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A new edible cricket species from Africa of the genus *Scapsipedus*

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Abstract

A new cricket of the genus *Scapsipedus* is described from Kenya. The distribution, acoustic behavior, including call and courtship song, mitochondrial sequences, and data on the biology of that new species are given. This edible cricket is a very promising species for mass production for food and feed.

Key words: Orthoptera, new species, bioacoustics, insect farming

Introduction

Whereas crickets are widely cultivated for human consumption and/or for inclusion in livestock feeds in many regions of the world (van Huis, 2013; Lundy & Parrella, 2015), cricket farming for food and feed is relatively new in Kenya and Africa at large (Ayieko *et al*., 2015). Recently, researchers from Kenya have been testing local cricket species under various rearing conditions for evaluating mass production protocols. Among the most promising was a species of *Scapsipedus* Saussure, 1877. *Scapsipedus* species are quite variable in size and coloration but can be differentiated by examination of male genitalia (Otte & Cade, 1984; Gorochov, 1988). Thirteen species of *Scapsipedus* Saussure, 1877 have been recorded from Africa, and none corresponded to the species that is widely farmed in the Nyanza region and other parts of Kenya, where some organizations have trained several youth and women groups to empower them with this entrepreneurial activity. In the present manuscript, this species is described as *Scapsipedus icipe* **n. sp.** Additionally, data is provided on its distribution in Kenya, its acoustic behavior, and its mitochondrial cytochrome oxidase 1 (CO1) barcode.

Material and methods

Samples. The new taxa described in the present paper are based on specimens recently collected in coastal, central and western regions of Kenya. All samples were collected using pitfall traps.

Measurements. The measurements have been performed on dry specimens or specimens stored in 70% ethanol.

Material repository. MNHN, Muséum national d'Histoire naturelle (France); NMK, the National Museums of Kenya; SH, collection of Sylvain Hugel, Strasbourg, France.

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Molecular characterization. Representative cricket samples collected from the wild at Nyamira (Nyam), situated in the Western highlands (1250–2100 m above sea level) of Kenya bordering Homabay County to the north, Kisii County to the west, Bomet County to the south east and Kericho County to the east and *icipe*, Kasarani, Nairobi reared colony were surface sterilized. Genomic DNA was extracted from individual right hindlegs using the Isolate II genomic DNA Kit (Bioline, London, UK), following manufacturer's instructions. The purity and concentration of the resultant extracted DNA was determined using Nanodrop 2000/2000c Spectrophotometer (Thermo Fischer Scientific, Waltham, Massachusetts, USA). Polymerase chain reaction (PCR) was performed to amplify the cytochrome oxidase I (COI) using LepF1 5' ATTCAACCAATCATAAAGATATTGG 3' and LepR1 5' TAAACTTCTGGATGTCCAAAAAATCA 3' (Hajibabaei *et al*., 2006) primers. The Polymerase chain reaction (PCR) was carried out in a total reaction volume of 20 µl containing 5X My *Taq* reaction buffer (Bioline, London, UK) (5 mM dNTPs, 15 mM $MgCl_2$, stabilizers and enhancers), 0.5 pmol μl^{-1} of each primer, 0.5 mM $MgCl_2$, 0.0625 U μl^{-1} My *Taq* DNA polymerase (Bioline, London, UK) and 15 ng.μl^{-1} of DNA template. This reaction was set up in the Nexus Mastercycler gradient (Eppendorf, Germany), using these cycling conditions: initial denaturation for 2 min at 95°C, followed by 40 cycles of 30 sec at 95°C, 45 sec annealing at 52°C and 1 min at 72°C, then a final elongation step of 10 min at 72°C. The target gene region was 700 base pairs. The amplified PCR products were resolved through a 1.2% agarose gel. DNA bands on the gel were analyzed and documented using KETA GL imaging system trans-illuminator (Wealtec Corp, Meadowvale Way Sparks, Nevada, USA). Successively amplified products were excised and purified using Isolate II PCR and Gel Kit (Bioline, London, UK) following the manufacturer's instructions. The purified samples were shipped to Macrogen Inc Europe Laboratory, the Netherlands, for bi-directional sequencing.

Sequence data analyses. The successful sequences were assembled and edited using Chromas Lite Version 2.1.1 (Thompson *et al*., 1997) and Geneious Version 8 (Kearse *et al*., 2012). For conclusive identification of the species, similarity searches were conducted by querying the consensus sequences via Basic Local Alignment Search Tool (BLAST) at the GenBank database hosted by National Centre of Biotechnology Information (NCBI). In addition to this, query was also done in BOLD (Barcode of Life Database). Pairwise nucleotide sequence divergences and overall transition/transversion ratio were calculated using Kimura 2-parameter (K2P) distance model (Kimura, 1980) in MEGA version 7 (Kumar *et al*. 2016).

***Scapsipedus icipe* Hugel & Tanga, n. sp.**

Figures 1–15, Table 1–2.

Scapsipedus icipe Hugel & Tanga nov. sp., here described.

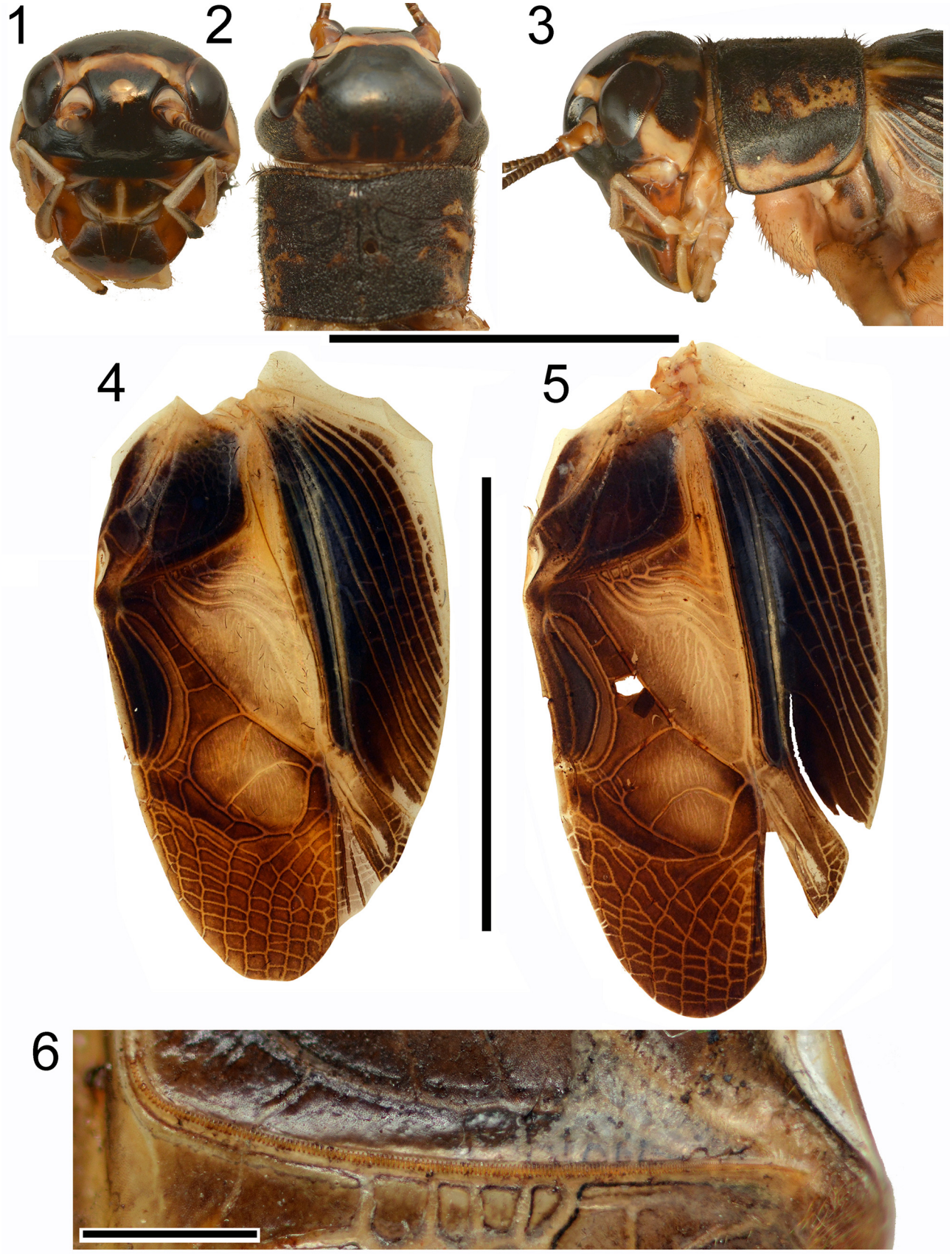
Distribution. Kenya (Figure 15).

Holotype. Male. *icipe* Duduville campus, Kasarani, Nairobi, Kenya, Lat. 01° 13' 14.6" S, Long. 036° 53' 44.5" E, Alt. 1612 m, 04th March 2014, Tanga Mbi leg, NMK.

Paratypes. Males. same as holotype, 2♂ NMK, 3♂ MNHN, 3♂ coll. SH. **Females:** same as holotype, 2♀ NMK, 3♀ MNHN, 3♀ coll. SH.

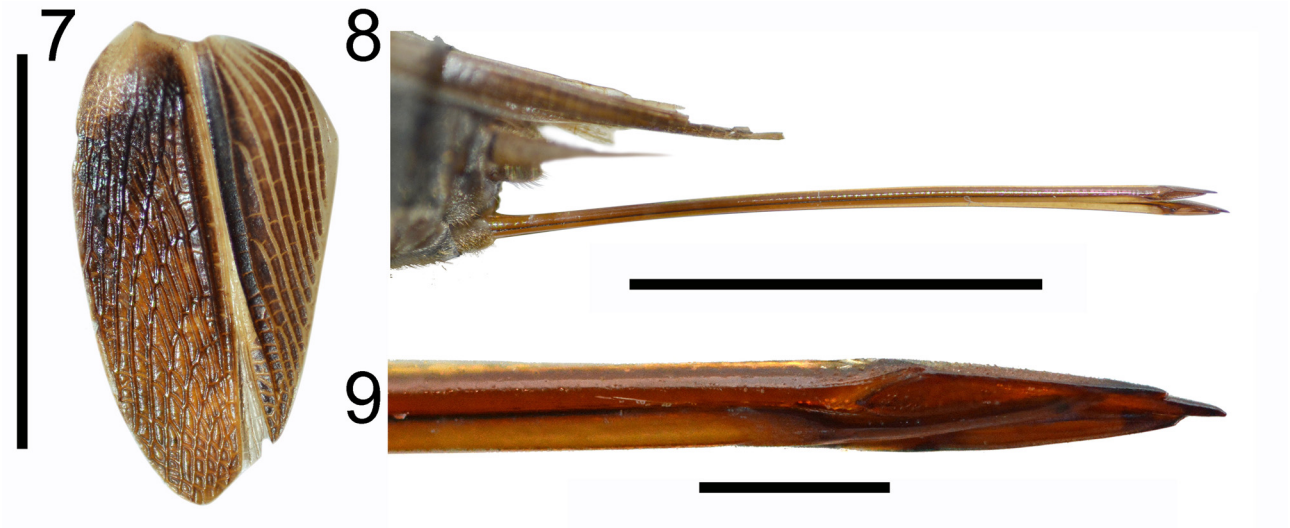
The species was also found in other localities (not included in the *seria typica*), as illustrated in Figure 15.

Diagnosis. Size large for the genus (body length >21 mm); head moderately but distinctly widened below the eyes (frontal view, Fig. 1; unlike *S. latus* Gorochov, 1988 where it is strongly widened; unlike *S. mjagkovi* Gorochov, 1988 and *S. thesigeri* Gorochov, 1993 where it is weakly widened), head black with a distinct yellow band between the eyes, no continuous lighter line around upper and posterior part of the eye (unlike *S. latus*, *S. steinbergi* Gorochov, 1988, *S. amplus* Gorochov, 1988), genae with a yellow ventral line from the posterior part of the eye to mandibles and posterior margin of head (side view, Fig. 3; similar to *S. latus,* somewhat similar to *S. thesigeri*; unlike *S. mjagkovi, S nigriceps* Chopard, 1954*, S. flavomarginatus* (Chopard, 1934), *S. obscuripes* (Chopard, 1962) which have no yellow pattern on genae, occiput with a fine yellow band from the top of the eye to the posterior margin of the head (side view, Fig. 3; unlike *S. nigripes* and *S. flavomarginatus* which have uniformly black heads, unlike *S. mjakovi* and *S. amplus* which have discontinuous bands, and unlike other species that have different patterns). Male genitalia similar to *S. mjagkovi*, distal projection of pseudepiphallus narrowing regularly at the basis (not strongly narrowing unlike *S. mjakovi* and *S amplus*, in dorsal view, Fig. 12), terminal part blunt-V-shaped (in dorsal view, Fig. 12; unlike all other species) widened ventral lobe of pseudepiphallus (the part with

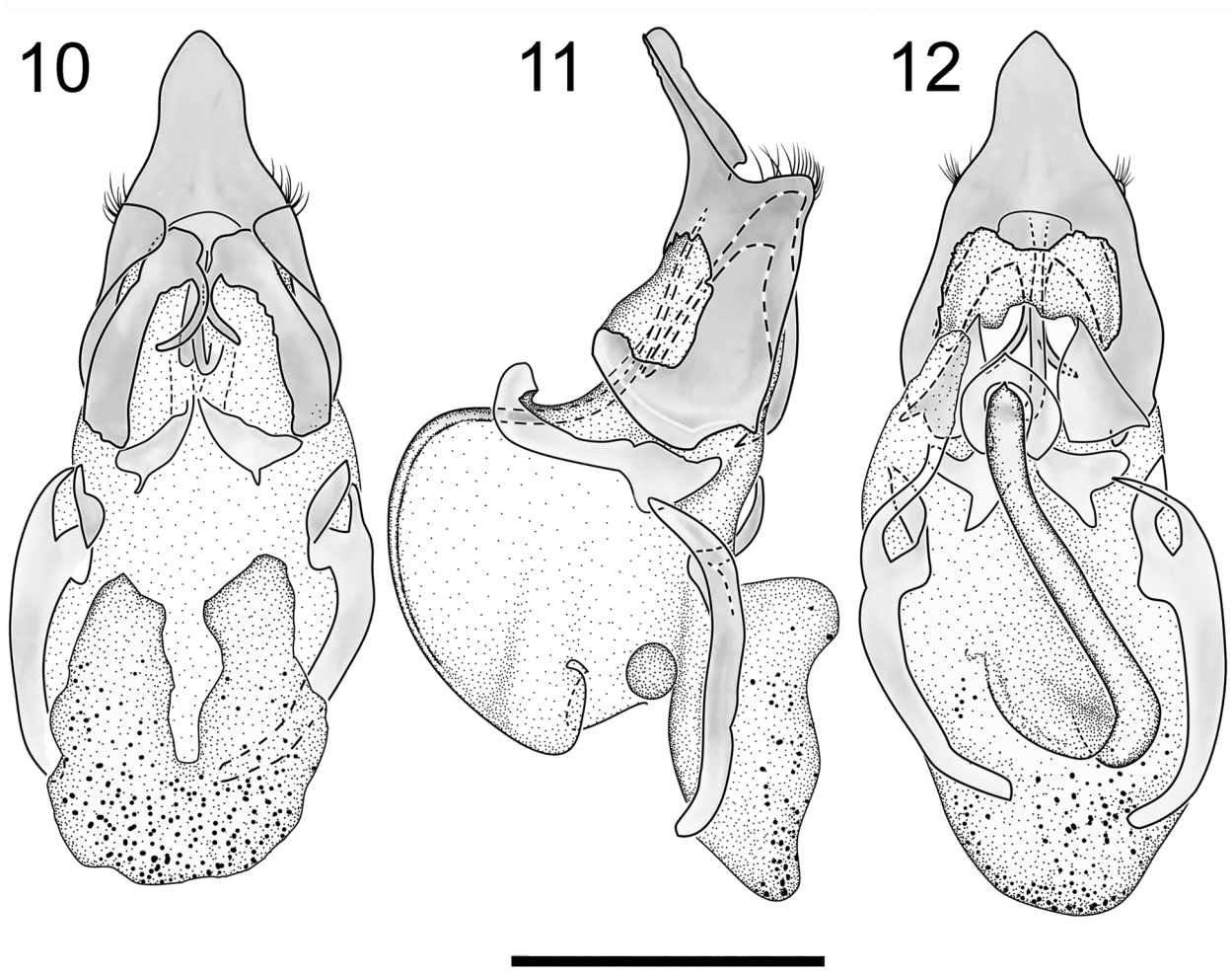


FIGURES 1–6. ***Scapsipedus icipe*** **n. sp. male.** 1: face in front view. 2: head and pronotum in dorsal view. 3: head and pronotum in sideview. 4, 5: right forewing in dorsal view (two paratypes). 6, stridulatory file of paratype illustrated in 5. Scale 1–5: 10 mm; scale 6: 1 mm.

setae) rounded apically (side view, Fig. 11; as in most species but unlike *S. mjagkovi* where it is obliquely truncated). Pseudepiphallic parameres with a particularly wide lateral anterior branch, and with a particularly short median anterior branch that hardly reaches the mid length of the lateral branch (unlike all other species, including *S. amplus*, the latter of which shares with *S. icipe* a paramere with a very wide lateral anterior branch; ventral view, Fig. 10).



FIGURES 7–9. *Scapsipedus icipe* n. sp. female. 7: Right forewing dorsally. 8: ovipositor in left side view. 9: detail of ovipositor tip. Scale 7–8: 10 mm; scale 9: 1 mm.



FIGURES 10–12. *Scapsipedus icipe* n. sp. male genitalia. 10: ventral view. 11: left side view. 12: dorsal view. Scale: 1 mm.

Description. Size large for the genus (Table 1). **Head** moderately but distinctly widened below the eyes (frontal view, Fig. 1); black with a distinct yellow band between the eyes, genae with a yellow ventral line from posterior part of the eye to mandibles and posterior margin of head (side view, Fig. 3), occiput with a fine yellow band from the top of the eye to the head posterior margin, with two faint sub-median lines (dorsal view, Fig. 2); mandibles brown, palpi yellow with black apex; clypeus dark with a yellow sagittal line and ventral margin; labrum black; eyes of average size. **Pronotum** slightly larger anteriorly; anterior margin inconspicuously convex, posterior margin inconspicuously concave; disk of pronotum black with inconspicuous transverse light patterns on the middle, with well distinct lateral band on the distal two thirds, these bands are spotted with black (dorsal and side views, Fig. 2, 3); lateral lobes with a yellow pattern on the ventral margin (side view, Fig. 3). **Legs.** Fore and midlegs of light color, femora often with black spots on proximal and distal ends, tibiae often infuscate near knees; hind femur brown with dark knee, hind tibia black. **Wings.** Forewings not reaching the end of abdomen; dark, with a lighter area around harp in males (Fig. 4, 5), near the fold in females (Fig. 7). Male file with ca. 170–190 teeth; teeth density increasing distally (Fig. 6); area with numerous setae distally to the file (ventral view, Fig. 6). Hindwings often present. **Abdomen** black dorsally with yellow spots, yellow ventrally. **Male genitalia**. Pseudepiphallus with a relatively narrow distal projection, distal projection narrowing regularly at the basis, terminal part blunt-V-shaped (in dorsal view, Fig. 12), ventral lobe of pseudepiphallus with setae, relatively acute, rounded apically (side view, Fig. 11). Pseudepiphallic parameres without posterior branch, with particularly wide lateral anterior branch, with particularly short median anterior branch hardly reaching the mid length of the lateral branch (ventral view, Fig. 10). Ovipositor much longer than hind femur, moderately curved (Fig. 7–9).

TABLE 1. Measurements of *Scapsipedus icipe* **n. sp.**

		Body L	Head L	Head W	IO W	Thx L	Thx W	T 1 L	F 1 L	T 2 L	F 2 L	T 3 L	F 3 L	FW L	OVP L
♂	Holotype	25.2	3.8	5.8	3.3	3.9	5.5	4.8	4.8	4.9	4.7	9.2	11.8	11.9	-
	Average	24.0	3.3	6.2	3.3	4.2	5.8	5.0	4.9	5.2	5.0	9.6	12.5	11.8	-
	Min	22.9	2.7	5.8	3.1	3.9	5.5	4.8	4.8	4.9	4.7	9.2	11.8	11.5	-
	Max	25.2	3.8	6.8	3.5	4.6	6.0	5.2	5.0	5.8	5.4	9.9	13.1	12.1	-
♀	Allotype	23.0	2.8	4.9	2.9	4.2	5.8	4.7	4.8	5.1	5.2	10.2	13.5	12.4	17.9
	Average	22.9	3.0	5.1	2.9	4.1	5.7	4.4	4.7	5.1	4.9	9.7	13.0	11.5	17.4
	Min	21.3	2.8	4.9	2.8	4.0	5.4	4.2	4.5	4.9	4.6	9.1	12.7	10.6	16.6
	Max	24.5	3.2	5.2	3.1	4.2	5.8	4.7	4.8	5.4	5.2	10.2	13.5	12.4	17.9

All values are in mm. F: femur; FW: forewing; IOW: inter ocular width; L: length; Ovp: ovipositor; T: thorax; W: width.

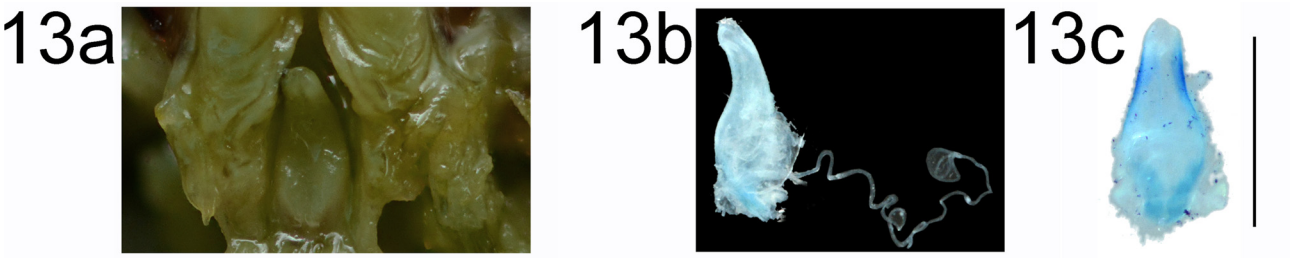


FIGURE 13. ***Scapsipedus icipe*** **n. sp. female copulatory papilla.** 13a: ventral view *in situ* (posterior on top). 13b: same, ex situ, left side view (posterior on top). 13c: same, *ex situ*, ventral view (posterior on top). Scale: 1 mm.

Acoustic behavior (Figures 13–14). The call of *S. icipe* **n. sp.** (Fig. 13) is made up of more or less regularly repeated sentences containing five syllables, the first syllable having a lower amplitude than the four others. At 28±1°C, 50 ± 8% RH and photoperiod of L12: D12, sentences are repeated every 2.70 ± 0.51 s (min: 2.04; max: 4.19 s; 4 specimens), these last 356 ± 116 ms (min: 228 ms; max: 704 ms; 4 specimens), the frequency peaks at 5.77 ± 0.04 kHz (min: 5.69; max: 5.84; 4 specimens). The courtship song of *S. icipe* **n. sp.** (Fig. 14) consists of irregularly repeated sequences, usually containing repeated pairs of syllables; the frequency peaks at similar frequency as the call.

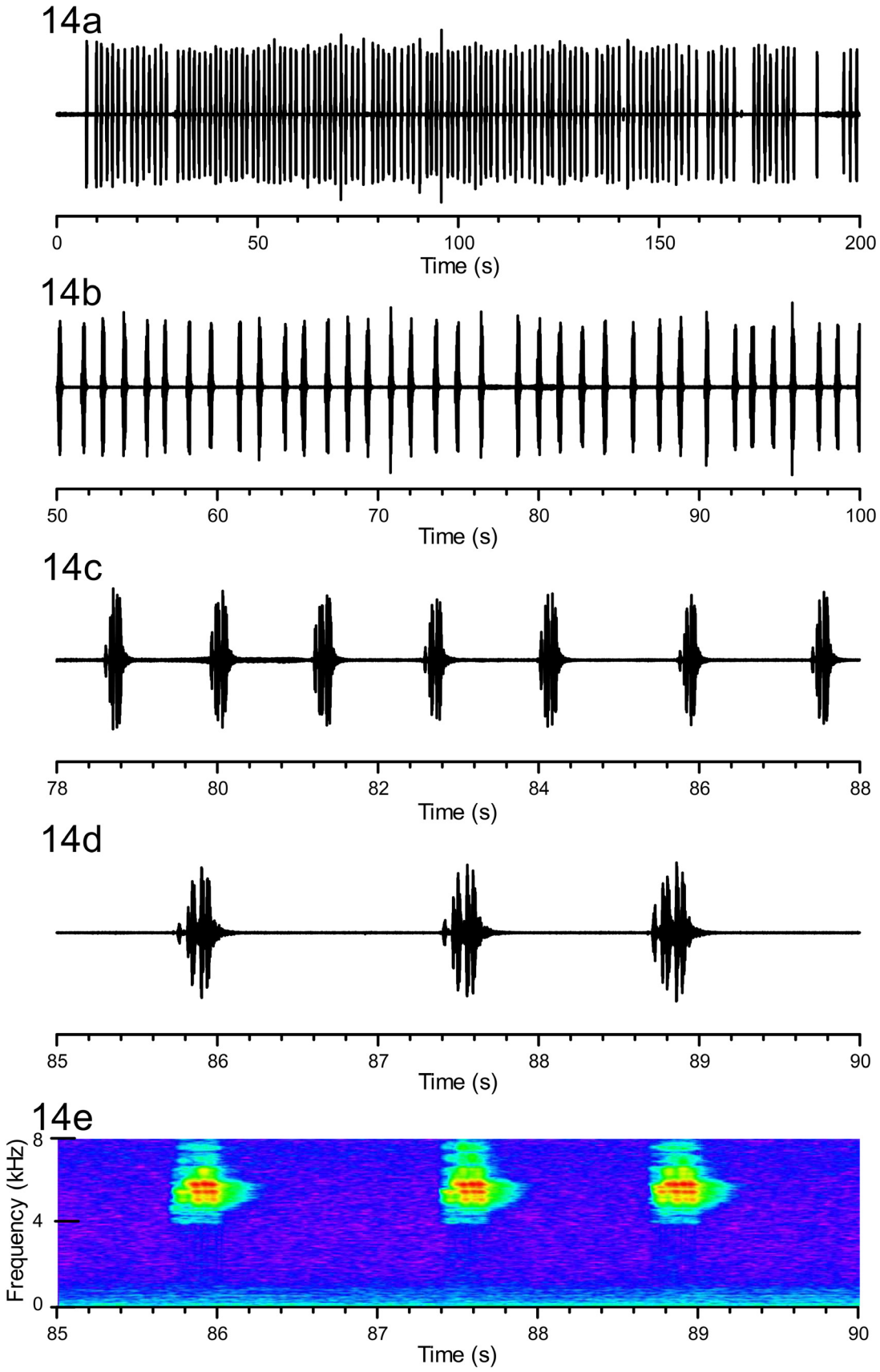


FIGURE 14. Calling song of *Scapsipedus icipe* n. sp. 14a–14d: oscillograms of the same specimen. 14e: power spectrum of the trace in d. Temperature: 28°C.

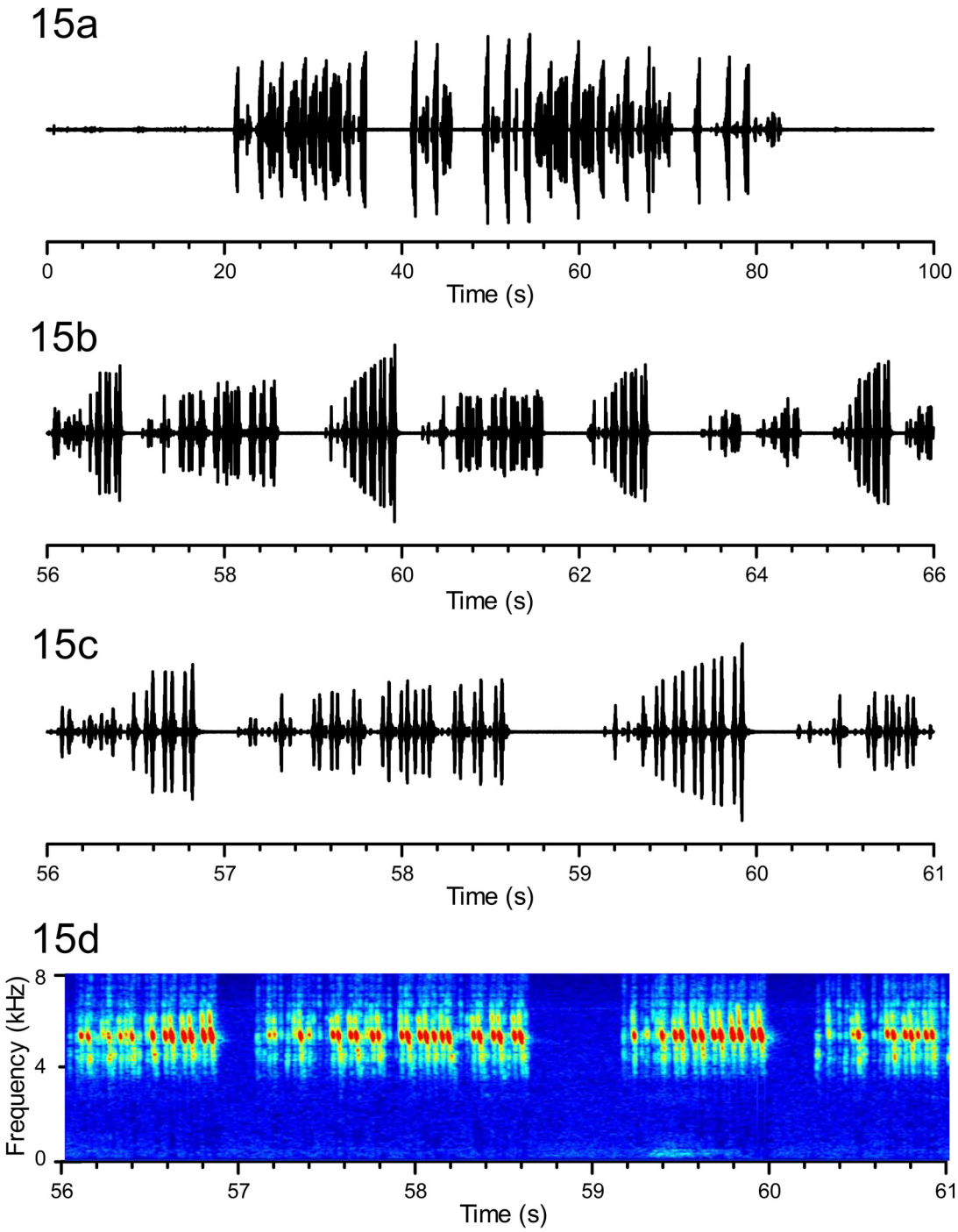


FIGURE 15. Courtship song of *Scapsipedus icipe* n. sp. 15a–15c: oscillograms of the same specimen. 15d: power spectrum of the trace in c. Song produced in presence of 3 females.

Molecular data. No sequences of *Scapsipedus* spp were available in databases. The percentage identities obtained from GenBank for the samples to the closest linking relative was 90% to *Gryllus bimaculatus* isolate 088 (MF046161.1) with query coverage of ≥ 99%. *Scapsipedus icipe* sequences have been deposited in Genbank (Accession number MH923437-MH923446).

Etymology. This new cricket is named after the type locality, International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus, Nairobi, Kenya.

Biology. This species is very widespread and inhabits various zones from Lowlands (19 m a. s. l) to highlands (2672 m a. s. l). Adults occur throughout the year, commonly in and close to human settlements where they hide

under and between stones, waste places, logs, dry grasses or leaves and in cracks (crevices) in the earth during daytime especially in open degraded forest landscapes. It is also found in grassland habitats. Although, preliminary studies have shown that the species is easy to breed in captivity, the biology of this species is largely unknown. Males are territorial and will fight off other males but allow any number of females to coexist in the same shelter. Females lay their eggs into humid soil and the pinhead crickets hatch in 13–14 days at 28±1°C, 60±5% RH and a photoperiod of L12: D12.

Remark. This species has been reared for three years in the research facility at the International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus, Nairobi, Kenya. It has been demonstrated through several research activities that it is a very promising species for mass rearing for food and feed. The best rearing conditions have been extensively tested and will be published in different journals elsewhere (Magara *et al* submitted).

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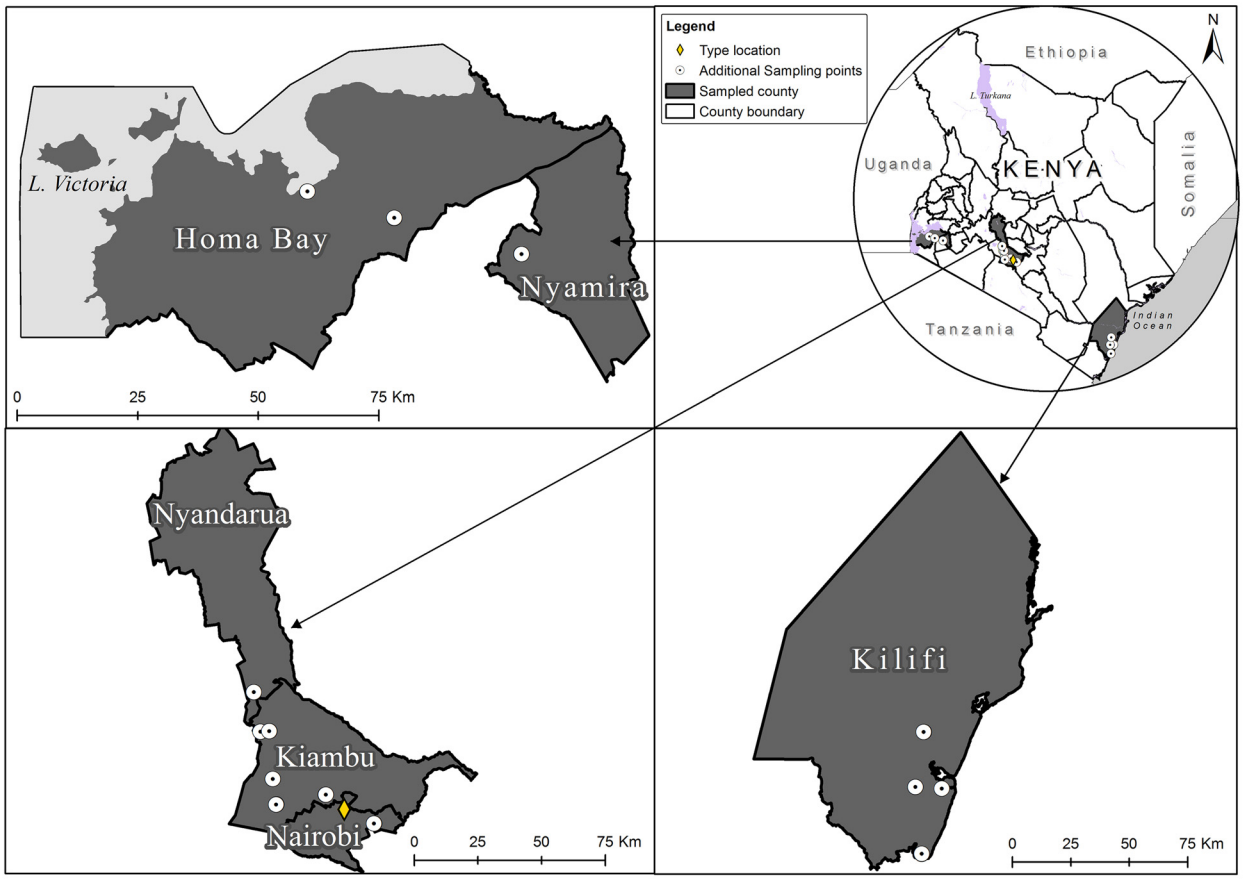

FIGURE 16. Distribution of *Scapsipedus icipe* n. sp. in six counties in Kenya. Yellow diamond-shaped symbol indicates the type locality.

Conflict of interest

The authors have declared that no competing interests exist.

Acknowledgments

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TABLE 2. Estimates of divergence between sequences

	Ex-Nyam-2	Ex-Nyam-4	Ex-colony-4	Ex-colony-5	Ex-colony-1	Ex-colony-3	Ex-colony-2	Ex-Nyam-3	Ex-Nyam-5	Ex-Nyam-1	MF046161.1_*G. bimaculatus*
Ex-Nyam-2	0.000										
Ex-Nyam-4	0.000	0.000									
Ex-colony-4	0.000	0.000	0.000								
Ex-colony-5	0.005	0.005	0.005	0.000							
Ex-colony-1	0.040	0.040	0.040	0.035	0.000						
Ex-colony-3	0.015	0.015	0.015	0.010	0.038	0.000					
Ex-colony-2	0.007	0.007	0.007	0.012	0.043	0.017	0.000				
Ex-Nyam-3	0.017	0.017	0.017	0.012	0.048	0.022	0.025	0.000			
Ex-Nyam-5	0.012	0.012	0.012	0.007	0.043	0.017	0.020	0.005	0.000		
Ex-Nyam-1	0.012	0.012	0.012	0.007	0.043	0.017	0.020	0.005	0.000	0.000	
MF046161.1_*G. bimaculatus*	0.105	0.105	0.105	0.105	0.138	0.117	0.114	0.105	0.110	0.110	0.000
JX897403.1_*A. domseticus*	0.145	0.145	0.145	0.150	0.190	0.157	0.151	0.150	0.156	0.156	0.141

The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al*. 2004). The analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 413 positions in the final dataset. Evolutionary analyses were conducted in MEGA version 7 (Kumar *et al*. 2016).