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Identification of sand frogs (Anura: Pyxicephalidae: *Tomopterna*) from Kenya with the description of two new species

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Abstract

African sand frogs in the genus *Tomopterna* presently include 13 species. These are known to be highly cryptic and morphologically similar. Despite increased effort in the recent past, the taxonomy of the group is still unresolved and some populations e.g. in Kenya have remained largely unstudied. This paper starts to address this gap using molecular, advertisement call and morphological comparisons. We test the boundaries of the Kenyan species based on mitochondrial 16S rRNA sequence data. Two new species are recognised and described: *Tomopterna wambensis* **sp. nov**. and *Tomopterna gallmanni* **sp. nov**. Further molecular and advertisement call studies of *Tomopterna* populations in Kenya are recommended, especially for those populations previously identified as *T. cryptotis* and *T. tandyi*.

Key words: Amphibia, Kenya, new species, *Tomopterna wambensis*, *Tomopterna gallmanni*

Introduction

Sand frogs in the genus *Tomopterna* Dumeril and Bibron, 1841 occur widely in the sandy areas of Africa. Presently 13 named species are recognized: *T. cryptotis, T. damarensis,T. delalandii, T. elegans, T. kachowskii, T. krugerensis, T. luganga, T. marmorata, T. milletihorsini, T. monticola, T. natalensis, T. tandyi* and *T. tuberculosa* (Frost 2013). A recent study of the relationships of the group (Dawood & Uqubay 2004) indicated that there were at least two undescribed species present among their samples, one from Beira (Mozambique) and the other from Shankara (Namibia). Recent work undertaken on this genus includes descriptions of new species (Channing & Bogart 1996, Dawood & Channing 2002, Channing *et al*. 2004), studies on sand frog tadpoles (Haas *et al*. 2006), and phylogeny determination (Dawood *et al*. 2002, Dawood & Uqubay 2004). *Tomopterna* is recognized within a morphologically heterogenous but geographically coherent family, the Pyxicephalidae of Bonaparte, 1850, on the basis of molecular evidence (Frost *et al*. 2006).

These frogs are cryptic in nature and they do not present obvious morphological distinctions. Furthermore, they are widely distributed. As such, the need to investigate species boundaries and relationships using data other than morphology has been emphasized (Dawood *et al.* 2002, Zimkus & Larson 2011).

The Kenyan populations have variously been identified as *T. cryptotis* and *T. tandyi* but these identifications require more robust data to validate. In the current study, we aim to identify *Tomopterna* species from Kenya using molecular data.

Materials and methods

Sampling. Fieldwork was conducted by DVW in Kenya between August 2009 and June 2010. At least three specimens were obtained from each locality. Muscle tissue was obtained using flame sterilized equipment and preserved in 95% ethanol. Five samples from Turkana were obtained from Museo di Storia Naturale dell'Università di Pavia (MCNPV), Italy, and two samples were derived from the NMK tissue collection. The

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sampling locations are presented in Fig. 2. Published sequences were retrieved from GenBank (www.ncbi.nim.nih.gov) for comparative analysis. *Pyxicephalus* (including *Aubria*) forms a sister taxon of the Pyxicephalinae of Dubois (2005), the "southern African clade" of Van der Meijden *et al*. (2005): *Amietia, Anhydrophryne, Arthroleptella, Cacosternum, Natalobatrachus, Strongylopus* and *Tomopterna* (Frost *et al.* 2006). All the samples used in the study are listed in Appendix 1 with details of their collecting localities.

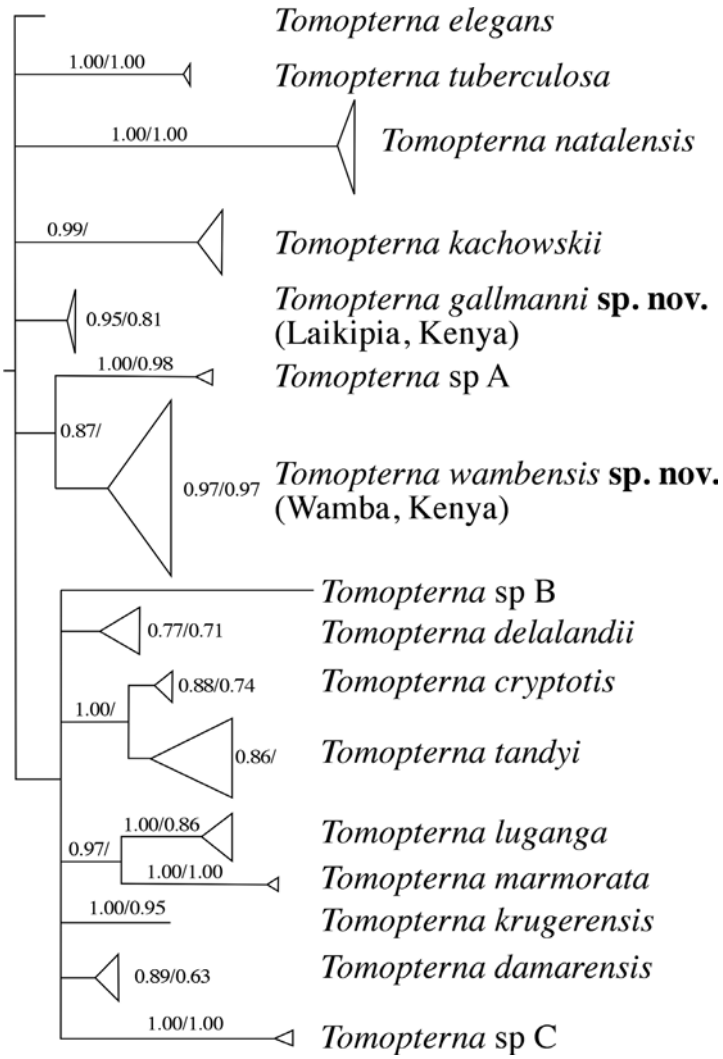

FIGURE 1. Phylogeny of *Tomopterna* based on 16S rRNA sequences. Support for the tree is indicated as PP/ML bootstrap. Values less than 60% are not shown. The specimens associated with each species are listed in Appendix 1.

Advertisement calls were recorded using a digital Sony camera DSC-W130, from Laikipia (voucher NMK A/5039/1) and Wamba (voucher NMK A/5152/1) with an air temperature of 24 °C.

Laboratory analysis. We used the mitochondrial 16S rRNA gene to infer a preliminary molecular phylogeny of the group for the purpose of delimiting species. Other studies have also used this and related markers since they are traditionally accepted as reliable in investigating the species level relationships among amphibians (Dawood & Uqubay 2004, Zimkus & Larson 2011).

We used macerated muscle tissue and followed standard procedure according to Hillis *et al.* (1996) to extract DNA. Tissues were digested using standard Proteinase-K protocol, and DNA was extracted using phenol-chloroform (Hillis *et al*. 1996). A 550 bp fragment of the mt 16S gene was amplified using the primers 16SaR-F and 16SbR-R of Simon *et al*. (1994), as modified by Bossuyt & Milinkovitch (2000) annealing at 51 °C. We used Fast Taq ready mix (Kapa Biosystems) for PCR, using the manufacturer's recommended protocol; an initial denaturing step of 1 minute at 95 °C, followed by 35 cycles of denaturing for 10 seconds at 95°C, annealing for 10 seconds, and extension for 1 second at 72 °C. There is no final extension step. The 550 bp product was sequenced at the University of Stellenbosch Central Analytical Facility.

Morphology. Measurements were based on materials deposited at the National Museums of Kenya as listed in Appendices 1 and 2. In total 24 features as described by Zimkus & Larson (2011) were used in statistical morphological comparisons. Measurements were taken to the nearest 0.1 mm by DVW using digital calipers. The following measurements were used to describe the species: snout-vent-length (SVL), head width (HW), head length (HL), tympanum diameter (TD), snout length (SL), width of the internarial space (INS), snout-nostril length SNL), eye diameter (ED), width of the interorbital space (IOS), eyelid-nostril length (ENL), humerus length (HL), radioulna length (RL), manual digit lengths (MDI–IV), femur length (FL), tibiofibula length (TL), pedal digit lengths (PDI–V), inner metatarsal length (IMT), leg length (LEG) and foot length (FOT). The last two measurements were only used for specific morphometric comparisons of the holotypes but not all material was examined and therefore they were excluded from statistical analysis.

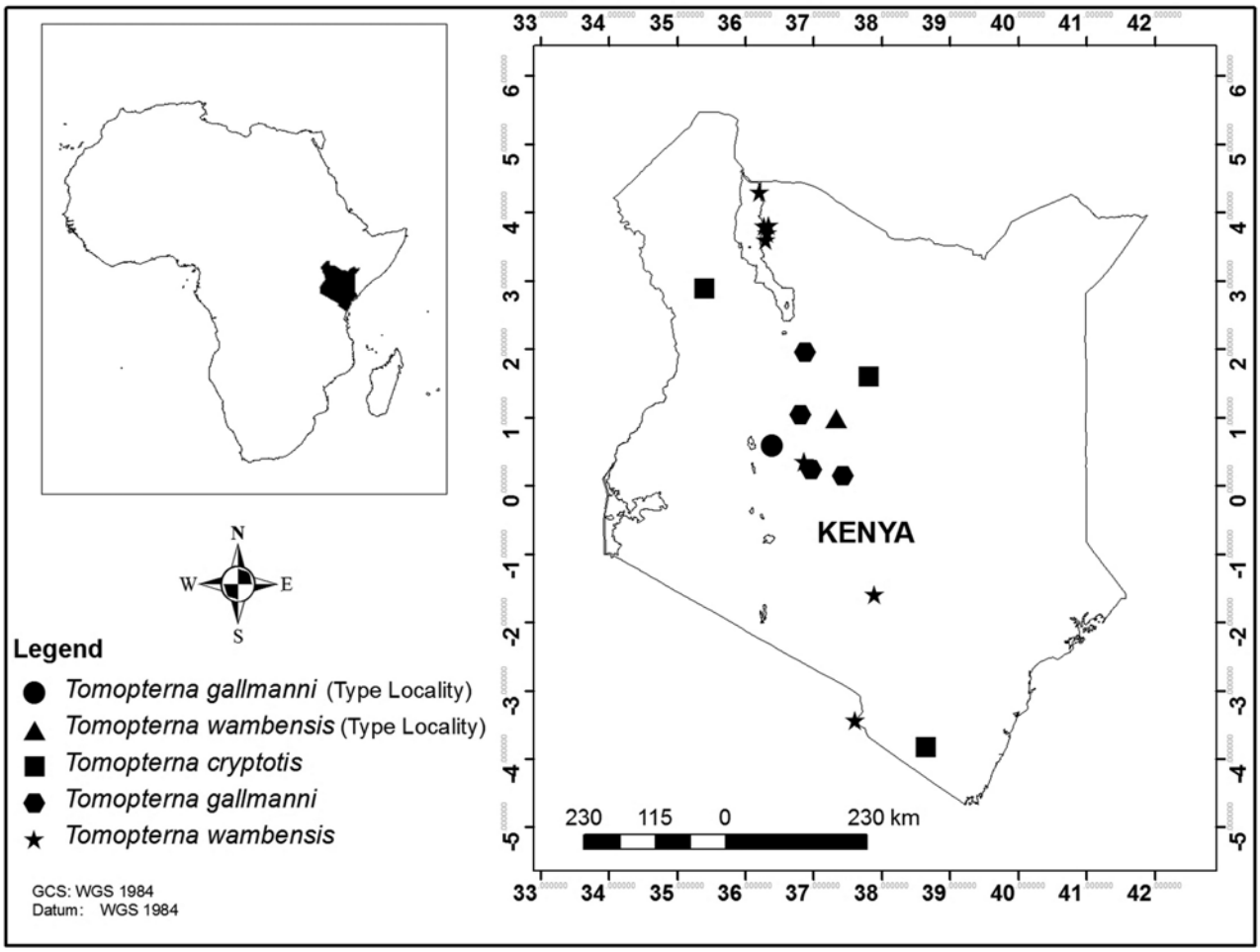

FIGURE 2. Map showing the sampled populations of *Tomopterna* species from Kenya. Type localities are marked by a circle - Laikipia Nature Conservancy (*T. gallmanni* **sp. nov**.) and a triangle - Wamba (*T. wambensis* **sp. nov**.). *T. cryptotis* populations are shown by solid squares.

Data Analysis. Sequences were trimmed and concatenated using BioEdit 7.0 (Hall 1999), then aligned using Clustal W2 (2.0.12) as implemented in MEGA 5 (Tamura *et al*. 2011) with default settings. jModelTest 0.1.1 (Posada 2008, Guindon & Gascuel 2003) was used to determine the appropriate model of evolution under AIC. The final dataset used in computation of pairwise distances and other analyses consisted of 414 sites including gaps (indels) for the 16S rRNA sequences. The aligned 64 sequences were input into MrBayes 3.2, (Huelsenbeck & Ronquist 2003) and run for 10 million generations, with three attempted swaps each iteration, with the temperature set at 0.1, and using the GTR + G + I model. Two independent runs were analysed, each with one hot and three cold chains. The default burn-in value of 25% was used. To determine percentage genetic variation within and between species, uncorrected *p*-distances were calculated in PAUP* 4b10 (Swofford 2002).

We computed a maximum likelihood bootstrap using GARLI Genetic Algorithm for Rapid Likelihood

Inference (Zwickl 2006) and grid computing (Cummings & Huskamp 2005) through The Lattice Project (Bazinet & Cummings 2008), which includes clusters and desktops in one encompassing system (Myers *et al*. 2008). A web service for GARLI was developed (available at http://www.molecularevolution.org/, Bazinet & Cummings 2011) that uses a special programming library and associated tools (Bazinet *et al*. 2007). Following the general computational model of a previous phylogenetics study (Cummings *et al*. 2003), which used an earlier grid computing system (Myers & Cummings 2003), we distributed the required files among hundreds of computers where the analyses were conducted asynchronously in parallel. Post-processing of the phylogenetic inference results used DendroPy (Sukumaran & Holder 2010) and the R system for statistical computing (R Development Core Team 2011). 1000 replicates were used following procedures adopted in similar phylogenetic studies (e.g. Dawood *et al.* 2002). Tree support is shown as posterior probability and maximum liklihood bootstrap.

The morphological data analysis was completed using Principal Components Analysis (PCA) as implemented in the software STATISTICA 6.0. All the measurements were log transformed before the analysis.

Results

Genetic variation and phylogeny. The consensus BI tree is shown in Fig. 1, with the terminals collapsed. Each terminal on the tree represents a species; the specimens represented are listed in Appendix 1. There is insufficient information in the 16S sequences to resolve the deeper relationships, but *Tomopterna* populations sampled from Kenya occurred in two distinct species, that were confirmed by high *p*-distances. We obtained high bootstrap values and posterior probabilities (PP) for terminal nodes in the maximum likelihood (ML) and Bayesian (BI) results respectively. Most of the terminal nodes for the populations sampled from Kenya showed values of >80% for bootstrap support and >95% for posterior probabilities. *T. wambensis* **sp. nov**. was supported by a bootstrap value of 97% and PP of 97%. *T. gallmanni* **sp. nov**. was distinguished with a bootstrap support of 81% and PP of 95%. *T. wambensis* **sp. nov**. formed a sister grouping with *Tomopterna* sp. A from Ayoun el-Atrouss.

T. wambensis **sp. nov**. and *T. gallmanni* **sp. nov**. differed from each other by a mean uncorrected *p*-distance of 4.1%. The genetic differences observed between *T. wambensis* **sp. nov**. and other species of *Tomopterna* ranged from 3.9–8.9%. On the other hand, observed inter-specific mean uncorrected *p*-distances for *T. gallmanni* **sp. nov**. ranged between 3.1–7.5% (Table 1).

Principal Component Analysis (PCA). PCA of 24 morphometric characters (Table 3) was performed using two species separated by molecular data as an *a priori* grouping variable, for a dataset consisting of male measurements only. We also included *T. cryptotis* specimens from Laisamis, Taita Hills and Western Lake Turkana in the analysis. The first two factors accounted for 54.3% of the variance (Fig. 3). Based on the loadings of the first four factors, the characters that contributed most significantly to the overall variance were snout vent length, head width, head length, radioulna length, manual digits (I and II), and toe IV (Factor score > 0.7). *T. gallmanni* **sp. nov**. which was the largest among the taxa included in the PCA was clearly separated from the rest of the materials along Factor 1. As size correction was not implemented prior to the analysis, the differentiation of *T. gallmanni* in Factor 1 is probably mainly caused by the standard length difference of this taxon from the rest of materials. Our *T. cryptotis* samples from Taita Hills, Laisamis and Western Lake Turkana were morphologically most similar to *T. wambensis* **sp. nov**. (and may in fact be the new species) although we have no calls or molecular data to confirm this (Fig.3). The taxonomic status of these specimens will be re-evaluated when additional material is available. At the moment, no *T. cryptotis* sequences are available from Kenya.

Advertisement calls. The two species were distinguishable on the basis of call length and repetition rate (Fig. 5). The advertisement call of *T. wambensis* **sp. nov.** consists of a series of high-pitched rapidly repeating notes. There is a typical note rate of 7.2–10.9 s^{-1}. The call shows two harmonics with mean frequencies of 1135 Hz and 2253 Hz, with a fainter third harmonic at 3372 Hz. The second harmonic is emphasised. Each note has a duration of 0.028 s. The advertisement call of *T. gallmanni* **sp. nov.** consists of a series of notes, each consisting of one or two pulses, which resemble a slow knocking. Males call antiphonally, with a typical note rate of two individuals of 5.5–6.5 s^{-1}. The individual note rates in a chorus vary from 2.7–3.3 s^{-1}. Each note is brief, about 0.012 s long, with two distinct harmonics. The mean frequency of the harmonics is 980 Hz and 1960 Hz with a fainter third harmonic at 2940 Hz. The second harmonic is emphasised.

On the basis of the phylogenetic relationships, genetic distances as well as advertisement call differences

observed, these two clades *T. wambensis* **sp. nov**. and *T. gallmanni* **sp. nov**. are recognised as new taxa. The distribution of Kenyan populations sampled in this study is shown in Fig. 2.

TABLE 1. Uncorrected mean percent genetic divergence of *Tomopterna* species using partial mitochondrial 16S rRNA. The species formed separate clades. *T. wambensis* and *T. gallmanni* sequences were determined during this study.

Taxa (Number of samples in analysis)	*T.* sp. Shankara	*T. elegans*	*T.* sp. Ayoun el Atrouss	*T. kachowskii*	*T. gallmanni*	*T. wambensis*	*T. tuberculosa*	*T. natalensis*	*T. delalandii*	*T. cryptotis*	*T. tandyi*	*T. luganga*	*T.* sp. Beira	*T. damarensis*	*T. krugerensis*
T. sp. B. Shankara (1)	__														
T. elegans (1)	6.3	__													
T. sp. A. Ayoun el Atrouss (2)	8.4	4.5	__												
T. kachowskii (5)	8.2	4.4	6.1	__											
T. gallmanni (5)	6.5	4.2	4.4	4.8	__										
T. wambensis (12)	7.5	4.3	4.8	4.5	4.1	_									
T. tuberculosa (2)	7.5	4.8	5.9	6.3	4.7	5.8	__								
T. natalensis (7)	10.4	7.3	8.6	8.3	7.5	8.9	8.0	__							
T. delalandii (4)	5.9	2.8	4.6	5.9	3.9	4.1	4.5	7.4	__						
T. cryptotis (3)	7.5	3.6	5.9	5.1	3.9	4.4	6.5	7.6	3.2	__					
T. tandyi (6)	7.5	3.7	5.9	5.6	3.7	4.3	6.5	7.7	3.3	1.6	__				
T. luganga (4)	8.2	4.8	7.1	6.6	5.2	5.9	7.9	9.4	4.2	4.9	4.7	__			
T. sp. C. Beira (2)	8.9	5.7	8.0	8.1	6.2	6.9	7.5	9.8	5.2	5.9	6.5	6.4	__		
T. damarensis (2)	6.7	2.5	5.1	4.7	3.1	3.9	5.8	7.3	2.2	2.2	2.8	4.3	6.1	__	
T. krugerensis (2)	6.5	3.8	6.7	6.2	4.6	4.7	6.3	8.0	4.0	4.1	4.9	5.6	6.2	3.3	__
T. marmorata (2)	7.4	5.7	8.0	7.5	6.3	6.0	8.1	9.2	5.4	6.2	6.4	4.8	7.2	5.3	5.2

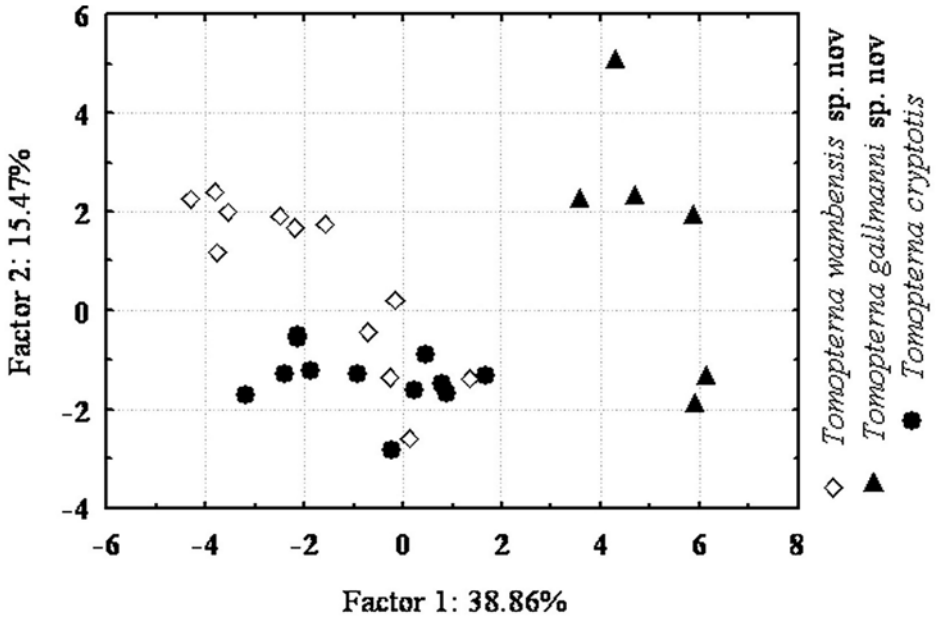

FIGURE 3. Scatter plot using the first two factor loadings based on 24 morphological characters for sand frogs sampled from Kenya. See text for discussion of similarity of Kenyan samples of *T. cryptotis* and *T. wambensis*.

TABLE 2. Factor loadings from a Principal Component Analysis of 24 morphological characters from species of *Tomopterna* sampled from Kenya.

	Factor 1	Factor 2	Factor 3	Factor 4
SVL	0.854252	0.309835	0.158625	-0.125230
HW	0.833614	0.189011	0.303543	0.120385
HL	0.523600	0.252270	0.709300	-0.009180
TD	0.525419	0.063580	-0.242390	-0.562400
SL	0.694976	0.126613	-0.124180	-0.269890
INS	0.484387	0.370693	-0.449110	0.090112
SNL	0.656443	0.026030	0.154063	0.073814
ED	0.324333	0.676755	0.185695	0.296743
IOS	0.619159	0.076033	-0.323750	0.112327
ENL	0.425349	-0.014030	0.472164	-0.581700
HL	0.682201	-0.491520	0.003904	-0.170570
RL	0.855676	0.120532	0.032882	0.003976
MDI	0.342532	-0.765730	-0.030600	0.438817
MDII	0.336810	-0.709260	-0.083400	0.251401
MDIII	0.545638	-0.096990	-0.145050	0.607035
MDIV	0.030564	-0.591800	-0.348160	-0.244100
FL	0.909510	-0.085470	-0.017310	0.028308
TL	0.915172	0.232795	-0.004260	0.049214
PDI	0.493317	-0.419730	-0.158890	-0.592610
PDII	0.034195	-0.698310	0.300334	-0.215110
PDIII	0.676045	-0.070940	-0.596730	0.127290
PDIV	0.872014	-0.258560	0.016646	0.154625
PDV	0.583537	-0.407360	0.513898	0.245053
IMT	0.582545	0.477465	-0.371020	-0.166060
Explained variance	9.326495	3.713758	2.307378	2.113381
Proportion of Total variance	0.388604	0.154740	0.096141	0.088058

***Tomopterna wambensis* sp. nov. Wasonga & Channing**
Wamba Sand Frog (Fig. 4)

Holotype. NMK A/5152/1, adult male, from Sodor water pan in farmlands near Wamba township, Isiolo County, Kenya (0° 59' N; 37° 20' E), collected 5 May, 2010 by Domnick V. Wasonga, Michael Roberts and Victoria Zero.

Paratypes. NMK A/5152/2–3, adult males and NMK A/5152/4–5 adult females collected together with the holotype; NMK A/5057/1–6 adult males collected from temporary pond at Ikave Secondary School, Kitui County, Kenya (1° 35' 20" S; 37° 54' 0" E, 948 m) collected 31 October, 2009 by P.K. Malonza; NMK A/5149/1–3 and NMK A/5150/1–2, adult males collected from a murrum pit near the Air Strip at Mpala Research Center, Laikipia County, Kenya (0° 21' 12" N; 36° 52' 20" E) collected 3 May 2010 by Domnick V. Wasonga, Michael Roberts and Victoria Zero; MCNPV-CA334 adult (not sexed) from open fields 17 km south of Allia Bay, Marsabit County, Kenya (03° 42' N; 36° 20' E), collected 7 July, 2005 by U. Ziliani, R. Sindaco and E. Razzetti; MCNPV-CA250 adult (not sexed) from open fields near Illeret township on the eastern shores of Lake Turkana (04° 18' N; 36° 13' E) collected 10 January, 2005 by U. Ziliani; MCNPV-CA252 adult (not sexed) from Karsa Well on the eastern shores of Lake Turkana, Marsabit County, Kenya (03° 36' N; 36° 18' E) collected 11 January, 2005 by E. Baucompagni; MCNPV-CA337 adult (not sexed) from Lugga Daudi sand river in Sibiloi National Park, eastern Lake Turkana, Marsabit County, Kenya (03° 49' N; 36° 21' E) collected in June 2005 by U. Ziliani, R. Sindaco and

E. Razzetti; NMK A/4323 adult (not sexed) from Bura Hasuma sand river, Marsabit County Kenya (03° 48' N; 36° 17' E) collected 5 July 2005 by U. Ziliani, R. Sindaco and E. Razzetti; NMK A/5145/1–2 adult males from roadside water puddles near Kitobo Forest, Taita-Taveta County, Kenya (03° 26' 29" S; 37° 37' 13" E) collected 11 April, 2010 by P.K. Malonza, B. Bwong' and J. Nyamache.

Material used for genetic analysis. NMK A/5057/1 adult male from Ikave, Kitui, NMK A/5149/1–3 adult males from Mpala Research Center, NMK A/5152/1 adult male from Wamba, NMK A/5145/1 adult male from Kitobo Forest, MCNPV-CA334, MCNPV-CA250, MCNPV-CA252, MCNPV-CA337, NMK A/4323 five unsexed adults from eastern Lake Turkana.

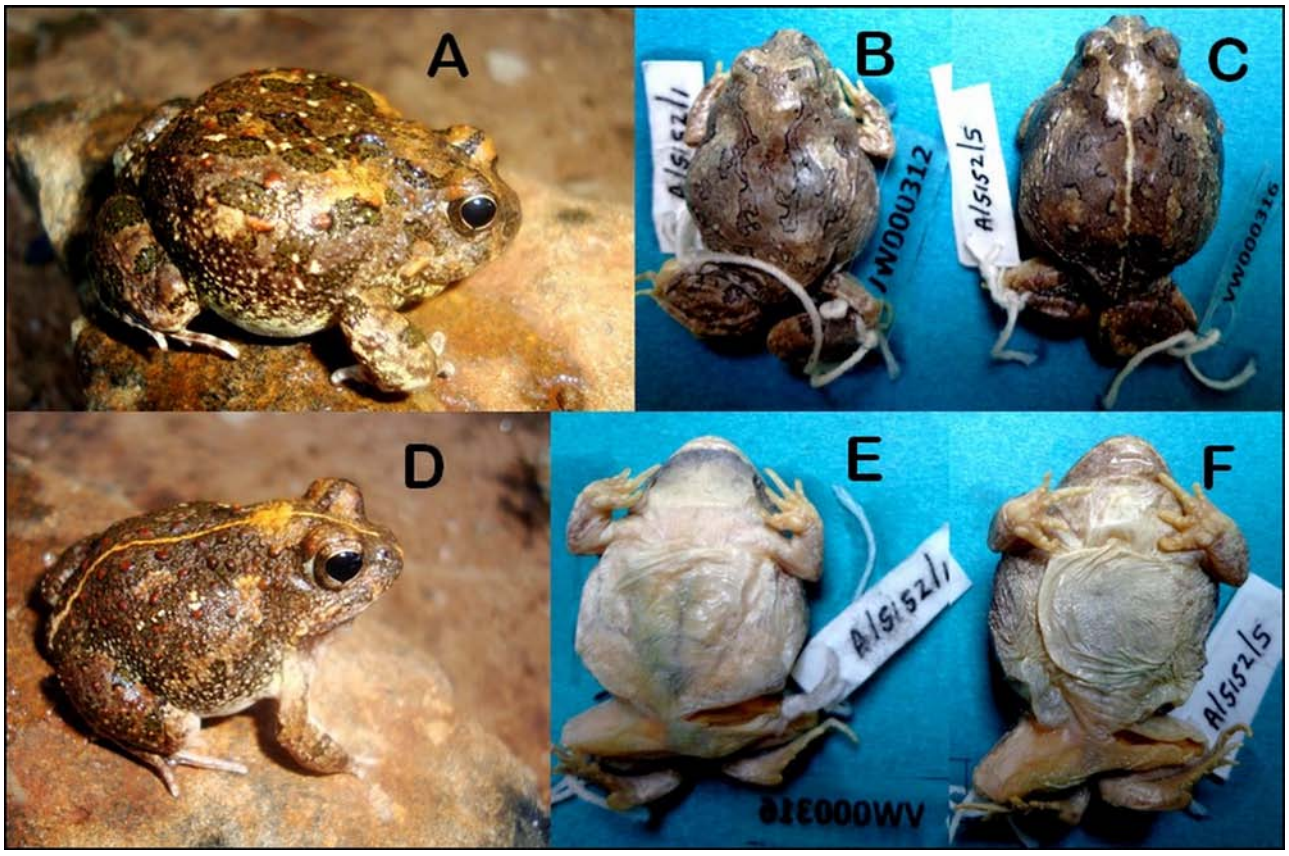

FIGURE 4. *Tomopterna wambensis* **sp. nov**.from Wamba, Kenya. Dorsolateral views in life (A) NMK A/5152/1, male and (D) A/5152/5, female, dorsal views in alcohol, (B) A/5152/1, male, and (C) A/5152/5, female, ventral views in alcohol, (E) A/5152/1, male and (F) A/5152/5, female.

Diagnosis. The phylogenetic analysis in this study has placed *T. wambensis* **sp. nov.** within the clade that includes all other *Tomopterna*, and we have no hesitation in assigning this taxon to the genus *Tomopterna*. The diagnostic characters and comparison with other members of the genus are presented in Appendices 3 and 4. The new species differs from all other *Tomopterna* species by a combination of morphological and advertisement call characteristics. These include divided subarticular tubercles, which are also found in *T. gallmanni* and *T. krugerensis*. The latter two species both have a harsh knocking-like advertisement call, unlike *T. wambensis.* The standard length (34.6–40.8, n=12) is much smaller compared to the recently re-described *T. monticola* (SVL 41.2, n=1) from Olengarua Village in Massai. Subarticular tubercles single and prominent. Webbing on hind toe is moderate; 2–2 ½ phalanges free of web on the forth digit. Glandular ridge below the tympanum broken into two unequal parts. Outer metatarsal tubercle absent; a weak whitish bump present instead (vs. prominent and strongly protruding in some members of the genus). Dorsum consists of greenish brown patches on a lighter background (Figs. 4A and D) contrasting with irregular dark markings and dark spots on a lighter ground colour in *T. krugerensis* and also varying from the scantily marked orange-brown to grey-brown of *T. marmorata*. Small reddish-brown warts present on dorsum (smooth in *T. krugerensis*, distinctive and prominent tubercles in *T. tuberculosa*). The advertisement call consists of a series of high-pitched rapidly repeated notes. The note rate is

7.2–10.9 s^{-1} (*T. krugerensis* covers entire frequency range with a click; *T. tandyi* 7–8 notes s^{-1}; *T. delalandii* 5–7 notes s^{-1}; *T. cryptotis* 9 notes s^{-1}). The call shows two harmonics with mean frequencies of 1135 Hz and 2253 Hz, with a fainter third harmonic at 3372 Hz. The second harmonic is emphasized (differing from *T. krugerensis* with multiple emphasized harmonics with dominant harmonic ranging from 1900–3100 Hz; *T. tandyi* 2500–2700 Hz; *T. delalandii* 1800–2200 Hz; *T. cryptotis* 3100–3800 Hz, *T. luganga* has the first harmonic emphasized 1050–1170 Hz). Each note has a duration of 0.028s (Fig. 5), varying from *T. krugerensis* 0.008–0.011 s; *T. tandyi* 0.04 s; *T. delalandii* 0.039 s; *T. cryptotis* 0.048 s.

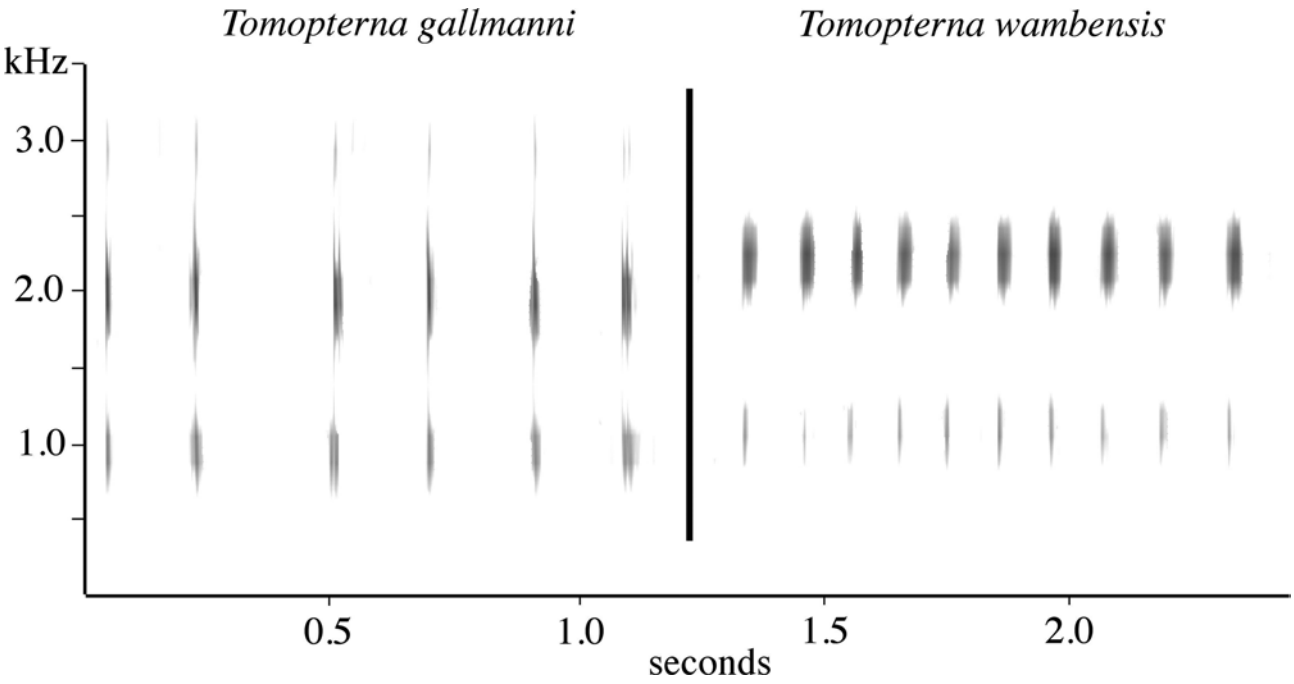

FIGURE 5. Chorus of *Tomopterna gallmanni* recorded at Laikipia, and a chorus of *Tomopterna wambensis* recorded at Wamba.

Description of Holotype. The body is stout and toad like; head comparatively small (HL/SVL 0.32, HW/SVL 0.41), not wider than trunk, not longer than wide (HL/HW 0.78); snout short (SL/HL 0.54), rounded in dorsal view, blunt in profile (Fig. 4A), slightly projecting beyond rounded lower jaw, narrower than long (SL/INS 1.47); canthus rostralis is moderately angled; loreal region concave; nostrils situated on slight projections of the canthus, much closer to the snout tip than the eye (ENL/SNL 1.19); eyes directed anterolaterally, slightly protruding, relatively small (ED/HL 0.44); eye diameter shorter than snout (ED/SL 0.81); interorbital distance is longer than upper eyelid (IOS/ED 1.33), and longer than internarial distance (INS/ED 0.84); tympanum visible externally, discontinuous glandular ridge present below, smaller than eye diameter (TD/ED 0.73); upper jaw without dentition; choanae small, round, partly hidden by upper jaw, located anteriorially on the roof of the mouth, vomerine teeth in two oblique rows between the choanae; tongue short and strongly notched behind, one side slightly larger (5.7 at widest part); median lingual processes present; vocal sac single, darkly pigmented; gular pouch flattened anteriorly into a U-shape on the lower lip margin. Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs, gular and abdomen smooth (Figs. 4B and E).

Fore limbs slender; hand moderately large (HND/SVL 0.24); tips of fingers not enlarged into discs; relative length of fingers: IV < I < II <III; subarticular tubercles single and distinct, with one on fingers I and IV, two on finger II and three on finger III; fingers without webbing; thenar tubercle distinct; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pad present on manual digit I.

Hind limbs stout, slightly more than half body length (LEG/SVL 0.78); tibio-tarsal articulation not reaching to level of tip of snout when legs are adpressed to body; tibiofibula short (TL/SVL 0.39), slightly longer than thigh (TL/FL 1.01); heels just reaching each other when knees are flexed and thighs are held laterally at right angle to body; foot shorter than tibiofibula (FOT/TL 0.98); relative length of toes: I<II<V<III<IV; toes without expanded discs; subarticular tubercles: one on toe I and II, two on toe III and V and three on toe IV; pedal webbing formula **I**1–1.5 **II**1.5^{+}–2 **III** 2–2 **IV**1.5^{-}–1**V**; inner metatarsal tubercle prominent and shovel-shaped; outer absent.

Colouration in life. The dorsal pattern consists of greenish brown patches on a lighter background. Reddish-brown dorsal warts spread to surface of head, often bordered with black, larger and more prominent towards the mid dorsum; lateral sides of head and scapular region grey, anterior border of eye black; a darker bar present between the eyes; dorsal stripe absent, white flecks present; ventrum cream, throat dark; hands and thighs show two and three transverse dark markings respectively. **Colouration in preservative**. Dorsal wart colouration is lost; the other patterns are visible.

Paratype variation. Females (SVL 42–43, mean 42.5, n=2) are larger than males (SVL 34.6–40.8, mean 37.6, n=12). Colouration of male paratypes is similar to that of the holotype. In some specimens, however, a white thin dorsal line reaching the snout is present in both sexes. Females have a speckled throat (Fig. 4F). The dorsal glands are relatively larger and scattered in male individuals compared to females with small dense dorsal glands. Measurements are given in Table 3.

Eggs and tadpoles. Unknown.

Habitat. The specimens were found mostly in open habitats including man-made (e.g. Wamba) and natural water points (e.g. Turkana and Kitobo Forest). Most of the breeding congregations were found in seasonal wetlands. Specimens were observed from within (ca. 10 cm deep) and outside the water bodies (ca. 2 m). Males called from similar positions, but maintained some distance from each other (>2 m). The following species were found sympatrically or syntopically with the new species: *Ptychadena anchietae, Ptychadena mascareniensis, Hildebrandtia ornata* and *Kassina senegalensis*.

Distribution. The collection locations of *Tomopterna wambensis* **sp. nov**. range from northern to south-eastern Kenya as indicated in Fig. 2. Occurrence in Tanzania is confirmed by a single specimen from Arusha, Tanzania as revealed by the molecular data. However, a possible range extension into Ethiopia is implied by the proximity of the northernmost record of the series at Illeret to the Kenya-Ethiopian border (< 20 km). The elevation range of collecting localities was between 725 m at Kitobo Forest and 1782 m at the Mpala Research Center.

Etymology. The specific name refers to the type locality, the village of Wamba in central Kenya.

Remarks. Based on the records of the type series across Kenya with at least one population in Tanzania, the species is considered widespread. We have recorded the species in both protected and unprotected areas. We presume that it is under no immediate threat and propose a Least Concern status under the current IUCN Redlist criteria.

***Tomopterna gallmanni* sp. nov. Wasonga & Channing**
Gallmann's Sand Frog (Fig. 6)

Holotype. NMK A/5039/1, an adult male from an artificial water pond, Dam Ya Colin, in Ol Ari Nyiro, Laikipia Nature Conservancy, Laikipia County, Kenya (0° 34' N; 36° 24' E) collected 3 August 2009 by Domnick V. Wasonga and Michael Roberts.

Paratypes. NMK A/5039/2, an adult male and NMK A/5039/3 an adult female collected together with the holotype; NMK A/5154/1, adult female and NMK A/5154/2 adult male collected from a pond at Mogwooni Ranch, Laikipia County, Kenya (0° 14.5' N; 36° 58.6' E) on 10 and 11 May, 2010 by Michael Roberts; NMK A/5045 adult male collected from wooded grassland near Ngare Ndare Forest, Lewa Wildlife Conservancy, Lakipia County, Kenya (0° 09'10" N; 37° 26'16" E); NMK A/5151/1-3, three sub-adults dug from the soil on the banks of a seasonal stream in Baawa village near Maralal, Isiolo County, Kenya (0° 02' 45" N; 36° 48' 55" E) collected 5 June, 2010 by Domnick V. Wasonga, Michael Roberts and Victoria Zero; NMK A/5159, subadult from the roadside, between Baragoi and South Horr, Samburu County, Kenya (01° 20.76' N; 36° 53.1' E) collected 20 June, 2010 by Michael Roberts.

Material used for genetic analysis. NMK A/5154/1, NMK A/5045 (one male from Mogwooni Ranch), NMK A/5039/2 (one male from Laikipia Nature Conservancy), NMK A/5151/1 (one sub-adult from Baawa Village, Maralal) and NMK A/5159 (sub-adult from Baragoi-South Horr Road).

Diagnosis. Based on both morphological similarities and phylogenetic affinities that place *T. gallmanni* **sp. nov.** within the clade that includes all other *Tomopterna*, we assign the new taxon to the genus *Tomopterna*. Appendices 3 and 4 give a summary of morphological features as well as interspecific comparisons. This species can be distinguished from other *Tomopterna* spp. by a combination of morphological and advertisement characters.

It has divided subarticular tubercles, which are found also in *T. krugerensis* and *T. wambensis.* The nostrils are midway between the eye and snout tip, a condition only shared with *T. cryptotis*. The advertisement calls of *T. krugerensis*, *T. wambensis* and *T. cryptotis* are quite different. The body is stout, morphologically similar to *T. cryptotis*. It is about the same size as *T. monticola* (SVL 41.2, n=1) with standard size ranging from 40.0–45.0 (n=6); but mainly differs from *T. monticola* which has a deeply notched tongue, finely serrated upper jaw, smaller head width, tympanum partially hidden (although this may be due to prolonged preservation of this material), shorter snout length, smaller tympanum diameter, nostril slightly closer to snout tip than eye and smaller inner metatarsal tubercle. The advertisement call consists of a series of notes, each consisting of one or two pulses, which resemble a rapid knocking, different from the slow repetitive "knock" of *T. krugerensis*. The typical note rate of two individuals is 5.5–6.5 s^{-1} (Fig. 5). The individual note rates in a chorus vary from 2.7–3.3 s^{-1}. This differs from *T. tandyi, T. delalandii* and *T. cryptotis* where the repetition rates are as high as 7–9 s^{-1}. Each note is brief, about 0.012 s long, with two harmonics (others being slightly longer e.g. *T. tandyi* and *T. cryptotis* 0.039–0.048 s). The mean frequency of the harmonics is 980 Hz and 1960 Hz with a fainter third harmonic at 2940 Hz. The second harmonic is emphasized (differing from *T. krugerensis* with multiple and higher frequency harmonics 1900–3100 Hz; *T. tandyi* 2500–2700 Hz; *T. delalandii* 1800–2200 Hz; *T. cryptotis* 3100–3800 Hz). Subarticular tubercles single and prominent. Webbing on feet is scanty; up to 2 ½ phalanges free of web on the forth digit. Glandular ridge below the tympanum asymmetric; continuous on the left side and interrupted on the right side (vs. *T. wambensis* **sp. nov**. where it is interrupted on both sides). Outer metatarsal tubercle absent; a weak whitish bump present instead. Dorsal pattern consists of irregular grey patches on a lighter background (Figs. 6A and D) contrasting with greenish brown patches on a lighter background in *T. wambensis* **sp. nov.**; irregular dark markings and dark spots on a lighter ground colour in *T. krugerensis* and also varying from the scantily marked orange-brown to grey-brown of *T. marmorata*. Scanty, slightly enlarged brown warts present on the dorsum spreading to dorsolateral surface, bordered with black edges (different from more denser, small reddish-brown warts present in *T. wambensis* **sp. nov**.; smooth in *T. krugerensis*, distinctive and prominent tubercles in *T. tuberculosa*). Ventral surface smooth but coarsely granular towards the vent and inner thighs (Figs. 6G and H).

Description of Holotype. Body is stout and toad like; head comparatively small (HL/SVL 0.28, HW/SVL 0.40), narrow compared to trunk, not longer than wide (HL/HW 0.70); snout short (SL/HL 0.55), rounded in dorsal view, blunt in profile (Fig. 6A), slightly projecting beyond moderately cusped lower jaw, narrower than long (SL/INS 1.56); canthus rostralis is moderately angled; loreal region almost straight; nostrils situated on slight projections of the canthus, approximately midway between the snout tip and the eye (ENL/SNL 1.06); eyes directed anterolaterally, protrusion less distinct, relatively small (ED/HL 0.47); snout longer than eye diameter (ED/SL 0.86); interorbital distance is shorter than upper eyelid (IOS/ED 1.25), and longer than internarial distance (INS/ED 0.75); tympanum distinct externally, vertically oval, discontinuous glandular ridge present below, smaller than eye diameter (TD/ED 0.70); upper jaw without dentition; choanae large, round, located anteriorly on the roof of the mouth and easily visible, vomerine teeth in two oblique rows between the choanae; tongue short and strongly notched behind, divided in fairly equal parts (6.0 at widest part); median lingual processes present; vocal sac single, darkly pigmented; gular flap absent, with three distinct longitudinal folds clearly visible (Fig. 6G).

Dorsal surfaces of head, trunk and limbs generally smooth; ventral surface of limbs, gular and abdomen smooth, but turning coarsely granular in the ventral area and thighs.

Fore limbs fairly thick; hand moderately large (HND/SUL 0.21); tips of fingers not enlarged into discs; relative length of fingers: IV<II<I<III; subarticular tubercles single and distinct, with one on fingers I and IV, two on finger II and three on finger III; fingers without webbing; thenar tubercle distinct; palmar tubercles absent; metacarpals without supernumerary tubercles; nuptial pad present on manual digit I.

Hind limbs stout, more than half body length (LEG/SVL 0.83); tibio-tarsal articulation not reaching to level of tip of snout when legs are adpressed to body; tibiofibula short (TL/SVL 0.40), slightly shorter than thigh (TL/FL 0.91); heels not reaching each other when knees are flexed and thighs are held laterally at right angle to body; foot longer than tibiofibula (FOT/TL 1.09); relative length of toes: I<II<V<III<IV; toes without expanded discs; subarticular tubercles: one on toe I and II, two on toe III and V and three on toe IV; pedal webbing formula **I** 1–1 **II** 1.5–1.5 **III** 1.5^{+}–1.5 **IV** 1.5^{+}–2**V**; inner metatarsal tubercle prominent and shovel-shaped; outer absent.

Colouration in life. The dorsal pattern consists of irregular grey patches on a lighter background. Scanty, brown warts present on the dorsum spreading to dorsolateral surface, bordered with black edges; darker bar present between the eyes, anteriorly bordered by a white patch extending to the snout; dorsal stripe absent, ventrum creamy

white, turning pinkish on the ventral surface and palms, soles of feet black; extended dark patch on the throat; hands and thighs show two and three transverse dark markings respectively. **Colouration in preservative**. Reddish dorsal wart and creamy colours on the ventral surface are lost; the other patterns are visible.

FIGURE 6. *Tomopterna gallmanni* from Laikipia Nature Conservancy, Kenya: dorsolateral views in life (A) NMK A/5039/1, male and (D) NMK A/5039/3, female; dorsal views in alcohol (B) NMK A/5039/1, male and (C) NMK A/5039/3, female; ventral views in alcohol (E) NMK A/5039/1, male and (F) NMK A/5039/3, female; ventral views in life (G) NMK A/5039/1, male and (H) NMK A/5039/3, female.

Paratype variation. The paratypes are similar to the holotype in measurements (Table 4). Female type specimen (SVL 55.9) is larger than males (SVL 40.0–45.0, mean 43.8, n=6). Thin, continuous white dorsal line from the snout to the vent in the female specimen, dorsolateral white steaks originating just behind the tympanum

running towards the thigh but not reaching it; in other male specimens dorsal white line present but interrupted near the head. Female throat almost clear, with only scant dark speckles (Fig. 6H). See measurements (in mm) of the type series of *Tomopterna gallmanni* in Table 3.

Eggs and tadpoles. Unknown.

Habitat. *T. gallmanni* specimens were found in habitats similar to those of *T. wambensis*. Large breeding congregations were observed in Laikipia Nature Conservancy. Solitary males were captured crossing the road during day time following a heavy downpour (at Lewa and near Baragoi). Calling males took solitary positions, in some cases in shallow edges of a water body. The following species were found sympatrically with *T. gallmanni*: *Ptychadena anchietae and Ptychadena mascareniensis*.

Distribution. The collecting records of *Tomopterna gallmanni* are mostly situated on the Laikipia Plateau of Kenya, with two northern populations at Maralal and Baragoi areas. Current data suggest a limited range, but with some populations known within privately protected property. The elevation of collecting localities ranged from 1314 m (Baragoi-South Horr Road) to 1952 m (Lewa Wildlife Conservancy).

Etymology. The species is named for the Gallmann Memorial Foundation for contributing funding for this research.

Remarks. Based on its wide distribution, and abundance at breeding sites, we propose a Least Concern status according to the current IUCN Redlist criteria.

Discussion

Currently, seven *Tomopterna* species are considered to be present in East Africa (Channing & Howell, 2006; Rödel & Hallerman, 2006) *T. cryptotis, T. luganga, T. tuberculosa*, *T. monticola, T. tandyi,* including *T. wambensis* **sp. nov**. and *T. gallmanni* **sp. nov**. with *T. kachowski* present in Ethiopia. Five of these species, *T. cryptotis, T. gallmanni* **sp. nov.**, *T. monticola, T. tandyi* and *T. wambensis* **sp. nov**. are present in Kenya. The identification of museum specimens remains a hindrance to understanding geographical patterns unless calls and molecular data are available (Channing & Howell 2006).

The phylogeny presented here is incompletely resolved. This could be attributed to the fact that the tree was inferred from a single gene only. Although other studies have used more markers e.g. 12S and 16S rRNA (Dawood & Uqubay 2004); 12S rRNA, valine-tRNA and 16S rRNA (Zimkus & Larson, 2011), here we were only attempting to identify Kenyan species. An unidentified taxon (here called *Tomopterna* sp A from Mauritania) as well as some sand frog populations from Kenya have been, or still remain, assigned to *T. cryptotis* (Dawood & Uqubay 2004; Channing & Howell 2006). In the current findings, *T. wambensis* **sp. nov**. was found to be morphologically similar to "*T. cryptotis"* from Kenya, but the lack of genetic data for the material prevents us determining possible synonymies. We did not make any morphological comparisons with *T.* sp. A from Ayoun el-Atrouss. Since no molecular data for *T. cryptotis* were available from Kenya at the time of this study, the relationship with the new taxon cannot be resolved with certainity. Results from a more recent molecular and morphological investigation by Zimkus & Larson (2011) had concluded that *T. cryptotis* might not be present in the horn of Africa contrary to earlier range estimations for this species. In fact, these findings seem to support Pickersgill (2007) in his earlier remarks that *T. cryptotis*, whose type locality is documented as "Catequero, Ponang Kuma (Dongwenna), and in the Kafitu Swamps" in Angola, might consist of cryptic species.

The mean genetic distances of *T. wambensis* **sp. nov**. and *T. gallmanni* **sp. nov**. from the other species of the genus ranges between 3.1% and 8.9%. They seem to be clearly separated from *T. cryptotis* and *T. tandyi* which are documented to be present in Kenya. There was a divergence of 4.4% and 4.3% between *Tomopterna wambensis* **sp. nov**. and *T. cryptotis* and *T. tandyi* respectively. Similarly, *Tomopterna gallmanni* **sp. nov**. had a divergence of 3.9% and 3.7% from *T. cryptotis* and *T. tandyi* respectively. The intra-sequence variation ranged from 0–0.05% and 0–0.02% within *T. wambensis* **sp. nov**. and *T. gallmanni* **sp. nov**. from 12 and five sequences used respectively. In spite of these observed differences, the polyploid nature of *T. tandyi* makes its molecular identification extremely challenging. The karyotype of the materials analyzed here was not determined to confirm polyploidy in any of the populations. Channing & Bogart (1996) based the distribution of *T. tandyi* on tetraploid records and field recordings of calls. To identify the materials available to us, we explored the latter approach.

TABLE 3. Measurements of males of *T. wambensis* **sp. nov.** and *T. gallmanni* **sp. nov.** including *T. cryptotis* from Kenya. Voucher status is indicated by H = holotype and P = paratype.

Catalogue No.	Taxa label	Status	SVL	HW	HL	TD	SL	INS	SNL	ED	IOS	ENL	HL	RL	MD I	MD II	MD III	MD IV	FL	TL	PD I	PD II	PD III	PD IV	PD V	TMT
NMK A/5152/1	*T. wambensis*	H	37.0	15.0	11.7	3.7	6.3	4.3	2.6	5.1	6.8	3.1	5.7	7.8	3.0	3.3	5.3	2.4	14.2	14.6	1.8	2.8	5.5	7.6	2.9	3.8
NMK A/5152/2	*T. wambensis*	P	38.0	15.2	10.9	4.0	5.5	4.2	2.9	5.7	6.4	3.1	5.5	7.2	2.8	3.2	5.2	2.7	14.4	14.2	1.9	2.9	5.5	7.4	3.0	3.8
NMK A/5152/3	*T. wambensis*	P	36.9	15.0	10.1	3.5	6.2	4.2	2.8	5.5	7.0	3.2	5.0	7.3	2.7	3.0	5.3	2.5	14.3	14.2	1.8	3.0	5.1	7.7	2.7	3.4
NMK A/5057/1	*T. wambensis*	P	40.8	15.7	12.3	4.4	6.0	4.4	3.0	4.8	7.0	3.2	8.1	7.2	3.9	3.5	5.6	3.2	16.5	15.3	2.3	3.0	5.8	7.9	4.9	3.5
NMK A/5057/2	*T. wambensis*	P	37.4	16.6	13.7	2.7	5.2	3.4	2.5	6.2	6.6	3.2	5.3	6.7	2.6	2.9	5.0	1.9	12.1	14.0	1.7	3.2	4.0	6.8	4.2	3.2
NMK A/5057/3	*T. wambensis*	P	37.4	15.5	13.2	3.9	5.8	3.4	2.8	5.9	6.7	3.3	5.0	6.9	2.8	2.5	4.9	2.0	13.1	13.8	1.4	3.2	4.0	6.8	4.4	3.1
NMK A/5057/4	*T. wambensis*	P	36.2	14.5	13.2	3.8	6.0	4.0	2.5	6.0	6.4	3.4	5.4	7.1	2.8	2.6	4.8	2.3	14.3	13.9	1.5	3.2	4.2	7.1	4.2	3.0
NMK A/5057/6	*T. wambensis*	P	38.3	15.6	13.6	3.0	5.8	3.8	3.2	5.2	6.0	4.0	4.8	7.2	2.6	2.4	4.9	2.9	13.2	13.8	1.6	3.4	4.3	6.8	4.3	2.8
NMK A//5149/1	*T. wambensis*	P	38.2	16.8	12.7	4.2	6.5	3.8	2.5	4.7	6.6	3.6	6.6	6.7	3.4	3.3	4.9	2.8	16.1	14.7	2.3	4.5	5.6	9.8	5.4	3.0
NMK A/5149/2	*T. wambensis*	P	38.3	16.7	10.6	4.0	6.0	4.0	3.1	5.2	6.7	3.7	6.1	7.1	3.5	3.5	5.7	3.0	14.7	14.4	2.1	3.5	5.0	8.6	4.6	3.2
NMK A/5149/3	*T. wambensis*	P	38.0	16.7	11.7	4.0	5.6	4.1	2.8	5.0	7.1	3.7	7.6	7.5	3.0	2.9	4.9	2.7	17.1	14.3	2.0	3.4	4.6	8.1	4.3	4.0
NMK A/5145/1	*T. wambensis*	P	34.6	14.9	10.9	4.4	6.5	4.1	3.5	4.8	6.8	3.5	6.5	8.1	3.3	2.8	5.3	2.3	14.5	14.1	2.0	3.6	5.6	8.3	3.9	3.1
NMK A/5039/1	*T. gallmanni*	H	45.0	18.1	12.7	4.2	7.0	4.5	3.2	6.0	7.5	3.4	5.9	9.5	3.1	2.9	5.6	2.1	19.6	17.8	2.0	3.8	5.9	9.2	4.9	4.3
NMK A/5039/2	*T. gallmanni*	P	44.9	18.2	13.5	4.5	7.4	4.4	3.9	6.8	7.5	3.6	6.6	9.1	3.7	3.4	5.9	2.5	18.0	18.0	1.8	3.1	6.1	10.3	5.2	4.1
NMK A/5482/2	*T. gallmanni*	P	40.0	18.6	14.8	3.5	6.4	4.6	3.1	6.6	7.1	3.7	7.3	8.2	3.5	3.3	5.7	2.0	16.4	17.4	2.0	2.8	5.8	10.0	4.5	3.7
NMK A/5482/5	*T. gallmanni*	P	44.7	19.7	14.1	3.7	6.5	4.7	3.6	6.7	7.3	3.3	5.5	8.8	2.8	2.5	6.0	2.0	18.8	17.6	1.3	2.5	6.1	10.0	5.4	4.0

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TABLE 3. (Continued)

Catalogue No.	Taxa label	Status	SVL	HW	HL	TD	SL	INS	SNL	ED	IOS	ENL	HL	RL	MD I	MD II	MD III	MD IV	FL	TL	PD I	PD II	PD III	PD IV	PD V	IMT
NMK A/5045	*T. gallmanni*	P	44.6	18.6	14.6	4.2	7.2	3.6	3.3	5.6	7.3	4.2	8.8	10.5	4.4	3.7	4.7	2.6	21.2	17.9	2.9	3.6	5.8	10.0	5.4	3.7
NMK A/5154/2	*T. gallmanni*	P	43.5	18.4	14.2	3.8	6.6	4.1	3.6	6.0	6.8	3.5	10.2	9.2	3.9	4.0	6.4	3.3	19.5	17.9	2.2	3.5	5.7	11.2	6.3	3.5
NMK A/853/3	*T. cryptotis*		37.2	16.5	12.6	3.1	5.5	3.8	3.4	6.0	6.7	3.1	6.0	8.2	4.4	4.3	5.6	2.7	17.7	14.9	1.7	3.3	5.1	8.6	5.5	2.9
NMK A/853/4	*T. cryptotis*		36.4	16.8	13.0	2.9	5.6	4.0	2.7	5.4	6.4	3.1	6.2	7.6	4.2	3.8	5.9	2.2	16.4	14.0	1.6	3.6	5.0	8.7	5.2	3.0
NMK A/4819/1	*T. cryptotis*		39.0	16.4	13.9	3.7	6.3	4.1	3.8	4.8	7.0	3.3	6.6	7.6	4.2	3.7	5.8	2.2	17.4	15.5	1.7	3.6	5.3	9.7	5.2	3.0
NMK A/4819/2	*T. cryptotis*		39.1	16.1	13.9	3.8	6.0	4.0	3.1	4.9	6.8	4.3	7.0	7.8	4.1	3.8	5.7	2.3	15.0	15.1	1.8	3.7	4.2	9.3	5.2	3.2
NMK A/4819/3	*T. cryptotis*		39.3	17.0	12.1	3.8	6.0	3.9	3.8	5.4	6.6	3.4	6.8	7.6	4.0	3.9	5.6	2.4	15.2	14.2	1.9	3.8	5.2	9.3	5.4	3.2
NMK A/4819/4	*T. cryptotis*		36.6	15.8	11.3	3.5	5.6	3.7	2.8	4.3	7.4	3.4	6.9	7.5	4.0	3.9	5.7	2.7	15.3	14.3	1.7	3.7	5.3	9.4	5.5	3.3
NMK A/4819/5	*T. cryptotis*		38.0	15.8	11.6	3.5	6.7	4.0	3.0	5.7	7.4	3.2	7.0	7.7	3.9	2.8	5.7	2.6	15.4	14.2	1.8	3.8	5.4	8.7	5.4	3.1
NMK A/1032/1	*T. cryptotis*		34.2	15.6	10.1	3.4	6.8	3.7	3.0	5.0	6.5	3.1	6.8	6.8	3.2	3.2	5.3	2.7	14.1	14.2	1.7	3.0	5.3	8.4	4.1	3.2
NMK A/1032/2	*T. cryptotis*		35.2	15.2	10.8	2.9	5.5	4.1	2.9	5.3	6.9	3.2	6.3	7.4	3.9	3.9	5.2	2.4	15.2	13.8	1.7	3.8	5.2	7.3	4.6	3.6
NMK A/1032/3	*T. cryptotis*		33.5	15.3	10.6	3.4	5.6	3.8	2.7	5.0	6.7	2.7	5.2	7.4	4.0	3.9	5.1	2.7	14.2	14.3	1.6	3.3	5.3	8.0	4.2	2.7
NMK A/1032/4	*T. cryptotis*		35.2	15.7	11.0	2.8	6.2	3.2	2.6	5.3	6.9	3.0	6.2	7.4	3.8	3.2	5.3	2.7	14.3	14.4	1.7	3.4	5.2	8.1	4.2	3.1
NMK A/1032/5	*T. cryptotis*		37.1	14.8	10.2	3.1	5.5	4.2	2.1	5.7	7.2	3.2	5.3	7.0	3.9	3.4	5.4	2.8	14.3	15.1	1.7	3.4	5.3	8.2	4.2	3.3

We found significant differences between the advertisement calls from Kenya and published calls for many other species. The calls of *T. wambensis* **sp. nov**. and *T. gallmanni* **sp. nov**. can be distinguished by note length, with *T. wambensis* **sp. nov**. notes being about twice the duration of the notes of *T. gallmanni* **sp. nov**. The note repetition rate of *T. wambensis* **sp. nov**. is slightly faster than that of *T. gallmanni* **sp. nov**. The new taxa are separated from *T. tandyi, T. luganga* and *T. cryptotis* on the basis of call length and repetition rate (Channing & Bogart, 1996; Channing *et al*. 2004). The advertisement call for *T. monticola* remains unknown.

On the basis of the available information, we found neither *T. wambensis* **sp. nov**. nor *T. gallmanni* **sp. nov**. to be synonymous with any of the East African *Tomopterna*. The recently rediscovered *T. monticola* by Rödel & Hallerman (2006) from "Massai area, creek Wasso-Njiro" [Ewaso Nyiro] in Kenya (not examined by us) requires genetic data from additional specimens in order for its status to be confirmed. No *Tomopterna* were available from Ewaso Nyiro and our comparisons are limited to the redescription of the holotype *T. monticola.* However, in the absence of a more positive sex determination of the holotype material of *T. monticola* as noted by Rödel & Hallerman (2006), our morphological comparisons as well as those noted under the redescription may require further corroboration.

It has been demonstrated by this and other studies that the identification of *Tomopterna* species requires a synergy of techniques other than morphology alone (Channing & Bogart 1996, Dawood & Channing 2002, Channing *et al.* 2004, Dawood *et al.* 2002, Dawood & Uqubay 2004). These findings also agree with more recent findings from a morphological analysis (Zimkus & Larson 2011) which suggested that unidentified specimens from east Africa may contain more cryptic taxa.

This study reaffirms the vital role of molecular data in determining species boundaries in morphologically similar species. Within the East African region (Ethiopia, Kenya, Somalia, Tanzania and Uganda), there are eight described sand frogs including the two additional species described here. In Kenya, it is important to note that large areas still remain to be studied and we recommend further sampling to confirm the status of *T. monticola* and ascertain the distribution of the newly described *T. wambensis* **sp. nov**. and *T. gallmanni* **sp. nov**.

Key to the species of *Tomopterna* from Kenya, Uganda and Tanzania

1	Vomerine teeth absent; more than 2.5 phalanges on toe IV free of web	*T. tuberculosa*
1'	Vomerine teeth present	2
2	Glandular ridge below tympanum a series of isolated glands	*T. tandyi*
2'	Glandular ridge below tympanum either a continuous or discontinuous ridge	3
3	Nostril closer to eye than to snout tip	*T. luganga*
3'	Nostril approximately equidistant or closer to snout tip than eye	4
4	Tympanum indistinct or not visible	*T. cryptotis*
4'	Tympanum visible	5
5	Ventral surface smooth but coarsely granular towards the vent and thighs	*T. gallmanni*
5'	Ventral surface generally smooth	6
6	Snout-vent length usually less than 40 mm, dorsal colouration greenish brown patches on a lighter background	*T. wambensis*
6'	Snout-vent length usually more than 40 mm, dorsal colouration greyish-brown with darker blotches, potentially symmetrical	*T. monticola*

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References

Bazinet, A.L. & Cummings, M.P. (2011) Computing the Tree of Life – Leveraging the Power of Desktop and Service Grids. *In: Proceedings of the Fifth Workshop on Desktop Grids and Volunteer Computing Systems* (PCGrid 2011), 1896–1902.

Bazinet, A.L. & Cummings, M.P. (2008) The Lattice Project: a grid research and production environment combining multiple grid computing models. *In:* Weber, M.H.W. (Ed.), *Distributed & Grid Computing - Science Made Transparent for Everyone. Principles, Applications and Supporting Communities.* Rechenkraft.net, Marburg, pp. 2–13.

Bazinet, A.L., Myers, D.S., Fuetsch, J. & Cummings, M.P. (2007) Grid services base library: a high-level, procedural application program interface for writing Globus-based Grid services. *Future Generation Computer Systems,* 23, 517–522. http://dx.doi.org/10.1016/j.future.2006.07.009

Bossuyt, F. & Milinkovitch, M.C. (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences USA,* 97, 6585–6590. http://dx.doi.org/10.1073/pnas.97.12.6585

Channing, A. & Bogart, J.P. (1996) Description of a tetraploid *Tomopterna* (Anura: Ranidae) from South Africa. *South African Journal of Zoology,* 31, 80–85.

Channing, A. & Howell, K.M. (2006) *Amphibians of East Africa*. Cornell University Press, New York, 418 pp.

Channing, A., Moyer, D.C. & Dawood, A. (2004) A new sand frog from central Tanzania (Anura: Ranidae: *Tomopterna*). *African Journal of Herpetology,* 53, 21–28. http://dx.doi.org/10.1080/21564574.2004.9635495

Cummings, M.P., Handley, S.A., Myers, D.S., Reed, D.L, Rokas, A. & Winka, K. (2003) Comparing bootstrap and posterior probability values in the four-taxon case. *Systematic Biology,* 52, 477–487.

Cummings, M.P. & Huskamp, J.C. (2005) Grid computing. *Educause Review*, 40, 116–117.

Dawood, A. & Channing, A. (2002) Description of a new cryptic species of African sand frog, *Tomopterna damarensis* (Anura: Ranidae), from Namibia. *African Journal of Herpetology,* 51, 129–134. http://dx.doi.org/10.1080/21564574.2002.9635468

Dawood, A., Channing, A. & Bogart, J.P. (2002) A molecular phylogeny of the frog genus *Tomopterna* in southern Africa: examining species boundaries with mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics and Evolution,* 22, 407–413. http://dx.doi.org/10.1006/mpev.2001.1060

Dawood, A. & Uqubay, S.M. (2004) A molecular phylogeny of the sand frog genus *Tomopterna* (Amphibia: Anura: Ranidae) based on mitochondrial 12S and 16S sequences. *African Zoology,* 39, 145–151.

Dubois, A. (2005) Amphibia Mundi. 1.1. An ergotaxonomy of recent amphibians. *Alytes Paris*, 23, 1–24.

Frost, D.R. (2013) Amphibian Species of the World: an Online Reference. Version 5.6. Electronic Database. American Museum of Natural History, New York, USA. Available from: http://research.amnh.org/herpetology/amphibia/ index.html (Accessed 19 April 2013)

Frost, D.R., Grant, T., Faivovich J., Bain, R.H., Haas, A., Haddad, C.F.B., De Sa, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler,W.C. (2006) Amphibian Tree of Life. *Bulletin of the American Museum of Natural History*, 297, 1–370. http://dx.doi.org/10.1206/0003-0090(2006)297[0001:tatol]2.0.co;2

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, 52, 696–704.

Haas, A., Mitgutsch, C., Hertwig, S., Dawood, A. & Channing, A. (2006) The tadpole of *Tomopterna luganga* Channing, Moyer & Dawood, 2004 (Anura: Ranidae). *Salamandra,* 42, 1–12.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series,* 41, 95–98.

Hillis, D.M., Mable, B.K., Larson, A., Davies, S.K. & Zimmer, E.A. (1996) Nucleic acids IV: Sequencing and Cloning. *In*: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular Systematics.* 2nd ed. Sinauer, Sunderland, MA, pp. 321–381.

Huelsenbeck, J.P. & Ronquist, F. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics,* 19, 1572–1574. http://dx.doi.org/10.1093/bioinformatics/btg180

Myers, D.S., Bazinet, A.L. & Cummings, M.P. (2008) Expanding the reach of Grid computing: combining Globus- and BOINC-based systems. *In:* Talbi, E.-G. & Zomaya, A. (Eds.), *Grids for Bioinformatics and Computational Biology, Wiley Book Series on Parallel and Distributed Computing.* John Wiley & Sons, New York, pp. 71–85.

Myers, D.S. & Cummings, M.P. (2003) Necessity is the mother of invention: a simple grid computing system using commodity tools. *Journal of Parallel and Distributed Computing,* 63, 578–589. http://dx.doi.org/10.1016/s0743-7315(03)00004-2

Pickersgill, M. (2007) *Frog Search. Results of Expeditions to Southern and Eastern Africa from 1993-1999. Frankfurt Contributions to Natural History Volume 28*. Edition Chimaira, Frankfurt am Mainz, 575 pp.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 1253–1256. http://dx.doi.org/10.1093/molbev/msn083

Rödel, M.-O. & Hallerman, J. (2006) Rediscovery and identity of *Phrynobatrachus monticola* Fischer, 1884. *African Journal of Herpetology*, 55, 69–74.
http://dx.doi.org/10.1080/21564574.2006.9635540

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, 87, 651–701.

Sukumaran, J. & Holder, M.T. (2010) DendroPy: A Python library for phylogenetic computing. *Bioinformatics,* 26, 1569–1571.
http://dx.doi.org/10.1093/bioinformatics/btq228

Swofford, D.L. (2002) PAUP* Phylogenetic analysis using parsimony (*and other methods) Version 4b10. Sinauer Associates, Sunderland, MA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28, 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121

Van der Meijden, A., Vences, M., Hoegg, S. & Meyer, A. (2005) A previously unrecognized radiation of ranid frogs in southern Africa revealed by nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 37, 674–685.
http://dx.doi.org/10.1016/j.ympev.2005.05.001

Zimkus, B.M. & Larson, J.G. (2011) Examining the molecular relationships of sand frogs (Anura: Pyxycephalidae: *Tomopterna*) and the resurrection of two new species from the Horn of Africa. *Zootaxa*, 2933, 27–45.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. dissertation, The University of Texas at Austin, 125 pp.

APPENDIX 1. A list of taxa used in the molecular analysis, their catalogue numbers, locality information and the GenBank accession numbers for the 16S rRNA sequences. Sequences generated as part of this study are marked by an asterisk (*).

Taxon	Voucher	Locality	GenBank 16S
Pyxicephalus adspersus	ZFMK 66446	KwaBonambi, South Africa	AF215505
Tomopterna cryptotis	no voucher	Bredell, South Africa	AF215506
Tomopterna cryptotis	AC 1154	Bloemfontein, South Africa	AY255099
Tomopterna cryptotis	AC 1100	Bubi, Zimbabwe	AY255090
Tomopterna damarensis	AC 1668	Khorixas, Namibia	AY255091
Tomopterna damarensis (as *T.* "cf. *tandyi*")	no voucher	no locality (Bossuyt *et al.* 2006)	DQ347301
Tomopterna damarensis (as *T.* "cf. *tandyi*")	ZFMK 66403	Khorixas, Namibia	AF215419
Tomopterna damarensis	no voucher	Khorixas, Namibia	DQ019610
Tomopterna delalandii	AC1134	Stellenbosch, South Africa	AY454372
Tomopterna delalandii	KTH 076	Anysberg, South Africa	EF136565
Tomopterna delalandii	AC 1114	Stellenbosch, South Africa	AY255085
Tomopterna delalandii	AC 944	Stellenbosch, South Africa	AY255086
Tomopterna elegans	MVZ:Herp:242739	Buq Village, Somalia	HQ700692
Tomopterna gallmanni	NMK A5045	Lewa Wildlife Conservancy, Kenya	JX088642*
Tomopterna gallmanni	NMK A5154/1	Mogwooni Ranch, Nanyuki, Kenya	JX088641*
Tomopterna gallmanni	NKM A5159	Road from Baragoi to South Horr, Kenya	JX088645*
Tomopterna gallmanni	NMK A5039/2	Laikipia Nature Conservancy, Kenya	JX088643*
Tomopterna gallmanni	NMK A5151/1	Maralal, Baawa Village, Kenya	JX088644*
Tomopterna krugerensis	AC 1506	Tembe Elephant Park, South Africa	AY255094
Tomopterna krugerensis	AC 1508	Tembe Elephant Park, South Africa	AY255098
Tomopterna luganga	AC 2063	Kigwembimbe, Iringa, Tanzania	AY547275
Tomopterna luganga	AC 2060	Dodoma, Tanzania	AY547276

.....continued on the next page

APPENDIX 1. (Continued)

Taxon	Voucher	Locality	GenBank 16S
Tomopterna luganga	AD 310	Tanzania	DQ017056
Tomopterna luganga	AD 309	Tanzania	AY751302
Tomopterna marmorata	AC 1387	Legalamectse Nature Reserve, South Africa	AF371233
Tomopterna marmorata	AC 1534	Zambezi Nkuku, Zambia	AY255084
Tomopterna natalensis	AC 1391	Mafefe Road, South Africa	AY255088
Tomopterna natalensis	AC 1451	Andries Vosloo Kudu Reserve, South Africa	AY255089
Tomopterna natalensis	no voucher	Itala Park, South Africa	AF215508
Tomopterna natalensis	ZFMK 68815	Port St Johns, South Africa	AF215509
Tomopterna natalensis	TM 84342	Tygerskloof Plantation, South Africa	AY205286
Tomopterna natalensis	ES 225	Kyalami, Johannesburg, South Africa	AY547277
Tomopterna natalensis	no voucher	unknown (Bossuyt *et al.* 2006)	AF215507
Tomopterna sp. A	ZFMK 76391	Ayoun el-Atrouss, Mauritania	AY255101
Tomopterna sp. A	ZFMK WB02	Mauritania	AY014838
Tomopterna sp. B	AC 1549	Shankara, Namibia	AY255095
Tomopterna sp. C	AC 1115	Beira, Mozambique	AY255092
Tomopterna sp. C	AC 1116	Beira, Mozambique	AY255093
Tomopterna kachowskii	AAU A2008-310	Akaki Wetland, Ethiopia	HQ700687
Tomopterna kachowskii	AAU A2008-317	Awash River, 40 Km SW of Addis Ababa, Ethiopia	HQ700688
Tomopterna kachowskii	AAU A2008-321	Awash River, 40 Km SW of Addis Ababa, Ethiopia	HQ700689
Tomopterna kachowskii	MVZ:Herp:241321	4 km north of Borama, Somalia	HQ700690
Tomopterna kachowskii	MVZ:Herp:241323	4 km north of Borama, Somalia	HQ700691
Tomopterna tandyi (as "*T. marmorata*")	no voucher	Mt Meru, Tanzania	AF215507
Tomopterna tandyi	AC 1567	Hardap Dam, Namibia	AF436073
Tomopterna tandyi	AC 1556	Grootfontein, Namibia	AF371234
Tomopterna tandyi	AC 2006	Adelaide, South Africa	AF436071
Tomopterna tandyi	AD 14	Blouberg Nature Reserve, South Africa	AF436072
Tomopterna tandyi	AD 231	Brak River, near Blouberg Nature Reserve, South Africa	AY838891
Tomopterna tuberculosa	AC 1670	Ongongo, Namibia	AY255087
Tomopterna tuberculosa	AC 1672	Ongongo, Namibia	AY255100
Tomopterna wambensis	NMK A5057/1	Ikave, Kitui, Kenya	JX088646*
Tomopterna wambensis	NMK A5149/1	Mpala Research Center, Kenya	JX088647*
Tomopterna wambensis	NMK A5149/2	Mpala Research Center, Kenya	JX088648*
Tomopterna wambensis	NMK A5149/3	Mpala Research Center, Kenya	JX088649*
Tomopterna wambensis	NMK A5152/1	Wamba (Sodor Water Pan), Kenya	JX088651*
Tomopterna wambensis	MCNPV CA337	Lagga Daudi, Lake Turkana, Kenya	JX088655*
Tomopterna wambensis	MCNPV CA334	17 km S of Allia Bay, Lake Turkana, Kenya	JX088650*
Tomopterna wambensis	MCNPV CA250	Illeret, Lake Turkana, Kenya	JX088653*
Tomopterna wambensis	MCNPV CA252	Karsa Well, Allia Bay, Lake Turkana, Kenya	JX088654*
Tomopterna wambensis	NMK A4323	Bura Hasuma, Lake Turkana, Kenya	JX088656*
Tomopterna wambensis	NMK A5145/1	Kitobo Forest, Kenya	JX088652*
Tomopterna wambensis	AC 2062	Arusha, Tanzania	AY547278

APPENDIX 2. Additional materials examined.

The following additional specimens were examined for morphological characters:

Tomopterna cryptotis: NMK A/4819/1–5 (adult males) Kasigau, Taita Hills, Taita-Taveta County, Kenya; NMK A/853/3–4 (adult males) Kakerongole, Turkana County, Kenya; NMK A/1032/1–5 Laisamis, Marsabit County, Kenya (adult males); AC 518–9 Bagani, Namibia; AC 552–3 Klain Hamakari farm, Namibia; AC 1385–6 Legalameetse Nature Reserve, South Africa: AC 1388–9 Hans Merensky Nature Reserve, South Africa; AC 3114 Oas Farm, Namibia; AD 226 Blouberg Nature Reserve, South Africa.

Tomopterna delalandii: AC 3222–4, AC 3228, AC 3229–31 Stellenbosch, South Africa; AC 3232–4 Noordhoek, Cape Town, South Africa; AC 3226 Rondebosch, Cape Town, South Africa; AC 3226 Rondevlei, Cape Town; AC 3227–8 Bellville, South Africa; AC 3235–6, AC 3237–8 Port Elizabeth, South Africa; AC 3239–40 St Francis Bay, South Africa.

Tomopterna krugerensis: AC 543–4 Okakarara, Namibia; AC 1500–3, AC 1507 Tembe Elephant Park, South Africa.

Tomopterna luganga: AC 2061 Dodoma, Tanzania.

Tomopterna marmorata: AC 1387 Legalamectse, South Africa.

Tomopterna natalensis: AC 1379–82, AC 1391–7 Mafefe Road, South Africa.

Tomopterna tandyi: AC 502, AC 510 Klein Hamakari, Namibia; AC 2358 Arusha, Tanzania; AC 2653–4 Naukluft Namibia; AC 1553–1563 Grootfontein, Namibia; AC 1566–1575 Hardap, Namibia.

Tomopterna tuberculosa: AC 1670–1672 Ongongo, Namibia; AC 2099 Kibebe Farm, Tanzania; AC 2302 Morogoro, Tanzania; AC 2062, Arusha, Tanzania; AC 2105, AC 2316 Tatanda, Tanzania.

APPENDIX 3. Morphological characters used to diagnose *Tomopterna* species.

1. Mean standard length, mature male: (1) 37 or less, (2) 38 - 44, (3) >44
2. Subarticular tubercles: (1) single, (2) completely or partially divided
3. Webbing, phalanges free on PDIV: (1) 1, (2) 2–3, (3) >3
4. Glandular ridge below the tympanum: (1) absent, (2) continuous, (3) discontinuous, (4) isolated glands
5. Tympanic membrane: (1) hidden, (2) scarcely visible, (3) distinct
6. Nostril position: (1) closer to snout, (2) mid-distance, (3) closer to eye
7. Canthus rostralis: (1) rounded, (2) moderately angled, (3) sharply angled
8. Tympanum: (1) absent (2) round, (3) ovoid
9. Outer metatarsal tubercle: (1) absent or weak whitish bump, (2) present
10. Inter-orbital bar: (1) absent, (2) interrupted (3) complete
11. Dorsal warts: (1) absent, (2) small, (3) distinct and prominent tubercles
12. Vertebral line: (1) absent, (2) present
13. Vomerine teeth: (1) absent, (2) present
14. Sexually mature males, gular colouration: (1) as venter, (2) sparsely mottled, (3) densely mottled (4) solid/dark
15. Palmer tubercles: (1) absent, (2) slightly prominent, (3) distinct
16. Ventral surface: (1) smooth, (2) coarsely granular near the vent and thighs
17. Barred limbs: (1) absent, (2) present
18. Asperities surrounding vent: (1) absent, (2) present

APPENDIX 4. A matrix of morphological characters and character states in *Tomopterna* species. Character descriptions are given in Appendix 3. Intra-species variation is denoted by a combination of states separated by a comma.

Character	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
T. cryptotis	1	1	3	2	1	2	1	1	1	2	1	1,2	2	1	1	1	2	1,2
T. damarensis	2	1	3	2	3	1	1	2	1	1	1	1	2	4	2	1	2	1
T. delalandii	2	1	3	4	3	1	3	3	2	3	1	1	1	4	3	1	2	2
T. elegans	2	1	2	1,2,3	3	1	3	2	2	1,3	2	1,2	2	3	3	1	2	1,2
T. gallmanni **sp. nov.**	2	2	2	2,3	3	2	2	3	1	2,3	3	1,2	2	2	2	1	2	2
T. kachowskii	2	1	3	2,3	3	1	1,2,3	2	2	1,3	2	1,2	2	4	3	2	2	2
T. krugerensis	2	2	3	2	2	1	3	3	1	3	1	1	2	4	3	2	2	1
T. luganga	2	1	2	3	2	1	2	3	1	1	2	1	1	4	3	1	2	1
T. marmorata	2	1	2	4	3	3	1	2	1	3	1	2	1		2	1	2	1
T. natalensis	1	1	3	2	3	1	1	2	1	1	1	1	2	4	1	1	2	2
T. tandyi	2	1	2	2	1	1	2	1	2	1,2,3	2	1	2	3	2	2	2	2
T. tuberculosa	2	1	3	1,3	2	3	1	2,3	1	3	3	1	1	4	3	1	2	2
T. wambensis **sp. nov.**	2	2	2	3	3	1	2	2	1	3	2	1,2	2	3	3	1	2	1