Journal of Economic Entomology, XX(XX), 2020, 1–13
doi: 10.1093/jee/toaa166
Research

Identification of Edible Short- and Long-Horned Grasshoppers and Their Host Plants in East Africa

Alfonce Leonard,[1,2,3] Fathiya M. Khamis,[1] James P. Egonyu,[1] Samuel Kyamanywa,[2] Sunday Ekesi,[1] Chrysantus M. Tanga,[1,] Robert S. Copeland,[1,4] and Sevgan Subramanian[1,5,]

[1]International Centre of Insect Physiology and Ecology, P.O. Box. 30772-00100 Nairobi, Kenya, [2]Department of Agricultural Production, Makerere University, Kampala, Uganda, [3]Tanzania Agricultural Research Institute (TARI)-Ukiriguru, Mwanza, Tanzania, [4]U.S. National Museum of Natural History, Smithsonian Institution, Washington, DC, [5]Corresponding author, e-mail: ssubramania@icipe.org

Subject Editor: Tong-Xian Liu

Received 26 March 2020; Editorial decision 5 July 2020

Abstract

There is a paucity of information on the edible grasshoppers and their host plants in East Africa. This study adopted morphological and molecular analysis to identify edible grasshoppers in Kenya and Uganda. The associated host plants were identified through molecular analysis of the gut contents of the grasshoppers. The cytochrome b and 16s gene primers were used for grasshopper DNA analysis; while *matK* gene primers were used for plant DNA analysis. All long-horned grasshoppers sampled were identified as *Ruspolia differens* (Serville) (Orthoptera: Tettigonidae); whereas short-horned grasshoppers were identified as *Acanthacris ruficornis* (Fabricius) (Orthoptera: Acrididae) and *Cyrtacanthacris tatarica* (L.) (Orthoptera: Acrididae). Host plants of *A. ruficornis* were *Achyranthes aspera* (L.), *Centella virgata* L.f. Drude, *Digitaria gayana* (Kunth), *Galinsoga quadriradiata* Ruiz and Pavon, and *Triumfetta pilosa* Roth; whereas those of *C. tatarica* were *Alysicarpus rugosus* (Willd.) DC and *Teramnus uncinatus* (L.) SW. Host plants of *R. differens* were *Ageratum conyzoides* (L.), *Citrus depressa* Hayata, *Cynodon dactylon* (L.), *D. gayana*, *Eragrostis mexicana* Hornem, *Eucalyptus saligna* SM., *Indigofera arrecta* Hochst. ex A. Rich., *Persicaria nepalensis* (L.), and *Sorghum halepense* (L.). Information on the host plants of edible grasshoppers can help in the development of their mass rearing protocols.

Key words: edible insect, Acrididae, Tettigoniidae, molecular identification, gut content analysis

Grasshoppers are among the most popular edible insects in East Africa (Kinyuru et al. 2012). The African short-horned grasshopper, *Acanthacris ruficornis* (Fabricius) (Orthoptera: Acrididae) are widely distributed throughout sub-Saharan Africa (Mungai 1987) and consumed in eastern and western Kenya (Kinyuru et al. 2012, Kinyuru et al. 2018). The long-horned grasshopper, *Ruspolia*, commonly called 'Nsenene', is an important food source in East Africa, especially among communities around Lake Victoria (Kinyuru et al. 2011, Kinyuru et al. 2018). In terms of nutrient content, *A. ruficornis* contains 50.5% protein, 15.3% fiber and 6.4% ash (Nginya et al. 2019), while 'Nsenene' contains 43–44% protein, 46–48% fat, 3% ash, and 4–5% fiber (Kinyuru et al. 2011). As an alternative protein, grasshoppers contribute to improved nutrition and food security, livelihoods, and employment in East Africa (Agea et al. 2008, Kinyuru et al. 2011).

The long-horned grasshoppers switch between a lone living 'solitary phase' (dry season) and a swarming or 'gregarious' phase (rainy season) and are known to swarm in large numbers (Bailey and McCrea 1978). Short-horned grasshoppers exist in solitary phase and they are usually collected by children for household consumption. The long-horned grasshopper swarms in grasslands and open bushes in tropical Africa and some Indian Ocean islands (Malinga et al. 2018a, GBIF 2019). People collect the swarming long-horned grasshoppers, making their availability as food, seasonal, and unreliable (Agea et al. 2008). The edible grasshoppers are highly valued and their trading is a source of income in Uganda. Approximately, the grasshopper traders in Uganda earn US$200 per season (Van Huis 2013). Optimizing mass rearing protocols for edible grasshoppers would ensure their year-round supply to enhance nutrition, food security, and livelihoods in East Africa (Malinga et al. 2018b).

The current knowledge on the identity of edible grasshoppers in Kenya and Uganda is poor. Mungai (1987) reported the occurrence of four species of the genus *Acanthacris*; *A. ruficornis*, *A. deckeni* (Gerstaecker), *A. elgonensis* (Sjöstedt), and *A. aithioptera* (Mungai) in Kenya which needs to be updated. On the other hand, there has been conflicting reports regarding the identity of

long-horned grasshoppers in East Africa. Matojo and Yarro (2010) reported the presence of *Ruspolia differens* (Serville) (Orthoptera: Tettigonidae) in Tanzania. However, Agea et al. (2008) reported the existence of *Ruspolia nitidula* (Scopoli) in Uganda, contradicting previous reports that the swarming edible grasshopper in Uganda is *R. differens* (Bailey and McCrea 1978). A combination of morphological and molecular techniques, such as DNA barcoding for species identification and delineation (Belshaw and Quicke 1997, Khamis et al. 2017), could aid in addressing this contradiction. Accurate species identification is critical in mass rearing of insects to avoid contaminations/mixing of different species (Shuker and Burdfield-Steel 2017) and facilitate the establishment of pure colonies and ensure the quality of grasshoppers produced.

Cytochrome c oxidase subunit 1 (COI) of the mitochondrial gene, a highly conserved protein-coding gene in animals, is the common DNA region used to identify and to compare species (Hebert et al. 2003). The COI has a good phylogenetic signal compared to other mitochondrial genes (Siepel et al. 2005). Despite the importance of COI gene in barcoding animals, COI genes of *R. differens* have not yet been sequenced and made available in the GenBank. There are other DNA gene regions that have been used in the identification of *Ruspolia* spp, which include 12s, 16s, and 18s genes (Matojo and Hosea 2013).

Grasshopper species have been reared in the laboratory using a combination of different substrates (Robinson and Hartley 1978, Miura and Ohsaki 2004). Such diets include a mix of seed heads of rice, finger millet and sorghum, wheat bran, wheat seedlings, chicken superfeed egg buster, puppy chow, rolled oat, and dog biscuit pellets (Hartley 1967, Malinga et al. 2018b). The choices of these rearing materials were based on diet acceptance and preference (Malinga et al. 2018a) and not based on information on food plants eaten by the insects in the wild, resulting in only about 38.1% survival (Malinga et al. 2018b). Field observation of insects feeding on plants cannot accurately unravel the interaction between the insects and their host plants (Matheson et al. 2008). With 'Nsenene' such observations are further difficult to make, considering their nocturnal feeding behavior (Opoke et al. 2019a). Precise identification of host plants consumed by insects is important to understand their nutritional ecology, speciation, and evolution (Jurado-Rivera et al. 2008).

Molecular analysis of gut content is gaining popularity as an efficient method for identifying host plants of insect herbivores (Cooper et al. 2016). These techniques have been used to identify the host range of many predatory insects and blood-sucking insects (Matheson et al. 2008). Molecules of hundreds of base pairs can be recovered despite oral enzymatic degradation after passing through the digestive channel of predatory insects or mammals (Jurado-Rivera et al. 2008). Plant DNA can stay inside the insect gut for up to 32 h without degrading through digestion (Matheson et al. 2008), highlighting the potential of gut content analysis for host plant identification. Information on the host plants of grasshoppers is critical to identify potential feeds for optimal productivity of captive colonies.

The current study deployed molecular and morphological tools to accurately identify edible short-and long-horned grasshoppers in East Africa, and their host plants through gut content analysis.

Materials and Methods

Sample Collection

Adults and nymphs of short-horned grasshoppers were collected in Kenya from Murang'a (S00°37′35.5″E36°53′50.9″; 1,905 m above sea level [a.s.l.]) in April 2017 and Kilifi (S03°33′31.8″E039°52′42.9″; 14 m a.s.l.) in May 2018; whereas, long-horned grasshoppers were collected in Uganda in November 2017 from Mbarara (S00°36′28.8″E030°39′08.8″; 1,439 m a.s.l), Masaka (S00°19′02.7″E031°45′35.2″; 1,212 m a.s.l), Hoima (N01°25′57.9″E031°21′29.4″; 1,119 m a.s.l), Kampala (N0°25′04.6″E32°33′51.8″; 1,208 m a.s.l), and Kabale (S01°16′11.5″E029°59′37″; 1,802 m a.s.l) (Fig. 1). Choice of sampling locations was based on the availability of edible grasshopper species in the location.

Short-horned grasshoppers were sampled using a sweep-net in the morning hours between 0800 and 1100 hours and in the afternoon between 1600 and 1800 hours when grasshoppers are not very active. A total of 100 grasshoppers were collected in Murang'a and Kilifi, Kenya. Fifteen grasshoppers from Murang'a and nine grasshoppers from Kilifi were randomly selected and preserved in dry ice (solid form of carbon dioxide) for identification. The rest of the grasshoppers were used for the establishment of laboratory colonies at the Animal Rearing and Containment Unit (ARCU) of the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi. Long-horned grasshoppers were collected from 2000 to 2100 hours, using Fluorescent bulbs placed on top of a wooden frame strategically to reflect directly on constructed silver iron sheets tied to a wooden frame and slanted into metallic drums. The fluorescent bulbs attract flying grasshoppers that hit on shining iron sheets and slide down into the collecting drums. Two kilograms of long-horned grasshoppers were collected from each site. About 78 insects were randomly selected from all sites for identification, of which 14 were from Hoima, 15 from Kabale, 13 from Kampala, 12 from Mbarara, and 24 from Masaka. Immediately after collection, grasshoppers were preserved in dry ice and transferred to *icipe*, where they were stored at –20°C for further analysis.

Morphological Identification

Samples of both long- and short-horned grasshoppers collected from the field were morphologically identified by comparing them with reference specimens maintained in the National Museums of Kenya. Further, the identity of the specimens was confirmed through observation for discriminating characters and running published taxonomic keys such as Mungai (1987) for genus *Acanthacris* (Orthoptera: Acrididae); Johnston (1956), Samejo and Sultana (2016) and Dirsh (1977) for *Cyrtacanthacris* and Bailey (1975) for genus *Ruspolia*.

Morphometric Assessment

Key microscopic characters of both long- and short-horned grasshoppers were measured using LEICA EZ4HD stereo microscope (Leica Microsystems Inc.) fitted with Leica LAS EZ computer software; while macroscopic features were measured using Absolute digimatic caliper (Mitutoyo (UK) Ltd). Samples of long-horned grasshoppers used for morphometric assessment were seven from Hoima, four from the colony reared at *icipe*, three from Kabale, three from Kampala, seven from Mbarara, and five from Masaka. Reference collection of *Ruspolia* (five specimens collected in Nairobi in 1958 and one specimen of *R. nitidula* with unknown collection site) from National Museum of Kenya were also included in the morphometric assessment of long-horned grasshoppers. Morphological features considered for long-horned grasshoppers included lengths of antennae, fastigium, prosternal protuberance, pronotum, metathoracic flaps, tarsus, tibia, femur, black marking on mid and hind leg tibia, tegmina, cercus and number of spines on tibia and femur.

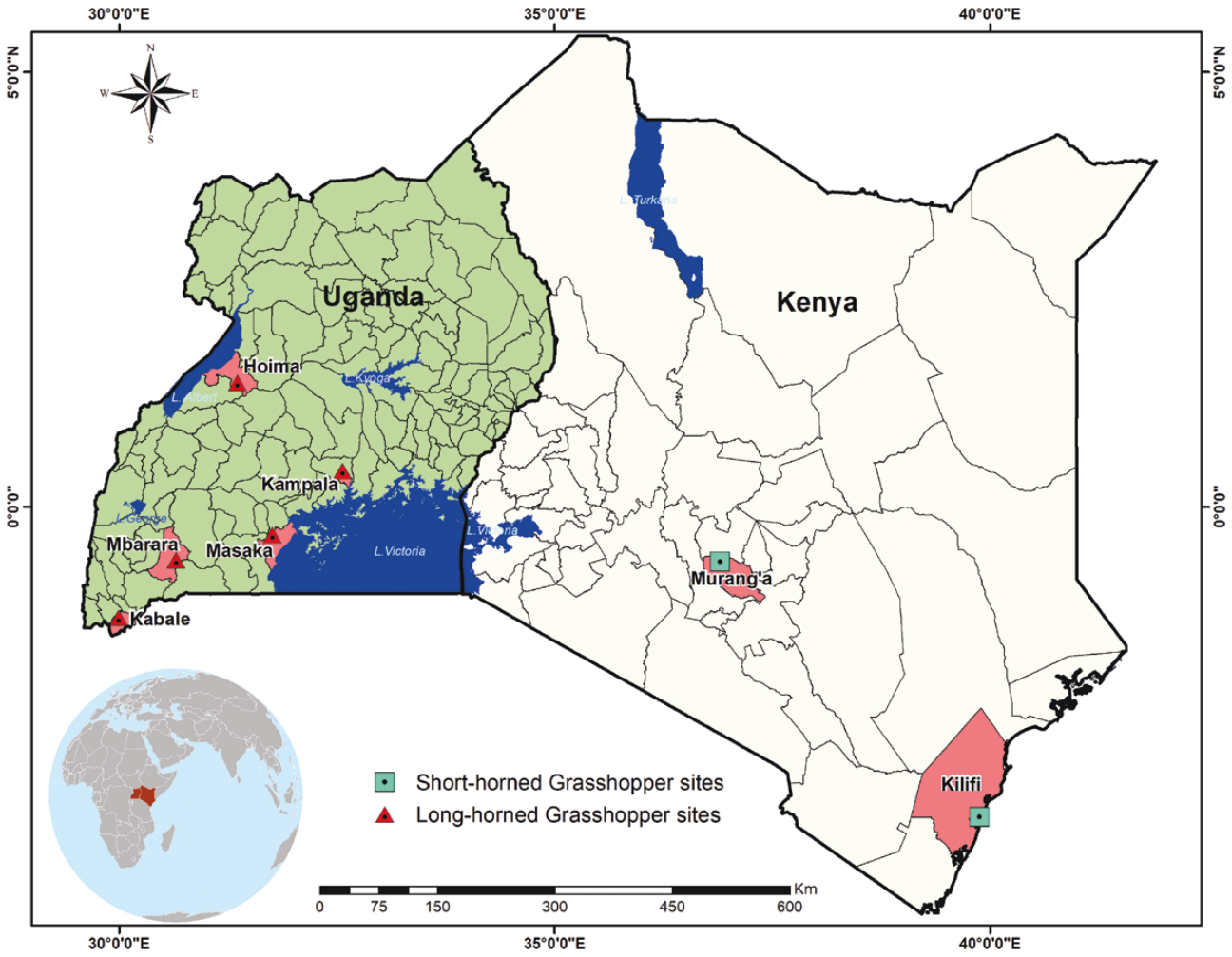

Fig. 1. Locations where grasshoppers were sampled in Kenya and Uganda.

Ten and 15 samples of short-horned grasshoppers from Kilifi and Murang'a, respectively, were subjected to morphometric assessment. Six specimens of *Cyrtacanthacris tatarica* (L.) (Orthoptera: Acrididae) reference collections (three collected in Lamu and three in Turkana in 1982 and 1968, respectively) and six specimens of *A. ruficornis* (preserved from a colony maintained in National Museum of Kenya in 1984) were included in the measurements. Morphological features considered for *A. ruficornis* and *C. tatarica* were lengths of pronotum, antennae, tarsus, tibia, femur, whole body, and tegmina.

Data Analysis

Morphometric data were tested for normality with Shapiro test in R studio (R Core Team 2013), whereby both numbers and length of morphometrics were normally distributed. Data sets were subjected to analysis of variance (ANOVA) in R studio (R Core Team 2013), Principle Component Analysis (PCA), a multivariate statistical procedure used to show patterns in measured correlated variables and Canonical Variate Analysis (CVA), for analyzing group structure in multivariate data using Pillai and Wilks tests (Khamis et al. 2012). Mahalanobis squared distances between grasshopper species were obtained through measuring distance between species based on means, variances, and covariances. *Ruspolia nitidula* was not involved in both Principal Component and CVA as only one specimen from collections was included.

Molecular Identification

DNA Extraction

Samples of the grasshoppers were surface sterilized with 70% ethanol to remove debris from the body surfaces (Avanesyan 2014). The body color of each long-horned grasshopper was recorded. The foregut, midgut, and hindgut of the sample were dissected to get the gut content. DNA was extracted from the right foreleg of each insect using ISOLATE II Genomic DNA Kit, while Plant DNA from the insect gut were extracted following the ISOLATE II Plant DNA Kit both from BIOLINE (Meridian Life Science Company) as per manufacturer's instructions.

Polymerase Chain Reaction for Species Identification

The cytochrome c oxidase I (COI) gene in long-horned grasshoppers was amplified using universal primers LepF1 5′ ATTCAACCAATCATAAAGATATTGG 3′ and LepR1 5′ TAAACTTCTGGATGTCCAAAAAATCA 3′ (Ivanova et al. 2007). The 16s gene in the long-horned grasshoppers was amplified using 16Sar 5′ CGCCTGTTTAACAAAAACAT 3′ forward and 16Sbr 5′ CCGGTCTGAACTCAGATCACGT 3′ reverse primers (Bucklin and Lajeunesse 1994). The cytochrome b gene in short-horned grasshoppers was amplified using primers of Cytb-J-1-933 5′ TCTTTTTGAGGAGCWACWGTWATTAC 3′ and Cytb-N-11367 5′ AATTGAACGTAAAATWGTRTAAGCAA 3′

(Belshaw and Quicke 1997). For the identification of plant species, the chloroplast maturase K gene (*matK*) was amplified using universal 3F_KIM_F 5′ CGTACAGTACTTTTGTGTTTACGAG 3′ and 1R_KIM_R 5′ ACCCAGTCCATCTGGAAATCTTGGTTC 3′ primers (Kar et al. 2015). The amplifications were in 30 µl final reaction volumes containing 5X My Taq buffer (Bioline, London, United Kingdom), 0.5 mol of each primer, 0.5 mM $MgCl_2$, 0.625 U of My Taq DNA polymerase (Bioline) and 15 ng ml^{-1} of DNA template. Polymerase chain reaction (PCR) amplification was done in a Mastercycler nexus gradient (Eppendorf) thermal cycler programmed, for initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 s, Cytb primers annealed at 52°C, Lep primers at 45°C, 16S primers at 56.4°C and *matK* primers at 49°C for 40 s, and extension at 72°C for 1 min. The last extension step was accomplished at 72°C for 10 min at the end of the amplification reaction. The PCR products were run on 1% agarose gels for 1 h at 80 V.

Sequencing, Identification and Phylogenetic Analysis of Plant and Insect DNA

PCR products were purified before sequencing using PCR kit from BIOLINE (Meridian Life Science Company) as per manufacturer's instructions. Purified PCR products were sent to Macrogen Europe (Amsterdam; Netherlands) for sequencing using Applied Biosystems 3730XL sequencer. Sequence editing was carried out using BioEdit software (Version 7.0.4) (Hall 1999). Consensus sequences were compared with known sequences of plant and insect species available in the GenBank database by performing nucleotide Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Maximum Likelihood analysis was conducted in MEGA 7 (Kumar et al. 2016) using the Tamura-Nei model (Tamura and Nei 1993) with 1,000 bootstrap values. GenBank accessions involved in analysis of COI gene were *Platycleis albopunctata* (Goeze) (FM882102.1) collected from Tanzania in 2008; *R. nitidula* (JQ824862.1), *R. nitidula* (JQ824864.1) *R. nitidula* (JQ824865.1), *Ruspolia yunnana* (Lian & Liu) (JQ793721.1), *R. yunnana* (JQ793722.1), *Ruspolia jezoensis* (Matsumura & Shiraki) (JQ793687.1), *R. jezoensis* (JQ793688.1), *Ruspolia liangshanensis* (Lian & Liu) (JQ793691.1), *Ruspolia dubia* (Redtenbacher) (JQ793671.1) all collected from China in 2012. GenBank accessions involved in the analysis of 16s gene were *R. differens* (FM882032.1) and *P. albopunctata* (FM882025.1) collected in Tanzania in 2008; *R. nitidula* (Z97618.1), *R. nitidula* (EF198443.1) and *R. jezoensis* (EF198442.1) collected from China in 2006, while GenBank accessions included in the analysis of the Cytb gene for the short-horned grasshoppers were *A. ruficornis* (KY981017.1), *Cyrtacanthacris aeruginosa* (KY981016.1), A. tatarica (MG993444.1) and *Schistocerca americana* (KY981030.1) collected in Texas, in 2017.

Table 1. Morphological identification results of long-horned 'Nsenene' and short-horned grasshoppers

Locality	Order	Family	Sub-Family	Species
Kampala	Orthoptera	Tettigonidae	Conocephalinae	*Ruspolia differens*
Masaka	Orthoptera	Tettigonidae	Conocephalinae	*Ruspolia differens*
Mbarara	Orthoptera	Tettigonidae	Conocephalinae	*Ruspolia differens*
Kabale	Orthoptera	Tettigonidae	Conocephalinae	*Ruspolia differens*
Hoima	Orthoptera	Tettigonidae	Conocephalinae	*Ruspolia differens*
Murang'a	Orthoptera	Acrididae	Cyrtacanthacridinae	*Acanthacris ruficornis*
Kilifi	Orthoptera	Acrididae	Cyrtacanthacridinae	*Cyrtacanthacris tatarica*

Results

Morphological Identification of Edible Grasshoppers

There was no significant difference between most morphometrics of long-horned grasshoppers' samples involved in this study and long-horned grasshoppers reference collections from National Museums of Kenya (Supp Table A1 [online only]); however, they varied significantly in size of mid-leg femur, antenna, prosternal protuberance, metathoracic flaps, and cercus. Samples of long-horned grasshoppers from all collection sites were morphologically identified as *R. differens* (Orthoptera: Tettigoniidae) (Table 1) based on the presence of the following characters of males; forewings not markedly pointed and length more than 40 mm (Fig. 2a and b); dorsal aspect of the stridulatory rib not distinctly crescentic, margins bulging (Fig. 2c, d, and f); number of teeth on the stridulatory rib between 60 and 100 teeth (Fig. 2g); presence of a notch between the fastigium of the vertex and frons and lateral lobes of the pronotum deeply invaginated (Fig. 2e). Short-horned grasshoppers from Murang'a were identified as *A. ruficornis* based on the strong lateral lobes of the trilobed subgenital plate; ochreous tegmina with dark brown oblique transverse markings all over and median dorsal carina of pronotum slightly arcuate (Fig. 3). Most morphometrics features of short-horned grasshoppers of both samples and reference collections varied significantly among males and females (Supp Table A2 [online only]). Tegmina length showed no significant difference among samples and reference collections of the same species from National Museum of Kenya. Short-horned grasshoppers collected from Kilifi were identified as *C. tatarica* based on the nature of the phallic complex of male specimens. Specimens of *Cyrtacanthacris* differed from the *Acanthacris* by the presence of simple and conical subgenital plate (Fig. 3e and f). The tegmina of *A. ruficornis* has oblique transverse brown markings and spots all over while that of *C. tatarica* has irregular dark maroon markings (Fig. 3a and c). *Cyrtacanthacris tatarica* has black spots on each segment of the abdomen unlike the abdomen of *A. ruficornis*, which lacks the dark spots (Fig. 3a and c). The pronotum of *A. ruficornis* has slightly arcuate median dorsal carina and dark spots on metazona while that of *C. tatarica* has flat dorsal carina, pale yellow lateral lobes, and dark brown fasciae (Fig. 3b and d).

PCA and CVA of Long-Horned Grasshoppers

Projection of morphometric data of the long-horned grasshoppers on the first two principal axes showed very low separation in terms of populations (Fig. 4). The contribution of the first two components to the total variance was 46.5% (PC1 = 33.4% and PC2 = 13.1%). The third, fourth, fifth, sixth, and seventh principal components contributed 10.5, 10.0, 7.1, 6.1, and 4.7%, respectively. All male insects clustered together on the positive side of the *x*-axis while females aggregated on the negative side of the same axis. The *R. differens* reference collections from the National Museum of Kenya aggregated together with other samples involved in this study.

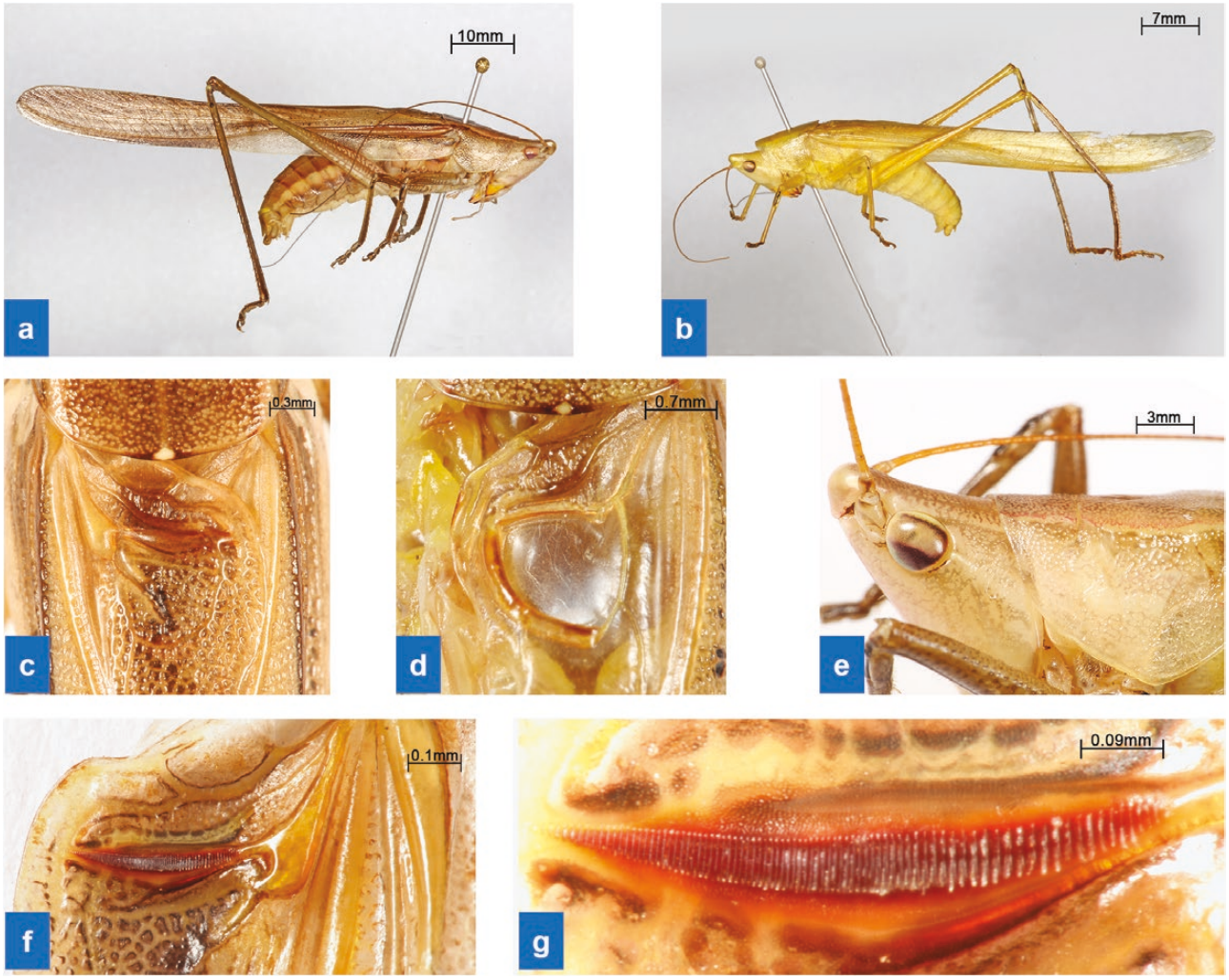

Fig. 2. Discriminating morphological characters of *Ruspolia differens* male. (a) Brown color form; (b) green color form (the specimen turned yellow during preservation in absolute ethanol); (c) Dorsal view of the stridulatory rib of the left male forewing; (d) Dorsal view of the mirror region of the right male forewing; (e) Blunt fastigium and lateral lobes of the pronotal lobe deeply invaginated, (f) Stridulatory rib not distinctly crescentic, both margins bulging; (g) Magnified image of the Stridulatory rib with around 80 teeth.

The multivariate analysis of variance (MANOVA) tests of both Pillai and Wilks of canonical discriminant indicated that there was no significant difference among the canonical variates (Pillai's trace = 3.67, $F_{1,6}$ = 1, P = 0.455; Wilk's lambda = 0.0005, $F_{1,6}$ = 1 and P = 0.221). The data showed a good pattern of separation on the first two canonical variates axes (Fig. 5). The CV1 and CV2 contributed a total variance of 84% (CV1=57.2 and CV=26.8%). The contribution of third, fourth, fifth and sixth canonical variates were 7.4, 4.3, 3.1, and 1.0%, respectively. Samples from *icipe* colony clustered together with *R. differens* reference collections. Samples from Mbarara and Kabale clustered together with samples from Kampala. Hoima and Masaka samples clustered closer at the positive side of the *Y*-axis (Fig. 5). Morphometrics divergence among population groups was compared by Mahalanobis distance (Table 2). The largest Mahalanobis squared distance was between samples from Kampala and *R. difference* reference collections from National Museums of Kenya (D^2 = 130.39) followed by samples from *icipe* colony and samples from Kampala (D^2 = 124.99) and the lowest Mahalanobis distances were between samples from Kabale and Mbarara (D^2 = 13.45) and between *icipe* colony and reference collections (D^2 = 14.07).

PCA and CVA of Short-Horned Grasshoppers

The first two Principal Component axes projected partial separation of morphometric data of the population. The contribution of the first two principal components was 84.25% (PC1 = 77.5% and PC2 = 6.7%) (Fig. 6), the third, fourth, and fifth contributed 5.6, 2.9, and 1.8%, respectively. The PCA separated males and females into two different groups whereby, males clustered in positive side of the *x*-axis, while females clustered in the negative side of the same axes. Male samples from Murang'a and *A. ruficornis* reference collections from National Museums of Kenya clustered together, while male samples from Kilifi clustered together with *C. tatarica* from reference collections. All female populations did not show clear separation from each other (Fig. 6).

Both Pillai and Wilks tests in MANOVA of canonical discriminant indicated significant difference among the two canonical variates (Pillai's trace = 1.33, $F_{1,3}$ = 1.6 P = 0.0463; and Wilk's lambda = 0.12, $F_{1,3}$ = 1.9, P = 0.011). The data projected better separation pattern on the first two canonical variate axes compared to PCA. These first two canonical variates contributed 96.1% (CV1 = 74.8% and CV2 = 21.3%) of the total variance (Fig. 7) and third canonical variate contributed 3.9%. Most

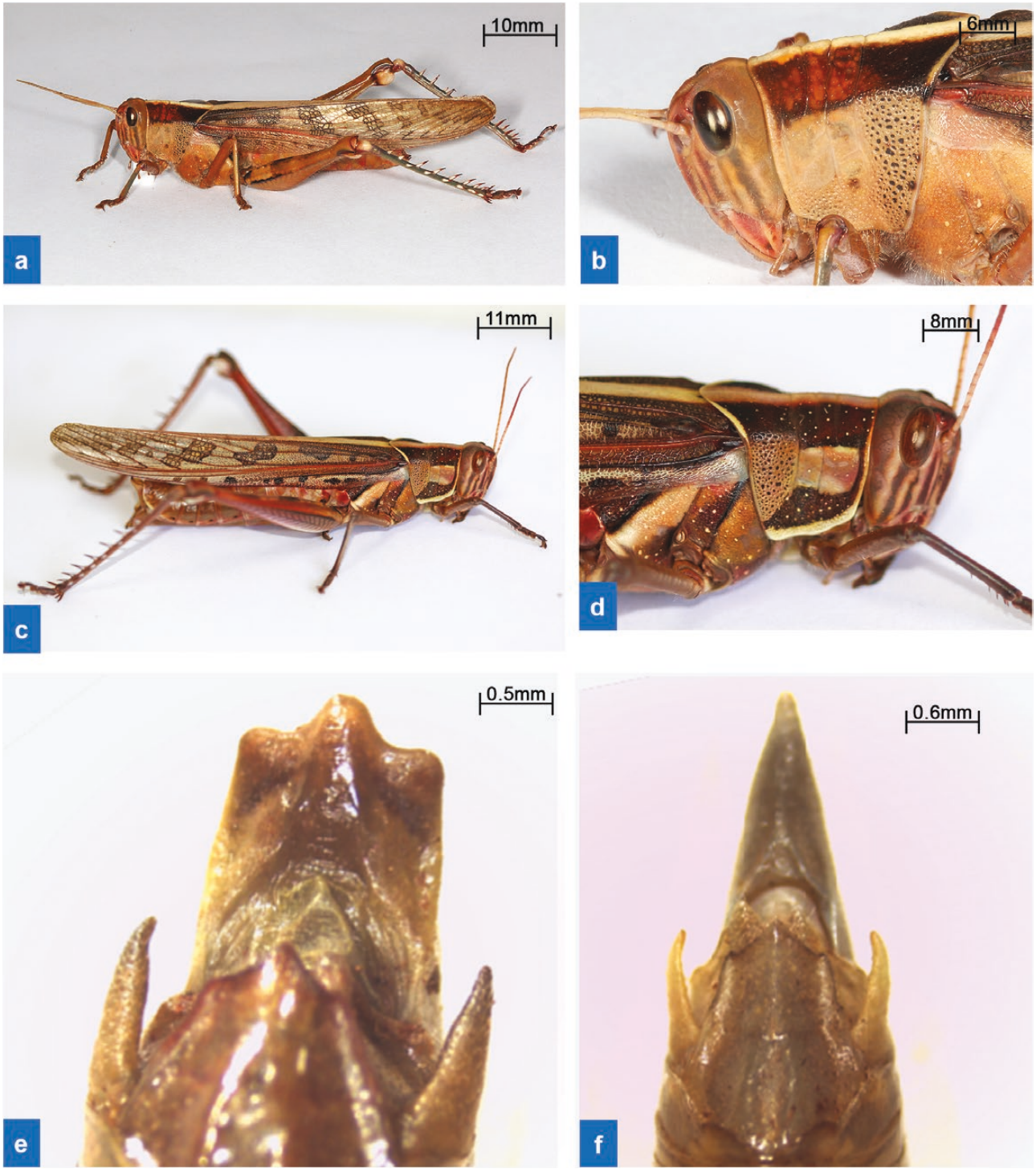

Fig. 3. Discriminating morphological characters of short-horned grasshoppers. (a) Whole body of *Acanthacris ruficornis*, the tegmina has oblique transverse brown markings and spots all over; (b) Pronotum of *Acanthacris ruficornis* with slightly arcuate median dorsal carina and dark spots on metazona; (c) Whole body of *Cyrtacanthacris tatarica* with flat median dorsal carina, irregular dark maroon markings on tegmina and black spots in each segment of abdomen, (d) Pronotum of *Cyrtacanthacris tatarica* with pale yellow lateral lobes and dark brown fasciae; (e) Subgenital plate of male *Acanthacris ruficornis* are relatively strongly trilobate; (f) Simple and conical subgenital plate of male *Cyrtacanthacris tatarica*.

samples from Murang'a clustered together with *A. ruficornis* reference collections in the positive side of the *x*-axis while most samples from Kilifi aggregated together with *C. tatarica* reference collections in the negative side of the *x*-axis. Mahalanobis distances showed low degree of divergence among insects involved in this study (Table 3). The highest Mahalanobis distance was between Kilifi samples and *A. ruficornis* reference collections (D^2 = 18.97), followed by Murang'a samples and *C. tatarica* reference collections (D^2 =17.82, Murang'a and Kilifi samples (D^2 =16.96). The smallest distances were between Murang'a samples and *A. ruficorns* reference collections (D^2 = 4.14) and Kilifi samples and *C. tatarica* reference collections (D^2 = 4.4).

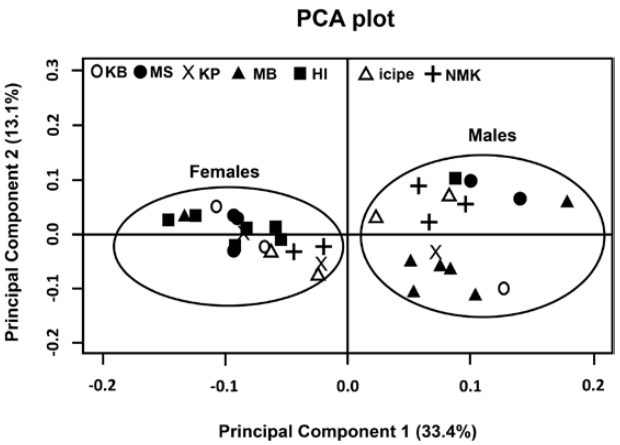

Fig. 4. Projection of morphometrics data of long-horned grasshoppers from different sites on the first two principal components. KB = Kabale, KP = Kampala, MB = Mbarara, MS = Masaka, *icipe* = International Centre of Insect Physiology and Ecology and NMK = National Museums of Kenya.

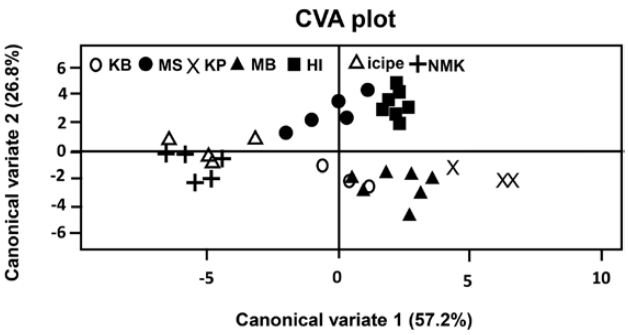

Fig. 5. Projection of morphometrics data of long-horned grasshoppers from different sites on the first two Canonical variates. KB = Kabale, KP = Kampala, MB = Mbarara, MS = Masaka, *icipe* = International Centre of Insect Physiology and Ecology and NMK = National Museums of Kenya.

Molecular Identification of Insects

The COI barcode sequences from Lep primers for long-horned grasshoppers (GenBank accessions MK635348 and MK635349) showed homology of (96–97%) with *R. nitidula* (JQ824865.1) from BLAST search; whereas the 16s sequences of the insects (GenBank accession MK639368) showed homology of (99.2%) with *R. differens* (FM882032.1) (Supp Table A3 [online only]). The Cytochrome b barcode sequences for short-horned grasshoppers collected from Murang'a (GenBank accession MK629459) showed the similarity of (98.19–100%) with *A. ruficornis* (KY981017.1) while samples collected from Kilifi (GenBank accession MK629458) showed the similarity of (97.2%) with *C. tatarica* (MG993444.1) from BLAST search.

Phylogenetic Analysis of Long-Horned and Short-Horned Grasshoppers

The phylogenetic tree of long-horned grasshoppers branched into two main branches. The first branch included samples from all sites analyzed in this study, *R. nitidula* (JQ824862.1), *R. nitidula* (JQ824864.1), *R. nitidula* (JQ824865.1), and *P. albopunctata* (FM882102.2); whereas the second branch comprised the rest of GenBank accessions (Fig. 8). The first branch was subdivided into two clades, the first comprised all samples involved in this study and the second included *R. nitidula* GenBank accessions and *P. albopunctata* (FM882102.2). The branch that had other GenBank accessions of the *Ruspolia* sequences was also subdivided into two Clades, whereby the first comprised *R. yunnana* GenBank accessions and the second involved *R. jezoensis* GenBank accessions, *R. liangshanensis* (JQ7936691.1) and *R. dubia* GenBank accessions. The clade with our samples was separated into two sub-clusters; the first cluster included *R. differens* (MK635348) and the second sub-cluster comprised *R. differens* (MK635349), KPG4, KPG11, MBB16 and MBG20. Green and brown color forms did not influence clustering of long-horned grasshoppers in the phylogenetic tree.

The phylogenetic tree of short-horned grasshoppers was paraphyletic with three main branches (Fig. 9). The first branch was subdivided into sub-clusters that included all samples collected from Murang'a and GenBank accession of *A. ruficornis* (KY981017.1), the second cluster included *Cyrtacanthacris aeruginosa* (KY981016.1) and *C. tatarica* (MG993444.1). The third group included all samples collected from Kilifi (*C. tatarica*). *Schistocerca americana* (KY981030.1) branched separately as an outgroup forming a sister clade relationship.

The overall mean genetic distance between samples from all sites in Uganda was 0.001. The genetic distance between long-horned grasshopper samples and *R. differens* GenBank accession (FM882032.1) ranged from 0.00061 to 0.00102, and it was the lowest distance compared to other GenBank accessions (Supp Table A4 [online only]). The distance between *R. nitidula* GenBank accessions (Z97618.1 and) and long-horned grasshopper samples ranged between 0.00192 and 0.00235; while these samples and *R. nitidula* (EF198443.1) ranged between 0.01054 and 0.0124; that of *R. jezoensis* (EF198442.1) ranged between 0.006 and 0.0134; and that of *P. albopunctata* (FM882025) ranged between 0.0022 and 0.00163.

Genetic distance between samples of short-horned grasshoppers varied depending on the collection sites. The genetic distance within samples collected from Kilifi ranged between 0.000 and 0.007; while the distance within samples collected from Murang'a ranged between 0.005 and 0.017. The genetic distance between Kilifi samples and *C. tatarica* (MG993444.1) GenBank accession was from 0.027 to 0.035. The genetic distance between samples collected from Murang'a and GenBank accession of *A. ruficornis* (KY981017.1) ranged between 0.005 and 0.022 (Supp Table A5 [online only]).

Molecular Identification of Host Plants

Out of 160 processed grasshopper samples, we successfully recovered plant DNA from 39 samples. All sequences of both short- and long-horned grasshoppers matched (by 97–100% identity) with sequences deposited in the GenBank. Seven host plants of *A. ruficornis*, two host plants of *C. tatarica* and nine host plants of *R. differens* were identified (Table 4). Host plants of *A. ruficornis* were *Achyranthes aspera* (L.), *Centella virgata* L.f. Drude, *Digitaria gayana* (Kunth), *Galinsoga quadriradiata* Ruiz and Pavon, and *Triumfetta pilosa* Roth. Those of *C. tatarica* were *Alysicarpus rugosus* (Willd.) DC and *Teramnus uncinatus* (L.) SW. Host plants of *R. differens* were *Ageratum conyzoides* (L.), *Citrus depressa* Hayata, *Cynodon dactylon* (L.), *D. gayana*, *Eragrostis mexicana* Hornem, *Eucalyptus saligna* SM., *Indigofera arrecta* Hochst. ex A. Rich., *Persicaria nepalensis* (L.), and *Sorghum halepense* (L.). *Digitaria gayana* was the most dominant host plant of *R. differens* and 20 out of 28 identified host plants belonged to this species (Table 5).

Table 2. Mahalanobis squared distance (D^2) between clusters separating long-horned grasshoppers

	Hoima	*icipe*	Kabale	Kampala	Mbarara	Masaka
icipe	64.99					
Kabale	38.49	46.05				
Kampala	48.67	124.99	55.47			
Mbarara	37.02	57.54	13.45	22.18		
Masaka	17.72	40.68	38.62	63.68	42.64	
National Museums	81.14	14.07	49.27	130.39	64.96	47.36

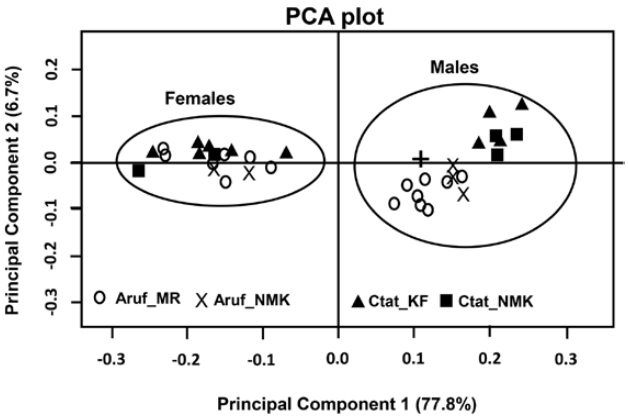

Fig. 6. Projection of morphometrics data of short-horned grasshoppers from different sites on the first two principal components. *Acanthacris ruficornis* (Aruf)_MR = Murang'a, Aruf_NMK = *Acanthacris ruficornis* reference collections from National Museums of Kenya, *Cyrtacanthacris tatarica* (Ctat)_KF = Kilifi and Ctat_NMK= *Cyrtacanthacris tatarica* reference collections from National Museums of Kenya.

Discussion

The primary objective of this study was to accurately identify species of edible grasshoppers and their associated food host plants in selected locations in Kenya and Uganda that can guide optimization of mass rearing protocols for edible grasshoppers. Based on the presence of the discriminating characters, all the long-horned grasshopper samples were identified as *R. differens*. In the wild, *R. differens* is highly variable with at least six polymorphic forms with very few intermediaries reported (Bailey 1978). Hence, we further confirmed the identity through morphometric and molecular assessments. Multivariate Analysis of the first two canonical variates (both Pillai and Wilks tests) showed no significant difference between long-horned grasshopper samples and *R. differens* reference collections from National Museums of Kenya, confirming their identity as *R. differens*. Length of pronotum, forewing/tegmen, and hind femur recorded for the samples in this study are within the range reported by Bailey (1975) for *R. differens*. Significant differences in some of the morphometric characters were observed between the male and female *R. differens*. The current results concur with other findings by Matojo and Yarro (2013), who reported morphological sex dimorphism in *R. differens*. High Mahalanobis squared distance observed between samples from different sites suggests population-associated morphological variations in *R. differens*. This variation might be attributed to ecological differences as CVA aggregated samples from Mbarara together with those from Kabale, which are not widely separated locations. Morphological characters such as deeply invaginated pronotum, the number of stridulatory ribs being 70–100 differentiates it from *R. nitidula*, whose pronotum is less

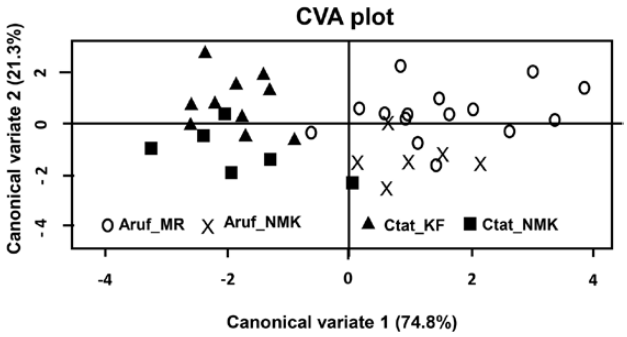

Fig. 7. Projection of morphometrics data of short-horned grasshoppers from different sites on the first two Canonical Variates. *Acanthacris ruficornis* (Aruf)_MR = Murang'a, Aruf_NMK = *Acanthacris ruficornis* reference collections from National Museums of Kenya, *Cyrtacanthacris tatarica* (Ctat)_KF = Kilifi and Ctat_NMK = *Cyrtacanthacris tatarica* reference collections from National Museums of Kenya.

invaginated and the number of stridulatory ribs range between 123 and 143 (Bailey 1975).

Molecular analysis of the COI gene region of *R. differens* samples in this study linked them to *R. nitidula* (JQ824865.1), however only at 96–97% similarity. We could not fix the identity of *R. differens* with the COI DNA barcodes, due to the absence of previous COI sequences of *R. differens* available in the GenBank. DNA sequences of *R. differens* have now been submitted to the GenBank to confirm COI gene barcoding and taxonomic identification. We further used 16s primers on six samples from each of two sub-clusters observed in COI DNA barcodes to amplify 16s gene. All the 16s gene sequences of the long-horned grasshoppers linked to *R. differens* (FM882032.1). The maximum likelihood model-based phylogenetic analysis of COI gene showed that the long-horned grasshoppers clustered separately from the closely related species *R. nitidula* and other species used in the analysis confirming the identity of swarming edible long-horned grasshoppers in East Africa as *R. differens* (Matojo and Hosea 2013) and not *R. nitidula* as suggested by Agea et al. (2008).

The phylogenetic tree clustered long-horned grasshopper samples into one clade divided into two sub-clusters. The first sub-cluster included samples from all sites, while the second included samples collected from Kampala and Mbarara, regardless of their color morph. The genetic distance of 16s gene observed in all long-horned samples was low, with an overall mean of 0.001, which is within the acceptable range for intraspecific variation. Virgilio et al. (2010) reported that the acceptable intraspecific genetic distance variation ranges between 0.00 and 0.076. This finding corroborates with Matojo and Hosea (2013), Bailey (1979), and McCrae (1982) who reported the long-horned grasshoppers present in East Africa as *R. differens*, which is widely distributed in Sub-Saharan Africa. *Ruspolia nitidula* is distributed in Asia, Europe, and Northern Africa (Bailey 1975).

Short-horned grasshoppers from Murang'a and Kilifi were morphologically identified as *A. ruficornis* and *C. tatarica*, respectively. Two species of short-horned grasshoppers were clearly distinguished based on morphological states such as dark spots on each abdomen of *C. tatarica* samples unlike *A. ruficornis* samples which had no dark spots, subgenital plates of samples from Murang'a were relatively strongly trilobed while those from Kilifi had simple and conical shaped subgenital plates as described by Dirsh (1977) and Mungai (1987). Pronotum structure was also considered as a distinct feature of the two species, whereby *A. ruficornis* had slightly arcuate median dorsal carina

Table 3. Mahalanobis squared distance (D^2) between clusters separating short-horned grasshoppers

	Murang'a	NMK (*A. ruficornis*)	Kilifi
NMK (*A. ruficornis*)	4.14		
Kilifi	16.96	18.97	
NMK (*C. tatarica*)	17.82	14.16	4.41

NMK, National Museums of Kenya.

and dark spots on metazoan, unlike *C. tatarica*'s pronotum with flat median dorsal carina, pale yellow lateral lobes, and dark brown fasciae. The identities of short-horned grasshoppers were supported by clustering of samples from Murang'a and Kilifi with *A. ruficornis* and *C. tatarica*, respectively, by both Principal Component and CVA. The Mahalanobis squared distance between our samples and the reference collections were low. Similar results on morphological identity of genus *Acanthacris* have been reported by Mungai (1987).

The Lep primers used with long-horned grasshopper did not amplify the gene region of interest in short-horned grasshoppers. The cyt b primers, which provided acceptable amplification of mitochondrial genome of short-horned grasshoppers were used. Based on sequences amplified by cyt b primers, samples of short-horned grasshoppers linked to *A. ruficornis* (KY981017.1) and *C. tatarica* (MG993444.1) for the samples collected from Murang'a and Kilifi, respectively. DNA barcodes of all short-horned grasshoppers corresponded to morphological (taxonomic) identification. These findings concur with Mungai (1987) and Reinhardt and Köhler (2014) who reported the distribution of *A. ruficornis* in central region of Kenya. *Cyrtacanthacris tatarica* has been reported in the coast of Somalia (Johnsen and Schmidt 1982), which is near the collection site of our samples (Kilifi). Phylogenetic analysis of cyt b gene of short-horned grasshoppers also clustered together with *A. ruficornis* GenBank accession and samples collected from Murang'a, while *C. tatarica* GenBank accession clustered with samples from Kilifi. The overall genetic distance of short-horned grasshoppers, 0.033 was high because it involved samples of two different species.

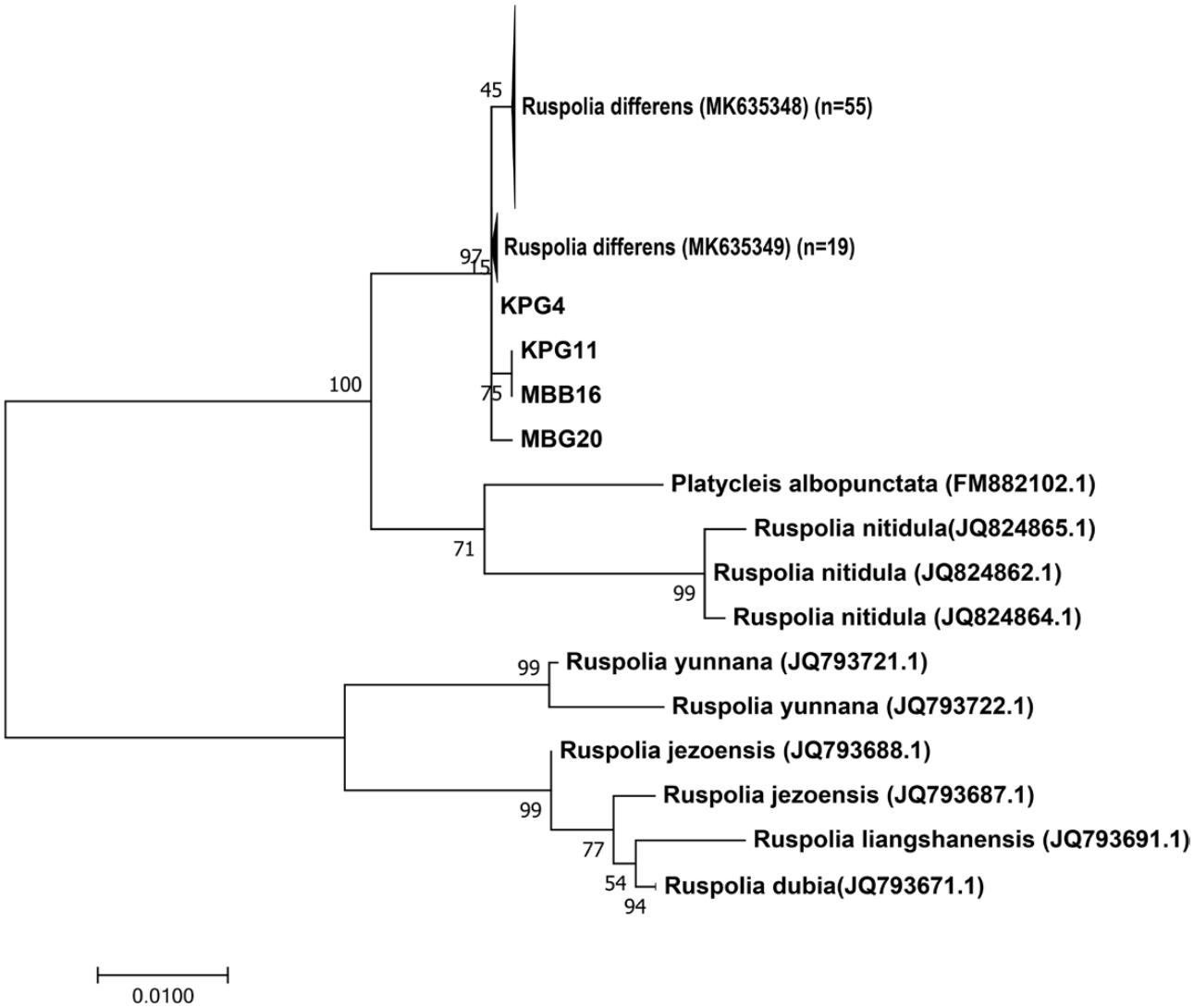

Fig. 8. Maximum likelihood tree showing the evolutionary relationship of COI gene of long-horned grasshopper samples from different locations in Uganda inferred by MEGA7. Bootstrap values based on 1,000 replicates are indicated at the branches. Numbers in brackets are GenBank accessions and n in brackets is number of samples. KPG = green samples from Kampala, MBG = green samples from Mbarara, MBB = brown sample from Mbarara and number after letters represent sample number.

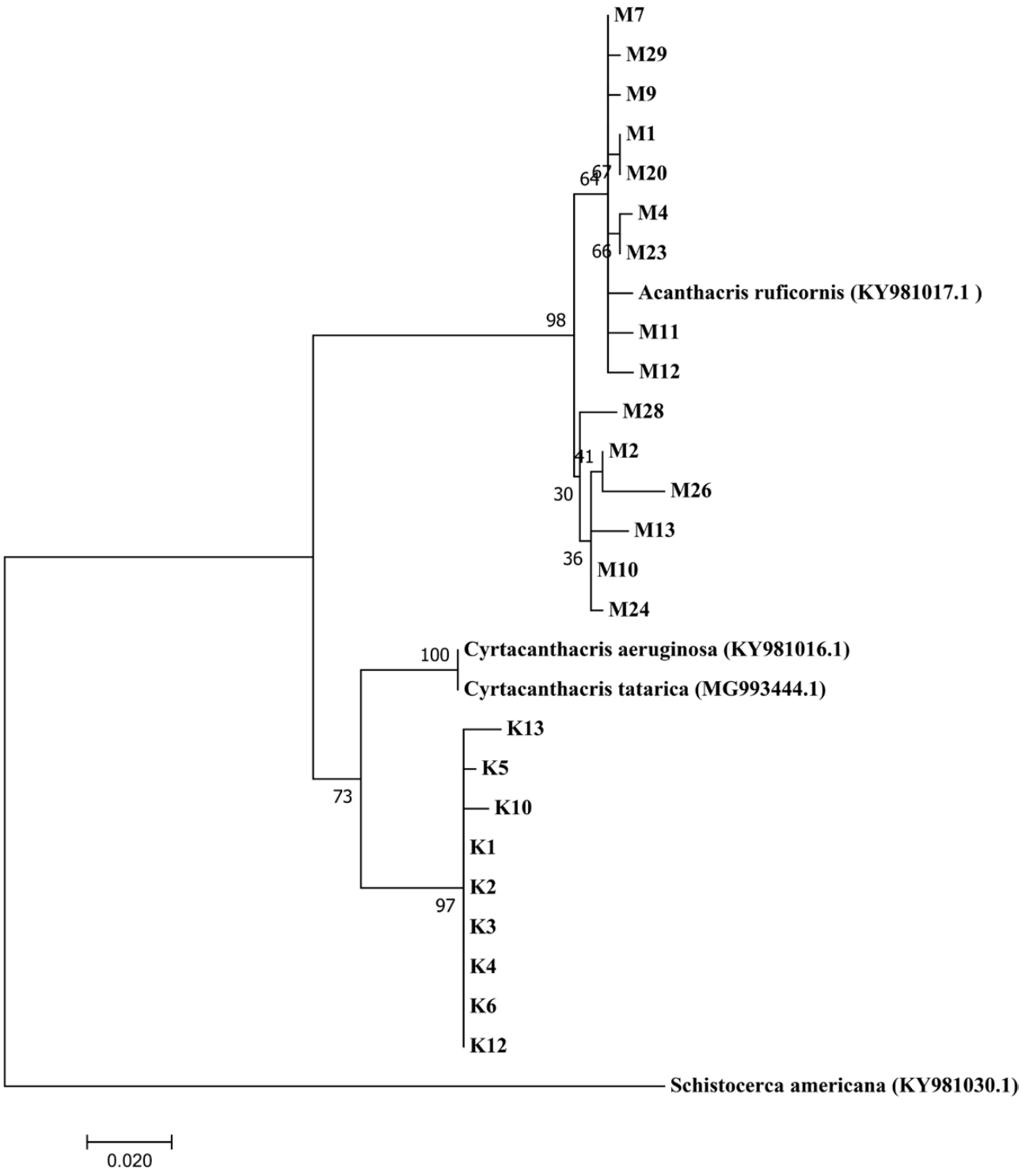

Fig. 9. Maximum likelihood tree showing evolutionary relationship of cytochrome b gene of short-horned grasshopper samples from different locations in Kenya inferred by MEGA7. Bootstrap values based on 1,000 replicates are indicated at the branches. A letter indicates the collection sites (M= Murang'a and K = Kilifi), the number indicate sample number and number in brackets indicate GenBank accession. MK629458 and MK629459 are GenBank reference accession numbers for Murang'a and Kilifi samples, respectively.

Correct identification of grasshopper host plants depends mostly on the taxonomic representations available at GenBank (Jurado-Rivera et al. 2008). This is made possible through diagnostic PCR amplifying plant materials consumed by herbivorous insects (Jurado-Rivera et al. 2008, Cooper et al. 2016). Sequences of 39 samples matched with the available sequences in GenBank with 97–100 similarity, this permitted direct identification of host plants up to species level. This agrees with the work of Avanesyan (2014), who previously reported successful host plants identification from gut contents of other grasshopper species. Our findings show that *A. ruficornis* and *C. tatarica* are polyphagous, feeding mostly on broad-leafed host plants. This finding corroborates a

Table 4. Host plants of short-horned grasshoppers

Site	Grasshopper	Voucher	Plant family	Plant species	GenBank accessions with similarity (%)
Murang'a	*Acanthacris ruficornis*	A9	Amaranthaceae	*Achyranthes aspera*	AY998117.1 (99)
		A2	Tiliaceae	*Triumfetta pilosa*	JF270979.1 (98)
		A1	Apiaceae	*Centella virgata*	KP110015.1 (99)
		A6	Apiaceae	*Centella virgata*	KP110015.1 (99)
		A12	Apiaceae	*Centella virgata*	KP110015.1 (98)
		A4	Asteraceae	*Galinsoga quadriradiata*	AY215801.1 (99)
		A13	Poaceae	*Digitaria gayana*	HE586102.1(98)
Kilifi	*Cyrtacanthacris tatarica*	KL9	Fabaceae	*Teramnus uncinatus*	EU717400.1 (98)
		KL10	Leguminosae	*Alysicarpus rugosus*	KX119350.1 (99)
		KL11	Fabaceae	*Teramnus uncinatus*	EU717400.1 (98)
		KL13	Fabaceae	*Teramnus uncinatus*	EU717400.1 (99)

Table 5. Host plants of long-horned grasshoppers

Site	Grasshopper	Voucher	Plant family	Plant species	GenBank Accessions with similarity (%)
Mbarara	*Ruspolia differens*	MB8	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		MB9	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		MB10	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		MB11	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		MB12	Poaceae	*Digitaria gayana*	HE586102.1 (98)
		MB21	Poaceae	*Digitaria gayana*	HE586102.1 (98)
		MB22	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		MB24	Poaceae	*Digitaria gayana*	HE586102.1 (98)
		MB26	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		MB29	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		3MB4	Poaceae	*Sorghum halepense*	MF159520.1 (98)
		3MB6	Fabaceae	*Indigofera arrecta*	KM896906.1 (100)
Hoima	*Ruspolia differens*	HI7	Poaceae	*Digitaria gayana*	HE586102.1 (98)
		HI10	Poaceae	*Digitaria gayana*	HE586102.1 (98)
		HI14	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		HI17	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		HI19	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		HI22	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		HI23	Poaceae	*Digitaria gayana*	HE586102.1 (98)
Hoima	*Ruspolia differens*	HI27	Poaceae	*Digitaria gayana*	HE586102.1(97)
Kabale	*Ruspolia differens*	KB7	Poaceae	*Digitaria gayana*	HE586102.1 (97)
		KB9	Poaceae	*Digitaria gayana*	HE586102.1 (98)
		2KB9	Asteraceae	*Ageratum conyzoides*	KX097918.1 (97)
		2KB10	Myrtaceae	*Eucalyptus saligna*	KM065288.1 (98)
		2KB12	Polygonaceae	*Persicaria nepalensis*	MH324414.1 (99)
		2KB15	Poaceae	*Cynodon dactylon*	HE591380.1 (99)
Masaka	*Ruspolia differens*	3MS10	Poaceae	*Eragrostis mexicana*	HE586093.1 (99)
		GP2	Rutaceae	*Citrus depressa*	AB626777.1 (99)

previous report by Reinhardt and Köhler (2014) that *A. ruficornis* prefers to feed on broad-leaved plants. We found that the insects fed on plants from eight families, namely Apiaceae, Asteraceae, Amaranthaceae, Tiliaceae, Poaceae, Nyctaginaceae, Leguminosae, and Fabaceae. The polyphagous behavior of the two species of short-horned grasshoppers is partly consistent with reports from species in a related genus, *Melanoplus*, which fed on plants from Poaceae and Asteraceae families (Avanesyan 2014). This study identified host plants of short-horned grasshoppers during wet season, while host plants of the insects during dry season need to be further assessed.

The host preference of *R. differens* has previously been investigated based on diet acceptance and preference only (Bailey and McCrea 1978, Malinga et al. 2018a, b, Opoke et al. 2019a). We have, for the first time, investigated host plants of the swarming long-horned grasshoppers by analyzing the contents of the insect guts, which is an indication of host plants consumed in the wild by the adult insects. Nine host plants of *R. differens* were identified from six families, namely Poaceae, Fabaceae, Asteraceae, Myrtaceae, Polygonaceae, and Rutaceae. Poaceae was the most identified family in most sites surveyed. *Digitaria gayana* was the most dominant among the identified host plants under Poaceae family. This finding partly corresponds with that of Bailey and McCrae (1978) that *Digitaria* and *Cloris* are host plants of *R. differens*. 'Nsenene' swarms are associated with long-distance migration (Opoke et al. 2019b), therefore identified host plants might be absent from the sample collection site as plants from the gut can stay up to 32 h before DNA degradation (Matheson et al. 2008). Opoke et al. (2019a) reported that grass species dominated by *Panicum maximum* Jacq. as the host plants of solitary phase of *R. differens*, through observation of plants on which *R. differens* were encountered in the night in Makerere University, Kampala. Gut content analysis, as in our study

combined with field observation of solitary phase of *R. differens* can add precision to information on their host plants. Identified plants are distributed in some parts of East Africa; hence can be available for use in mass rearing of the long-horned grasshoppers.

Conclusions

This study confirmed that the sampled swarming long-horned grasshoppers in Uganda were *R. differens*; while the edible short-horned grasshoppers collected in Murang'a and Kilifi were *A. ruficornis* and *C. tatarica*, respectively. The most dominant host plant recovered from the gut of long-horned grasshoppers was *D. gayana*; whereas short-horned grasshoppers were observed to be highly polyphagous with higher preference for broad-leaved plants. Precise identification of the edible grasshoppers and their host plants in this study can further contribute to optimizing mass rearing protocols for these edible grasshoppers and can be useful for quality control during production and of products in the market.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

Table A1. Means (±SE) of morphometric measurements of long-horned grasshoppers from different locations in Uganda and reference collections from National Museums of Kenya.

Table A2. Means (±SE) of morphometric length measurements (mm) of short-horned grasshoppers from different locations in Kenya and reference collections from National Museums of Kenya.

Table A3. Basic Local Alignment Search Tool results of 16s gene sequences of long-horned grasshoppers' samples collected from different locations in Uganda.

Table A4. Estimates of evolutionary divergence of 16s gene region sequences of long-horned grasshoppers and GenBank accessions sequences of their related species determined using p-distance model in MEGA 7.

Table A5. Estimates of evolutionary divergence of mitochondrial cytochrome b gene region sequences of short-horned grasshoppers and GenBank accessions sequences of their related species determined using p-distance model in MEGA 7.

Gene Sequencing

DNA sequences for species identification have been deposited in the GenBank (https://www.ncbi.nlm.nih.gov/WebSub/). The GenBank accession numbers for our samples are as follow: COI sequences (MK635348, MK635349), CytB sequences (MK629458, MK629459) and 16S sequences (MK639368).

Acknowledgments

This research was funded by Federal Ministry for Economic Cooperation and Development (BMZ) (ENTONUTRI–81194993), Netherlands Organization for Scientific Research, WOTRO Science for Global Development (NWO-WOTRO) (ILIPA–W 08.250.202), The Bioresources Innovations Network for Eastern Africa Development (BioInnovate Africa) (INSBIZ—Contribution ID No. 51050076), The Rockefeller Foundation (SiPFeed—2018 FOD 009), the Canadian International Development Research Centre (IDRC) and the Australian Centre for International Agricultural Research (ACIAR) (INSFEED—phase 2—Cultivate Grant No: 108866-001) through the International Centre of Insect Physiology and Ecology. The senior author Alfonce Leonard was supported by the German Academic Exchange Service (DAAD) In-Region Postgraduate Scholarship. We thank Levi Odhiambo Ombura and Maureen Adhiambo for their technical assistance. We also gratefully acknowledge the *icipe* core funding provided by UK Aid from the Government of the United Kingdom; Swedish International Development Cooperation Agency (SIDA); the Swiss Agency for Development and Cooperation (SDC); Government of the Federal Democratic Republic of Ethiopia and the Kenyan Government. The views expressed herein do not necessarily reflect the official opinion of the donors.

References Cited

Agea, J. G., D. Biryomumaisho, M. Buyinza, and G. N. Nabanoga. 2008. Commercialization of *Ruspolia nitidula* (Nsenene grasshoppers) in central Uganda. Afr. J. Food Agric. Nutr. Dev. 8: 319–332.

Avanesyan, A. 2014. Plant DNA detection from grasshopper guts: a step-by-step protocol, from tissue preparation to obtaining plant DNA sequences. Appl. Plant Sci. 2: 1300082.

Bailey, W. J. 1975. A review of the African species of the Genus *Ruspolia* Schulthess (Orthoptera: Tettigonioidea). Bull. Inst. found. Afr. Noire (Sci. nat). 37: 171–226.

Bailey, W. J. 1979. A review of Australian Copiphorini (Orthoptera: Tettigoniidae: Conocephalinae). Aust. J. Zool. 27: 1015–1049.

Bailey, W. J., and A. W. R. McCrae. 1978. The general biology and phenology of swarming in the East African tettigoniid *Ruspolia differens* (Serville) (Orthoptera). J. Nat. Hist. 12: 259–288.

Belshaw, R., and D. L. Quicke. 1997. A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). Mol. Phylogenet. Evol. 7: 281–293.

Bucklin, A., and T. C. Lajeunesse. 1994. Molecular-genetic variation of *Calanus pacificus* (Copepoda: Calanoida): Preliminary evaluation of genetic-structure and subspecific differentiation based on mtDNA sequences. Calif. Coop. Ocean. Fish. Invest. Rep. 35: 45–51.

Cooper, W. R., D. R. Horton, T. R. Unruh, and S. F. Garczynski. 2016. Gut content analysis of a phloem-feeding insect, *Bactericera cockerelli* (Hemiptera: Triozidae). Environ. Entomol. 45: 938–944.

Dirsh, V. M. 1977. The species and synonymy of the genus *Cyrtacanthacris* (Orth., Acrididae). Revista Española de Entomología 53: 35–50.

GBIF. 2019. *Ruspolia differens* (Serville, 1838) in GBIF Secretariat. GBIF Backbone Taxonomy. Checklist dataset https://doi.org/10.15468/39omei accessed via GBIF.org on 23 December 2019.

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

Hartley, J. C. 1967. Laboratory culture of a tettigoniid, *Homorocoryphus nitidulus vicinus* (Wlk.) (Orthoptera). Bull. Entomol. Res. 57: 203–205.

Hebert, P. D., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003. Biological identifications through DNA barcodes. Proc. Biol. Sci. 270: 313–321.

van Huis, A. 2013. Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58: 563–583.

Ivanova, N. V., T. S. Zemlak, R. H. Hanner, and P. D. Hebert. 2007. Universal primer cocktails for fish DNA barcoding. Mol. Ecol. Notes. 7: 544–548.

Johnsen, P., and G. H. Schmidt. 1982. Notes on, and a check-list of Acridoidea (Saltatoria) collected in Somalia (East Africa). Monitore zool. Ital. 16: 69–119.

Johnston, H. B. 1956. Annotated catalogue of African grasshoppers, Vol. 1. Cambridge University Press, Cambridge, United Kingdom.

Jurado-Rivera, J. A., A. P. Vogler, C. A. Reid, E. Petitpierre, and J. Gómez-Zurita. 2008. DNA barcoding insect–host plant associations. Proc. R. Soc. Lond. B. Biol. Sci. 276: 639–648.

Kar, P., A. Goyal, and A. Sen. 2015. Maturase K gene in plant DNA barcoding and phylogenetics, pp. 75–86. *In* M. A. Ali *et al.* (eds.), Plant DNA Barcoding and Phylogenetics. Lambert Academic Publishing, Saarbrücken, Germany.

Khamis, F. M., D. K. Masiga, S. A. Mohamed, D. Salifu, M. de Meyer, and S. Ekesi. 2012. Taxonomic identity of the invasive fruit fly pest, *Bactrocera invadens*: concordance in morphometry and DNA barcoding. PLoS One 7: e44862.

Khamis, F. M., I. Rwomushana, L. O. Ombura, G. Cook, S. A. Mohamed, C. M. Tanga, P. W. Nderitu, C. Borgemeister, M. Sétamou, T. G. Grout, *et al.* 2017. DNA barcode reference library for the African citrus triozid, *Trioza erytreae* (Hemiptera: Triozidae): vector of African citrus greening. J. Econ. Entomol. 110: 2637–2646.

Kinyuru, J. N., G. M. Kenji, S. N. Muhoho, and M. Ayieko. 2011. Nutritional potential of longhorn grasshopper (*Ruspolia differens*) consumed in Siaya district, Kenya. J. Agr. Sci. Tech. 12: 32–46.

Kinyuru, J. N., S. O. Konyole, G. M. Kenji, and C. A. Onyango. 2012. Identification of traditional foods with public health potential for complementary feeding in western Kenya. J. Food Res. 1: 148–158.

Kinyuru, J. N., D. Nyangena, E. Kamau, A. Nderitu, J. Muniu, C. Kipkoech, J. Weru, N. Ndung'u, and M. Mmari. 2018. The role of edible insects in diets and nutrition in East Africa, pp. 93–108. *In* A. Halloran *et al.* (eds.), Edible insects in sustainable food systems. Springer, New York, NY.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Malinga, G. M., A. Valtonen, V. J. Lehtovaara, K. Rutaro, R. Opoke, P. Nyeko, and H. Roininen. 2018a. Mixed artificial diets enhance the developmental and reproductive performance of the edible grasshopper, *Ruspolia differens* (Orthoptera: Tettigoniidae). Appl. Entomol. Zool. 53: 237–242.

Malinga, G. M., A. Valtonen, V. J. Lehtovaara, K. Rutaro, R. Opoke, P. Nyeko, and H. Roininen. 2018b. Diet acceptance and preference of the edible grasshopper *Ruspolia differens* (Orthoptera: Tettigoniidae). Appl. Entomol. Zool. 53: 229–236.

Matheson, C. D., G. C. Muller, A. Junnila, K. Vernon, A. Hausmann, M. A. Miller, and Y. Schlein. 2008. A PCR method for detection of plant meals from the guts of insects. Org. Divers. Evol. 7: 294–303.

Matojo, N. D., and K. M. Hosea. 2013. Phylogenetic relationship of the longhorn grasshopper *Ruspolia differens* Serville (Orthoptera: Tettigoniidae) from Northwest Tanzania based on 18S ribosomal nuclear sequences. J. Insects. 2013: 504285.

Matojo, N. D., and J. G. Yarro. 2010. Variability in polymorphism and sex ratio of the conehead *Ruspolia differens* Serville (Orthoptera: Conocephalidae) in north-west Tanzania. Int. J. Integr. Biol. 9: 131–136.

Matojo, N. D., and J. G. Yarro. 2013. Anatomic Morphometrics of the "Senene" Tettigoniid *Ruspolia differens* Serville (Orthoptera: Conocephalidae) from North-West Tanzania. ISRN. Entomology. 2013: 176342.

McCrae, A. W. R. 1982. Characteristics of swarming in the African edible bush-cricket *Ruspolia differens* (Serville) (Orthoptera, Tettigonioidea). J. East Afri. Nat. Hist. Soc. Natl. Mus. 178: 1–5.

Miura, K., and N. Ohsaki. 2004. Diet mixing and its effect on polyphagous grasshopper nymphs. Ecol. Res. 19: 269–274.

Mungai, M. N. 1987. The African grasshopper genus *Acanthacris* (Orthoptera: Acrididae: Cyrtacanthacridinae). J. Nat. Hist. 21: 807–823.

Nginya, E. S., J. O. Ondiek, A. M. King'ori, and J. M. Nduko. 2019. Evaluation of grasshoppers as a protein source for improved indigenous chicken growers. Livest. Res. Rural. Dev. 31: 2.

Opoke, R., P. Nyeko, G. M. Malinga, K. Rutaro, H. Roininen, and A. Valtonen. 2019a. Host plants of the non-swarming edible bush cricket *Ruspolia differens*. Ecol. Evol. 9: 3899–3908.

Opoke, R., G. M. Malinga, K. Rutaro, P. Nyeko, H. Roininen, and A. Valtonen. 2019b. Seasonal pattern in population dynamics and host plant use of non-swarming *Ruspolia differens* Serville (Orthoptera: Tettigoniidae). J. Appl. Entomol. 143: 371–379.

R Core Team, R. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/. Accessed 10 August 2018.

Reinhardt, K., and G. Köhler. 2014. A locust *Acanthacris ruficornis* (Fabricius, 1787) in a flower shop of Tübingen, Germany (Acrididae: Cyrtacanthacridinae). Articulata. 29: 93–97.

Robinson, D. J., and J. C. Hartley. 1978. Laboratory studies of a tettigoniid (Insecta: Orthoptera) *Ruspolia differens* (Serville): colour polymorphism. J. Nat. Hist. 12: 81–86.

Samejo, A. A., and R. Sultana. 2016. Comparative study on the various species of locusts with special reference to its population fluctuation from Thar Desert, Sindh. J. Entomol. Zool. Stud. 4: 38–45.

Shuker, D. M., and E. R. Burdfield-Steel. 2017. Reproductive interference in insects. Ecol. Entomol. 42: 65–75.

Siepel, A., G. Bejerano, J. S. Pedersen, A. S. Hinrichs, M. Hou, K. Rosenbloom, H. Clawson, J. Spieth, L. W. Hillier, S. Richards, *et al.* 2005. Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. Genome Res. 15: 1034–1050.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10: 512–526.

Virgilio, M., T. Backeljau, B. Nevado, and M. De Meyer. 2010. Comparative performances of DNA barcoding across insect orders. BMC Bioinf. 11: 206.