed a close similarity between the afrotemperate amphibian fauna (*Nectophrynoidea*, *Spiniphrynoidea*, *Altiphrynoidea*, and brevicitipids) of Ethiopia and Tanzania. They also noted the absence of these lineages in Kenya, which bisect these two areas. Despite many notable absences of taxa in the afrotemperate amphibian fauna of the Taita Hills of Kenya, the presence of a brevicitipid species links this area to the south (*Callulina* in the highlands of Tanzania alongside) and to the north in Ethiopia (*Balebreviceps*). Further attention should be paid to highlands of Kenya to investigate the links to montane areas in the north and south, which should allow a better understanding of the evolutionary history of the Eastern Afromontane area.

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## References

- Channing, A. and Howell, K. M. (2005) *Amphibians of East Africa*. Cornell University Press, Ithaca, 418 pp.
- Charif, R. A., C. W. Clark, and K. M. Fristrup. (2004) Raven 1.2 User's Manual. Ithaca, New York, U.S.A Cornell Laboratory of Ornithology.
- de Sá, R. O., Loader S. P. and Channing, A. (2004) A new species of *Callulina* (Anura: Microhylidae) from the West Usambara Mountains, Tanzania. *Journal of Herpetology* 38(2), 219–222.
- Frontier Tanzania, (2001) *West Kilombero Scarp Forest Reserve - Management and Summary Report*. Eds. Doody, K.Z., Howell, K.M. and Fanning, E. Report for the Udzungwa Mountains Management and Biodiversity Conservation Project. MEMA, Iringa, Tanzania. 78 pp.
- Howell, K.M. (1993) Herpetofauna of the eastern African forests. In: *Biogeography and Ecology of the Rain Forests of Eastern Africa*. Eds. Lovett, J.C. and Wasser, S.K. Cambridge University Press, Cambridge, pp. 173–201.
- Kocher, T.D., Thomas, W.K., Meyer, A., Pääbo, S., Villablanca, F.X. and Wilson, A.C. (1989) Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA* 86, 6196–6200.
- Largen, M.J. and Drewes, R.C. (1989) A new genus and species of brevicitipine frog (Amphibia Anura Microhylidae) from high altitude in the mountains of Ethiopia. *Tropical Zoology* 2, 13–30.
- Loader, S.P., Gower, D.J., Clarke, B.T., Howell, K.M., Doggart, N., Rödel, M.-O., de Sá, R.O., Cohen, B.L. and Wilkinson, M. 2004. Phylogenetic relationships of African microhylid frogs inferred from DNA sequences of mitochondrial 12S and 16S rRNA genes. *Organisms, Diversity and Evolution* 4, 227–235.
- Loader, S.P., Channing, A., Menegon, M. and Davenport, T. (2006) A new species of *Probreviceps* from the Eastern Arc Mountains, Tanzania. *Zootaxa* 1237, 45–60.
- Menegon, M., Doggart, N. and Owen, N. (2008) The Nguru Mountains of Tanzania, an outstanding hotspot of herpetofaunal diversity. *Acta Herpetologica* 3, 107–127.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B. and Kent, J. (2000) Biodiversity hotspots for

- conservation priorities. *Nature* 403, 853–858.
- Palumbi, S.R. (1996) The polymerase chain reaction. In: *Molecular systematics, 2nd edn.* Eds. Hillis, D.M., Moritz, C. and Mable, B.K Sunderland, MA: Sinauer Associates, pp. 205–247.
- Parker, H. W. (1934) *A monograph of the frogs of the family Microhylidae.* London: Trustees of the British Museum (Natural History), London. i–viii, 1–208.
- Schiøtz, A. (1981) The amphibia in the forested basement hills of Tanzania: a biogeographical indicator group. *African Journal of Ecology* 19, 205–207.
- Taylor, W. R. and Van Dyke, G. (1985) Revised Procedures for Staining and Clearing Small Fishes and Other Vertebrates for Small Bone and Cartilage Study. *Cybium* 9(2), 107–119.

## Appendix 1. Material examined

*Callulina kisiwamsitu*: BM 1982.591-597, BM 2002-45-47; field numbers RdS 917, RdS 930, RdS 931, RdS 934–940, MCZ A 13632, A13633, MHNG 2621.15-18.

*Callulina kreffti*: BM 1974.24-26, BM 1974.28-38, BM 1974.40-42, BM 1974.44, BM 2000-185-205, BM 2005.1508, ZMB 21777-8, ZMB 23341, MCZ A 13624-29, MCZ A 25492-93, USNM 200072, USNM 226754, MHNG 2609.99, MHNG ERPI 2640.098, MHNG ERPI 2640.099, CAS 162505.

*Callulina dawida*: NMK A 3647/3, A 3647/4, A 3913, A 3613/2, A 3647/5, A 3613/1, A 3647/2, A 3534/3, A 3534/2, A 3534/1, A 3619, A 3647/6, A 3647/1, A 1499/1, A 3617, A 3703, A 4266, A 4267, A 4268/2, A 4268/1, A 3535, and BMNH 2005.1574-75

**APPENDIX 2.** Details of molecular samples used in this study. Field Tag abbreviations are: MW, Mark Wilkinson Field Series, KMH, Kim M. Howell Field Series, and JM, John Measey Field Series. Locality abbreviations are: FR, Forest Reserve, NR.

No.	Species	Field No. of voucher, and Museum Accession No.	Locality	Genbank Accession
T424	<i>Hoplophryne rogersi</i>	KMH 23364, BM 2002.624	Nilo FR, East Usambara Mts. Tanzania	AY531834, AY531857, FJ998373
T453	<i>Phrynomantis bifasciatus</i>	MW 3842, BM 2005.1031	Mkomazi Game Reserve, Tanzania	AY531833, AY531856, FJ998374
T281	<i>Breviceps mossambicus</i>	MW 1826, BM 2005.1377	Sali FR, Mahenge Mts., Tanzania	AY531836, AY531859, FJ998375
T473	<i>Spelaeophryne methneri</i>	KMH 21547, BM 2000.216	Uluguru Mountains, Milawilila FR, Tanzania	AY531838, AY531861, FJ998376
T449	<i>Probreviceps m. rungwensis</i>	KMH 19141, BM 2005.873	West Kilombero Scarp F.R., Udzungwa Mts, Tanzania	AY531843 AY531866, FJ998377
T148	<i>Probreviceps m. macrodactylus</i>	KMH 16360, BM 2000.595	Amani N.R., East Usambara Mts., Tanzania	AY531851, AY531874, FJ998378
T447	<i>Callulina kisiwamsitu</i>	MW 3215, BM 2002.47	Ambangula FR, West Usambara Mts, Tanzania	AY531841, AY531864, FJ998379
T303	<i>Callulina kisiwamsitu</i>	MW 1968, BM 2002.45	Mazumbai FR, West Usambara Mts, Tanzania	AY531840, AY531863, FJ998380
T423	<i>Callulina kreffti</i>	KMH 23534, BM 2000.205	Nilo FR, East Usambara Mts., Tanzania	AY531842, AY531865, FJ998381
T519	<i>Callulina n. sp. dawida</i>	JM 1234, NMK A/4266	Taita Hills, Fururu FR, Kenya	FJ998382, FJ998383, FJ998384
T446	<i>Callulina n. sp. dawida</i>	MW 3197 NMKA 3913	Taita Hills, Ngangao FR, Kenya	FJ998385, FJ998386, FJ998387