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A new genus and species of toad from Mount Kenya illuminates East African montane biogeography

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ABSTRACT

Discoveries of new species can greatly impact our understanding of the biogeography of a region. For example, groups of amphibian lineages restricted to the Afrotemperate forests of Tanzania and Ethiopia are indicative of a shared biogeographical history of this highly discontinuous ecosystem. Curiously, many of these lineages are absent from the geographically intermediate Kenyan highlands. This phylogeographical interval is generally considered to be attributable to the younger, volcanic origins of much of the Kenyan highlands, and thus an amphibian fauna that is derived largely from recent colonization events rather than comprising older relicts. Contrasting with this view, here we report on the discovery of a single specimen of Bufonidae (true toad) from Mount Kenya. The specimen belongs to a species new to science and deserves recognition at the generic level owing to its notable molecular phylogenetic and morphological divergences from other described taxa. It is most closely related to the Tanzanian genera *Churamiti* and *Nectophrynoides*. The discovery of this new toad and its association with Afrotemperate species is significant because it links Kenya to the biogeographically more ancient Tanzanian mountains and supports the potential longevity of the Afrotemperate forests in Kenya. Broadly, it highlights that we are still adding major branches to the phylogeny of anurans.

Keywords: biogeography; Eastern Arc Mountains; East Africa Rift; Afromontane; Amphibia; Bufonidae; micro-computed tomography

INTRODUCTION

New species discoveries can alter our understanding of a group of species, its evolution, and more broadly, the biogeographical history of an area (e.g. Biju *et al.* 2003, Min *et al.* 2005, Clarke *et al.* 2007, Blackburn *et al.* 2019). With large gaps in our understanding of the spatial distribution of biodiversity, our current assumptions of how faunas and floras evolved over space and time are likely to change. These knowledge gaps are not taxonomically or spatially homogeneous. Despite being a charismatic group of vertebrates, the current number of documented amphibian species is likely to represent a vast underestimation of their true diversity (Wake and Vrendenburg 2008). In the last half-decade, nearly 150 species were described per year, with the trend for species descriptions on the rise (Streicher *et al.* 2020). New species discoveries and taxonomic revisions are therefore likely to be biogeographically insightful, particularly for regions that are historically understudied (e.g. Brown and Lomolino 1998). In comparison to other tropical regions, our understanding of the amphibian fauna of Sub-Saharan Africa remains notably incomplete (Streicher *et al.* 2020). Because of this, biogeographical hypotheses on the drivers of African species diversity remain uncertain.

The Afrotemperate forests represent a discontinuous high-elevation ecosystem that traces the entire eastern flank of continental Africa and parts of Central to West Africa. Although the continent of Africa has undergone extensive aridification over the Cenozoic, which has been suggested to have negatively impacted present-day species diversity of certain groups potentially more than in other tropical zones (Raven *et al.* 2020, Hagen *et al.* 2021), Afrotemperate forests stand out as pockets of

richness and endemism. As a result, many of these forests have been recognized as biodiversity hotspots (Myers *et al.* 2000). Although elevational limits vary depending on latitude, they share a similar climate and are likely to be refuges with relative environmental stability through time (Lovett *et al.* 2005). Sister-group relationships across these Afrotemperate locations, often across large distances separated by drier, low-lying savannah, have been documented in a wide variety of taxa (Bowie *et al.* 2004, Loader *et al.* 2014, Menegon *et al.* 2014, 2022, Grebennikov 2017). These regions might therefore once have been connected as recently as the mid- to late Pleistocene, but have become isolated as habitats have contracted in recent, drier periods (Migliore *et al.* 2020).

Not all Afrotemperate regions share the same geological history, however. The East African Rift (Fig. 1), an active continental rift zone, consists of both old (>25 Myr old) uplifted mountains, such as the Ethiopian Highlands, the Eastern Arc Mountains, and parts of Kenya (Baker *et al.* 1988, Lovett and Wasser 1993, Corti 2009), and more recent volcanic highlands, such as Mt. Kilimanjaro (~1 Myr old) and Mt. Kenya (~3 Myr old) (Grove 1983, Baker *et al.* 1988). Although all hold high-elevation areas of increased rainfall suitable for Afrotemperate forest, their biogeographical histories are likely to be distinct, given their different ages and the extent of humidity retention during drier cycles (Hamilton 1982). The relationships of Afrotemperate fauna and flora can inform the biogeographical history of this montane archipelago and elucidate how species diversity has accumulated across this unique, but heterogeneously formed ecosystem (e.g. Chartier *et al.* 2016).

Countries such as Tanzania and Ethiopia host some of the oldest stable mountain ecosystems in the eastern branch of the rift system (Lovett and Wasser 1993, Lovett *et al.* 2005, Corti 2009), and therefore also hold disproportionate amounts of diversity compared with areas such as Kenya, which is mostly recent and volcanic (e.g. Baker 1988, Lovett and Wasser 1993, Lovett *et al.* 2005, Burgess *et al.* 2007, Loader *et al.* 2014, Siu-Ting *et al.* 2014). The relatively lower species richness in Kenyan Afrotemperate zones has been noted in different taxonomic groups (Diamond and Hamilton 1980, Rodgers *et al.* 1982, Scharff 1992, Brühl 1997), and this paucity has been named the 'Kenyan interval' (Poynton 1999: 498). However, whether the Kenyan interval exists or is simply the consequence of proportionally lower sampling in the region is not clearly understood.

It is unclear how these volcanic highlands interact faunistically with the more ancient highlands to the north and south of Kenya. Palaeoenvironmental data show shifts between lowland and highland forest and savannah habitats, and tectonic and volcanic activity is more pronounced in Kenya (Hamilton 1982, Lamb *et al.* 2007, Scholz *et al.* 2007). This has meant that assemblages have been subject to heavy local species extinction and recolonization (Demos *et al.* 2014). This is particularly evident in organisms with high dispersal capabilities (e.g. Dijkstra 2006, Popp *et al.* 2008, Odinti *et al.* 2021). Other studies, however (e.g. Scharff 1992, Tolley *et al.* 2011, Menegon *et al.* 2014, Chartier *et al.* 2016), have outlined the prevalence of old Rift Mountain clades that include lineages with narrow ranges in Eastern Afrotemperate parts of Kenya, such as the Aberdare Mountains and Mount Kenya. Determining the phylogenetic relationships

of species occurring there can therefore help to disentangle these opposing biogeographical hypotheses on faunal origins.

Here, we report on a single specimen of an exceptional toad (Bufonidae) found during survey work on Mount Kenya. Based on morphological and molecular data, we show that the species is distinctive and deserves recognition at the genus level. Intriguingly, it shows a close relationship with eastern Afrotemperate bufonid genera and therefore connects phylogeographically the Afrotemperate region of Kenya with those of Ethiopia and Tanzania. The biogeographical significance of this new taxon is discussed.

MATERIALS AND METHODS

Specimen preservation and morphology

A single specimen was collected from the eastern flanks of Mount Kenya during a survey of the Chogoria Forest block in 2015 (−0.207056, 37.500667, elevation 2396 m; Fig. 1). The individual was recovered alive from a pitfall trap, but was in poor condition (Supporting Information, Fig. S1A–D). The specimen was euthanized using tricaine methanesulfonate (MS-222; Sigma Aldrich), fixed in 10% formaldehyde (formalin), stored in 70% ethanol, and deposited in the National Museums of Kenya (NMK). Tissue samples were taken from leg muscle before fixation and preserved in 96% ethanol.

The specimen was measured using Mitutoyo CD-6″ CS electronic callipers. Measurements were taken to the nearest 0.1 mm by S.P.L. Twenty-five measurements were taken, mainly (* noted if not) corresponding to standard amphibian morphometrics defined in detail by Watters *et al.* (2016), including: snout–vent length (SVL), snout–urostyle length (*SUL), head width (HW), head length (HL), body width (BW), thigh length (THL), tibia length (TL), tarsus length (TSL), internarial distance (IND), eye diameter (ED), interorbital distance (IOD), snout length (SL), head length (HL), nostril–lip distance (*NLD), eye–nostril distance (EN), forearm length (FLL), hand length (HAL), finger IV disc width (Fin4DW), subarticular tubercle width (*STW), greatest length of inner carpal tubercle (ICT), greatest length of outer carpal tubercle (OCT), length of inner metatarsal tubercle (IMC), length of outer metatarsal tubercle (OMC), toe IV length (Toe4L), and toe IV disc width (Toe4DW). Numbering of fingers is based on homology, vertebrate digits II–V (from inner to outer digits), as recommended by Watters *et al.* (2016).

For characterizing osteological features, the specimen was scanned at the Natural History Museum (NHM) with a Nikon (Metris) X-Tek HMX ST 225 System. Volumes were dissected digitally to create volumetric models of skeletal structures using AMIRA v.2020.3.1 (Thermo Fischer Scientific). Additional bufonid material was examined for comparison (Supporting Information, Table S1).

DNA extraction, amplification and sequencing

DNA was extracted from leg muscle tissue preserved in ethanol using Qiagen DNeasy tissue kits (Qiagen, Valencia, CA, USA). Subsequently, fragments from two nuclear markers (*CXCR4*, 711 bp; and *RAG1*, 933 bp) and three mitochondrial markers (12S, 377 bp; 16S, 513 bp; and *COI*, 840 bp) were amplified via PCR using Illustra puReTaq Ready-To-Go PCR beads (GE
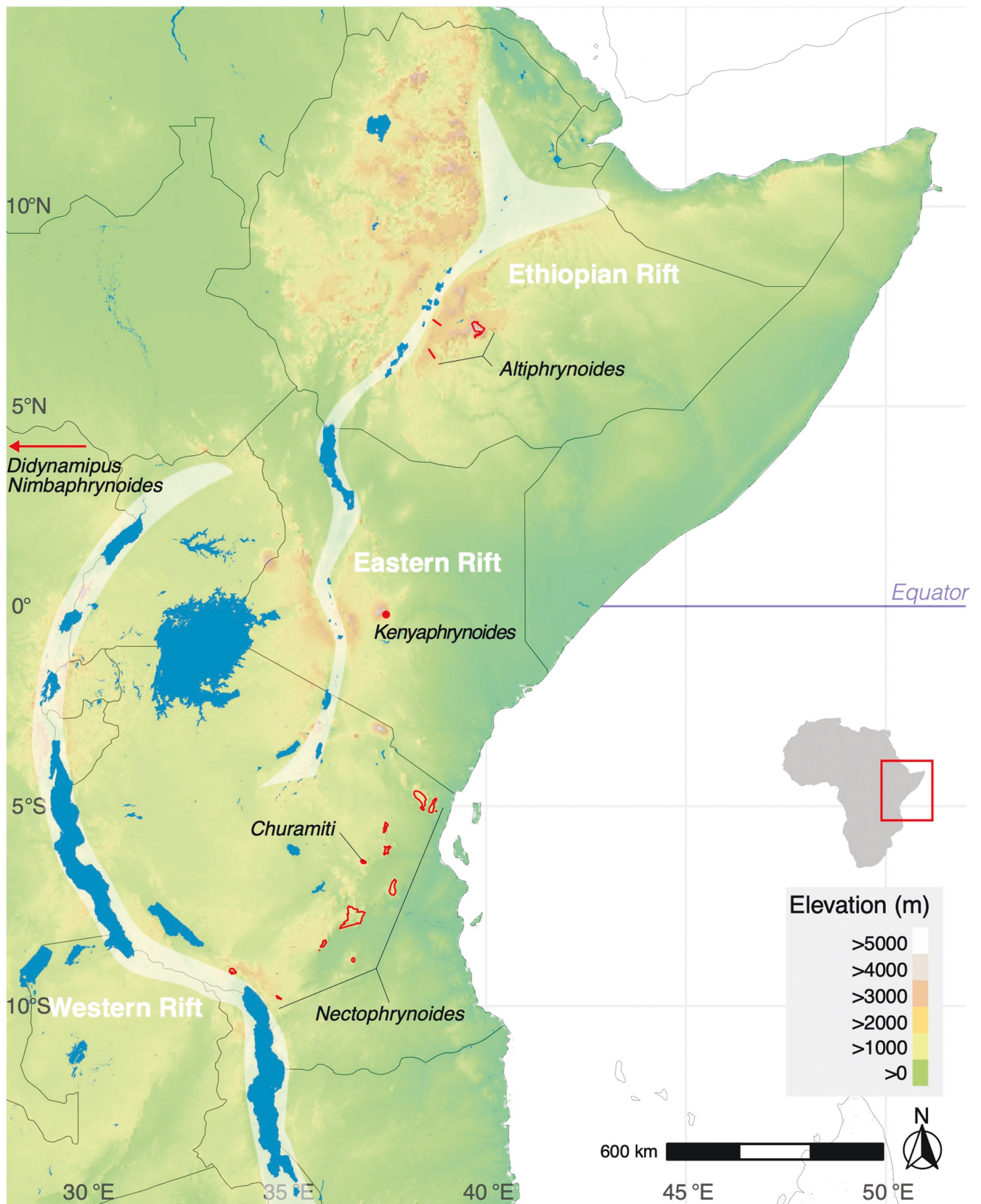
Figure 1. Map of Eastern Africa showing the distribution of ***Kenyaphrynoides vulcani*** and related bufonid genera (red polygons). Spatial data were obtained from the IUCN Red List (2022). Also shown are the approximate traces of the Western and Eastern branches of the East African Rift system (translucent white polygons).

Healthcare, Amersham, UK). Primers used and PCR conditions are given by Liedtke *et al.* (2017). The PCR products were visualized on 1% agarose gels, and successful amplifications were purified and sequenced at NHM molecular laboratories. Complementary strands were sequenced and subsequently proofread using the bioinformatics platform GENEIOUS PRO v.5.6.7 (created by Biomatters, available from https://www.geneious.com). Nucleotide sequence data were deposited in GenBank (OR211585, OR11684, OR116845, OR465047 and OR465049).

Alignment and phylogenetic reconstruction

To provide phylogenetic context, the newly generated sequences were aligned to those of other, closely related bufonids. We selected at least one representative of all genera of the relevant bufonid clades, based on the work by Liedtke *et al.* (2016), and single representatives of all available species of *Nectophrynoides* (11 of the 13 currently described species), the most likely closest relatives based on 16S barcodes. *Bufo bufo* (Linnaeus, 1758)was also included to serve as an outgroup. The resulting alignments contained 23 species, of which 22 had complete gene sets, with one (*Nectophrynoides frontierei* Menegon, Salvidio, and Loader, 2004) having only mitochondrial sequences. The full list of sequences is available as Supporting Information (Table S2). Sequences lists per gene fragment were processed using GENEIOUS PRO. Coding genes (*CO1*, *CXCR4*, and *RAG1*) were aligned with the MAFFT v.1.5.0 plug-in using the auto setting, and the alignment block was manually trimmed to the open reading frame. PASTA v.1.8.5 (Mirarab *et al.* 2015), a tree-based aligner, was used to align 12S and 16S using MAFFT as the aligner, OPAL as the merger, and FASTTREE as the tree estimator under a GTR+CAT model. Subproblems were limited to 12, with an iteration limit stop rule of 100. We then used IQ-TREE v.2.2.0 (Minh *et al.* 2020) to find the best partition scheme (treating codon positions of coding genes as independent partitions; Supporting Information, Table S3) and to reconstruct the phylogeny (MFP + MERGE mode with 1000 bootstrap iterations). The outgroup was fixed to be *Bufo bufo*. Trees were estimated based on a concatenated alignment of all five loci, and on subsets for the concatenated mitochondrial DNA (12S, 16S, and *COI*) or each of the two nuclear loci (*RAG1* and *CXCR4*).

IQ-TREE was also used to test explicitly four topological hypotheses, to assess whether inserting the new species in existing, closely related genera (based on 16S barcodes) would be a significantly better fit than assigning it to its own, basal lineage (i.e. H_0, the new species falling outside the *Churamiti*–*Nectophrynoides* clade). The alternative hypotheses tested were as follows: H_1, the new species falling in between *Churamiti* and *Nectophrynoides*; H_2, the new species and *Churamiti* forming a single sister clade to *Nectophrynoides*; and H_3, the new species falling inside the *Nectophrynoides* clade. We set 10000 RELL replicates and included weighted Kishino-Hasegawa (KH), weighted Shimodaira-Hasegawa (SH) and Approximately Unbiased (AU) tests as recommended by the software authors.

The proportions of pairwise sequence differences in the 16S alignment were calculated using the R package 'ape' v.5.7-1, ignoring sites where at least one of the pairs had missing data.

RESULTS

Molecular phylogenetics

Analysis of the 23-species, five-locus dataset of African bufonids does not place the Kenyan specimen within any existing genera, instead forming an independent phylogenetic branch, representing a sister lineage to the Eastern Arc clade comprising the genera *Churamiti* and *Nectophrynoides* (99% bootstrap support; Fig. 2). The new bufonid genus shows divergence patterns approaching those of other generic groupings in the dataset. The 16S locus shows a pairwise sequence distance of 7.56% when compared with *Churamiti* and 10.62% when compared with *Nectophrynoides wendyae* (Fig. 2). In comparison, the average pairwise distance within *Nectophrynoides* was 5.90% (SD: 2.02%) and the average distance between genera (excluding *Bufo bufo* and with *N. wendyae* Clarke, 1988 representing all *Nectophrynoides*) was 11.91 ± 2.31%.

The topological relationship between the new, Kenyan specimen and *Churamiti* remains to be validated. The AU topology test could only significantly reject the topology where this specimen falls inside *Nectophrynoides* (H_3; $P < 0.001$; Supporting Information, Table S4), but all other topological hypotheses had similar log likelihoods. Nonetheless, genetic loci appear to have shared histories, recovering the same most likely topologies when analysing the mitochondrial loci and the *RAG1* nuclear marker separately (*CXCR4* was generally uninformative; Supporting Information, Fig. S2).

External and skeletal morphology

The genetic data resolve the Kenyan specimen to be closely related to *Churamiti* and *Nectophrynoides*, but distinct from both. At the skeletal level, this is supported by the presence of eight presacral vertebrae, the lack of an ossified columella, a T-shaped terminal phalanx with a broad crossbar, giving it a more chisel-like shape, and an unossified sternum. Externally, thumb spines (probably modified nuptial pads; Fig. 3B) are present, the finger tips are spatulate shaped, eyelids are not glandular, forearms are without a large glandular mass, and toe tips are without lamellae on the ventral edge.

GENERIC AND SPECIES DESCRIPTION

Kenyaphrynoides gen. nov.

Nectophrynoides sp. (Spawls *et al.* 2019: 15). Bufonidae *incertae sedis* (Malonza and Bwong 2023: 516)

Type species: Kenyaphrynoides vulcani gen. et sp. nov. Liedtke, Malonza, Wasonga, Müller and Loader by original designation.

Etymology: The generic name is derived from the words 'Kenya', a reference to the country and to the eponymous mountain, from which the name for the country was originally derived, and 'phrynoides', meaning toad-like. For nomenclatural purposes, we consider it to be neutral.

Diagnosis: Kenyaphrynoides can be distinguished from other bufonid genera by the combination of the following characters: lack of continuous parotoid glands (similar in *Churamiti* and different from some *Nectophrynoides*); eyelids lack glandular
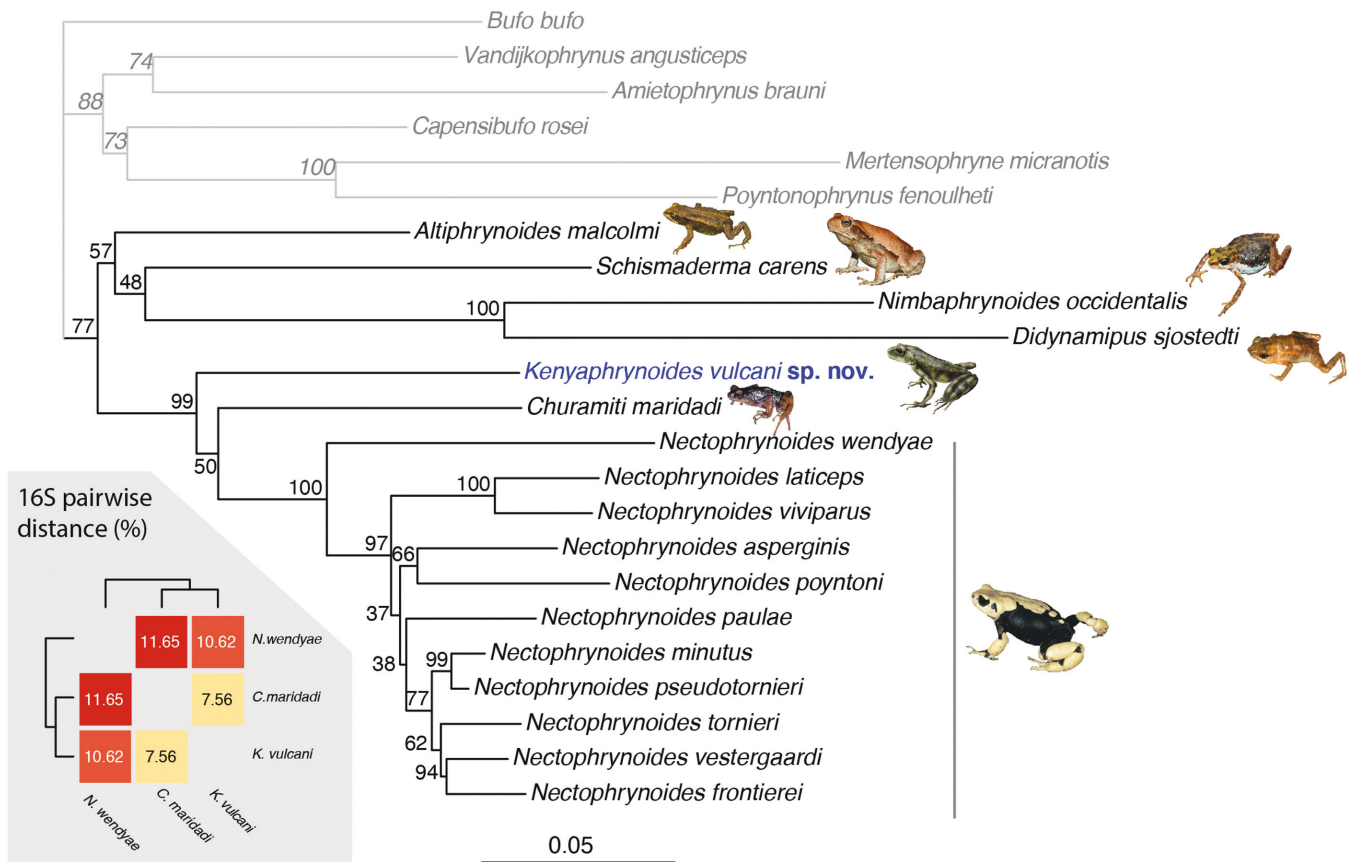
Figure 2. Maximum likelihood phylogeny of Afrotemperate dwarf bufonids and relatives. Branch support refers to bootstrap values, and branch lengths represent the number of nucleotide substitutions per nucleotide site (see scale bar). Grey inset shows uncorrected 16S genetic pairwise distances.

masses (glandular in *Churamiti*); lack of tympanum; forearms without a large glandular mass (present in *Churamiti* and some *Nectophrynoides*); spatulate toe tips (differently shaped in *Churamiti* and *Nectophrynoides*); toes with marginal webbing; toe tips without lamellae on ventral edge (present in *Churamiti*); nuptial spines on thumbs in males (similar to *Nimbaphrynoides*, absent in *Churamiti* and *Nectophrynoides*); distinct green/brown dorsal coloration and cream ventrum (somewhat similar in *Churamiti*, different in *Nectophrynoides*); eight presacral vertebrae (seven in *Churamiti*, eight in *Nectophrynoides*); tips of terminal phalanges broadly T-shaped, chisel-like (T-shaped with slender, curved crossbar in *Churamiti*, more knob-like or slightly T-shaped in *Nectophrynoides*); an unossified sternum (ossified in *Churamiti*, not ossified in *Nectophrynoides*); and lack of an ossified columella (absent in *Churamiti*, present in *Nectophrynoides*).

Distribution: *Kenyaphrynoides* is only known from Chogoria Forest Block on Mount Kenya.

Mount Kenya forest toad

Kenyaphrynoides vulcani sp. nov.

Holotype: NMK A6217, male. Collected on 9 September 2015 in Mount Kenya Forest in Chogoria Forest Block, Tharaka-Nithi

County (0°12′25.4″S, 37°30′02.4″E, elevation 2396 m; Fig. 1) by Domnick V. Wasonga and Joash Nyamache.

Description of holotype: For all measurements, see the Supporting Information (Table S1). Medium-sized male frog (45.2 mm SUL, 42.9 mm SVL), body elongate. Body width 15.0 mm, head width 15.7 mm. Arms and legs relatively long, gracile (Figs 3, 4). Outline of head in dorsal and ventral view oval, snout blunt. Eyes not visible in ventral view. Canthus rostralis flat, obliquely directed. In dorsal view, snout distinctly raised bulge, starting from anterior edge of the eye along to each nostril [canthus rostralis]. Lateral snout margins slightly expanded and curved at margin of canthus rostralis and midline of head, resembling upper parts of two parallel, sausage-shaped structures from eye to snout. Nostrils laterally directed, 3.5 mm apart, much nearer to apex of snout (one-quarter of total distance from snout tip to eye) than eye. Ventrally, chin region relatively smooth, with small glands present on jaw line. Tongue present, without any papillae. Choanae present on anterior part of roof of mouth. Eyes large (4.6 mm). No tympanum visible. Interorbital distance (5.4 mm) almost twice the internarial distance (3.5 mm).

Dorsal body surface with glandular masses symmetrically distributed along the midline. Dorsum glandular, particularly around the parotoid region anteriorly and laterally, glands not coalesced into recognizable parotoid gland. Glandular masses circular, ≤1.5 mm, shown as darker or lighter coloured areas on dorsum. Ventral skin
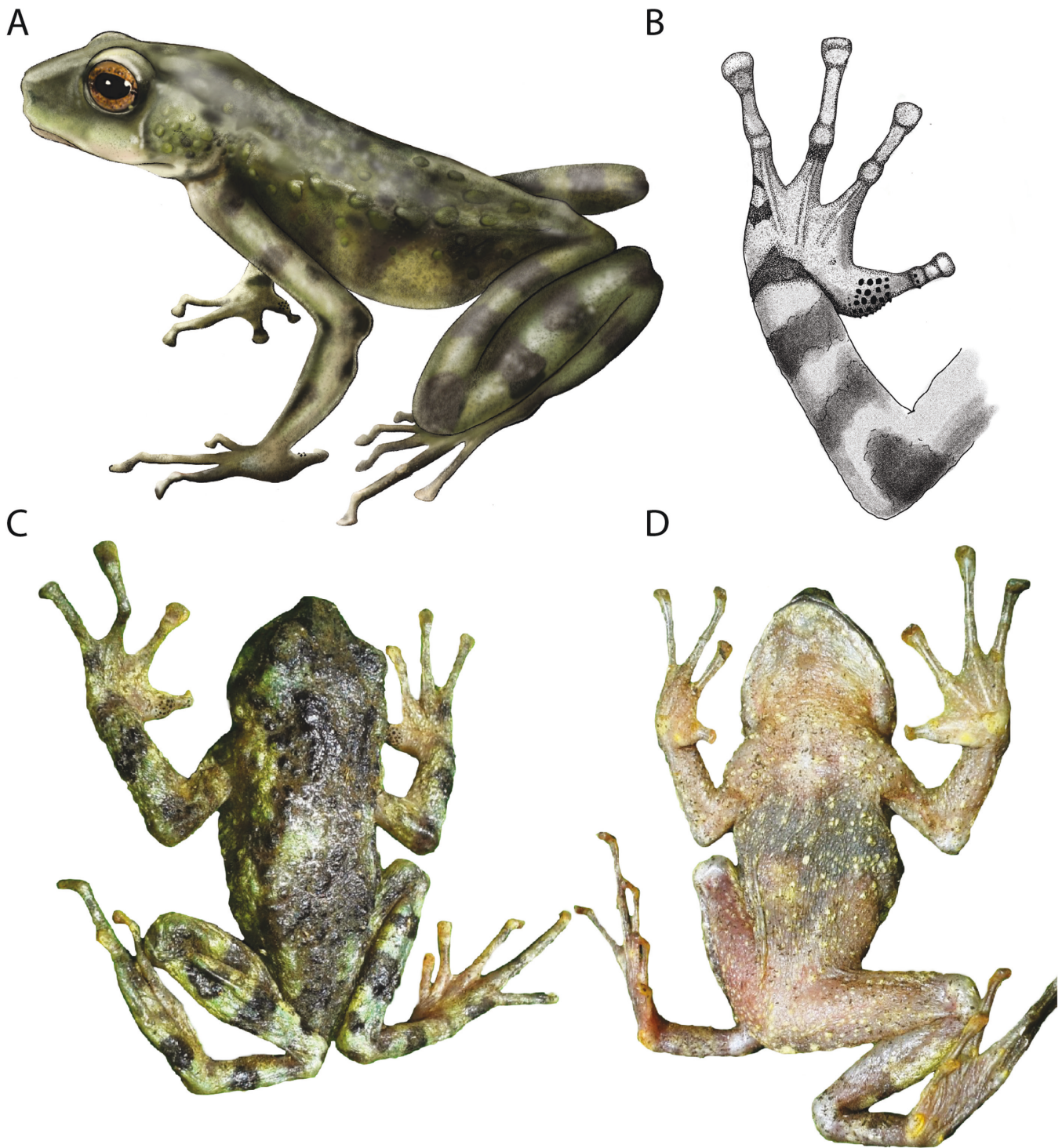
Figure 3. Artistic rendition of ***Kenyaphrynoides vulcani*** (A). Drawing of a dorsal view of the left hand of the holotype (B), and dorsal (C) and ventral (D) photographs of the holotype prior to preservation.

granular. Vent relatively smooth, lacking ornamentation or spines, ventrally displaced, with overhanging dorsal skin flap. A lateral incision on the ventral side, and on ventral side of the right thigh, with ~15 mm × 3.5 mm strip of skin and musculature missing.

Arms moderate in size, with slightly thickened forearms, barred brown and cream. Fingers moderately long, lacking webbing, with expanded spatulate tips on fingers III–V. Finger II blunted and somewhat flattened, but tip not expanded. Palm

with enlarged basal tubercle. Thin, elongated tubercle at base of thumb (finger II). Smaller tubercles present on each finger joint. Finger II shorter than III, IV, and V; III and V equal in length, finger IV longest (15.8 mm). Black spines resembling nuptial pads with papillary epidermal projections (see Luna *et al.* 2018) on dorsal surface of first joint on finger II, ~20 in total, grouped but not overlapping (Fig. 3B). A smaller number of spines coalescing at base of first finger tip. No spines on ventral surface.

Figure 4. Lateral (A), dorsal (B), and ventral (C) views, and ventral aspect of right foot (D) and left hand (E) of the holotype of *Kenyaphrynoides vulcani*.

Legs long, with tibiotarsal articulation reaching level of snout. With a larger, broadly oval inner tubercle at base of toe I and a smaller, more elongated outer tubercle at base of toe V. Inner tubercle about double the size of outer tubercle (see Fig. 4D). Toes relatively long, thin, lacking webbing apart from a thin fringe at the base of each toe. Toe IV longest (23.7 mm), with III, V, II, and I in descending order of length. Tips of toes slightly swollen and expanded, spatulate in shape.

Specimen mottled brown and cream dorsally, with darker brown mottling anteriorly. Snout dark brown. Legs barred brown and cream. Ventral side cream.

Coloration of species in life: The dorsum dark brown, with light green patches. Light green coloration more dominant on lateral sides. The background colour of upper surfaces of arms and legs light green with three dark bands (two on femur and one on tibia, ~8 mm in thickness). Venter beige, with pinkish coloration on groin, chest, and neck. Throat whitish, especially margins of jaws. Prominent white-tipped glandular warts scattered across vent, fewer on limbs.

Skeletal morphology: Skull nearly as long as wide, with bluntly pointed snout formed by nasals and premaxillae (Fig. 5A). In lateral view, dorsal side of skull tapers slightly towards tip of snout, ending abruptly, with alary processes of premaxillae nearly vertical. Jaw articulation slightly below anterior end of otic capsule. Dorsally exposed part of nasal longer than wide, forming anterior-most border of orbit. Edges of the nasal, especially anteriorly and posteriorly, irregular. Sphenethmoid single, poorly ossified, almost completely exposed dorsally.
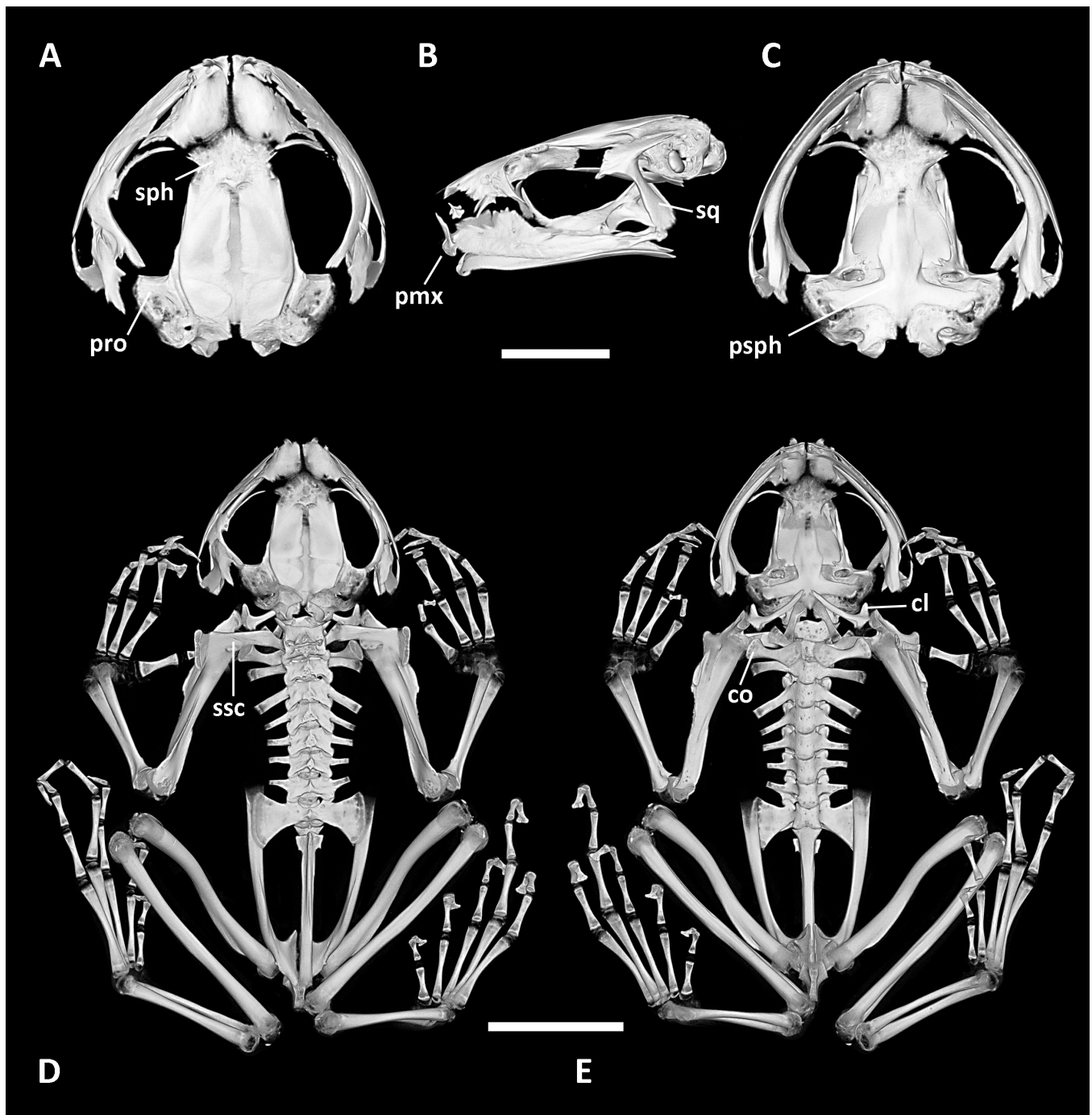
Figure 5. Dorsal (A), lateral (B), and ventral (C) view of the skull, and dorsal (D) and ventral (E) view of the whole skeleton of the holotype of *Kenyaphrynoides vulcani*. Features indicated are the cleithrum (cl), coracoid (co), premaxilla (pmx), prootic (pro), sphenethmoid (sph), squamosal (sq), and suprascapula (ssc).

Frontoparietal with somewhat irregular medial edge; both nasal and frontoparietal not in contact with their antimeres medially. Prootic poorly ossified. Ossified columella or operculum absent. Premaxilla toothless, alary process relatively broad and short (about half as wide as long), widely separated from nasal and about as long as pars dentalis. Palatine shelf of premaxilla with deep, V-shaped medial notch. Septomaxilla small, with median notch, slim, pointed dorsal process, and more broad, blunt posterior process. Maxilla toothless, terminating posteriorly somewhat before otic region. The quadratojugal relatively thin, with an expanded, knob-shaped posterior end, not in direct contact with maxilla. Pterygoid relatively broad and stout, with more slender posterior ramus extending ventrally towards jaw articulation to about level of end of squamosal; a slightly shorter, broader medial ramus extends towards, but terminates well before, the prootic. Palatine slender, slightly curved, isolated rod on right side of skull, slightly longer and contacting pterygoid on left side. Vomer relatively short, stout, with weakly developed, triangular lateral process. Cultriform process of parasphenoid broadest immediately anterior of prootic and gradually narrowing anteriorly,

ending with irregularly shaped, broadly rounded tip that narrowly overlaps with posterior edge of sphenethmoid ventrally. Lateral processes of parasphenoid slightly more than twice as long as wide, angled backwards by ~10°. Squamosal triradiate: a long ventral ramus increases in width towards jaw articulation, ending there in a broadly rounded arch; an otic ramus slightly longer than half the ventral ramus, tapering to a point slightly above midline of otic fenestra; a short, broad zygomatic ramus about half the length of otic ramus.

Eight presacral vertebrae, with transverse processes on presacral vertebrae 2–8. These are directed anterolaterally on vertebra 2, slightly posterolaterally on vertebra 3, posterolaterally on vertebrae 4 and 5, and roughly laterally on vertebrae 6–8. Sacral diapophyses broadly triangular, about twice as wide laterally as medially, with the lateral width about double the width of centrum. Urostyle relatively straight and even, with slight, rounded dorsal keel on its anterior half.

Pectoral girdle comprises clavicle, coracoid, scapula, and suprascapula; ossified sternum and omosternum absent. Clavicle slender, gently curved, extending medially almost to midline of pectoral girdle, without contact with its antimer. Coracoid relatively broad, with enlarged lateral and medial ends, with medial end being ~2.5 times the width of narrowest part of coracoid. Scapula directed dorsolaterally and relatively stout, with greatest width being about two-thirds its length. Suprascapula rather robust, slightly longer than scapula, distinctly L-shaped. Slightly offset positions of coracoids in cross-section suggest an arciferal girdle, but exact anatomy is difficult to assess from uncontrasted computed tomography scan.

Pelvic girdle consists of ilium, ischium, and pubis, with pubis only weakly ossified. Ilium, ischium, and pubis not sutured or fused; acetabulum not completely ossified. Ilium and ischium seem well ossified; pubis smallest and seemingly less well ossified; no crest on shaft of ilium.

The humerus is subtly longer than radioulna, with low dorsal crest extending medially from its lateral end for about two-thirds its length; with broad flange-like crest anteromedially, extending for about one-quarter to one-third the length of humerus. Carpal elements only poorly ossified; phalangeal formula 2-2-3-3 on manus; terminal phalanges broadly expanded, T-shaped terminally on fingers III–V, less pronounced on finger II. T-shape with broad crossbar, giving it a more chisel-like appearance. Femur and tibiofibula near equal in length. A small and a tiny sesamoid are present at ankle joint. Tibiale and fibulare equal in length, tibiale subtly more slender in width. Phalangeal formula 2-2-3-4-3 on pes, with distal tips of distal phalanges broadly expanded, but less extensively than in manus.

Habitat and natural history: The specimen was collected in September, during the dry season, and midday temperatures were relatively hot. It was collected in a pitfall trap in a moist indigenous montane forest with streams nearby (Supporting Information, Fig. S1). The habitat had a good amount of dry leaf litter, moss-covered tree trunks, and fallen logs. The nights and better parts of the morning had heavy mist cover, and the forest received some amount of rain in the afternoons within the week when the specimen was found.

The forest and moorlands of Mount Kenya have been sampled for amphibians in various locations (see Malonza 2015).

In brief, surveys in 2008, 2009, 2015, 2017, and 2019 sampled forest blocks including Naro Moru, Chogoria, Irangi, Sirimon, and Kamweti between elevations of 1550 and 3392 m and yielded 84 anuran individuals in total (Malonza 2015, Malonza and Bwong 2023). *Kenyaphrynoides vulcani* (one individual) was found within the Chogoria forest with five other species: *Hyperolius cystocandicans* Richards and Schiøtz, 1977 (seven), *Amietia nutti* (Boulenger, 1896) (two), *Phrynobatrachus* sp. (one) *Hyperolius montanus* (Angel, 1924) (five), and *Amietia wittei* (Angel, 1924) (three). Surveys across all forest blocks gave cumulative numbers of specimens including: *Amietia nutti* (seven), *Amietia wittei* (17), *Cacosternum kinangopensis* Channing and Schmitz, 2009 (12), *Hyperolius cystocandicans* (32), *Hyperolius montanus* (Angel, 1924) (15), *Kenyaphrynoides vulcani* (1), and *Phrynobatrachus* sp. (1). It is interesting to note the relative rarity of *K. vulcani* in comparison to most other species collected on Mount Kenya. Whether this reflects the difficulties in observations, or a small, potentially declining population is difficult to say but requires further research.

No observations of the breeding biology of *K. vulcani* were made other than the presence of nuptial spines on the single specimen that was collected.

Vocalizations: Not known.

Etymology: The name *vulcani* is in reference to the more recent, volcanic origins of Mount Kenya, the only locality from which this species is known. The species name is a noun in the genitive form ('of the volcano').

Common name: Kenyan Volcano toad.

DISCUSSION

Based on morphology and genetics, the newly discovered specimen is distinct from any known amphibian and requires the description of a new genus and species: *Kenyaphrynoides vulcani* (Fig. 3A–D). The new species is sister to a clade of Tanzanian montane toads comprising *Nectophrynoides* and *Churamiti* and therefore forms part of the Afrotemperate toad clade that also includes Ethiopian and Central/West African genera (*Altiphrynoides*, *Nimbaphrynoides*, and *Didynamipus*), in addition to the widespread shrubland and savanna genus *Schismaderma*.

The molecular data available suggest a sister relationship with the Tanzanian genera, although deeper nodes in this clade are not completely resolved. Resolving older nodes for the family Bufonidae appears to be particularly difficult, with previous studies pointing to a burst of diversification experienced early on in the history of the clade (Van Bocxlaer et al. 2010). Based on phylogenetic node ages from the study by Liedtke et al. (2017), the *Kenyaphrynoides* lineage is likely to be ~20 Myr old. It therefore represents the first palaeoendemic Afrotemperate toad lineage from Kenya. This is significant because most other Anura lineages known from these highlands are not deeply divergent from lineages typical of East African highland forests elsewhere (usually belonging to the same genus). Brevicipitidae and Petropedetidae are the only other two truly Afromontane anuran groups with records from Kenya (Loader et al. 2014, Siu-Ting et al. 2014, Bwong et al. 2017). The discovery of *K. vulcani*

corroborates the existence of old forest connections between the volcanic mountains of Kenya and the ancient crystalline mountains of Tanzania, thought to have shared only a recent biogeographical history. It is not clear what biogeographical event linked Mount Kenya to, and separated it from, the Eastern Arc, but there are multiple climate changes in the Cenozoic impacting forest cover (Couvreur *et al.* 2008).

The estimated age of divergence of *Kenyaphrynoides* and *Churamiti/Nectophrynoides* (~20 Mya) and the age of Mount Kenya (~3 Mya) is a notable discrepancy and one that is a current biogeographical conundrum. The faunal and floral biogeography of the volcanic Kenyan highlands is clearly complex, and their relationship with other blocks in the East African Rift system continues to be intriguing (Spawls and Rotich 2013). Although recent studies continue to find examples of Pleistocene colonization events of Mt. Kenya (e.g. Bryja *et al.* 2017), our discovery marks an important addition to the comparatively fewer known cases of palaeoendemics with likely Miocene origins, such as chameleons (Tolley *et al.* 2011) and forest vipers (Menegon *et al.* 2014).

With only a single specimen it is difficult to infer much about the biology of this species. Based on phylogeny, *K. vulcani* is closely related to both *Churamiti maridadi* and *Nectophrynoides* spp., the former a species assumed to breed in open water bodies based on ovarian clutch characteristics (Channing and Stanley 2002; Liedtke *et al.* 2014, 2017), whereas the latter comprises a group of viviparous (lecithotrophic) species. With the only known specimen of *K. vulcani* being a male, no inferences of its potential breeding biology based on ovarian egg sizes or numbers are possible. The male specimen of *K. vulcani* has thumb spines on its inner finger (Fig. 3B), which are present in other bufonid taxa both in open water breeders and viviparous ones, such as in *Mertensophryne micranotis* and *Nimbaphrynoides occidentalis* (e.g. Sandberger *et al.* 2017). Interestingly, nuptial pads are fairly enlarged in some phytotelm-breeding taxa, involving thumb spines, such as in *Crossodactylodes* (Leptodactylidae; Cochran 1955, Barata *et al.* 2013), *Hoplophryne* (Barbour and Loveridge 1928, Noble 1931), some species of *Kalophrynus* (Matsui 2009), and *Osteopilus wilderi* (Dunn, 1925) (Luna *et al.* 2018). Although these thumb spines are not informative for assessing its exact type of breeding biology, if nuptial ornaments are seasonal, as in related toad species (Sandberger-Loua *et al.* 2017), their presence might indicate the breeding season to be around the time the specimen was collected (i.e. September). Both *Churamiti* and *Nectophrynoides* are arboreal taxa. Given that distally expanded toe tips are indicative of a climbing nature (Kamermans and Vences 2009), *K. vulcani* might share similar arboreal habits. Further detailed ecological research will be required on this enigmatic species before its breeding biology can be inferred. Targeted survey work is also needed to assess its conservation status because its likely restricted distribution would make it very vulnerable to environmental change.

SUPPLEMENTARY DATA

Supplementary data is available at *Zoological Journal of the Linnean Society* online.

CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY

All data are made publicly available. DNA sequences are deposited in GenBank (see accession numbers in Supporting Information, Table S2), and skeletal scans are deposited in MorphoSource (see accession numbers in Supporting Information, Table S1). Morphological data are provided in Supporting Information, Table S5.

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix 'http://zoobank.org/'. The LSID for this publication is: urn:lsid:zoobank.org:pub:2BBB2C42-F482-45D4-BC6E-A55C3D06D207. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central and LOCKSS.

REFERENCES

Baker BH, Mitchell JG, Williams LAJ. Stratigraphy, geochronology and volcano-tectonic evolution of the Kedong–Naivasha–Kinangop region, Gregory Rift Valley, Kenya. *Journal of the Geological Society* 1988;**145**:107–16.

Barata IM, Santos MT, Leite FS *et al*. A new species of *Crossodactylodes* (Anura: Leptodactylidae) from Minas Gerais, Brazil: first record of genus within the Espinhaço Mountain Range. *Zootaxa* 2013;**3731**:552–60.

Barbour T, Loveridge A. A comparative study of the herpetological faunae of the Uluguru and Usambara Mountains, Tanganyika Territory with descriptions of new species. *Memoirs of the Museum of Comparative Zoology. Cambridge, Massachusetts* 1928;**50**:87–265.

Biju SD, Bossuyt F. New frog family from India reveals an ancient biogeographical link with the Seychelles. *Nature* 2003;**425**:711–4.

Blackburn DC, Giribet G, Soltis DE, Stanley EL. Predicting the impact of describing new species on phylogenetic patterns. *Integrative Organismal Biology* 2019;**1**:obz028.

Bowie RCK, Fjeldså J, Hackett SJ *et al.* Systematics and biogeography of double-collared sunbirds from the Eastern Arc Mountains, Tanzania. *The Auk* 2004;**121**:660–81.

Brown JH, Lomolino MV. *Biogeography*, 2nd edn. Sunderland: Sinauer Press, 1998.

Brühl C. Flightless insects: a test case for historical relationships of African mountains. *Journal of Biogeography* 1997;**24**:233–50.

Bryja J, Šumbera R, Peterhans JCK *et al.* Evolutionary history of the thicket rats (genus *Grammomys*) mirrors the evolution of African forests since late Miocene. *Journal of Biogeography* 2017;**44**:182–94.

Burgess N, Butynski TM, Cordeiro NJ *et al.* The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation* 2007;**134**:209–31.

Bwong BA, Nyamache JO, Malonza PK *et al.* Amphibian diversity in Shimba Hills National Reserve, Kenya: a comprehensive list of specimens and species. *Journal of East African Natural History* 2017;**106**:19–46.

Chartier M, Dressler S, Schönenberger J *et al.* The evolution of afro-montane *Delphinium* (Ranunculaceae): morphospecies, phylogenetics and biogeography. *Taxon* 2016;**65**:1313–27.

Channing A, Stanley W. A new tree toad from the Ukaguru Mountains, Tanzania. *African Journal of Herpetology* 2002;**51**:121–128.

Clarke JA, Ksepka DT, Stucchi M *et al.* Paleogene equatorial penguins challenge the proposed relationship between biogeography, diversity, and Cenozoic climate change. *Proceedings of the National Academy of Sciences of the United States of America* 2007;**104**:11545–50.

Cochran DM. Frogs of Southeastern Brazil. *Bulletin of the United States National Museum* 1955;**206**:1–423.

Corti G. Continental rift evolution: from rift initiation to incipient break-up in the Main Ethiopian Rift, East Africa. *Earth-Science Reviews* 2009;**96**:1–53.

Couvreur TL, Chatrou LW, Sosef MS *et al.* Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biology* 2008;**6**:54.

Demos TC, Peterhans JCK, Agwanda B *et al.* Uncovering cryptic diversity and refugial persistence among small mammal lineages across the Eastern Afromontane biodiversity hotspot. *Molecular Phylogenetics and Evolution* 2014;**71**:41–54.

Diamond AW, Hamilton AC. The distribution of forest passerine birds and Quaternary climatic change in tropical Africa. *Journal of Zoology* 1980;**191**:379–402.

Dijkstra KDB. The *Atoconeura* problem revisited: taxonomy, phylogeny and biogeography of a dragonfly genus in the highlands of Africa (Odonata, Libellulidae). *Tijdschrift voor Entomologie* 2006;**149**:121–44.

Grebennikov VV. Phylogeography and sister group of *Lupangus*, a new genus for three new flightless allopatric forest litter weevils endemic to the Eastern Arc Mountains, Tanzania (Coleoptera: Curculionidae, Molytinae). *Fragmenta Entomologica* 2017;**49**:37–55.

Grove AT. *Evolution of the Physical Geography of the East African Rift Valley Region.* Cambridge, Massachusetts: Academic Press, 1983.

Hagen O, Skeels A, Onstein RE *et al.* Earth history events shaped the evolution of uneven biodiversity across tropical moist forests. *Proceedings of the National Academy of Sciences of the United States of America* 2021;**118**:e2026347118.

Hamilton AC. *Environmental History of East Africa: a Study of the Quaternary.* London: Academic Press, 1982.

IUCN. The IUCN Red List of Threatened Species. Version 2022-2. 2022. https://www.iucnredlist.org (1 June 2023; date last accessed).

Kamermans M, Vences M. Terminal phalanges in ranoid frogs: morphological diversity and evolutionary correlation with climbing habits. *Alytes* 2009;**26**:117–52.

Lamb HF, Bates CR, Coombes PV *et al.* Late Pleistocene desiccation of Lake Tana, source of the Blue Nile. *Quaternary Science Reviews* 2007;**26**:287–99.

Liedtke HC, Müller H, Hafner J *et al.* Interspecific patterns for egg and clutch sizes of African Bufonidae (Amphibia: Anura). *Zoologischer Anzeiger - A Journal of Comparative Zoology* 2014;**253**:309–15.

Liedtke HC, Müller H, Rödel MO *et al.* No ecological opportunity signal on a continental scale? Diversification and life-history evolution of African true toads (Anura: Bufonidae). *Evolution* 2016;**70**:1717–33.

Liedtke HC, Müller H, Hafner J *et al.* Terrestrial reproduction as an adaptation to steep terrain in African toads. *Proceedings of the Royal Society B: Biological Sciences* 2017;**284**:20162598.

Loader SP, Ceccarelli SF, Menegon M *et al.* Persistence and stability of Eastern Afromontane forests: evidence from brevicipitid frogs. *Journal of Biogeography* 2014;**41**:1781–92.

Lovett JC. The oldest rainforests in Africa: stability or resilience for survival and diversity? In: Purvis A, Gittleman JL, Brooks T (eds.), *Phylogeny and Conservation.* Cambridge: Cambridge University Press, 2005, 198–229.

Lovett JC, Wasser SK. *Biogeography and Ecology of the Rain Forests of Eastern Africa.* Cambridge, England: Cambridge University Press, 1993.

Luna MC, McDiarmid RW, Faivovich J. From erotic excrescences to pheromone shots: structure and diversity of nuptial pads in anurans. *Biological Journal of the Linnean Society* 2018;**124**:403–46.

Malonza PK. Patterns of reptile and amphibian species richness along elevational gradients in Mt Kenya. *Dong wu xue yan jiu = Zoological research* 2015;**36**:342–7.

Malonza PK, Bwong BA. *A Field Guide to the Reptiles and Amphibians of Kenya.* Frankfurt am Main: Edition Chimaira, 2023.

Matsui M. A new species of *Kalophrynus* with a unique male humeral spine from Peninsular Malaysia (Amphibia, Anura, Microhylidae). *Zoological Science* 2009;**26**:579–85.

Menegon M, Loader SP, Marsden SJ *et al.* The genus *Atheris* (Serpentes: Viperidae) in East Africa: phylogeny and the role of rifting and climate in shaping the current pattern of species diversity. *Molecular Phylogenetics and Evolution* 2014;**79**:12–22.

Menegon M, Lyakurwa JV, Loader SP *et al.* Cryptic diversity in pygmy chameleons (Chamaeleonidae: *Rhampholeon*) of the Eastern Arc Mountains of Tanzania, with description of six new species. *Acta Herpetologica* 2022;**17**:85–113.

Migliore J, Lézine AM, Hardy OJ. The recent colonization history of the most widespread *Podocarpus* tree species in Afromontane forests. *Annals of Botany* 2020;**126**:73–83.

Min MS, Yang SY, Bonett RM *et al.* Discovery of the first Asian plethodontid salamander. *Nature* 2005;**435**:87–90.

Minh BQ, Schmidt HA, Chernomor O *et al.* IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 2020;**37**:1530–4.

Mirarab S, Nguyen N, Guo S, Wang LS, Kim J, Warnow T. PASTA: Ultra-large multiple sequence alignment for nucleotide and amino-acid sequences. *Journal of Computational Biology* 2015;**22**:377–386.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. Biodiversity hotspots for conservation priorities. *Nature Methods* 2000;**403**:853–858.

Noble GK. *The Biology of the Amphibia.* New York and London: McGraw-Hill, 1931.

Onditi KO, Demos TC, Peterhans KJ *et al.* Historical biogeography, systematics, and integrative taxonomy of the non-Ethiopian speckled pelage brush-furred rats (*Lophuromys flavopunctatus* group). *BMC Ecology and Evolution* 2021;**21**:89.

Popp M, Gizaw A, Nemomissa S *et al.* Colonization and diversification in the African 'sky islands' by Eurasian *Lychnis* L. (Caryophyllaceae). *Journal of Biogeography* 2008;**35**:1016–29.

Poynton JC. Distribution of amphibians in Sub-Saharan Africa, Madagascar, and Seychelles. In: Duellman WE (ed.), *Patterns of Distribution of Amphibians.* Baltimore: Johns Hopkins University Press, 1999, 483–539.

Raven PH, Gereau RE, Phillipson PB *et al.* The distribution of biodiversity richness in the tropics. *Science Advances* 2020;**6**:eabc6228.

Rodgers WA, Owen CF, Homewood KM. Biogeography of East African forest mammals. *Journal of Biogeography* 1982;**9**:41–54.

Sandberger-Loua L, Müller H, Rödel MO. A review of the reproductive biology of the only known matrotrophic viviparous anuran, the West

African Nimba toad, *Nimbaphrynoides occidentalis*. *Zoosystematics and Evolution* 2017;**93**:105–33.

Scharff N. The linyphiid fauna of eastern Africa (Araneae: Linyphiidae)—distribution patterns, diversity and endemism. *Biological Journal of the Linnean Society* 1992;**45**:117–54.

Scholz CA, Johnson TC, Cohen AS *et al.* East African megadroughts between 135 and 75 thousand years ago and bearing on early-modern human origins. *Proceedings of the National Academy of Sciences of the United States of America* 2007;**104**:16416–21.

Siu-Ting K, Gower DJ, Pisani D *et al.* Evolutionary relationships of the Critically Endangered frog *Ericabatrachus baleensis* Largen, 1991 with notes on incorporating previously unsampled taxa into large-scale phylogenetic analyses. *BMC Evolutionary Biology* 2014;**14**:44.

Spawls S, Mathews G. Kenya: A Natural History. New York, Bloomsbury USA; 2013.

Spawls S, Wasonga DV, Drewes RC. *The Amphibians of Kenya.* London, England: Bloomsbury Publishing, 2019.

Streicher JW, Sadler R, Loader SP. Amphibian taxonomy: early 21st century case studies. *Journal of Natural History* 2020;**54**:1–13.

Tolley KA, Tilbury CR, Measey GJ *et al.* Ancient forest fragmentation or recent radiation? Testing refugial speciation models in chameleons within an African biodiversity hotspot. *Journal of Biogeography* 2011;**38**:1748–60.

Van Bocxlaer I, Loader SP, Roelants K *et al.* Gradual adaptation toward a range-expansion phenotype initiated the global radiation of toads. *Science* 2010;**327**:679–82.

Wake DB, Vredenburg VT. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 2008;**105**:11466–73.

Watters JL, Cummings ST, Flanagan RL *et al.* Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. *Zootaxa* 2016;**4072**:477–95.