Viability and fertility of hybrid New Zealand tree weta *Hemideina* spp. (Orthoptera: Anostostomatidae)

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Abstract

Natural hybridization between species provides an opportunity to study the mechanisms that maintain independent lineages and may help us understand the process of speciation. The New Zealand tree wētā species *Hemideina thoracica* produces F_1 hybrids where it lives in sympatry with two closely related species: *Hemideina crassidens* and *Hemideina trewicki*. This study looked at the viability and fertility of F_1 hybrid wētā between *H. thoracica* and *H. crassidens* that were collected from the wild and kept in captivity. The hybrids appeared to have normal viability from the late juvenile stage, with all male wētā maturing at a late instar. Male F_1 hybrids displayed normal mating behavior and one male produced offspring in captivity. In contrast to Haldane's rule, female F_1 hybrids appeared to be infertile; they refused to mate and did not produce eggs. No evidence of *Wolbachia* infection was identified in any of the three North Island *Hemideina eina* species.

Key words

Haldane's rule, Hybridization, introgression, sexual exclusion, Wolbachia

Introduction

Natural hybridization between species provides an opportunity to study the mechanisms that maintain independent lineages and may help us understand the process of speciation (Butlin 1987, Barton and Gale 1993). Hybrid animals often have lower fitness than individuals of their parent species (Harrison 1993 and references therein) as a result of incompatible genetic combinations, such as Dobzhansky-Muller incompatibilities (Dobzhansky 1937, Muller 1942, Bolnick and Near 2005), mismatched chromosomes (Shaw and Wilkinson 1980, Searle 1993), incompatible cellular organelles (e.g. mitochondria: Ellison and Burton 2008) and symbiotic bacteria (e.g. intracellular *Wolbachia* infections: Bordenstein et al. 2001; gut bacteria: Brucker and Bordenstein 2013). However, low hybrid fitness also arises through natural and sexual selection when an intermediate phenotype is a disadvantage (Svedin et al. 2008).

If hybrids are viable they might nevertheless have limited fertility. If fertility of F_1 hybrids is very low, fertility levels usually

improve in subsequent generations of backcross hybrids (Mallet et al. 1998, Mallet 2005, Descimon and Mallet 2009), and even limited fertility provides the possibility for introgression that can have important consequences for species interactions. Adaptive alleles that arise in one population/species can be transferred to another through hybridization, known as adaptive introgression, as seen in wing coloration in Heliconius butterflies (Pardo-Diaz et al. 2012). In some cases, when hybridization reduces fertility a unimodal hybrid (or tension) zone forms. Unimodal hybrid zones are geographically constrained; with most individuals in the zone having mixed ancestry, and the width of the hybrid zone depending on hybrid disadvantage and dispersal of the species (Jiggins and Mallet 2000). If hybridization is more limited, a bimodal hybrid zone may result, where parental forms overlap and predominate with a few individuals of mixed ancestry amongst them. Bimodal hybrid zones are typically associated with assortative mating so, in tree weta, reproductive character displacement is a likely outcome that increases assortative mating (Dieckmann and Doebeli 1999, Jiggins and Mallet 2000). Where two species compete for the same resources, hybridization may prevent one from successfully out-competing the other. Alternatively sexual exclusion, where one species (often the males of that species) out-competes the other for mates, can limit fitness through reproductive interference (Gröning and Hochkirch 2008), as observed between the tetrigids: Tetrix ceperoi and Tetrix subulata (Hochkirch et al. 2007).

Tree wētā (Orthoptera: Anostostomatidae: *Hemideina*) are a genus of seven nocturnal arboreal insects, with high morphological and ecological similarity (Field and Bigelow 2001, Dewhurst 2012, Bulgarella et al. 2014). They are hypothesized to have speciated in allopatry during the Pliocene or earlier (Trewick and Morgan-Richards 2005), and they now have broadly parapatric distributions (Trewick and Morgan-Richards 1995, Bulgarella et al. 2014). Hybridisation in the wild between the New Zealand tree wētā *Hemideina thoracica* (White) and both *Hemideina crassidens* (Blanchard) and *Hemideina trewicki* Morgan-Richards has recently been confirmed with genetic data, but so far only F_1 hybrids have been confirmed (Mckean 2014, Mckean et al. 2016). These three *Hemideina eina* species each have distinct karyotypes (i.e. different numbers of

chromosomes with some differing in size and shape; Morgan-Richards 1995, 1997, 2000, Mckean et al. 2015). Karyotype differences are generally seen as presenting barriers to gene flow by disrupting meiosis and rendering F, hybrids infertile. However, some tree wētā species naturally comprise multiple chromosome races that are capable of interbreeding in the wild (Morgan-Richards 1997, 2000, Morgan-Richards et al. 2000, Morgan-Richards and Wallis 2003). The apparent tolerance of chromosome rearrangements displayed in this orthopteran lineage might influence fertility of interspecies hybrids. Karyotype, mtDNA haplotypes, and alleles at four nuclear DNA loci were found to differentiate parent populations of H. thoracica and H. trewicki in a large area of sympatry in Hawke's Bay. These markers (except mtDNA, which is maternally inherited) were heterozygous in individuals who were phenotypically intermediate in abdominal coloration (orange rather than yellow or brown), abdominal bands (faint rather than striking or non-existent), abdominal stripe (a series of spots rather than a stripe or the absence of a stripe) and the number of spines on the prolateral hind tibia (typically between the three spines seen in H. thoracica and the four in H. crassidens/H. trewicki, with a half-sized medial spine on each leg being common, or three spines on one leg and four on the other). A similar situation was seen in the Manawatu area of sympatry between H. thoracica and H. crassidens, where karyotype, mtDNA and three nuclear DNA markers were found to differentiate the two species (with some introgression detected relative to allopatric populations). All individuals, which had an intermediate phenotype (the same phenotype as for *H. thoracica* × *H. trewicki* hybrids), were heterozygous for these markers (Mckean et al. 2016). Whether hybridization occurs between H. crassidens and H. trewicki is currently unknown due to the morphological similarities of these two species, and unknown distribution boundaries due in part to clearance of native forest where the two are hypothesized to have historically met (Trewick and Morgan-Richards 1995). A lack of gene flow suggests that H. thoracica × H. trewicki hybrids, which are found at a frequency of 1% of weta in sympatry, are infertile, but genetic and morphological data suggest a low, but potentially significant, level of introgression between H. thoracica and H. crassidens, where hybrid frequency is ~3 in every 100 weta (Mckean et al. 2016).

Introgression is the signal of past hybridization, and an ability to successfully hybridize might be of fundamental importance to the future of a species, while climates and environments continue to change (Grant and Grant 1993, Allendorf et al. 2001, Becker et al. 2013, Taylor et al. 2015, Sivyer et al. 2018). There is evidence that *H. crassidens* formerly occupied much of central North Island that is now the range of H. thoracica (Bulgarella et al. 2014). Isolated populations of H. crassidens remain in regions of high elevation, which suggests they are adapted to colder environments. With global warming, H. crassidens might continue to be displaced, but this depends on the ecological and sexual interaction between species. Adult tree weta often form harems in tree cavities during the summer and autumn (Wehi et al. 2013), and mixed species harems in areas of overlap suggest that species recognition is not complete (Trewick and Morgan-Richards 1995, Wehi et al. 2017). Of F, hybrids collected in the wild, the majority had an H. thoracica father and H. crassidens mother (Mckean et al. 2016), which suggests sexual exclusion by H. thoracica males; as in *Hemideina* spp. the females do not appear to actively choose their mates. Although females will sometimes resist mating, resistance times are similar whether mating occurs or not (Field and Jarman 2001). This gives rise to the possibility that hybridization may be an important factor in the coexistence/exclusion of these species.

Haldane (1922) observed that where one sex is absent, rare, or infertile in F_1 hybrids, it is usually the heterogametic sex. In tree wētā this is the male, as tree wētā, like most Orthoptera, have an XO sex determination system where females have two copies of the sex chromosome and males one (White 1940, Morgan-Richards 1997, Morgan-Richards and Wallis 2003). Based on this, if there is a difference between the sexes, we would expect male F_1 hybrids to have lower viability and/or fertility than female F_1 hybrids.

Given their apparent tolerance of karyotype variation, the high degree of infertility in weta might have another source. Wolbachia is an endosymbiotic intracellular bacteria that infects a large proportion of the arthropod and nematode phyla (Werren et al. 2008). In arthropods, Wolbachia is estimated to infect about 65% of species (Hilgenboecker et al. 2008) including many grasshoppers and crickets (Werren and Windsor 2000, Mandel et al. 2001, Bella et al. 2010). Wolbachia is known to manipulate the reproductive biology of many of its hosts to its own advantage (Werren et al. 2008). Some of the currently known host-reproductive manipulations include male killing, induction of parthenogenesis, feminization of genetic males, forced production of haploid individuals in haplodiploid systems, and cytoplasmic incompatibility (Werren 1997). Wolbachia is hypothesized to have a role in arthropod speciation via induction of cytoplasmic incompatibility (Werren 1998 and references therein). Wolbachia infections appear to be responsible for maintaining hybrid zones between the well-studied grasshopper subspecies Chorthippus parallelus parallelus and Chorthippus parallelus erythropus via two different forms of cytoplasmic incompatibility (Bella et al. 2010). Although recently detected in New Zealand insects (Bridgeman et al. 2018), it is not known whether the tree weta lineage (with many hybrid zones; Morgan-Richards and Wallis 2003) contains this intracellular parasite.

Here, we describe the viability and fertility of hybrids between *Hemideina thoracica* and *H. crassidens*, using F_1 hybrids collected in the wild and held in captivity. We sought evidence of *Wolbachia* infections to assess whether this common intracellular parasite has potential to limit reproductive compatibility among these wētā species.

Methods

Sampling and captive conditions.-Eleven F, hybrid tree weta were captured from native forest in Turitea Valley (\$40.47184, E175.60943) and Kahutawera Valley (S40.431725, E175.674595), Manawatu, New Zealand (Fig. 1, Table 1). Hybrid identity was tested and confirmed using genetic markers for eight of the 11 individuals which died or were euthanized during the course of this study, and were preserved as specimens in alcohol (Table 1; F, hybrids; Morgan-Richards 1995, Mckean et al. 2016). The other three putative hybrid individuals were assumed to be F, hybrids as their phenotypes were completely consistent with the F, hybrids that had been genetically identified. No cryptic hybrids were identified in previous studies of tree weta from this population (Trewick and Morgan-Richards 1995, Morgan-Richards and Gibbs 2001, Bulgarella et al. 2014, Mckean et al. 2016). Live weta were held in individual containers at a constant temperature of 14°C. They were given a suitable daytime roost cavity made from harakeke (Phormium tenax) flower stalk, and were fed palatable leaves from at least three native plant species each week and 80% soy protein pellets (Griffin et al. 2011).

Body size of F_1 *hybrids.*—No significant difference in body size between adult females of the two parent species has been found in



Fig. 1. Distribution of the three North Island New Zealand species of tree wētā (*Hemideina*) and an *H. thoracica* × *H. crassidens* F_1 hybrid. The distributions of the species were taken from Morgan-Richards and Wallis (2003) and Morgan-Richards (2000).

Table 1. Sampling information, size and results for mating behavior in both sexes and egg production in hybrid females.

Wētā	Sample	Location	Genetically Confirmed Hybrid	Sex	Age	Tibia Length (mm)	Instar at Maturity	Behavior	Age since Maturity (Final Molt)	Eggs
Hybrid 1	Live	Kahutawera valley	Yes	М	Adult	23.63	10	Normal; mated*	NA	NA
Hybrid 2	Live	Turitea valley	Yes	М	Adult	24.01	10	Normal; mated*	NA	NA
Hybrid 3	Live	Turitea valley	Yes	М	Adult	23.62	10	Normal; mated*	NA	NA
Hybrid 4	Live	Kahutawera valley	Yes	F	Adult	22.92	10	Resisted Mating+	6 months	No
Hybrid 5	Live	Kahutawera valley	No	F	Adult	21.26	10	Resisted Mating+	4 months	No
Hybrid 6	Live	Kahutawera valley	No	F	Adult	23.76	10	Partial Resistance+	3 months	No
Hybrid 7	Preserved	Kahutawera valley	Yes	F	Adult	21.26	10	NA	6 months	No
Hybrid 8	Preserved	Kahutawera valley	No	F	Adult	22.29	10	NA	3 months	No
Hybrid 9	Preserved	Kahutawera valley	Yes	М	Juvenile	16	10	NA	NA	NA
Hybrid 10	Live	Kahutawera valley	Yes	М	Sub-adult	18.42	10	NA	NA	NA
Hybrid 11	Preserved	Kahutawera valley	Yes	М	Adult	21.11	10	NA	NA	NA

Legend: *See Table 3, + See section 'Mating Behavior' in methods.

this zone of sympatry (Mckean et al. 2016). To detect signs of impaired growth (hybrid inviability) the hind tibia length of both dead and living adult hybrids was measured with electronic calipers and compared via ANOVA to wild adult females of both parent species measured in a previous study (15 *H. thoracica* and 19 *H. crassidens;* Mckean et al. 2016), and to a separate sample of adult males from both species (25 *H. thoracica* and 22 *H. crassidens*), that were sampled from the same locations as the hybrids. Hind tibia length is a reliable proxy for body size in tree wētā (Minards et al. 2014, Bulgarella et al. 2014.) The sex of hybrids and instar at maturity for male wētā were both recorded. Maturity is determined in tree wētā by the shape and size of the cerci or ovipositor. Tibia length data for each sex were compared via ANOVA.

Mating behavior.-Six hybrid weta (three males, three females) were provided with one potential mate of each parent species, on different nights, in a Perspex tank ($60 \text{ cm} \times 60 \text{ cm} \times 60 \text{ cm}$) (Table 1). Mating trials were observed for 30 min in the evening when tree wētā are most active (Kelly 2006a). For male wētā, successful transfer of spermatophores was recorded as well as attempts to mate, defined as curling the abdomen to position for mating. Other mating behavior prior to this, such as following the female or rapid twitching of the palps that indicated the male had scented the female, and running the palps over the female's abdomen, were recorded (Field and Jarman 2001 and references therein). As male mating behavior has been well described elsewhere, the male F, hybrids' behavior was compared to what is known from previous work which details the parental species' behavior. Female tree wētā do not appear to actively choose or approach male wētā (Field and Jarman 2001 and references therein), so their acceptance or active resistance to mating was recorded. Resistance was defined as any behavior that appeared to obstruct mating attempts by the male including moving away, stridulating (a defensive/aggressive gesture in tree weta; Field 2001, Field and Glasgow 2001), and biting and kicking the male to dislodge him. Acceptance was defined as the female staying still and allowing copulation to be initiated and completed, as evidenced by the successful transfer of a spermatophore.

Egg production.—Females of both parent species begin producing eggs as soon as they reach maturity (N.E.M. personal observation, >50 females 2012–2013). Eggs inside the ovarioles of mature females typically vary in developmental stage and range from very small undeveloped yellow eggs through to large black mature eggs with a thick outer casing (Griffin 2011). After laying, the embryo case expands and turns from black to brown and eventually yellow (Stringer 2001). Four F, hybrid adult females and 18 H. crassidens females were given soil slightly deeper than the length of the ovipositor to lay eggs in (Table 2). Conditions were otherwise the same as detailed in captive conditions above. After approximately 100 days (StDev = 35.9) the eggs laid were removed and counted. Each weta was euthanized, dissected and the number of unlaid mature eggs counted under a dissecting microscope. Additional data were obtained from a preserved hybrid female euthanased before she laid eggs (n=5 in Table 2).

Male fertility.—Two adult F_1 hybrid males, which were adults at the time of the study, were each provided with virgin females of both parent species, as above (Table 3). They were observed until a mating occurred and then left together in the tank overnight. Female wētā were removed the next morning and placed in a container with a layer of soil slightly deeper than the length of the

Table 2. Average number of eggs +/- standard deviation for *H. crassidens* females vs. F, hybrid females.

	Sample size	Age since maturity (days)	Eggs (unlaid)	Eggs (laid)	Eggs (total)
H. crassidens	18	201 +/- 70.7	26 +/- 30.9	65 +/- 32	91 +/- 26.5
F ₁ Hybrids	5	139 +/- 47.8	0	0	0

Table 3. Results of captive breeding experiments with F_1 hybrid *H*. *thoracica* × *H*. *crassidens* fathers and mothers of both parent species. Growth of eggs was both physical expansion and changing color from black to brown or yellow.

Male	Female	No. Eggs Laid	Growth	Hatched
	H. crassidens	50	Yes	0
Hybrid 1 x	H. crassidens	35	Yes	0
	H. thoracica	111	Yes	0
	H. thoracica	37	Yes	4
Hydrid 2 X	H. crassidens	-	-	-

ovipositor. After a period of oviposition the female was removed, the eggs counted and placed back into the soil. As little is known about triggers for embryo growth and hatching in wētā, the eggs were stored outside, exposed to the ambient winter temperature fluctuations experienced by the wild population from which they were derived. Expansion and hatching were recorded the following summer (approximately 9 months after laying).

Wolbachia detection.-Two methods were used to obtain evidence of infection by the bacteria Wolbachia: amplification of DNA sequences using Wolbachia specific Polymerase Chain Reactions (PCR) primers, and whole genome sequencing and alignment to a reference Wolbachia genome. For amplification of specific Wolbachia DNA sequences, DNA was extracted from three tree wētā specimens representing each of the three North Island species (H. thoracica, H. crassidens and H. trewicki). Tissue was taken from the hind femur and testes or ovariole of each tree weta specimen and DNA isolated using a salting out method (Trewick and Morgan-Richards 2005). Wolbachia-specific primers (Appendix 1) were used in PCR with weta DNA, and DNA from an introduced gregarious parasitoid wasp (Nasonia vitripennis) known to be infected with Wolbachia as a positive control. Standard PCR conditions for these primers were followed (Braig et al. 1998, Heddi et al. 1999, Baldo et al. 2006) (Appendix 1). PCRs were repeated to rule out problems with reaction conditions. One PCR product longer than the expected Wolbachia fragment from the CoxA primer pair was amplified. This long DNA fragment was sequenced at the Massey Genome Service with a capillary AB13730 Genetic Analyzer (Applied Biosystems Inc.), and then visualized and trimmed in Geneious 6.1.7 (Biomatters LTD; Kearse et al. 2012) software. The resulting 269 bp sequence was compared to public databases using the Basic Local Alignment Search Tool (BLAST) algorithm on the NCBI website.

Total genomic DNA from two tree wētā specimens (an *H. thoracica* male collected from the Kahutawera Valley and an *H. crassidens* male collected from a South Island population) were separately processed through parallel, high-throughput sequencing (Illumina HiSeq 2500) for a separate phylogenetic study (Dowle 2013). Briefly, DNA was extracted from a single male individual (testes tissue), fragmented, prepared using the ThruPLEX DNA-seq Kit (Rubicon Genomics) and used to generate 100 bp paired-end

sequence on a Hi-Seq 2000 (BGI). This resulted in 5,191,884 100 bp paired-end sequences 200 bp apart for the H. thoracica specimen and 17,434,429 100 bp paired-end sequences for the H. crassidens specimen. An annotated reference Wolbachia genome was obtained from New England Biolabs (http://tools.neb.com/ wolbachia, originating from infection of Brugia malayi; Foster et al. 2005). Reads were trimmed to remove index sequences using solexaQA (Cox et al. 2010) before mapping to the Wolbachia genome using the default settings with Bowtie 2 (Langmead and Salzberg 2012). Results were visualised with Tablet v1.7.0_35 (Milne et al. 2010). Sequences that matched parts of the Wolbachia genome were compared with published data using the NCBI (National Library of Medicine) GenBank BLAST search algorithm to determine their similarity to Wolbachia DNA sequences from other hosts. This enabled us to determine whether the sequences came from the Wol*bachia* genome or another related bacterial species, which could be determined by sequence similarity.

Results

Phenotype F_1 *hybrids.*—Hybrids were identified by genetic markers and intermediate phenotypes, and no morphologically cryptic hybrids were identified (Mckean et al. 2016). The sex ratio of F, hybrids in our small sample was even (five females, six males). All but two hybrid weta examined were adults (or reached adulthood in captivity - two weta) providing no evidence of reduced hybrid viability. There was no significant size difference between adult F₁ hybrid females and adult females of the two parent species from the same location with ANOVA; F = 2.575, P = 0.09 (Fig. 2A), however male F, hybrids were significantly larger than males of either parent species (ANOVA; F = 8.969, P = 0.00049; Fig. 2B). The five adult male hybrids matured at the tenth instar as determined by comparing their hind tibia lengths to data of weta trimorphism in Hemideina crassidens (Kelly and Adams 2010, Bulgarella et al. 2015). Although one male did not reach maturity (Hybrid 10; Table 1), as a ninth instar sub-adult he would have been an adult at the tenth instar, as determined by growth/size charts from previous studies (Spencer 1995, Kelly and Adams 2010).

Mating behavior.—All three F_1 hybrid males mated with females of both species (Table 1). Each male exhibited normal and similar mating behavior to females of both species he was housed with (Field and Jarman 2001 and references therein), and was accepted by females of both species. In contrast, two of three hybrid females actively resisted mating. The third allowed the *H. thoracica* male to begin copulation several times, but then dislodged him and proceeded to bite him and display other resistance behaviors. She allowed mating to occur once with the *H. crassidens* male, and then resisted all subsequent mating attempts, and was the only hybrid female wētā that was observed to accept a spermatophore.

Fertility.—None of the five female F, hybrids contained eggs in any stage of development when killed and dissected as adults. This contrasts with 18 H. crassidens females that each laid and/or contained an average of 91 eggs (Table 2). Females that were mated to the hybrid males laid 35-111 eggs (except one H. crassidens female that died soon after mating with Hybrid 2). Some eggs from every female showed signs of expansion after 6 – 8 months, with many eggs increasing in size and changing color from black to light brown or yellow (Table 3). Four eggs by male Hybrid 2 and his H. thoracica female mate expanded and then hatched to produce offspring. The nymphs were inferred to be phenotypically normal, as no obvious morphological differences were seen under a dissecting microscope. The color of nymphs is uniformly grey (dorsal) and yellowish white (ventral) at this stage regardless of species, so no inferences could be drawn about eventual color phenotype (whether the F_2 generation look the same as F_1 , or resemble the wētā of the parent species). No other eggs hatched during the study, including the eggs produced by the control weta (Table 3).

Wolbachia.—The fbpA and Wol16S primers failed to amplify a DNA fragment when used with tree wētā DNA, but produced a DNA fragment with the positive control (a wasp know to be infected with *Wolbachia*). The Wsp and CoxA primers gave a series of weakly amplified DNA fragments longer than that expected from the *Wolbachia* genome. A consistent DNA fragment amplified with the CoxA primers was 200 bp longer than the positive control.



Fig. 2. A. Tibia length of adult female F_1 hybrids compared with adult females from the two parent species, showing no significant difference; **B.** Tibia length of F_1 hybrid males compared with males of the two parent species, showing a significant difference: p-value = 0.0001.

No close sequence match was found when compared to DNA sequences on the database Genbank, including *Wolbachia* sequences.

None of the > 17 million H. crassidens next-generation short read DNA sequences mapped to the Wolbachia genome. However, eight 100 bp DNA sequences from genomic H. thoracica DNA shared similarity with Wolbachia. Six identical DNA sequence reads mapped to one location, all with the same ten mismatches. The other two reads mapped to a different location on the Wolbachia genome, differing at nine sites (mismatches). However, the paired-end for all eight of these sequence reads (100-300 bp downstream from the putative-bacteria DNA sequence) did not map to the Wolbachia genome sequence. Comparing the putative Wolbachia sequences to the Genbank database identified these sequences as: 1) 93% similarity with the 16S rRNA gene from various members of the Chlamydiae phylum, with six of these matches belonging to the Rhabdochlamydia genus, and 2) 93% match for three 28S gene fragments from Simkania negevensis, which also belongs to the Chlamydiae phylum. As similarity with Wolbachia sequences was lower (90-91%), it is likely that the H. thoracica weta was infected with a bacteria species from the chlamydia family, not closely related to Wolbachia. Both the 16S and 28S rRNA genes are highly conserved among bacteria, and of the > 22 million DNA short-sequences from the weta none mapped to Wolbachia-specific regions of the Wolbachia genome. A separate study of other Orthoptera confirmed that this level of data was sufficient for detection of Wolbachia infections (Bridgeman et al. 2018).

Discussion

The size of H. thoracica \times H. crassidens hybrids fell within the normal range expected for the parent species (with males at the larger end), and many hybrids were found as adults in the wild, therefore we have no positive evidence of hybrid inviability or abnormal development. There could be some inviability early in development, during the pre-hatching or early instar phases, but it appears that at least by the time F, hybrids have reached the larger instars (5th to 7th), they are as successful as a typical weta of either parent species. Female tree weta all mature at the tenth instar but males can mature at the eighth, ninth or tenth instar (Spencer 1995, Kelly and Adams 2010), resulting in a wide size range of adult males. All F, hybrid males in this study matured at the tenth instar, which may be important in understanding their reproductive success (if any). Male tree weta compete for females via competition for tree cavities (resources) that females use as refuges during the day (Spencer 1995, Field 2001). Tenth instar males have much larger mandibles than eighth instar adult males, which are used during male-male competition, but which also limit their mobility, leading to the hypothesis that smaller males actively search for and mate with females which are away from their tree cavities foraging at night (Field 2001, Kelly 2004, Kelly 2006a, Kelly and Adams 2010). If male F, hybrids all mature at a later instar, it is unclear whether this is an advantage or disadvantage for reproductive success. As the ratios of other male weta maturing at different instars may play a part in determining success in controlling harems, more research into reproductive success among males in this location is needed to determine the outcome of these 10th instar hybrid males.

Our observations of mating were limited to experimental pairs (rather than harems, which are common in the wild; Wehi et al. 2013) and this might have influenced the behaviors exhibited and observed. Female wētā were not given a choice of mate, which could have also influenced mating behavior. However, mating behavior appeared to be normal for our limited sample of F, hybrid males when paired with adult H. thoracica and H. crassidens females. One of the hybrid males had been found in the wild with a harem consisting of two adult H. crassidens females. This male produced offspring in captivity, hence it is likely that this male, along with at least some others, are behaving in the wild in a manner typical of males from the parent species. One significant limitation of this study is the lack of control mating crosses and mating behavior comparisons for parental species from the same populations, as previous mating studies were conducted with Hemideina crassidens from southern populations (Field and Jarman 2001, Kelly 2006b, c). There have been few studies of H. thoracica mating behavior (Wehi et al. 2013) and there is a general lack of understanding of mating outcomes in this lineage (Field and Jarman 2001). Unfortunately, the triggers for embryo development and hatching are unknown for tree weta, making laboratory crosses difficult and prone to failure, as evidenced by the control weta embryos failing to hatch. Therefore no inferences can be drawn about the success of these crosses relative to parental crosses, but given that laboratory crosses are sometimes successful when virgin females are mated to single males, it does not appear that sperm competition has to take place in these species to induce fertility in females (Morgan-Richards 2000, Stringer 2001, present study), and can probably be ruled out as an explanation for lack of fertility.

In contrast to the males, the female F_1 hybrids did not show typical mating behavior, but this may be irrelevant to fertility if they cannot produce eggs. The lack of egg production in all five F_1 female hybrids is probably biologically important, despite the small sample, because it contrasts with that observed in adult *H. crassidens* females kept in the same conditions (Table 2). A lack of eggs was also never observed in more than 50 mature parent females of both species that were dissected (N.E.M. personal observation, 2012–2013). The absence of eggs suggests that F_1 females may typically be infertile, whereas at least some F_1 male hybrids are fertile, as was evident from the offspring produced in captivity.

Male F, hybrids being partially fertile while females are infertile contrasts with the usual variation between the sexes in reduced fertility (Haldane's rule) and may be of interest for future research. Haldane's rule applies across many animal taxa, including others with a XO sex determination system (Haldane 1922, and one analysis found that it applied in 99% of 223 cases of sex-specific hybrid sterility and 90% of 115 cases of sex-specific hybrid inviability (Laurie 1997). Infertility as opposed to inviability appears to be the most normal sex-skewed outcome, as heterogametic infertility is known to outnumber heterogametic inviability about 10:1 in Drosophila and mammals (Wu and Davis 1993). The mechanism behind Haldane's rule is still unclear although two main hypotheses are X chromosome to autosome imbalance, and incompatibilities between the sex chromosomes. It is also possible that there are multiple causes underlying this phenomenon, but with so few sex determination systems for comparison a conclusive inference is elusive (Coyne 1985, Wu and Davis 1993, Turelli 1998). There is also evidence that the genetic basis of inviability in heterogametic hybrids differs from the genetic basis for infertility (Coyne 1985). Exceptions such as this may eventually shed light on why this rule applies so well to the majority of species. There are some contradictions to Haldane's rule in other XO systems, such as the field crickets Teleogryllus oceanicus and Teleogryllus commodus (Moran et al. 2017), although

whether contradictions to Haldane's rule are more common in XO systems is unknown.

One question remaining unanswered in the present study is where the barriers to reproduction are. As bimodal hybrid zones are typically associated with pre-mating rather than post-mating barriers (Jiggins and Mallet 2000 and references therein), the situation here is somewhat unusual. As there does not appear to be assortative mating between these species pairs (Field and Jarman 2001, Morgan-Richards et al. 2001, Wehi et al. 2017), it suggests that barriers are more likely to be the result of genetic constraints. It is not known at what stage the production of F₁ hybrids is limited, but as intermediate forms are far less common than expected if the species were freely interbreeding (Mckean et al. 2016) some reproductive constraint must operate. Barriers are hypothesised to be at the post-mating pre-zygotic stage or early in development, and it is possible that the weta use unknown behavioral mechanisms to limit interbreeding. A bimodal hybrid zone in two species of chrysomelid beetles (*Chyrsochus cobaltinus* and *C. auratus*) also involves stronger post-zygotic barriers than pre-zygotic barriers (Peterson et al. 2005), so the association of assortative mating and bimodal hybrid zones has exceptions. A later study of these same beetles also showed a significant sex-bias in the production of offspring (most had mtDNA haplotypes and hence mothers from one species), despite mating occurring in both directions in the wild, and offspring in both sex-pairings being produced in equal numbers and with equal viability in laboratory crosses at the first instar (Monsen et al. 2007). The proposed explanation was asymmetric post-mating pre-zygotic barriers, or possible asymmetric inviability later in development. As H. thoracica and *H. crassidens* appear to exhibit both a bimodal hybrid zone in the apparent absence of pre-mating barriers, and also a sexbiased production of F₁ offspring, there may be some similarities in the mechanisms causing reproductive isolation in these disparate species pairs. Examples such as these may give insights into how bimodal hybrid zones are typically formed and maintained.

Female infertility would prevent mtDNA passing the species boundary (introgressing), and this may explain why no evidence of mtDNA introgression has been seen in previous studies (Bulgarella et al. 2014, Mckean et al. 2016), despite evidence of a low level nuclear DNA and possible phenotypic introgression (Mckean et al. 2016). The low number of F, hybrids seen in the wild suggests that these wetā are forming a bimodal hybrid zone (Mckean et al. 2016), and with reduction in fertility of at least a 50% (due to female infertility), production of hybrids is probably costly. The most likely outcome in this scenario would appear to be reproductive character displacement or niche divergence limiting hybridization and loss of reproductive potential, as loss of reproductive compatibility allowing merging of the species would be unlikely with such a significant reduction in fertility (Dieckmann and Doebeli 1999, Jiggins and Mallet 2000). However, if one species uses sexual exclusion to monopolize mates of the other, it could enable for the range expansion of this species. Given that most F_1 hybrids (8/9) were shown to have a H. thoracica father, this may in part explain how H. thoracica has been able to displace H. crassidens from much of its former range as climate has warmed since the last glacial maximum (Bulgarella et al. 2014). Introgression of adaptive alleles (e.g. cold tolerance) could enable continued expansion of H. thoracica, otherwise the hybrid zone is likely to settle where environmental selection and mate competition are at equilibrium. Further work to determine if male F, hybrids are more likely to successfully reproduce with H. thoracica or H. crassidens females would be valuable, because a bias at this point could influence introgressive asymmetry.

Neither of the two methods employed here provided evidence of *Wolbachia* infection in *Hemideina*. The primer pairs that amplify DNA from the common *Wolbachia* supergroups that infect arthropods (Simoes et al. 2011), and the mapping software used with NGS were sensitive enough to detect infection by another bacterial parasite, so it is highly likely that these wētā did not contain *Wolbachia* although other New Zealand Orthoptera do (Bridgeman et al. 2018). A *Chlamydia*-like infection was detected in one of the wētā in this study however, and as this bacteria also functions as an intracellular parasite (Wyrick 2000), it may be of interest. Wētā make good candidates for sexually transmitted diseases, as they generally have some level of promiscuity and have overlapping adult generations (Knell and Webberley 2004).

Our sample of hybrid individuals was small, due to the low frequency of hybrids in the wild (Mckean et al. 2016), however, the results are biologically significant (i.e. F, males having at least some fertility, the infertility of female F, hybrids, thus being an exception to Haldane's Rule), and raise questions about future interaction and survival of these species. In summary, both male and female F, hybrids are capable of reaching maturity, and although Wolbachia is not involved in limiting hybridization, there is at least a 50% (probably higher) reduction in F, hybrid fertility due to female infertility, which might have a strong limiting effect on introgression in the wild. There appears to be a contrast between complete failure by female F, hybrids to produce eggs and partial fertility of some male hybrids, suggesting this system provides another exception to Haldane's rule that in interspecific hybrids the heterogametic sex (in this case males; XO) will have lower fertility than the homogametic sex (female Hemideina XX).

Tree wētā are an interesting group for evolutionary studies, in part because they appear to have a high tolerance for chromosome rearrangement that leads to many intraspecific hybrid zones. Much remains unknown about wētā biology, particularly with regard to species coexistence and production of hybrids where these wētā meet in sympatry lends an extra layer of complexity to the situation. Given that these species meet in different zones of sympatry across the country (and in different species combinations), there is the possibility that different mechanisms have, or will, evolve in different areas, which could be another promising area for further study.

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Appendix 1

Table 4. Information for wētā DNA amplification with Wolbachia-specific PCR primers.

X	6	Forward / Reverse	
Locus	source	Primers	
Wolbachia surface protein (wsp)	Braig et al. 1998	Wsp81F / Wsp691R	
Fructose-bisphosphate aldolase (fbpA)	Baldo et al. 2006	fbpAF1 / fbpAR1	
Cytochrome c oxidase, subunit I (coxA)	Baldo et al. 2006	CoxAF1 / CoxAR1	
Wolbachia specific portion of	II-11:	W-licer / W-licer	
16S ribosomal RNA gene (wol16S)	Heddi et al. 1999	woll68F / woll68K	

Revision of Aresceutica (Orthoptera: Acrididae: Catantopinae) with comments on related genera

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Abstract

The East African forest genus *Aresceutica* Karsch is revised; there are now four valid species, including *A. nguruensis*, **sp. n.** from south eastern Tanzania. *Aresceutica* is extremely similar to the West African genus *Serpusia*; it differs only in the form of the prosternal tubercle and the detail of the phallic morphology. Two new combinations are proposed: *Ptemoblax lemarineli* Bolívar, 1911 = *Aresceutica lemarineli* (Bolívar, 1911), **comb. n.** and *Aresceutica vansomereni* Kevan, 1956 = *Duviardia vansomereni* (Kevan 1956), **comb. n**.

Key words

East Africa, grasshopper, taxonomy

Introduction

The genera Aresceutica Karsch, 1896, Serpusia Karsch, 1891, Pseudophialosphera Dirsh, 1952, and Vesevacris Dirsh, 1959, are morphologically similar, flightless Catantopine grasshoppers, found in the leaf litter or on the understory herbaceous vegetation of wet forests in equatorial Africa. Their great external similarity led Johnston (1956) to group the first three of these genera (Veseyacris was described later), together with Segellia Karsch, 1891, Auloserpusia Rehn, 1914, Pteropera Karsch, 1891, Coenona Karsch, 1896 and Serpusilla Ramme, 1931 in a subtribe Serpusiae. Dirsh (1975) retained Johnston's (1956) subtribe, but transferred it to a new subfamily Apobolinae, which has not found favor with subsequent authors. Otte (1995) reduced Dirsh's (1975) Apobolinae to a tribe (Apoboleini Johnston, 1956) of the Catantopinae, but transferred all the genera of the Serpusiae to an uncertain position within the Catantopinae. The OSF (Cigliano et al. 2018) has, however, restored these genera to a "genus group" Serpusiae Johnston, 1956, thus reestablishing Johnston's (1956) original arrangement. The specific composition of these genera has been rather unstable, partly due to the difficulty of distinguishing between them. Thus, Ptemoblax

insidiosus Bolívar, 1908 was synonomized with *Serpusia succursor* Karsch, 1896 by Johnston (1956); *Ptemoblax lemarineli* Bolívar, 1911 was reassigned to *Serpusia* without explanation by Dirsh (1955).

While editing unpublished manuscripts by the late N.D. Jago, the present authors (C.H.F.R. and C.H.) came across the following statements: "Dirsh (1970) included Aresceutica lemarineli in the genus Serpusia s. lat. (however, examination of material from West Africa shows that, on the basis of male genital morphology, Serpusia should contain only S. opacula Karsch and S. succursor (Karsch) (W. Zaire to Cameroon and S.E. Nigeria). Moreover, Serpusia catamita Karsch (Togo, Ghana) and another new species from Guinea and Liberia should be removed from Serpusia and placed in a new genus). This makes the genus Aresceutica almost entirely East African, though A. lemarineli is also common in eastern Zaire. All genera and species in this group of closely related insects are reliably identifiable using male genitalia, other characters, e.g. black varnish on tegmina, green coloration, size, etc., being open to considerable variation. Note that a publishing error in Dirsh (1965) added to the confusion by interchanging the figures of Serpusia lemarineli with that of Aresceutica subnuda.'

Jago (1970) had earlier discussed the very close relationships between the genera of the Serpusiae, and even considered the possibility of synonomizing *Serpusia* in part with *Auloserpusia* and in part with *Aresceutica*.

In this paper we follow Jago's (1970) lead, and have revised the genus *Aresceutica*. We also compared the male internal genitalia of *lemarineli* with those of all known *Aresceutica* spp. and with those of *Serpusia opacula*, the type species of its genus, and of *S. succursor*. We conclude that *lemarineli* is not a *Serpusia*, but is indeed a member of *Aresceutica*, and formally transfer it to that genus. *Aresceutica vansomereni* Kevan, 1956, on the other hand, differs from the rest of the genus, and we here transfer it to *Duviardia* Donskoff, 1985, on the basis of its phallic structures. We further describe a new species of *Aresceutica* from the Nguru Mountains of Tanzania. We have not examined critically the other genera of the Serpusiae, nor other West African species currently ascribed to *Serpusia*.

Materials and methods

Aresceutica species were collected in Kenya and Tanzania by C.H. and by N.D.J., and Serpusia species (including S. lemarineli) by C.H.F.R. in Uganda and Cameroon. Further Cameroon material was kindly provided by C. Oumarou Ngoute. The provenance of the specimens used for phallic preparations is shown in Table 1.

Phallic preparations used standard methods: the dried pinned specimens were rehydrated in water before dissection, the extracted phallic complexes were macerated in 8% KOH and extraneous tissue removed, and then neutralized in 5% acetic acid, followed by staining in acid fuchsin. Drawings were made under 25× magnification with a Wild M5 microscope equipped with a drawing tube, digitized, and then refined in PHOTOSHOP C5 (Adobe). Measurements were taken using the stereo microscope with a graticule eyepiece and a digital stage (Mitutoyo) reading to 0.01 mm.

In all the figures of phalli in this article, the epi- and endophallic sclerites are shown in darker shading, the ectophallic sclerites in lighter shading. The ejaculatory and spermatophore sacs are indicated with coarse stipple, other membrane with light stipple.

Results

Aresceutica Karsch, 1896 Figs 1–6

Type species.—Aresceutica subnuda Karsch, 1896

Description.—(modified from Dirsh 1965) Male. Medium size. Cuticle finely or moderately roughened. Antennae slender, their length longer than, equal to, or shorter than length of head and pronotum combined. Fastigium of vertex short, flat or slightly concave, apex truncate. Frons slightly oblique; frontal ridge with straight sides along whole length, slightly produced forward between the antennae. Pronotum sub-cylindrical, disc crossed by transverse sulci 2-4, sometimes (*A. lemarineli*) weakly depressed just behind transverse sulcus 4; median carinula weak to fairly distinct; metazona much shorter than prozona. Anterior and posterior margins of pronotum straight or weakly convex, often slightly emarginate in midline. Prosternal tubercle subconical, antero-posteriorly compressed, tilting somewhat backwards. Tegmina lateral, strap-like, partially covering tympanum. Tympanum present. Legs slender; external apical spine of hind tibiae

Table 1. Provenances of specimens used for phallic preparations.

absent; arolium enlarged. Medial carina well marked in proximal 7 abdominal segments, obsolete distally. Male supra-anal plate elongate, angular. Cercus simple, acutely pointed. Subgenital plate short, subconical.

Female. Ovipositor long, with slender valves curved at apices.

Key to species in the genus Aresceutica (males)

- brownish near knee, apically orange-red, spines orange red, black tipped (N.E. TANZANIA, East and West Usambara Mts.; S.E. KENYA, Shimba Hills).....subnuda Karsch, 1896 Antennae longer than head and pronotum together. Pronotal lateral 2A. lobe black or dark brown, usually not polished. Light ventro-posterior patch sometimes present, variable in clarity and distinctness, extending from rear margin of lobe to third transverse sulcus. Tegminal apex rounded, extends barely into second abdominal tergite. Underside of thorax and abdomen dull ochreous yellow, usually suffused with crimson. Hind femur mottled dark brown, with no distinct light patches on outer face, lower outer and lower inner areas crimson; inner area mostly black, crimson ventrally and basally. Hind tibia brown by knee, distally crimson, spines colored correspondingly, black tipped (Eastern D.R. CONGO; N.W. RWANDA; Southern and Western UGANDA; Western KENYA, Kakamega forest).....
 -lemarineli (Bolívar, 1911), comb. n. Antennae same length as that of head and pronotum combined. Pronotal disc with pair of dorso-lateral, shallowly incurved, pale lines, which approach each other most closely at level of transverse sulcus 2. Tegminal tips rounded, just overlapping base of abdominal segment 2. Episternum T3 clearly marked in light ochre. Underside of

Genus	Species	Country	Locality	Collection date	Collector
	subnuda Karsch, 1891	Tanzania	Lutindi Mental Hospital	Jan. 2016	C. Hemp
	subnuda Karsch, 1891	Kenya	Shimba Hills Nat. Reserve, Makadara Picnic Site	Jun. 1981	M. Ritchie
	subnuda Karsch, 1891	Tanzania	Sigi, nr. Tanga	Dec. 1955	No data
	morogorica Dirsh, 1954	Tanzania	Forest above Morningside, Uluguru Mts.	Jan. 2016	C. Hemp
Arossoutica	morogorica Dirsh, 1954	Tanzania	Mangula Gate, Udzungwa Mountains	Jan. 2015	C. Hemp
Alesceutica	morogorica Dirsh, 1954	Tanzania	Bunduki Forest Reserve, Uluguru Mts.	Oct. 1964	N.D. Jago
	<i>nguruensis</i> sp. n.	Tanzania	Forest above Turiani, Nguru Mts.	Jan. 2016	C. Hemp
	<i>nguruensis</i> sp. n.	Tanzania	Forest above Turiani, Nguru Mts.	Nov. 1964	N.D. Jago
	lemarineli (Bolívar, 1911)	Uganda	Mabira Forest, Buganda	Mar. 2013	C.H.F. Rowell
	lemarineli (Bolívar, 1911)	Uganda	Mpanga Forest, Buganda	Mar. 1998	C.H.F. Rowell
Cantonala	opacula (Karsch, 1891)	Cameroon	Ongot, C. Cameroon	Month? 2014	C. Oumarou Ngoute
Serpusia	succursor (Karsch, 1896)	Cameroon	S. Bukundu Forest.	Oct. 1971	T.E. Rowell
5 . 1.	vansomereni (Kevan, 1956)	Kenya	Mt Kenya, montane forest	Mar. 2003	C. Hemp
Duviatala	vansomereni (Kevan, 1956)	Kenya	Karen	Oct. 1999	C. Hemp

3.

Females are best identified by their associated males.

1. Aresceutica morogorica Dirsh, 1954 Figs 2H–I, 3 urn:lsid:Orthoptera.speciesfile.org:TaxonName:56202

Recognition.-Male. Frons, clypeus, labrum, mouthparts, ochraceous yellow, speckled black dorsally. Fastigium, vertex, inter-ocular space, greyish-ochraceous with dark speckle. Genae ochraceous with dark speckle anteriorly, darkening rearwards to solid black at posterior margin. Disc of pronotum dark brown to black. A pair of ochraceous stripes run the length of the pronotal disc, most narrowly separated at level of second transverse sulcus, and continue rearwards on to the vannal area of the tegmina. Pronotal lateral lobes glossy black, with a posterio-ventral pale ochraceous patch tapering forward to end on prothoracic episternum. Meso- and metathoracic pleura black, metathoracic episternum striped with ochre. Tegmina black apart from the lighter vannal region mentioned above. Abdominal tergites in midline ochraceous with black speckle, laterally black. Pro- and mesothoric legs mottled olive green and ochre. Hind femur: dorsal inner and outer faces ochraceous brown. Outer area suffused blackish, with black chevron markings. Ventral margin of outer area bears two ochraceous patches, the basal patch being twice the length of the distal patch; in some individuals there is a third patch on the dorsal margin of the outer area. Ventral inner and outer faces of femur crimson. Internal face black. Hind knees brown. Hind tibia brown proximally, shading to crimson distally. Spines brown with black tips.

Female. Similar to male but larger, darker and generally more dully colored. Ochraceous areas of male are light brown in female. Pronotal disc is devoid of light stripes. Tegmina often bear one or two shiny black patches.

Note: The color pattern of *A. morogorica* is very similar to that of *Veseyacris*.

Measurements. —

Size (mm)	Males $(N = 8)$	Females $(N = 8)$
Posterior femur length	12.12-12.48	15.4-17.71
Posterior femur depth	2.93-3.07	3.98-4.25
Total length*	18.5-22.53	25.1-32.9

(*frons to tip of abdomen)

Distribution.—TANZANIA: Uluguru Mts, above Morogoro, and Udzungwa Mountains National Park.

2. Aresceutica subnuda Karsch, 1896

Figs 2J–K, 4

urn:lsid:Orthoptera.speciesfile.org:TaxonName:56198

Description.—Male. Frons, clypeus and labrum, light ochre brown with darker speckles. Pronotal disc dark brown, pitted and roughened overall. A pair of irregular orange brown stripes run along lateral margins of pronotal disc and continue onto anal area of tegmina. Rear margin of pronotal metazona straight or slightly convex, with a minute emargination in the midline. Side of pronotum polished dark-brown to black; light ventro-lateral patch tapers forward to end on prothoracic episternum. Metathoracic episternum striped in lighter ochraceous color. Tips of tegmina reach to posterior margin of tympanum or to midpoint of abdominal segment 2; black varnished regions common on tegmina in front of R1/Sc veins and in R /M areas near tip. Underside of thorax and abdomen dull ochraceous yellow, with no red suffusion.

Two clear pale creamy spots on lower part of outer area of hind femur; basal one four times longer than deep. Lower inner and lower outer areas of hind femur dull dark crimson. Inner area of hind femur black. Hind tibiae dull brownish near knee, apically orange-red, spines orange red, black tipped.

Measurements.-

Size (mm)	Males $(N = 6)$	Females $(N = 6)$
Posterior femur length	11.46-13.2	16.81-19.2
Posterior femur depth	2.42-3.2	3.8-4.3
Total length*	18.65-19.80	26.6-31.13

(*frons to tip of abdomen)

Distribution.—TANZANIA: West and East Usambara Mts., Tanga region. KENYA: Shimba Hills.

3. Aresceutica nguruensis sp. n.

Figs 1E-G, 5

http://zoobank.org/5AA3AEAC-68F1-4278-9111-DAFC4395FDE0 urn:lsid:Orthoptera.speciesfile.org:TaxonName:502927

Holotype male.—TANZANIA, Nguru Mts, forest above Turiani, Mhonda. ii. 2017 (C. Hemp). NHML.

Allotype female.—Same data as holotype.

Paratypes.—TANZANIA, Nguru Mts., forest above Turiani, (5-7) xi. 1964, (N.D. Jago). 1 male (NHML). TANZANIA 2 males, 4 females, same data as holotype but March (2 females), June (1 male, 2 females) and November 2017 (1 male) (Hemp coll. 2 males, 5 females, TANZANIA, same data as holotype but January, 2018 (Hemp coll.).

Description.—Male. (See key). Antennae basally brown, distally black, longer than head and pronotum combined. Head and mouthparts generally ochraceous yellow with sparse black speckle, but genae suffused with black. Pronotal disc finely and densely pitted but not matte, uniform brown with no paler longitudinal stripes. Rear margin of metanotum almost straight, hardly at all emarginate in midline. Pronotal lateral lobe polished dark brown, almost black; posterio-ventral corner occupied by a triangular wedge of light brown which tapers forward to end on the prothoracic episternum. Meso and metathoracic pleura dark brown with irregular black markings; metathoracic episternum with a weak lighter stripe. Tegmen dark brown, polished black along the costal margin, often also on medio-medial area. Abdominal tergites ochraceous dorsally, black laterally. Cerci black. Hind femur as in *A. morogorica*, but pale patches on outer area are deeper, extending upwards to midline of femur. Hind tibiae and tarsi red. Fore and middle femora ochraceous, often touched with green apically.

Aresceutica nguruensis is very similar to A. morogorica, differing only in details of coloration, in having longer antennae, and being slightly larger.

Measurements.-

Size (mm)	Males $(N = 3)$	Females $(N = 5)$
Posterior femur length	13.5-14.29	19.0-20.09
Posterior femur depth	3.21-3.24	4.45-4.63
Total length*	20.9-23.02	27.7-33.02

(*frons to tip of abdomen)

Distribution.—TANZANIA: Nguru Mts., above Turiani.

4. Aresceutica lemarineli (Bolívar, 1911), comb. n. Figs 1A–D, 6 urn:lsid:Orthoptera.speciesfile.org:TaxonName:502928

Synonomy: *Ptemoblax lemarineli* Bolívar, 1911: 298 (holotype female, Zaire [= Democratic Republic of the Congo]; in MRAC Tenvuren KMMA).

Serpusia lemarineli (Bolívar, 1911): Dirsh 1955; Johnston 1956. Ptemoblax vicinus Rehn, 1914: 137 (holotype – female, Zaire, Semliki Valley; in MfN). Synonymized with Serpusia lemarineli by Johnston (1956).

Description.-Male. (See key). Antennae basally yellow-brown, distally black, longer than head and pronotum together. Head and mouthparts generally ochraceous yellow with dense black speckle, but genae suffused with black. Pronotal disc densely pittted, uniform brown, usually with no paler longitudinal marks. Medial carina of pronotum relatively well developed, disc surface behind fourth sulcus clearly depressed. Rear margin of metanotum weakly convex, slightly emarginate in midline. Pronotal lateral lobe dark blackish brown; posterio-ventral corner occupied by a triangular wedge of lighter color which extends forward along ventral margin to level of third sulcus. Meso- and metathoracic pleura dark brown, with a variable degree of lighter color striping the episterna and epimera. Tegmen dark brown, polished black along the costal margin. Tip of tegmen rounded or rounded acute, variable between individuals; extends just into second abdominal tergite, partially covering tympanum. Abdominal tergites mainly ochraceous dorsally, with black pigment laterally only in segments A1-4. Underside of abdomen and thorax ochraceous, usually heavily suffused with red. Cerci suffused black. Hind femur brown with blackish green suffusion and no pale patches on outer area. Internal ventral area of hind femur red. Internal face of hind femur red basally, black distally. Hind tibia brown proximally, red distally. First tarsal segment red dorsally, other tarsal segments brown. Fore and middle femora ochraceous, often touched with green apically.

Female. Similar to male, but larger and darker in coloration.

Measurements.-

Size (mm)	Males (N = 2)	Females $(N = 1)$
Posterior femur length	13.3,12.25	16.69
Posterior femur depth	3.08, 2.98	3.9
Total length*	21.01, 19.21	28.62

(*frons to tip of abdomen)

Distribution.—Eastern D.R. CONGO; N.W. RWANDA; Western and Southern UGANDA; Western KENYA (Kakamega Forest only).

Phallic structures in Aresceutica (Figs 3-6)

Aresceutica males have a highly characteristic phallus, with little variation between the species. It seems unlikely that the species could be distinguished on the basis of phallic characters alone, though a larger sample size might disclose some specific regularities. The phallus is short, compact and robust, with several unusual features. A glance at these figures will convince that the phallus of *lemarineli* does not differ significantly from those of the previously recognized *Aresceutica* species.

Epiphallus (Figs 3A–C, 4A, 5A–C, 6A–B).—The epiphallus is a narrow bridge connecting two lateral plates which bear small hooked ancorae on their anterior margins, and broad, rounded, widely separated, lobe-like lophi on their posterior margins. Lateral oval sclerites are present, roughly triangular in shape in most species, though oblong and strap-like in *A. lemarineli*. The epiphallic membrane runs backwards from the epiphallus to anchor on the zygoma and the edges of the ectophallic rami; there are no post-epiphallic sclerites, but in some individuals there is a weak oblong thickening of the membrane ventrolaterally, anterior to the the cingular rami.

Ectophallus (Figs 3D-E, 4B-C, 5D-E, 6C-D).—The cingulum has a well-sclerotised zygoma, which bears short, curved cingular apodemes, giving a horseshoe shaped appearance in dorsal view (these are distinctly different from the longer, more orthogonally arranged apodemes of Serpusia - see below). The posterior margin of the zygoma forms a projecting ridge, seen as a short beaked projection in lateral view. The ectophallic rami are well developed, roughly oblong in lateral view; they extend to the ventral midline of the phallus, and meet but do not fuse at their ventral extremities. From their posterior margins an ectophallic sheath arises, forming two closely juxtaposed structures: a) a thick semitransparent membrane that covers the aedeagal valves ventro-laterally as they emerge from between the rami, and b) an opaque reinforced ectophallic sheath which envelops the tips of the aedeagal valves. The arch of the cingulum is well developed and forms a saddlelike sclerite on the posterior end of the spermatophore sac below the zygoma. It is rigidly connected to the inner surface of the zygoma by two oblique columns, which have to be cut through to extract the endophallus from the cingulum. Ventrolaterally, there are also connections between the arch and the inner surfaces of the rami. The cingular valves are short and deep, and at their extremities are fused with the inner surface of the ectophallic aedeagal sheath into which they protrude. The anterior margins of the rami bear a fringe of filamentous chitinous material; this fringing material runs from the ventral margin of the ramus upwards along its anterior margin to extend along the ventral edge of the cingular apodemes. A similar fringe is present on the dorsal edge of the proximal endophallic sclerites, and seems to be characteristic of the genus; we do not recall seeing it in other genera.

Endophallus (Figs 3F, 4D–F, 5F–G, 6E–F).—The anterior endophallic apodemes are short and concave, widely separated and arranged at nearly 90° to the long axis of the phallus. The gonopore processes are long and thin, and run backwards to end nearly halfway to the flexure. The ejaculatory sac is large and capacious, roughly spherical. The dorsal surface of the basal endophallic sclerites is



Fig. 1. A–D. Aresceutica lemarineli comb. n.: A. Male; B. Female, lateral; C. Female, dorsal, alive; D. Female, ventral view, to show characteristic red suffusion; E–G. Aresceutica nguruensis, sp. n.: E. Male holotype, lateral; F. Male paratype, lateral; G. Female paratype, alive.

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Fig. 2. H–I. Aresceutica morogorica: H. Male, alive; I. Female, alive; J–K. Aresceutica subnuda: J. Male alive; K. Male and female, in cop., alive; L–M. Duviardia vansomereni: L. Male alive; M. Male and female, in cop., alive.

"ornamented" with a chitinous fringe, as mentioned in the preceding paragraph. The flexure is fine and flexible, S-shaped in lateral view, and connects to the endophallic valves, which are simple slender curved sclerites running beneath the arch and inserting into the ectophallic aedeagal sheath. Like the cingular valves, their tips do not lie free, but instead are fused to the inner wall of the aedeagal sheath. In this genus it seems that the functional aedea-

gal valves are composed primarily of ectophallic sheath material, rather than of the actual endo- and ectophallic sclerites, which are incorporated into the lateral walls of the sheath.

The complexity of the phallic anatomy within the Serpusiae, and especially of the aedeagus, was previously examined by Jago (1970), in his study of the genus *Auloserpusia*. To elucidate the aedeagal structure he was forced to use the technique of serial microscope sections,



Fig. 3. *Aresceutica morogorica*, phallus. **A.** Epiphallus, axial view; **B.** Epiphallus, and oval sclerites, dorsal view; **C.** Epiphallus, lateral view; **D.** Phallic complex after removal of epiphallus, lateral view. The arrow indicates the cut edge of the epiphallic membrane, where the epiphallus was removed; the remaining epiphallic membrane has dropped down behind the zygoma. In the other figures of this series (i.e. Figs 4–6) this membrane has been dissected off; **E.** As in D, but dorsal view; **F.** Endophallus and arch. The region where the connection between arch and zygoma has been cut is arrowed. The lighter shading indicates the ectophallic aedeagal sheath.



Fig. 4. *Aresceutica subnuda*, phallus. **A.** Epiphallus and oval sclerites, axial view; **B.** Phallic complex after removal of epiphallus and epiphallic membrane, lateral view. x: cut edge of epiphallic membrane. y: aedeagal sheath. z: chitinous fringe to ectophallic ramus and basal endophallic sclerites; **C.** As in B, but dorsal view; **D.** Endophallus and arch of cingulum, lateral view. w: cut attachment of arch to zygoma; **E.** As D, but dorsal view; **F.** As D, but ventral view.



Fig. 5. Aresceutica nguruensis sp. n., phallus. A. Epiphallus, axial view; B. Epiphallus and oval sclerites, dorsal view; C. Epiphallus, lateral view; D. Phallic complex after removal of epiphallus and epiphallic membrane, lateral view; E. As in D, but dorsal view; F. Endophallus and arch, lateral view; G. As in F, but dorsal view.



Fig. 6. Aresceutica lemarineli, comb. n., phallus. A. Epiphallus, axial view; B. Epiphallus, dorsal view; C. Phallic complex in lateral view, with epiphallus and epiphallic membrane removed; D. As in C, but dorsal view; E. Endophallus and arch, lateral view; F. As in E, but course of dorsal and ventral aedeagal valves inside the aedeagal sheath indicated.

and presented serial transverse sections of the aedeagal region of *Auloserpusia poecila* Jago, 1970 and remarked on its complexity. He wrote "Penis apex therefore consists of a triple sheath: a core of median penis valve and cingular valve, surrounded in turn by sheath derived from ventral side of penis valves and an outer double membranous part attached to posterior side of cingular arch. Penis valves often attached by cuticular material to the outer sheath".

His diagrams support our conclusions in the above description, including the fusion of the aedeagal sclerites with the surrounding ectophallic sheath, and the connections between the arch and the cingular rami. He considered some elements of the aedeagal sheath to be derived from lateral lobes of the arch. We were unable to confirm this with simple dissection techniques.

Comparison with Serpusia spp. (Figs 7-8)

The genera *Serpusia* and *Aresceutica* are essentially indistinguishable in their outer morphology. Jago (1970) wrote of them "both the above genera must be defined together" and gave only a single morphological description covering both genera. We find that only the prosternal tubercle separates the two genera reliably. In both species of *Serpusia* this is short, acutely conical and vertical, whereas in all species of *Aresceutica* it is longer, subconical, antero-posteriorly compressed, and tilted somewhat backwards. We therefore examined the phallic anatomy to see whether these are more discriminatory.

The phallic complexes of *S. opacula* Karsch, 1891 (type species of the genus) and of *S. succursor* Karsch, 1896 are shown in Figs 7, 8. These are the two species said by Jago (1970) to be "true" *Serpusia* (see Introduction), and they have very similar phalli, close to those of *Aresceutica*, but differing from the latter in a number of details:

- and presented serial transverse sections of the aedeagal region of 1. The complex as a whole is more elongate and less robust than *Auloserpusia poecila* Jago, 1970 and remarked on its complexity. He that of *Aresceutica*.
 - 2. The endophallic apodemes are more deeply cupped, and from their ventral margins two channels with a U-shaped cross section run ventrally and posteriorly to form the gonopore processes. The gonopore processes of *Aresceutica* lack this channeled profile, and are longer and more slender, extending further rearwards. As a consequence, the ejaculatory sac, which is bounded by the gonopore processes, is situated more anteriorly in *Serpusia* than in *Aresceutica*, where it is distinctly ventral in position under the basal endophallus.
 - 3. The flexure of the endophallus is very slender in *Aresceutica*, but in *Serpusia* it is thread-like and quite difficult to see at all if the preparation is not stained to visualise the sclerifications.
 - 4. The cingular apodemes of *Serpusia* are long and more or less straight, running roughly parallel to each other distally. They are not curved to form a horseshoe-like profile in dorsal view as in *Aresceutica* (compare Fig. 8C with Fig. 4C or Fig. 5E).
 - 5. The anterior margin of the cingular arch under the zygoma is broadly emarginate in the midline in *Serpusia*, whereas that of *Aresceutica* is straight.
 - 6. The cingular valves are slender and pointed, not short and laterally flattened as in *Aresceutica*. They run along the upper margin of the endophallic valves, but end at the level where the latter enter the aedeagal sheath.
 - 7. The endophallic valves are long and slender, wide and dorsoventrally flattened basally, but laterally compressed and narrow apically. They extend almost to the tip of the ectophallic aedeagal sheath, and are the major component of the aedeagus.



Fig. 7. *Serpusia opacula*, phallus. **A**. Epiphallus, axial view; **B**. Epiphallus, dorsal view; **C**. Phallic complex after removal of epiphallus and epiphallic membrane, lateral view; **D**. As in C, but dorsal view. In this preparation the cingular apodemes are broken off; intact, they resemble those of *S. succursor* (Fig. 8), being long and straight and almost parallel; **E**. Endophallus and arch, lateral view; **F**. As in E, but dorsal view. In E and F, the arrows indicate cut margins of the arch where it has been freed from the inner surface of the zygoma.



Fig. 8. *Serpusia succursor*, phallus. **A.** Epiphallus, axial view; **B.** Epiphallus, dorsal view. **C.** Phallic complex after removal of epiphallus and epiphallic membrane, dorsal view. The two cross hatched areas are the zones of attachment of the the arch to the inner surface of the zygoma; **D.** As in C, but lateral view; **E.** Endophallus and arch in lateral view; **F.** As in E but dorsal view. The arrows indicate cut margins of the arch where it has been freed from the inner surface of the zygoma; **G.** As F, but ventral view. In E, F and G the ejaculatory sac has been removed to show the detail of the gonopore processes.

- 8. A complex ectophallic aedeagal sheath envelops the aedeagal valves as in *Aresceutica*, but is much less robust and is closely appressed to the endophallic valves, to which it is attached but not so extensively fused as in *Aresceutica*. In the intact phallus the projecting aedeagus of *Serpusia* therefore looks much more slender than that of *Aresceutica*, due to the wider ectophallic sheath of the latter.
- 9. The epiphalli of the two genera are similar, but the ancorae of *Serpusia* are stronger and are slanted inwards towards the mid-line, whereas those of *Aresceutica* point directly forwards (compare Figs 6B and 3B with Figs 7B and 8B).

The systematic position of *Duviardia vansomereni* (Kevan, 1956), comb. n. (Figs 2L-M, 9)

Kevan (1956:20) described *Aresceutica vansomereni* from the Aberdare Mts. of Kenya (holotype male, Kenya, Aberdare Mts., Katamayu; in BMNH London). This species differs from the other members of *Aresceutica* in several external features, as was noted by Kevan in his description; he wrote "it differs in its stouter appearance, less strongly compressed and differently shaped prosternal tubercle, in the shape of the tegmina and in the coloration of the male".

Examination of the phallus (Fig. 9) shows that it differs from those of *Aresceutica* species, but is similar to that described by Donskoff (1985) for *Duviardia oubitai*. This resemblance had been previously noted by Jago (unpublished MS). The external morphology

of *vansomereni* fits the generic diagnosis of *Duviardia* given by Donskoff (1985) very well, allowing for the fact that the latter was based on only a single species (*D. oubitai*). The supranal plate is not trilobed at its extremity, but this feature is only very weakly seen even in the type species *oubitai*, as shown in Donskoff's (1985) figure, and is absent in *D. fishpooli* Grunshaw, 1990. This character, together with the midline indentations of the anterior and posterior pronotal margins described by Donskoff (1985), seem to be specific to *D. oubitai*, and not properly part of the diagnosis of the genus.

The phallus of *Duviardia vansomereni* is closely similar to those of *D. fishpooli* and *D. oubitai*, and it differs from that of *Aresceutica* in several ways:

- 1. The aedeagus consists of two pairs of valves (ectophallic and endophallic), both of which are well separated and not fused to an ectophallic sheath at their tips;
- 2. The arch sclerite is connected to the inner surface of the zyoma by a single medial sclerification;
- 3. The gonopore processes are very short, causing them to be "éloigné de la flexure", as described by Donskoff (1985).
- 4. The flexure of the endophallus is wide and robust, not thread-like.
- 5. The epiphallus is exactly like that figured for the other two species, and has considerably more robust ancorae than seen in *Aresceutica*.

On the basis of these observations, we formally transfer *van-somereni* from *Aresceutica* to *Duviardia*.



Fig. 9. *Duviardia vansomereni* (comb. n.), phallus. **A.** Entire phallic complex, lateral view. Fine stippling indicates remaining portions of the epiphallic membrane; **B.** As in A, but dorsal view. The epiphallus is twisted out of its normal position in this preparation; **C.** As in A, but entire epiphallic layer removed; **D.** As in C, but after removal of ectophallic cingulum and rami, showing endophallus and ectophallic arch. The arrow indicates the cut edge of the arch sclerite. **E**, **F**, **G**. Epiphallus in axial, dorsal and lateral views.

Discussion

Summarizing, comparison of the two genera *Aresceutica* and *Serpusia* indicates that they are very closely related, but distinct; they can be separated by the form of the prosternal tubercle externally and by the detail of the phallus internally. Biogeographically, the two genera are also distinct. *Aresceutica* currently contains four species and is confined to East Africa, with *A. lemarineli* extending into the Eastern Congo. *Serpusia* s. str. occurs in the Western Congo, Cameroon and Eastern Nigeria, and similar genera are found still further West into Guinea and Ghana.

1 mm

Duviardia now contains three described species, and is known to extend from Ivory Coast to Kenya and Tanzania, though not yet recorded from all the intervening countries; Jago (op. cit.) however considered it likely that several other known equatorial taxa are in fact members of this relatively new genus too.

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Preliminary study on the diversity of Orthoptera from Kuala Belalong Field Studies Centre, Brunei Darussalam, Borneo

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Abstract

The Orthoptera, comprising grasshoppers, crickets, and katydids, is diverse and species rich in tropical Southeast Asia, including the island of Borneo. However, not every part of Southeast Asia is equally well sampled and studied. This includes Brunei Darussalam, specifically at the Kuala Belalong Field Studies Centre (KBFSC) within the Ulu Temburong National Park. We present here an annotated and illustrated checklist of Orthoptera from the primary dipterocarp forest around Kuala Belalong based on three field trips in 2016 and 2017. We provide notes on their taxonomy (including how each species was identified) and natural history of species. In total, 72 species were recorded, representing eight of the 16 monophyletic orthopteran superfamilies. In total, 73.6% of all species recorded were singletons and doubletons, indicating that many species are probably rare. The collection led to the discovery of ten species new to science already published separately, with more expected to be described from pending material and confirmation. More species, including undescribed ones (at least four new species), are expected with continued sampling effort. Despite the 21 day-long surveying efforts from three trips around KBFSC, we believe that the species list provided here is non-exhaustive and only a preliminary one.

Key words

checklist, diversity, natural history, Southeast Asia, species richness, taxonomy

Introduction

Orthoptera is an order of insects consisting of grasshoppers (suborder Caelifera), and crickets and katydids (suborder Ensifera). These are among the most diverse (around 28,000 species worldwide) and common terrestrial macro-invertebrates (Cigliano et al. 2018). There are about 2,000 species from Southeast Asia (Tan et al. 2017a). While some species are notoriously considered as agricultural pests (Willemse 2001), these species represent a small proportion of the overall diversity of orthopterans. In fact, far more species could be found hiding in the pristine forests from Southeast Asia.

Southeast Asia is made up of numerous biodiversity hotspots (Myers et al. 2000), yet, the knowledge of orthopteran diversity in this region is not completely understood, with some regions less surveyed than others (Tan et al. 2017a). So, although species new to science are continuously being described from this region, even more can be expected with further intensive surveys into unchartered parts of Southeast Asia.

On the island of Borneo, pockets of forests remained unstudied for orthopterans. This includes Brunei Darussalam, particularly in the Temburong District (Fig. 1). Within the primary dipterocarp forest of Ulu Temburong National Park lies the Kuala Belalong Field Studies Centre (KBFSC) (Fig. 2A). KBFSC, located along Sungai (= river in Malay) Belalong (Fig. 2B), is under the charge of Universiti (= University, in Malay) Brunei Darussalam. Sungai Belalong forms part of the Sungai Temburong drainage basin which is 1,100 km² and is the third largest catchment in Brunei Darussalam. While a huge range of biodiversity surveys were conducted in the field studies center since its opening in 1990, orthopteran diversity around KBFSC remained understudied and overlooked. There was no concerted effort to sample and examine the orthopterans there.

In 2016 and 2017, surveys were conducted around the forests of Kuala Belalong with the objectives of discovering species new to science, collecting new material for taxonomic treatments, and making revisions of poorly known or problematic taxa, as well as to present an annotated checklist of Orthoptera from KBFSC. While new species discovery and taxonomic treatments were published separately in taxonomic journals (Tan and Wahab 2017a, b, Tan et al. 2017c, d), we present here the first annotated and illustrated checklist of Orthoptera from KBFSC based on the collections made in 2016 and 2017. We provide references and notes on the taxonomy (including how each species was identified) and natural history of each species, whenever possible. While some of the species could not be promptly identified owing to the insufficient material, we aim to use this publication as a baseline for future work on the taxonomy of Orthoptera from Brunei Darussalam and Southeast Asia.

Materials and methods

Surveys were conducted by the first author (MKT) in the primary lowland and ridge dipterocarp forests (Fig. 2C) surrounding the KBFSC, Ulu Temburong National Park, Brunei Darussalam (Fig. 1) in September 2016, January 2017, and July 2017. Two main sites were surveyed: Ashton Trail (including the route to Sungai Mata Ikan) located behind the field studies center (Fig. 2D), as well as slightly downstream where the Ulu Ulu Resort and Canopy Tower of the Ulu Temburong National Park are located (Fig. 2E). Opportunistic collecting and sweep-netting were conducted during daytime (0830 to 1500 hours) and/or during night time (1930 to 2300 hours) (Fig. 2F). Light-trapping was also conducted by switching on the corridor lights on the laboratory building facing Sungai Belalong. Whenever possible, acoustic recordings of calling songs were done using the video-recording function of a Canon EOS 500D digital SLR camera or with a digital sound recorder (Olympus WS-750M, frequency response 40–21,000Hz). The specimens were preserved in absolute analytic-grade ethanol and later pinned and dry-preserved. A single hind leg was preserved in absolute analytic-grade ethanol for future molecular work.

Habitus images were made with a Canon EOS 500D digital SLR camera with a compact-macro lens EF 100mm f/2.8 Macro USM. Close-up images of morphological features (including male genitalia) were done using a Canon EOS 50D digital SLR camera with a macro photo lens MP-E 65mm f/2.8 USM (1-5x). Canon Macro Twin Lite MT-24EX and Canon Macro Ring Lite MR-14EX were used for lighting and flash. Image editing was accomplished using Adobe Photoshop CC 2014. Scales were given with the images. Acoustic analysis was done using the open source R package WarbleR (Araya-Salas and Smith-Vidaurre 2017) or Sound Ruler software.

Specimens were identified by the authors using relevant, published keys and descriptions (see remarks for each species) and by comparisons of voucher specimens and type specimens (sometimes only images available). Where doubtful, the identities of the orthopteran specimens were verified by orthopterists: Andrej V. Gorochov (for Grylloidea), Sigfrid Ingrisch (for Tettigonioidea), Tony Robillard (for Eneopterinae), Luc Willemse (for Acridoidea), Josip Skejo (for Tetrigoidea), Josef Tumbrinck (for Tetrigoidea), Sergey Storozhenko (for Tetrigoidea), and Xingbao Jin (for Tettigonioidea).



Fig. 1. Map of the island of Borneo with the dot indicating the location of KBFSC in Brunei Darussalam.



Fig. 2. Environment of KBFSC and surrounding habitats: A. View of the KBFSC from Sungai Belalong; B. View of Sungai Belalong from KBFSC; C. Afternoon view of the canopy on the ridge along Ashton Trail; D. Sungai Mata Ikan, a small forest stream near KBFSC; E. Morning view of the canopy at Ulu Temburong National Park; F. Dusk time at the Canopy Tower.

Classification and nomenclature of species were based on the Results and discussion Orthoptera Species File (OSF) Online Version 5.0/5.0 (Cigliano et al. 2018). The families, subfamilies, and genera are arranged alphabetically for ease of reference. All specimens collected will eventually be deposited in Institute for Biodiversity and Environmental Research (IBER), Universiti Brunei Darussalam and Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum (former Raffles Museum of Biodiversity Research), National University of Singapore.

Overall, 72 species representing nine families were collected between 2016 and 2017 (Table 1). The species from KBFSC represent eight of the sixteen monophyletic orthopteran superfamilies (sensu Song et al. 2015), thus covering 50% of the lineages in the orthopteran phylogeny. As expected, the monophyletic suborder Ensifera is represented with more species (53 species, 73.6%) than monophyletic suborder Caelifera (19 species, 26.4%). The

	Ranking	Nomenclature	Species Richness
1.	Order	Orthoptera	
1.1.	Suborder	Caelifera	
1.1.1.	Superfamily	Acridoidea	5
1.1.1.1.	Family	Acrididae	
1.1.2.	Superfamily	Tetrigoidea	13
1.1.2.1.	Family	Tetrigidae	
1.1.3.	Superfamily	Trigonopterygoidea	1
1.1.3.1.	Family	Trigonopterygidae	
.2.	Suborder	Ensifera	
.2.1.	Superfamily	Grylloidea	21
.2.1.1.	Family	Gryllidae	
.2.1.2.	Family	Mogoplistidae	
.2.2.	Superfamily	Gryllotalpoidea	1
.2.2.1.	Family	Gryllotalpidae	
1.2.3.	Superfamily	Rhaphidophoroidea	1
1.2.3.1.	Family	Rhaphidophoridae	
1.2.4.	Superfamily	Stenopelmatoidea	5
1.2.4.1.	Family	Gryllacrididae	
1.2.5.	Superfamily	Tettigonioidea	25
1.2.5.1.	Family	Tettigoniidae	

Table 1. A summary of the coverage of orthopteran superfamilies and families that were represented in the collection in KBFSC.

most speciose groups are the Tettigonioidea (34.7%), followed by Grylloidea (29.2%), and Tetrigoidea (18.1%). On the other hand, Trigonopterygoidea and Rhaphidophoroidea are only represented by a single species each. So far, ten species new to science had been described based on material from the collection. From the collection, 38 species (52.8%) recorded were singletons, whereas 15 species (20.8%) recorded were doubletons. This represents 73.6% of all species recorded, indicating that many species in the forest are likely to be rare and difficult to collect. More species, including undescribed ones (at least four new species), are expected with continued sampling effort. Despite the 21 day-long surveying efforts from three trips, we believe that the species list provided here is non-exhaustive and only a preliminary one.

Checklist

Order Orthoptera Suborder Caelifera Superfamily Acridoidea Family Acrididae

1. Craneopsis sp. Fig. 3

Remarks.—We tentatively identified this species to be close to *C. olivacea* Ramme, 1941. Both adults and nymphs of the species were found on *Citrus limon* at KBFSC. The grasshoppers feed on the leaves, and appear to prefer the older leaves to the younger ones. We identified our species based on the key to species by Willemse (1956) as well as images of type specimens (courtesy of L. Willemse). To date, this genus is endemic to Borneo. The male phallus was previously unexamined. Virtually nothing is known about the life history of these elusive grasshoppers.

2. *Perakia* sp. Fig. 4A

Remarks.—We tentatively identified this species to be near to *P. borneensis* Willemse, 1936. This cryptic grasshopper was found on tree branches in the dipterocarp forest. The brown and elongated habitus of the grasshopper enables it to camouflage on branches. Interestingly, they were only encountered after the rain, perhaps suggesting that it is a canopy species, brought down to the understory by the rainfall. We identified our species based on the generic key by Willemse (1956). We only have females and can only identify the species name tentatively. Identification was based mainly on color patterns.

3. Traulacris sp. Fig. 4B

Remarks.—A female specimen was identified using the generic key by Willemse (1956). The species is very similar to *Meltripata* Bolívar, 1923 but differs by: head distinctly elevated above level of pronotum and frontal ridge barely projecting, and female subgenital plate quadrate with a mucronate projection on apical margin. Only two species are currently known from this Bornean-endemic genus, but our specimen differs in coloration from both species, particularly in the hind femur and general body coloration, as well as tegmen length (Willemse 1957). Our specimen may represent an undescribed species, but more material is needed for confirmation.

4. Traulia azureipennis (Serville, 1838)

Remarks.—This species was found near the forest edge. It was identified based on the description of the color in Willemse (1957) and comparison with type images from OSF (Cigliano et al. 2018).

5. Traulia borneensis Willemse, 1921 Fig. 4C

Remarks.—The two *Traulia* species were found co-occurring in similar habitat, although this species can also be found in more forested areas of the Canopy Walk. This species was identified based on the description of the color in Willemse (1957) and comparison with type images from OSF (Cigliano et al. 2018).

Superfamily Tetrigoidea Family Tetrigidae Subfamily Cladonotinae

1. Boczkitettix borneensis (Günther, 1935) Fig. 5A

Remarks.—This species was fairly common in the forest floor of Kuala Belalong, usually camouflaging among the leaf litter or forest trails. We used the key by Tumbrinck (2014) for identification and the material was also verified by J. Tumbrinck and J. Skejo.

2. Epitettix punctatus Hancock, 1907 Fig. 5B, C

Remarks.—This species was fairly common in the forest floor of Kuala Belalong, usually camouflaging among the leaf litter or forest trails. We used the key by Tumbrinck (2014) to identify to genus. Subsequently, type images and description were used for comparison. Identification was also verified by J. Tumbrinck and J. Skejo.

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Fig. 3. Acrididae: *Craneopsis* cf. *olivacea*: A. Male adult; B. Nymph; C, D. Female adult; E. Male genitalia in dorsal (top and bottom left inset), ventral (bottom right inset) views.

3. Potua morbillosa (Walker, 1871) Fig. 5D, E

Remarks.—We found this pygmy grasshopper on dead logs and tree trunks along Ashton Trail. We used the key by Tumbrinck (2014) to identify to genus. Subsequently, type images, and description were used for comparison. Identification was also verified by J. Tumbrinck and J. Skejo.

Subfamily Discotettiginae

4. Discotettix belzebuth (Serville, 1838) Fig. 5F

Remarks.—This species was found on the wooden planks and ground along the trail to the Canopy Tower. We compared our specimens with type images from OSF (Cigliano et al. 2018). Identification was also verified by J. Skejo.

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Fig. 4. Acrididae: A. Perakia nr. borneensis; B. Traulacris sp.; C. Traulia borneensis.



Fig. 5. Tetrigidae: A. Boczkitettix borneensis; B, C. Epitettix punctatus; D, E. Potua morbillosa; F. Discotettix belzebuth; G, H. Lamellitettigodes contractus; I, J. Pseudoparatettix lineatus. Scale bars: 1 mm.

Subfamily Metrodorinae

5. Lamellitettigodes contractus (Bolívar, 1887) Fig. 5G, H

Remarks.—A single specimen (a female) collected from the boardwalk en route to the Canopy Tower represents the first record of this species in Brunei Darussalam and Borneo. This species was identified by J. Tumbrinck.

6. Pseudoparatettix lineatus (Hancock, 1907) Fig. 5I, J

Remarks.—Unlike most ground-dwelling pygmy grasshoppers, this species was found on foliage of understory plants. This species was identified by J. Tumbrinck.

Subfamily Scelimeninae

7. Falconius clavatus Bolívar, 1898 Fig. 6A, B

Remarks.—These pygmy grasshoppers were collected near the sandy bank of the Sungai Belalong at KBFSC. We compared our specimens with type images from OSF (Cigliano et al. 2018). Identification was also verified by J. Tumbrinck and J. Skejo.

8. Falconius deceptor Günther, 1938 Fig. 6C

Remarks.—A single female specimen was found attracted to light. We compared our specimen with type images from OSF (Cigliano et al. 2018). Identification was also verified by J. Tumbrinck and J. Skejo.

9. Loxilobus sp. 1 Fig. 6D

Remarks.—This pygmy grasshopper was found near small drains near the forest edge at KBFSC. The species was identified by J. Tumbrinck.

10. Loxilobus sp. 2 Fig. 6E

Remarks.—This pygmy grasshopper was also found near small drains near the forest edge at KBFSC. The species was identified by J. Tumbrinck.

11. Scelimena sp. Fig. 6F, G

Remarks.—We tentatively identified this species to be near to *S. dentiumeris* (Hancock, 1907). This large pygmy grasshopper is a powerful flyer that was collected along the sandy river bank along Sungai Belalong at KBFSC. The type specimen of the species was from Brunei Darussalam. We compared our single female specimen with type images from OSF (Cigliano et al. 2018).

12. Thoradonta nodulosa (Stål, 1861) Fig. 6H, I

Remarks.—We compared our single female specimen with type images from OSF (Cigliano et al. 2018). Identification was also verified by J. Tumbrinck and J. Skejo. This is also plausibly the first record of this species in Borneo (Cigliano et al. 2018).

Subfamily Tetriginae

13. Coptotettix sp. Fig. 6J

Remarks.—This pygmy grasshopper was found near small drains near the forest edge at KBFSC, probably because they are hydrophilic. The species was identified by J. Tumbrinck.

Superfamily Trigonopterygoidea Family Trigonopterygidae

1. Systella platyptera (Haan, 1842) Fig. 7

Remarks.—We found numerous individuals on the forest understory near the Canopy Tower. We compared our specimens with images from OSF (Cigliano et al. 2018) for identification.

> Suborder Ensifera Superfamily Grylloidea Family Gryllidae Subfamily Eneopterinae

1. Cardiodactylus pelagus Otte, 2007 Fig. 8A–D

Remarks.—These crickets are sometimes encountered among vegetation in the understory of the dipterocarp forest. The nymphs can sometimes be found on the forest floor. We compared our specimens with images from Robillard et al. (2013). Identification was verified by T. Robillard.

> 2. Nisitrus vittatus (Haan, 1844) Fig. 8E–H

Remarks.—This species was found abundantly around the station at the forest edge. During the day, the males can be heard calling on plant foliage. We also observed them feeding on flowers (Tan et al. 2017b). We compared this species with other congeners from Malay Peninsula and Borneo with help from T. Robillard. Tentatively, we considered the specimens from Kuala Belalong as *Nisitrus vittatus*, which can also be found in Malay Peninsula and Sumatra (Robillard and Tan 2013). There are some minor differences in acoustic and morphological characters between our Bornean specimens and those from other parts of Southeast Asia. We need more material from Borneo to better understand the species.

Subfamily Gryllinae

3. Gymnogryllus unexpectus trusmadi Gorochov, 2011 Fig. 9A–C

Remarks.—The understory can be filled with the loud male calling song of this large cricket during dusk (around 7 pm). The male lives in a burrow and produces loud and high-pitched (peak frequency = 4.2 kHz) trilling (mean pulse intervals = 9.1 ms) calls. As they can be quite abundant, the amount of noise produced can be very loud and heard a distance away. Specimens from Kuala Belalong resemble the images and description by Gorochov (2011) and identification was verified by A.V. Gorochov.

4. *Loxoblemmus* sp. Fig. 9D, E

Remarks.—This ground cricket was found co-occurring with two species of *Velarifictorus* among short grasses and herbaceous plants at the Ulu Ulu resort. Despite similarities in habitus, this genus differs from the latter by the head morphology and male genitalia.



Fig. 6. Tetrigidae: A, B. Falconius clavatus; C. Falconius deceptor; D. Loxilobus sp. 1; E. Loxilobus sp. 2; F, G. Scelimena nr. dentiumeris; H, I. Thoradonta nodulosa; J. Coptotettix sp. Scale bars: 1 mm.

5. *Mimicogryllus* sp. Fig. 9F–I

Remarks.—This rare and beautiful black cricket was encountered only once on the forest floor of the dipterocarp forest. A.V. Gorochov assisted with the identification of this species and this could represent an undescribed species (see Gorochov 1994). However, more material would be necessary before species description can be done.

6. Velarifictorus (Velarifictorus) aspersus aspersus (Walker, 1869)

Remarks.—This species is one of the two species of the cosmopolitan genus *Velarifictorus* Randell, 1964 (Modicogryllini) found in

Kuala Belalong. Male genitalia resemble that of the species illustrated in Ingrisch (1998a). This species is widespread in Southeast Asia, and can be found in Thailand, Singapore, and Peninsular Malaysia (Ingrisch 1998a, Tan 2012, Tan and Kamaruddin 2014, 2016, Dawwrueng et al. 2017). Refer to Tan et al. (2017c) for more details on the calling songs and species distribution.

7. Velarifictorus (Velarifictorus) temburongensis Tan et al., 2017

Remarks.—This species was found near river banks, among short grasses and herbaceous plants. It was identified as undescribed and the description was subsequently published in Tan et al. (2017c). This represents the first species of the genus *Velarifictorus* to be described from Borneo.



Fig. 7. Trigonopterygidae: Systella platyptera male adults.

Subfamily Landrevinae

8. Duolandrevus (Bejorama) lambir Gorochov, 2017

Remarks.—Prior to the description, we could only tentatively identify the male specimen to be close to *D. luzonensis* (Tan and Wahab 2017a), which was subsequently considered as a new species described from Lambir Hill National Park by Gorochov (2017a).

9. Duolandrevus (Eulandrevus) kawataredoki Tan & Wahab, 2017

Remarks.—One of the two Landrevinae newly described from the collection; this cricket can be commonly heard after dusk in the dipterocarp forest, often while hiding among dead leaves hanging on branches. They were also observed to feed on dead leaves. Refer to Tan and Wahab (2017a) for species description and calling song description.



Fig. 8. Gryllidae: Eneopterinae: *Cardiodactylus pelagus*: A. Male; B. Female; C, D. Male genitalia in ventral and dorsal views respectively; *Nisitrus vittatus*: E, F. Male; G, H. Male genitalia in dorsal and ventral views respectively.

10. Endodrelanva nympha Tan & Wahab, 2017

Remarks.—One of the two Landrevinae newly described from the collection; this cricket was found among branches of trees in the dipterocarp forest. Refer to Tan and Wahab (2017a) for species description.

Subfamily Podoscirtinae

11. Aphonoides sp. Fig. 10A

Remarks.—A single specimen (a female) was collected from a tree trunk in the dipterocarp forest. It resembles congeners of *Aphonoides* by: inner tympanum open, but outer one obliterated, tegminal *Sc* with several normal branches, ovipositor with apex rounded, and with large teeth on hind and ventral surfaces (Gorochov 2006). Many species from Borneo were described without the females, so determination of the species name is not possible without the males.

12. Sonotrella (Megatrella) sp. Fig. 10B

Remarks.—Our female specimen may belong to *Sonotrella* (*Megatrella*) *optima* Gorochov, 2002 or *Sonotrella* (*Megatrella*) *remota* Gorochov, 2002 both known from Borneo. Only the males of both species are known and females are very similar among congeners.

Subfamily Pteroplistinae

13. Pteroplistes sp. Fig. 10C, D

Remarks.—A single specimen (a female) was spotted camouflaging with the tree trunk of an old tree in the dipterocarp forest. It has characters diagnostic of the genus: large inner and small outer tympana of fore tibia and long ovipositor (Gorochov 2004). Two species have been described from Borneo (i.e. *P. borneoensis*



Fig. 9. Gryllidae: Gryllinae: *Gymnogryllus unexpectus trusmadi*: A. Male; B. Calling song; C. Male genitalia in lateral view; *Loxoblemmus* sp.: D. Male; E. Female; *Minicogryllus* sp.: F. Male; G–I. Male genitalia in ventral, dorsal, and lateral views respectively.

Gorochov, 2004 and *P. lagrecai* Gorochov, 2004), neither of which has had the female described (Gorochov 2004). It is therefore difficult to affirm the species name.

Subfamily Trigonidiinae

14. Amusurgus or Metiochodes sp.? Fig. 10E

Remarks.—Only a single specimen (a female) was collected. The species is characterized by tegmen pubescence, presence of only inner tympanum (very large), and ovipositor with apical valves broader than stem but with acute teeth apically.

15. Amusurgus or Paratrigonidium sp.? Fig. 10F

Remarks.—Distinct from all other Trigonidiinae, we, however, only collected females, making it nearly impossible to differentiate them among closely related genera. Tegmen not pubescent and abdomen distinctly humped dorsally.

16. *Paratrigonidium* sp. Fig. 10G

Remarks.—The male can be distinguished from the other Trigonidiinae above by the developed stridulatory apparatus, and foretib-

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Fig. 10. Gryllidae: Podoscirtinae: A. Aphonoides sp.; B. Sonotrella (Megatrella) sp.; Grylloidea: Pteroplistinae: C, D. Pteroplistes sp.; Trigonidiidae: Trigonidiinae: E. Amusurgus or Metiochodes sp.?; F. Amusurgus or Paratrigonidium sp.?; G. Paratrigonidium sp. ia with inner and outer tympana. The male genitalia also resemble that of a typical *Paratrigonidium*. More specimens will, however, be needed to ascertain the genus and species. This group is probably in need of further taxonomic revision.

Family Phalangopsidae Subfamily Phalangopsinae

17. Parendacustes (Minizacla) mulu Gorochov, 2017 Fig. 11A–D

Remarks.—Juveniles and adults were found on the tree trunks of old trees in the dipterocarp forest. They were well camouflaged among the ridges of the tree trunk. A.V. Gorochov assisted with the identification of this species which he recently described from Mulu National Park, which is relatively close to KBFSC (Gorochov 2017b, c).

18. *Terrozacla borneo* Gorochov, 2014 Fig. 11E–G

Remarks.—This species was fairly common in the forest floor of Kuala Belalong, usually camouflaging among the leaf litter or forest trails. T. Robillard assisted with the identification of this species. We compared the genitalia of our specimens with images from Gorochov (2014). This species can be found in Sabah, Sarawak, and now Brunei Darussalam.

Subfamily Phaloriinae

19. Phaloria (Papuloria) tristis Gorochov, 2014 Fig. 11H–K

Remarks.—The males produce trilling calling songs after dusk, often on foliage of trees. They are quite common in the dipterocarp forest. The genitalia of our specimens resemble that of the type specimen, described from Mulu National Park (Gorochov 2014).

Family Mogoplistidae Subfamily Mogoplistinae

20. *Cycloptiloides* sp. Fig. 12A

Remarks.—These small scaly crickets are usually cryptic but were found hopping around on the forest floor. Only females and nymphs were collected but male genitalia is needed for species identification (Ingrisch 2006). This represents the first record of this genus in Borneo (Ingrisch 2006, Cigliano et al. 2018). Because this genus has rarely been studied (Ingrisch 2006), the species from Brunei could be an undescribed species.

21. Ectatoderus sp. Fig. 12B

Remarks.—We tentatively identified this species to be close to *E. angusticollis* Chopard, 1969. The cricket was found among foliage and branches of understory trees. The key to Southeast Asian species was used for identification but this genus is likely to be paraphyletic and taxonomic work is needed (Ingrisch 2006).

Superfamily Gryllotalpoidea Family Gryllotalpidae

1. Gryllotalpa (?) sp. Fig. 12C

Remarks.—We recorded the calling songs heard in the evening sporadically across the forest floor, even though we did not manage to collect any specimen. The calling song is characteristic of congeners from the region. This species tends to call near dead and rotten logs at around 6:45–7pm and the male burrows clearly resemble that of congeners.

Superfamily Rhaphidophoroidea Family Rhaphidophoridae

1. *Rhaphidophora* (?) sp. Fig. 12D–F

Remarks.—This group is speciose and can be hard to identify for Southeast Asian representatives (A.V. Gorochov, in litt.).

Superfamily Stenopelmatoidea Family Gryllacrididae

1. Asarcogryllacris (Pseudolarnaca) sp. Fig. 13A–E

Remarks.—Our single specimen (a male) resembles the drawing of the habitus of *A. genualis* (Walker, 1869) from Borneo. The abdominal apex (particularly the medial apical sclerotized processes of ninth abdominal tergite) also resembles that in Gorochov (2005). It clearly differs from the only subcongener, *A. (P.) nigroscutata* (Brunner von Wattenwyl, 1888) from Java, by color patterns and abdominal apex.

2. Capnogryllacris (?) (Capnogryllacris) sp. Fig. 13F

Remarks.—We tentatively identified our female specimen to be close to *C. superba* (Brunner von Wattenwyl, 1888) using an old key (Murphy 1973). This species is characterized by red hind wings.

3. Melaneremus (?) sp. Fig. 13G–J

Remarks.—This is among the most common Stenopelmatoidea that was found in the understory of the Kuala Belalong forest. Unfortunately, we could only tentatively identify our specimens to this genus using an old key (Murphy 1973). However, our specimens are also different in shape of male abdominal apices from described species of the genus. Until a revision of the genera of Asiatic Gryllacridinae is completed, the determination of the species is problematic (A.V. Gorochov, in litt.).

4. Phlebogryllacris (?) sp. Fig. 14A–D

Remarks.—This stunningly red raspy cricket was found among foliage in the understory. Without comparison with the type specimen, we could only only tentatively identify our single specimen (a male) to be close to *P. venosa* (Walker, 1869) as it appears superficially similar and was described from Sarawak close to Brunei Darussalam.


Fig. 11. Phalangopsidae: *Parendacustes (Minizacla) mulu*: A. Male; B, C. Male genitalia in dorsal and ventral views respectively; D. Male tegmen; *Terrozacla borneo*: E. Female; F. Male; G. Nymph; *Phaloria (Papuloria) trista*: H. Male; I. Female; J, K. Male genitalia in dorsal and ventral views respectively. Scale bar: 1mm.



Fig. 12. Mogoplistidae, Gryllotalpidae, and Rhaphidophoridae: A. *Cycloptiloides* sp.; B. *Ectatoderus* cf. *angusticollis*; C. *Gryllotalpa* (?) sp. (frequency in kHz); D. *Rhaphidophora* (?) sp. male; E, F. Male abdominal apex. Scale bars: 1mm.



Fig. 13. Gryllacrididae: A. Asarcogryllacris (Pseudolarnaca) cf. genualis male; B. Face; C. Dorsal view of head and pronotum; D, E. Abdominal apex; F. Capnogryllacris (Capnogryllacris) cf. superba; G. Melaneremus (?) sp. mating pair; H. Male adult; I. Male stridulatory file; J. Male subgenital plate. Scale bars: 1mm.



Fig. 14. Gryllacrididae: A. Phlebogryllacris (?) cf. venosa male; B. Face; C. Male abdominal apex; D. Male subgenital plate; E. Phryganog-ryllacris sp.

5. *Phryganogryllacris* sp. Fig. 14E

Remarks.—We tentatively identified our specimens to this genus using an old key (Murphy 1973) and compared with images of the genus from Gorochov (2005). This genus is characterized by a cup-shaped ninth abdominal tergite, posteriorly with spines pointing downward far apart laterally, and tenth abdominal tergite without spine-like process.

Superfamily Tettigonioidea Family Tettigoniidae Subfamily Conocephalinae

1. Viriacca modesta Gorochov, 2013 Fig. 15A

Remarks.—This species was found on the foliage of understory plants in the dipterocarp forest. We used key to genera of Agraecini by Ingrisch (1998b) to identify our specimens. Subsequently we compared our specimens with species descriptions in Ingrisch (1998b) and Gorochov (2011, 2013). This species was described from Mulu National Park.

Subfamily Lipotactinae

2. Lipotactes alienus Brunner von Wattenwyl, 1898 Fig. 15B

Remarks.—This katydid is not uncommon in the understory of Kuala Belalong forest. However, curiously, we have yet to encounter the males. Unfortunately, the male of this species has also not been described. Our females correspond to the description by Ingrisch (1995) and images from OSF (Cigliano et al. 2018) by color patterns, shapes of ovipositor, and subgenital plate.

Subfamily Meconematinae

3. Alloteratura (Alloteratura) belalongensis Tan et al., 2017

Remarks.—This is one of the four new species of Meconematini katydids collected and described from Kuala Belalong. This new species is very similar to *A*. (*A*.) *karnyi* Kästner, 1932 from northern Sumatra (Kästner 1932, Gorochov 1998a) and Thailand (see Sänger and Helfert 2000) but could belong to several different subspecies of the same species owing to the similarities in morphology (Tan et al. 2017d). We need more material around Southeast Asia and study of their population genetics to confirm species limits. Refer to Tan et al. (2017d) for the new species description.

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Fig. 15. Tettigoniidae: A. Viriacca modesta; B. Lipotactes alienus; C. Asiophlugis longiuncus; D. Neophisis (Indophisis) cf. longipennis or curvata; Mecopoda elongata: E. Female; F. Calling song.

4. Asiophlugis longiuncus Gorochov, 2013 Fig. 15C

5. Kuzicus mirabilis Tan & Wahab, 2018

Remarks.—This species was found on foliage of the dipterocarp forest understory at night. Usually, a few individuals (mix of adults and nymphs) were found on a single large leaf. Only the female was collected, and was compared with the original description (Gorochov 2013). This species was described from Mulu National Park.

Remarks.—This species was encountered on foliage of the dipterocarp forest understory at night. Refer to Tan and Wahab (2018a) for the new species description.

6. Leptoteratura (Rhinoteratura) chela Tan et al., 2017

Remarks.—This species was encountered on foliage of the dipterocarp forest understory at night. Refer to Tan et al. (2017d) for the new species description.

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7. Neophisis (Indophisis) sp.

Fig. 15D

Remarks.—We tentatively identified this species to be close to either *N. longipennis* Jin, 1992 or *N. curvata* Jin, 1992. This species was found fairly frequently on foliage of the dipterocarp forest understory at night.

8. Pulchroteratura huiqing Tan et al., 2017

Remarks.—This species was quite frequently encountered on foliage of the dipterocarp forest understory at night. The peculiar shape of the abdominal apex led us to consider this to be a completely different genus. Refer to Tan et al. (2017d) for the new species description.

9. Pseudoteratura (Pseudoteratura) kenuan Tan et al., 2017

Remarks.—Refer to Tan et al. (2017d) for the new species description.

Subfamily Mecopodinae

10. Mecopoda elongata (Linnaeus, 1758) Fig. 15E, F

Remarks.—This large katydid can be heard calling at night among shrubby bushes. The calling song resembles the train call (see Diwakar and Balakrishnan 2007). Sometimes, they were also attracted to light.

Subfamily Phaneropterinae

11. Baryprostha bellua Karsch, 1891 Fig. 16A

Remarks.—The only specimen (a female) we encountered was found on the forest floor of the dipterocarp forest along Ashton Trail. It is probable that it resides in the canopy but had dropped down to the forest floor. We used the key to the species of *Bary*-*prostha* from Ingrisch (1990) and images of the type specimen from OSF (Cigliano et al. 2018) for identification.

12. Casigneta bisinuata Karny, 1926 Fig. 16B

Remarks.—This species was found attracted to light, thus appeared around the station. We compared our male specimens with images of type specimens from OSF (Cigliano et al. 2018).

13. Hemimirollia gracilis (Karny, 1925) Fig. 16C

Remarks.—Only one male specimen was collected during light trapping. We used the key to genera of Mirolliini by Ingrisch (2011) and compared our specimen with the descriptions of the two other congeners.

14. Holochlora sp. Fig. 16D

Remarks.—One male specimen was collected during light trapping. Upon freezing the katydid, a horsehair worm (Nematomorpha)

emerged from the abdominal apex. This parasite is not uncommon among katydids found near the water (i.e. Sungai Belalong). The Nematomorpha was preserved in ethanol. The katydid bears the male diagnostic characters of *Holochlora*: male tenth abdominal tergite with two apical lobes, area around left stridulatory file strongly swollen (Liu and Kang 2007).

15. *Mirollia* sp. Fig. 16E

Remarks.—We tentatively identified this species to be close to *M. abnormis* Karny, 1926. One female specimen was collected from the foliage on a tree near the Canopy Tower. We used the key to genera of Mirolliini by Ingrisch (2011) and compared our female specimen with the descriptions. Male specimens are needed to examine the titillators and confirm the species identity.

16. *Liotrachela* (?) sp. Fig. 16F

Remarks.—This species was found attracted to light and we postulate that it is also a canopy species. This species is superficially similar to *Holochlora* but the tenth abdominal tergite in males does not extend backwards into two lobes (Ingrisch 2002), and it possesses dorsal spines on fore tibia (Webber et al. 2003). We tentatively identified the specimens as belonging to this genus although it is not currently known from Borneo (Cigliano et al. 2018). More material is needed for verification.

17. Liotrachela hyalina Karny, 1926 Fig. 16G

Remarks.—We compared our male specimen with the original description (Karny 1926) and images of type specimen from OSF (Cigliano et al. 2018): male tenth abdominal tergite, cerci, and subgenital plate resemble our specimen. This species was described from Java, suggesting that our male specimen represents the first record of this species and genus in Borneo.

Remarks.—We tentatively identified the species to be close to *P. marginata* Brunner von Wattenwyl, 1878. One female was collected from a light trap. We recognized this species as *Phygela* owing to the tegmen with Rs and M angled at each cross vein and becoming obliterated in a series of large polygonal cells which occupy the radial field beyond the middle; and presence of a small lateral lobe between the subgenital plate and pileolus (this makes it different from an otherwise similar *Pseudopsyra* Hebard, 1922). Our specimen appears to differ from the female of *P. marginata* from Borneo by a more elongated ovipositor and a stouter subgenital plate, but further examination of the three described species from Southeast Asia is needed to confirm species identity.

19. *Rectimarginalis* sp. Fig. 16I

Remarks.—We tentatively identified this species to be similar to *R. profunda* Liu & Kang, 2007. We compared our single specimen (a male) with the descriptions of all species of *Rectimarginalis* Liu & Kang, 2007 (see Liu and Kang 2007) and images of the type specimen from OSF



Fig. 16. Tettigoniidae: Phaneropterinae: A. Baryprostha bellua; B. Casigneta bisinuata; C. Hemimirollia gracilis; D. Holochlora sp.; E. Mirollia cf. abnormis; F. Liotrachela (?) sp.; G. Liotrachela hyalina; H. Phygela cf. marginata; I. Rectimarginalis nr. profunda; J. Sympaestria lampra.

(Cigliano et al. 2018). Although *R. profunda* is not found in Borneo, our specimen resembles this species in its tenth abdominal tergite, subgenital plate, and genitalia, and is more similar to *R. profunda* than to its congeners found closer to or in Borneo [i.e. *R. fuscospinosa* (Brunner von Wattenwyl, 1891) and *R. ensis* (Haan, 1843), respectively]. There are some small differences in the male tenth abdominal tergite and genitalia. We require more material for verification since only one specimen was collected from light trapping.

20. Stictophaula bruneii Tan & Wahab, 2017

Remarks.—Only one specimen (a male) was collected during light trapping, perhaps suggesting that this is a canopy species rarely encountered in the forest understory. Extensive work recently on this Southeast Asian genus (see Ingrisch 1994, Gorochov 1998b, Gorochov and Kang 2004, Gorochov and Voltshenkova 2009) and images of type specimen from OSF (Cigliano et al. 2018) allowed



Fig. 17. Tettigoniidae: Pseudophyllinae: A. Olcinia dentata; B. Phyllomimus sp.; C. Tympanophyllum sp.

us to confirm that our specimen was undescribed. Full description of the new species and a key to all species can be found in Tan and Wahab (2017b).

21. Sympaestria lampra Hebard, 1922 Fig. 16J

Remarks.—This species is among the more common species of orthopterans attracted to light. They are, however, not encountered during opportunistic surveys in the understory, which is indicative of this being a canopy dweller. We used original descriptions (Hebard 1922) and type images from OSF (Cigliano et al. 2018) to identify our specimens.

22. Tapiena paraincisa Tan & Wahab, 2018

Remarks.—Our male specimen, collected during light trapping, resembles *Tapiena* (Tan et al. 2015). It is however unique in the shape of cercus and tenth abdominal tergite, and also differs from *T. incisa* Karny, 1923 from Sarawak. Refer to Tan and Wahab (2018b) for the new species description.

Subfamily Pseudophyllinae

23. Olcinia dentata de Jong, 1939 Fig. 17A

Remarks.—Only one specimen (a female) was collected during light trapping, perhaps suggesting that this is a canopy species rarely encountered on the forest understory. The crenulated costal margin of the tegmina distinguish our specimen from allied genera from the region (such as *Sathrophyllia* Stål, 1874 and *Tegra* Walker, 1870) (de Jong 1939, 1960, Bresseel and Vermeersch 2017). We also used the keys by De Jong (1960) and Bresseel and Vermeersch (2017) to identify our specimen.

24. *Phyllomimus* sp. Fig. 17B

Remarks.—This species was found on the understory leaves, and can frequently be heard calling. Upon approaching the katydid, it flattened its body and extended its tegmina to camouflage against the background of the leaf. Patterns on the tegmina resemble epiphylls that were found commonly on the leaves of the trees in the forest around KBFSC (notice the leaf of Fig. 17B).

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25. *Tympanophyllum* sp. Fig. 17C

Remarks.—This species was found on the understory leaves. This genus is similar to *Chondroderella* in habitus and shape of tegmen, but differs by tegmen with Sc and R separated at the base and showing some short cross-veins between them (instead of lying closely appressed). Only a single specimen (a male) was collected but we need both male and female to compare with congeners and identify the species.

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Paraplangia sinespeculo, a new genus and species of bush-cricket, with notes on its biology and a key to the genera of Phaneropterinae (Orthoptera: Tettigonioidea) from Madagascar

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Abstract

Madagascar is a well-known hotspot of biodiversity. However, many Orthoptera, and especially the Tettigonioidea, belong to little-studied groups. Here we describe a new genus and species of bush-cricket reared from fieldcollected eggs. *Paraplangia sinespeculo* gen. nov., sp. nov. belongs to Phaneropterinae and shares diagnostic characteristics with members of the tribe Amblycoryphini and its African subtribe Plangiina stat. nov. *Paraplangia*, which has a chromosome number of 31 X0, differs from other African members of the tribe and subtribe such as *Eurycorypha* and *Plangia*, which both have 29 X0. In addition to morphology, we describe the male calling song, female acoustic response, and mating behavior. As calling song, the male produces two series of short syllables. At the end of the second series the female responds with signals of similar duration and spectral composition as the male sounds (peak about 8-9 kHz). To make future identification easier, a key to all genera of Phaneropterinae found in Madagascar is presented.

Key words

Amblycoryphini, bioacoustics, chromosomes, duetting, Orophus

Introduction

The island of Madagascar is well known for its richness of endemic species. It is considered to be a very important hotspot of biodiversity due to several factors. Madagascar is a very large island, which is large enough to minimize the risk of extinction once a species has established. It is close enough to the mainland to receive occasional continental immigrants, but is far enough to allow a long, independent evolution of the fauna (MacArthur and Wilson 1967). Madagascar was formerly part of a continent (for a review see Vences et al. 2009). It separated from Gondwana about 135 million years ago (e.g. Briggs 2003, Yoder and Nowak 2006, McIntyre et al. 2017), so parts of its fauna and flora may be of Gondwanan origin. Then, for some time Madagascar and India drifted north-eastwards together, until their connection also broke (about 90 million years ago) and Madagascar remained isolated in the Indian Ocean. It was, however, never very far from Africa, and even non-flying mammals seem to have crossed this channel several times (Ali and Huber 2010). Therefore, the time at which the Malagasy taxa diverged from their closest relatives varies greatly, and there are many publications dealing with this question for different groups of animals and plants. However, even more groups, especially insect groups, are still nearly completely unstudied.

One of these groups is bush-crickets (katydids; Tettigonioidea). Most studies on this group in this region were conducted before 1914, when the last comprehensive paper appeared (Carl 1914). Only very recently have some papers on selected groups been published (Massa 2017a,b,c,d, Ünal and Beccaloni 2017). There are no published data on the biology of any taxon (except some observations in Ünal and Beccaloni 2017). At present, four subfamilies of Tettigonioidea are represented by several species each in Madagascar, and Listroscelidinae is represented by a single species (all data according to Cigliano et al. 2018, abbreviated OSFO). The four more common subfamilies have quite different biogeographic patterns in terms of their Malagasy fauna. Pseudophyllinae and Meconematinae are represented with relatively few species each and only endemic species or genera are known. All Pseudophyllinae (except the phyllophorine-like genus Aspidonotus) belong to the tribe Simoderini which consists of species that occur exclusively in Australia and Madagascar. If this tribe was confirmed as a monophyletic unit, it would be a strong indication for a Gondwanan origin of the group. The subfamily Conocephalinae has many species in Madagascar and most genera are endemic, but there are also a few endemic species of the widespread genera Conocephalus and Ruspolia and even non-endemic species [Conocephalus maculatus (Le Guillou, 1841) ("Madagasian Region" Pitkin 1980), Pseudorhynchus hastifer (Schaum, 1853) and the swarm-producing (see Bailey and McCrae 1978) Ruspolia differens (Serville, 1838)]. Most conocephaline genera belong to the tribe Euconchophorini, which is endemic to Madagascar and some surrounding islands. Gorochov (1988) considered this group of short-winged species as a sister group to all other world-wide distributed Conocephalinae. However, in a

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recent molecular study (Hemp et al. 2015) the Euconchophorini representative appeared basally to the studied Agraeciini from Africa, India and Australia, but within Conocephalinae. In any case, a member of this Agraeciini/Euconchophorini-complex seems to have arrived in Madagascar relatively long ago.

The largest Malagasy subfamily is Phaneropterinae (at present ca. 40 species; Table 1). Madagascar has a few species in common with continental Africa (Phaneroptera sparsa, Tylopsis bilineolata, T. irregularis, and Eurycorypha cereris), and several genera have species which are endemic to Africa as well as to Madagascar - most of them also with endemics on other islands in the Western Indian Ocean (Eurycorypha, Plangia, Parapyrrhicia, Trigonocorypha). One genus with one endemic species to Madagascar has most of its species distributed in Asia (Holochlora). Thus, two thirds of the 22 phaneropterine genera are endemic to Madagascar. Most phaneropterine species are long-winged. There have probably been several independent colonization events of Madagascar, mostly from Africa [as with the flying mammals (bats), although some of which seem to have come directly from Southeast Asia; Bates et al. 2006]. For genera endemic to Madagascar, the closest relatives are expected to be found in Africa.

Considering the low intensity of research, the number of species and genera known at present is certainly only a small percentage of that actually occurring there or which had, sadly, occurred there in the past (see Goodman and Jungers 2014). Therefore, one of the authors (KGH) seized the opportunity to rear nymphs of an unidentified species from Madagascar. After these animals molted successfully to adults, they had to be identified (see key below) and, not completely unexpectedly, they were identified as belonging to a new genus and species. Since they lived for quite some time in the laboratory, we were able collect data which were not previously known from any other Malagasy bush-cricket, and which are hopefully a starting point for similar studies *in situ*.

Methods

The animals were held in plastic containers, differing in size depending on the size of the animals, and fed with *Taraxacum of-ficinale*, replaced daily.

Measurements.—Total body length, lateral aspect, refers to the midline length of the insect from fastigium verticis to tip of abdomen including the subgenital plate. In females, the ovipositor is not included in the measurement of the body length. Measurements of ovipositor are taken laterally in a straight line from tip to base disregarding the curvature.

To obtain the mass data, living animals and spermatophores were weighed to the nearest 2 mg (balance Tanita Professional Mini 1210– 100).

Acoustics.—The male calling song was recorded in the laboratory using a digital bat detector (Pettersson D1000X) with a sampling rate of 100 kHz. Duets were recorded in stereo using a Sony ECM-121 microphone (frequency response relatively flat up to 30 kHz according to own tests) and an Uher M645 microphone connected to a personal computer through an external soundcard (Transit USB, "M-Audio"; 44.1-kHz sampling rate).

Song measurements and spectrograms were obtained using Amadeus II and Amadeus Pro (Martin Hairer; http://www.hairersoft.com). Oscillograms of the songs were prepared using Turbolab (Bressner Technology, Germany). All recordings were made at temperatures between 22 and 25 °C. The singers were caged in plastic tubes or gauze cages with microphone fixed or hand-held at distances between 5 (duet) and 80 cm.

Acoustical terminology.—Tettigonioids produce their songs by repeated opening and closing movements of their tegmina. The sound resulting during one cycle of movements is called a syllable; opening and closing hemisyllables can often be differentiated (Ragge and Reynolds 1998). Syllable duration: time period measured from the first impulse to the last; syllable period: time period measured from the first impulse to the first impulse of the next syllable; impulse: a simple, undivided, transient train of sound waves (here: the damped sound impulse arising as the effect of one tooth of the stridulatory file). Typically, after some time the same or a similar pattern of syllables and pauses is repeated. This grouping, often separated from the next by a silent interval, is called a song unit.

Chromosomal analysis.—Paraplangia sinespeculo (two males CH8239, CH8240) and Orophus cf. tessellatus (Saussure, 1861) [Costa Rica; obtained from https://www.saltatoria.info/ arten%C3%BCbersicht-a-z-species-a-z/orophus-tesselatus/] (one male CH7705 and one female CH7707) were used for chromosomal analyses. Preparations were obtained from testes and ovaries, incubated in hypotonic solution (0.9% sodium citrate), fixed in ethanol: acetic acid (3:1), and crushed in 45% acetic acid. Cbanding was carried out using the method of Summer (1972) and the silver staining method (AgNO₃) for localization of the nucleolus organizer regions (NORs) was performed as previously reported (Warchałowska-Śliwa and Maryańska-Nadachowska 1992). Fluorescence in situ hybridization (FISH) with ribosomal 18S rDNA and telomeric (TTAGG), DNA probes were conducted following the protocol described by Warchałowska-Śliwa et al. (2009). Chromosomes were studied with a Nikon Eclipse 400 microscope with a CCD DS-U1 camera and NIS-Elements BR2.

Results

The specimens of the new species were identified using the key given below (based on Karsch 1889, Brunner von Wattenwyl 1891, Carl 1914, Ragge 1980, OSFO). See also Table 1 for additional data. Using Ragge's (1980) key for all African Phaneropterinae with open tympana (excluding Madagascar) one would end up with *Plangia*.

Key to the genera of Phaneropterinae of Madagascar and the islands in the western Indian Ocean

1	Fore coxa without spine 2
_	Fore coxa with spine
2	Occurring in Madagascar
_	Occurring in Mauritius onlyArantia (two species)
3	Tegmen shorter than 30 mm (observed 25), hind wings not longer
	than tegmina Paracosmophyllum atrodelineatum
_	Tegmen longer than 30 mm (observed 37), hind wings longer than
	tegmina Nesoscirtella polita
4	One or both tympana of the fore tibia closed
_	Both tympana of the fore tibia open
5	Both tympana closed
_	Only anterior tympanum closed
	Holochlora (mauritiana Mauritius, biloba Madagascar)

6	Side keels of the pronotum modified (crenulated or serrate or with tubercles etc.) (possibly all members of Trigonocoryphini)7
_	Side keels of the pronotum smooth or indistinct
7	Hind wings hidden under (shorter than) tegmina
-	Hind wings longer than tegmina9
8	Male supra-anal plate not modified Cosmozoma (4 species)
-	Male supra-anal plate enlarged and three-lobed
	Sikoriella (bimaculata)
9	Side keels with 4 large tubercles Polygamus (2 species)
-	Side keels of pronotum crenulated or with small, irregular and indis-
	tinct tubercles10
10	Side keels of pronotum dark, crenulated Trigonocorypha (maxima)
-	Side keels of pronotum not dark, with small, irregular and indistinct
	tubercles
11	Tegmina narrow [ratio length/width >3.8; observed (3.8 M.p.*-)
	4.3-7.0]
-	Tegmina broad (ratio length/width <3.8; observed 2.1–3.6)
12	Hind wings less than half of the length of the tegmina
	Eucatopta heringi
-	Hind wings longer than tegmina
13	Dorsal side of fore tibia rounded14
-	Dorsal side of fore tibia flat or furrowed15
14	Margins of pronotum rounded (=side keels indistinct)
	Parapyrrhicia (5 species)
-	Margins of pronotum evident (=side keels smooth but distinct)
15	Tegmina long (>25 mm; observed 29–46 mm)16
-	Tegmina quite short (<25 mm; observed 14–21 mm) 17
16	Tegmina long (>32 mm; observed 34–46 mm)
_	Tegmina short (<32 mm; observed 29 mm)Agennis (parallelinervis)
17	Last tergite of male unmodified, ovipositor short (4.0–4.7 mm), teg-
	mina unicolored
-	Last tergite of male modified, ovipositor long (7.5–8.0 mm), teg-
10	Male subgenited plate with long up, and resurved enpendices
18	Male subgenital plate with long, up- and recurved appendices
	Anonispora appendicular
-	Facticity variation parrower than econyce 20
19	Fastigium verticis narrower than scapus
-	Pasugium venues as wide of wider man scapus
20	Decembring in Madagascai, tegninia wide (ratio fengui/widui = 5)
	Occurring on the Souchelles, terming narrow (ratio length/width -
-	Decenting on the seychenes, tegnina narrow (ratio length/width =
21	Ever elongate fastigium verticis at least twice as broad as scapus
21	Eyes clongate, lastigium ventes at least twice as broad as scapus
_	Ever circular or oval fastigium verticis less than twice as broad as
	scapus 22
22	Fore femore ventrally armed ventral edge of ovinositor evenly
~~	rounded 23
_	Fore femora ventrally unarmed ventro-posterior edge of ovipositor
	slightly curved its dorsal ending bent frontally (horizontal)
	Darablanaia gon n
23	Legs flattened laterally Dlangia (3 species)
_	Legs not flattened laterally Madaoascarantia (alholinoata)
	-0

* Only *Mimoscudderia paulyi* Massa, 2017 with unusually wide tegmina.

Tribe Amblycoryphini Brunner von Wattenwyl

Brunner von Wattenwyl (1891) combined several genera in Amblycoryphini using the key character "fastigium as wide or wider than scapus". This may well be a convergently evolved character, but as long as no better grouping is available, it is useful for placing and finding similar genera. The group included and still includes (even after the splitting by Cadena-Castañeda 2014, 2015) African and American genera which were separated in Brunner von Wattenwyl (1891)'s key but not by Cadena-Castañeda (2014). According to the preliminary molecular tree (Mugleston et al. 2013), *Amblycorypha*, the type genus, is deeply nested among New World genera from different tribes. In other molecular studies, *Eurycorypha* and *Plangia* are closely related (Mugleston 2016), with *Isopsera* nearby (Liu Cx unpubl.). *Indogneta* Ingrisch & Shisodia, 2002, which also has a broad fastigium, certainly belongs to Isopserae (see Kang et al. 2014), and *Isopsera* has ant-like nymphs like *Eurycorypha*.

We also include *Corycomina* Karsch, 1896 because of its similarity to some *Eurycorypha* (*E. flavescens* was considered as member of *Corycomina* Karsch, 1896; see Massa 2017c).

Subtribe Plangiina Cadena-Castañeda, 2015, stat. n. (formerly genus group Plangiae)

When splitting up Amblycoryphini, Cadena-Castañeda (2015) listed the similarities between Amblycoryphini s.s. and his Plangiae. They had all generic characters in common except the eye shape. We consider this single trait to be insufficient for excluding *Plangia* and *Monteiroa* from the tribe, especially since Plangiae obviously do not have any similarities to other phaneropterine genera, but share e.g. nymphal properties with *Eurycorypha*, the largest genus of the tribe (see Hemp in preparation). Despite some doubts, we retain the group as a subtribe and include *Madagascarantia* Massa, 2017, which is a sister genus to *Plangia, Pseudoplangia* Massa, 2014, and *Paraplangia* gen. n.

After having seen photos of one of the syntypes of *Plangia albolineata* (described as *Turpilia albo-lineata*), it turned out that *Madagascarantia bartolozzii* is conspecific with this species. Since *Madagascarantia* is considered as generically distinct from *Plangia*, we propose the following taxonomical corrections.

Madagascarantia albolineata (Brunner von Wattenwyl, 1878), comb. n.

syn. n. Madagascarantia bartolozzii Massa, 2017

Paraplangia Heller, gen. n.

http://zoobank.org/F80FFF6B-DDAB-4836-8F16-045E6EC76F27 urn:lsid:Orthoptera.speciesfile.org;TaxonName:502224

Type species of the genus.—Paraplangia sinespeculo sp. n., here designated.

Description.—Large size, short head, wide round eyes, fastigium verticis as wide as or slightly wider than scapus of antennae, in contact with fastigium frontis, fronto-genal carinae very indistinct. Antennae shorter than tegmina. Pronotum without lateral carinae, length shorter than height, two small pits at two-thirds point on midline; prozona hardly separable from metazona, anterior margin straight, posterior margin rounded, with evident lateral excisions where wings are inserted. Prothoracic spiracle slit-like, very

Table 1. List of the known phaneropterine species from Madagascar and some islands in the Western Indian Ocean together with some selected measurements. Abbreviations: Mad = Madagascar, Mau = Mauritius, Com = Comoros, Sey = Seychelles, B.v.W. = Brunner von Wattenwyl. Measurements in mm (taken from original sources).

		Distribution		Length of		Width of	Ratio width tegmen/	Ratio length/width	
Species	Mad.	Mau.	Com.	Sey.	pronotum	tegmen	tegmen	length pronotum	of tegmen
Agennis parallelinervis B.v.W., 1891	•				4.8	29	4.5	0.9	6.4
Anchispora appendiculata B.v.W., 1891	•				6.2	46	13	2.1	3.5
Arantia (Arantia) dentata Saussure, 1899		•			7.5	48	10	1.3	4.8
Arantia (Euarantia) mauritiana Saussure, 1899 ^a		•			8.5	60	19	2.2	3.2
Cosmozoma coelebs Carl, 1914	•				9	62	18	2.0	3.4
Cosmozoma doenitzi Karsch, 1889	•				6	37	11.5	1.9	3.2
Cosmozoma sikorae B.v.W., 1891	•				5	30	10	2.0	3.0
Cosmozoma vespertilio Carl, 1914	٠				10	65	21	2.1	3.1
Cosmozoma voluptaria B.v.W., 1891	•				8.2	43	17	2.1	2.5
Eucatopta heringi Karsch, 1889	•				3.5	12.5	3	0.9	4.2
Eurycorypha brevipennis Karsch, 1889	•				5	21	10.2	2.0	2.1
Eurycorypha brunneri Brancsik, 1893	•				5.5	35	14	2.6	2.5
Eurycorypha cereris (Stål, 1857)	•				5	28	9	1.8	3.1
Eurycorypha prasinata Stål, 1874	•			•	5	30	10	2.0	3.0
Holochlora biloba Stål, 1874 ^b	•	•?			8	52	14	1.8	3.7
Holochlora mauritiana Massa, 2017		•			7.2	48.1	12.2	1.7	4.0
Madagascarantia albolineata (B.y.W., 1878)	•				9	48	18	2.0	2.7
Megotoessa insulana Karsch, 1889	•				9	47	18	2.0	2.6
Mimoscudderia modesta Carl, 1914	•				5	39	7.5	1.5	5.2
Mimoscudderia paulvi Massa, 2017	•				4.7	45.6	12.1	2.6	3.8
Mimoscudderia picta Carl. 1914	•				5.5	41	7	1.3	5.9
Mimoscudderia spinicercata Massa, 2017	•				4.5	34.2	6.8	1.5	5.0
Nesoscirtella polita Carl, 1914	•				6	37	11	1.8	3.4
Paracosmothyllum atrodelineatum B.y.W., 1891	•				6.5	25	9.5	1.5	2.6
Paraphylloptera relicta Carl, 1914	•				6	39	13	2.2	3.0
Paraplangia sinespeculo sp. n.	•				6.5	42.5	11.7	1.8	3.6
Paratyrrhicia dentites Saussure, 1899	•				5	34	_	_	
Paratyrrhicia insularis Chopard, 1958			•		4.5	23.5	5.5	1.2	4.3
Paratyrrhicia longitodex Massa, 2017	•				4.2	26.6	4.2	1.0	63
Paratyrrhicia madagassus (Karsch, 1889)	•				5	28.5	6.5	13	4 4
Paratyrrhicia virilis Carl, 1914	•				5	35	-	_	
Pelerinus rostratus (B v W. 1878)				•	6	38	11	1.8	35
Phaneroptera sparsa Stål, 1857 °	•				34	17	2.8	0.8	6.1
Planoja outtatipennis Karsch, 1889	•				6	27.5	8	13	3.4
Plangia ovalifolia Bolívar. 1912	•?			•	4 5	29	10	2.2	2.9
Plangia segonoides (Butler, 1878)	•				8	42.5	18.4	2.2	2.3
Polygamus macropherus Carl. 1914	•				9	61	16	1.8	3.8
Polygamus mucropierus Garl, 1914	•				6	50	12.5	2.1	4.0
Sikoriella himaculata Carl 1914	•				5	28	10	2.0	2.8
Symmetroraggea detravata Massa 2017	•				4	26.3	54	1.4	4.9
Symmetroraggea dirempta (Karsch 1889)	•				4 5	20.5	4 5	1.1	6.4
Trigonocorriba marima Carl 1914	•				6	53	15	2.5	3.5
Tylopsis hilineolata (Serville 1838) d	•				35	20	35	1.0	5.5
Tylopsis ounicounin (Servine, 1898)	•				з. <u>э</u>	30.6	3.5	1.0	87
Yonodovus annilatus (ByW 1891)					-+	17 5	2,5	0.8	7.0
Xenodoxus nobilis Carl. 1914 f	•				2 75	20	3.6	13	5.6

^a status doubtful, perhaps error in type locality (see Hollier and Heads 2015)

^b not *biloba* B.v.W = *Holochlora indica* Kirby

^c data Ragge 1956

^d data B.v.W. 1878

^e data from foto in OSFO

^f data Massa 2017a

long, reaching nearly up to metanotum. Ventral edge of paranota rounded. Tegmina much wider than pronotal length. Right tegmen of male without clearly defined mirror. Hind wings longer than tegmina.

Fore coxae armed, fore femora unarmed, several spinules positioned ventrally on fore tibiae, furrowed proximally, rounded distally, dorsal side rounded or flat or very slightly furrowed (rounded and slightly furrowed on left and right leg of the same specimen), without dorsal spurs. Tympana open on inner and outer side. Mid femora ventrally with 1-2 spinules, mid tibiae with about ten spinules. Hind femora armed ventrally, lower genicular lobe with spine on both sides. Hind tibiae armed ventrally and dorsally, furrowed on all sides. Hind tibiae longer than femora.

Meso- and metasterna with two lobes each, rounded.

Female.—Ovipositor short, curved, but not evenly; ventro-posterior edge of the lower valve only slightly curved, without teeth, dorso-posterior edge sharply bent inwards, this part and distal half of the upper valve serrated. Proximal quarter of right tegmen (dorsal area) with transverse veins bearing small teeth.

Diagnosis.-Paraplangia differs from most African and Malagasy phaneropterine genera with open tympana by its fastigium. Being about as wide as scapus, the fastigium is wider than in most genera, but clearly narrower than in Eurycorypha and Monteiroa. Its width is similar to that of Plangia and Madagascarantia. These two genera, however, have evenly curved ovipositors. In Paraplangia, the fore femora are also unarmed [a character occasionally found within otherwise armed genera: e.g. Arantia (Hemp and Massa 2017), but used by Brunner von Wattenwyl (1891) to differentiate genera], which is not observed very often in large species. The paranota are relatively narrow for Plangiina, only about two thirds as wide as high. Paraplangia has other unusual characters, like a stridulatory file with relatively few teeth of varying size, which is quite different from that known in Plangia and Madagascarantia. Its right male tegmen lacking a glossy mirror is not known in any of the similar genera.

Derivatio nominis.—Para, Greek = nearby; *Plangia*, another Plangiina genus. *Paraplangia* feminine.

Paraplangia sinespeculo Heller, sp. n. http://zoobank.org/03B56614-F8D3-40C1-8652-EFE6F2C80077 urn:lsid:Orthoptera.speciesfile.org:TaxonName:502225

Material examined and depository.—Holotype \Diamond , allotype \Diamond and 1 paratype \Diamond . All pinned, original labels "MADAGASCAR: Mitsinjo Forest Reserve, near Moramanga (18°57′S, 48°13′E), 1 i - 31 xii 2014, coll. Giesse". "Holotype *Paraplangia sinespeculo*" [red handwritten label]. Holo- (CH8239) and allotype (CH8241) in Museum für Naturkunde, Berlin, Paratype (CH8240) in Collectio Heller. One hind leg of CH8240-1 separate in pure ethanol in Collectio Heller.

Sound files are deposited at OSFO and bio.acousti.ca (see also Suppl. materials 1, 2).

Measurements.—(In mm) Males. Body length: 27.5–28.2; pronotum length: 6.8–7.4; pronotum height: 6.9–7.0; hind femur: 19.5–20.0; hind tibiae: 21.9–23.5; tegmina: 43.2–43.8; length of hind wings: 46.4–46.6; tegmina width: 14.3–14.7. Female. Body length: 29.9; pronotum length: 7.8; pronotum height: 7.0; hind

femur: 20.9; hind tibiae: 22.0; tegmina: 43.6; length of hind wings: 47.6; tegmina width: 17.2; ovipositor 13.0.

Diagnosis.—As for genus (sole species).

Description.—Male. Habitus and color: Large bush-crickets, predominantly green with a weak yellow mid line on head and pronotum (Fig. 1; not visible in dried specimens). Anterior (costal) edge of tegmen in basal half to two thirds with white spots. Head, pronotum and thoracic sternites (Fig. 2) as for genus.

The stridulatory area of the left tegmen green, with distinct but not elevated stridulatory vein (Fig. 3A), of right tegmen weakly green or white, with several irregular veins, without glossy mirror (Fig. 3B). The stridulatory vein beginning at the distal end with a series of ca. 15 small teeth, increasing slowly in size. At the same place, the file starting to be elevated above the tegmen level. Having reached the highest point, there are ca. 10 widely spaced large teeth. After five of them, tooth size and spacing continuously decrease again. The file then ends in about 10 small to very small teeth (Fig. 3D).

Fore coxae armed with a long spine. Fore femora unarmed, fore tibiae with 2–3 inner ventral spines and 3 distal ones, superiorly mainly flat (see above). Mid femora with 2 very small spinules, mid tibiae ventrally with about 7 spines each on outer and inner side. Hind femora distally with about 7 outer and 2-4 inner ventral spines, hind tibiae straight, longer than femora, in cross-section square, with many spines on all four edges (about 15 on each ventral, about 25 on each dorsal side).

Abdomen.—Subgenital plate long, tapering into a deeply incised caudal part, bearing long styli (Fig. 4A). Cerci relatively long, slightly in-curved at tip, with a short, strong, hook-like outer and a rounded inner spine (Fig. 4A–B). No sclerotized titillator.

Female.—Color and general habitus like male. Ovipositor as described for genus (Fig. 5A-B). Subgenital plate short, triangular and at apex slightly incised, side sclerites with strong and incurved lower edge (Fig. 4C), probably the anchor point for the male cerci during mating.

Derivatio nominis.—Sinespeculo (Latin = without mirror). To be treated as noun in the nominative singular.

Eggs.—Mature eggs were taken from the female after her death and preserved in ethanol. They show the flat, ovoid shape, typical for phaneropterines (length 5.7 ± 0.2 mm, width 2.75 ± 0.06 mm, n = 4; Fig. 1B). The collector wrote "we found the eggs under leaves".

Nymphs.—The specimens were obtained as relatively small nymphs (probably stage 2 or 3). At that time they were nearly completely green with few brown markings (not looking like the small nymphs of *Plangia satiscaerulea;* see Hemp in preparation). Soon they showed a yellow midline running from head to mid of abdomen and they developed a brown pattern at the back of the abdomen, similar to that seen in older *Eurycory-pha* nymphs (Hemp in preparation; Fig. 1C–E). The pronotum had a middle keel like the adults in *Tropidonotacris* Chopard, 1954 (Hemp et al. 2014) or *Pelecynotum* Piza, 1967 (OSFO). The males became adult at 9th and 13th May, the female slightly later, at 20th May. The female died on 30th June due to an infection with Nematomorpha and all were prepared.



Fig. 1. Habitus. A. Adult male; B. Egg (lateral and ventral view, scale 5 mm); C. Nymph, 3rd March; D. Nymph, 30th March; E. Female nymph, 10th May, 10 days before imaginal molt.



Fig. 2. Head and pronotum. A. Face (head frontal); B. Pronotum lateral; C. Fastigium verticis and pronotum dorsal; D. Meso- and metasterna, head to the left.



Fig. 3. A, B. Left and right, respectively, stridulatory areas in male and C. Female tegmina (scale 5 mm); D. Male stridulatory file (distal end to the left; scale 1 mm).

Acoustics.—The male calling song consisted of song units, repeated in interval of many minutes as long as the female did not respond. Each song unit (163 recorded) contained two series of syllables, the first with 10.7 \pm 1.0 syllables (mean \pm SD; range 8-12; n = 21), the second only with 4.2 \pm 0.9 (range 3-5; Fig. 6). The second series started 9.3 \pm 0.7 s (range 8.3-10.7 s) after the beginning of the first and both series were separated by a silent interval of 4.2 \pm 0.4 s (range 3.7-5.3 s). The intervals between the syllables ranged from 400 to 619 ms (487 \pm 66 ms), measured in the second half of the first series. In amplitude modulation, both series were decrescendo or without change in loudness. The syllables were very short, less than 5 ms (Fig. 7A), consisting of few, often hardly separable impulses.

Like in most phaneropterines (Heller et al. 2015), the female that was ready to mate reacted to the male song with its own acoustic signals. It always answered after or at the end of the second series. The first response syllable of its response was registered 2.7 \pm 0.6 s after the beginning of the second male series (range 1.7-4.2 s; n = 74) and 0.55 \pm 0.33 s after a male syllable (range 0.04-2.8 s; n = 73). The female response was quite variable; the simplest answer consisted of one impulse, but she could also make two impulses at relatively large intervals, long (about 8) series of impulses with short intervals or mixtures with many impulses at varying intervals (Fig. 6B–C; see Suppl. materials 1, 2: duets 1 and 2).

The carrier spectrum of male and female song is relatively narrow-banded with its maximum at about 8.4 ± 0.7 kHz (n = 3; range 10 db below peak 7.3-10.2 kHz) in the male and 8.7 ± 0.2 kHz (n = 3; range 10 db below peak 6.9-10.9 kHz; Fig. 7) in the female.

Mating behavior.—Both males (body mass 1178 mg and 1236 mg) copulated with the female (body mass 2536 mg, 2528 mg) at an interval of 3 days (21st and 24th June). They mated in the morning



Fig. 4. Subgenital plate and cerci. A. Male subgenital plate and cerci, ventral; B. Male cercus, dorso-lateral view (dorsum to the left); C. Female subgenital plate with lateral sclerites, ventral view.



Fig. 5. A, B. Ovipositor of *Paraplangia sinespeculo*; and other Plangiina for comparison; C. *Plangia graminea* (Serville, 1838); D. P. guttatipennis Karsch, 1889; E. P. karschi Chopard, 1954; F. P. multimaculata Hemp, 2017; G. P. nebulosa Karsch, 1890; H. P. satiscaerulea Hemp, 2017; I. P. variacantans Hemp, 2017; J. Madagascarantia albolineata (syntype); K. Pseudoplangia laminifera (Karsch, 1896). Sources OSFO: D, E, G, J, K; Hemp 2017: C; Hemp et al. 2015: F, H, I.

(about 9:00–10:00; they were placed together the evening before, but did not mate) with mating durations of a few minutes and transferred spermatophores of 130 and 151 mg (mean of male loss and female gain), thus about 12% of the male body mass. They were slightly smaller than in *Plangia multimaculata* (17%; Hemp et al. 2015). Both spermatophylaces showed relatively irregularly formed central parts protruding anteriorly, in addition to the symmetrical lateral basal parts (Fig. 8).

Chromosomes.—Both analyzed species, the African Paraplangia sinespeculo and Orophus cf. tessellatus from Costa Rica, showed a diploid chromosome number of 2n = 31 karyotype in the male with an X0 and 32 in the female (*O. cf. tessellatus*) with XX sex determination system. Fifteen pairs of acrocentric chromosomes gradually decreased in size; the sex chromosome (X) was the largest element in the karyotype (Fig. 9A-D). After FISH with 18S rDNA, silver staining, and C-banding, the results demonstrated coincidence between the localization of major ribosomal genes and active NOR as well as the position of C-bands. In individuals of both species, a single large rDNA cluster per haploid genome was detected, located on the first pair of autosomes near the distal region (Fig. 9B – left corner) and active NOR (Fig. 9B) as well as C-band (Fig. 9A).



Fig. 6. Oscillograms of: A. the spontaneous male calling song, and B, C. of two-channel recordings of male-female duets (B: Female response with few impulses; C: With many impulses).



Fig. 7. Oscillograms of: A. A male syllable and; B. A female response together with power spectra (Hanning window, 512 points, mean of 12-ms-section of the song).

In contrast, in *Orophus* cf. *tessellatus*, FISH revealed a paracentromeric signal on the fourth pair of autosomes (Fig. 9D) coincident with thick C-bands (Fig. 9C) and active NOR (not shown). The signals produced by FISH with the $(TTAGG)_n$ probe were stronger in *O*. cf. *tessellatus* (Fig. 9D) than those observed in *P. sinespeculo* (hardly visible).

Discussion

Paraplangia sinespeculo belongs to a phaneropterine morphotype which is widespread in many tropical areas. Such medium to large species with long wings of intermediate width and white markings at the costal edge of the tegmina are found in central America (e.g. Lamprophyllum, Philophyllia; Fontana et al. 2008, and



Fig. 8. Female with spermatophore after mating.

Syntechna; Cadena-Castañeda 2014), Australia (e.g. *Paracaedicia*; Rentz 2010), China (e.g. *Sinochlora*; Kang et al. 2014), and Africa (e.g. some *Arantia*; Hemp and Massa 2017). They are unlikely to be closely related and do not seem to possess any other special characters in common. Their specific ecological adaptations are unknown for all of them. They may live in trees with similar leaf shape or spend the day at special roosting sites where the shape of the tegmina essentially improves the camouflage among the leaves.

However, some morphological similarities may have been overlooked. Many species do not have any or have only weakly developed mirrors on the right wing (checked in OSFO for members



Fig. 9. Chromosomes of: A, B. *Paraplangia sinespeculo* and; C, D. *Orophus* cf. *tessellatus* stained using different techniques: C-banding (A, C), silver staining (B), and FISH with both 18S rDNA (green) and telomeric DNA (red) probes (B – left corner and D). Diakinesis and the insert in the left corner mitotic large chromosome (A), diplotene and FISH with 18S rDNA in the left corner mitotic chromosome (B): arrows and the insert chromosome demonstrated distally located thin C-band and single NOR coincide with cluster of 18S rDNA on the first pair of autosome. Arrows in spermatogonial (C) and female metaphases (D) indicate near paracentromeric located C-bands and 18S rDNA loci on the fourth pair of chromosomes. X indicates sex chromosome.

of the above-mentioned genera; only the morphologically very diverse Arantia seems to also be variable in this character: A. fasciata has a large distinct mirror, while some other Arantia species have small and/or indistinct ones, and A. mauritiana does not have one at all; Hemp and Massa 2017). The mirror is an important structure for sound radiation and is considered as typical for bushcrickets (e.g. Montealegre-Z et al. 2017). However, its systematic distribution and size have never been studied in detail. In some species it is a typical 'mirror', a large, thin, transparent membrane covering a significant portion of the tegmen's width, while in others it is hardly discernible at all (for examples see e.g. fig. 5 in Heller et al. 2014). In Amblycoryphini (Old and New World species) and some other phaneropterines (see above), it is often, if not always, weakly developed or even missing. On the contrary, in many Poreuomenini, another African tribe which occurs together with Amblycoryphini and Phaneropterini (according to OSFO), it is large and transparent. Correlations with song characteristics are unknown. Unfortunately, in many descriptions of new species, even in recent ones, size and shape of the mirror are often not mentioned nor illustrated.

Amblycoryphini are well known for the complexity of their songs, as in the type genus *Amblycorypha* (e.g. Walker 2004). Some species can produce up to four different syllable types. This complexity results obviously from different neuro-muscular programs used for the stridulatory movements. The stridulatory files can differ in tooth number between species (Walker 2004), but do not show any irregularities (see front page of Science, Walker and Dew 1972). Also the song of another New World species, *Orophus conspersus* (Brunner von Wattenwyl, 1878), contains several different song elements (Taliaferro et al. 1999), produced with a simple file (see Cadena-Castañeda 2014). The calling songs of two African *Plangia* spe-

cies, however, are relatively simple (Hemp et al. 2015). They consist of short di- or tri-syllabic echemes or of single syllables, sometimes combined into small series. In contrast, Paraplangia males produce a relatively long song which is answered by the female at its end, suggesting that the latter evaluates the preceding syllable pattern. The very short syllables would seem to be suitable as trigger for a very fast female response (e.g. Heller et al. 2018), but there is no strict temporal correlation between the female response and any parameter of the male song. The female responded with a relatively long and variable delay of about 0.5 s after a syllable (mostly the last) of the second series of the male, so the female signal may be interpreted by eavesdropping male rivals as an additional male syllable. Also the carrier frequency and the intervals between additional female responses would fit into this pattern. However, the female sometimes produced quite fast response series with unknown function. The male file with its low teeth number and abrupt change in tooth density differs from all genera mentioned above.

Paraplangia has a relatively broad fastigium verticis. This character is generally rare in long-winged phaneropterines worldwide as well as in Africa (e.g. in *Monteiroa* and *Pseudoplangia*; Ragge 1980), but surprisingly frequent in Madagascar (genera *Eurycorypha, Madagascarantia, Plangia, Paraplangia*). It is unknown if the reasons are phylogenetic, biogeographical, ecological or accidental. In any case, the commonness is not based on a Malagasy radiation. All these genera are currently included in Amblycoryphini. Surprisingly, they also share a flat (only in some *Plangia* and a few *Eurycorypha* very slightly sulcate; own unpublished observations) or rounded dorsal surface of the fore tibiae, which is also uncommon among Phaneropterinae. In the amblycoryphine genera *Amblycorypha* and *Orophus*, fore and middle tibia are dorsally sulcate (Brunner von Wattenwyl 1878). In size, habitus of adults and nymphs, and shape of the male subgenital plate, *Paraplangia* shows surprising similarities to *Gonatoxia* (Hemp in preparation), but differs in width of fastigium and structure of the tympana.

Representatives of the two Amblycoryphini genera examined in this study, Paraplangia and Orophus, have a karyotype including 31 (male) or 32 (female) acrocentric chromosomes with X0 (male) and XX (female). This chromosome number coincides with previous studies, which revealed that more than 50 genera of phaneropterines found in the Palaearctic region, South America, East Africa and India are characterized by such basic/ancestral karyotypes (e.g. for review see Warchałowska-Śliwa 1998, Hemp et al. 2014). It should be noted that this chromosome number, morphology (except for the bi-armed X chromosome) and sex determination system was found also in Canadian specimens of Amblycorypha oblongifolia (De Geer, 1773; Beaudry 1973). However, this plesiomorphic chromosome number is reduced to 29 (X0) in the African genera *Plangia* (Hemp et al. 2015) and *Eurycorypha* (Hemp et al. 2013 and unpublished data Warchałowska-Śliwa) as a result of a tandem fusion between two pairs of autosomes. Additionally, in comparison to Paraplangia and Orophus with an ancestral acrocentric X chromosome, pericentric inversions modified the position of the centromere and changed the morphology of the X chromosome from acrocentric to bi-armed in A. oblongifolia and some species of Plangia and Eurycorypha. In the Amblycoryphini chromosomes described in this paper as well as in that of Eurycorypha and Plangia (Hemp et al. 2013, 2015, respectively) one 18S rDNA FISH locus (per haploid genome) coincides with a single active NOR and C-band segment, independently from the number of chromosomes in the set. However, in these species, different patterns of the location of rDNA/NOR were observed. The single 18S rDNA loci revealed by FISH are located distally on the largest autosome in the Malagasy Paraplangia (2n = 31, present study) and the African Plangia (2n = 29, Hemp et al. 2015), but were found in the paracentromeric region of a medium-sized chromosome in the Costa Rican Orophus (2n = 31; present paper) and in an interstitial region on a short autosome in African species of Eurycorypha (Hemp et al. 2013). The cytogenetic study presented here constitutes another step to better understanding of chromosome organization and the evolution of Phaneropterinae species and genera.

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Supplementary material 1

Authors: Klaus-Gerhard Heller, Claudia Hemp, Bruno Massa, Maciej Kociński, Elżbieta Warchałowska-Śliwa

- Data type: WAV file
- Explanation note: Male-female-duet 1 (2-channel-recording) with female response consisting of few impulses (microphone male UHER M645, female SONY ECM-121, T=25°C).
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Supplementary material 2

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Data type: WAV file

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The fishing mantid: predation on fish as a new adaptive strategy for praying mantids (Insecta: Mantodea)

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Abstract

Observations in unmanipulated, semi-natural conditions were made of a single individual of the praying mantid *Hierodula tenuidentata*, while hunting and capturing an unusual prey for this kind of insect, guppy fish, *Poecilia reticulata*. This repetitive fishing behavior, recorded daily, is reported here for the first time and discussed in relation to the adaptive behavioral plasticity of praying mantids. We speculate regarding learning from experience as a hunting strategy.

Key words

behavior, evolution, India, learning, vision

Introduction

Mantids can be considered generalist predators but they are known to feed mostly on other insects, especially on fly-like insects (Prete et al. 1999). Vertebrates as prey for mantids are historically considered "anomalous data" based on anecdotal records, mostly from caged or in some way "induced" encounters between mantids and small birds, lizards, frogs, newts, mice, snakes and turtles (reviewed in Kevan 1985, Prete and Wolfe 1992, Costa-Pereira et al. 2010).

Several observations in the literature (i.e. Butler 1949, Hildebrand 1949, Prescott 1968) describe the predation of birds by mantids. However, McCormick and Polis (1982) point out that these observations are inconclusive since the birds captured by mantids were ultimately freed by concerned ornithologists. It is, however, stated that large praying mantids have the ability to kill small birds like hummingbirds and brown creepers. Recently, passerine birds trapped in mist nets have also been reported as occasional prey for mantids (Copete 2006). A recent overview of mantid predation on birds suggests that this behavior may be frequent in mantids, having already been recorded for 12 large-sized mantid species (including the genus *Hierodula*) in 13 different countries world-wide, preying on over 24 species of birds, with a marked preference for hummingbirds attracted to artificial birdfeeders (Nyffeler et al. 2017). Moreover, even if without scientific validation, it is not difficult to find several pictures and reports on the internet of caged (induced or manipulated) encounters/fights between mantids and a variety of small vertebrates.

Materials and methods

A single, 5.6 cm large male specimen of *Hierodula tenuidentata* Saussure, 1869, unequivocally recognizable by a white antennomerus on the right antenna, was occasionally observed in a private roof garden in Karnataka, India, preying on guppy fish, *Poecilia reticulata* Peters, 1859 (Fig. 1). The predations were observed every day for five consecutive days from the 7th to the 11th of March 2017 during periodical scans of the habitat from sunset at 6:30 pm to 12:30 am. Therefore, they were unmanipulated and occurred naturally without interference. About 40 fish [mostly *Poecilia reticulata*, few *Danio rerio* Hamilton, 1882 and one *Hypostomus plecostomus* (Linnaeus, 1758)] were contained in an artificial pond made in an earthen planter of 58 cm diameter, under natural light conditions. The mantid was able to reach the fish by perching on leaves of water lilies (*Nymphaeaceae*) and water cabbage (genus *Pistia* Linnaeus, 1785) growing on the surface of the pond.

The garden is located on top of a building about 5 m from the ground and containing about 15 potted plants. Other natural prey for mantids like wasps, butterflies, spiders, etc., have been frequently observed on these plants and in the garden which, even if mostly artificial, can be considered a semi-natural and functional habitat for a mantid like *Hierodula*, which is well-accustomed to gardens and urban areas (Kurosawa et al. 2003, Leong 2009).

Results

During the five days, the mantid was observed capturing and devouring a total of nine guppy fish. In seven cases, the mantid started eating from the tail (Fig. 2). On a single occasion, he started from the head and on another, from the top side. On the first four of the five days, the mantid was observed to hunt and devour



Fig. 1. The artificial pond with the male of Hierodula tenuidentata eating a Poecilia reticulata. Photo by R. Puttaswamaiah.

two fish. The second fish was hunted within 10–30 mins of consuming the first one. After the fifth day, the mantid disappeared and was not observed again at the pond.

Discussion

Fish do not move like lizards, locusts, hummingbirds or flies. They swim under the usual hunting field of a mantid and are separated by the barrier of water. Some other invertebrates like spiders, and especially fishing spiders, can hunt in water, but the origin of this adaptation occurred more than once in their evolution (Nyffeler and Pusey 2014), and is not well understood. Our observations in this fishing mantid – a unique case at the moment – may indicate the origin of this phenomenon.

Despite the limitations, our observations raise three important fields for speculation. First, this case confirms that in their natural habitat, mantids can and do feed on vertebrates, even on fish. In this case, of the approximately 40 individual fish present in the pond, nine of them were eaten by the mantid within a span of five days, showing the potential for a single invertebrate to have a strong impact on the fish community and, since guppies, like many other small fish, are active predators of aquatic insects, indirectly on the whole pond ecosystem.

Second, although the compound eyes of most mantids are appositional, sensitive to movement, and adapted to vision mostly in daylight (Kral 2012), this male was able to see and catch the fish under the water at night and to overcome refractive challenges. The

fish were caught near the water's surface, always after sunset, sometimes late at night and, in general, in low light [compact fluorescent lamp (CFL)] conditions. Male mantids are known to be very active during the night (Battiston et al. 2010), but these fishing events suggest further visual abilities of mantids that should be investigated.

Third, the predation was not just occasional, but repeated. This scenario, from a more speculative perspective, suggests the possibility that the insect learned from the experience where and what to hunt. Mantids are known to use aversive learning to avoid toxic prey (Carle et al. 2018), a first basic learning ability shared by many predators. This case, however, suggests a further step to a more articulated cognitive process, including the ability to learn not only from a single stimulus but from different environmental clues and experiences, for elaborate new hunting strategies.

Many mantids, including *Hierodula*, are known to be sit-andwait predators (Prete et al. 1999), and there is evidence that at least some species carefully choose their habitat and hunting field (Battiston and Fontana 2010, Watanabe et al. 2013, O'Hanlon et al. 2014). Remembering the prey's abundance at a particular site in relation to their ease of capture and their nutritional content, could be one important factor of this choice and may indirectly influence individual fitness. This should be investigated in further studies.

Acknowledgments

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Fig. 2. *Hierodula tenuidentata* eating *Poecilia reticulata* from the tail while the fish is still alive and breathing in the water. Photo by R. Puttaswamaiah.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Supplementary material 1

Author: Roberto Battiston, Rajesh Puttaswamaiah, Nayak Manjunath

Data type: JPEG file

Explanation note: The mantid eating another fish from the head.

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Supplementary material 2

Author: Roberto Battiston, Rajesh Puttaswamaiah, Nayak Manjunath

Data type: JPEG file

Explanation note: The mantid resting under a leaf during the day. Copyright notice: This dataset is made available under the Open

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Build it and they will come: grasshoppers check-in to a grassland bee hotel

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Abstract

A five-floor bee hotel was constructed using wooden pallets in an area of urban grassland in Ipswich, United Kingdom. Within one month of construction, two grasshopper species were observed using the hotel, with nymphs in shaded, uncut grass at the base, while adults were observed on all five floors and the roof. On the fourth floor, a stridulating field grasshopper, *Chorthippus brunneus*, was sighted on two separate occasions. Further research is required to determine whether bee hotels may be an effective 'shade' refuge for grasshoppers in open grassland, while also providing an effective platform for stridulation.

Key words

Acrididae, biodiversity, bug hotel, conservation, urban

Introduction

In urban areas, bee hotels are considered to be part of the solution for reversing pollinator declines linked to loss of foraging and nesting habitats (Moenen 2012, MacIvor and Packer 2015). Many bee hotels specifically focus on providing cavities for a range of above-ground bee species to nest in (Gaston et al. 2005). To provide these nest sites, various materials are used including bundles of dry stems and holes drilled in wood (Lee-Mäder et al. 2010, Vickruck and Richards 2012). However, concern has been expressed about the efficacy of these hotels in conserving the intended bee species due to parasitism of nests (MacIvor and Packer 2015). The apparent use of bee hotels by other taxa is also not well reported in the scientific literature. It is with this latter point in mind that the usage of a bee hotel by grasshoppers (Orthoptera: Acrididae) was studied in an urban grassland in the UK. The findings are discussed in relation to the biology of the grasshopper species observed and further research is suggested.

Methods

Bee hotel construction.—A bee hotel was constructed from wooden pallets (Fig. 1) in a well-drained grassland area at the Environment Agency's Ipswich office in Suffolk, United Kingdom (Ordnance Survey grid reference: TM 19277 42710).

The bee hotel was constructed on 21 June 2018 using five wooden pallets (each 80×120 cm, 13 cm height) placed on top of each other to create a five-floor structure. Within the gaps in the pallets, oak (*Quercus robur*) branches and cut reeds (*Phragmites australis*) were placed in bundles. The bottom story was left unfilled to allow vegetation to grow through the hotel creating a more natural habitat for insects. The hotel was in full sun for most of the day, with shade provided by overhanging vegetation (bramble, *Rubus fruticosus*) on a first-floor 'gallery' created by the base pallet being larger than those above. On the roof of the fifth-floor pallet, plastic plant pots of shingle were placed to create nesting sites for bees along with dried shrub leaves to provide cover from rain. Holes were drilled in the wooden pallets to create nesting sites for solitary bees.

Grasshopper and microclimate surveys.—The bee hotel was inspected on seven occasions (for around 15 mins each time) from 12 July to 1 August 2018 with a thorough inspection of basal vegetation, all floors (and roof) and the vegetation on the 'gallery.' To determine whether the frequency of grasshopper observations was significantly different than expected, a one-way Chi-square (χ^2) goodness of fit test was undertaken.

On 30 July (1600 h) a thermometer was used to record the ambient air and compare it with microclimatic temperatures of the five floors and roof of the bee hotel. The ambient air temperature at 1 m from the ground was compared to the temperature of the five floors and roof of the bee hotel. To compare the microclimate of the main fill materials, the mean temperature of the reed (floors two and four) and oak branch (floors three and five) bundles was compared using a Student's t-test.

Results and discussion

On 12 July (1600 h), less than one month after construction, a field grasshopper, *Chorthippus brunneus*, late instar nymph was seen in shade on the second floor and a lesser marsh grasshopper, *Chorthippus albomarginatus*, was observed singing from grass at the base of the first-floor pallet. A male *C. brunneus* was also seen stridulating from the fourth floor. A further stridulating male of this species was sighted singing from a reed bundle on the fourth floor on 1 August.

On 13 July (1630 h), a further three grasshoppers were sighted on the hotel. Two adult male *C. brunneus* were seen in grass at the base of the first-floor pallet, while a nymph of the same species jumped from an adjacent bramble leaf onto the second floor. The mean microclimatic temperature for the five floors and roof (25 °C) was lower than the ambient air temperature at 1 m above ground (27 °C). Therefore, grasshoppers may be using the bee hotel as a source of shade from excessively hot microclimatic temperatures in the sparse grassland in an unusually hot and dry spell similar to the summer of 2003 (Gardiner and Hassall 2009). This kind of behavioral thermoregulation to avoid overheating is well known in Orthoptera (Willott 1997).

Furthermore, mean temperature difference (from ambient air temperature) in the reed bundles of floors two and four (-2.5 °C) was significantly different (t = 3.68, P = 0.03) from that of the oak branch bundles of floors three and five (-1.3 °C). This suggests that the fill material for the pallet layers could have a key influence over the usage of the bee hotel by insects as a shade refuge from high temperatures, with bundles of reed being particularly cool. Unfortunately, there were not enough observations in this small study to statistically test the issue, although only two grasshoppers were sighted on the warmer oak bundles compared to seven on the cooler reed (Fig. 2). We suggest further detailed experimental design with replication to test the efficacy of bee hotels in providing habitat for grasshoppers in grassland. These studies should allow meaningful statistical analysis to be undertaken.

The observation of grasshoppers stridulating from the fourth floor suggests that the hotel may also form an effective platform from which to broadcast mating calls, perhaps providing an advantage over enclosed vegetation (Robinson and Hall 2002). It is unlikely that grasshoppers will use the hotel for feeding due to the dry nature of the reed in the bundles and presence of woody vegetation (Gardiner and Hill 2004). However, shaded grass at the base of the hotel may provide a suitable feeding environment of 'live' vegetation away from the high temperatures of the open grassland. The grassy vegetation growing through the 'gallery' may also be a suitable feeding environment for grasshoppers. However, the distribution of grasshoppers on the hotel was not significantly different from that expected (Chi-square $\chi^2 = 10.5$, d.f. 5, P = 0.06) with the lower two floors (including the 'gallery') having the most observations (Fig. 3).

Additional observations were undertaken in August 2018 on a grassland bee hotel in Dedham, UK (approx. 16 km south-west of Ipswich bee hotel). A meadow grasshopper, *Chorthippus parallelus*, and long-winged conehead, *Conocephalus fuscus* (Orthoptera: Tetti-goniidae), were observed in basal vegetation while the former species was also sighted on the roof. These observations would seem to concur with those from Ipswich that bee hotels and their immediate grassland surroundings have some value for Orthoptera.

In conclusion, further experimental research is required to determine whether bee hotels may be an effective 'shade' refuge in open grassland with little cover, while also providing an effective platform for stridulation. Replicated studies from a wide range of bee hotels would further aid the design to promote use by nontarget insects such as grasshoppers.

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Figure 1. The bee hotel. Photo credit: T. Gardiner.



Figure 2. Male field grasshopper, *Chorthippus brunneus*, on fourth-floor reed bundle, observed stridulating. Photo credit: T. Gardiner.



Figure 3. Frequency of observed grasshoppers on the five floors and roof of the bee hotel.

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Morphometric variation among males of *Orphulella punctata* (De Geer, 1773) (Acrididae: Gomphocerinae) from different biomes in Brazil

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Abstract

The objective of the present study was to examine variation in the shape and size of pronotum, hind femur, and head in the males of *Orphulella punctata* (De Geer, 1773) from three different Brazilian biomes: the Cerrado, the Atlantic Forest, and the Pantanal. A total of 150 specimens were analyzed from three populations. The results of MANOVA indicated significant differences (p<0.01) in the shape of the analyzed structures of *O. punctata* from the different biomes. The results of ANOVA demonstrated significant differences (p<0.05) in the size of all analyzed structures. Pearson's correlation analyses among the different structures and the environmental variables revealed that the shape of pronotum, hind femur, and head (dorsal view), as well as the size of pronotum and head (dorsal and lateral views) varied with the geographic longitude, while the shape of hind femur and head (dorsal view) showed a significant negative correlation with size. Results indicated that the shape and size of the analyzed structures, in general, were influenced by the geographical variables.

Key words

geometric morphometrics, hind femur, Orthoptera, population analysis, pronotum, shape, size

Introduction

Grasshoppers are characterized by diverse forms and colors, and a range of ecological and economic importance (Sperber et al. 2012) particularly due to their potential for causing damage to agriculture, as certain grasshopper species are phytophagous with defoliating behaviors (Carrano-Moreira 2015). Among such species, *Orphulella punctata* (De Geer) shows a wide geographical distribution ranging from Mexico to Argentina (Cigliano et al. 2018). This species is common in both native and anthropogenic areas and is considered a pest that causes minor damages to agricultural crops in Brazil (Guerra et al. 2012).

Gomphocerine grasshoppers may be identified by characters of their external morphology (Otte 1979). However, in this subfamily as well as in other grasshopper groups, individuals of the same species occupying different geographical locations may exhibit

morphological variation in several structures of their external morphology (Pocco et al. 2014). Geometric morphometrics is a technique that has been used widely in studies conducted on morphological variations at the intraspecific level throughout the geographical distribution range of species (Rattanawannee et al. 2012, Zelditch et al. 2012).

Geometric morphometrics is a tool for detecting morphological variation among the organisms through the identification of landmarks and the subsequent evaluation of the relationships between these landmarks and certain other variables, such as environmental or geographical variables (Klingenberg 2013). This technique also allows the detection of significant differences in the shape and size of body structures with the objective of revealing intraspecific and interspecific variation that may be associated with evolutionary and ecological factors (Bower and Piller 2015). Therefore, this technique has become useful for the identification of variation among the populations of a given species (Oleksa and Tofilski 2015).

Geometric morphometrics also enables the analysis of correlations between the form and size of structures and the patterns of distribution of individuals across various geographical areas as well as with the patterns of diversification of their life histories. As morphometric variations among insect populations are generally associated with differences in geographical and environmental variables, this methodology has proved to be very useful in this group of organisms (Nunes et al. 2012, Romero et al. 2014, Prado-Silva et al. 2016).

Although geometric morphometrics has been demonstrated to be effective in analyzing morphological variation in insects, this methodology has been underused in studies of Orthoptera (Song and Wenzel 2008, Song 2009, Bidau et al. 2012, Cisneiros et al. 2012). In the present paper we analyze morphometric variation in males of *O. punctata* belonging to populations collected from three different biomes in Brazil.

Material and methods

Study area.—The specimens were collected from three Brazilian biomes: the Cerrado, the Pantanal, and the Atlantic Forest. Study

sites were located in: Serra da Bodoquena, Bonito, MS (21°15'56"S, 56°42'10"W); Estrada Porto Cercado, located between river Bento Gomes and the Pantanal Advanced Research Base (BAPP) of the Federal University of Mato Grosso, Poconé, MT (16°18'55.01"S, 56°32'33.64"W); and the Fazenda Baixa de Areia, Highway BA-026, Serra da Jiboia, Varzedo, BA (12°57'41.9"S, 39°26'54.9"W) (Fig. 1).

According to the Brazilian Ministry of Environment (MMA), the Cerrado and the Atlantic Forest are considered biomes of great interest as they are rich in biodiversity and contain several endemic species. The Pantanal is characterized by pronounced wet seasons and is the smallest biome in the country, occupying just 1.76% of the total land area (MMA 2017).

Sampling.—In order to perform the morphometric analyses, a total of 150 *O. punctata* males (50 individuals from the Cerrado, 50 from the Atlantic Forest, and 50 from the Pantanal) were included in the study, except for the analysis of femur structure, where only 34 individuals from the Cerrado biome were included. All the specimens were deposited in the Laboratory of Ecology and Taxonomy of Insects (LETI), Biological Sciences Section, Center for Agricultural, Environmental and Biological Sciences (CCAAB), Universidade Federal do Recôncavo da Bahia (UFRB).

Analyses.-The pronotum (left lateral view, Fig. 2A), hind femur (left lateral view, Fig. 2B), and head (left lateral and dorsal views, Fig. 2C, D) were photographed using Zeiss Discovery V20.0 stereo microscope with an attached camera. The TPS series of programs (Rohlf 2006) were used for the analysis of shape and size, and the images were converted in the TPSUtil program (Rohlf 2010). The landmarks and semi-landmarks (Fig. 2) were inserted using the TPSDig2 program (Rohlf 2006). The Cartesian coordinates were subjected to Procrustes superimposition, and Principal Component Analysis (PCA) and discriminant analysis were performed using MorphoJ software (Klingenberg 2011). MANOVA was used for analyzing the differences in the shape of the structures and ANOVA was used for analyzing the differences in the size (centroid size) of the structures. Pearson's correlations were performed in order to evaluate the relationships between the shape and size of the structures and the geographical variables (latitude, longitude, and altitude), using Past software v. 2.16 (Hammer et al. 2001). Additionally, a cluster analysis was performed using the same program by applying the UPGMA method, and the cophenetic correlation coefficient was calculated.

Results

Analysis of pronotum shape in O. punctata.—MANOVA results revealed significant differences (p<0.01) in the pronotum shape among the *O. punctata* males from the different biomes that were analyzed. The results of Hotelling's test (T²) showed that the *O. punctata* males from the Atlantic Forest differed significantly in comparison to the males from the Pantanal and the Cerrado.

The first four components explained 84% of the total variation in the pronotum shape. The first PCA explained 40%, the second one explained 24.8%, the third one 13.6%, and the fourth 5.6%. The greatest variation in the pronotum shape was observed in the dorsal and ventral posterior regions (Fig. 3A, B).

The first PCA also revealed that the specimens from the Pantanal site differed in comparison to the populations from the other biomes (Fig. 3C). Specimens from the Pantanal site were located on the positive axis of PCA 2 and exhibited the greatest distortion in the posterior ventral region of pronotum which



Fig. 1. Collection sites for *Orphulella punctata* (De Geer, 1773): Cerrado, Atlantic Forest and Pantanal.



Fig. 2. Lateral view of *Orphulella punctata* (De Geer, 1773). A. Pronotum: 10 anatomical points; B. Femur: 18 anatomical points; C. Lateral view of the head: 16 anatomical points; D. Dorsal view of the head: 18 anatomical points. Black circles represent the landmarks and white circles represent the semi-landmarks.

expanded, as indicated by the Thin-Plate spline. On the other hand, this structure contracted in the individuals collected from the Cerrado and Atlantic Forest sites (Fig. 3C).

Procrustes distance matrix showed a significant difference (p<0.01) in pronotum shape between the three biomes (Table 1).

Table 1. Procrustes distance of *Orphulella punctata* pronotum shape among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0004**	0.0001**
Atlantic Forest	0.0270	0	0.0001**
Pantanal	0.0622	0.0534	0
** : : : ()	(10)		

hich ** significant (p<0.01).



Fig. 3. Scatter plot of the Principal Components Analysis (PCA) of *Orphulella punctata* (De Geer, 1773) pronotum shape in populations collected from the Cerrado, Atlantic Forest, and Pantanal. **A**. Thin-plate spline of the positive (+) and **B**. negative (-) axes of PCA 2; **C**. PCA plot.

The UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 95% corroborated the above-mentioned results of PCA, demonstrating that the population from the Pantanal was distantly related to the group that was constituted by the populations from the Atlantic Forest and the Cerrado (Fig. 4).

Analysis of the shape of the hind femur.—MANOVA results demonstrated significant differences in the shape of hind femur among the populations from the different biomes (p<0.01). The first four PCAs accounted for 78.5% of the total variation in femur shape: the first PCA explained 54.8%, the second one explained 13.3%, the third one 6.1%, and the fourth 4.3%. It was possible to verify differences in the femoral structure among the *O. punctata* populations from the analyzed biomes where specimens from the Cerrado biome were separated from individuals from the other two biomes.

On the positive axis of PCA 1, a contraction in the proximal region (points 2 and 17) of the femur in the individuals from the Pantanal and an expansion in the medial region (points 5, 6, 14, and 15) could be observed (Fig. 5A, C). The inverse of this occurred on the negative axis, i.e. an expansion in the proximal region and a contraction in the medial region of the femur (Fig. 5B).

The Procrustes distance matrix revealed significant differences in shape of the hind femur (p<0.01) among the studied populations (Table 2).

Similar to the PCA plot (Fig. 5C), UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 100% showed that the population from the Cerrado biome was distantly



Fig. 4. Similarity dendrogram for the pronotum in *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 97.1%.



Fig. 5. Scatter plot of the Principal Components Analysis (PCA) from *Orphulella punctata* (De Geer, 1773) femur shape in populations collected in the Cerrado, Atlantic Forest, and Pantanal. A. Thin-plate spline of the positive (+) and B. negative (-) axes of PCA 1; C. PCA plot.

Table 2. Procrustes distance of *Orphulella punctata* femur shape among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0001 * *	0.0001**
Atlantic Forest	0.0321	0	0.0073**
Pantanal	0.0218	0.0113	0

** significant (p<0.01).

related to the group constituted by the populations from the Atlantic Forest and the Pantanal (Fig. 6).

Analysis of the shape of the head in dorsal view.—MANOVA results revealed significant differences in the shape of head in dorsal view among the *O. punctata* populations from the different biomes (p<0.01). The PCA showed that the first four components explained 77.6% of the total variation: the first PCA explained 44.9%, the second one explained 13.9%, the third one 11.3%, and the fourth 7.5%. The major variations in the shape of head in dorsal view occurred in the distal region of the head (near the pronotum), in the medial portion, and in the fastigium. The greatest distortion occurred in the anterior and medial regions of the head, which expanded on the positive axis, as indicated in the deformation grids (Fig. 7A), and contracted on the negative axis (Fig. 7B).

The second axis of the PCA revealed that the Cerrado population was slightly distinct from the populations of the other biomes (Fig. 7C).



Fig. 6. Similarity dendrogram for the femur from *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 86.83%.



Fig. 7. Scatter plot of the Principal Components Analysis (PCA) from *Orphulella punctata* (De Geer, 1773) dorsal head shape in populations collected in the Cerrado, Atlantic Forest, and Pantanal. A. Thin-plate spline of the positive (+) and B. negative (-) axes of PCA 2; C. PCA plot.

Results from the Procrustes distance matrix demonstrated significant differences (p<0.01) in shape of the head in dorsal view among the populations (Table 3).

The UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 97% corroborated the PCA results, showing that the population from the Cerrado biome was distantly related to the group constituted by the populations from the Atlantic Forest and the Pantanal (Fig. 8).

Analysis of the shape of the head in lateral view.—MANOVA results indicated that the shape of the head in lateral view differed significantly among the *O. punctata* populations from the different biomes (p<0.01). The PCA demonstrated that the first four components explained 67.6% of the total variation in this trait. The first principal component explained 28.5%, the second one explained 20.6%, the third one 12.1%, and the fourth 6.4%. The greatest variation in the shape of the head in lateral view was

Table 3. Procrustes distance of *Orphulella punctata* shape of the dorsal view of the head among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal			
Cerrado	0	0.0001 * *	0.0001**			
Atlantic Forest	0.0187	0	0.0115*			
Pantanal	0.0262	0.0141	0			

* significant (p<0.05), ** significant (p<0.01).



Fig. 8. Similarity dendrogram for the head in dorsal view for *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 79.2%.



Fig. 9. Scatter plot of the Principal Components Analysis (PCA) from *Orphulella punctata* (De Geer, 1773) lateral head shape in populations collected in the Cerrado, Atlantic Forest, and Pantanal. A. Thin-plate spline of the positive (+) and B. negative (-) axes of PCA 2; C. PCA plot.

observed near the occipital suture and in the eye. Although a uniform distribution occurred, the major distortion was observed in the regions just mentioned, which contracted on the positive axis (Fig. 9A) and expanded on the negative axis (Fig. 9B). The second axis of the PCA did not represent sufficient differences to separate the populations from the different biomes (Fig. 9C).

The Procrustes distance matrix revealed significant differences in the characteristics analyzed (p<0.01), indicating differences in the shape of head in lateral view among the populations (Table 4).

The UPGMA with 10,000 permutations and a cophenetic correlation coefficient value of 92% showed that the population from the Cerrado biome formed an independent branch in the dendrogram separated from the group constituted by the Pantanal and the Atlantic Forest populations (Fig. 10).

Analysis of the size of pronotum, hind femur, and head in Orphulella punctata.—ANOVA analyses demonstrated significant differences in size among the populations from the different biomes (p<0.01)

Table 4. Procrustes distance of *Orphulella punctata* shape of the lateral view of the head among three Brazilian biomes. Values in lower half of distance matrix and significance in top half of the distance matrix; 10,000 permutations.

	Cerrado	Atlantic Forest	Pantanal
Cerrado	0	0.0001**	0.0001**
Atlantic forest	0.0360	0	0.0013**
Pantanal	0.0343	0.0257	0

** significant (p<0.01).



Fig. 10. Similarity dendrogram for the lateral view of the head in *Orphulella punctata* populations from the Cerrado, Atlantic Forest, and Pantanal by the UPGMA method. The permutation test was carried out with 10,000 replicates and a cophenetic correlation coefficient of 98.8%.
for all the analyzed structures. Tukey's test confirmed that pronotum and head in lateral view were larger in individuals from the Cerrado (Fig. 11A, D). The Atlantic Forest population had a larger hind femur in comparison to individuals from the other biomes (Fig. 11B), and the largest size of the head in dorsal view was found in the Pantanal (Fig. 11C).

Correlation analyses between the shape and size of body structures and the geographical variables.—Pearson's correlation analyses between the shape and size of the pronotum, hind femur, and head in *O. punctata*, and the geographical variables (latitude, longitude, and altitude) showed a significant negative correlation between shape × longitude, size × latitude, and size × longitude of the pronotum. A negative correlation was observed between shape × latitude, shape × longitude, and shape × size of the hind femur. The head in dorsal view revealed a negative correlation between size × longitude and shape × size; while the head in lateral view showed a negative correlation between shape × longitude, size × latitude, and size × longitude (Table 5). These results indicated that the morphology of pronotum, hind femur, and head varied in relation to the geographical variables.

Discussion

Among the evaluated structures in O. punctata, the hind femur and the head in the population from the Cerrado biome differed in comparison to the other populations from the remaining two biomes. The pronotum of the Pantanal population was significantly different from the other two populations. Nunes et al. (2007), while studying Melipona scutellaris Latreille (Hymenoptera: Apidae), suggested that variation among geographically isolated populations might occur due to the existence of a barrier that prevents frequent migrations of individuals. For example, in bees, wing shape is influenced by geographical distance (Nunes et al. 2012, Lima et al. 2014, Prado-Silva et al. 2016). In a previous study, the relationship between shape and geographical distance was highlighted, suggesting that individuals distributed across different latitudes, longitudes, and altitudes might present differences that are associated with adaptations to local conditions (Berner et al. 2004). Similar patterns were observed in the present study, where the correlation analyses indicated that both the shape and the size of the morphological structures analyzed herein might be influenced by the geographical variables.



Fig. 11. Analysis of the size of the A. pronotum, B. femur, C. head in dorsal view and D. head in lateral view. Similar letters indicate that these biomes are statistically equivalent in relation to the size of the pronotum, femur, dorsal, and lateral view of the head by Tukey's test (p<0.05).

Table 5. Correlation between the shape and size of the pronotum, femur, and dorsal and lateral views of the head of *Orphulella punctata* with latitude, longitude, and altitude.

		R	Р
Pronotum	Shape × Latitude	-0.0968	0.2383 ^{ns}
	Shape × Longitude	-0.2295	0.0047**
	Shape × Altitude	-0.1091	0.1837 ^{ns}
	Size × Latitude	-0.3044	0.0001 * *
	Size × Longitude	-0.2368	0.0035**
	Size × Altitude	0.2308	0.0044**
	Shape × Size	-0.1516	0.0639 ^{ns}
Femur	Shape × Latitude	-0.6112	0.0000 **
	Shape × Longitude	-0.4583	0.0000 **
	Shape × Altitude	0.4414	0.0000 **
	Size × Latitude	0.5311	0.0000 **
	Size × Longitude	0.6829	0.0000 **
	Size × Altitude	-0.0160	0.8544 ^{ns}
	Shape × Size	-0.4916	0.0000 **
Dorsal Head	Shape × Latitude	0.0681	0.4075^{ns}
	Shape × Longitude	-0.0316	0.7007^{ns}
	Shape × Altitude	-0.1518	0.0639 ^{ns}
	Size × Latitude	-0.0934	0.2551 ^{ns}
	Size × Longitude	-0.1876	0.0214*
	Size × Altitude	-0.0651	0.4281 ^{ns}
	Shape × Size	-0.2587	0.0013**
Lateral Head	Shape × Latitude	-0.1412	0.0847^{ns}
	Shape × Longitude	-0.1590	0.0518*
	Shape × Altitude	0.0488	0.5526 ^{ns}
	Size × Latitude	-0.3526	0.0000 **
	Size × Longitude	-0.2239	0.0058**
	Size × Altitude	0.3272	0.0000**
	Shape × Size	0.0080	0.9225 ^{ns}

* significant (p<0.05), ** significant (p<0.01), ^{ns} not significant.

Intraspecific variation may complicate taxonomic studies, especially when the variation occurs in the structures that have been used as taxonomic characters for identification, as is the case of the pronotum and head (fastigium) in *O. punctata* (Otte 1979). Intraspecific variation may also occur in the internal structures in grasshoppers, as demonstrated by Song and Wenzel (2008) in a study on the male genitalia morphology among three populations of *Schistocerca lineata* Scudder.

Species that exhibit a wide geographical distribution tend to present both geographical variations and polymorphism, which may lead to incorrect identifications (Pocco et al. 2014). O. punctata is widely distributed from Mexico to Argentina, with polymorphism as a common observation among its populations (Dixon 2005). Liebermann (1947) considered that O. punctata may be one of the acridid species with greatest variation in size and color. Rehn (1906) was the first to report the existence of variation in the structure and color among individuals belonging to different populations collected from diverse regions of Brazil and the U.S. Virgin Islands. In 1916, the same author (Rehn 1916) remarked on the existence of a number of variations among the populations of this plastic and widespread species collected from three different northern states of Brazil. Rehn (1918) then reported the existence of variation in the head and pronotum of the individuals belonging to two populations from Para, Brazil. Bruner (1913) also reported the existence of variation in size among the individuals of O. punctata collected from Peru.

Hebard (1923) described a remarkable variability of this species, especially in size, pronotal contour, and coloration, which has often led to misidentifications generating unusually complex synonymies (including the description of *O. punctata* populations as different species placed in different genera such as *Oxycoryphus* and *Stenobothrus*). Cigliano et al. (2018) reported 19 synonyms of *O. punctata*.

In a taxonomic review of this group, Otte (1979) considered that *O. punctata* is easily misidentified with *O. aculeata* Rehn in central Mexico, and with *O. losamatensis* Caudell and *O. concinnula* (Walker) in Central and South America. The same author none-theless mentioned the importance of the hind femur and shape of the pronotum as the characteristics for distinguishing *O. punctata* from the remaining species of *Orphulella*. However, and according to the results obtained in the present study, the hind femur and pronotum also displayed variations among the populations from different biomes, and hence other structures and/or methods of identification are required for the purpose of a correct identification of this species.

In addition to the structures suggested by Otte (1979) as diagnostic characters for identification of O. punctata, the present study showed that the head also presented intraspecific variation, reinforcing the needs for other structures to be used in the identification of this species. Intraspecific variations in the morphological characters, such as head, pronotum, femur, body, and wings, have also been reported in studies conducted on the populations of Chromacris speciosa (Thunberg) collected from two locations in Pernambuco, Brazil (Cisneiros et al. 2012). Also, analyses of the body size and the wings of Trilophidia annulata (Thunberg) (Orthoptera: Oedipodinae) demonstrated significant differences among the populations collected from different kinds of environments in China (Bai et al. 2016), which corroborates the occurrence of variations in individuals of the same species living in different biomes. Whitman (2008) demonstrated that body size in Orthoptera varies both between and within species, mainly as a result of environmental factors.

It is concluded that the shape and the size of the analyzed structures of *O. punctata* vary among biomes, indicating the possible influence of environmental conditions on the variations in the morphology of this species. The geometric morphometrics analyses conducted in this study indicated that it is possible to separate the populations from different biomes by the shape and size of their various body parts.

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Microhabitat segregation among three co-existing species of grasshoppers on a rural meadow near Seoul, South Korea

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Abstract

Microhabitat segregation among grasshopper species in Asia has not been well studied. We determined the differences in the use of substrates by three common North East Asian grasshopper species co-existing on a natural meadow near Seoul, South Korea. While many *Oedaleus infernalis* individuals were found on the ground, *Acrida cinerea* and *Atractomorpha lata* were usually observed on plants. *Acrida cinerea* was mostly observed on the grass *Zoysia japonica* (Poaceae) and *Atractomorpha lata* was mostly found on plants from the family Asteraceae. This is the first study to provide quantitative information about microhabitat differences among some common grasshoppers in rural habitats of continental North East Asia. Future studies should focus on determining the mechanisms that produce such ecological segregation.

Key words

Acrida cinerea, Atractomorpha lata, ecology, microhabitat, Oedaleus infernalis

Introduction

Microhabitat selection is important for small ectothermic animals including insects (Ahnesjö and Forsman 2006, Gardiner and Hassall 2009), and for herbivores with specific diets (including those that sequester unpalatable chemicals from plants for their own protection against predators; Sword et al. 2000, Sword 2002). Different species of grasshoppers have different diets (Joern 1979, Otte 1981, Chu 2002) and they move between sunlit and shaded areas to control their body temperature (Pielou 1948, Ahnesjö and Forsman 2006). Grasshoppers also choose microhabitats that provide better camouflage (Eterovick et al. 1997). Microhabitat segregation among co-existing species of grasshoppers has been studied in North America and Europe (Isely 1937, Joern 1979, 1982, Gardiner and Hassall 2009), but rarely studied in Asia (Tan et al. 2017, T. Gardiner pers. comm.). Here, we provide basic information on microhabitat segregation among three common grasshopper species in South Korea (Park and Kim 2011) and Japan

(Yoshioka et al. 2010). We chose to study the three species that were the most common at our study site: *Acrida cinerea* (Thunberg, 1815), *Atractomorpha lata* (Mochulsky, 1866), and *Oedaleus infernalis* Saussure, 1884. From classical ecological theory (Hardin 1960) and based on previous studies on grasshoppers (Isely 1937, Joern 1979, 1982, Gardiner and Hassall 2009, Tan et al. 2017), we expected that they would differ in their ecological niches.

Materials and methods

The observation site (37°24.07'N, 126°44.62'E) was comprised of a 10,000 m² lush grassland adjacent to Soraepogu Ecological Park, with an abundance of plants belonging to Asteraceae, especially Artemisia princeps and Aster pilosus. A hiking path crossed the meadow and each side of the trail was covered with a 1-m wide band of Zoysia japonica. To determine microhabitat segregation among the three species, we used a modified point-sampling technique (Joern 1982). A researcher moved very slowly through the grassland (including the 1-m wide band of Zoysia japonica) and noted the location of each detected individual grasshopper and the plant species (or ground) it was sitting on (or just jumped from). Each grasshopper species has distinctive morphology making the identification of species in the field relatively easy (Storoženko and Paik 2007, Kim 2013). Observations were carried out for four days from the end of August to the beginning of September 2017 and resulted in 327 grasshopper presence records. Plants were classified into four structural types which roughly aligned with plant family: Stem-plants, usually Asteraceae, consisted of one straight stem with leaves emanating to all sides; Low-vegetation plants, usually Zoysia japonica (Poaceae), were short and formed relatively dense cover; and Tall-grass included tall (50-150 cm) Poaceae with long and thin grass leaves. Other records of grasshoppers on rarely observed plant species were put into the category Others. Observations on the ground were classified into the Ground category. Plant structural type was closely correlated with plant family, so they are not independent. We performed two analyses,

one utilizing plant family as the dependent variable and the other utilizing structural type, as two alternative and correlated analyses of substrate use by grasshoppers. We used the Fisher's exact test (function *fisher.test* from the stat-package in R; Mangiafico 2015) to statistically test the null hypothesis of no differences among the three grasshopper species in their use of substrates. This test was most appropriate because our data were in frequency tables with a small number of records in some of the cells.

Results and discussion

The three grasshopper species differed significantly in their association with different plant families (Fig. 1A; Fisher's exact test P < 0.001). While many individuals of *Oedaleus infernalis* were found on the ground, most *Acrida cinerea* and *Atractomorpha lata* were observed on Poaceae (usually *Zoysia japonica*) and Asteraceae (usually *Artemisia princeps* or *Aster pilosus*), respectively (Table 1). The three grasshopper species also differed significantly in their association with different plant structural types (Fig. 1B; Fisher's exact test P < 0.001). *Acrida cinerea* was most often observed on **Low-vegetation** structure plants (mostly Poaceae), *Atractomorpha lata* was mostly found on **Stem-plants** (mostly Asteraceae), and *Oedaleus infernalis* was largely observed on the **Ground**.

Atractomorpha lata utilizes host plants belonging to various families including Asteraceae, Convolvulaceae, and Fabaceae (Tanaka 2008). In this study, many Atractomorpha lata individuals were observed on Asteraceae. This is consistent with previous findings that Artemisia princeps (Asteraceae) is one of the best host plants for growth and survival of Parapodisma subastirs grasshoppers (Miura and Ohsaki 2004a, b, 2006). As we did not determine the relative abundance of different plant species at the study site, we cannot directly evaluate the host plant preferences of each species. However, we can focus on microhabitat differences between the three species at the same location.

While Atractomorpha lata was found on Asteraceae as well as other plant families, Acrida cinerea was mostly observed on Poaceae (usually Zoysia japonica). Acrida spp. grasshoppers are known to prefer grass as a food resource (Haldar et al. 1995). We also hypothesize that these differences in host plant associations may be linked to specific thermal microhabitat. Zoysia japonica on the research site had recently been trimmed and so the grass was shorter than normal, which might have contributed to an increase in surface temperature due to exposure to sunlight (Gardiner and Hassall 2009). Considering *Acrida cinerea*'s relatively large body size (especially in females), actively seeking sunlit locations may be beneficial for effectively warming up the body (Pielou 1948, Ahnesjö and Forsman 2006).

Grasshoppers from Oedipodinae are generally known to favor bare ground (Otte 1981, Craig et al. 1999, Chu 2002, Capinera et al. 2004). Therefore, it is not surprising that individuals of Oedaleus infernalis in our study were often observed on the bare soil exposed to sun. This preference might have provided thermal benefits, especially to females, which are relatively heavy (4-5 times heavier than Atractomorpha lata and male Acrida cinerea). Warming up their heavy bodies is easier in hot locations on the ground. The body color of each grasshopper species seems to be well adapted to its own microhabitat. Oedaleus infernalis usually has a light brown body with dark brown stripes (Kim 2013), providing camouflage on typical ground coloration in the natural habitat. Conversely, the color of Acrida cinerea and Atractomorpha lata is usually green (Tanaka 2008, Pellissier et al. 2011), making it hard to recognize the individual grasshoppers against green plant parts. Additional camouflage is provided by the resting posture of Atractomorpha lata. With their hind legs tightly pressed against their body and pointy tips of head and wings visibly protruding, the individual grasshopper resembles a narrow green leaf. Acrida cinerea also have

 Table 1. Number of individuals of the three grasshopper species

 observed on ground and plants. The category "Others" includes Fa

 baceae, Lamiaceae, Onagraceae, Polemoniaceae and Cannabaceae.

Substrate	Grasshopper			Total
	A. cinerea	A. lata	O. infernalis	
Ground	6	4	53	63
Asteraceae	18	116	7	141
Poaceae	51	22	28	101
Others				
Fabaceae	0	8	2	10
Lamiaceae	1	4	0	5
Onagraceae	0	3	1	4
Polemoniaceae	1	0	1	2
Cannabaceae	0	1	0	1
Total	77	158	92	327



Fig. 1. The use of different types of substrates by the three grasshopper species. A. Substrates divided according to taxonomy; B. Substrates divided according to vegetation structure.

pointy head tips contributing to their camouflage while sitting on Isely FB (1937) Seasonal succession, soil relations, numbers, and regional plant stems or grass leaves.

In summary, we documented microhabitat segregation among three common Asian grasshopper species and we hypothesized that food and microclimatic preferences, as well as phylogenetic history, might have contributed to the observed differences. These differences coincide with the differences between species in adaptations to camouflage their bodies in their respective microhabitats. Future experiments should determine if active preferences for specific habitats are responsible for the observed segregation, and if interspecific competition affects the segregation.

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Introduced Japanese burrowing cricket (Orthoptera: Gryllidae: Velarifictorus (Velarifictorus) micado) range continues to expand in North America

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Abstract

Japanese burrowing cricket, *Velarifictorus* (*Velarifictorus*) *micado* (Saussure, 1877), was introduced into the eastern United States in 1959 and has since increased its distributional range to include the eastern Great Plains and the northern United States. Although generally thought of as an urban species associated with human habitation, some specimens are now being captured in more remote areas, which is attributed to this species sometimes being macropterous and dispersing through flight. Public data sources such as BugGuide and iNaturalist were found to be sound, passive tools for identifying the expanding range of this species in the Americas. Collection and observation in atypical habitats suggest that potential ecological impacts may be occurring.

Key words

citizen science, crickets, geographic distribution, invasive species, singing insects

Introduction

Japanese burrowing cricket, Velarifictorus (Velarifictorus) micado (Saussure, 1877), is native to Asia and was first found in the United States in 1959 in the District of Columbia (Alexander and Walker 1962; Fig. 1). The source of the early introductions is not entirely clear, and it may have been introduced as discarded or escaped fishing bait, or via ornamental plants. Since that first discovery, V. micado became widely distributed and established in the eastern and southeastern United States (Walker 1977, Peck et al. 1992). Although additional reports and accounts of this species can be found on multiple internet-based sites, no updates on its range in the United States have been published since the previously referenced studies. Here, I present additional distributional records for this species in North America, which show that its geographic range has continued to increase both northward and westward in the United States. Although this species is generally not considered to be invasive or destructive (Center for Invasive Species and Ecosystem Health 2018), the range expansion documented here suggests it may be more invasive than previously thought. Potential ecological impacts associated with this species have not been documented but, as noted by Bowles and Bowles (2015), im-

pacts resulting from some non-native species introductions may be complex and not easily described in tangible terms.

Generalized distribution maps previously constructed for V. micado and presented on public information sources, such as Encyclopedia of Life (2018), Mobugs (2018), Singing Insects of North America (2018), Songs of Insects (2018), and in field guides (Capinera et al. 2004) show an approximate distribution South of latitude 40°N and East of longitude -92°W. Based on collections I made during a National Park Service BioBlitz in southeastern Missouri in 2014, and during routine insect monitoring in Arkansas in 2017, it was clear that the range of this species extended beyond that typically presented. In addition to my own collecting data, I searched two common internet-based naturalist platforms for photographs of V. micado occurring outside this previously described range. They included BugGuide (https://bugguide. net/) and iNaturalist (https://www.inaturalist.org/). These crowdsourced portals aggregate photographs and collection data of insects and other arthropods found within the United States and elsewhere. The purpose of this study is to estimate the increased geographic range of Japanese burrowing cricket, V. micado, in the United States.

Methods

I collected specimens by hand (which I later pinned) and by using black-light traps (which I later preserved in 70% ethyl alcohol). Records from photographed specimens were taken from BugGuide.net and iNaturalist.org. Both websites include several photographs of V. micado that were taken at numerous locations in the United States. Another public database, iDigBio (idigbio.org), yielded only previously published records and those data are not presented here. I did not physically examine the specimens listed on BugGuide or iNaturalist, and I could not determine if specimens were deposited in museums or may no longer exist. I examined each photograph listed on those sites and compared them to published descriptions of the species (Alexander and Walker 1962, Capinera et al. 2004). The photographs unambiguously depict V. micado. Sex of specimens was determined where practical. While not a substitution for examination of physical specimens, photographic records are an important and valid means to learn

more about the distribution and phenology of species. Although photographs and accompanying information can be deleted or modified by the submitter at any time, archival web services, such as the WayBack Machine (archive.org/web/), maintain copies of those sites taken at multiple instances in time. Numerous other photographs of *V. micado* are posted on BugGuide and iNaturalist, but they fell well within the previously reported range and therefore are not included here. Three specimens of Japanese burrowing cricket listed on iNaturalist from Mexico (photos#8364819, #10828284, #9960828) could not be confirmed and are not addressed further here.

Collection acronyms are as follows: HTLN (National Park Service, Heartland Inventory and Monitoring Network, c/o Missouri State University, Department of Biology, Springfield, Missouri), BUG (BugGuide.net) and INAT (iNaturalist.org). Data in brackets [] were added by the author.

Results

I collected two specimens by hand (1 male, 1 female), and three by black-light/alcohol pan trap (2 females, 1 nymph). Numerous additional records were taken from BugGuide (2018) and iNaturalist (2018). Collection data follow. Some contributors of photos used an identifier rather than a formal name. In those instances where the collectors' names could not be determined, I have placed the collector's identifier in quotation marks.

Collection data.—**UNITED STATES: Arkansas: Marion Co.**, Buffalo National River, Buffalo River @ Clabber Creek, 36.127858N, -92.545530W, 13–14.vii.2017, D. E. Bowles and C. Cheri, black-light, female [macropterous] (HTLN); **Newton Co.**, Buffalo National River, Buffalo River @ Carver Access, 35.983355N, 93.038055W, 13–14.vii.2017, D. E. Bowles and C. Cheri, blacklight, nymph (HTLN); **Searcy Co.**, Buffalo National River, Buffalo River @ Tyler Bend, 35.988611N, 92.767222W, 21–22.ix.2017, D. E. Bowles and C. Cheri, black-light, female (HTLN). **Missouri: Newton Co.**, George Washington Carver National Monument, prairie, 36.989341N, -94.357135, 27.ix.2014. D. E. Bowles, at large, 1 male, 1 female (HTLN).

Records from BugGuide and iNaturalist.—UNITED STATES: Arkansas: Benton Co., Bella Vista, 21.ix.2015, female, EmilyK, photo#1153123 (BUG), same, but Centerton, 5.ix.2017, female, John Moreno, photos#1462556, 1462550 (BUG), same, but Lowell, 29.ix.2015, female, Mabel Serrano, photo#1147293 (BUG); Montgomery Co., Ouachita National Forest, 34.633261N, -93.581269W, 16.x.2014, female, Michael Skvarla, photo#4308701 (INAT); Newton Co., Buffalo National River, 36.037828N, -93.341024W, 8.ix.2013, female, Michael Skvarla, photos#4309265, 4309202 (INAT), same, but nr. Ponca, Buffalo National River, Steel Creek, 11.ix.2013, Michael Skvarla, photos#840796, 840794 (BUG); Washington Co., Fayetteville, 18.ix.2015, male, Christ Kortis, photo#1141234 (BUG), same, but Fayetteville, 5.ix.2015, female, Layton McCullars, photo#1145100 (BUG), same, but 5.x.2015, female, Zac Jones, photo#1153873 (BUG), same, but 11.x.2015, female, "anbroshu", photo#1154284 (BUG), same, but U of A, 11.x.2015, male, L. Dave Smith, photo#1149209 (BUG), same, but 12.x.2015, female, leaddiso, photo#1154233 (BUG), same, but 8.ix.2017, female, Cody Seymour, photos#1462488, 1462487 (BUG), same, but 19.ix.2017, female, "ngbadger", photo#1462604 (BUG). Delaware: New Castle Co., Bear, 29.viii.2007, female,

Ted Kropiewnicki, photo#141710 (BUG), same, but 31.vii.2010, female, photos#442224, 442225 (BUG), same, but 6.x.2012, female, Ted Kropiewnicki, photos#846874, 846872 (BUG). Illinois: Will Co., New Lenox, 41.490497N, -87.989875W 25.vii.2016, male, Erik Attaway, photo#1277770 (BUG), photo#3909356 (INAT). Iowa: Dubuque Co., Dubuque, 11.ix.2017, female, "Maria320414", photo#1439725 (BUG). Kansas: Johnson Co., Overland Park, 30.viii.2008, female, Andrew Williams, photo#263440 (BUG), same, but 22.viii.2015, male, "NYKZ", photos#1126696, 1126695 (BUG), same, but 19.ix.2008, female, Andrew Williams, photo#263440 (BUG), same, but 27.ix.2015, 2 females, "NYKZ", photo#1130919 (BUG); Sedgwick Co., Wichita, 16.ix.2010, female, Terry Allison, photo#458181 (BUG). Michigan: Wayne Co., Dearborn, 6.ix.2013, 2 females, 1 male, Kyle Kandilian, photo#837714 (BUG). Missouri: Boone Co., 38.946228N, -92.446573W, 3.ix.2017, male, "lfelliott", photo#7773805 (INAT), same, but 38.94626N, -92.44653W, 8.x.2016, female, "Ifelliott", photo#4337141 (INAT), same, but 38.94626N, -92.44653W, 19.x.2016, female, "lfelliott", photo#4449513 (INAT); Buchanan Co., 39.739062N, -94.829944W, 20.x.2017, male, "chinquapin", photo# 8571138 (INAT); Camden Co., Osage Beach, 38.127219N, -92.65221W, 21.viii.2017, female, "firewolfspirit", photo#7595682 (INAT); Franklin Co., Pacific, 28.ix.2009, female, James C. Trager, photo#1488545 (BUG), same, but 17.ix.2009, male, James C. Trager, photo#1488544 (BUG), same, but 38.487353N, -90.801582W, 22.ix.2017, SND, "jigglytuff", photo#8038422 (INAT), same, but Shaw Nature Preserve, 38.476137N, -90.822875W, 23.ix.2017, female, "eviemae", photo#8058408 (INAT), same, but 38.443384N, -90.808629W, 3.xi.2017, male, "natric4u", photo#8668506 (INAT); Nodaway Co., Maryville, 40.35448N, -94.885222W, 30.viii.2017, male, Evan Grimes, photo#7722519 (INAT); St. Louis Co., St. Louis, 38.602747N, -90.254968W, 17.viii.2017, male, Alex Wild, photo#7532527 (INAT), same, but 38.45059N, -90.217275W, 20.viii.2017, female, "gcwarbler", photo#7672768 (INAT). New Jersey: Burlington Co., Camp Creek Run, Marlton, 28.vii.2010, male, John R. Maxwell, photos#436070, 436069 (BUG); Camden Co., Atco, 19.viii.2012, female, Yurika Alexander, photo#692452 (BUG); Essex Co., Newark, 40.779999N, -74.174721W, 20.x.2017, male, "carlosm", photo#8515960 (INAT); Morris Co., Madison, 40.775912N, -74.431043W, 20.ix.2015, female, "iumer", photo#2080090 (INAT). New York: Kings Co., New York, 40.700821N, -73.99596W, 20.viii.2015, male, Stanton B., photo#2091504 (INAT); Nassau Co., New York, 40.817325N, -73.761986W, 13.ix.2017, male, "sflee8", photo#7900218 (INAT); New York Co., New York City, Central Park, 24.ix.2006, female, Marie Winn, photo#79642 (BUG), same, but 25.ix.2006, male, Marie Winn, photo#79639 (BUG), same, but 40.817982N, -73.948642W, 25.x.2017, female, "mdoom"_1eg, photo#8549726 (INAT), same, but 40.818805N, -73.948372W, 25.x.2017, male, "eshika"_1eg, photo#8549352 (INAT), same, but 40.819008N, -73.9479W, 2.ix.2017, female, J. Rigby, photo#8527361 (INAT), same, but 40.81721N, -73.949246W, 2.ix.2017, female, "anasalazar", photo#7756003 (INAT), same, but 40.818722N, -73.94671W, 13.ix.2017, male, Isabel G., photo#7905290 (INAT); Queens Co., Broad Channel/Jamaica Bay Wildlife Refuge, 6.ix.2014, male, Seth Ausubel, photos#991914, 991915 (BUG), same, but Forest Park, 28.ix.2013, male, Antonio Liberta, photo#1030267 (BUG); Richmond Co., Staten Island, 13.iii.2017, female, Loubara, photo#1346734 (BUG), same, but 40.508153N, -74.218004W, 8.ix.2016, female, Maya, photo#4904975 (INAT); Tompkins Co., Cornell Campus, Ithaca,



Fig. 1. Velarifictorus (Velarifictorus) micado (Saussure, 1877), nymph. Source: USGS Bee Inventory and Monitoring Lab, Public Domain.

27.viii.2015, 2 males, Brandon Woo, photos#1129586, 1129587, 1129588, 1129589 (BUG), same, but 5.x.2015, male, Brandon Woo, photo#1151601 (BUG), same, but 17.vii.2016, [misidentified as female] male, Brandon Woo, photo#1260224 (BUG), same, but 3.viii.2016, female, Brandon Woo, photo#1271221 (BUG), same, but male, Brandon Woo, photos#12883225, 1271221 (BUG), same, but 14.viii.2016, male, Brandon Woo, photos#12883225, 1277132 (BUG), same, but 28.viii.2016, female, Brandon Woo, photo#1286518 (BUG), same, but 27.viii.2016, female, Brandon Woo, photos#12883225, 1283223 (BUG), same, but 30.viii.2016, female, Brandon Woo, photo#1286519 (BUG), same, but 15.ix.2017, male, female, Brandon Woo, photos#1445747, 1445748 (BUG), same, but 16.ix.2016, male, Brandon Woo, photos#1293464, 1293463, 1277132 (BUG), same, but 17.ix.2015, male, Brandon Woo, photo#1141372 (BUG), same, but 42.446889N, -76.481167W, 18.ix.2015, male, Brandon Woo, photo#10074532 (INAT); Westchester Co., Elmsford, 12.ix.2017, male, Preston W., photo#1439404 (BUG), same, but Bronxville, 24.ix.2010, female, Andrew V.F. Block, photo#461274 (BUG), same but 40.952106N, -73.772022W, 19.IX.2017, male, D. Ruby, photo#7996228 (BUG). Ohio: Lucas Co., Toledo, 41.689971N, -83.615041W, 23.ix.2017, male, J. Witter, photo#8065789 (INAT). Oklahoma: Okmulgee Co., Henryetta, 7.vii.2005, male, Charles Schurch Lewallen, photo#23583 (BUG). Texas: Dallas Co., De-Soto, 23.ix.2008, female, Gacko, photo#284824 (BUG), same, but Dallas, 32.704863N, -96.852556W, 7.x.2017, female, "butterflies4fun", photo#8303510 (INAT); Harris Co., Russ Pitman Park, Houston, 15.vi.2012, male, John Schneider, photo#659597 (BUG), same, but Houston, 29.760048N, -95.597783W, 9.x.2016, female, Dan Johnson, photos#4309355, 4309355, 4309355



Fig. 2. Map showing the commonly projected distribution of *Velarifictorus* (*Velarifictorus*) *micado* (Saussure, 1877) in the United States of America and itscurrent known distribution. The shaded area indicates the previously reported range, triangles represent data collected by the authors, and circles represent data generated by public sources. Records are shown only for those locations that fall outside the previously reported range.

(INAT), same, but 29.761588N, -95.605404W, 9.x.2016, SND, Dan Johnson, photos# 4309354, 4309354 (INAT), same, but 29.760773N, -95.599563W, 3.x.2016, female, Dan Johnson, photo#4265317 (INAT), same, but 29.75917N, -95.594202W, 2.x.2016, female, Dan Johnson, photos#4257670, 4257670 (INAT); **Parker Co.**, Weatherford, 13.ix.2016, female, Tzila"Z" Duenzel, photos#1291788, 1291786, 1291787 (BUG).

Discussion

The range of V. micado has broadly expanded in the United States since it was first introduced, including a western expansion of the species range into the plains of Iowa, Oklahoma, and Texas, and a northern expansion into Illinois, Michigan, New Jersey, and New York (Fig. 2). The present known range of the species in the United States now encompasses approximately 43°N, -98°W. Since V. micado is thought to be distributed primarily via ornamental plants (Walker 1977), it is unknown if some of the records presented here represent localized, temporary introductions, or breeding populations. Most specimens were collected/ observed from June through early November, and the majority were observed during August and September. Although most of the records reported here are from cities and urban areas, several specimens were collected in rural areas, including the Buffalo National River, Arkansas, and a restored prairie at George Washington Carver National Monument in southwest Missouri. Other specimens were photographed in the Ouachita National Forest, Arkansas. Those specimens are arguably less likely to have been introduced via ornamental plants and may have dispersed there via flight. Dispersal by flight cannot be ruled out since some individuals can be macropterous. Notably, one female collected at Buffalo National River, Arkansas, was macropterous. Since this species overwinters in the egg stage, it also is possible that some breeding populations may become established in northern areas (Alexander and Walker 1962).

V. micado is now occupying ecological habitats previously unreported for the species. In the southeastern United States and throughout its native range in Asia, this species primarily inhabits mesic habitats including grassy fields and wet, wooded and partially wooded areas (Walker 1977). However, it's occurrence on the Great Plains (Fig. 1) suggests it may be adapting to drier grassland habitats. Walker (1977) suggested that inadequate soil moisture might limit the spread of *V. micado* in Florida, which may ultimately limit its spread into drier areas of the western United States.

Using public data does present some concerns. For example, Carlson et al. (2012) noted that passive surveillance tools such as BugGuide may have an inherent bias because records are added haphazardly in contrast to active, professional sampling efforts that often target specific taxa. As illustrated by this paper, however, passive surveillance is certainly preferable to the absence of sustained professional surveillance. Using internet-based tools such as BugGuide and iNaturalist can be especially useful for tracking certain species readily identified through photographs. Such sources can provide much greater coverage than one or a few individual scientists can practically accomplish (Marshall 2008, Epps et al. 2014, Michonneau and Paulay 2015, Geneviève et al. 2018). Indeed, citizen-generated distributional data or 'digital collecting' based on photographs can serve as a valuable tool to study the ranges of selected taxa, including medically important and nonnative, invasive species, and species of conservation concern (Marshall 2008). Other studies have successfully used BugGuide locality data to augment professionally collected distributional data (Carlson et al. 2012, Epps et al. 2014, Bowles et al. 2015, Brunke 2016, Hoebeke et al. 2017, Wheeler 2018).

In addition to public data sources such as BugGuide and iNaturalist, BioBlitz-type events using citizen scientists can be instrumental for further defining the ranges of species by using groups of interested naturalists to collect specimens for identification by taxonomic experts (Gimmel and Ferro 2010, Plumb 2014, Wiedenmann et al. 2014, Hinsey and Johnson 2015, National Park Service 2016). BioBlitzes and rapid biological inventories, including those for invertebrates, are becoming increasingly popular. They can provide important species occurrence records when properly documented through preservation and curation, or through photographs. BioBlitz events and other citizen scientists' collections, when coupled with internet platforms such as BugGuide and iN-aturalist, present potentially powerful passive surveillance tools that help provide a practical and relatively low-cost means to help "bridge the gap" between broad based biodiversity inventories and intensive biodiversity monitoring and research. Interestingly, *V. micado* has a distinctive calling song which allows for its identification in the field (Walker 1977). Training volunteers to search for this species using its call may be a way to further delineate its range.

Conclusions

The range of introduced Japanese burrowing cricket in the United States has increased beyond previously published accounts to include remote and atypical habitats. This information suggests that the Japanese burrowing cricket may be more invasive than previously thought. BioBlitzes involving citizen scientists and internet-based public data sources present passive but sound tools for documenting the occurrence and spread of easily identified species.

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Gryllacrididae (Orthoptera: Ensifera) in southern Africa

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Abstract

Although Gryllacrididae are a largely southern hemisphere insect family, they are relatively poorly represented in southern Africa, with three genera (*Ametroides* Karny, 1928, *Glomeremus* Karny, 1937, and *Stictogryllacris* Karny, 1937) and ten species and subspecies recorded from the region. All *Ametroides* and *Glomeremus* species are wingless while those of *Stictogryllacris* are long-winged. All species are arboreal and nocturnal, returning by day to characteristically silk-spun shelters between leaves. Here, we present a diagnosis, key to genera, and high-quality images to assist in identification of Gryllacrididae. By compiling all published information in one place, we hope to facilitate future researchers to investigate this poorly known group.

Key words

aptery, diagnosis, genera, macroptery, Stenopelmatoidea, silk production, taxonomic key

Introduction

Gryllacrididae (leaf-rolling or raspy crickets) are a cosmopolitan, though largely southern hemisphere (about one-third of the world's 600-odd species are known from Australia; Rentz 1997), family of Stenopelmatoidea with only a few representatives in southern Africa. But since the group is poorly studied there, having been last revised by Karny (1929), the fauna is quite likely to be significantly richer than assumed. Three genera with a total of ten species and subspecies have been recorded from the region.

Gryllacridids are robust, non-jumping crickets with stout, spiny legs. The exceptionally long antennae are rolled or curled around the body when the cricket is at rest (Figs 1–2). The southern African species are medium-sized (adults about 15 mm long), pale brown with soft bodies. *Ametroides* Karny, 1928 and *Glomeremus* Karny, 1937 (Figs 1–3) species are totally wingless while *Stictogryllacris* Karny, 1937 species have long tegmina and functional hind wings (Figs 4–5). Mature females have an ovipositor

of roughly body-length (Figs 2, 5). In *Stictogryllacris* the tegmina are about twice the length of the abdomen and protrude a considerable distance beyond the tip of the body (Figs 4–5). As far as is known, all southern African species are arboreal and nocturnal and build shelters by spinning leaves together; elsewhere soil-burrowing species are known in which the silk is used to stabilize the surrounding soil (Morton and Rentz 1983). Gryllacridids produce often loud sounds (the origin of the common name "raspy crick-ets" coined by Rentz (1996), specifically for Australian species) either during defensive displays or while drumming on the substrate in intra-specific communication. Sound is produced by rubbing spines on abdominal tergites against tubercles on the inner surface of the hind legs (Field and Bailey 1997). All species lack tympana so sound communication is via surface vibration.

Silk-production has evolved at least 23 times in 17 orders of insects (Sutherland et al. 2010), sometimes multiple times in larger orders (twice in Neuroptera, twice in Coleoptera, three times in Diptera, and six times in Hymenoptera). Silk production has apparently evolved three times in Orthoptera: twice in different clades of Anostostomatidae – once in *Lezina* Walker, 1869 species, which occur in southwestern Asia and in northern and northeastern Africa, and once in *Cnemotettix* Caudell, 1916 of western North America (Vandergast et al. 2017) – and in Gryllacrididae where it is universal, and clearly monophyletic (Walker et al. 2012, Vandergast et al. 2017). Although silk is used during some stage of the life-cycle of all of these insect groups, in most it is produced only during a short-lived process or during only one life-stage. In only two orders, Embioptera and Orthoptera, however, is silk produced throughout the life-cycle of its members.

Members of the Gryllacrididae have certain unusual and unique characteristics, the foremost of which is the ability to produce silk, while another, recorded in some *Glomeremus*, is that they feed on nectar which is imbibed through a network of special maxillary microtrichiae that connect the maxilla and mandibles by capillary tubes. This adaptation essentially deviates from the typical biting and chewing mouthparts in Orthoptera to functionally one of sucking or fluid-feeding (Hugel et al. 2010, Krenn et al. 2016). Due to this adaptation to nectar-feeding, one species of Gryllacrididae from La Réunion in the Mascarene Islands, *Glomeremus orchidophilus* Hugel et al., 2010, evolved to become the only known orthopteran pollinator (Micheneau et al. 2010).

Silk is produced in one of three positions on the insect body: in the labium (as modified "salivary" glands), Malpighian tubules, or in a variety of dermal glands. The latter include silk-secreting accessory sex glands. Labial glands account for most examples of silk-production in insects such as the ubiquitous silk-spinning in Lepidoptera larvae. Dermal glands follow in terms of frequency of occurrence, while the production of silk by Malpighian tubules is rare (Sutherland et al. 2010). Examples of dermal gland production are found in Embioptera of all stages and ages in which the glands are situated in the prothoracic tarsomeres. The main function of the silk is for lining residence tunnels amongst debris and under bark where they live. Sexual accessory glands as are found in male Archaeognatha Börner, 1904 and Zygentoma Börner, 1904, function to spin silken threads that lead females to a spermatophore or silken mats on which spermatophores are deposited. The most familiar examples of Malpighian tubules producing silk are known from Neuroptera larvae, all of which spin pupal cocoons with silk.

Gryllacridids of both sexes are capable of spinning silk from soon after hatching until they die and the function is exclusively for construction of day-time shelters. These may be completed within 24 hours and are returned to repeatedly unless damaged, apparently by following pheromone trails. Crickets seal themselves into the shelters by closing the entrance with a silken flap through which they cut an access hole with the mandibles to enable them to emerge to forage. This is done repeatedly after every emergence and return to the shelter. Adjacent leaves may be pulled together and held with the tarsi while they are spun together (Fig. 6). The shelters are thought to function mainly in anti-predator defense, although in soil-frequenting species in arid regions, protection against desiccation is presumed (Walker et al. 2012). Most species are omnivorous although predation of sessile insects and spiders has been recorded (Hale and Rentz 2001).

The diameter of each silk strand produced increases with age – in the Australian species, *Apotrechus illawarra* Rentz, 1990, the diameter of a strand increases threefold between early and late instar crickets (Walker et al. 2012). Silk strands are produced by the labial glands from which a droplet of fluid issues and is formed when the labium is touched against the substrate then drawn away from the droplet (Figs 7–8). Single strands are spun from one substrate and attached to another. This is done repeatedly, resulting in thicker fibers, or individual strands are added together or crossed to form a film. Where fibers touch, they stick together, eventually forming a mat. Silk is added to the existing mats over time until the inside of the shelter is more or less covered in silk sheets (Walker et al. 2012; see Fig. 1).

In this short communication, we review the limited information available regarding southern African Gryllacrididae in the hopes that future researchers will be encouraged to study this elusive, but fascinating, group. We present a key and images to genera found in southern Africa, which will hopefully assist in future identifications of southern African Gryllacrididae.

Methods

Detailed photographs illustrating elusive Gryllacrididae behaviors were taken by H. de Klerk opportunistically from 1985–2017 during his hiking trips throughout southern Africa's natural areas using predominantly Nikon equipment. Most Gryllacrididae were encountered at night by carefully surveying surrounding vegetation until movement of antennae was observed and an individual was spotted. All images were taken *in situ* with either a 105 mm or a 200 mm Nikkor macro lens with flash illumination using multiple flashes.

Results and discussion

Diagnosis of southern African Gryllacrididae.—Gryllacrididae are most easily confused with Stenopelmatidae but do form a distinct monophyletic clade (Vandergast et al. 2017). Southern African Stenopelmatidae tend to be more robust in appearance and have shorter and thicker antennae than southern African Gryllacrididae. Gryllacridids are characterized by depressed and soft tarsi with prominent lateral lobes (Hale and Rentz 2001). Female stenopelmatids have reduced, flap-like ovipositors (see Weissman and Bazelet 2013, fig. 7), whereas southern African Gryllacrididae have long, sword-like ovipositors typical of Ensifera. Adult male Stenopelmatidae have lateral hooks on the anal plate (tenth tergite + epiproct, see Weissman and Bazelet 2013, fig. 6), while Gryllacrididae males



Fig. 1. *Glomeremus* sp. 1. From the Drakensberg, South Africa, illustrating the long, curled antennae and the silk matting on the inner surface of the leaf shelter. Body length about 15 mm.



Fig. 2. *Glomeremus* sp. 2. From the mountains of the southwestern Cape Province, South Africa. Body length about 15 mm.



Fig. 3. *Glomeremus* sp. 3. A mating pair from the southwestern Cape Province, South Africa. The female is on top, the male below her. Spermatophores, produced by the male during copulation, and characteristic of Ensifera, are clearly visible. Body length about 15 mm. Photo by C. S. Bazelet.



Fig. 4. *Stictogryllacris lyrata* from the northern face of the Soutpansberg range in South Africa. Body length about 15 mm.



Fig. 5. *Stictogryllacris* sp. (*lyrata*?) from Highveld riverine habitat in Malolotja Nature Reserve, Eswatini (Swaziland). Body length about 15 mm.



Fig. 6. The same individual as in Fig. 5; grasping two leaves with fore tarsi and pulling them together while attaching them with silk strands.

have an enlarged ninth tergite at their abdominal apex (Gorochov 2001). All southern African Stenopelmatidae are obligatorily apterous, while Gryllacrididae can be either macropterous or apterous, depending on the species. Gryllacrididae have mouthparts specially adapted for silk production, which include well-developed maxillae and labial palps with specialized structures, while Stenopelmatidae have simple chewing mouthparts with well-developed, long, but unmodified labial palps. Both gryllacridids and stenopelmatids in southern Africa may have pegs interior to the hind femur used for femoro-abdominal stridulation, and both groups lack tympana on the fore tibiae. Furthermore, gryllacridids have heart-shaped heads when viewed head-on; stenopelmatids do not.

Ametroides (no images available) is an African genus with two of the 13 species found in southern Africa – the rest are restricted to central Africa. Males and females are totally without tegmina or hind wings.

Glomeremus (Figs 1–3) is the largest gryllacridid genus in Africa, with a total of 18 species spread across sub-Saharan Africa and some Mascarene Islands. Six species and subspecies are known from southern Africa. Both sexes lack both fore- and hind wings.

Stictogryllacris (Figs 4–8) has nine species, seven from central Africa and two from southern Africa. All species are fully-winged.



Fig. 7. The same individual as in Fig. 5; spinning silk strands to secure leaves for shelter construction.



Fig. 8. The same individual as in Fig. 5; spinning silk strands to secure leaves for shelter construction.

Key to the southern African genera of Gryllacrididae; adapted from Karny (1929)

- 1 Tegmina and wings fully developed... Stictogryllacris Karny (Figs 4–5)

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Conspecific mortality cues mediate associative learning in crickets, *Acheta domesticus* (Orthoptera: Gryllidae)

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Abstract

Many terrestrial and aquatic animals learn associations between environmental features and chemical cues of mortality risk (e.g. conspecific alarm pheromones or predator-derived cues), but the chemical nature of the cues that mediate this type of learning are rarely considered. Fatty acid necromones (particularly oleic and linoleic acids) are well established as cues associated with dead or injured conspecifics. Necromones elicit risk aversive behavior across diverse arthropod phylogenies, yet they have not been linked to associative learning. Here, we provide evidence that necromones can mediate associative olfactory learning in an insect by acting as an aversive reinforcement. When house crickets (Acheta domesticus) were forced to inhabit an environment containing an initially attractive odor along with a necromone cue, they subsequently avoided the previously attractive odor and displayed tolerance for an initially unattractive odor. This occurred when crickets were conditioned with linoleic acid but not when they were conditioned with oleic acid. Similar aversive learning occurred when crickets were conditioned with ethanol body extracts composed of male and female corpses combined, as well as extracts composed of female corpses alone. Conditioning with male body extract did not elicit learned aversion in either sex, even though we detected no notable differences in fatty acid composition between male and female body extracts. We suggest that necromone-mediated learning responses might vary depending on synergistic or antagonistic interactions with sex or species-specific recognition cues.

Key words

fatty acid, habitat selection, insect learning, mortality risk, necromone

Introduction

In both terrestrial and aquatic environments, animals utilize chemical cues to detect and avoid prevailing mortality risks (Dicke and Grostal 2001, Ferrari et al. 2010). Predominant chemical cues include those derived from predators (e.g. kairomones) or from conspecifics (e.g. alarm cues). Organisms may avoid risky environments either by responding to risk cues directly or by avoiding environmental stimuli which the organism has learned to associate with a reliable cue of risk (Ferrari et al. 2010). Associative learning may greatly expand the environmental footprint of perceived

danger, thus allowing animals to engage greater caution or avoid settings where cues of risk vary in space and time.

Learned risk avoidance via association with chemical cues has been experimentally demonstrated in a wide range of animal taxa, including insects (Wisenden et al. 1997, Dukas 1998, Nomikou et al. 2003, Yao et al. 2009), molluscs (Dalesman et al. 2006), crustaceans (Hazlett et al. 2002, Yao et al. 2009), fish (Magurran 1989, Chivers and Smith 1994), and amphibians (Polo-Cavia and Gomez-Mestre 2014). Protocols for assessing this type of learning usually consist of at least two phases. First, the animal is "conditioned" in an environment containing a novel stimulus (e.g. a food odor or a cue derived from a novel predator) paired with an innately recognized chemical risk cue (common examples include killed conspecifics or recognized predator cues) to serve as an aversive reinforcement. This is followed by a testing phase in which the animal re-experiences the stimulus without the reinforcement. Despite the applicability of this learning protocol across diverse taxa (Ferrari et al. 2010), the specific chemical nature of the cues that may mediate learned responses has rarely been considered.

For invertebrates, "necromone" cues might provide a chemical basis for learning about risks associated with conspecific mortality. Necromones are a class of chemicals released from dead or injured animals that elicit risk aversive behavior (e.g. alarm, avoidance, or hygienic behaviors) in living conspecifics (Wilson et al. 1958, Rollo et al. 1994, 1995, Yao et al. 2009). This concept traces back to E. O. Wilson et al.'s (1958) famous discovery on the chemical nature of "necrophoric" behavior in ants (i.e. the transport of dead individuals from the nest to the refuse pile). Wilson et al. (1958) showed that material treated with either acetone corpse extracts of Pogonomyrmex badius workers or oleic acid alone resulted in the same necrophoric behavioral response. Since Wilson et al. (1958), remarkable evidence for aversive behavioral responses to body extracts or chemical traces of dead conspecifics has accumulated across broad invertebrate taxa (Wilson et al. 1958, Rollo et al. 1994, 1995, Nilsson and Bengtsson 2004a,b, Yao et al. 2009, Green 2009, Chouvenc et al. 2012, Ulyshen and Shelton 2012, Sun and Zhou 2013, Aksenov and Rollo 2017). In eusocial insects, necromone recognition elicits undertaking or burial behavior (Wilson et al. 1958, Chouvenc et al. 2012, Ulyshen and Shelton 2012), whereas solitary and gregarious species respond by avoiding associated habitats (Rollo et al. 1994, 1995, Yao et al. 2009). Cockroaches, for instance, are strongly repelled from shelter habitats containing necromone cues (Rollo et al. 1994, 1995).

Chemical analyses have revealed that the major aversive components found in cockroach body extracts are (as discovered by Wilson et al. (1958) for ants) unsaturated fatty acids (Rollo et al. 1994). Oleic and linoleic acids were both highly repellent (Rollo et al. 1994). More recent studies have confirmed that unsaturated fatty acids alone (oleic and/or linoleic) elicited aversive behaviors in the same fashion as extracts of dead bodies. This has been documented in species ranging from primitive Collembola (Nilsson and Bengtsson 2004a) and Psocoptera (Green 2009) to the Blattodea (Rollo et al. 1994, Chouvenc et al. 2012), termites (Chouvenc et al. 2012), crickets (Aksenov and Rollo 2017), and even terrestrial isopods (Yao et al. 2009). Given the ancient divergence of insects and isopods, it appears that fatty acid necromone recognition is conserved across phyla that shared a common ancestor more than 400 million years ago. However, despite their broad taxonomic distribution and strength of responses, fatty acid necromones have not been considered as potential mediators of associative learning.

We recently extended necromone recognition to the house cricket, *Acheta domesticus* (L.) (Aksenov and Rollo 2017). Consistent with necrophobic behavior (Rollo et al. 1994, Yao et al. 2009), body extracts of cricket corpses as well as oleic and linoleic acids were all highly repellant to adult male and female crickets. Like many insects (Papaj and Prokopy 1989, Dukas 2008), crickets are capable of a remarkable degree of associative learning mediated by ecologically relevant cues (Matsumoto and Mizunami 2000, Wessnitzer et al. 2008), and *A. domesticus* has served as a useful model for olfactory learning and its underlying neurobiology (Cayre et al. 2007).

Here, we test whether conspecific body extracts as well as authentic unsaturated fatty acids can serve as aversive chemical reinforcements for associative learning in *A. domesticus*. Specifically, we tested the effectiveness of five different "necromone" cues in mediating olfactory learning: oleic acid, linoleic acid, and alcohol body extracts of male, female or combined male-female cricket corpses.

Methods

Experimental animals.—Adult *Acheta domesticus* were obtained from a large, genetically heterogeneous breeding population housed in an acrylic terrarium (78 cm × 56 cm × 39 cm) maintained at 30°C with a 12 h light/12 h dark photoperiod. Cardboard egg cartons and paper towels provided shelter. Chick feed pellets (Quick Feeds[®]) and water (soaked cellulose sponges) were provided *ad libitum*. All crickets used in learning experiments were less than 14 d past their imaginal molt.

Chemical preparation.—The "necromones" tested in the learning assay were oleic acid (Sigma-Aldrich cat# 364525), linoleic acid (Sigma-Aldrich cat# L1626), and ethanol extracts of adult cricket corpses. Cricket body extracts were obtained from mature crickets euthanized by freezing at -20°C and placed in vials containing 95% ethanol (1 mL of ethanol per cricket). Each batch of body extract was prepared using at least 10, but no more than 15, dead individuals. Vials were stored in a dark chamber at room tempera-

ture for five days to allow for extraction of body constituents into the alcohol, after which bodies were removed. Extracts were then stored at 4°C. Three types of cricket body extract were prepared: all-female extract (F Ex), all-male extract (M Ex), and a combined male-female extract consisting of equal numbers of crickets from each sex (MF Ex). This is in accordance with previous methods for obtaining repellant body extracts of crickets (Aksenov and Rollo 2017), cockroaches (Rollo et al. 1994, 1995), caterpillars and woodlice (Yao et al. 2009).

GC-MS analysis of body extracts.—Ethanol body extracts for the gas chromatography-mass spectrometry (GC-MS) analysis were prepared as described above. A male body extract and a female body extract were made, each using five individual corpses. For sample preparation, 50 μ L of the ethanol extract was dried under a gentle stream of nitrogen gas and reconstituted in 1 mL methanol (2.5% H₂SO₄) and 10 μ L 0.12 mg/mL stearic acid-d₃₅ (internal standard for GC-MS analysis). The samples were incubated at 80°C for 1 h and analyzed by GC-MS immediately.

GC-MS analyses were performed using an Agilent 6890N gas chromatograph (Santa Clara, CA, USA), equipped with a DB-17ht column ($30m \times 0.25mm$ i.d. $\times 0.15\mum$ film, J & W Scientific) and a retention gap (deactivated fused silica, 5 m $\times 0.53$ mm i.d.), coupled to an Agilent 5973 MSD single quadruple mass spectrometer. The derivatized cricket extract (1 µL) was injected using an Agilent 7683 autosampler in splitless mode. The injector temperature was 250 °C and carrier gas (helium) flow was 0.7 mL/min. The transfer line was 280 °C and the MS source temperature was 230 °C. The column temperature was set at 50 °C, raised to 300 °C at 8 °C/ min, and held there for 15 min. After a five-minute solvent delay, mass spectra were acquired using electron ionization (EI) in full-scan mode. Fatty acid methyl esters were identified using NIST Mass Spectral Search Program version 2.0f (score > 800).

Learning assay.—Adult male or female crickets were individually tested under an olfactory learning paradigm consisting of three stages. First, in an initial olfactory preference test, crickets were monitored for their total amount of time spent attending to a favorable scent (vanilla) and an unfavorable scent (peppermint). Second, crickets underwent a conditioning period in which they were presented one of the five necromone cues (either oleic acid, linoleic acid, F Ex, M Ex, or MF Ex) in combination with the vanilla scent. For the control treatment, the conditioning period consisted of the vanilla scent in combination with evaporated 95% ethanol in place of a body extract or acid. Third, crickets underwent a postconditioning olfactory preference test that was identical to the initial preference test. Throughout all experiments, each cricket was used only once. Therefore, all experimental crickets had no prior experience with necromone cues.

All olfactory preference tests were conducted in an enclosed circular arena (diameter = 29 cm; height = 29 cm), containing three vanilla-scented and three peppermint-scented filter papers (4 cm × 4 cm) equally spaced and oriented vertically along the inside base of the arena in an alternating fashion. Each filter paper was coated with 300 μ L of either vanilla or peppermint solution (Clubhouse*, diluted 6-fold with water).

In each initial or post-training preference test, a single cricket was placed in the center of the circular arena and allowed to roam freely after the alcohol had completely evaporated. Each cricket was transferred to the arena in a plastic cylindrical vial (9.5 cm height, 2.5 cm width). Each test lasted for 10 min and was recorded with an overhead camera and digital video recorder (Diginet^{*}). To control for spatial bias, the circular arena was manually rotated 180 degrees at 5 min after the initiation of each test to reverse the orientation of the filter papers relative to possible external cues visible outside the arena. Each ten-minute recording was subsequently scored for the total amount of time each cricket spent perching on vanilla- versus peppermint-scented papers.

During the conditioning period, each cricket was exposed to a piece of filter paper (4 cm × 12 cm) coated with 450 µL of vanilla extract solution and one of five necromone cues (oleic acid, linoleic acid, F Ex, M Ex, or MF Ex). Body extracts were applied at a dose of 0.45 body equivalents (b.e.) per filter paper, and oleic and linoleic acids were applied at 5 b.e. per filter paper. For body extracts, 1 b.e. was equivalent to 1 mL of ethanol extract (i.e. the estimated amount of material extracted from one cricket corpse). For oleic and linoleic acids, 1 b.e. was equivalent to approximately 4.65 mg and 7.9 mg respectively (estimated from fatty acid and lipid analyses of adult A. domesticus: see Grapes et al. 1989). Acids were dissolved in sufficient ethanol to ensure uniform application to the filter papers. In the control treatment, each cricket was conditioned with vanilla scent in combination with ethanol instead of body extract or acid. In all cases, ~30 min was allowed for the alcohol to entirely evaporate. Crickets were subjected to conditioning immediately after initial olfactory preference testing, and training lasted for 22 h. Post-conditioning olfactory preferences were tested immediately after the conditioning period.

Statistics.—An index of olfactory preference was created to reflect each cricket's time spent perching on a vanilla (T_v) or peppermint (T_p) scented cue in each preference test by applying the following calculation:

$$(T_v - T_p) \div (T_v + T_p)$$

where a positive index (0 to 1) indicated preference for vanilla, and a negative index (0 to -1) indicated preference for peppermint.

All data analyses were performed in R Studio version 1.1.456 (R Studio Team 2016). Results of the learning assay were analyzed using a linear mixed effects model in the "lme4" package (Bates et al. 2014). Olfactory preference index was used as the response variable. Predictor variables included sex, necromone treatment (Control, F Ex, M Ex, MF Ex, oleic acid, and linoleic acid), and preference test (initial and post-conditioning) as fixed effects. Also included were the two-way interactive effects between treatment and sex, treatment and test, and sex and test, as well as the three-way interactive effect between treatment, sex, and test. Individual was included in the model as a random effect. Statistical significance was recognized as p < 0.05.

Results

Learning assays.—During each preference test, crickets typically spent approximately one minute exploring the scented filter papers along the inside wall of the arena before selecting one to perch on. Crickets typically changed their perch several times over the duration of the test. Crickets displayed a strong inherent preference for vanilla over peppermint, inferred from perching durations in initial trials. Vanilla preference was 3.2-fold greater than peppermint in females (chi-square: p < 0.001) and 1.8-fold greater in males (chi-square: p < 0.001). Indeed, initial preference indices remained consistent among females and males

throughout experimental trials (see Fig. 1). Similar preferences were obtained for the cricket *Gryllus bimaculatus* by Matsumoto and Mizunami (2000).

Relative to the control treatment (n = 16), crickets conditioned in the MF Ex treatment (n = 25) showed a significant change in olfactory preference from vanilla to peppermint between initial and post-conditioning trials (Treatment × Preference Test interaction: t = -3.095, df = 97, p = 0.002; Fig. 1). Similarly, for crickets conditioned in the linoleic acid treatment (n = 19), there was a significant change in olfactory preference from vanilla to peppermint between initial and post-conditioning trials (Treatment × Preference Test interaction: t = -3.020, df = 97, p = 0.003; Fig. 1). Crickets conditioned in the F Ex treatment (n = 15) also showed a significant change in olfactory preference from vanilla to peppermint, relative to controls (Treatment × Preference Test interaction: t = -2.634, df = 97, p = 0.010; Fig. 1), and this preference change was greater in females than in males (Treatment \times Preference Test \times Sex interaction: t = 2.136 df = 97, p = 0.035). Significant changes in olfactory preference were not observed for crickets conditioned in the M Ex treatment (Treatment × Preference Test interaction: n = 15, t = -1.457, df = 97, p = 0.148) or oleic acid treatment (Treatment × Preference Test interaction: n = 20, t = -0.216, df = 97, p = 0.829) relative to the control treatment (Fig. 1). There were no significant interactions between treatment and sex or sex and preference test.

Chemical analysis.—GC-MS analysis showed that the fatty acid profiles of F Ex and M Ex cricket body extract samples were simi-



Fig. 1. Changes in olfactory preference index for adult crickets (*Acheta domesticus*) conditioned to associate a favorable vanilla olfactory scent with one of five necromone cues: all-female cricket body extract (F Ex), all-male extract (M Ex), combined male-female extract (MF Ex), oleic acid (OA), and linoleic acid (LA). In the control treatment, crickets were conditioned with ethanol instead of a necromone cue. Positive indices indicate vanilla preference and negative indices indicate peppermint preference. A significant change in preference index between initial and post-conditioning tests (relative to the control) indicates learning associated with a necromone cue (* p < 0.05, ** p < 0.01). All preference indices were derived from perching durations (i.e. total time spent attending to either the vanilla or peppermint cue during the preference test). Bars indicate standard error.

Compound	Male Extract (M Ex)	Female Extract (F Ex)	
Myristic acid	0.43	0.53	-
Palmitic acid	27.14	27.19	
Palmitoleic acid	1.10	1.96	
Stearic acid	14.15	11.33	
Oleic acid	18.23	20.69	
Linoleic acid	51.43	52.60	
γ-Linoleic acid	1.04	0.90	
Paullinic acid	0.53	0.31	

Table 1. Fatty acid methyl ester (FAME) analyses of adult female (F Ex) and male (M Ex) *Acheta domesticus* body extracts. Normalized peak areas of detected FAME. Four strongest peaks detected are in bold corresponding to: palmitic, stearic, oleic, and linoleic acids.

lar (Table 1). Samples consisted of bodies from five individuals to minimize bias resulting from potential variability among individual crickets. Strong peak areas were revealed corresponding to linoleic, oleic, stearic, and palmitic acids. In males, the acids were present in a ratio of linoleic:palmitic:oleic:stearic = 51.43:27.14:18.23:14.15. The female acid ratio was 52.60:27.19:20.69:11.33. The mean acid ratio for males and females combined was 52.01:27.16:19.46:12.74. It was not possible to distinguish between *cis* and *trans* isomers of the compounds.

Discussion

Learned responses to cues from dead or injured conspecifics are widespread (Dicke and Grostal 2001, Ferrari et al. 2010), but there has been little consideration of which chemicals may mediate these responses. Here, we examined a potential role for necromone cues as aversive chemical mediators of olfactory learning in an insect. Male and female *A. domesticus* showed learned aversion to an initially favorable odor after being conditioned to associate the favorable odor with a necromone cue. This was observed when crickets were conditioned with either body extracts of female crickets or body extracts of both sexes combined. In contrast, learning responses were not observed when crickets were conditioned with body extracts of male crickets (Fig. 1) even though male and female body extracts did not appear to differ in fatty acid composition (Table 1).

Next, we tested whether learning could be mediated by oleic and linoleic acids as necromone cues. Compelling reports suggest that unsaturated fatty acids (particularly oleic and linoleic) elicit aversive behavior associated with death recognition in a variety of invertebrate species (Wilson et al. 1958, Rollo et al. 1994, 1995, Nilsson and Bengtsson 2004a, Yao et al. 2009, Green 2009, Chouvenc et al. 2012) including A. domesticus (Aksenov and Rollo 2017). Chemical analyses of cockroach corpse extracts identified the active repellent fraction as oleic and linoleic acids. Chemical profiles obtained for A. domesticus here (Table 1) confirm strong prevalence of these fatty acids in our crickets. In this study, learning responses were observed when crickets were conditioned with linoleic acid but not when they were conditioned with oleic acid (Fig. 1). Our results suggest that cue strength, complexity or synergism (additive effects of multiple repellant or species-specific cues) or antagonism (masking of repellant cues by sex-specific attractants) might play important roles in mediating learned responses to conspecific mortality cues.

Our central finding that crickets modify their olfactory preferences following learned association with conspecific body extracts indicates that prior experience may impact behavioral decisions even when direct cues of risk are not apparent (i.e. the environment itself becomes perceived as risky). Similar examples of this form of associative learning mediated by conspecific mortality cues have been demonstrated in other insect species, suggesting that this could be a widespread phenomenon. Dukas (1998), for instance, demonstrated that Drosophila larvae learn to avoid odors associated with crushed conspecifics. Damselfly larvae reared in the absence of their natural predators responded to chemical cues from injured conspecifics, but not the predator-derived cues (Wisenden et al. 1997). However, after being exposed to conspecific and predator cues simultaneously, the larvae learned to respond directly to predator-derived cues. In a related study, damselfly larvae initially failed to respond to chemical stimuli from predators but did so after they had been exposed to chemical stimuli from predators that had been feeding on conspecifics or familiar heterospecifics (Chivers et al. 1996). Similarly, adult female whiteflies (Bemisia tabaci) learned to avoid ovipositing on plants harboring predatory mites that ate whitefly eggs and larvae (Nomikou et al. 2003). Although such learning responses were not explicitly linked to any type of alarm or avoidance chemistry, we suggest that necromones should be considered given their phylogenetic pervasiveness and strength of responses throughout the invertebrates (Yao et al. 2009, Sun and Zhou 2013).

Interestingly, the collembolan, Sinella curvisetaed no evidence of a conditioned response to predator cues from wolf spiders (Pardosa milvina) after pairing with crushed conspecifics (Sitvarin et al. 2015). While this negative result could be attributable to differences in experimental design (e.g. duration of the conditioning period), it is also possible that the wolf spider cues were not perceived as environmentally relevant to the Collembola, as they did not respond to the cues initially. In contrast, the conditioned stimulus utilized in our experiment (the vanilla olfactory cue) was initially attractive to both male and female crickets. An alternative possibility could be that A. domesticus has an evolved propensity for forming learned associations with olfactory cues. Indeed, olfactory learning has previously been reported in A. domesticus (Scotto-Lomassese et al. 2003) and other Gryllidae (Matsumoto and Mizunami 2000, 2002), and the neural mechanisms mediating this type of learning are well characterized in Acheta (Cayre et al. 2007).

Despite the strong presence of both oleic and linoleic acid in male and female cricket body extracts, learning responses were only observed for linoleic acid. This seems consistent with previous data demonstrating that both sexes showed weak initial aversion to habitats treated with oleic acid, but aversion to linoleic acid-treated habitats was stronger (Aksenov and Rollo 2017). Additionally, cricket body extracts tended to elicit stronger and more immediate aversion than either oleic or linoleic acid (Aksenov and Rollo 2017). It seems likely that the relative strength of responses to body extracts arises via cue synergism (i.e. combinatorial effects of necromone cues or their synergism with species-specific recognition cues).

Further evidence for cue synergism was obtained when Yao et al. (2009) tested body extract repellency on a number of related species and found that extracts from one species also repelled others, but extracts of conspecifics were most repellant. Chouvenc et al. (2012) obtained similar evidence of cue synergism by showing that corpse burial by termites (*Pseudacanthotermes spiniger*) was elicited by material treated with conspecific body extract or a combination of chemicals found in the extract, but not when these chemicals were tested individually. Additionally, oleic acid elicited building behavior in the termite *Reticulitermes virginicus* but only when applied to imitation corpses of conspecifics, suggesting that synergism of chemical and tactile cues might also be important (Ulyshen and Shelton 2012).

Lack of learning responses when either male or female crickets were conditioned with body extracts of male crickets suggests that the F Ex and M Ex extracts differ in chemical composition. However, this is unlikely to reflect sex differences in necromone fractions given that the unsaturated fatty acid profiles of F Ex and M Ex did not notably differ (Table 1). One explanation could be that sex-specific extracts contain compounds relevant to sex such as pheromones or cuticular hydrocarbon signatures (Warthen and Uebel 1980, Hardy and Shaw 1983, McFarlane et al. 1983, Tregenza and Wedell 1997) that might have antagonistic (i.e. attractive) effects on aversive necromone cues. However, we must note that the quantity of body extract used in current experiments was low relative to the quantities shown to completely repel crickets in previous experiments (Aksenov and Rollo 2017). Therefore, another possible explanation could be that attractive components (e.g. sex-specific pheromones) may predominate in lower quantities of extract by overriding repellant fractions. Further research is required to understand complexities such as quantity of aversive fractions, duration of exposure, or antagonism by sex-specific cues.

The learning reported here occurred following a single conditioning event (i.e. crickets learned to avoid the vanilla olfactory cue after experiencing it together with the body extract reinforcement only once). Such "one-time learning" has been consistently reported for learned responses to chemical alarm cues in aquatic species (for review, see Ferrari et al. 2010), and in many cases, the learned association can persist long after it has been formed (Suboski 1990). Such robust learning seems particularly appropriate given the potential consequences of predation or disease. Albeit the reinforcing period employed in our study was extensive (22 hours), environments treated with alcohol body extracts have been repeatedly shown to remain highly repellant over similar durations (Rollo et al. 1994, Yao et al. 2009, Aksenov and Rollo 2017), suggesting that such an extended reinforcement period could be generally appropriate in this context.

The olfactory cues used to differentiate between environments in this experiment are somewhat artificial. It remains to be seen whether such learned aversion could have important impacts in natural settings. It seems possible that these conditions could somewhat resemble vegetation with characteristic scents (e.g. pine, cedar, mint, flowering herbs), which often occurs in patches. A foraging or dispersing animal could benefit by avoiding or increasing vigilance in environments previously identified as risky. In principle, this could limit the potential range of environments that organisms exploit (e.g. foraging, oviposition, shelter selection, etc.) and may even extend to foraging decision tradeoffs between environmental quality and mortality risk (see Lima and Dill 1990). Indeed, it has been noted that risk aversion can lead to prey populations shifting to habitats of suboptimal quality (Werner et al. 1983).

Recognition of conspecific alarm or avoidance cues (including necromones) does not require the evolution or maintenance of multiple recognition systems for diverse risks, which might include predators, pathogens, or toxins. Associative learning broadens the range of risks and environments that can be avoided and may facilitate adaptive responses to risks varying in ecological space and time. For instance, necromone recognition could facilitate learned avoidance of generalist or even introduced predators (Nunes et al. 2013, Polo-Cavia and Gomez-Mestre 2014) that may not be innately recognized by prey. Perhaps even aversion to environmental toxins (e.g. Surinov 2007, Rollo et al. 2014) or poisonous foods (Bernays 1993) could be reinforced by necromones (or other cues) associated with ill, injured, or dead conspecifics.

In summary, we provide evidence that fatty acid necromones may serve as aversive chemical mediators of olfactory learning, thereby extending aversion to potentially risky environments. Given that both learning and necromone recognition are highly conserved, we suggest that such associative learning could impact foraging and habitat distribution across wide insect phylogenies, and such questions are amenable for study in a wide range of organisms. Particularly important outstanding questions pertain to how learning outcomes might be complexly mediated by synergistic or antagonistic interactions among various repellant or attractive cues as well as the broader ecological relevance of such learning.

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Ethology of the cricket *Endecous* (*Endecous*) *chape* Souza-Dias & de Mello, 2017 (Orthoptera: Grylloidea: Phalangopsidae) I: Agonistic and reproductive behavior

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Abstract

The mating behaviors of crickets, especially those related to agonistic encounters and oviposition, are poorly known. For example, only 10 of the 1005 valid species of Phalangopsidae have been studied to some extent. Here, we describe the reproductive behavior of Endecous (Endecous) chape, characterizing the actions involved in agonistic encounters, mating behaviors (female attraction, pair-formation, courtship, mating, and postcopulatory behavior), and oviposition. We recorded and timed agonistic, mating, and oviposition behaviors in staged trials. The male-male interactions of E. chape ranged in aggressiveness from low intensity (only antennal interaction) to high intensity (reciprocal fights). In the mating behavior, males courted females through antennation of the females' abdomen and cerci, followed by production of acoustic signals (with the exception of two males). Copulation occurred with females positioned above males (as is typical of Phalangopsidae), with an average elapsed time of 684.13 s, which is shorter than in other Phalangopsidae. We observed oviposition behavior only when three gravid females were placed together in an arena. Here, we contribute new knowledge of phalangopsid cricket behavior and provide useful information for understanding the evolution of reproductive behaviors. New characters described here can be used in phylogenetic analysis and for future studies about sexual selection and natural history.

Key words

aggressiveness, ethology, Luzarinae, mating behavior, Parque Nacional do Iguaçu

Introduction

From a behavioral viewpoint, crickets (Orthoptera: Grylloidea) use all the main classes of sensory perception for intraspe-

cific communication (chemical, acoustic, tactile, and visual), and present specialized aggressive and hierarchical behaviors as well as a high degree of territoriality when compared to other non-social invertebrates (Alexander 1962). Male-male agonistic behavior, for example, is primarily related to territoriality, and dominance is established through antennal contact, stridulation, and fights (Alexander 1961, Khazraïe and Campan 1997, Prado 2006, Wilson et al. 2010). Cricket reproductive behavior includes female attraction by means of the calling song and sexual recognition by antennation (Hardy and Shaw 1983, Balakrishnan and Pollack 1997), which triggers courtship and copulation (Alexander 1966, Alexander and Otte 1967, Alexander 1975, Otte and Cade 1976, Otte 1992, Kortet and Hedrick 2005).

During courtship, males communicate with females through stridulation (Alexander 1966, Zefa et al. 2008), antennation (Prado 2006, Funk 2016), and body vibration (Bell 1980, Zefa et al. 2008, Souza-Dias et al. 2015). Copulation begins when a male inserts his spermatophore duct into the female copulatory papilla for sperm transfer (Alexander and Otte 1967). Post-copulatory behavior includes ingestion of the spermatophore by males (Zefa et al. 2008) or females (Alexander and Otte 1967, deCarvalho and Shaw 2010), and male guarding to prevent the female from either removing the spermatophore or mating with other males (Alexander and Otte 1967, deCarvalho select a suitable oviposition site (Evans 1983) using visual and olfactory cues (Huber et al. 1989, Sugawara 1993).

Although the main points of Grylloidea agonistic and reproductive behavior have been extensively studied in the last decades (Alexander and Otte 1967, Bell 1980, Evans 1988, de Mello and dos Reis 1994, Prado 2006, Funk 2016, Lunichkin et al. 2016), few studies have focused on the family Phalangopsidae. To date, 10 of the 1005 species of Phalangopsidae have had their reproductive behavior described (Alexander and Otte 1967, Dambach and Lichtenstein 1978, Boake 1984, de Mello and dos Reis 1994, Nischk and Otte 2000, Gnaspini and Pelegatti-Franco 2002, Prado 2006, Zefa et al. 2008, Souza-Dias et al. 2015, Lunichkin et al. 2016), and only three species have had their agonistic behavior studied (Boake 1984, de Mello and dos Reis 1994, Prado 2006). This diversity in the behavioral repertoire is likely to be expanded when other species have been studied, particularly since Phalangopsidae are widely distributed, occupying different strata of tropical and subtropical forests, litter, rock crevices, and caves (Desutter-Grandcolas 1995, Bolfarini and Bichuette 2015, Souza-Dias et al. 2017, Cigliano et al. 2018), and also have diverse morphological variation in structures related to reproduction (Prado 2006, de Mello 2007, Zefa et al. 2008, Lunichkin et al. 2016).

Even with few species studied, the Phalangopsidae present a wide behavioral repertoire among the crickets. For example, males of *Nemoricantor maya* (Hubbell, 1938) produce courtship songs that simultaneously keep the female close and intimidate competing males (Boake and Capranica 1982, Boake 1984). In *Phaeophilacris spectrum* (Saussure, 1878), males and females do not have tympana, so communication during courtship occurs by movement of the air produced by the tegmina of males (Heinzel and Dambach 1987). However, females of *Vanzoliniella sambophila* de Mello & Cezar dos Reis, 1994, do not copulate if the male does not drum the substrate with his forelegs (de Mello and dos Reis 1994). Furthermore, males of *Eidmanacris corumbatai* Desutter-Grandcolas, 1995, have metanotal glands that produce secretions offered as nuptial gifts to females during mating (Prado 2006).

Within the Phalangopsidae, Endecous is an exclusively Neotropical genus, and is one of the most diverse genera of Luzarinae. The genus includes three subgenera and 18 species, all of which inhabit leaf litter associated with rock gullies, burrows, caves, and any natural cavities (Souza-Dias et al. 2014, Zefa et al. 2014, Bolfarini and Bichuette 2015, Souza-Dias et al. 2017). These crickets are generalists, have cavicolous and straminicolous populations, and, so far, three troglobitic species have been recognized (Souza-Dias et al. 2017). Recently we collected and described the cricket Endecous (Endecous) chape Souza-Dias & de Mello, 2017, which is abundant in the leaf litter of the Atlantic Forest of Western Paraná and Santa Catarina States, southern Brazil (Souza-Dias et al. 2017). Here, we describe the agonistic (male-male interactions) and reproductive (male-female interaction) behaviors of this cricket, highlighting pair-formation, courtship, post-copulatory, and oviposition behaviors.

Methods

Study area and sampling methods.—Nymphs and adults of Endecous chape were sampled in the Parque Nacional do Iguaçu (Iguaçu National Park), Foz do Iguaçu municipality, Paraná state, Brazil, between November 2015 and March 2016. The Iguaçu National Park is one of the largest fragments of Atlantic Forest protected in Brazil, and the largest conservation unit that protects the Atlantic Semideciduous Forest (a phytophysiognomy of the Atlantic Forest). Moreover, the Iguaçu National Park protects areas with Ombrophylous Mist Forest (Araucaria forest) and is considered a world heritage site by UNESCO (UNESCO 1986). The regional climate lies within humid subtropical mesothermal, with a mean annual temperature and rainfall of 19°C and 1600 mm, respectively (Guimarães et al. 2003).

Specimens (23 adults and 76 nymphs) were collected using nocturnal active searching on the Iguaçu National Park trails – Cataratas main trail (25°41.013'S, 54°26.385'W) and Poço Preto trail (25°37.735'S, 54°27.831'W). The national authorization for collection was issued by the Instituto Chico Mendes de Conservação da Biodiversidade ICMBio (SISBio 46964). The species studied is not considered endangered or protected.

Individuals were isolated in circular plastic vials (10 cm height and 15 cm diameter), with paper filter as substrate. Water, in open dishes (0.5 cm height and 2 cm diameter) filled with cotton, and food (fish food flakes) were offered *ad libitum*. We also reared nymphs to adulthood in these conditions. Specimens were acclimated for at least 15 days in an acclimatized room at 23°C, 75% relative humidity, and a 12:12h light/dark photoperiod before experiments.

Laboratory trials.—To observe agonistic encounters and mating behaviors, adult crickets were randomly paired (male-male or male-female) in clear glass arena boxes (20 cm length, 15 cm width, and 15 cm height) with filter paper substrate. For all encounters we used different individuals so that each individual contributed only to a single set of observations. Individuals were placed on opposite sides of the arena, isolated under plastic tea cups for 2 min, with simultaneous cup removal. After observations were completed for each encounter, the arena was cleaned with 98° ethanol and dried for 15 min to eliminate odors, and the filter paper substrate was replaced. All encounters were recorded with a digital camera (Canon® PowerShot SX210) at a resolution of 1280x720 at 30 frames per second.

For the observations of male-male interactions, we staged 25 encounters between pairs of males with all behaviors observed and recorded during 15 min. For mating behavior observations, we staged 22 encounters between 44 randomly selected male-female pairs. We included in our analysis only the encounters that resulted in copulation (n = 15 of 22). Since behavior in captivity may not predict natural behavior under field conditions (Fisher et al. 2015), observations were interrupted five minutes after they began if no courtship behavior was observed. For the same reason, after courtship began we waited 15 min from the beginning of copulation. If there was no copulation, observations were interrupted. Observations continued for 20 min after the copulations to verify post-copulatory behavior.

Oviposition behavior was observed for 15 copulated females. Trios of females were placed in the glass arena with wet sand as substrate. This combination was used because in previous observations with one (n = 5) or two females (n = 4), they did not oviposit. Oviposition behavior was observed for 60 min for one of the three females. After oviposition, we removed the sand from the arena and counted the eggs by sifting through the sand.

All individuals used in our experiments were fixed in undiluted ethanol fuel (Szinwelski et al. 2012, Szinwelski et al. 2013) and deposited in the Laboratório de Orthoptera of Universidade Estadual do Oeste do Paraná (Unioeste).

Behavioral analysis.—We timed the repertoire of behavioral elements (e.g. stridulation, antennation, copulation, and fights) during each behavioral unit (agonistic encounters, mating behaviors, and oviposition) that comprised *E. chape* agonistic and reproductive behavior. We report means, standard deviations and ranges for all behavioral elements observed, as well as the number of oviposited eggs. Based on these estimates, we created an ethogram which describes the sequence of mating behaviors.

Results

Male-male interactions.—After removing the tea cups, males remained almost motionless for $35.62 \text{ s} \pm 12.04 (11 - 64 \text{ s}, n = 25)$ and then began to pass their antennae and foreleg tarsi over their mouth parts (grooming behavior). Males then walked through the arena touching the substrate with their antennae. The first contact between males was by antennation in a face-to-face position (Fig. 1A), followed by antennal lashing. This behavior and face-to-face position occurred in all interactions and before any other behavior.

After antennation, we observed a sequence of aggressive behaviors and a sequence of same-sex sexual behavior (Bailey and Zuk 2009). From the least to the most aggressive, the behaviors observed were: (i) males remaining immobile or with few movements throughout the arena, without contact (n = 12); (ii) antennal contact with emission of song by one male (Fig. 1B) while the other male (subordinate) went to the corner and the dominant remained in the center (n = 2); (iii) antennal contact followed by reciprocal fight with subsequent escape of one male while the other male remained in the center of the arena making slight movements (n = 4); (iv) the same behavior presented in (iii), plus song emitted by the winning male in the center of the arena (n = n)4) (Fig. 1D). We observed same-sex sexual behavior in three pairs, characterized by: reciprocal "courtship" with males touching each other with antennae and forelegs, as observed in a male-female courtship, followed by partial exposure of the genitalia and spermatophore production (Fig. 1C). In two of these cases we did not observe fights or escape (n = 2), but in one case, after exposing the spermatophores, both males fought and emitted songs, followed by one male retreating to the corner of the arena.

Except for the first behavior (i), all males lifted their bodies a few times during observations. All fights were brief (8.37 s \pm 2.72

(4 - 15 s, n = 8)), mutual, and consisted of fast hindleg kicks, bites, and tarsal pushes. Songs were emitted with the tegmina lifted around 80° in relation to the body axis. When male/male courtship occurred, all males lifted their abdomen and moved the cerci and forelegs. The dominant males were those which remained in the center of the arena, walking and touching the substrate with their antennae while the subordinate male was at the corner, almost motionless.

Mating behavior.—After starting the experiment, both male and female walked through the arena touching the substrate with antennae and maxillary palpi. Males scavenged the substrate more actively than females (91.73 s \pm 97.4 (3 – 427 s, n = 15) and 29.86 s \pm 23.2 (0 – 72 s, n = 15), respectively). While scavenging the substrate, both repeatedly passed their antennae and foreleg tarsi over their labial and maxillary palpi, labrum, clypeus, and mandibles, as well as rubbed their hindleg tarsi against the cerci (grooming). The first contact between male and female occurred through mutual antennation on any body part while making rapid movements of the antennae. The elapsed time until first antennal contact was 119.86 s \pm 164.31 (7 – 651 s, n = 15), and antennation between them lasted 17.46 s \pm 19.37 (3 – 68 s, n = 15).

After antennal contact, 13 of 15 males initiated courtship by placing themselves next to the female (Fig. 2A) and touching her tergites, ovipositor, or cerci with their antennae or forelegs. At the same time, males elevated their tegmina about 80° in relation to their horizontal axis (Fig. 2B) and emitted intermittent acoustic signals. Two males did not produce acoustic signals and immediately assumed the mating position after antennation. When acoustic signals were produced, females touched male cerci or tergites with her antennae, sometimes kicking the male's antennae and body with her hindleg, while males stridulated intermittently. During this time, the male slowly neared the female and performed a series



Fig. 1. Male-male interactions of *Endecous chape*. **A**. Antennation in face-to-face position; **B**. One male stridulating while the other lifts up his body; **C**. Same-sex sexual behavior, with both males producing a spermatophore; **D**. Male in the center of arena lifting up his body and stridulating, while the other moves to the corner.



Fig. 2. *Endecous chape* mating behavior. A. Courtship position; B. Male stridulating next to the female; C. Exposure of the spermatophore; D. Copulation position; E. Copulation; F. End of copulation; G. Female dragging the male in end-to-end position; H. Male removing the spermatophore.

of sudden but mild anteroposterior vibrations with his body and touched the female's abdomen, cerci, and hind femora with his hind tarsus. Males also moved their cerci up and down and slightly lifted the abdomen. During the courtship stridulation, six males partially everted their genitalia and slowly positioned themselves to show their backs to the female, exposing the spermatophore (Fig. 2C). Nine males exposed the spermatophore when they were in the mating position. The time between spermatophore production (since genitalia exposition) and complete exposition was 1356 s \pm 41.95 (1296 – 1402 s, n = 15). Males stopped emitting acoustic signals when they assumed the mating position.

Females touched male cerci and abdomen with their mouthparts or antennae regardless of whether or not the male had exposed the spermatophore. Then males walked backwards with the abdomen touching the substrate, raising their tegmina about 130° to the horizontal body axis, positioning himself underneath the female and assuming the copulation position (Fig. 2D). After assuming this position, the mean time for mating to start was $80.2 \text{ s} \pm 81.57 (12 - 304 \text{ s}, n = 15)$. During the copulation positioning, females moved their subgenital plates downwards and males engaged their phallic complex (pseudoepiphallus) with the females' genitalia. During copulation (Fig. 2E), males performed upward and lateral movements of the cerci. Eight females moved away spontaneously from the copulation position while males remained attached, deterring the female's escape. Mating ended when females detached from males, assuming no specific position (Fig. 2F). In six observations, females walked or jumped and assumed an end-to-end position, dragging males (Fig. 2G). In these cases, females freed themselves from males using their hindlegs. Copulation duration was $684.13 \text{ s} \pm 563.16 (182 - 2276 \text{ s}, \text{ n} = 15)$. The elapsed mating time between sexual recognition by antennation to couple separation was $1072.86 \text{ s} \pm 717.10 (194 - 2837 \text{ s}, \text{ n} = 15)$.

After the couple separated, males retained the spermatophore and removed it using their hind tibial spines (Fig. 2H); three males tried to remove it with mandibles, bending their bodies, without success. After spermatophore removal, the male ate it (n =11) or left the spermatophore on the substrate (n = 3). One male retracted his genitalia along with the spermatophore. Post-copulation songs were emitted by seven males right after the couple separated. In two observations, males began another courtship, stridulating and producing another spermatophore. Those cases were observed for 50 min, and the females were not receptive, so subsequent matings were not observed.

Oviposition behavior.—When released in the arena with wet sand substrate, females stayed still for 77.21 s \pm 16.36 (42 – 112 s, n = 15). Afterwards, they started to walk through the arena touching the substrate with antennae, maxillary, and labial palpi, also ingesting substrate particles. Then one female lifted her body with her hindlegs and positioned her ovipositor 40° in relation to the substrate (Fig. 3A). Females that displayed oviposition behavior pressed their ovipositor tips against the sand surface and walked backwards to penetrate the substrate. Afterward, they lifted their bodies up and down, always redirecting their ovipositor at another angle (not exceeding 75°) (Fig. 3B), and repeatedly penetrated the substrate. Finally, they buried their ovipositors until the subgenital plate contacted the substrate (Fig. 3C).



Fig. 3. *Endecous chape* oviposition behaviors. **A.** Female pressing the tip of ovipositor against the sand surface at a 40° angle; **B.** Female redirecting the ovipositor at another angle (about 70°); **C.** Female burying the ovipositor; **D.** Female with the ovipositor penetrated, remaining immobile.

When the ovipositor penetrated the substrate, females remained motionless for $295.43s \pm 26.86$ (245 - 362 s, n = 15; Fig. 3D) and then slightly lifted the abdomen tip. After this behavior, we observed that eggs passed through the ovipositor's valves. During egg laying, females partially lifted the ovipositor with subsequent penetration. The re-penetration occurred several times with the ovipositor always deflected at another angle. The oviposition behavior lasted 2340 s \pm 441 (1632 - 2938 s, n = 15). In total, 1235 eggs were oviposited, with each female laying 88 ± 20 (45 - 156, n = 15) eggs.

Discussion

Antennal contact.—The antennal contact observed in E. chape prior to agonistic and mating behavior has been reported in previous studies (Alexander and Otte 1967, Boake 1984, de Mello and dos Reis 1994, Prado 2006). This is a crucial behavior that allows intraspecific, sexual, and age profile recognition between individuals (Rence and Loher 1977, Tregenza and Wedell 1997). Thus, antennal contact is an important driver of reproductive behaviors and has been reported for all cricket species studied to date. Agonistic, courtship, or other reproductive processes do not start before antennal contact (Alexander and Otte 1967). Those interactions were preceded by grooming in E. chape, probably allowing the removal of substances that may obstruct olfactory sensilla, impregnating such parts with contact pheromones to recognize partner fitness (Rence and Loher 1977, Balakrishnan and Pollack 1997, Tregenza and Wedell 1997, Böröczky et al. 2013, Sakura and Aonuma 2013).

Male-male interactions.—The male-male interactions of *E. chape* involve multiple levels, from antennation to agonistic interactions with reciprocal fights to same-sex sexual behavior. Different levels of aggressiveness are common for other cricket species (Alexander 1961), and are related to a male's fight performance (Rillich et al. 2007), based on age, size (Alexander 1961), or prior contacts (Adamo and Hoy 1995).

Similar male/male antennation and body lifting as those of *E. chape* were also described for *Eidmanacris corumbatai* (Prado 2006). These behaviors, along with body size and antennal lashing, as observed in *Phaeophilacris spectrum* (Dambach and Lichtenstein 1978), may be a visual clue for a male to decide to not fight, increasing his chances of survival, avoiding injury/damage, and saving energy (Parker 1974). The wide range of agonistic behaviors presented by *E. chape* indicate that crickets have elaborate systems to define dominance and subordination.

The same-sex sexual behavior of male *E. chape* may occur due to failure in sexual recognition (Bailey and Zuk 2009, Bailey and French 2012) that can occur through pheromones left by females during previous matings (Thomas and Simmons 2009), or because postures or motor patterns of males are similar to females, inducing male's courtship or copulation (Wendelken and Barth 1985).

Mating behavior.—After pair formation, males of *E. chape* interacted with the females through several channels of communication, including stridulation, body vibration, and antennation. Although those interactions are common in the communication system during cricket courtship (Alexander 1960, 1962), little is known about what type of message is transmitted to the female. In some cricket species, those signals can send information about the genetic quality of the male to the female (Gray and Cade 1999). The intensity of the emission of these signals by the male may be relat-

ed to the female's age and experience in low densities, i.e. younger females and/or females that have experienced low density tend to be more selective (Tinghitella 2014), requiring male displays that meet or exceed minimum requirements (Boake 1984) like body vibrating, spermatophore exhibition, and sound production.

Antennation in *E. chape* is different than that of *E. corumbatai* and the second copulation phase of *Nemoricantor maya*, in which the male whips his antennae without touching the female (Boake 1984, Prado 2006). In *E. chape*, antennation is identical to that of *Adelosgryllus rubricephalus* (Zefa et al. 2008), *Eidmanacris meridionalis* (personal observation), and the first copulation phase of *Nemoricantor maya* (Boake 1984), where males and females touch each other quickly. A lack of data for other species of Phalangopsidae does not allow further comparisons.

Body vibration promotes substrate waves (Bell 1980), which are perceived by the females through the subgenual organs (Alexander and Brown 1963) and can provide information about the adaptive value of males, quality of environments that they occupy, or the presence of predators (Alexander and Otte 1967, Dambach 1972, de Mello and dos Reis 1994, Lunichkin et al. 2016). However, these assumptions have not yet been tested. In a specific case, females of *Vanzoliniella sambophila* (Phalangopsidae) will only mate if the male drums the substrate with his forelegs, producing vibrations in dry leaf litter (de Mello and dos Reis 1994).

Exposition of the spermatophore may be associated with sexual selection, allowing females to evaluate male genetic qualities since large males tend to produce larger spermatophores (Sakaluk 1985), representing good nutritional status or mating effort associated with fertilization success (Wedell 1994). Furthermore, larger males generate more fertile daughters (Simmons 1987) and more competitive sons (Simons and Roff 1994). Larger spermatophores may benefit females and increase their fecundity, since they present more accessory substances capable of increasing prostaglandin levels in females, leading to higher rates of oviposition (Loher et al. 1981). However, this has not yet been tested for Luzarinae crickets, including *Endecous*.

For the Phalangopsidae species whose mating behavior has been described (except Phaeophilacris bredoides Kaltenbach, 1986 and *P. spectrum*), the spermatophore always remains with the male after copulation (Alexander and Otte 1967, Dambach and Lichtenstein 1978, Boake 1984, Gnaspini and Pelegatti-Franco 2002, Prado 2006, Zefa et al. 2008, Lunichkin et al. 2016). Phalangopsidae males removing the spermatophore with the hind tibial spines is reported here for the first time, since generally males bend their body and pick up the spermatophore with their mouthparts, as occurs in Adelosgryllus rubricephalus Mesa & Zefa, 2004 (Zefa et al. 2008), or rub the end of their abdomen against the substrate, as in Nemoricantor maya (Alexander and Otte 1967, Boake 1984) and Strinatia brevipennis Chopard, 1970 (Gnaspini and Pellegatti-Franco 2002). Males that ingest the spermatophores can use the nutrients to produce other spermatophores, allowing subsequent copulas (Zefa et al. 2008), but this hypothesis has not been tested yet. However, males of E. chape do not always eat the spermatophore. We hypothesize that due to the latency between the end of copulation to the beginning of the next one, there may be little selective pressure to reacquire nutrients.

In most Phalangopsidae species whose copulation behavior has been studied, the male inclines his tegmina over his head exposing the dorsal region of the thorax. In *Eidmanacris corumbatai* and *Endecous itatibensis* Rehn, 1918 this allows the female to access the metanotal gland opening. This opening secretes substances (nuptial gift) that are ingested by the female (Gnaspini and Pelegatti-Franco 2002, Prado 2006), probably giving the males additional time to transfer more sperm to the female copulatory papilla, and transferring nutrients to females that may indirectly benefit the offspring (Boggs 1995, Poiani 2006). On the other hand, the metanotal gland is not present in *E. chape, A. rubricephalus* (Zefa et al. 2008), *P. bredoides* (Lunichkin et al. 2016), *P. spectrum* (Dambach and Lichtenstein 1978), *V. sambophila* (de Mello and dos Reis 1994), *Pizacris zefai* (Mews & Sperber, 2010) (Souza-Dias et al. 2015), and *N. maya* (Alexander and Otte 1967, Boake 1984). In these cases, the inclined tegmina can act as support for the females to remain in the copula position, since they are much larger than the males. Alternatively, it may be a plesiomorphic behavior (Alexander and Otte 1967).

In some observations, females of E. chape tried to interrupt copulation early by forcing their hindlegs against male bodies or jumping, and the males in turn avoided the escape of the females perhaps due to the strong connection promoted by the phallic sclerites. This male strategy to prevent the female from escaping during copulation may occur in *E. chape* due to high competition for females, since the sex ratio is apparently very male-biased (Fianco M, 2018, unpublished data). In Luzarinae, male phallic claspers (pseudepiphallic parameres) used to hold the female copulatory papilla and keep the female attached during copulation are relatively common (de Mello 2007, Souza-Dias and Desutter-Grandcolas 2014, Souza-Dias et al. 2015, 2017). This type of behavior seems to be well adapted in Aracamby de Mello, 1992 (Phalangopsidae) whose males bear claspers in the paraprocts that grab the female during copulation, preventing her from aborting the copulation (de Mello 2007).

Oviposition behavior.—The fact that we did not observe oviposition when one or two females were in the arena may be related to the lack of adequate conditions and/or lack of correspondence to their natural environment, evidenced by the long time which females spent exploring the substrate. In addition, the observation time (60 min) may have restricted such observations, with females still combing the substrate searching for suitable locales for oviposition and not laying eggs during the observation period. Contrarily, we observed oviposition when three females were put together into the arena. Therefore, these individuals may have experienced the "group effect", either as a numeric effect and/or synergistic interindividual effect, as reported in vertebrates (Krams et al. 2009) and invertebrates (Salzemann and Plateaux 1988, Avilés and Tufiño 1998, Le Goff et al. 2010, Lihoreau and Rivault 2008). Another hypothesis is that E. chape is a gregarious cricket, meaning that it lives in larger groups, as reported by Boake (1984) for N. maya. The presence of more individuals might be related to defense against predation of both eggs and nymphs, as well as an increased rate of nymph development and the probability of offspring surviving to maturity. However, this has not yet been tested for crickets.

All oviposition behaviors presented by *E. chape* have been reported in other Gryllidae species (Destephano et al. 1982, Evans 1983, Sugawara 1993). Some behaviors, like females combing the substrate, may be interpreted as a search for an oviposition site that offers adequate temperature (Destephano et al. 1982) and moisture, as indicated by Boake (1984) with *N. maya*, and tested by Farias-Martins et al. (2017) with *Ubiquepuella telytokous* Fernandes, 2015 (Phalangopsidae), among other qualities that are important choices for offspring success. Similarly, numerous insertions of the oviposition site or a way to lay more eggs, which both ensure greater reproductive success.

Conclusion

Most of the behaviors observed in other Phalangopsidae species during reproductive encounters were also observed in *E. chape.* The behavioral sequences that we observed and quantified show a rich diversity in the reproductive behaviors of both males and females, as well as diverse communication channels. Studies like this are important for a better understanding of the evolution of Grylloidea behaviors, especially Phalangopsidae, from both a phylogenetic and a behavioral-evolutionary perspective. In addition, these features may be useful in distinguishing cryptic species and are models for future sexual selection studies.

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Acoustic profiling of Orthoptera: present state and future needs

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Abstract

Bioacoustic monitoring and classification of animal communication signals has developed into a powerful tool for measuring and monitoring species diversity within complex communities and habitats. The high number of stridulating species among Orthoptera allows their detection and classification in a non-invasive and economic way, particularly in habitats where visual observations are difficult or even impossible, such as tropical rainforests. Major sound archives were queried for Orthoptera songs, with special emphasis on usability as reference training libraries for computer algorithms. Orthoptera songs are highly stereotyped, reliable taxonomic features. However, exploitation of songs for acoustic profiling is limited by the small number of reference recordings: existing song libraries represent only about 1000 species, mainly from Europe and North America, covering less than 10% of extant stridulating Orthoptera species. Available databases are fragmented and lack tools for song annotation and efficient feature-based searching. Results from recent bioacoustic surveys illustrate the potential of the method, but also the challenges and bottlenecks impeding further progress. A major problem is time-consuming data analysis of recordings. Computer-aided identification software exists for classification and identification of cricket and grasshopper songs, but these tools are still far from practical for field application.

A framework for acoustic profiling of Orthoptera should consist of the following components: (1) Protocols for standardized acoustic sampling, at species and community levels, using acoustic data loggers for autonomous long-term recordings; (2) Open access to and efficient management of song data and voucher specimens, involving the Orthoptera Species File (OSF) and Global Biodiversity Information Facility (GBIF); (3) An infrastructure for automatized analysis and song classification; and (4) Complementation and improvement of Orthoptera sound libraries using OSF as the taxonomic backbone and repository for representative song recordings. Taxonomists should be encouraged, or even obliged, to deposit original recordings, particularly if they form part of species descriptions or revisions.

Key words

acoustic monitoring, data repositories, Orthoptera Species File, sound libraries, standardization

Introduction

A considerable number of animal species produce species-specific sounds for communication, indicating their presence acousti-

cally. Among the most impressive examples are tropical rainforest insects, producing a huge variety of audible signals, while only very few can actually be seen (Riede 1993).

There is a long tradition in ornithology of identifying birds by their songs (Parker 1991). Acoustic assessment forms part of regular censusing (reviewed by Brandes 2008), or targeted searches for flagship species such as the Ivory Woodpecker (Swiston and Mennill 2009). Efficiency and reproducibility of human observers can be increased considerably by using powerful directional microphones in combination with cheap portable sound recording devices and bat detectors, allowing monitoring of high frequency or even ultrasound signals (reviewed by Obrist et al. 2010, p. 79). Several research groups developed sophisticated autonomous sound recording and automated classification techniques, facilitating monitoring and inventorying of birds (Haselmayer and Quinn 2000, Celis-Murillo et al. 2009; but see Hutto and Stutzman 2009, for a discussion of limitations), whales (Širović et al. 2009), bats (Jennings et al. 2008), frogs (Hu et al. 2009), crickets (Riede 1993, Nischk and Riede 2001, Riede et al. 2006), bushcrickets (Penone et al. 2013) and grasshoppers (Chesmore and Ohya 2004, Gardiner et al. 2005).

Due to their small size, high species diversity, strong population fluctuations, and cryptic lifestyles, insects are particularly difficult to monitor, requiring expensive and frequent sampling of specimens (Gardner et al. 2008). The species-specific songs of Orthoptera enable detectability by acoustic monitoring. With the help of adequate equipment, recordings can be used for discovery of hitherto undescribed, "new" species, detection of endemics, non-invasive mapping of species abundances and ranges (Penone et al. 2013), and rapid assessment of community structure and species turnover (Forrest 1988), particularly in complex habitats with low visibility (Riede 1993, Diwakar et al. 2007, Schmidt et al. 2013). At present, information on phenology, activity patterns, abundance, and community structure is only available for a very small number of insects, but is urgently needed to document potentially dramatic effects of climate change and changing land use patterns on insect communities (Garnas 2018, Maurer et al. 2018). The high number of stridulating species among the Orthoptera is both an opportunity and a challenge for compiling these highly needed datasets by acoustic profiling.

Besides species discovery, the potential of acoustic monitoring for Environmental Impact Assessments and Red Listing of Orthoptera is evident. Cordero et al. (2009) recognized and mapped the rare and endangered silver-bell cricket *Oecanthus dulcisonans* Gorochov, 1993 by its song. On the island of Réunion, several endemic crickets are indicator species for native forest, and acoustic monitoring was applied successfully to survey a reforestation program (Hugel 2012). The strong high-frequency components of bushcricket songs allow separation from ambient noise by highpass filtering. Due to their strong ultrasound components, Penone et al. (2013) were able to map singing specimens along roadsides in France, using ultrasound bat recorders.

Several bioacoustic monitoring studies focusing on Orthoptera applied (semi-)automatic identification (Fischer et al. 1997, Gardiner et al. 2005) illustrating the potential of the method. However, there are severe challenges impeding further progress. Among these are the lack of baseline data (Lehmann et al. 2014) for the respective region where acoustic monitoring is planned. Lists of candidate species are missing for most regions of the world, even for the comparatively well-known European fauna. Another bottleneck is the lack of well-curated song reference libraries, which will be the main topic of this paper.

Comprehensive song libraries are paramount for acoustic profiling of entire communities, either machine-based or relying on human expertise. At present, there is not even a simple identification tool for unknown Orthoptera songs. The vision is to upload a sound recording to a data warehouse portal and search for similar acoustic patterns, comparable to the Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990), available as a tool in genetic databases (e.g. National Center for Biotechnology Information (NCBI), http://blast.ncbi.nlm.nih.gov/Blast.cgi). However, this requires comprehensive databases. Upload of sequence data to Gen-Bank is a pre-requisite for publication in peer-reviewed journals (see editorial policies for data sharing and submission guidelines of major journals, e.g. https://journals.plos.org/plosgenetics/s/ submission-guidelines#loc-accession-numbers). As a result, we now have comprehensive repositories for gene sequences. As will be shown below, Orthoptera song libraries are far from comprehensive. An editorial policy of obligatory submission of original sound files to selected sound libraries would rapidly improve coverage of existing sound repositories, which is a necessary condition for progress of computer-aided species identification.

This article explores several acoustic archives and their pros and cons as a possible repository for song reference recordings, based on data-mining of existing online sound repositories for Orthoptera songs. By analyzing the lessons learnt, I present a strategic framework for establishing acoustic profiling as a core element of future automatized monitoring schemes, targeting all vocalizing animals within entire soundscapes.

Present knowledge of Orthoptera songs and coverage in sound repositories

The analysis of insect sounds started with simple, descriptive verbal descriptions and musical annotation, pioneered by Scudder (1868) for North American and Yersin (1854) for European grasshoppers (reviewed by Ragge and Reynolds 1998: p. 64). Faber (1953) focused on their function for intraspecific communication, with elaborate verbal transcriptions of songs and entire behavioral sequences, including optical displays. Research about female attraction – phonotaxis – elicited by these stereotyped songs has a long history, reviewed by Weber and Thorson (1989). Some crick-

ets and several gomphocerine grasshopper species were used as model organisms for sophisticated neuroethological and biological experiments to unravel underlying neural circuitry (for *Gryllus bimaculatus*, *G. campestris*: Weber and Thorson 1989, Schöneich et al. 2015; for Acrididae: Roemer and Marquart 1984, Helversen and Helversen 1998, Ronacher and Stumpner 1988).

It is now widely demonstrated that most Orthoptera songs are inborn, stereotyped and species-specific, providing reliable taxonomic features. Most species exhibit a maximum of only three distinct song types: calling, courtship, and rival song, depending on the behavioral context. Striking differences in calling song structure of morphologically similar species helped taxonomists to diagnose and describe "cryptic species", many of which cannot be determined without a sound recording. In a seminal paper, Walker (1964) reviewed studies on songs and taxonomy of North American Orthoptera, searching for eventual cryptic species. He concluded that "approximately one-fourth of the species of gryllids and tettigoniids of the eastern United States had never been recognized or had been wrongly synonymized." (l.c., p. 346). His discovery and description of "virtuoso katydids" (uhleri group of the genus Amblycorypha: Walker 2004a) corroborated this prediction.

Regional faunistic surveys including songs were pioneered by Pierce (1948) and Alexander (1956) for North American crickets, Otte and Alexander (1983) for Australian crickets, and Heller (1988) for European Tettigonioidea. Each of these studies provided graphic representations and comparative analysis of acoustic signatures for hundreds of species, highlighting pronounced interspecific differences in frequency composition and temporal structure.

Original recordings are available for only a small fraction of these pioneer studies, as analog tapes or on CD (see below). In any case, most authors published basic song parameters and graphic representations revealing frequency composition (spectrograms and power spectra) and temporal structure (oscillograms, cf. Fig. 1). These parameters could eventually be used as preliminary proxies in annotated repositories, and later be supplemented by song recordings.

An adequate analysis of Orthoptera songs cannot be achieved by the unaided human ear but requires visualization and temporal analysis by signal analysis software. A wide variety of programs is now available for personal computers (for an extensive list see Obrist et al. 2010), including the RavenViewer plug-in for the Firefox web-browser, allowing online analysis (Fig. 1).

Particularly for tropical Orthoptera, reliable species identification is only possible by determination of a collected voucher specimen, which often turns out to be an unknown species in need of taxonomic description. Therefore, most tropical Orthoptera are caught and recorded in captivity, to establish a reliable cross-reference between voucher specimen and recording. Besides essential parameters like time, recordist, etc. (cf. Table II in Ranft 2004), temperature must always be annotated because temporal patterns of Orthoptera songs depend considerably on temperature ("Dolbear's law": Dolbear 1897, Frings and Frings 1962).

Older recordings and state of digitization.—The history of analog recordings starts in 1889, and major archives of wildlife recordings go back to the 1940s (for a historical synopsis see Ranft 2004). Targeted recording of individual specimens with directional microphones and portable (albeit heavy) tape recorders was the standard methodology during the 20th century, resulting in impressive analog tape archives which often remained with the re-


Fig. 1. Web-based sound analysis tool for the Macaulay Sound Library, Cornell Lab (https://www.macaulaylibrary.org). Macaulay Library provides more than 400,000 playable audio files (http://macaulaylibrary.org/index.do), and even permits spectrographic online visualization using RavenViewer as a free browser plugin (http://www.birds.cornell.edu/brp/software/sound-analysis-tools). The example shows a recording of a Virtuoso katydid by T. Walker, who provided most of the Orthoptera sound recordings for this sound library. For further details, see text.

searcher. There is a high risk of loss of these valuable collections due to deterioration and misplacement (Marques et al. 2014).

Microphones and recording apparatuses varied widely due to considerable technological changes during the last decades, evolving from analog tape recorders to digital recording. The frequency spectrum of many Orthoptera reaches far into the ultrasound, with the recently described, hitherto highest-pitched katydids of the Neotropical genus Supersonus reaching up to 150 kHz (Sarria-S et al. 2014). During the 20th century, analog recording of ultrasound song components required special microphones and expensive high-speed tape recorders (see materials and methods in Morris 1980, Morris and Beier 1982, and Morris et al. 2018). Today, common digital recorders with built-in microphones and 96 kHz sampling rate cover a frequency range up to 30 kHz with sufficient quality. In addition, there is an increasing number of ultrasound recording devices and "bat detectors", reaching far into the ultrasound up to 300 kHz (see Obrist et al. 2010, p. 79), facilitating classification of tettigoniid songs in the field.

Since the 1990s, most monographs compiling Orthoptera songs were backed up by recordings on CD, serving as potential acoustic determination guides and targeting a wider audience. Compilations are available for most European (Ragge and Reynolds 1998), Italian (Fontana et al. 2002), Central European (Bellmann 1993), Australian Orthoptera (Rentz 1996), and Costa Rican katydids (Naskrecki 2000). A comprehensive compilation of Japanese Orthoptera songs on two CDs forms part of an illustrated guide to Orthoptera (Murai 2015). Note that a CD is already a digitized recording, usually of high quality. Due to copyright rules, most of these recordings are not publicly available. Nevertheless, they usually can be used for research purposes, analysis and feature extraction.

Several well-organized sound libraries house more than hundreds of thousands of catalogued analog tape recordings of vocalizing animals, such as the Tierstimmenarchiv Berlin (http://www. tierstimmenarchiv.de/), British Library Sound Archive's wildlife collection (https://www.bl.uk/collection-guides/wildlife-and-environmental-sounds), or the Macaulay Library of Sounds (Cornell Lab (2017) http://macaulaylibrary.org/). The latter provides more than 402,720 playable audio files, and even permits spectrographic online visualization using RavenViewer as a free browser plugin (cf. Fig. 1). With more than 40,000 animal sound recordings, the Borror Laboratory of Bioacoustics archive (http://blb.osu.edu/database/; Ohio State University) is among the smaller archives, but contains important historic Orthoptera recordings by R. Alexander and D. Borror, including the few available recordings of North American grasshoppers (Acrididae).

Besides the major sound archives reviewed below, there are important regional archives (reviewed for Latin America by Ranft 2004 in Annex II) and new initiatives such as the sound library of the Museum National d'Histoire Naturelle (La sonothèque: https://sonotheque.mnhn.fr/). A list of links to major sound libraries is provided by the International Bioacoustic Council (IBAC 2018).

Digital availability of sound recordings.—Digitization of existing analog recordings in most major sound archives is under way, but there are distinct policies on use and access via the World Wide Web (Baker et al. 2015). At present, most major sound archives provide searchable catalogues of all audio, offering public access to and download options for digitized recordings, under varying license agreements. In some cases, scientific re-use is limited because sound files are made available in compressed formats such as mp3 (ISO/IEC 11172-3:1993).

The following comparison of major sound archives focuses on the number of accessible Orthoptera songs, number of species, and taxonomic compatibility with the Orthoptera Species File (OSF; Cigliano et al. 2018), as well as user-friendliness of web interfaces. Connectivity with the Global Biodiversity Information Facility (GBIF: http://www.gbif.org) was analyzed by a GBIF query for "Orthoptera", adding "audio" multimedia type as additional filter criterion. The number of Orthoptera recordings and species for these major sound archives is summarized in Table 1, including comments on accessibility and particular issues. Archives differ considerably in taxonomic and geographic coverage. Most archives have several recordings for each species, and each archive has strengths and weaknesses summarized in the last column.

While all databases allow extraction of the number of Orthoptera recordings, information about the number of species was not always available. Therefore, it was queried from a table downloaded from GBIF (2015). A close inspection reveals three major contributors: Borror Lab, Animal Sound Archive (= TSA), and ZFMK DOR-SA. Note that GBIF accesses data providers dynamically and the number of records is increasing daily. While the GBIF (2015) dataset contained 3973 occurrences, a more recent Orthoptera/Audio search (GBIF 2017) resulted in 4803 occurrences from 119 species.

Major sound libraries focus on vertebrates, particularly birds, containing few insect recordings. In contrast, SINA (Walker 2004b), OSF (Cigliano et al. 2018) and SYSTAX (SysTax 2017) focus exclusively on Orthoptera. The SYSTAX-DORSA (2017) virtual museum is a repository dedicated to Orthoptera types, song recordings, pictures, and voucher specimens from German institutions and private collections. This database includes 2229 type specimens documented by approximately 25,000 images (Fig. 2). As part of a major digitization initiative funded by the German Research ministry, analog tapes from widely scattered institutional and private sound archives have been digitized (Ingrisch et al. 2004) and made accessible at http://www.systax.org and via GBIF (2017). The digitization of historic analog tapes of ultrasound recordings was particularly challenging, because the appropriate tape recorders for their reproduction are becoming rare.

In summary, accessibility of Orthoptera song recordings in any format is extremely limited. With a total of 26,000 described Orthoptera species of which a (conservatively!) estimated 10,000 are able to stridulate, we have web access to song recordings for about 1000 species, i.e. coverage of a meagre 10% of all stridulating Orthoptera species. Adding another 1000 songs scattered in publications, CDs, books and private collections, we might have song

Archive ¹	N Orthoptera	N taxa	Taxa	Geographic focus Orthoptera	Issues and special features			
	recordings fauna							
Macaulay	9,282	262 ²	All animals; Ensifera	North America	+ Raven viewer for sound visualization			
Cornell Lab					+ Basket function for download, annotations			
					(+) GBIF federation with issues			
					 no voucher cross–reference 			
					- Temperature missing or comment only			
SYSTAX-	8,669 ³	550	Orthoptera	Europe (Ecuador, South East	+ Additional user interfaces via Europeana			
DORSA				Asia) ⁴	(+) GBIF federation with issues			
					+ Additional user interfaces			
					- uploads difficult; completed archive			
					- Temperature in commentary			
Tierstimmen-	1,093	66	All animals; Orthoptera	World–wide, mainly Europe	+ Full GBIF federation			
archive					 no voucher cross-reference 			
					 temperature missing or hidden in text 			
BioAcoustica5	2,358	556	Orthoptera	World–wide, mainly Europe	+ Graphic display of standard sound analysis			
					+ Rapidly growing, allowing user uploads			
					(+) GBIF federation with issues			
Borror Sound	1,761	119	All animals; Orthoptera	North America, Australia	+ full GBIF federation			
Archive								
SINA	n.a.	$(440)^{6}$	Ensifera	North America	+ Species fact sheets with sonagrams and songs for download			
					+ full tables of song parameters for download ⁷			
					+ cross-reference to voucher			
					 no database query interface 			
					- GBIF			
Orthoptera	n.a.	776 ⁸	Orthoptera	World-wide	+ well-curated, up-to-date taxonomic backbone			
Species File					(+) providing links to additional resources			
					 temperature hidden in commentary 			
GBIF ⁹	4,803	119	Orthoptera	World-wide	- double-entries of specimens from distinct data providers but			
					identical primary source			

Table 1. Digitized Orthoptera songs in major sound archives and databases. For further details on issues and special features see text.

¹ See References for web addresses and extraction date. ² Calculated using GBIF download GBIF Occurrence Download 10.15468/dl.xsud5i ³ Riede K, Ingrisch S, Jahn O (2013). ⁴ See map at http://www.gbif.org/dataset/72309d40–0c1f-47d6–8008–33e687b7df7a ⁵ http://bio.acousti.ca/analyses. ⁶ Estimate using complex OSF search for North American Ensifera AND link, most links leading to SINA species fact sheets. Note that not all SINA pages contain a sound recording. ⁷ Full workbook: http://entnemdept. ifas.ufl.edu/walker/Buzz/g610ms3.htm showing temperature–dependence of song parameters. ⁸ Including subspecies. ⁹ GBIF Occurrence Download doi:10.15468/dl.psq6q1 accessed via GBIF.org on 03 Nov 2017



Fig. 2. The SYSTAX database. Screenshot of the new SYSTAX user interface, to be released under www.systax.org. A search for the Neotropical tettigoniid genus *Anaulacomera* recovers several sound recordings from a voucher specimen of a hitherto undescribed species, documented by photographs. Faceting allows searching by images or sounds exclusively.

data for about 2000 species, which is still only 20% of all known stridulating species. If we assume that another 20,000 Orthoptera species still remain to be described (again, a conservative estimate, cf. Stork et al. 2015), we get an idea of the daunting task ahead!

Accessibility of new digital recordings.—The amount of multimedia data documenting animal songs is growing exponentially thanks to Passive Acoustic Monitoring (PAM) and citizen science efforts (cf. August et al. 2015, Di Minin et al. 2015). In addition, behavior and song recordings can be found on YouTube (see Olivero and Robillard 2017, for cricket behavior "in the wild from You-Tube") or as digital supplementary material for scientific journals. In a letter to Science, Toledo et al. (2015) suggested that scientific journals require deposition of sound files used in publications. Submitting sound as additional online material for publications is certainly a step forward, but will lead to further fragmentation, with valuable sound recordings hidden as supplementary material behind journal paywalls, or distributed over a wide variety of online repositories such as Figshare, Dryad, etc. Instead, a long-term, sustainable archival strategy should be centered around memory institutions, which in general have a longer half-life than states or private companies. Therefore, Riede and Jahn (2013) suggested that researchers submit sound recordings and well-annotated corpora to a few well-established memory institutions, comparable to common practice in genetics.

Traditional targeted song recordings of individual Orthoptera species have now been complemented by acoustic profiling using entire soundscapes (*sensu* Schafer 1994). Soundscapes are recorded routinely for environmental monitoring (Szeremeta and Zannin 2009) or military uses (Ferguson and Lo 2004). A huge number of recordings is generated by PAM. Following a definition of Marques et al. (2013), PAM "refers loosely to methods using sounds made by animals to make inferences about their distribution and occurrence over space and time." (l.c., p. 290). There is a rapidly increasing number of acoustic monitoring initiatives recording overall soundscapes by Autonomous Recording Units (ARUs), using custom-built or commercial equipment. Acoustic monitoring by microphone arrays is a rapidly developing field, allowing exact 3D mapping of the position of songsters, reviewed by Blumstein et al. (2011). PAM focusses either on endangered vertebrate species or entire soundscapes.

Soundscape projects generally do not even try to identify or assess species compositions, but rather measure overall indices. Sueur et al. (2008b) applied signal analysis to entire soundscapes recorded at Tanzanian coastal forests, measuring entropy as a surrogate for biodiversity richness. Further recordings were made at biodiversity hotspots in New Caledonia and French Guiana (reviewed in Sueur et al. 2014). Such overall bioacoustic indices do not provide information about actual Orthoptera species presence and diversity, but informative snippets could be extracted (Riede and Jahn 2013, Lehmann et al. 2014). This means that post-hoc analysis for Orthoptera presence/absence at an ever-increasing number of acoustic monitoring sites is possible, if soundscape recordings would be made available for re-analysis.

The generated data volume is huge, and in most cases not publicly accessible or, as is the case for microphone arrays, not stored at all. Terabytes of acoustic recordings are stored on researchers' hard disks, with a high risk of getting lost, thereby impeding the chance for re-analysis. Only a small number of projects maintain servers to release soundscape recordings for re-analysis. Maintenance and release of soundscape data will provide opportunities and future challenges, as well as valuable data sources for orthopterists, because most PAM recordings from rainforests are dominated by insects, and Orthoptera in particular (Aide et al. 2017). At present, the Purdue soundscape server provides unlimited access to an impressive number of high-quality recordings (Pijanowski et al. 2011, Purdue Sound Ecology Project 2015). The extensive soundscape collection of Krause (2017) is commercial, but nevertheless available for Orthoptera song data mining.

Improving data coverage and requirements for data sharing

Improving data coverage.—The number of species covered by each database presented in Table 1 is not cumulative because there is a strong overlap between DORSA, Tierstimmenarchiv, and OSF, with a strong focus on European species. Exact numbers on SINA (Walker 2004b) are not available, and not every link from OSF to SINA leads to a sound recording. SINA is restricted to North American Ensifera, while Caelifera remain uncovered, apart from some very few historic acridid recordings from the Borror sound archive. For the time being, the best available documentation of North American acridid songs are verbal descriptions and musical annotations by Scudder (1868) and spectrogram figures published by Otte (1981). In light of the incomplete coverage of available sound libraries, filling the gaps for Orthoptera species without any song recording should have highest priority. Because OSF (Cigliano et al. 2018) is a taxonomic hub for all Orthoptera taxa, uploading at least one recording per species would be the most straightforward and efficient way to monitor progress of Orthoptera song coverage and store at least one song recording and/or parameter for each species. The orthopterist community is small and given the excellent communication between OSF curators and authors, the easiest way to increase the OSF song repository would be by proactive encouragement of authors to deposit their available recordings in OSF.

For most species with a SYSTAX-DORSA recording, a "typical" song has already been transferred to OSF, which presently contains songs for 818 species and subspecies (Cigliano et al. 2018). With a considerable number of recordings imported from DORSA, OSF has a similar bias towards European species. The addition of songs from newly described species will sooner or later compensate this imbalance, but incorporation of songs from newly described species grows slowly: According to a "complex search" ("sounds" AND "description date >=2014", extracted 6/9/2015) in OSF, from the 857 recent species described since 2014, only eight sound recordings found their way into OSF: two Neoxabea spp. and three Oecanthus spp. described in Collins et al. (2014), Tettigonia balcanica Chobanov and Lemonnier-Darcemont, 2014 (Chobanov et al. 2014), and two *Typophyllum* spp. described by Braun (2015). For others (e.g. Walker and Funk 2014, Hemp et al. 2015, Baker et al. 2017) the publications contain detailed song descriptions, while the songs are either deposited outside OSF, or are not accessible at all. However, OSF already contains links, e.g. to Walker and Funk's (2014) recordings, and it would be a comparatively easy task to transfer additional songs to OSF. Likewise, editors of Orthoptera song CDs (e.g. Rentz 1996, Naskrecki 2000) are actively involved in the enrichment of OSF and are probably disposed to contribute their CD recordings.

Problems of data sharing and file exchange.—At present, federated bioacoustics datasets downloaded from GBIF have issues result-

ing from unresolved problems between data providers and GBIF. Macaulay (Scholes 2015), Systax (2017) and BioAcoustica (Baker and Rycroft 2017) are registered, citable GBIF data providers, but occurrences disappear once the multimedia audio filter is applied.

In addition, downloading sound files from currently available repositories leads to disintegration of sound file and sound metadata. The safest way to avoid such disintegration is to store metadata within the sound file – typically, a spoken announcement by the recordist often contains information about time, place, temperature, microphone, and recording conditions. However, if this information is clipped for the sake of signal clarity and detectability, a downloaded sound file cannot be attributed to its source and metadata. For SYSTAX-DORSA sound files, the Soundminer software (http://store.soundminer.com/) was used to annotate metadata, showing species name and source when displayed on most devices (Fig. 3).

Embedding metadata within the sound file creates redundancy which can be used to restore or cross-check the links between the original database storing the metadata and the multimedia object.

Future needs: a data warehouse for bioacoustic data

A combination of features from all databases reviewed here probably describes best the requirements for an ideal Orthoptera song data warehouse. In particular:

- · Baseline collection data such as recordist, time, and locality.
- Cross-reference to voucher specimen, if available: repository (e.g. museum collection), unique identifier (collection number), identifier, and baseline data.
- If no voucher specimen is available, an image, video and comments on taxonomic reliability by naming the identifier.
- Comprehensive metadata for each recording, in particular temperature, microphone with frequency characteristics and distance from specimen, and preferably sound intensity at a given distance.
- User-friendly upload and query interface for input.

On the output side users need:

- · Advanced search functions.
- Basket function for download of selected songs and/or corpora, including metadata.

Optional requirements include online visualization of sound files (spectrogram/oscillogram), generation of bioacoustic factsheets, and flexible tools for annotation of song parameters.

Building on these basic features, a bioacoustics workbench could provide efficient, reciprocal connection to taxonomic (OSF) and specimen-based federated specimen databases (GBIF).

None of the existing databases fulfill all these requirements. Therefore, the way forward is interoperability and the federation of existing multimedia databases. Commercial or community multimedia providers like the pioneering peer-to-peer filesharing program Napster (https://en.wikipedia.org/wiki/Napster), iTunes, or SoundCloud (https://soundcloud.com/) demonstrate that efficient, user-friendly data management and federation of sound files is feasible, but not designed for scientific use, requiring annotation, citability and sustainability of repositories. GBIF federates specimen data. It allows filtering for audio data, providing multimedia links, but without any interface for direct listening or bulk download via shopping basket functions. However, GBIF is evolving rapidly and is attentive to users' needs. Among the existing sound libraries, BioAcoustica (http://bio.acousti.ca/, Baker et

Soundminer Edit Mark/Tag View Database DAW										
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Filename	Duration	TrackTitle	Description	Artist	Category	CDTitle	Library	a 5 2		
CI51057.wav	00:31	Metrioptera (Roeseliana) roeselii roeselii (Hagenbach, 1822)	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Montenegro, Montenegro, between Durdjevica Tara and Kosanica: Lab recording: date recorded;	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	Artist S. Ingrisch BitDepth		
CIs0012.wav	00:22	Barbitistes yersini Brunner v. Wattenwyl, 1875	(c) Foundation Zoological Research Museun (c) Koenig (ZFMK); License: CC BY; Montenegre Lice Durmitor, Komarnica: Kljestina; Lab recordi and date recorded: 7/25/1988; temperature: 24 tem	Foundation Zoolo nse: CC BY; Monte Kosanica; Lab re perature: 30!C; lig	luseum Koenig (ZFMK); sgro, between Durdjevica Tara corded: 8/20/1989; or heat; recorder: Kenwood	S. Ingrisch	24 Category Orthoptera CDTitle			
CIs0013.wav	00:34	Barbitistes ocskayi Charpentier, 1850	(c) Foundation Zoological Research Museun KNB Koenig (ZFMK); License: CC BY; Montenegro Durmitor, Canyon of Susica; Lab recording; date recorded: 7/25/1988; temperature: 281c;	e			S. Ingrisch	DORSA Sound Collection (ZFMK) Channels		
CIs0014.wav	00:14	Barbitistes ocskayi Charpentier, 1850	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Montenegro, Durmitor, Canyon of Susica; Lab recording; date recorded: 7/25/1988; temperature: 28IC;	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	CreationDate 2012-07-08 17:09:35 +0000 Description (c) Foundation Zoological Research		
CIs0017.wav	00:15	Poecilimon nonveilleri Ingrisch & Pavicevic, 2010	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Montenegro, Durmitor, Durdevica Tara; Lab recording; date recorded: 7/25/1988; temperature: 28IC;	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	Museum Koenig (ZFMK); License: CC BY; Montenegro, Montenegro, between Durdjevica Tara and Kosanica; Lab recording; date recorded: 8/20/1989;		
CIs0019.wav	00:33	Poecilimon nonveilleri Ingrisch & Pavicevic, 2010	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Montenegro, Durmitor, Durdevica Tara; Lab recording; date recorded: 7/25/1988; temperature: 28IC;	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	temperature: 3012; light: 60 W builb for heat; recorder: Kenwood KX880HX; microphone: AKG D202; tape: M-MX90; tape no.: KW10:00.00-06.00; filter: microphone filter below 100 Hz		
CIs0022b.wav	00:25	Arcyptera fusca (Pallas, 1773)	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Serbia, Vodice; field recording; date recorded: 7/26/1988; temperature: 28.5IC; light: sunshine; recorder:	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	Duration 00:31 Filename		
CIs0023.wav	00:35	Ephippiger ephippiger ephippiger (Fiebig, 1784)	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Serbia, Rudnik: Ugrinovci; Lab recording; date recorded: 7/26/1988; temperature: 24!C; light: (+);	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	CIS1057.wav Index 0 Keywords		
CIs0030.wav	00:25	Isophya clara Ingrisch & Pavicevic, 2010	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Serbia, Vodice; Lab recording; date recorded: 7/26/1988; temperature: 22.5/C; recorder: SonyWM-D3;	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	CIS 1057 wav Library S. Ingrisch		
CIs0032b.wav	00:20	Pholidoptera frivaldskyi (Herman, 1871)	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Serbia, Partizanske Vode; Lab recording; date recorded: 7/27/1988; temperature: 24/C; recorder:	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	ModificationDate 2013-04-26 15:57:40 +0000 Popularity 0		
CIs0003.wav	00:31	Poecilimon schmidti (Fieber, 1853)	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Croatia, Gespanschaft Virovitica-Podravina, Jankovac; Lab recording; date recorded: 7/25/1988;	S. Ingrisch	Orthoptera	DORSA Sound Collection (ZFMK)	S. Ingrisch	Rating 0 ReleaseDate		
		Poecilimon affinis affinis (Frivaldsky, 1867)	(c) Foundation Zoological Research Museum Koenig (ZFMK); License: CC BY; Croatia, Gespanschaft Virovitica-Podravina, Jankovac; Lab recording; date recorded: 7/25/1988;			DORSA Sound Collection (ZFMK)		1969-12-31 23:00:00 +0000 SampleRate 96000 ScannedDate		
CIs0009.wav	00:18	Barbitistes yersini Brunner v.	(c) Foundation Zoological Research Museum	S. Ingrisch	Orthoptera	DORSA Sound Collection	S. Ingrisch	2013-05-25 03:56:39 +0000		
Found:548 in tes	st		%			Time: J	Selected:1 00:30.62 Duration: 00:3	Metadata Transfer History		
						· · · · · · · · · · · · · · · · · · ·	·····			
E:\DORSA neu sortiert\CI snippets\CIS1057.wav										

Fig. 3. Embedding metadata within sound files. Metadata were embedded within wav and mp3 fields directly from the SYSTAX database using Soundminer software (http://store.soundminer.com/). Metadata are visible within most mp3-players, displaying the species name as "TrackTitle" and the recordist as "Artist" (Courtesy: S. Ingrisch).

al. 2015) comes closest to the requirements outlined above due to its modular design using cutting-edge technology.

A scheme illustrating elements and workflows of a bioacoustics data warehouse is presented in Fig. 4. A fully developed bioacoustic workbench should allow seamless integration of entire soundscape recordings (as generated by PAM) and tools for managing acoustic scenes, with software for annotation and identification of acoustic snippets (cf. Riede and Jahn 2013), and reference corpora generated from targeted recordings with taxonomically identified voucher specimens.

A well-designed data warehouse infrastructure is the only way to organize efficient workflows between taxonomists (providing reference sound libraries) and computer scientists developing algorithmic recognition tools. Ideally, code and documentation of recognizer software should be publicly accessible through the (virtual) data warehouse, together with the sound libraries and references to voucher specimens. For the time being, it is suggested to establish OSF as a taxonomic backbone to host at least one song recording per species, which would allow for verifying completeness of bioacoustic coverage of singing Orthoptera species. Every sound file could be associated with a unique Life Science Identifier (LSID), comparable to Digital Object Identifiers (DOI), facilitating the necessary cross reference between names, multimedia files, voucher specimens, and eventually genetic sequences. However,

at present, a functional LSID architecture is jeopardized by lack of standards (cf. Table 1 in Guralnick et al. 2015).

The way forward: algorithms for acoustic profiling

Well-documented, comprehensive song libraries are the prerequisite for the next logical step, which is acoustic profiling of entire communities. This is particularly promising for lesser known tropical faunas, where acoustic recording could accelerate species assessment. Up to now, overall analysis of Orthoptera communities based on entire soundscapes are still limited to very few sites. Lehmann et al. (2014) used ARUs in the Hymettos mountain range, Greece. Tropical Orthoptera communities have been assessed in the Western Ghats, India (Diwakar et al. 2007), Panama (Schmidt et al. 2013) and Amazonian Ecuador, the latter based exclusively on ethospecies (Riede 1993). Evidence that ethospecies can be reliably attributed to well-defined morphospecies was provided by systematic recording of captured individuals in Ecuadorian lowland and mountain rainforests (Nischk and Riede 2001).

There is a fundamental difference between: 1) automatic classification and identification of individual recordings, consisting of high-quality sound signals of an unknown Orthoptera songster, or; 2) recognition of Orthoptera songs "hidden" within overall soundscape recordings. The two problems are quite distinct, and

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Fig. 4. A data warehouse for sound management. The scheme illustrates elements and workflow for acoustic profiling of Orthoptera. Songs are sampled either by recording individual songsters (Targeted Recordings), or entire acoustic scenes, each of which could contain several Orthoptera songs. Targeted recordings are treated like specimens, with time and locality stamps and, preferably, a voucher specimen. All databases listed in Table 1 are designed to store individual recordings. These distributed databases could be federated via ABCD-or Darwin-protocol. Soundscapes require distinct data management of large multimedia files. Orthoptera songs could be extracted manually or semi-automatically as sound snippets, and eventually be identified (ID) manually, or using automatic sound recognition algorithms (ASR). Many snippets can be extracted from each scene, resulting in a one-to-many relationship between scenes and snippets.

the latter requires additional, complex processing steps. Therefore, they are discussed separately in the following sections.

Classification of individual recordings.—For individual recordings, song parameters such as pulse rate and carrier frequency can be easily extracted by basic sound analysis software. These parameters might be sufficient to identify species using a traditional taxonomic key (Ragge and Reynolds 1998, p.83) based on acoustic features. Benediktov (2015) analyzed a calling community of the orthopteran (Tettigoniidae and Gryllidae) community from an agrocenosis in eastern Bulgaria by straight-forward interpretation of spectrograms, showing that valuable information can be extracted from overall recordings "manually", without complex computer algorithms. Such direct comparisons of song parameters with available feature datasets was classified as a "brute force" approach by Tacioli et al. (2017).

More complex software for Orthoptera song identification is based on Artificial Neural Networks (ANN) and Hidden Markov Models (HMM) which are widely used in automatic human speech recognition (Mustafa et al. 2017). Because ANNs have to be trained by a set of training recordings, and later be tested on another validation set, this approach is only possible for identification of species with at least ten recordings of distinct specimens.

Dietrich et al. (2004) used ANNs and temporal fusion to classify 31 Orthoptera songs from the DORSA database (Ingrisch et al. 2004). Potamitis et al. (2006) used the SINA repository and some additional resources to test automatic identification of insects using speech recognition tools. In a follow-up publication, Ganchev and Potamitis (2007) applied a hierarchic classification scheme, with identification accuracy that exceeded 99% at suborder and family levels. Chaves et al. (2012) used Costa Rican katydid songs from the Naskrecki (2000) CD for sound parameterization using Mel Frequency Cepstral Coefficients and subsequent classification based on HMM, resulting in high accuracy of identification.

Riede et al. (2006) annotated Grylloidea from the SYSTAX-DOR-SA files with essential parameters such as carrier frequency and pulse rate. They applied a batch routine, using segmentation and feature extraction modules developed by Dietrich et al. (2004) to annotate song parameters for hundreds of recordings from 53 species.

Tacioli et al. (2017) reviewed basic principles of existing animal sound identification software and implemented a user-friendly, downloadable software (Wildlife Sound Identification Software (WASIS) http://www.naturalhistory.com.br/wasis.html). At present, the underlying reference database contains recordings from Neotropical birds and amphibia, but it should be possible to use this promising approach for Orthoptera song recognition, as well.

Data-mining soundscapes.—Identification of individual species in soundscapes is a much harder task because of noise and highly variable microphone distances from songsters. As a first step, Regions of Interest (ROIs) – sound signals probably containing a song – have to be identified and filtered. In a second step, these

ROIs can eventually be treated and classified like individual recordings. A considerable number of publications report successful algorithmic identification of sets of bat (Jennings et al. 2008), bird (Potamitis et al. 2014), and frog (Hu et al. 2009) species within field recordings from certain sites. As with individual song recognition software, these algorithms have to be trained, requiring a considerable number of training recordings, preferably from the respective area.

Most recognition software was developed for birds, based on extensive corpora of overall soundscape recordings and high numbers of individual, labelled species recordings used for training and testing. Knight et al. (2017) provide an overview of underlying principles and performance benchmarking of five readily available species recognition programs. Among these programs, the template-based MonitoR software (Katz et al. 2016) is particularly promising, because it is a package implemented in R (https:// www.r-project.org/), a free software project becoming increasingly popular among biologists. In addition, R contains the seewave package (Sueur et al. 2008a), designed for sound analysis and synthesis. Users familiar with R can modify or combine it with other R packages (Sueur 2018). Ovaskainen et al. (2018) developed Animal Sound Identifier (ASI), an interesting toolbox running on Matlab. Unlike most previous approaches, ASI locates training data directly from the field recordings and thus avoids the need for pre-defined reference libraries.

Phillips et al. (2018) present an impressive method of reducing audio data to six orders of magnitude, facilitating the interpretation of environmental audio. By clustering vectors of acoustic indices, they were able to attribute clusters to dominant sound sources, such as birds, cicadas, or Orthoptera. They were able to determine Orthoptera calling date and time of day within a huge dataset of 26 months of recordings. With this pre-processing, it should be easy to extract relevant Orthoptera snippets and eventually store them as "ethospecies" (*sensu* Riede 1993) for future identification.

To facilitate multiple use of sound files for improving algorithms, the respective sound files should be tagged and labelled as a corpus. A wide variety of well-documented corpora is available to be used in computational linguistics and speech recognition. A speech corpus is a well-defined set of speech audio files (Harrington 2010), and a pre-requisite for reproducible results in classifier and recognizer development. Well-curated corpora are not yet available in bioacoustics (cf. Riede and Jahn 2013), which hampers progress of computer-aided analysis.

Discussion

Otte and Alexander (1983) were the first to point out the enormous potential of communicative signal analysis for understanding the systematics and taxonomy of Orthoptera:

"It must be clear at this point that those systematists who utilize communicative signals and isolating mechanisms as their principal means of locating and recognizing species are not simply studying biology as well as morphology, or simply using a wide variety of characters, as is commonly and justifiably considered desirable in bio-systematic work. Their entire approach, their methods of analysis, and their interpretations of particular kinds of data are all different. Further, and probably most important, their possibilities for rapid and accurate systematic coverage are unparalleled. For this reason, the groups of animals for which these techniques are possible ought to present unique opportunities for breakthroughs in biogeography and in the study of speciation and other evolutionary phenomena." (1.c., p. 5). Three decades later, bioacoustic characters of Orthoptera songs frequently form part of species descriptions, taxonomic revisions (e.g. Anatolian *Chorthippus* species: Mol et al. 2003), as well as phylogenetic studies (Desutter-Grandcolas 2003, Nattier et al. 2011), being a well-established element of a comprehensive, "integrative" taxonomy (Dayrat 2005, Schlick-Steiner et al. 2010).

To mobilize the full potential of sound repositories for biodiversity research, innovative query tools are needed. The vision is to upload a sound recording to a data warehouse portal and search for similar acoustic patterns, comparable to BLAST (Altschul et al. 1990), available as a tool in genetic databases (e.g. NCBI). The potential of such innovative tools will be further enhanced by federated access to distinct sound archives, using one portal with a unified query tool. As a next step, applications running on portable computers could allow classification and identification of songs in the field. Such an infrastructure sounds demanding, but its elements are already available.

Thanks to the rapid technological evolution of hard- and software, complex Artificial Intelligence tools for recognition of human speech, music and animal sounds are now available for personal devices such as smartphones. Commercial programs and apps such as Shazam (for music recognition: https://play.google. com/store/apps/details?id=com.shazam.androidandhl=en_US) or Alexa (for human speech recognition https://play.google.com/ store/apps/details?id=com.amazon.dee.appandhl=de) are wellknown examples. Evidently, speech recognition is of considerable military and economic interest, which means that large parts of on-going research are not accessible to the research community. This might be the reason that animal sound recognition lags far behind the performance of the above-mentioned commercial products.

PAM in combination with computer-aided algorithms could lead to major progress in species monitoring and discovery. Lomolino et al. (2015) highlight the potential of these ecoacoustic surveys for biogeography. However, most of the terabytes resulting from PAM are only used to calculate soundscape indices, to be used in landscape ecology (cf. Ross et al. 2018). Ferreira et al. (2018) compared six soundscape indices with sonotype richness in a species-rich Brazilian tropical savanna. A sonotype is equivalent to the acoustic morphospecies (Aide et al. 2017) or ethospecies (Riede 1993). It is recognizable as an individual vocalization, but not necessarily supported by a reliable species identification. Ferreira et al. (2018) showed that the majority of sonotypes could not be attributed to birds. They criticize the bias of several indices on avifauna and emphasize the need to include insects and anurans in ecoacoustics.

While there has been considerable progress in bird song recognition and the labelling of large audio datasets, a comparable milestone has not yet been reached for Orthoptera. This is probably due to insufficient coverage in sound libraries. Orthoptera songs have been documented for less than 20% of described species. Reference sound libraries are missing not only for tropical regions, but are incomplete even for well-known faunas, e.g. North American grasshoppers, despite comprehensive literature including detailed description of communication and spectrograms of songs (Otte 1981). In addition, complex software for training ANNs requires several recordings for each species. Therefore, for simple logical reasons, neither queries nor sophisticated software will produce useful results with reference libraries containing only 1 or 0 recordings for each species.

It must be doubted that self-organizing scientific routine procedures will suffice to establish the necessary infrastructure sketched here. A strong commitment for data sharing as part of **Acknowledgements** good scientific practice is needed, preferably under the leadership of the respective scientific societies such as IBAC or the Orthopterists' Society, together with representatives from major sound archives. The OSF (Cigliano et al. 2018) provides an authoritative taxonomic backbone and tools for the upload and retrieval of sound files. At present, OSF database managers and editors of the Journal of Orthoptera Research encourage submission of sound files together with manuscripts, but there is no obligation. In contrast, submission of gene sequence data to the NCBI is a pre-requisite for publication, resulting in rapid population of gene banks and impressive advances in molecular biology.

Despite promising first results, an efficient connection and data flow between sound archives, museum collections, advanced computational tools and users has not yet been established. Close cooperation of biologists with computer engineers is needed to cope with the data deluge generated by PAM. Again, well-curated and documented song libraries are a prerequisite to exploit bioacoustic Big Data for further biodiversity assessments.

Basically, an efficient acoustic sampling strategy should consist of the following components:

- 1. Protocols for standardized acoustic recording, at species and community level, using acoustic data loggers for autonomous long-term recordings.
- 2. Open access to and efficient management of sound recordings, song data, and voucher specimens, involving the Orthoptera Species File (OSF: Cigliano et al. 2018) as a taxonomic backbone, and the Global Biodiversity Information Facility (GBIF) for federation of distinct biodiversity multimedia databases.
- An infrastructure for automatic analysis and song classification for on-the ground and web-based analysis, including web2.0 applications for user communities and citizen science.
- 4. A strategic framework for future inventorying and monitoring efforts, including geographic priorities.

Components 1 and 3 involve the entire terrestrial bioacoustics research community, requiring considerable effort to overcome fragmentation between distinct bioacoustic subgroups, clustering around distinct taxa (e.g. frogs, birds, etc.). In contrast, 2 and 4 focus on Orthoptera and are feasible, eventually serving as a model for other species groups.

Conclusions

A recent commentary paper by Deichmann et al. (2018) entitled "It is time to listen" called for a systematic monitoring of rainforest soundscapes. Singing insects are the principal component of these soundscapes. Orthoptera songs are characterized by well-defined signal parameters such as carrier frequency and pulse rate. Acoustic profiling techniques have much to offer, from rapid assessment and species discovery of acoustically active species in remaining wilderness areas to continuous monitoring in managed landscapes. Their full potential can only be developed by cooperative data sharing. At present, an increased wealth of digitized bioacoustic data leads to confusing fragmentation: without the creation of a data warehouse infrastructure, bioacousticians will lose an excellent opportunity to exploit potential synergies from on-going soundscape monitoring initiatives and contribute to urgently needed biodiversity assessments. Likewise, without willingness for data sharing, the newly emerging field of ecoacoustics will generate fragmented soundscape monitoring projects.

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