Neoxabea mexicana sp. nov. (Gryllidae: Oecanthinae): A new species from Mexico and a key for *Neoxabea* in North and Central America

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Abstract

A new species of tree cricket, *Neoxabea mexicana* **sp. nov.**, is described from northeast Mexico. Although it has morphological similarities to two other species found in Mexico, there are distinguishing characters, such as a well-developed tubercle on the pedicel, black markings on the maxillary palpi, one of the two pairs of spots on the female wings positioned at the base of the wings, stridulatory teeth count, and the pulse rate of the male calling song. The calling song description and pre-singing stuttering frequencies are provided. Character comparisons that rule out other species in the genus are presented. The common name given to this new species is Mexican tree cricket. Sound recordings and video are available online. We also make some clarification of the status of *Neoxabea formosa* (Walker, 1869), described as *Oecanthus formosus*, and present a key of *Neoxabea* in North and Central America.

Keywords

Introduction

A photograph posted in October 2018 (Fig. 1) on Naturalista (2020) of a female tree cricket in northeast Mexico led to the investigation of the new species described here. According to the current listing on the Orthoptera Species File (OSF) online (Cigliano et al. 2020), only two of the thirteen species of *Neoxabea* have been documented in Mexico—*N. bipunctata* (De Geer, 1773) and *N. formosa* (Walker, 1869). A 2020 photograph (Naturalista 2020, iNaturalist 2020) from Bosques del Rey, Guadalupe, Nuevo Leon, Mexico, showed a male tree cricket that lacked spines on the hind tibiae and had areas of dark staining on the tegmina. Subsequent findings of another male and female (Figs 2, 3) from the exact locality as the photograph of the female in 2018 led to further photographs, song recordings, and the collection of specimens. By ear, the recordings (Naturalista 2020, iNaturalist 2020) revealed the same slow clicking sound sometimes made by other species in this genus, but the trilling portion of the song had a richer musical quality than the buzzy sound of *N. bipunctata*.

The morphology and song data of *N. bipunctata* are well documented and thus easily compared to this new species. The lack of data for *N. formosa*, along with its complicated history, presented challenges. In his description of *Oecanthus formosus* Walker, 1869, a female specimen from the collection of M. Salle was designated as the holotype (Walker 1869). In the preface of F. Walker's description, J.E. Gray (Keeper of Zoology, which then included entomology) explained that letters before any specimen "denote the specimens now contained in the British Museum," and F. Walker listed the specimen as "*a* – Mexico. From M. Salle's collection."

Kirby (1906) catalogued much of the Insect Collection in the British Museum (Natural History), and listed *O. formosus* as a synonym of *N. bipunctata*, adding ** to indicate that the type was present in the collection. In his 1966 examinations of Oecanthinae, T. Walker indicated that the *N. formosa* holotype was missing from the British Museum. The British Museum, at Great Russell Street, London, originally included both human and natural history collections, but in 1881 the Natural History Department was transferred to a new building in South Kensington and referred to as the British Museum (Natural History). In 1963, this was legally separated from the British Museum, though it retained the name BM(NH) until 1992, when it was formally renamed as the Natural History Museum (NHM), referred to more recently in publications as NHMUK.

In November 2020, after requesting confirmation from NHMUK that the type specimen of *Oecanthus formosus* was missing, as cited in the OSF website (Cigliano et al. 2020), Ben Price, Senior Curator at the NHMUK, located the holotype of *O. formosus*. The number on the specimen "[18]56–143" refers to the Accession Register and confirms the data of this specimen as "From Mexico. Purchased from Cuming. Collected by M. Sallé on & around the volcano of Orizaba." Orizaba is located in Veracruz, Mexico. T. Walker (1967) noted a female specimen with a label of "type ?" from the British Museum and wrote that if any question is associated with the specimen being a type, it should not be designated as such. He also pointed out that the female might eventually prove to be other than *N. formosa*. He indicated that there were two species present among the specimens regarded as

N. bipunctata, and that the females of *N. formosa* could not be distinguished from those of *N. bipunctata.*

Thirteen specimens from Mexico and Brownsville, Texas, were examined by T. Walker in four major collections (Walker 1967). He determined that males have 56–63 stridulatory teeth and included that data on a graph with several other species of *Neoxabea* (Walker 1967). The Florida State Collection of Arthropods (FSCA) holds five specimens labeled as *N. formosa* (Fig. 6A–E), including two male and three female specimens from "Brownsville, Texas, USA" and "Atoyac, Veracruz, Mexico". The specimen presented in Figure 6A has tubercles on both the pedicel and scape and a dark line on the palpi. The three female specimens, faded from age (Fig. 6C–E), have a single spot mid wing on each side and a fainter pair of spots at the base of the wings.

A photograph taken by NC in September 2019 (Fig. 7A, B) shows two of the six specimens held in the collection at the Academy of Natural Sciences in Philadelphia labeled as *N. formosa.* Figure 7A represents a female collected in Brownsville, Texas, and Figure 7B a female collected in Veracruz, Mexico. The Veracruz specimen has two pairs of spots that are centrally positioned, and the Brownsville specimen has one of the two pairs of spots positioned at the base of the wings.

This paper describes a new species of *Neoxabea* that has a stridulatory teeth count that falls within the range of some of the male specimens labeled as *N. formosa* that T. Walker examined in 1966. The original species of *N. formosa* remains intact, with the only known specimen being the holotype, a female, at the NHMUK in South Kensington, and which was collected at the volcano Orizaba in Veracruz, Mexico (Fig. 17E). A key for five species of *Neoxabea* known to occur in North and Central America is provided.

Materials and methods

Collection methods.—Individuals were found on an outdoor illuminated ceiling and walls.

Type verification.—Keys from Walker (1967) and the Singing Insects of North America (SINA) (2020) were used to verify the genus. Specimens were examined for the absence of spines on the hind tibiae.

Song recordings and analyses.—Songs of an actively roaming captive male were recorded using a Huawei P30 Pro cellphone held as close to the singing tree cricket as possible. The cellphone was tested for calibration by recording a file from SINA and comparing the pulse rate and frequency to the original file. Raven Lite 2.0 was used to prepare waveforms to count pulse rates, and spectrograms were used to determine carrier frequency.

Habitat.—Individuals were found on the private property of CGV-M in Guadalupe, Monterrey, Nuevo Leon, in northeast Mexico. Native vegetation on the property includes *Tecoma stans* (L.) Juss. ex Kunth; *Senna alata* (L.) Roxb.; *Asclepias curassivica* L.; *Lantana camara* L.; *Poliomintha bustamanta* B. L. Turner; *Croton ciliatoglandulifer* Ortega; *Ruellia simplex* C. Wright; *Merremia dissecta* (Jacq.) Hallier f. (POWO 2020).

Guadalupe sits at the base of Cerro de La Silla, and the private property sits approximately 800 meters from the start of vegetation of the mountain. Species of vegetation that predominate on the mountain are *Vachellia rigidula* (Benth.) Seigler & Ebinger; *Vachellia farnesiana* (L.) Wight & Arn.; *Cordia boissieri* A. DC.; and *Opuntia* spp. At higher altitudes, the following are more frequent species: *Havardia pallens* (Benth.) Britton & Rose; *Helietta parvifolia*

(A. Gray) Benth.; *Caesalpinia mexicana* (A. Gray, 1861) E. Gagnon & G. P. Lewis, 2016; *Leucaena pulverulenta* (Schltdl.) Benth; and a naturalized tree from southern Mexico—*Leucaena leucocephala* (Lam.) de Wit. In the highest zone, *Quercus fusiformis* Small and *Q. canbyi* Trel. occur (Cerro de La Silla 2009, Alanís-Flores et al. 2010).

Climate.—Temperatures of the spots where tree crickets were singing were measured using a Steren thermometer, Model TER-150, with a range of -10 to 50°C.

Preservation of specimens.—Specimens were preserved in 70% ethyl alcohol.

Morphological measurements.—Measurements were made after the specimens were euthanized by freezing or by immersion in 70% ethyl alcohol. Total body length refers to the midline length from the tip of the labrum to the apex of the subgenital plate, not including antennae, tegmina, limbs, or cerci. Tegminal width was measured at the widest section, while resting atop the abdomen of the male. Pronotal length was measured along the medial line of the pronotum. Female ovipositors were measured from the base at the distal abdomen to the tip. Photographs and measurements of the ovipositor, cerci, and metanotal gland, as well as counts of the stridulatory teeth, were made with the aid of an AmScope Stereoscope, magnification WF10× to 2× to 4× (photographs made using a Huawei P30PRO cellphone camera with a Leica Vario-Summilux-H1.6-3.4/16-125 ASPH lens). Comparison photographs of *N. bipunctata, N. cerrojesusensis,* and *N. ottei* were made with a Canon PowerShot S5 IS.

Male genitalia.—The male genitalia complex was extracted and photographed using a Huawei P30PRO cellphone camera with a Leica Vario-Summilux-H1.6-3.4/16-125 ASPH lens. Copulatory blades (Fulton 1915), which are also known as pseudepiphallus (Chopard 1961) or lophi medians (Desutter 1987), are situated just above the subgenital plate. The blades can sometimes be seen if the male's subgenital plate is lowered, including spontaneously on live males.

Results

Neoxabea mexicana Collins & Velazco-Macias, sp. nov.
http://zoobank.org/170F61F8-6BA5-4D58-AACE-19B51DE91EE2
Figs 1–5, 8–14

Type-specimen.—**Holotype** ♂ (Fig. 2), alcohol vial. Bosques del Rey, Guadalupe, Nuevo Leon, Mexico. 25°38.21'N, 100°12.26'W, elevation ca 580 masl, urban area adjacent to Cerro de La Silla, C.G. Velazco-Macias & J.G. Velasco-Castañon leg., 15 X 2020.

Holotype description.—Face, head, and pronotum brown. Pedicel and scape brown, each with one protruding tubercle (Fig. 5); remainder of antennomeres translucent whitish with black staining. Palpi light brownish with black line on each segment (Fig. 4). Eye color brown. Wing color brown and whitish or cream. Ventral abdomen brownish with scattered staining (Fig. 9). Dorsal abdomen median terga projections rounded (Fig. 10). Tarsi, tibiae, and femora with translucent pale yellowish tan with no lines or spotting. Tympanal membrane on fore tibiae with whitish rim. Cerci loosely S-curved and brown (Figs 9, 11). Total length 17.0 mm; tegminal length 12.0; tegminal width 4.0; pronotal length 3.0; distal pronotal width 2.0; hind femur length 8.0; cerci 1.5; stridulatory file length 2.0 mm. Right tegminal stridulatory teeth total 55.

Figs 1–5. *Neoxabea mexicana* **sp. nov.** adults: **1.** ♀ habitus; **2.** Holotype ♂ habitus; **3.** ♀ with larger amount of cream coloring; **4.** Black line on palpi; **5.** Pedicel and scape tubercles.

Type material.—Located October 2020 on private property in Bosques del Rey, Guadalupe, Nuevo Leon, Mexico. 2 ♂ and 1 ♀ deposited at Universidad Nacional Autónoma de Mexico (UNAM), one ♂ of these designated as the holotype for this species.

The genus *Neoxabea* was determined as the Nuevo Leon specimens lacked spines on the hind tibiae (Figs 1–3). Males display large amounts of dark staining on the tegmina (Fig. 2). Females (Figs 1, 3) display a pair of blackish spots on the lateral edges of brown wings with areas of cream and a pair of less obvious spots embedded in the dark brown areas of the wings at the base. Both sexes have a black line on each segment of the maxillary palpi (Fig. 4) and a protruding tubercle on both the pedicel and scape (Fig. 5). The female in Figure 3 actively mated with one of the two males found on the same property (video in Suppl. material 1).

Male paratype (n=1).—Total body length 17.0 mm, tegminal length 12.5, tegminal width 5.5, pronotal length 3.5, distal pronotal width 3.0, proximal pronotal width 2.2, hind femur length 9.0, cerci 2.25. Stridulatory file length 2.0. Right tegminal stridulatory teeth total 53.

The metanotal gland, color of the ventral abdomen, cerci, distal abdominal terga projections, ovipositor, and copulatory blades are presented in Figs 8–13, respectively. The metanotal gland scutum to scutellum (Walker 1967) ratio is 1:0.6. Long setae cover the ends of two curved structures situated on both sides of the scutellum that project upward into the center orifice of the gland

Fig. 6. Florida State Collection of Arthropods specimens labeled as *N. formosa*. **A.** ♂ from Atoyac, Veracruz, Mexico; **B.** Same ♂ lateral view; **C.** ♀ from Veracruz; **D.** ♀ from Brownsville, Texas, USA; **E.** ♀ from Brownsville.

(Fig. 8). The gland area resembles that of *Neoxabea femorata* Walker, 1967 in figure 25 of Walker (1967); however, the scutum of *N. mexicana* **sp. nov.**, is twice as wide as long and has a straight upper edge. Figure 10 provides a closer inspection of the shapes of all eight abdominal terga projections. The most distal (eighth) projection is much smaller in size than the other seven. The cerci are robust and loosely S-shaped (Figs 9, 11, 12). The copulatory blades (main lobes of pseudepiphallus) touch at the midline while in a rested position and hook upward (Fig. 13A–C). Photos of additional structures in Suppl. material 2.

Female (n=2).—One female was not euthanized and only partially measured. Latticed vein pattern on wings with a single large dark pair of spots at lateral edges mid-wing (Figs 1, 3), and a second pair of spots at the base of the wings. The degree of brown and cream varies. Total body length 16.0 mm, pronotal length 2.5–3.0, distal pronotal width 2.0–2.5, proximal pronotal width 1.5–2.0, hind femur length 7.5–8.2, cerci 2.0, ovipositor length 5.0. The tip of the ovipositor (Fig. 12) does not extend beyond the tips of the hindwings.

Morphological differences.—The maxillary palpi of *N. mexicana* **sp. nov.** have a longitudinal black line (Fig. 4), which is ruddy on *N. bipunctata*. The numerous darkened areas on the male tegmina of *N. mexicana* **sp. nov.** are not found on *N. bipunctata*. Female *N. mexicana* **sp. nov.** have a pair of black spots mid wing, and a pair of spots at the base of the wings. *N. bipunctata* also has a total of two pairs of spots, but the proximally placed spots are not at the base of the wings. The tubercle on the pedicel of *N. mexicana* **sp. nov.** is well-developed, while there is generally a very small dimple on the pedicel of *N. bipunctata*. See other species comparisons in Fig. 17A–R and Table 1.

Song.—Calling song recordings were made in captivity by CGV-M (Suppl. material 3, 4). Waveform review revealed that this species has bursts of trilling with 36 pulses per second at 26.6°C (Fig. 14). Pulse rates of the songs at different temperatures by this species and three other *Neoxabea* species occurring in North and Central America were plotted on a pulse rate vs temperature graph (Fig. 15). Although only a single recording with an accurate temperature for *N. ottei* and two recordings for *N. cerrojesusensis* are available, several additional recordings were analyzed, and the pulse rate never dropped below 80 pulses per second with am-

Table 1. Non-matching characters of 13 described species of *Neoxabea* when compared to *N. mexicana* **sp. nov.**

Neoxabea species	Characters not matching *N. mexicana* sp. nov.	Sources
N. ottei (Collins & van den Berghe, 2014)	< 25 stridulatory teeth; females w/ 4 centrally located wing spots	Collins et al. (2014) (p. 165)
N. astales (Walker, 1967)	< 30 stridulatory teeth; no abdomen terga projections	T. Walker (1967) (fig. 28)
N. bipunctata (DeGeer, 1773)	< 30 stridulatory teeth	T. Walker (1967) (p. 786)
N. cerrojesusensis (Collins & van den Berghe, 2014)	< 30 stridulatory teeth; male tegmina with minimal staining	Collins et al. (2014) (p. 165)
N. obscurifrons (Bruner, 1916)	< 30 stridulatory teeth; drab coloring	T. Walker (1967) (fig. 23)
N. femorata (Walker, 1967)	< 40 stridulatory teeth; striped hind femora	T. Walker (1967) (fig. 28)
N. trinodosa (Hebard, 1928)	75–80 stridulatory teeth	T. Walker (1967) (fig. 28)
N. brevipes (Rehn, 1913)	Spotting on limbs; song > 54 pps at 19°C	T. Walker (1967); Zefa et al. (2018)
N. enodis (Walker, 1967)	Bold cephalic pattern; no abd terga projections	T. Walker (1967) (fig. 16)
N. lepta (Walker, 1967)	Long, thin, straight cerci; no abd terga projections	T. Walker (1967) (fig. 6)
N. meridionalis (Bruner, 1916)	Distinct tegmina staining; no abd terga projections	T. Walker (1967) (fig. 12)
N. formosa (Walker, 1869)	Females with four centrally located spots on wings	F. Walker (1869)
N. quadrula (Walker, 1967)	Cerci with large swelling; 35 stridulatory teeth	T. Walker (1967) (figs 9, 28)

Fig. 7. Academy of Natural Sciences in Philadelphia specimens labeled as *N. formosa*. **A.** ♀, Brownsville, USA; **B.** ♀, Coatepec, Veracruz, Mexico.

bient temperatures above 22°C. Permit limitations allowed only two nights of recordings. The pulses per second rate of *N. mexicana* **sp. nov.** is less than half of that for *N. bipunctata, N. ottei,* and *N. cerrojesusensis.* Spectrograms review revealed a calling song carrier frequency of 3.6 kHz at 26.6°C and 3.4 kHz for the pre-singing stuttering (Fig. 16). When compared to the frequencies of the other three *Neoxabea* species mentioned above, *N. mexicana* **sp. nov.** had a more marked change in the frequency of the stuttering versus the frequency of the singing.

Calling song tone comparison.—The tone of the trilling portion of the song of *N. mexicana* **sp. nov.** is more musical and flute-like
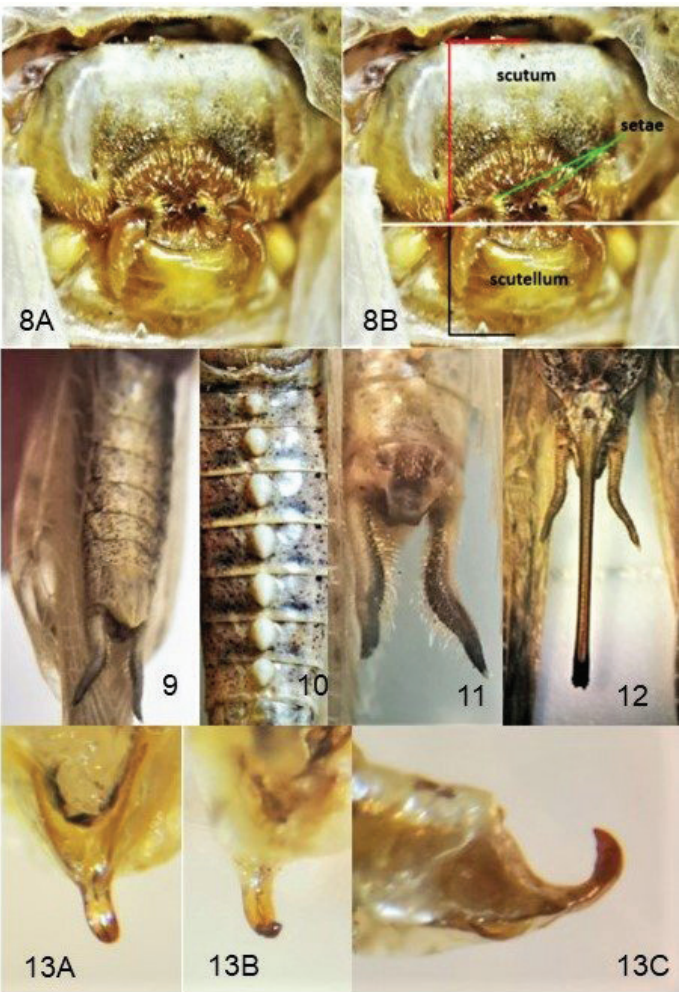
Figs 8–13. Morphology of *N. mexicana* **sp. nov. 8A.** Metanotal gland; **8B.** Labeled metanotal gland; **9.** Ventral abdomen color and subgenital plate on live male; **10.** All eight projections on dorsal abdominal terga of male; **11.** Dorsal view of male cerci; **12.** Ventral view of female ovipositor; **13A.** Ventral view of copulatory blades (main lobe of pseudepiphallus) of holotype male; **13B.** Dorsal view; **13C.** Lateral view.
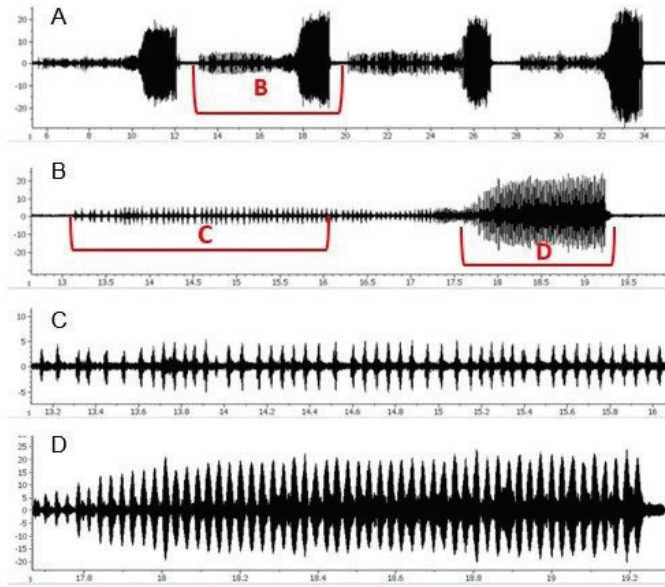
Fig. 14. Waveforms of the calling song of *N. mexicana* **sp. nov.** at 26.6°C (after applying 2 kHz high pass filtering); **A.** Six bursts of singing in 50 sec.; **B.** One burst of singing with warm up stuttering before next burst of singing; **C.** One second of warm up stuttering showing a total of 17 pulses (click sounds); **D.** One second of singing (36 pulses per second).

than the buzzy sound of the faster song of *N. bipunctata*. Both species have a pre-trilling stuttering, but the stuttering of *N. mexicana* **sp. nov.** is longer in duration, more consistently present, and was found to be more flute-like in tone even at moderate temperature (22.7°C). *Neoxabea mexicana* **sp. nov.** has a 200 Hz difference between the frequency of the stuttering and the song trilling, while the frequency of *N. bipunctata* remains constant.

Key to *Neoxabea* species in North and Central America

Fig. 15. Comparing calling song pulse rates of *N. mexicana* **sp. nov.** to *N. bipunctata*, *N. ottei*, and *N. cerrojesusensis*. Data for recordings in Suppl. material 5.
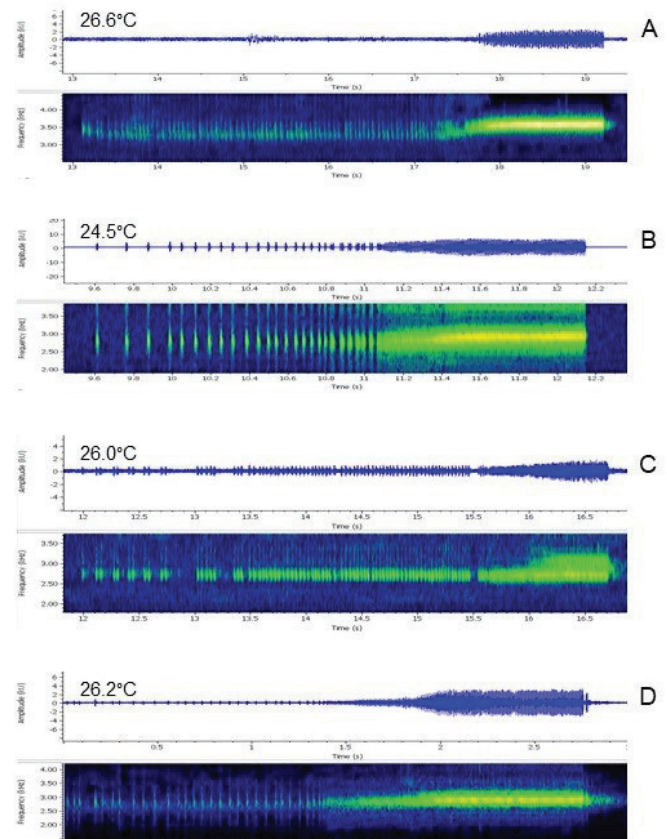
Fig. 16. Waveforms and spectrograms highlighting stuttering and singing. **A.** *N. mexicana* **sp. nov.**; **B.** *N. bipunctata* (Source: SINA); **C.** *N. cerrojesusensis*; **D.** *N. ottei*.

Neoxabea species comparisons.—The 13 described species of *Neoxabea* can be ruled out as *N. mexicana* **sp. nov.** with non-matching characters, as in Table 1.

Fig. 17. Images of adult wing patterns, palpi markings, and pedicel tubercles for species in the *Neoxabea* key. **A.** *mexicana* **sp. nov.** ♀; **B.** *cerrojesusensis* ♀; **C.** *bipunctata* ♀; **D.** *ottei* ♀; **E.** *formosa* ♀; **F.** *mexicana* **sp. nov.** ♂; **G.** *cerrojesusensis* ♂; **H.** *bipunctata* ♂; **I.** *ottei* ♂; **J.** *bipunctata*; **K.** *mexicana* **sp. nov.**; **L.** *formosa*; **M.** *cerrojesusensis*; **N.** *ottei*; **O.** *mexicana* **sp. nov.**; **P.** *cerrojesusensis*; **Q.** *ottei*; **R.** *bipunctata*.
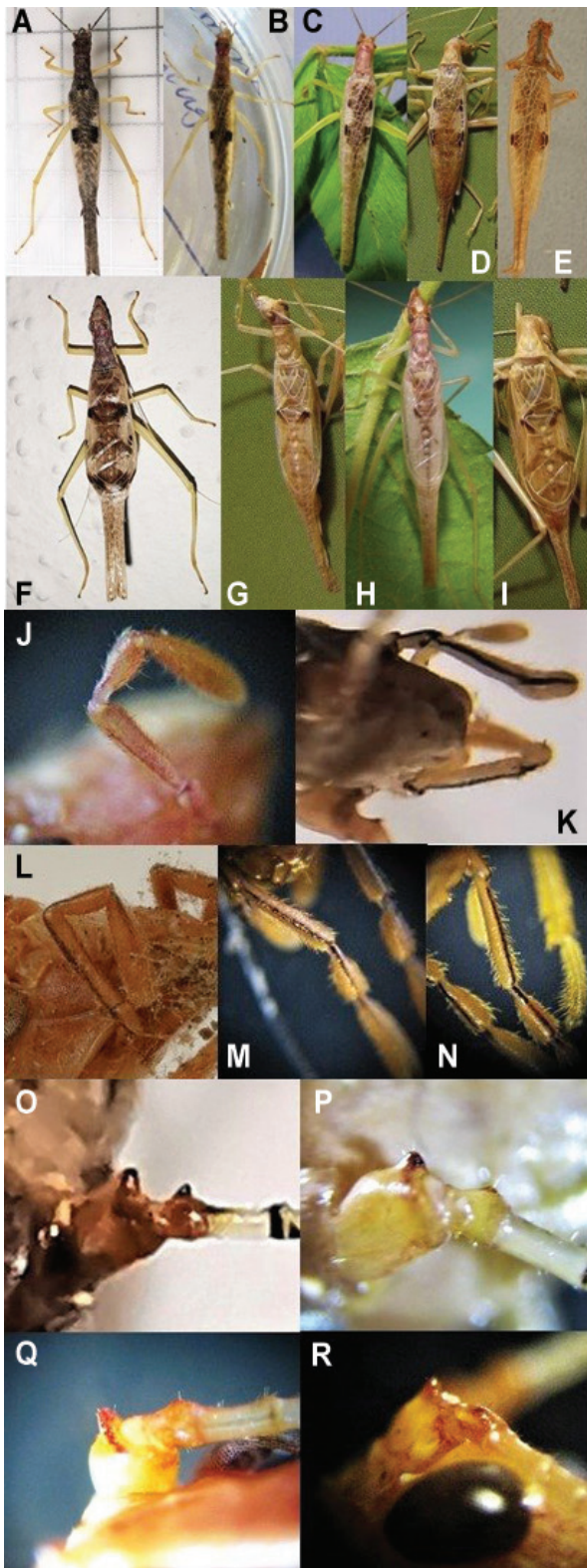
Discussion

This investigation revealed the following characters for *Neoxabea mexicana* **sp. nov.**: well-developed tubercles on the pedicel as well as the scape; a black line on all segments of the maxillary palpi; a large amount of dark staining on the male tegmina, and one pair of dark spots on the wings of females located at the base. These characters, along with the calling song rate and stridulatory teeth count, are distinctly different from those in *N. bipunctata*, which is also reported from Mexico. While the warm-up stuttering and the singing phase of *N. bipunctata* remain at a consistent frequency, the song of *N. mexicana* **sp. nov.** consistently shows a rise from the stuttering phase to the singing phase of up to 200 kHz.

In this paper, we focus on comparisons to *N. bipunctata*, since that species is well studied and documented as occurring in Mexico. Comparisons to other species of *Neoxabea* in Table 1 and the key to *Neoxabea* of North America and Central America confirm the diagnosis of a new species. The dark lines on the maxillary palpi of the holotype of *N. formosa* are distinctly different from the fainter ruddy lines on *N. bipunctata*. The ventral abdomen is too degraded from age to check for the two dark lines described by F. Walker. Suppl. material 6 provides photos of *N. formosa*. Since a species described from Nicaragua, *N. ottei*, also has females with four spots on the wings in the same positions as those on the *N. formosa* holotype, these two species cannot currently be separated without examining males of *N. formosa*. The only known locality of *N. formosa* is a dormant volcano, Orizaba, which is the third highest in North America. This habitat could easily be a location with uncommonly encountered species.

Our recommendation for male specimens currently labeled as *N. formosa* in collections is to treat them as either *N. formosa* or *N. mexicana* **sp. nov.** until a living male can be verified as *N. formosa*. Female specimens labeled as *N. formosa* with two pairs of centrally located spots on the wings cannot currently be distinguished from *N. ottei*, but the intensity of the line on the maxillary palpi can be used for differentiating between *N. bipunctata* and *N. formosa*.

Acknowledgements

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Supplementary material 1

Author: Carlos Gerardo Velazco-Macias

Data type: Movie (mov. file)

Explanation note: Mating pair of *N. mexicana* sp. nov..

Link: https://doi.org/10.3897/jor.26.62000.suppl1

Supplementary material 2

Author: Carlos Gerardo Velazco-Macias

Data type: Image (jpg. file)

Explanation note: Structures of *N. mexicana* sp. nov.: **A.** Holotype right wing stridulatory file with teeth; **B.** Female tympanal membrane; **C.** Male tympanal membrane with front limb claw; **D.** Spermatophore; **F.** Ovipositor.

Link: https://doi.org/10.3897/jor.26.62000.suppl2

Supplementary material 3

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Data type: SWF file

Explanation note: Recording of captive male.

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Supplementary material 4

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Data type: SWF file

Explanation note: Recording of captive male.

Link: https://doi.org/10.3897/jor.26.62000.suppl4

Supplementary material 5

Author: Nancy Collins

Data type: Excel spreadsheet

Explanation note: Graph of recording data for four species of *Neoxabea*.

Link: https://doi.org/10.3897/jor.26.62000.suppl5

Supplementary material 6

Author: Ben Price

Data type: Image (jpg. file)

Explanation note: Photographs of the female holotype for *N. formosa* – taken at the Natural History Museum in the UK.

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Embryo-to-embryo communication facilitates synchronous hatching in grasshoppers

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Abstract

Synchronous hatching within single egg clutches is moderately common in locusts and other insects and can be mediated by vibrational stimuli generated by adjacent embryos. However, in non-locust grasshoppers, there has been little research on the patterns of egg hatching and the mechanisms controlling the time of hatching. In this study, the hatching patterns of six grasshoppers (*Atractomorpha lata*, *Oxya yezoensis*, *Acrida cinerea*, *Chorthippus biguttulus*, *Gastrimargus marmoratus*, and *Oedaleus infernalis*) were observed under various laboratory treatments. Under continuous illumination and a 25/30°C thermocycle, the eggs of these grasshoppers tended to hatch during the first half of the daily warm period. Eggs removed from egg pods and cultured at 30°C tended to hatch significantly earlier and more synchronously when kept in groups vs. singly. In general, eggs hatched earlier when egg group size was increased. Egg hatching was stimulated by hatched nymphs in some species, but not in others. In all species, two eggs separated by several millimeters on sand hatched less synchronously than those kept in contact with one another, but the hatching synchrony of similarly separated eggs was restored if they were connected by a piece of wire, suggesting that a physical signal transmitted through the wire facilitated synchronized hatching. In contrast, hatching times in the Emma field cricket, *Teleogryllus emma*, which lays single, isolated eggs, were not influenced by artificial clumping in laboratory experiments. These results are discussed and compared with the characteristics of other insects.

Introduction

Most grasshopper species deposit their eggs a few centimeters underground in a foamy egg pod that can contain as many as 200 clumped eggs, depending on species (Uvarov 1977, Stauffer and Whitman 1997). In temperate zones, the eggs typically overwinter and then hatch in the spring. Field observations suggest that different species tend to hatch at different times of the day, and some species exhibit hatching synchrony such that the majority of eggs in a single egg pod hatch more or less simultaneously (Uvarov 1977, Smith et al. 2013).

The daily hatching time in grasshoppers is thought to be controlled by environmental factors such as daily photoperiod and temperature cycles, as observed in other insects (Tauber et al. 1986, Danks 1987, Saunders 2002). For example, eggs of the desert locust *Schistocerca gregaria* (Forskål, 1775) hatched around dawn in the field (Ellis and Ashall 1957) and during the low-temperature period of a thermocycle or during the dark phase of a photoperiod in the laboratory (Padgham 1981, Nishide et al. 2015a, b). In contrast, eggs of the migratory locust *Locusta migratoria* (Linnaeus, 1758) and the lubber grasshopper *Romalea microptera* (Beauvois, 1817) (Chen 1999, Smith et al. 2013, Nishide et al. 2017a) hatched during the day or during warm periods of thermocycles. Because the eggs of most grasshopper species are laid underground where light might not penetrate, it is possible that the eggs use changes in soil temperature rather than photoperiod to control hatching time. For example, *S. gregaria* eggs removed from a pod and exposed to light-dark cycles under constant temperature hatched mainly during the dark period (Padgham 1981, Nishide et al. 2015a, b). However, they hatched during the light and dark periods at similar frequencies when they were covered with a layer of sand or kept in naturally laid pods deposited underground (Nishide et al. 2015b), suggesting that the light had not reached the eggs buried in the sand. Species-specific hatching times may have evolved to maximize the survival of the fragile fresh hatchlings, which are susceptible to predation and environmental extremes (Uvarov 1977, Smith et al. 2013). Overall, however, the timing of hatching and the degree of synchronous hatching are relatively understudied in grasshoppers.

Synchronous hatching within a single egg pod was originally hypothesized to be triggered by a thermal threshold mechanism, whereby the eggs are ready to hatch but require a certain temperature to do so. In this scenario, rising temperatures in the spring heat the soil, and synchronous hatching is induced on the day when the soil at the level of the buried eggs finally exceeds the species-specific threshold temperature (Smith et al. 2013). However, this proposed mechanism cannot explain how synchronous hatching can occur in grasshoppers with 5- to 11-cm-long egg pods buried vertically, because the threshold temperature would reach only the top eggs. Likewise, it does not explain what triggers

synchronous hatching in warm climate grasshoppers where soil temperatures typically remain above proposed hatching-threshold temperatures.

Recently, a new mechanism controlling synchronized hatching was discovered. In *S. gregaria*, *L. migratoria*, and the Bombay locust *Nomadacris* (also known as *Patanga*) *succincta* (Johannson, 1763), eggs kept in contact with one another hatched synchronously, while those kept separately hatched asynchronously (Nishide and Tanaka 2016, Tanaka 2017, 2021, Tanaka et al. 2018, Sakamoto et al. 2019). However, the latter also hatched synchronously when connected by a piece of wire, suggesting that a physical stimulus transmitted through the wire was involved in the synchronized hatching. In *L. migratoria*, sound recordings of the vibrations emitted by an embryo influenced the hatching time of other eggs, again, suggesting that vibrations from hatching eggs can stimulate hatching in nearby eggs. Communication by vibration is reasonable, considering that grasshopper eggs typically touch one another in the tightly packed egg pod. How many other grasshopper and insect species employ this mechanism is unknown.

In the present study, I document the hatching behavior of six grasshopper species in response to thermocycles, number of eggs in the group, presence of early hatched nymphs, and vibrations transferred through a wire. To explore the taxonomic breadth of the vibration response, I also tested to see if the eggs of a cricket that lays eggs singly would hatch synchronously if artificially placed in a group. This paper describes the results of these observations and compares them with those previously reported for other insects.

Materials and methods

Insects.—Five species of grasshopper – the longheaded grasshopper *Atractomorpha lata* (Motschilsky, 1866), the Oriental longheaded grasshopper *Acrida cinerea* (Thunberg, 1815) the bow-winged grasshopper *Chorthippus biguttulus* (Linaeus, 1758), the band winged grasshopper *Gastrimargus marmoratus* (Thunberg, 1815) and *Oedaleus infernalis* Saussure, 1884 – were collected in Tsukuba, Ibaraki, Japan from August to October of 2017 and 2018. Egg pods of the rice grasshopper *Oxya yezoensis* Shiraki, 1910 were collected in Tsukuba in September 2017 and in paddy fields in Kuroishi, Aomori, Japan in May 2018 and sent to Tsukuba, where experiments were performed. All species are of the family Acrididae, except for *A. lata*, which is of the family Pyrgomorphidae. Adults of each species were reared under outdoor conditions on various host plants, such as *Bromus catharticus*, *Artemisia indica* var. *maximowiczii*, and *Miscanthus sinensis* in nylon-screened cages (22 × 39 × 43 cm) in which a plastic cup (volume: 340 ml) filled with moist sand (10–15% water by wt) was placed as the oviposition substrate. *L. migratoria* and *C. biguttulus* are bivoltine and produce non-diapause eggs in early summer but diapausing eggs in the fall. For the five species, I used overwintering, diapausing eggs, which, in nature, remain in the egg stage for several months. Laid egg pods were kept outdoors until December and then stored in a refrigerator (7°C) for 2–5 months until used. The eggs of all five species appeared to have entered diapause at the end of the anatrepsis stage, by the arrival of winter, and were ready to hatch upon transfer to warm conditions in late January. In contrast, eggs of *A. lata* are known to have no diapause and overwinter in a state of quiescence (Y. Ando, pers. comm.), but were maintained as above. All of these species occur in grasslands in Japan and hatch in the spring when semimonthly mean soil temperatures measured every 60 min at a depth of approximately 3 cm at an exposed site in

Tsukuba ranged from 12.8 to 28.8°C from early April to late July in 2020 (Tanaka, S. pers. obs.).

For the experiments, eggs of all species were handled in the same way: each egg pod was washed with chlorinated tap water; the eggs were separated from the pod and individually placed on wet tissue paper in a 9-cm plastic Petri dish until used. They were maintained at 30 ± 1°C under continuous illumination in incubators. The compound eyes could be seen through the chorion several days before hatching. The number of eggs per pod varied from ~ 10 in *C. biguttulus* to more than 100 in *A. cinerea*.

Hatching under thermocycles.—Eggs of each species were kept either singly or in a group in pits on moist non-sterilized white sand (~ 15% moisture content by wt; Brisbane White Sand, Hario Co. Ltd., Japan) in a 9-cm Petri dish with a transparent lid and exposed to a thermocycle of 25/30°C under continuous illumination at least 5 days before hatching, unless otherwise mentioned. The eggs were incompletely covered with sand. The time required for the hatching rhythm of each species to be entrained by the thermocycle is unknown, but it was assumed that 5 days was sufficient based on previous studies with other grasshopper species (Nishide et al. 2015a, b; Tanaka 2021). The dishes were photographed from above with a digital camera every half-hour until no more hatching was observed. Hatching times were later recorded. The number of eggs that hatched every half-hour was recorded each day and then pooled for the 2–5 days of the experiment.

Effect of egg group size on hatching time.—Eggs from each pod were divided into treatments that differed in the number of grouped eggs: 1 vs. 2, 2 vs. 4, or 4 vs. 10, except for *C. biguttulus*, in which only two treatments (1 vs. 15 eggs) were prepared because fewer eggs were available for this species. The eggs in a group were held in a sand pit in a plastic Petri dish, and singly kept eggs were held in sand pits in another dish, as described earlier. The hatching time of eggs was recorded under continuous illumination and temperature (30 ± 1°C). Mean hatching times of the various treatment groups were calculated and compared. For each species, a value of 5 h was assigned to the mean hatching time of the largest group to standardize comparisons between different group sizes.

Effect of hatched nymphs on the hatching times of late-hatching eggs.—Whether the hatching time of an egg was influenced by the presence of an early-hatching nymph was determined under 30 ± 1°C and continuous illumination by treating pairs of eggs from the same pod in three different ways: 1) two eggs placed horizontally and in contact with one another on moist sand in a well of plastic 24-well plates (Thermo Fisher Scientific KK, Tokyo, Japan), 2) eggs separated by 2–3 mm on sand, and 3) eggs separated by a stainless steel wire screen that kept hatchlings from physically touching unhatched eggs. The hatching times were determined as described earlier, and the hatching intervals of eggs in pairs were calculated. Because photographs were taken every half-hour, 0.5 h was added to the hatching interval of two eggs and, thus, the minimum hatching interval was 0.5 h.

Stimuli inducing synchronized hatching.—To determine the stimuli responsible for synchronized hatching when two eggs are kept in contact, pairs of eggs from the same pod in each species were treated in three different ways: (1) eggs horizontally placed in contact with one another on sand in the same well, (2) those separated
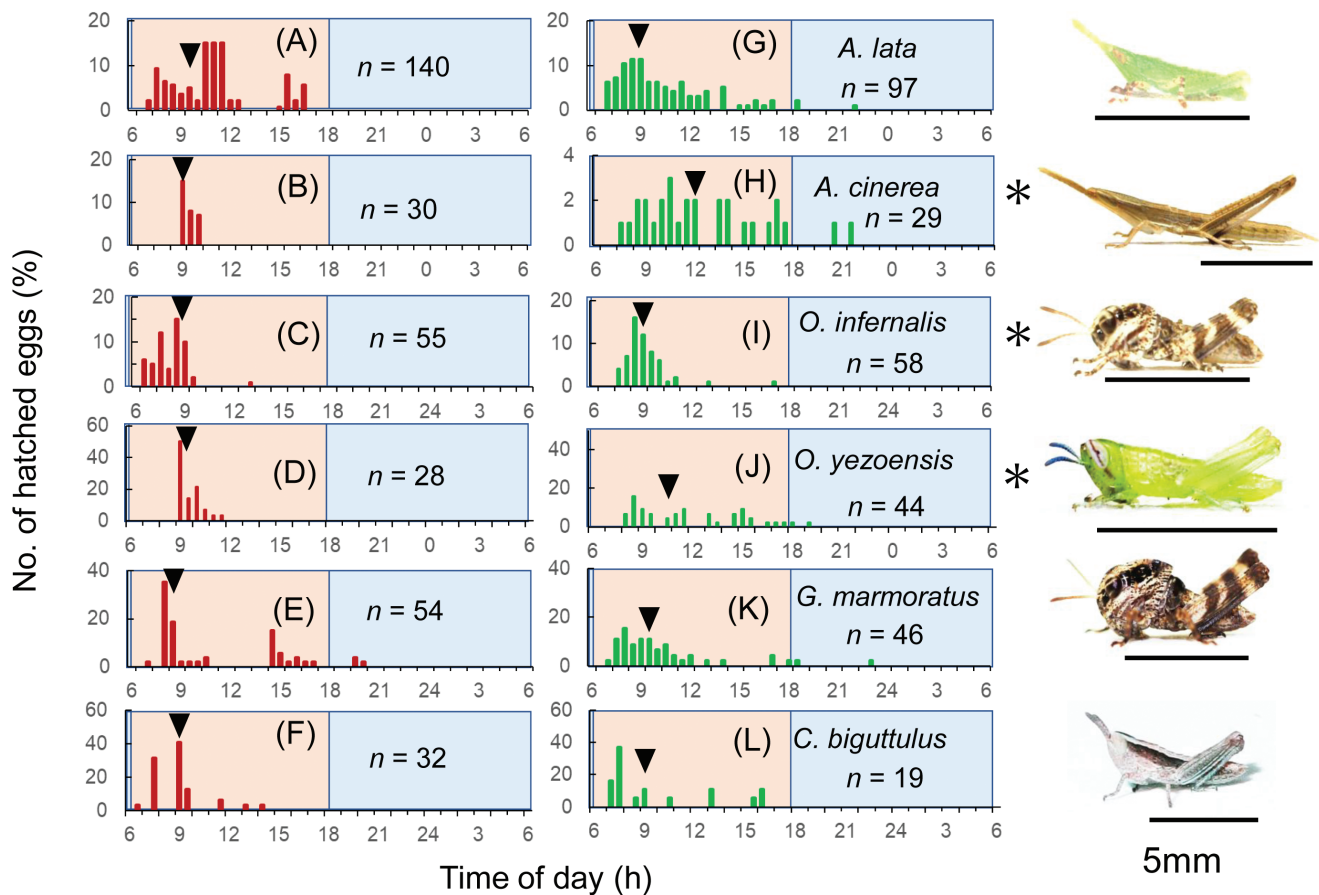
Fig. 1. Hatching activity of eggs kept in a group (**A–F.**) and those kept singly (**G–L.**) in a thermocycle of 30 (orange) and 25 °C (blue) under continuous illumination in the six indicated grasshopper species. The numbers of eggs that hatched over 2–5 days were pooled and plotted against the time of day. Black arrows indicate the medians. Asterisks indicate a significant difference between the two treatments by the Mann Whitney *U*-test at the 5% level. The photographs on the right show hatchlings of respective species. Scale bars: 5 mm.

by 2–5 mm, and (3) those similarly separated but connected by a piece of stainless steel wire (diameter, 0.1 mm; length, 0.7 cm). All treatments were done for all grasshoppers except for the two species in which the separation of eggs did not show a marked effect on the hatching intervals. In the last two species, the eggs in (2) were separated by a wire screen, and those in (3) were separated by a screen but connected by a piece of stainless steel wire placed through the screen separator. Connecting wires were laid on top of the two eggs (Fig. 4). The hatching times were determined as described above, and the hatching intervals of eggs in pairs were determined.

Effect of clumping of cricket eggs on the hatching time.—More than 20 adults of the Emma field cricket, *Teleogryllus emma* (Ohmachi & Matsuura, 1951), were collected in Tsukuba in August and September 2018, and allowed to lay eggs in moist sand in plastic cups at room temperature. The cups containing the eggs were then kept outdoors until February, when the eggs were ready to hatch when transferred to warm conditions (Tanaka, S. pers. obs.). The eggs were separated from the sand by washing with cold tap water and divided into two batches; 5 groups of 10 eggs were placed either as groups or singly in sand pits in 9-cm plastic Petri dishes. The dishes were then incubated at 30 ± 1 °C under continuous illumination with 10 days required for the eggs to hatch. The hatching times of the eggs were recorded.

Statistical analyses.—The hatching times were compared using ANOVA, Tukey's multiple comparison test, or *t*-test. The proportions of eggs that hatched synchronously were compared with the χ^2-test. The comparisons of hatching intervals were made with the Steel-Dwass test or the Mann-Whitney's *U*-test. These analyses were performed using a statistics service available at http://www.gen-info.osaka-u.ac.jp/MEPHAS/kaiseki.html. Descriptive Statistics were presented in Excel (Microsoft Office 365) or StatView (SAS Institute Inc., NC, USA). Differences were judged as significant when *p* < 0.05.

Results

Hatching under thermocycles.—The hatchlings of each species had a characteristic body shape, size, and color (Fig. 1). Most eggs of all tested species hatched during the high-temperature phase of the thermocycle regardless of whether they were grouped or singly, and the majority hatched during the first half of the thermophase (Fig. 1). However, the variance of hatching times was significantly smaller in treatments with grouped eggs vs. single eggs in all species (*F*-test; *p* < 0.05 each) except for *G. marmoratus* (*F*-test; *p* = 0.22). The Mann Whitney *U*-test indicated a significant difference in hatching time of day between the two treatments in *A. cinerea*, *O. infernalis*, and *O. yezoensis* (asterisks in Fig. 1) but not in *A. lata*, *G. marmoratus*, or *C. biguttulus*.
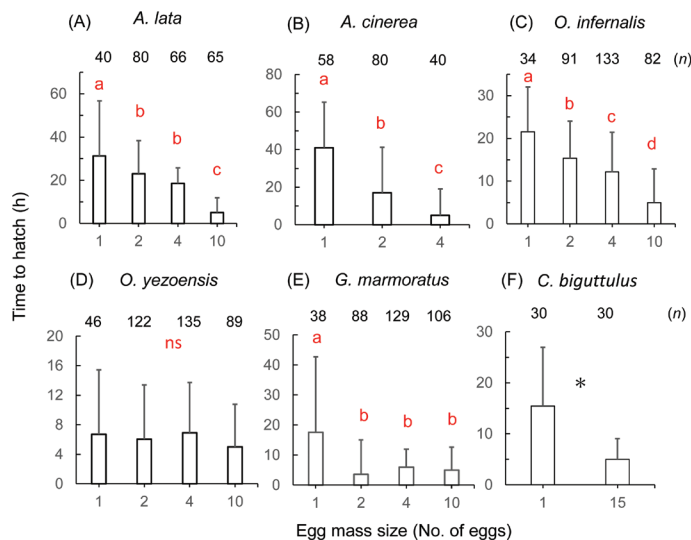
Fig. 2. Relationship between number of eggs in a group and mean hatching times in six grasshopper species under continuous illumination and 30°C temperature. For each species, hatching times were normalized by assigning a value of 5 h to the mean hatching time of the largest group. n (number of eggs in each treatment) is given above each histogram. Different letters indicate significant differences in mean values at the 5% level using the Tukey's multiple test (**A–E.**) or the t-test (**F.**). ns indicates no significant difference.

Effect of egg group sizes on hatching time.—The relationship between number of eggs in a treatment and hatching time varied depending on the species. Eggs hatched earlier as the number of eggs in the group increased from 1 to 4 or 10 in *A. lata*, *A. cinerea*, and *O. infernalis* (Fig. 2A–C), but not in *O. yezoensis*, where group size did not influence hatching time (Fig. 2D). In *G. marmoratus*, hatching time was significantly longer in the eggs kept singly than those kept in groups (Fig. 2E; Tukey's multiple test; $p < 0.05$); however, no significant difference was observed among the grouped (2, 4, or 8 eggs) treatments ($p > 0.05$). In *C. biguttulus*, the hatching time was significantly longer in the eggs kept singly than those kept in a group of 15 eggs (Fig. 2F; Tukey's multiple test; $p < 0.05$).

Effect of hatched nymphs on the hatching times of later-hatching eggs.— The mean hatching interval of two eggs was significantly larger in eggs separated by a few millimeters than those kept in contact with one another, but it was further increased when the eggs were separated by a screen in *A. lata*, *A. cinerea*, *O. infernalis*, and *O. yezoensis* (Fig. 3A–D; Steel-Dwass test; $p < 0.05$ each), suggesting that the early-hatched nymph stimulated the hatching of the later-hatching egg. In contrast, in *G. marmoratus* and *C. biguttulus*, no significant difference was observed in the mean hatching interval between the eggs kept in contact with one another and those kept separated, although the hatching interval for those kept in contact with one another was significantly shorter than those kept with a screen separator (Fig. 3E, F; Steel-Dwass test; $p < 0.05$ each).
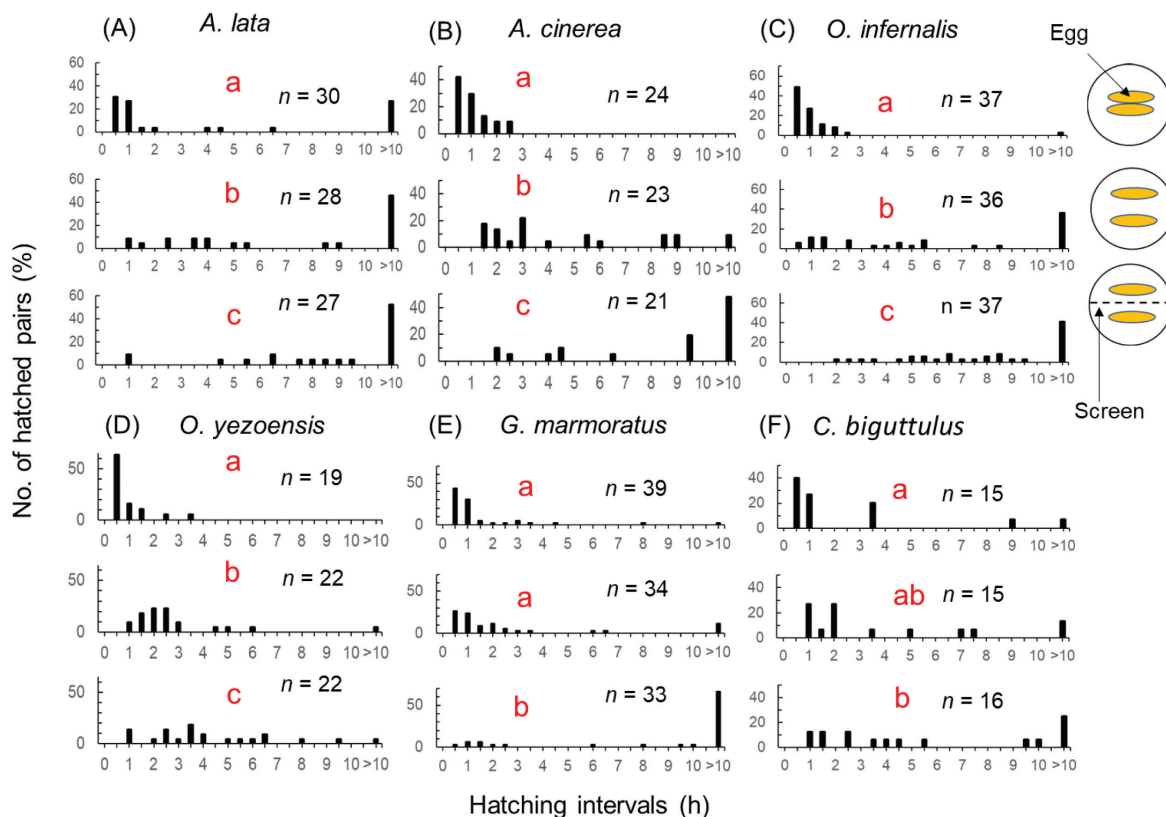
Fig. 3. Hatching intervals of two eggs kept in contact with one another (top panel), separated by 3–5 mm (middle panel), or separated by a screen (bottom panel) in the six indicated grasshopper species. Eggs were maintained under continuous illumination and 30°C temperature (**A–F.**). Different letters indicate significant differences in mean values at the 5% level using the Steel-Dwass test. Diagram on the right shows how the eggs were arranged in wells.
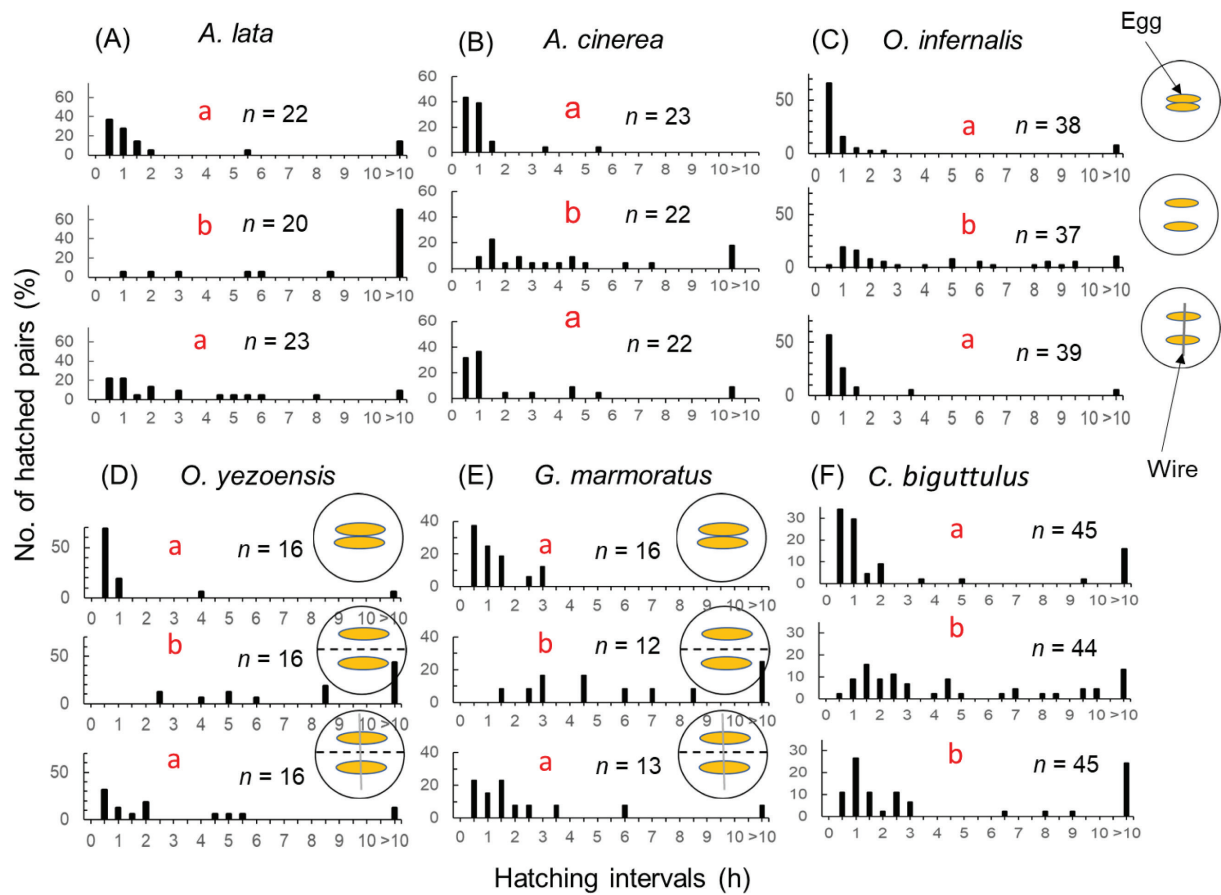
Fig. 4. Hatching intervals of two eggs kept in contact with one another (top panel), separated by ~5 mm (middle panel) with or without a screen,or connected by a piece of wire (bottom panel) at 30°C under continuous illumination in the six indicated grasshopper species (**A–F.**). Different letters indicate significant differences in mean values at the 5% level by the Steel-Dwass test. Diagrams in panels show how the eggs were arranged in wells.

Stimuli inducing synchronized hatching.—The hatching interval of two eggs that were kept in contact with one another was significantly shorter than those that were separated by a few millimeters (Fig. 4A–C; Steel-Dwass test; $p < 0.05$) but similar to those separated but connected by a wire in *A. lata*, *A. cinerea*, and *O. infernalis* ($p > 0.05$). In *O. yezoensis* and *G. marmoratus*, the effect of egg separation on the hatching interval was small (Fig. 3D, E). Thus, the eggs in these species were separated by a screen (middle panels in Fig. 4 D, E) or connected by a piece of wire through the screen separator (bottom panels). The results were similar to those obtained in the above three species. In *C. biguttulus*, the hatching mean interval for the eggs separated without a separator was significantly larger than for those kept in contact with one another (top and middle panels in Fig. 4F; Steel-Deass test; $p < 0.05$), but the connection of eggs by a piece of wire did not significantly reduce the mean hatching interval (bottom panel in Fig. 4F; $p > 0.05$). The proportion of pairs that hatched with a < 1-h hatching interval in the eggs connected by a piece of wire was 37.8%, which was significantly smaller than the value for the eggs kept in contact with one another (61.4%; $\chi^2 = 4.95$; $p < 0.05$). However, the value was significantly larger than that in the separated eggs (11.1%, $\chi^2 = 8.66$; $p < 0.05$). This result suggested that physical signals transmitted through the wires stimulated other eggs to hatch synchronously, as observed in the other species tested.

Effect of clumping of cricket eggs on their hatching time.—The Emma field cricket showed no significant difference in the mean hatching time (*t*-test; $p = 0.12$) and its variance (*F*-test; $p = 0.07$) between the eggs kept in a group of 10 eggs and those kept singly (Fig. 5). The mean hatching intervals were 37.6 and 35.3 h in those kept in a group and those kept singly, respectively. This difference was insignificant (Mann-Whitney *U*-test; $p > 0.05$; $n = 5$ each). These results imply that the clumping of eggs did not induce synchronized hatching in this cricket.

Discussion

Although the egg pods of many grasshopper species hatch more or less synchronously (Uvarov 1977), the mechanism controlling such behavior has only recently been discovered (Tanaka 2017, 2021, Tanaka et al. 2018, Sakamoto et al. 2019). The present research demonstrates that synchronous hatching is strongly influenced by the number of eggs in a group and provides evidence that vibrational stimuli from hatching eggs induce hatching in adjacent eggs. These results imply egg-to-egg communication. Below, I first discuss the time of day of hatching, followed by synchronicity. These two factors are interrelated, but separate. The former is a property of the population, whereas the latter is a property of individual egg pods.
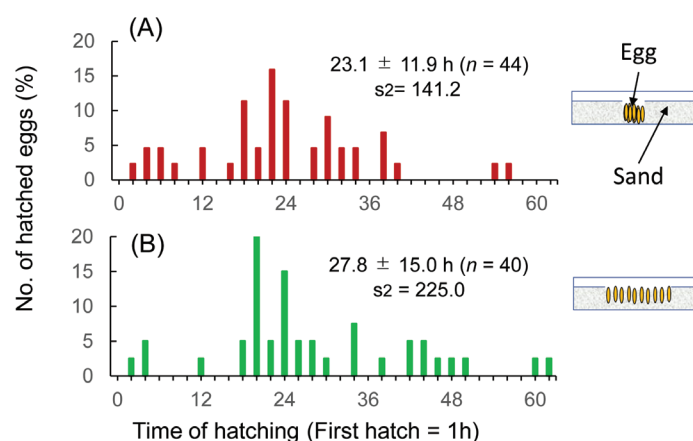
Fig. 5. Hatching activity of the eggs of *Teleogryllus emma* kept in a group (**A.**) and those kept singly (a distance of approximately 5 mm) (**B.**) at 30°C under continuous illumination. The hatching times for 5 groups of 10 eggs were pooled and calculated by designating the time of the first hatching egg as 1 h. The mean hatching time ± SD (sample size) is given in each panel. s^2 indicates the variance. Diagrams on the right show the experimental setup.

In nature, each grasshopper species tends to hatch at a specific time of day, depending in part on local habitat and current weather (Uvarov 1977, Smith et al. 2013, Nishide et al. 2017a). Many species hatch in the morning as temperatures rise (Nishide et al. 2017a), whereas other species tend to hatch before or at dawn, at midday, at night, throughout the day, or throughout a 24-h period (Ellis and Ashall 1957, Smith et al. 2013). Of the 10 grasshopper species listed in Table 1, eight hatched during the warm period of thermocycles in the laboratory under continuous illumination, whereas *S. gregaria* eggs hatched during the cool period (Nishide et al. 2015a, Tanaka 2021). These results strongly suggest that the thermoperiod controls the time of day of hatching in grasshoppers. In contrast, *N. succincta* eggs hatched during either period (Tanaka 2021) (Table 1).

Hatching time is thought to have evolved to maximize hatchling survival against predators and weather extremes. Hatching at the wrong time of day can be lethal. For example, mid-day hatching would be lethal for grasshoppers living in hot deserts because desert soil temperatures can exceed 65°C (Whitman 1987), which would instantly kill tiny hatchlings. This may be why some hot-desert grasshoppers hatch at night or in the early morning. For example, desert *S. gregaria* hatch around dawn, the coolest, most humid time of day (Ellis and Ashall 1957, Nishide et al. 2017b, Tanaka 2021). Conversely, for cold-climate grasshoppers, hatching in the late afternoon or evening could be dangerous when falling night temperatures incapacitate hatchlings. This may be why temperate-zone grasshoppers tend to hatch during the day, especially during mid- to late morning (Smith et al. 2013, Tanaka 2021). Indeed, all six of the temperate-zone grasshoppers tested in this paper tended to hatch during the first half of the warm phase in the laboratory (Fig. 1). These six species inhabit grasslands in Japan and hatch in the spring as daily temperatures rise.

Table 1. Summary of hatching behavior and responses to external stimuli in grasshopper species and some other insects.

| Species | Hatching time under thermocycles | More eggs | | Stimuli from hatched nymph | Vibration from wire | References |
		Increased synchrony	Shorter hatching time	Shorter hatching time	Increased synchrony	
Orthoptera: Acrididae						
Locusta migratoria L. 1758	Warm period	+	+	+	+	(Nishide et al. 2015a, Sakamoto et al. 2019, Tanaka 2021)
Schistocerca gregaria Forskål, 1775	Cool period	+	+	+	+	(Nishide et al. 2015a, Tanaka 2021)
Nomadacris succincta Johannson, 1763	Both periods	+	+	+	+	(Tanaka 2021)
Atractomorpha lata Mochulsky, 1866	Warm period	+	+	+	+	This study
Oxya yezoensis Shiraki, 1910	Warm period	+	-	+	+	This study
Acrida cinerea Thunberg, 1815	Warm period	+	+	+	+	This study
Oedaleus infernalis Saussure, 1884	Warm period	+	+	+	+	This study
Gastrimargus marmoratus Thunberg, 1815	Warm period	+	△	-	+	This study
Chorthippus biguttulus L. 1758	Warm period	+	+*	+	+	This study
Orthoptera Romaleidae						
Romalea microptera Palisot de Beauvois, 1817	Warm period	n.d.	n.d.	n.d.	n.d.	(Smith et al. 2013)
Orthoptera: Gryllidae						
Teleogryllus emma Ohmachi & Matsuura, 1951	n.d.	-	-	n.d.	n.d.	This study
Hemiptera: Pentatomidae						
Nezara viridula L. 1758	n.d.	+	+	n.d.	n.d.	(Kiritani 1964)
Halyomorpha halys Stål, 1855	n.d.	+	+	-	+	(Endo et al. 2019, Tanaka and Kotaki 2020)
Lepidoptera: Crambidae						
Chilo suppressalis Walker, 1863	n.d.	+	+	-	n.d.	(Morimoto and Sato 1962)

*Comparison between a group of 15 and singly kept eggs only.

+, present; -, not present; △, single vs. group; n.d., not determined.

Cuticle physiology may have also influenced grasshopper hatching times. This is because hatchlings require time for their integument to harden before they can actively move or feed (Harano et al. 2009). Morning hatching may be advantageous for spring-hatching species because it allows their exocuticle to harden rapidly during the warming day, allowing them time to seek night roosts and to forage before the cold nightfall prohibits movement or feeding. This is seen in *Romalea microptera*, where morning-hatchlings fed that afternoon, but afternoon-hatchlings could not feed until the next day, ~ 20 h after hatching (Rackauskas et al. 2006). In the present study, *N. succincta* hatched during both the warm and cool thermoperiods (Table 1), possibly because this species evolved in a warm humid subtropical environment where moisture and temperature are usually suitable for hatching throughout the 24-h cycle (Japan Meteorological Agency). However, the hatching patterns of these species need to be confirmed in the field.

Although synchronous hatching in grasshoppers has long been known (Uvarov 1977), the mechanisms controlling such behavior have only recently been discovered (Tanaka 2017, Tanaka et al. 2018, Sakamoto et al. 2019, Tanaka 2021). The present research confirms that the number of eggs in a group can strongly influence synchronous hatching, which implies that vibrational stimuli from hatching eggs can induce hatching in adjacent eggs.

Table 1 summarizes the hatching characteristics of various insects. Hatching occurred earlier in eggs kept in a group than in eggs kept separately in all grasshopper species tested, in two true bugs, and one moth (Table 1). The way in which eggs responded to different egg group sizes varied with species. Most grasshoppers (7 species) hatched earlier as the egg group size increased, whereas the eggs of *O. yezoensis* kept in different group sizes showed no significant variation in the hatching time. Other insect species, including the rice stem borer *Chilo suppressalis* Walker, 1863 (Morimoto and Sato 1962), the southern green stink bug *Nezara viridula* (Linnaeus, 1758) (Kiritani 1964), and the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Tanaka and Kotaki 2020), also showed a tendency to hatch earlier with increasing egg group size.

In addition to those mentioned earlier, other insects, such as a cockroach (Provine 1976, 1977) and a mantis fly (Dorey and Merritt 2017), also hatch simultaneously from the egg case or egg group. Overall, synchronized hatching appears to be widespread in insects. However, little is known about the mechanisms controlling synchronized hatching and how they evolved. In nine true bug species tested, five species hatched less synchronously when the eggs were removed from the group and kept separated, whereas four species did not show such a difference, although they all laid eggs as groups (Kiritani 1964, Endo and Numata 2017). In contrast, all nine grasshopper species tested to date hatch more synchronously when kept in a group than when kept singly (Table 1). The difference in the response to egg condition between grasshoppers and true bugs may relate to differences in the duration of their egg stages. Because the incubation period in the true bugs is only 4–7 days at 25°C, their hatching is completed in 3–9 h irrespective of whether the eggs are kept in a group or separated. In other words, because of rapid and uniform physiological development, they hatch more or less synchronously whether or not the eggs are grouped. In contrast, the incubation period (excluding the diapause period) in the grasshopper species lasted 15 –> 50 days at 30°C in the laboratory, and the eggs from the same pod required 2–5 days to complete hatching when removed from the pod and kept separated individually. This indicates a much larger individual variation in the incubation period in the grasshoppers than in the bugs and, thus, it is necessary for the grasshopper species to develop a controlling mechanism if synchronized hatching is important.

This paper confirms previous studies suggesting that vibrational signals from siblings can induce synchronous hatching in some insects (Tanaka 2017, Tanaka et al. 2018, Sakamoto et al. 2019, Tanaka 2021). In the migratory locust, signals from nearby hatching eggs can induce hatching in adjacent embryos that differ in age by more than a day, suggesting that developing eggs reach a stage where they "wait" for hatching signals from podmates (Tanaka 2017, Tanaka et al. 2018). A similar mechanism is known for the desert and Bombay locusts, although the differences in age range that allow eggs to hatch synchronously depend on the species (Tanaka 2021). In migratory locusts, vibrations are emitted by movement of the embryonic abdomen (Sakamoto et al. 2019, http://www.eje.cz/attachments/000076.avi). In the present study, in six grasshopper species, eggs separated by several millimeters hatched sporadically compared with those kept in contact with one another. However, some of those similarly separated hatched synchronously (< 1 h) when connected by a piece of wire (Fig. 4), as observed in the above-mentioned three locust species (Tanaka 2021). Together, these varied results strongly suggest that grasshopper species can use vibrational signals to control the hatching time for synchronized hatching.

The specific time at which the signals are produced by the six grasshoppers tested in this study is currently unknown. In addition to the vibrational signals generated by an embryo, other physical signals from hatching eggs, egg shell cracking, vermiform nymphs wiggling through the egg mass to reach the surface, or new hatchlings walking on the surface could also be involved.

In the present study, the eggs of the Emma field cricket failed to hatch synchronously when artificially kept in a group. This cricket requires a total of ~15 days of incubation at 30°C (excluding diapause) and does not lay eggs as a group. This result is reasonable in view of the fact that in nature, the eggs of this cricket are laid individually in soil and do not hatch synchronously, and neither nymphs nor adults aggregate. Perhaps vibration-induced hatching synchrony has been selected for only in species that lay grouped eggs and benefit from synchronous hatching. Two lady beetles, *Epilachna sparsa orientalis* and *E. vigintioctomaculata*, lay eggs as groups. The former lays eggs so they touch and the latter lays eggs that do not touch one another. Morimoto (1965) observed that egg hatching from groups was completed in 1.8–2.8 h in the former and 4.5–10.5 h in the latter. Although the mechanism responsible for synchronized hatching is unknown in these two species, it is possible that the eggs of *E. s. orientalis* achieve synchronized hatching through a physical stimulus such as vibration transmitted through contact with one another. Morimoto noted that the well-synchronized hatching pattern observed in *E. s. orientalis* might be related to their strong tendency to aggregate as hatchlings compared to the other beetles.

Many grasshoppers and other insects form tight aggregations in the 1st instar (Fig. 6), which may or may not continue into later stages (Uvarov 1977, Costa 2006, Hatle and Whitman 2001). In the desert locust, both solitarious and gregarious hatchlings show similar degrees of aggregative behavior (Guershon and Ayali 2012), although the aggregating tendency in solitarious hatchlings usually weakens in later instars (Uvarov 1977). The advantages and disadvantages of aggregative behavior have been extensively studied and may include diluting individual predation risk, group defense, overcoming plant defenses via group efforts, increased

Fig. 6. Aggregations of *Nomadacris succincta* hatchlings at 09:46 on May 25, 2018 on Minami-Daito Island, Japan (Photographed by Masanari Aizawa). This grasshopper aggregates tightly as 1st instars only during the nymphal stage.

thermoregulation or desiccation resistance, shelter building, etc. (Edmunds 1974, Vulinec 1990, Ruxton et al. 2004, Costa 2006). As such, synchronized hatching may have evolved in part to facilitate immediate aggregation in newly hatched insects.

To understand the mechanism underlying synchronized hatching and its evolution, more species of insects that produce eggs in a group with different lengths of embryonic stage should be examined. Grasshopper species would be ideal insects to use to explore this subject.

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Perception and knowledge of grasshoppers among indigenous communities in tropical forest areas of southern Cameroon: Ecosystem conservation, food security, and health

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Abstract

The increased attention given to health, food security, and biodiversity conservation in recent years should bring together conventional scientists and indigenous people to share their knowledge systems for better results. This work aims to assess how grasshoppers are perceived by the local people in southern Cameroon, particularly in terms of food, health, and landscape conservation. Villagers were interviewed individually using a rapid rural assessment method in the form of a semi-structured survey. Nearly all people (99%) declared that they are able to identify local grasshoppers, generally through the color of the insect (80%). Crop fields were the most often cited landscape (16%) in terms of abundance of grasshoppers, with forest being less mentioned (8%). In general, villagers claimed that grasshopper abundance increased with forest degradation. Grasshoppers were found during all seasons of the year but noted to be more abundant during the long dry seasons. People found grasshoppers both useful and harmful, the most harmful reported being *Zonocerus variegatus*, an important crop pest. Cassava is the most attacked crop with 75–100% losses. Industrial crops, such as cocoa, coffee, and bananas, were not cited as being damaged by grasshoppers. The most effective conventional method cited for the control of pest grasshoppers is the use of pesticides (53%) with, in most cases (27%), a 75–100% efficiency. The traditional method of spreading ash was also often cited (19%), with an estimated efficiency of 25–75%. Biological methods were neither cited nor used by the villagers. Most of them (87%) declared that they eat grasshoppers; some sold these insects in the market (58%) and some used them to treat diseases (11%).

Keywords

Introduction

Sustainable development is now emerging as an alternative to conventional development as a way to reduce poverty in the Third World (UN 2019). A sustainable development perspective is consistent with the need to conserve ecosystems and agrosystems for better development (Ulluwishewa 1993, Andres and Bhullar 2016). In the last century, ecosystem transformations due to agricultural intensification and rapid industrial and urban development have imposed pressures on biological diversity, such that there is an urgent need to create interest and awareness regarding functional biodiversity (Rastogi and Kumar 2009, Rastogi 2011), biodiversity conservation (Kearns 2010), and the economic resources provided by biodiversity (Nijkamp et al. 2008). Accelerated exploitation of natural resources in the Third World leads to environmental degradation and loss of biodiversity, which, combined with the harmful effects of climate change, threatens to reverse decades of development efforts and have a negative impact on agriculture, health, settlement, and infrastructure in developing countries (Thornton et al. 2011). As a Sub-Saharan African country, Cameroon is seriously affected because it depends mainly on rain-fed agriculture. Due to the current effects of climate change, the livelihoods of local farmers are vulnerable to unpredictable floods, prolonged droughts, and related famine, pests, and diseases, thus calling for adaptive strategies to be undertaken (Akinnagbe and Irohibe 2014, Torquebiau et al. 2016). Early warning systems have proven to be indispensable in preparedness for such climatic consequences (Tadesse et al. 2008, Singh and Zommers 2014). This increased attention to climate change and landscape degradation is bringing together both conventional scientific and indigenous communities to share their knowledge systems (Nakashima et al. 2012). Historically and to date, local communities in different parts of the world have continued to rely on indigenous knowledge to conserve the environment and deal with natural disasters (Iloka 2016). However, various people now consider that, especially in Africa, the knowledge of indigenous people should be included when designing adaptations to natural disasters and particularly to climate change (Robinson and Herbert 2001, Joshua and Jürgen 2013).

Indigenous knowledge can be defined as a set of strategies, practices, tools, explanations, beliefs, intellectual sources, and other values accumulated through time by indigenous communities without interference or involvement of hegemonies or external forces (Emeagwali and Sefa-Dei 2014). The knowledge of indigenous communities has been accumulated through generations of living in a given environment and allows the members of these communities to live in harmony with nature; this knowledge provides valuable tools for food security, health, education, environmental conservation, and the reduction of the degradation of natural resources. To a certain extent, indigenous knowledge allows for the foreseeing of hot weather, periods of seeding, and anticipation of the rainy season (Mwaura 2008). The use and application of appropriate indigenous knowledge systems can promote environmental conservation and aid in the management of disasters in terms of disaster prevention, mitigation, recovery, prediction, early warning, preparedness, response, and rehabilitation (Mwaura 2008). In Africa, the indicators used by indigenous knowledge systems include temperature variation, astronomical observations, plant phenology, and the behavior of birds, amphibians, reptiles, and insects (Mwaura 2008). In Tanzania, various environmental and astronomical means have been used to predict rainfall, including plant phenology and the behavior and movement of animals such as birds and insects (Chang'a et al. 2010). In Uganda, indicators for the onset of the dry season include the appearance and movement of insects, specifically butterflies, red caterpillars, western honeybees (*Apis mellifera* Linnaeus, 1758), and bush-crickets (*Ruspolia baileyi* Otte, 1997; Joshua and Jürgen 2013). In Ghana, the presence of the bird *Butastur rufipennis* Sundevall, 1850 would indicate an imminent invasion of crops by locusts (Owusu 2010). According to Mwaura (2008), many people of Africa use indigenous knowledge on insects' behavior, such as grasshoppers, to protect forests used for rituals, i.e., forests that have trees or animals considered sacred or totems.

Grasshoppers are one of the more diverse taxa in the world (Zhang 2011). While some species are harmful, many are not, but all grasshopper species are a crucial link in food chains (Badenhausser 2012), playing an important role in the recycling and equilibrium of natural ecosystems (Hao et al. 2015). The decline in grassland bird species has been shown to have a positive correlation with an increase in grasshopper densities (Bock et al. 1992). Grasshoppers are a major component in the diet of grassland birds, and studies have shown that there is a direct decrease in birds when grasshoppers are less abundant (Bock et al. 1992). Grasshoppers are also an important food source for other fauna in grassland ecosystems (Latchininsky et al. 2011), such as shrews, moles, bats, armadillos, and anteaters (Srivastava et al. 2009). They are also a food source for many people in the world (Paul et al. 2016). Several authors have reported that grasshoppers and crickets, especially *Hieroglyphus africanus* Uvarov, 1922, *Acanthacris ruficornis citrina* (Serville, 1838), *Zonocerus variegatus* (Linnaeus, 1758), *Ornithacris cavroisi* (Finot, 1907), *Brachytrupes membranaceus* (Drury, 1770), *Oxya cyanoptera* Stal, 1873, *Cyrtacanthacris aeruginosa* (Stoll, 1813), *Ornithacris turbida* (Walker, 1870), and *Anacridium melanorhodon* (Walker, 1870), are the insects predominantly eaten by humans in Nigeria, Cameroon, Benin, and in many other parts of Africa because of their high protein content (Banjo et al. 2006, Riggi et al. 2013, Meutchieye 2019, Zabentungwa et al. 2020). According to De Conconi and Moreno (1988), grasshoppers are also used by many people throughout the world in the preparation of traditional medicines used to cure certain diseases; *Sphenarium* spp., *Taeniopoda* sp., and *Melanoplus* sp. are used to treat kidney diseases and intestinal sickness. Nevertheless, most of the indigenous knowledge on grasshoppers has not been documented and remains the secret of the local populations of Africa, especially in Cameroon. The aim of this study is to assess the indigenous knowledge and perception of communities of South Cameroon on the local forest grasshoppers, especially as it pertains to (1) the use of grasshopper diversity to predict the level of forest degradation; (2) pest grasshoppers, damage to crops, and known and/or used methods to control these pests; and (3) use of grasshoppers in medicine, culture, and as a food source.

Materials and methods

Study site.—This study was conducted in villages in the forest areas of the southern Cameroon plateau (between 3°27'N, 11°32'E and 4°10'N, 11°49'E). This area covers almost 42% of Cameroon and is a vast plateau of about 650 m a.s.l., belonging to a strip of plateau that forms the north and west edges of the Congo basin (Westphal et al. 1981). It is dominated by a Guinean climate with four seasons: a long dry season (mid-November to March), a short rainy season (April to June), a short dry season (July to August), and a long rainy season (September to mid-November). Precipitation ranges from 1500 to 2000 mm per year (Santoir and Bopda 1995). These forests are characterized by the dominance of Sterculiacae and Ulmacae, which have great expansion potential, with the undergrowth being invaded by herbaceous plants such as Maranthacae and Acanthacae (Westphal et al. 1981). In these ecosystems, the forest cover is not uniform, as it is regularly degraded because of the economic exploitation of wood and the practice of slash and burn agriculture. The resulting vegetation after degradation are the less diversified fallowlands, dominated by *Chromolaena odorata* (L.) R.M.King & H.Rob., 1970, *Ageratum conizoides* L., 1753, *Synedrella nodiflora* (L.,) Gaertn, and *Imperata cylindrica* (L.) P.Beauv. Plantain and cocoyam, cassava, yam, maize, and groundnuts are the main food crops (Westphall et al. 1981), while industrial crops include cocoa, coffee, sweet banana, and oil palm (Santoir and Bopda 1995). In the southern Cameroon plateau, our surveys were conducted in four regions (Center, South, East, and Littoral) with the following eight divisions: Mbam and Inoubou (villages investigated: Tchekos, Biabetom, Bokito, Dang, Bygna, and Goufe), Mbam and Kim (village investigated: Ngoro), Mefou and Akono (villages investigated: Ongot and Ngoumou), Nyong and Kelle (villages investigated: Memel, Elale, and Bof Makak), Mvilla (villages investigated: Adoum, Mekam, Mang, Djop, and Biyeyem), Valley of Ntem (villages investigated: Ngutadjap, Aloum, Meko, Akonangui, and Olamze), Sanaga Maritime (village investigated: Ngambe), and High Nyong (village investigated: Ngoyla) (Fig. 1).

Surveys and data analysis.—A total of 341 people were interviewed individually in the 24 villages selected. Rapid rural appraisal methods (RRA) (Chambers 1981, Polidoro et al. 2008, Sattout et al. 2008) were used between January and July 2017; interviews were conducted using a semi-structured survey form. Thirty-one questions were asked to each participant: two questions about personal information (origin, age, sex, and background); eleven questions on the respondents' general knowledge of grasshoppers, the influence of forest degradation on grasshopper diversity, and on the potential use of these insects to trace the disruption level of forests due to human activities; nine questions on harmful grasshoppers and the methods used to control pest species; and nine questions on the importance of grasshoppers to the local popula-
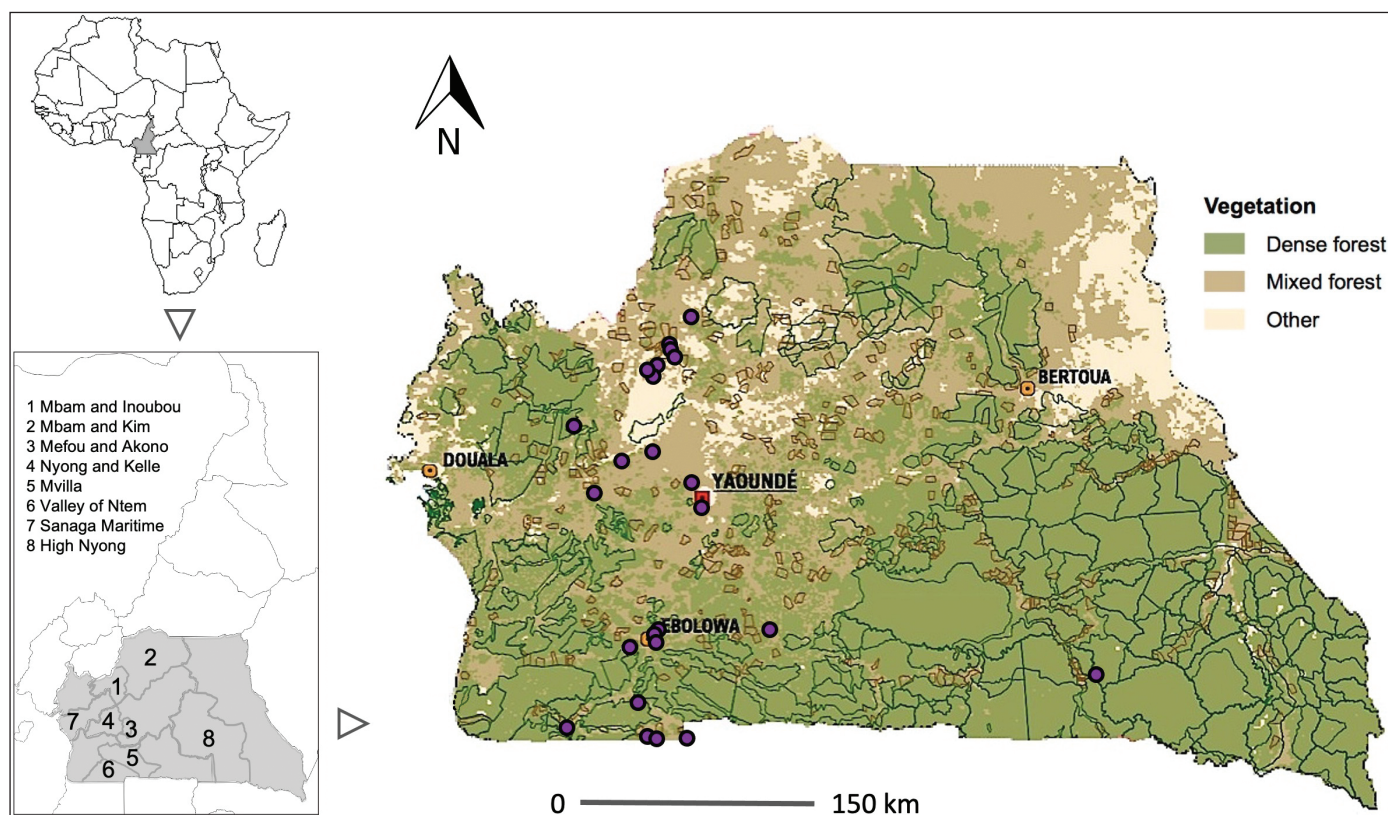
Fig. 1. Study sites in Cameroon.

tion as a food source or for commerce, medicine, traditional rites, and magic (See supplementary file). The interviews were done in French or in the common language of the area with the help of a local translator. Assessment of the recognition of grasshopper species by the local people was facilitated by the use of pictures of many species from the area around the villages. All frequencies (calculated using EXCEL version 2016) were compared using the Chi² found using the Kruskal-Wallis test in PAST version 4.03 (Hammer et al. 2001). The Mann-Whitney test was used with the same software for two samples. Differences were considered significant at a probability less than 0.05.

Results

Socio-demographic characteristics of the surveyed people

The population studied consisted of 58.9% males (201 respondents) and 41.1% females (140 respondents) (Table 1). Most respondents were 18–30 years old (40.1%), followed by 31–40 years (22.6%), 51–60 years (14.7%), 41–50 years (12.3%), and over 60 years (10.3%). Most (53.4%) had a high school level education, while 29.6% had a primary level education and 11.4% had a university level education. A small number of respondents (5.6%) never went to school at all.

Grasshopper recognition by local people

Only one respondent said he did not know what a grasshopper is. In general, in all the villages, the respondents said that they know these insects (99.7%) from their personal experiences (50.4%) or from school (48.7%). Some (33.7%) got their experience from their neighbors and 18.8% from the media. The peo-

ple surveyed said that they used general coloring (80.1%), form (66.9%) or odor (30.2%) to recognize grasshoppers, with color predominating in some divisions, and form predominating in others (Table 2).

Landscapes reported as habitat for grasshoppers

The data shows that most villagers reported that grasshoppers were in all landscapes (79.8%) (Table 3). However, some villagers (16.4%) reported that crop fields hosted grasshoppers more often, while a few (9.7%) thought grasshoppers were mainly in fallow lands (9.7%).

The grasshoppers were called by many names, depending on the village and language: "Etandak" in the Beti language (Mefou and Akono, Mvilla and Valley of Ntem divisions), "Gomatataï" and "Ketataï" in the Bafia language (Mbam and Inoubou division), "Kanè" in the Mvouté language (Mbam and Kim division), "Ndenga" in the Bassa language (Nyong and Kelle and Sanaga Maritime), and "Atjembeka" in the Nvjem language (High Nyong division).

In general, species-specific names do not exist in these villages, with the exception of *Z. variegatus*, called "Mbakssana" in the Beti language and "Ikadjala" in the Nvjem language. However, 23 species were recognized by the local people : *Parapetasia femorata* Bolívar, 1884, *Dictyophorus karschi* (Bolívar, 1904), *Mazea granulosa* Stål, 1876, and *Gemeneta terrea* Karsch, 1892 in forest; *Odontomelus kamerunensis* Ramme, 1929, *Cyphocerastis tristis* Karsch, 1892, and *Eupropacris coerulea* (Drury, 1770) in fallow, crop fields, and forests; *Pteropera balachowskyi* Donskoff, 1981 and *Pteropera mirei* Donskoff, 1981 in fallow lands and forest; *Zonocerus variegatus* (Linnaeus, 1758), *Oxycatantops spissus* (Walker, 1870), *Taphronota ferruginea* (Fabricius, 1781), *Chirista compta* (Walker, 1870), and

Table 1. Socio-demographic characteristics of respondents in the investigated divisions. Each value represents a frequency in % (number of respondents); N = size of the sample; *p* value = probability; χ² = value of the Kruskal-Wallis test. The letters a, b, and c represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

Comparison parameters	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ²	*P* value	Total
Sex											
Male	58.9(50)	43.3(13)	48.5(16)	64.5(20)	59.0(36)	73.7(28)	40.0(12)	78.8(26)	14.2	0.006	58.9(201)
Female	41.1(35)	56.7(17)	51.5(17)	35.5(11)	41.0(25)	26.3(10)	60.0(18)	21.2(7)	14.2	0.006	41.1(140)
χ²	3.9	1.8	0.04	3.8	2.9	12.6	1.8	16.6			15.3
p value	0.02	0.1	0.8	0.02	0.04	<0.001	0.1	<0.001			<0.001
N	85	30	33	31	61	38	30	33			341
Age (in years)											
18 – 30	36.5(31)a	56.7(17)a	42.4(14)a	6.4(2)a	57.4(35)a	36.8(14)a	23.3(7)ab	51.5(17)a	21.9	< 0.001	40.1(137)a
31 – 40	21.2(18)b	16.7(5)b	18.2(6)b	35.5(11)b	16.4(10)b	23.7(9)ab	46.6(14)b	12.1(4)b	9.4	0.01	22.6(77)b
41 – 50	17.6(15)b	3.3(1)b	9.1(3)b	9.7(3)a	6.6(4)b	21.1(8)ab	16.7(5)a	9.1(3)bc	3.4	0.2	12.3(42)c
51 – 60	11.8(10)b	10.0(3)b	30.3(10)ab	19.4(6)ab	9.8(6)b	10.5(4)b	6.7(2)a	27.3(9)b	5.7	0.03	14.7(50)c
Above 60	12.9(11)b	13.3(4)b	0.0(0)c	29.0(9)b	9.8(6)b	7.9(3)b	6.7(2)a	0.0(0)c	6.1	0.002	10.3(35)c
χ²	10.1	15.9	11.1	5.8	32.8	6.1	9.7	15.5			61.3
p value	< 0.001	< 0.001	< 0.001	0.02	< 0.001	0.01	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341
Background											
unschooled	8.2(7)b	0.0(0)c	6.0(2)b	6.5(2)c	1.6(1)c	0.0(0)c	0.0(0)c	21.2(7)a	3.8	0.001	5.6(19)a
Primary	34.1(29)a	16.7(5)a	39.4(13)a	29.0(9)a	18.0(11)a	36.8(14)a	40.0(12)a	24.2(8)a	7.3	0.01	29.6(101)b
High school	45.9(39)a	76.7(23)ab	36.4(12)a	54.8(17)b	68.9(42)b	52.7(20)a	46.7(14)a	45.5(15)b	47.6	< 0.001	53.4(182)c
University	11.8(10)b	6.6(2)ab	18.2(6)ab	9.7(3)c	11.5(7)a	10.5(4)b	13.3(4)b	9.1(3)a	0.8	0.9	11.4(39)d
χ²	24.8	33.0	7.3	13.7	53.7	19.7	12.9	6.7			142.7
p value	< 0.001	< 0.001	0.004	< 0.001	< 0.001	< 0.001	< 0.001	0.007			< 0.001
N	85	30	33	31	61	38	30	33			341

Acanthacris ruficornis (Fabricius, 1787) in fallow lands and crop fields; *Heteropternis thoracica* (Walker, 1970) and *Pyrgomorpha vignaudii* (Guérin-Méneville, 1849) near houses, in fallow lands, and in crop fields; *Spathosternum pygmaeum Karsch*, 1893, near houses and in fallow lands; *Gymnobothrus temporalis* (Stål, 1876), *Abisares viridipennis* (Burmeister, 1838), *Catantops stramineus* (Walker, 1870) and *Afroxyrrhepes obscuripes* Uvarov, 1943, only in fallow lands; *Atractomorpha acutipennis* (Guérin-Méneville, 1844) and *Eucoptacra anguliflava* (Karsch, 1893) near houses, in fallow lands, in crop fields, and in forest (Appendix 1).

Abundance of grasshoppers in different landscapes, forests, and seasons

Crop fields were cited most often as having an abundant number of grasshoppers (45.7%), while fallow areas were cited as having only moderate levels (38.7%) (Fig. 2). Grasshoppers were seen as being less abundant to rare in forests (37–38.4%) and rare near houses (49.3%). In general, respondents reported that the abundance of grasshoppers increased with degradation of the forest. They recognized that grasshoppers were generally rare in pristine forests (63.9%) and, in severely degraded forests, they noted low (27.3%), moderate (41.3%) or high (8.2%) abundance levels. In general, respondents reported that grasshoppers were present in all seasons but more abundant during the dry season than in the rainy season. Mainly, high grasshopper abundance was reported during the long dry season (39.3%), with lower levels during the short dry season (16.7%) and the long rainy season (11.7%). Grasshoppers were considered to be least common during the short rainy season, the rarest categories being predominant (31.4%).

Perception of grasshoppers by local people

In all the divisions visited, the respondents recognized grasshoppers as both useful and harmful in Mbam and Kim (100%), Mefou and Akono (94%), Nyong and Kelle (93.5%), Mvilla (91.8%), Sanaga Maritime (90%), Mbam and Inoubou (88.2%), High Nyong (63.6%), and Valley of Ntem (50%) (Fig. 3A). Grasshoppers were reported as only harmful in seven of the eight divisions studied: Valley of Ntem (34.2%), High Nyong (12.2%), and Sanaga Maritime (10%) had the high frequencies of this response, with the four others presenting a low frequency. Only the respondents of the divisions Mbam and Kim and Nyong and Kelle did not recognize grasshoppers as harmful. Grasshoppers were reported as only useful more often by some people in the High Nyong (21.2%) and Valley of Ntem (13.2%) divisions.

Harmful effects of grasshoppers.—In general, in all the divisions, the most harmful action of grasshoppers reported by respondents was damage to crops (Fig. 3B). Some people cited wounds due to the spines of grasshoppers in Valley of Ntem (28.9%), Mbam and Inoubou (5.9%), and in Mvilla (1.6%). Skin irritation was only reported in Mbam and Inoubou (9.4%).

Grasshoppers cited as crop pests.—In all divisions visited, respondents recognized all grasshopper species as crop pests (51%) (Table 4). However, in these divisions, only *Zonocerus variegatus* was cited as a crop pest having a significant economic impact by a high proportion of respondents (33.1%). With the exception of *Oxycatantops spissus* (3.5%) and *Pyrgomorpha vignaudii* (1.5%), all other grasshoppers were cited as crop pests by less than 1% of the respondents and only in the areas of Mbam and Inoubou, and Mvilla.

Table 2. Recognition of grasshoppers by local people. Frequency in % (number of respondents); N = size of the sample; *p* value = probability; χ^2 = value of the Kruskal-Wallis test. The letters a, b, and c represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

Comparison parameters	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ^2	*p* value	Total
Knowledge of grasshoppers											
Yes	100.0(85)	100.0(30)	100.0(33)	100.0(31)	100.0(61)	100.0(38)	100.0(30)	97.0(32)	0.08	0.2	99.7(340)
No	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)	3.0(1)	0.08	0.2	0.3(1)
χ^2	126.8	44.3	45.8	45.7	90.8	56.3	44.3	44.4			506.3
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341
Where knowledge was gained											
School	47.1(40)a	90.0(27)a	36.4(12)a	71.0(22)a	21.3(13)a	28.9(11)a	76.7(23)a	54.5(18)a	43.3	< 0.001	48.7(166)a
Media	25.9(22)b	23.3(7)b	15.2(5)b	19.3(6)b	1.6(1)b	7.9(3)b	50.0(15)b	15.2(5)b	17.8	< 0.001	18.8(64)b
Neighbor	24.7(21)b	10.0(3)b	18.2(6)ab	54.8(17)a	18.0(11)a	60.5(23)c	53.3(16)ab	54.5(18)a	32.7	< 0.001	33.7(115)c
Personal experience	61.2(52)a	33.3(10)b	63.6(21)c	32.3(10)b	90.2(55)c	26.3(10)a	23.3(7)c	21.2(7)b	68.6	< 0.001	50.4(172)a
χ^2	23.7	30.2	14.9	14.7	84.1	16.2	12.8	13.2			67.2
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341
Recognition of grasshoppers											
Form	83.5(71)a	93.3(28)a	66.7(22)a	90.3(28)a	32.8(20)a	60.5(23)a	70.0(21)	45.5(15)	47.6	< 0.001	66.9(228)a
Color	94.1(80)b	70.0(21)b	54.5(18)a	80.6(25)a	96.7(59)b	86.8(33)b	53.3(16)	63.6(21)	27.1	< 0.001	80.1(273)b
Odor	43.5(37)c	20.0(6)c	27.3(9)b	19.4(6)b	18.0(11)c	18.4(7)c	40.0(12)	45.5(15)	13.8	0.003	30.2(103)a
χ^2	36.2	26.7	7.9	24.8	63.6	26.9	4.0	2.2			57.7
p value	< 0.001	< 0.001	0.004	< 0.001	< 0.001	< 0.001	0.7	0.2			< 0.001
N	85	30	33	31	61	38	30	33			341

Table 3. Landscapes reported as habitats for grasshoppers in the divisions studied. Frequency in % (number of respondents); N = size of the sample; *p* value = probability; χ^2 = value of the Kruskal-Wallis test. The letters a, b, and c represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

| Landscapes	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ^2	*p* value	Total
All landscapes	82.4(70)c	70.0(21)c	84.8(28)c	100.0(31)a	100.0(61)a	68.4(26)c	63.3(19)c	48.5(16)c	26.1	< 0.001	79.8(272)c
Forest	2.4(2)b	3.3(1)a	12.1(4)b	0.0(0)b	0.0(0)b	7.9(3)b	26.7(8)ab	24.2(8)b	6.1	< 0.001	7.6(26)a
Fallow	9.4(8)ab	6.7(2)ab	12.1(4)b	0.0(0)b	0.0(0)b	15.8(6)b	16.7(5)ab	24.2(8)b	5.7	0.002	9.7(33)a
Crop fields	14.1(12)a	20.0(6)b	9.1(3)ab	0.0(0)b	0.0(0)b	21.1(8)b	36.7(11)b	48.5(16)c	22.3	< 0.001	16.4(56)b
House	16.5(14)a	16.7(5)ab	0.0(0)a	0.0(0)b	0.0(0)b	10.5(4)b	10.0(3)a	3.(1)a	4.9	0.001	7.9(27)a
χ^2	107.8	26.0	47.1	73.9	154.9	28.2	17.0	14.5			398.6
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341
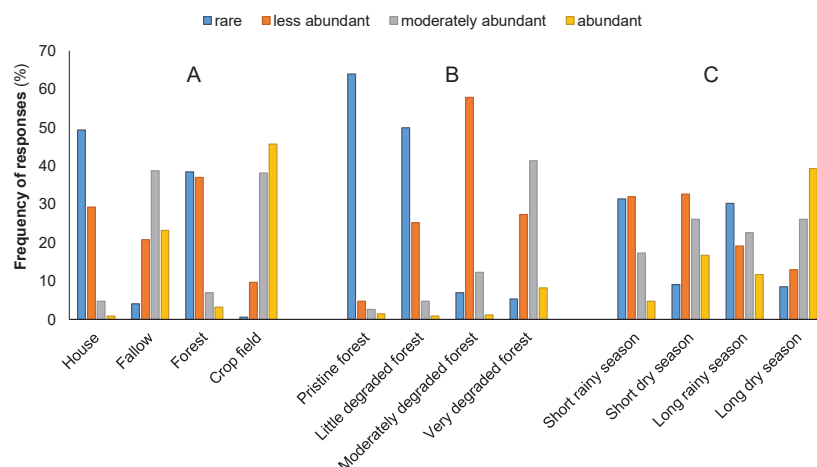
Fig. 2. Abundance of grasshoppers in different landscapes (**A**), forests (**B**), and seasons (**C**).
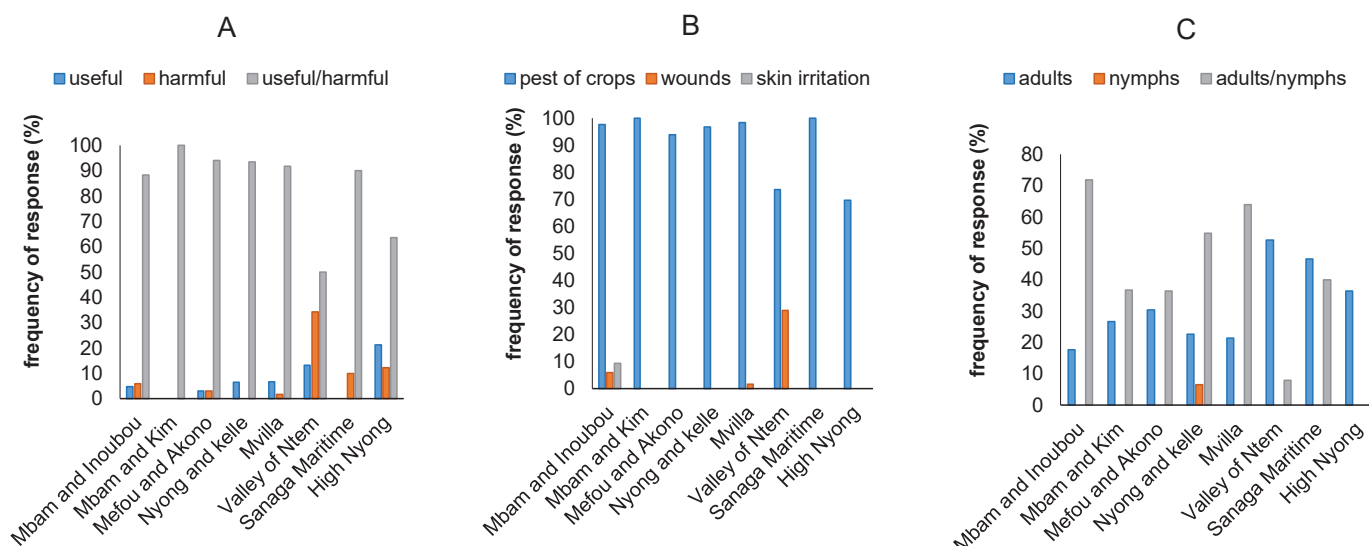
A

B

C

Fig. 3. Perception of grasshoppers by local people: general perception (**A**), harmful effects of grasshoppers (**B**), and development stage of pest grasshoppers (**C**).

Table 4. Pest grasshoppers cited by local people. Frequency in % (number of respondents); N = size of the sample; *p* value = probability; χ^2 = value of the Kruskal-Wallis test. The letters a, b, c, and d represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

Grasshoppers species	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ^2	*p* value	Total
All the species	67.1(57)a	63.3(19)a	42.4(14)a	51.6(16)a	32.8(20)a	31.6(12)a	73.3(22)a	42.4(14)a	24.2	< 0.001	51.0(174)a
Zonocerus variegatus	28.2(24)b	53.3(16)b	30.3(10)b	38.7(12)a	55.7(34)b	31.6(12)a	13.3(4)b	3.0(1)b	25.5	< 0.001	33.1(113)b
Taphronota ferruginea	2.4(2)c	0.0(0)c	0.0(0)c	0.0(0)b	0.0(0)c	0.0(0)b	0.0(0)c	0.0(0)b	0.2	0.5	0.6(2)c
Acanthacris ruficornis	1.2(1)c	0.0(0)c	0.0(0)c	0.0(0)b	1.6(1)cd	0.0(0)b	0.0(0)c	0.0(0)b	0.05	0.9	0.6(2)c
Atractomorpha acutipennis	1.2(1)c	0.0(0)c	0.0(0)c	0.0(0)b	3.3(2)cd	0.0(0)b	0.0(0)c	0.0(0)b	0.2	0.6	0.9(3)cd
Pyrgomorpha vignaudii	1.2(1)c	0.0(0)c	0.0(0)c	0.0(0)b	6.6(4)d	0.0(0)b	0.0(0)c	0.0(0)b	0.6	0.06	1.5(5)cd
Oxycatantops spissus	1.2(1)c	16.7(5)c	6.1(2)c	0.0(0)b	6.6(4)d	0.0(0)b	0.0(0)c	0.0(0)b	2.6	< 0.001	3.5(12)d
χ^2	97.8	41.2	18.4	27.7	48.5	16.2	40.2	14.9			258.7
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341

Developmental stage of crop pests.—Except in the Sanaga Maritime (12%), Valley of Ntem (7.9%), and High Nyong (3%) divisions, the most frequent pest grasshopper stages reported by respondents were both adults and nymphs (Fig. 3C). However, a few respondents in the Nyong and Kelle division reported that grasshoppers were only harmful during the nymphal stage (6.5%).

Crops cited as most often damaged by pest grasshoppers.—The crops most cited by the respondents as affected by pest grasshoppers were cassava (*Manihot esculenta* Crantz, 1766) (60.1%), corn (*Zea mays* L., 1753) (58.1%), groundnut (*Arachis hypogaea* L., 1753) (35.5%), and okra (*Abelmoschus esculentus* (L.) Moench, 1794) (27.9%) (Table 5). Less cited were green vegetable (15.5%), cucumber (*Cucumis sativus* L., 1753) (9.1%), sweet potato (*Ipomoea batatas* (L.) Lam., 1793) (7.3%), bean (*Phaseolus vulgaris* L., 1753) (6.2%), macabo-cocoyam (*Xanthosoma sagittifolium* (L.) Schott, 1832) (5.3%), and bitter leaf (*Vernonia amygdalina* Delile) (2.3%). No other crops were cited.

Impact of pests on crops productivity.—Cassava was most cited (32.3%) as suffering high losses (75–100%) due to pest grasshop-

pers, followed by corn (12.6%), green vegetable (12.3%), and groundnut (8.2%) (Fig. 4). The loss of 50–75% of crops was most cited in the same plants, while a loss of about 25–50% was more often reported in corn (29.3%) than in cassava (14.7%). High levels of damage (75–100%) were rarely reported for cucumber (3%), macabo-cocoyam (3%), and bitter leaf (1.2%).

Methods known and used to control pest grasshoppers.—The conventional grasshopper control methods cited by respondents were insecticides, weeding, picking by hand, and the use of improved seeds (Table 6). Insecticides were the most cited (52.5%), but many thought they were little used due to their high cost. Weeding (4.5%) and picking (13.5%) grasshoppers by hand were less cited but most used by the villagers. Improved seeds were rarely cited (0.6%), and biological methods were not cited at all. Among the traditional methods, the most cited and used by the villagers was spreading ash (18.8%), smoke (7.9%), or litter (2.3%) on crops. Other traditional methods were rarely mentioned or used.

Efficiencies of the methods used to control pest grasshoppers.—Insecticides were considered to be most effective in removing grass-
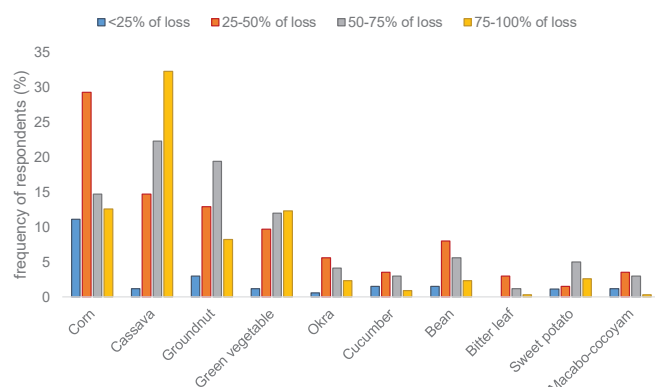
Fig. 4. Impact of pest activities on the productivity of crops.

hoppers, with 26.7% of respondents saying that insecticides can remove 75–100% of the grasshoppers in crop fields, although 1.5% said they were not very effective, removing less than 25% (Fig. 5). Weeding and picking grasshoppers by hand seems to be less effective, as such methods were claimed only by 2.1% and 8.2% of the people, respectively, to remove 25–50% of the grasshoppers in the crops. Improved seeds were rarely used (0.3%) but, according to the villagers, they guarantee an efficiency of up to 75–100%. A few people (0.9–3.8%) expressed a belief that the use of smoke can remove up to 50–100% of pest grasshoppers in the crop fields, but some (1.5–2.1%) purported it to have less efficiency. A slightly higher proportion (2.3–10.6%) of respondents said ash can efficiently (25–75%) control pest grasshoppers in crop fields. Other traditional methods of control were rarely mentioned and cited as having an efficiency less than 50%.

Table 5. Crops cited by local people as damaged by pest grasshoppers. Frequency in % (number of respondents); N = size of the sample; p value = probability; χ^2 = value of the Kruskal-Wallis test. The letters a, b, c, d, e, and f represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

Crops	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ^2	p value	Total
Corn	67.1(57)a	83.3(25)a	48.5(16)a	90.3(28)a	55.8(34)a	21.1(8)a	50.0(15)a	45.5(15)a	36.2	< 0.001	58.1(198)a
Cassava	81.2(69)b	66.7(20)a	48.5(16)a	71.0(22)ab	73.8(45)b	28.9(11)a	40.0(12)a	30.3(10)ab	44.5	< 0.001	60.1(205)a
Groundnut	43.5(37)c	13.3(4)b	30.3(10)ab	16.1(5)c	59.0(36)a	34.2(13)a	30.0(9)ab	21.2(7)bc	23.5	< 0.001	35.5(121)b
Green vegetables	27.1(23)d	16.7(5)b	3.0(1)c	19.4(6)c	19.7(12)c	0.0(0)b	10.0(3)b	9.1(3)bc	8.8	0.002	15.5(53)c
Okra	20.0(17)de	66.7(20)a	21.2(7)b	48.4(15)bd	31.1(19)c	23.7(9)a	16.7(5)b	9.1(3)bc	24.4	< 0.001	27.9(95)d
Cucumber	12.9(11)e	10.0(3)c	3.0(1)c	0.0(0)e	8.2(5)c	15.8(6)a	3.3(1)c	12.(4)bc	1.8	0.5	9.1(31)e
Bean	5.9(5)ef	3.3(1)d	6.1(2)c	12.9(4)f	9.8(6)c	0.0(0)b	3.3(1)c	6.1(2)c	1.0	0.4	6.2(21)ef
Bitter leaf	3.5(3)f	0.0(0)d	3.0(1)c	0.0(0)e	0.0(0)d	0.0(0)b	0.0(0)c	12.(4)bc	8.7	< 0.001	2.3(8)g
Sweet potato	0.0(0)f	0.0(0)d	6.1(2)c	32.3(10)d	9.8(6)c	0.0(0)b	16.7(5)b	6.1(2)c	1.9	0.02	7.3(25)ef
Macobo-cocoyam	0.0(0)f	0.0(0)d	0.0(0)c	6.5(2)f	19.7(12)c	0.0(0)b	6.7(2)b	6.1(2)c	5.4	< 0.001	5.3(18)f
χ^2	190.4	86.5	29.9	79.8	108.5	19.7	23.8	13.9			437.6
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341

Table 6. Methods for controlling pest grasshoppers cited and used by local people. Frequency in % (number of respondents); N = size of the sample; p value = probability; χ^2 = value of the Kruskal-Wallis test. The letters a, b, c, d, and e represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

Methods of control	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ^2	p value	Total
Conventional methods											
Insecticide	69.4(59)a	50.0(15)a	24.2(8)a	29.0(25)a	37.7(23)a	57.8(22)a	46.6(14)a	39.4(13)a	28.8	< 0.001	52.5(179)a
Weeding	7.1(6)b	0.0(0)b	21.2(7)a	3.2(1)b	0.0(0)b	0.0(0)b	0.0(0)b	3.0(1)b	3.9	< 0.001	4.5(15)b
Picking	2.4(2)b	0.0(0)b	42.4(14)b	38.7(12)c	19.7(12)c	7.9(3)b	0.0(0)b	9.1(3)b	21.5	< 0.001	13.5(46)c
Improved seed	2.4(2)b	0.0(0)b	0.0(0)c	0.0(0)b	0.0(0)b	0.0(0)b	0.0(0)b	0.0(0)b	0.1	0.5	0.6(2)d
χ^2	81.1	16.7	8.9	36.3	17.9	26.4	14.6	9.9			173.6
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341
Traditional methods											
Smoke	5.9(5)a	30.0(9)a	3.0(1)	0.0(0)a	3.3(2)	10.5(4)a	13.3(4)a	6.1(2)	6.1	< 0.001	7.9(27)a
Ashes	36.5(31)b	33.3(10)a	3.0(1)	35.5(11)b	1.6(1)	0.0(0)b	20.0(6)a	12.1(4)	25.0	< 0.001	18.8(64)b
Litter	1.2(1)c	13.3(4)a	0.0(0)	0.0(0)a	0.0(0)	0.0(0)b	10.0(3)ab	6.1%(2)	1.9	0.003	2.3(10)c
Paracetamol	1.2(1)c	0.0(0)b	0.0(0)	0.0(0)a	0.0(0)	0.0(0)b	0.0(0)b	0.(0)	0.03	0.9	0.3(1)d
Cow dung	0.0(0)c	0.0(0)b	0.0(0)	0.0(0)a	0.0(0)	0.0(0)b	3.3(1)ab	0.0(0)	0.08	0.3	0.3(1)d
Hot pepper water	0.0(0)c	23.3(7)a	0.0(0)	0.0(0)a	0.0(0)	0.0(0)b	0.0(0)b	0.0(0)	4.5	< 0.001	2.1 (7)ce
Bell sounds	0.0(0)c	0.0(0)b	0.0(0)	6.5(2)c	0.0(0)	0.0(0)b	0.0(0)b	6.1(2)	0.6	0.02	1.2(4)ce
Tobacco leaf water	0.0(0)c	0.0(0)b	0.0(0)	0.0(0)a	0.0(0)	0.0(0)b	0.0(0)b	3.0(1)	0.08	0.2	0.3(1)d
χ^2	28.5	13.3	0.1	10.0	0.2	1.4	3.2	1.3			29.3
p value	< 0.001	< 0.001	0.5	< 0.001	0.2	< 0.001	0.002	0.2			< 0.001
N	85	30	33	31	61	38	30	33			341

Importance of grasshoppers cited by local people

Grasshoppers were reported as used mainly as food (86.8%), but also sold at markets (57.7%), used as fishing bait (13.2%), or to treat diseases (10.9%) (Table 7). Grasshoppers are eaten and commercialized in all the divisions studied, used to treat diseases in five divisions, and as fishing bait in four. A very few respondents mentioned using grasshoppers as a charming medium (1.8%), as being important for ecosystem balance (0.9%), or considered them to be biological control agents against weeds (0.3%) or a pollinator agent (0.3%).

Types of grasshoppers eaten and commercialized.—*Zonocerus variegatus* and *Oxycatantops spissus* (Fig. 6) were the two grasshopper species most often reported as eaten and sold in the markets (Table 8). Orthopteran species coming from the families Tettigonidae and Gryllidae were also eaten-fried or braised and marketed fresh or fried.

Diseases treated with grasshoppers.—*Zonocerus variegatus* (Fig. 6A) was cited by the respondents as used to treat a wide variety of diseases including spleen pain, burns, tuberculosis, angina, malaria and several others (Appendix 2). *Atractomorpha acutipennis* (Fig 6C) was crushed to treat disease of the baby's fontanelle and sighting this grasshopper was a sign of luck in hunting, while *Oxycatantops spissus* (Fig 6B) was used in treatment of some diseases and as a charming medium (Appendix 2).
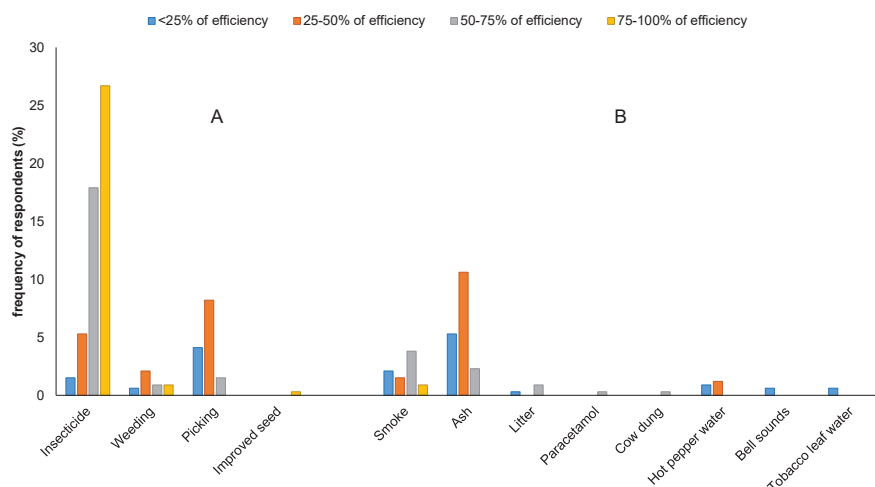

Fig. 5. Efficiency of the methods used to control pest grasshoppers: conventional methods (**A**) and traditional methods (**B**).

Table 7. Importance of grasshoppers cited by local people. Frequency in % (number of respondents); N = size of the sample; *p* value = probability; χ^2 = value of the Kruskal-Wallis test. The letters a, b, c, and d represent the results of the Mann-Whitney test for two samples in the same column; the same letter indicates non-significant differences between the values.

Uses	Mbam and Inoubou	Mbam and Kim	Mefou and Akono	Nyong and Kelle	Mvilla	Valley of Ntem	Sanaga Maritime	High Nyong	χ^2	*p* value	Total
Food	89.4(76)a	100.0(30)a	93.9(31)a	83.8(26)a	95.1(58)a	57.8(22)a	90.0(27)a	78.8(26)a	13.8	< 0.001	86.8(296)a
Commerce	57.6(49)b	76.7(23)b	72.7(24)b	77.4(24)a	32.8(20)b	34.2(13)b	66.7(20)b	72.7(24)a	29.6	< 0.001	57.7(197)b
Treat diseases	17.6(15)c	0.0(0)c	6.1(2)c	32.2(10)b	3.3(2)c	21.1(8)bc	0.0(0)c	0.0(0)b	11.2	< 0.001	10.9(37)c
Charming medium	2.4(2)d	0.0(0)c	0.0(0)c	0.0(0)c	0.0(0)c	10.5(4)bc	0.0(0)c	0.0(0)b	1.2	0.04	1.8(6)d
Fishing bait	16.5(14)c	0.0(0)c	0.0(0)c	0.0(0)c	21.3(13)b	36.8(14)ab	0.0(0)c	12.1(4)c	14.3	< 0.001	13.2(45)c
Pollinator agent	1.2(1)d	0.0(0)c	0.0(0)c	0.0(0)c	0.0(0)c	0.0(0)cd	0.0(0)c	0.0(0)b	0.03	0.9	0.3(1)d
Biological control	1.2(1)d	0.0(0)c	0.0(0)c	0.0(0)c	0.0(0)c	0.0(0)cd	0.0(0)c	0.0(0)b	0.03	0.9	0.3(1)d
Ecosystem balance	0.0(0)d	0.0(0)c	0.0(0)c	0.0(0)c	0.0(0)c	7.9(3)c	0.0(0)c	0.0(0)b	0.6	0.001	0.9(3)d
χ^2	193.3	107.3	102.8	86.9	140.2	33.5	84.9	81.8			764.6
p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
N	85	30	33	31	61	38	30	33			341

Table 8. Grasshoppers and other Orthopterans cited by local people as eaten and sold commercially.

| Species/Family	Consummation forms	Commercialization forms	Divisions
Zonocerus variegatus	fried or braised		Mbam and Inoubou, Mvilla, Valley of Ntem, High
		fresh or fried	Nyong, Mefou and Akono, Nyong and Kelle
Oxycatantops spissus	fried or braised	fresh	Mbam and Inoubou
Tettigonidae	fried or braised	fresh or fried	All the divisions
Gryllidae	fried or braised	not sold	Mbam and Inoubou
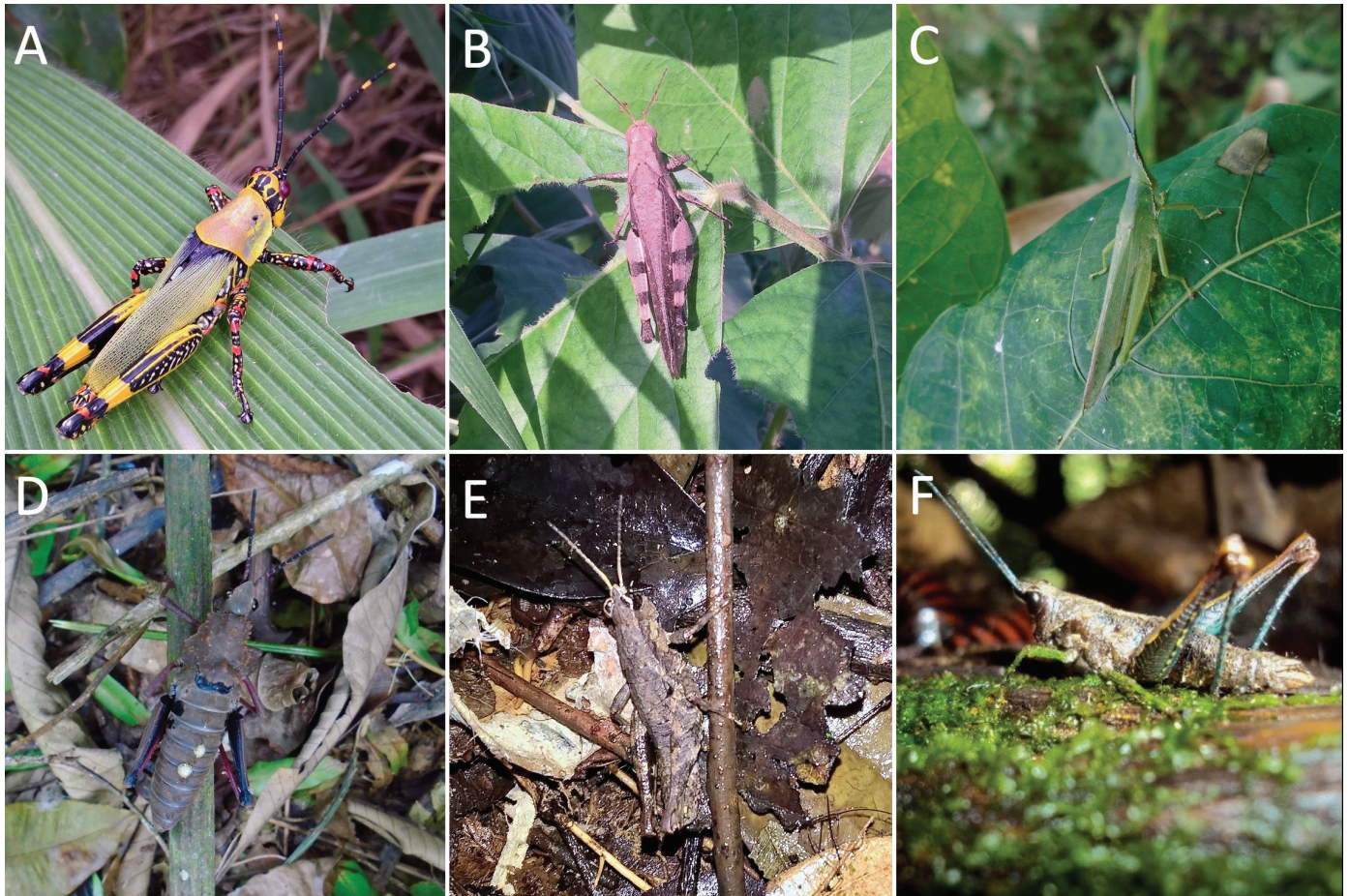
Fig. 6. Some grasshoppers mainly used/cited by local people: *Zonocerus variegatus* (pest of crops, fallowland species, use as food and to treat diseases) (**A**), *Oxycatantops spissus* (pest of crops, fallowland species, use as food and to treat diseases) (**B**), *Atractomorpha acutipennis* (pest of crops, species of forest edge and fallow, use to treat diseases) (**C**), *Parapetasia femorata* (forest species, use as indicator to characterize forest ecosystems) (**D**), *Mazea granulosa* (forest species, use as indicator to characterize forest ecosystems) (**E**), *Gemeneta terrea* (forest species, use as indicator to characterize forest ecosystems) (**F**).

Discussion

Our data allow an assessment of the perception of grasshoppers by local people in Southern Cameroon, particularly in terms of food, health, and landscape conservation. Grasshoppers are known to be herbivore insects common to grassland ecosystems worldwide (Lockwood et al. 2000, Branson et al. 2006). Most of the respondents interviewed (99.7%) thought grasshoppers were found in all landscapes.

Our study shows that the local people understand that the abundance of grasshoppers increases with the degradation or opening of forests: grasshoppers were reported to be rare in pristine forests and abundant in much degraded forests, which is consistent with Badenhausser's (2012) conclusion that abundance increases with environmental degradation. According to Latchininsky (1996), in the ex-USSR, some forest Orthopteran species (especially *Tetrix tartara subacute* Bey-Biento, 1951, *Acrida oxycephala* (Pallas, 1771), *Duroniella gracilis* Uvarov, 1926, *Duroniella kalmyka* (Adelung, 1906), and *Mesasippus kozhevnikovi iliensis* Mistshenko, 1951) become rare after forest degradation; this author noted an increase in grassland species in the degraded areas, especially of *Sphingonotus maculatus* Uvarov, 1925, *Sphingonotus halocnemi* Uvarov, 1925, *Sphingonotus satrapes* Saussure 1884, and *Sphingoderus carinatus* (Saussure, 1888). Hao et al. (2015) suggested that, apart from steppes and deserts, the abundance of grasshoppers was almost the same in other ecosystems. Unlike our work, Joshi et al. (1999) reported that in India, species diversity and richness were higher in less disturbed sites, followed by replanting environments and severely disturbed environments. These differences show that the behavior of grasshoppers related to the opening of the environment depends on the eco-climatic zones and the structure of the vegetation. The changes to ecosystems strongly affect grasshoppers' behavior, as grasshoppers use plants as both food and habitat (Latchininsky et al. 2011, Oumarou Ngoute et al. 2020). The challenge is to predict the potential responses of the grasshoppers, in a given ecosystem, to global environmental change.

The respondents said grasshoppers were present in all climatic seasons, but more abundant during the dry seasons. Kijazi et al. (2013) claimed that indigenous peoples in Nigeria used insect abundance and movement to predict the onset of the dry season, and Joshua and Jürgen (2013) reported that the appearance of the bushcricket *Ruspolia baileyi* Otte, 1997 is known to indicate the dry season in western Uganda. Poubom et al. (2005) and Oladele et al. (2014) recorded the abundant activity of *Z. variegatus* during the dry seasons in Cameroon and Nigeria.

In general, respondents recognized grasshoppers as being both useful and harmful. They reported that all species consume crops, but only *Z. variegatus* was recognized as a pest with economic impact. According to Poubom et al. (2005), the majority of farmers in Cameroon consider insects to be pests, and after the green mite *Mononychellus tanajoa* (Bondar, 1938), it is the stinky grasshopper *Z. variegatus* that is responsible for most of the damage observed on crop leaves, especially during the dry seasons. The farmers in the study said that *Z. variegatus* is a polyphagous pest and that its damage has increased over the past 10 years as forest destruction has increased (Poubom et al. 2005). Oladele et al. (2014) found that in Nigeria, grasshoppers were reported as the main pest followed by beetles and butterflies; the grasshopper *Z. variegatus* is known to be polyphagous in this area and can devastate fields of vegetables during the dry season. In the same country, Okunlola and Ofuya (2010) reported that *Z. variegatus* is the third most frequent crop pest after *Dysdercus superstitiosus* (Fabricius, 1775) (Hemiptera) and *Sylepta derogata* (Fabricius, 1775) (Lepidoptera).

Our data reveal that the villagers felt that insecticides were the most effective method to control pest grasshoppers, but because of the high cost of chemical insecticides, most farmers used weeding and picking by hand; biological and ecological methods were not mentioned. Worldwide, most locust and grasshopper management programs still rely on chemical pesticides (Zhang et al. 2019). Suggested products, pros and cons, and doses are regularly made publicly available by the FAO Pesticide Referee Group (FAO 2014). In recent years, a very remarkable advance has been the use of biopesticides, prepared with the fungus *Metarhizium acridum*, as important components of management programs and with good efficacy (Zhang et al. 2019). However, in our study, traditional methods of grasshopper control were the most used, which is consistent with the results of Joshua and Jürgen (2013), who reported that the majority of African farmers still depended on indigenous pest management approaches. In Nigeria, according to Oladele et al. (2014), 76.7% of farmers use cultural or traditional methods due to the unavailability and high cost of chemical insecticides. In the same country, Okunlola and Ofuya (2010) found that 76% of farmers were aware of indigenous methods for the control of vegetable pests. Our study shows that the most-used traditional methods were spraying smoke and spreading ash and litter on crops. Respondents reported that smoke can be up to 75–100% effective in repelling grasshoppers, but the control of *Z. variegatus* comes down to hand picking and human consumption. Poubom et al. (2005) reported that in most regions of Cameroon, the traditional methods of grasshopper control were manual collection or capture of edible species that provide additional food for families. Some local people in southern Cameroon collect grasshopper species (mainly *Z. variegatus*) as a food source for poultry. Page (1978) recommends plowing to control *Z. variegatus*. When females have laid their eggs in clumps in the soil, plowing brings the eggs to the surface and causes them to dry out. If this practice was adopted by all farmers, populations of *Z. variegatus* may be greatly reduced and damage minimized (Modder 1994). In Uganda, the natives use extracts of natural plants to control crop pests, specifically *Capsicum frutescens* L., 1753, *Tagetes* spp., *Nicotiana tabacum* L. 1753, *Cypressus* spp., *Tephrosia vogelii* Hook.f., 1849, *Azadirachta indica* A.Juss., 1830, *Musa* spp., *Moringa oleifera* Lam., 1785, *Tithonia diversifolia* (Hemsl.) A.Gray, 1883, *Lantana camara* L., 1753, *Phytolacca dodecandra* L'Her., *Vernonia amygdalina* Delile, *Aloe* spp., *Eucalyptus* spp., *Cannabis sativa* L., 1753, *Coffea* sp., and *Carica papaya* L., 1753 (Joshua and Jürgen 2013). Extracts with insecticidal properties come from the roots, stems, leaves, or flowers of these plants; they have a low spectrum of action, are easily usable,

and have few residues capable of accumulating in animal or plant tissues. However, many farmers have reported that some botanical formulations take a long time to prepare and are not easy to apply, especially on a large scale (Mugisha-Kamatenesi et al. 2008).

Most of the respondents (85.9%) in our study said grasshoppers are used as food in addition to Tettigonidae and Gryllidae, and the grasshopper species consumed by the local people are *Z. variegatus* and *O. spissus*. Orthoptera species are used as food in many parts of the world, such as Australia, India, South America, and Africa (van Huis 2003, Srivastava et al. 2009, Mitsuhashi 2016, Niassy et al. 2016, Jongema 2017, Tchibozo and Lecoq 2017). Gullan and Cranston (2010) reported that most of the edible insects used worldwide come from a relatively small number of orders, including crickets, grasshoppers, and locusts. According to Riggi et al. (2013), Coleoptera are the most commonly consumed, and Orthoptera are the second group of insects consumed in Africa, specifically grasshoppers such as *Hieroglyphus africanus* Uvarov, 1922, *Acanthacris ruficornis citrina* (Serville, 1838), *Ornithacris cavroisi* (Finot, 1907), locusts [*Locusta migratoria* (Linnaeus, 1758)], and crickets [*Brachytrupes membranaceus* (Drury, 1773)] which have a nutritional quality superior or similar to that of the meat products currently available. Insects as food are not inferior to other sources of protein, such as other animals or plants (Xiaoming et al. 2010). A recent analysis of the nutrient composition of *Z. variegatus* from Nigeria showed high values of protein, crude lipids, and minerals (potassium, sodium, and calcium) (Anaduaka et al. 2021). Our study shows that *Z. variegatus* and *O. spissus* are also sold in the markets. Bronwyn (2013) noted that grasshoppers are eaten and sold in the markets of Dimapur and Kohima, India: the legs, wings, and viscera are removed, and they are fried in oil with ingredients such as onion, bamboo, ginger, and salt. Pemberton and Yamasaki (1995) reported that grasshoppers appear on restaurant menus in Japan.

Zonocerus variegatus, *Atractomorpha acutipennis*, and *Oxycatantops spissus* are used by local people to treat spleen pain, burns, tuberculosis, angina, malaria, stomachaches, and anal tingling. Grasshoppers are considered to have therapeutic value in Australia, India, South America, and Africa (Srivastava et al. 2009). De Conconi and Moreno (1988) reported that most of the insects (such as grasshoppers and locusts) sold in the markets in Mexico are also used as diuretics, analgesics, anesthetics, or aphrodisiacs. The species *Sphenarium* spp., *Taeniopoda* sp., and *Melanoplus* sp. are used to treat kidney diseases and intestinal disorders (the hind legs of grasshoppers are crushed and mixed with water, then drunk as a powerful diuretic). Locusts of the species *Schistocerca* spp. are helpful in cases of postnatal anemia and in pulmonary diseases, asthma, and chronic cough. The legs of the crickets *Acheta domesticus* (Linnaeus, 1758) are crushed and mixed with water and drunk as a diuretic for dropsy edema (De Conconi and Moreno 1988). Lawal and Banjo (2007) reported that the grasshoppers *Z. variegatus* and *Zabalius lineolatus* (Stal, 1873) are used to treat childhood illness and injuries in Nigeria. Some species are involved in the magic and mystical treatment of diseases in Mexico: *Brachytrypes* sp. crickets are used to treat bleeding in women before delivery (De Conconi and Moreno 1988).

All these examples demonstrate that, as in many regions of the world, diverse use is made of grasshoppers in southern Cameroon, whether as a food source, remedies, or as indicators of environmental change. In addition, numerous local solutions (of varying effectiveness) are used to control pest species. It is important to continue to identify, understand, and develop this traditional knowledge as a possible source or at least partial solution to some of the environmental changes currently underway.

Acknowledgements

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Appendix 1

Grasshoppers recognized by local people in the different landscapes; + indicates species recognized as present by villagers.

Sub-Familly	Grasshoppers species	House	Fallow	Crop	Forest	Divisions
Acridinae	*Chirista compta*		+	+		Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Mvilla, Valley of Ntem, Sanaga Maritime
	Gymnobothrus temporalis		+			Mbam and Inoubou, Mefou and Akono, Mvilla, Valley of Ntem
	Odontomelus kamerunensis		+	+	+	Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Nyong and Kelle, Mvilla, Valley of Ntem
Catantopinae	*Abisares viridipennis*		+			Mbam and Inoubou
	Catantops stramineus		+			Nyong and Kelle, Mvilla, Valley of Ntem
	Europacris coerulea		+	+	+	Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Nyong and Kelle, Mvilla, Valley of Ntem
	Gemeneta terrea			+		Mbam and Kim, Mefou and Akono, Mvilla
	Mazea granulosa			+		Mbam and Kim, Mefou and Akono, Mvilla, Valley of Ntem

Sub-Familly	Grasshoppers species	House	Fallow	Crop	Forest	Divisions
Catantopinae	*Oxycatantops spissus*		+	+		Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Nyong and Kelle, Mvilla, Valley of Ntem, Sanaga Maritime
	Pteropera balakoswki		+		+	Mbam and Inoubou, Mefou and Akono, Valley of Ntem
	Pteropera mirei		+		+	Mbam and Inoubou, Mefou and Akono, Mvilla
Coptacrinae	*Cyphocerastis tristis*		+	+	+	Mbam and Inoubou, Mbam and Kim, High Nyong
	Eucoptacra anguliflava	+	+	+	+	Mbam and Inoubou, Mvilla
Cyrtacantacridinae	*Acanthacris ruficornis*		+	+		Mbam and Kim, Mvilla
Spathosterninae	*Spathosternum pygmaeum*	+	+			Mbam and Inoubou, Mbam and Kim, Mvilla, Valley of Ntem
Oedipodinae	*Heteropternis thoracica*	+	+	+		Mbam and Inoubou, Mbam and Kim, Mvilla, Valley of Ntem
Pyrgomorphinae	*Atractomorpha acutipennis*	+	+	+	+	Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Nyong and Kelle, Mvilla, Valley of Ntem, High Nyong, Sanaga Maritime
	Dictyophorus karschi				+	Mvilla, Valley of Ntem, Mbam and Kim
	Parapetasia femorata				+	Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Nyong and Kelle, Mvilla, Valley of Ntem, High Nyong
	Pyrgomorpha vignaudii	+	+	+		Mbam and Inoubou, Mbam and Kim, Mvilla, Valley of Ntem, Sanaga Maritime
	*Taphronota **ferruginea***		+	+		Mefou and Akono, Mvilla, Valley of Ntem
	Zonocerus variegatus		+	+		Mbam and Inoubou, Mbam and Kim, Mefou and Akono, Nyong and Kelle, Mvilla, Valley of Ntem, High Nyong, Sanaga Maritime
Tropidopolinae	*Afroxyrrhepes obscuripes*		+			Mbam and Inoubou

Appendix 2

Diseases cited by local people as treated using grasshoppers.

Grasshoppers species	Diseases/cultural uses	Preparation	Posology	Divisions
Zonocerus variegatus	Spleen pain	Crush the grasshopper and extract its oil	Scarify the patient with spines of the grasshopper's legs and rub oil extracted	Mbam and Inoubou, Mvilla
	Spleen pain	Remove head and viscera of the grasshopper, wash with boiling water, and cook in cucumber dishes	Eat in three days	Mbam and Inoubou
	Spleen pain	Crush the grasshopper and mix with water	Purge the patient with the solution	Valley of Ntem
	Scabies and burns	Crush the grasshopper and mix with red palm oil	Rub on the scabies or burns	Valley of Ntem
	Belly swollen of children	Remove head and viscera of the grasshopper, wash with boiling water, fry, and mix with red palm oil	Eat once daily until disease regression	Mbam and Inoubou
	Tuberculosis	Remove head and viscera of the grasshopper, wash with boiling water, and cook in cucumber dishes	Eat daily until disease regression	Mbam and Inoubou
	Angina	Crush the head and viscera grasshopper and mix with "the king of grass" Algeratum conizoides	Rub on the throat every day during illness	Mvilla
	Malaria	Put the grasshopper on the child so the child is stung by its spines		High Nyong
	Burn	Burn and crush the grasshopper and mix it with a little water	Rub on the wound	Mefou and Akono
	Anal itching of children of 2 to 3 years old	Crush head and viscera of the grasshopper and mix with "the king of grass" Algeratum conizoides	Purge the patient with the solution	Nyong and Kelle
	Anal itching of children of 2 to 3 years	Sting three times the anus of child with the spines of grasshopper		Nyong and Kelle
Atractomorpha acutipennis	« abobo » disease of the baby's fontanelle	Crush grasshoppers	Rub on the fontanelle	Valley of Ntem
	luck		Viewed in the forest by a hunter reflects a successful hunt	Mefou and Akono
Oxycatantops spissus	Burns and painful menstruation of women	Remove head and viscera of the grasshopper, wash with boiling water, and cook in dishes or cucumber sauces	Eat during the period of menstruation	Mbam and Inoubou
	Spleen pain	Scarify the child at the hip with the spines of grasshopper's legs		Mvilla
	Charming medium	Remove grasshopper viscera and cook it with smoked freshwater fish in cucumber dishes	Give food only to the person you want to charm	Mbam and Kim

Supplementary material 1

Author: Charly Oumarou Ngoute

Data type: Survey sheet

Explanation note: Grasshoppers Survey sheet. This survey sheet was used in the villages to collect informations about grass-hoppers.

Link: https://doi.org/10.3897/jor.30.64266.suppl1

A new species of pygmy mole cricket (Orthoptera: Tridactylidae) from the Lake Wales ridge of Florida and new records of *Ellipes eisneri* from the northern Brooksville ridge

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Abstract

Pygmy mole crickets (Orthoptera: Tridactylidae) are usually associated with the edges of ponds and streams, but in the sandy uplands of Florida, at least two lineages of these insects have evolved to live in xeric scrub and sandhill habitats. Very little work has been done with scrub tridactylids since they are tiny and often difficult to collect. In this paper, the pygmy mole cricket *Ellipes deyrupi* **sp. nov.** is described from the northern Lake Wales Ridge of Florida. It is sympatric at all known locations with another scrub tridactylid, *Neotridactylus archboldi* Deyrup & Eisner, but has not been found co-occurring with the closely related *Ellipes eisneri* Deyrup. The habits of this new species are convergent with both *N. archboldi* and *E. eisneri*. In addition, new records of *Ellipes eisneri* are presented, extending this species' known distribution to the Northern Brooksville Ridge. Both species of scrub *Ellipes* are found in restricted geographic ranges and suffer from a lack of study and recognition. Conservation implications for these two species are discussed.

Introduction

The pygmy mole crickets, or Tridactylidae (Orthoptera: Caelifera: Tridactyloidea), are a family of small, burrowing orthopterans with a worldwide distribution (Deyrup and Eisner 1996). There are currently seven species known in the Nearctic region, with five recorded in Florida (Günther 1975, 1977). Three of these, *Neotridactylus apicialis* (Say, 1825), *Ellipes minuta* (Scudder, 1862), and *E. gurneyi* Günther, 1977, are fully winged, widespread in the southern USA, and occur in wet, sandy areas along water, as is typical for most members of the family. The other two species, *N. archboldi* Deyrup & Eisner, 1996, and *E. eisneri* Deyrup, 2005, are flightless and endemic to ridge systems of central Florida, where they occur in open patches of bare sand within scrub (*N. archboldi*) or sandhill (*E. eisneri*) habitats. They are able to occur in these xeric environments because of the presence of a subsurface layer of algae in the bare patches of sand they inhabit. This pro- vides them with a source of food that is transiently available when rains occur, at which time the insects come to the surface and construct feeding burrows (Deyrup and Eisner 1996). Their habitat and food restrictions, as well as their flightless nature, mean that populations of scrub tridactylids are often isolated from each other, both within and between different ridge systems.

The Florida scrub is a unique and threatened habitat restricted to a series of ridges and dunes formed from Miocene, Pliocene, or Pleistocene shorelines, during which time the rest of the state was underwater (Deyrup 1989). These ridges served as biotic refuges during glaciation events, and today serve as ecological islands, where isolated populations of many species have diverged into distinct species. Despite being located in a subtropical region with high precipitation, the scrub is a rather dry environment owing to the excessive draining of water through its fine-grained sands (Menges 1999). Because of these conditions, it supports a distinct community of organisms specially adapted to drought. Florida scrub is characterized by low evergreen oaks (*Quercus* spp.) and sand pine (*Pinus clausa* (Chapm. ex Engelm.)) Vasey ex Sarg.), often with the ericaceous shrub *Ceratiola ericoides* Michx., interspersed with patches of bare white sand where many endemic herbaceous plants and lichens occur (Menges 1999). Sandhill, or 'high pine,' is another xeric habitat that is found on the Florida sand ridges but which also extends into other southeastern states (Myers 1985). This environment has a canopy of longleaf pine (*Pinus palustris* Mill.) and turkey oak (*Quercus laevis* Walter) and is more densely herbaceous than scrub, with abundant *Aristida* spp. grasses dominating (Menges 1999). Both scrub and sandhill are fire-maintained ecosystems, with sandhill burning more frequently than scrub (Myers 1985).

The oldest and longest of Florida's sand ridges, and the one with the highest levels of endemism, is the Lake Wales Ridge (LWR), which extends in central Florida from just south of the Orlando area to just north of Lake Okeechobee (Deyrup 1990). The LWR contains a distinctive type of habitat, called yellow sand scrub, which is an intermediary between classic scrub and sandhill (Deyrup and Carrel 2012). This habitat occurs on higher elevated sites with yellow sand and large populations of scrub hickory

(*Carya floridana* Sarg.) and can vary from dense to more open scrub. There appears to be several arthropod species restricted to yellow sand scrub, including the Highlands tiger beetle (*Cicindelidia highlandensis* Choate, 1984), a candidate for federal protection (Deyrup and Carrel 2012). The LWR is one of the best studied ridges since it is the home of the Archbold Biological Station (ABS), which has conducted research on LWR endemic organisms since 1941. A total of 91 species of arthropods are known to be restricted to scrub habitat on the LWR (Deyrup and Carrel 2012), including six described species of Orthopterans.

Most Florida scrub endemic arthropods are poorly known due to their small sizes, restricted geographic ranges, and habitat specializations (Deyrup 1990), which are often such that only targeted collecting will reveal individuals. The scrub tridactylids are particularly difficult to locate, since they are only reliably found at or near the surface of the sand within a few hours after a rain. Their presence can sometimes be predicted in periods of drier weather, however, by the presence of small, excavated mounds of sand (tumuli) created when the insects burrow vertically downward in patches of open sand. Even when populations are located, individuals are a challenge to capture due to their impressive leaping abilities. Deyrup and Carrel (2012) completed an extensive sampling effort of LWR endemic arthropods across 23 different protected scrub preserves. In the course of that work, specimens of an unusual tridactylid were collected on the east side of the north-central LWR that clearly represented an undescribed species. The species was recognized as new by Deyrup (pers. com.) but has remained undescribed until now.

Methods

Collection.—Initial specimens of the new species were collected in yellow bowl traps (always following rain) during a 2009 survey of arthropods endemic to the Lake Wales Ridge. Additional material was collected by the author in August 2018 at the Tiger Creek Preserve and in August 2019 at two roadside sites east of Lake Marion. These two localities, as well as roadside sites on the Brooksville Ridge that might harbor *Ellipes eisneri* populations, were located using Google Maps. The author made several trips to the Brooksville Ridge during June and July 2018 in search of *E. eisneri*, but dry conditions kept the insects deep underground and out of reach. On July 28 and 29, 2018, the weather was more favorable, and specimens of *E. eisneri* were collected at five sites. All tridactylid specimens were collected either by carefully digging up the raised burrows immediately after rain or by sifting dry sand around the tumuli.

Determination.—Specimens of *E. eisneri* were identified using Deyrup (2005). No other known tridactylid species has the abbreviated wings and pale color pattern of *E. eisneri*, except for the new species described below. The new species is a member of the genus *Ellipes* Scudder, 1902, due to the extreme reduction of the hind tarsus. Its pale white and brown coloration distinguishes it from all other species of the genus, except for *E. albicollatus* Günther, 1977 and *E. eisneri*. *E. albicollatus* is described as having the caudal margin of the pronotum all white, with a white spot in the middle of the anal field of the tegmen. This species differs in other aspects of its coloration as well as in the shape of the female subgenital plate and is also known only from Brazil. Range, as well as the characters given below, will separate *E. eisneri* and the new species.

Measurements and terminology.—Measurements were made to the nearest 0.5 mm by hand using a ruler and dissecting microscope

(Wild Heerbrugg, Heerbrugg, Switzerland). Terminology follows Deyrup (2005).

Photographs and maps.—Live nymphs and adults of both species were photographed in situ using a Canon EOS Rebel T3 with an attached Canon 100 mm macro lens and an external flash (Sunpak Auto 383 Super connected with a CowboyStudio 4 Channel Wireless Hot Shoe Flash Trigger & Receiver). Collected nymphs and adults were photographed in a whitebox studio setup using the same equipment, with the addition of Canon extension tubes (EF25 and EF12 II) to the camera. Images of the habitat and tumuli were taken with an iPhone. Images of preserved specimens were made using a Canon EOS 6D with attached MPE 65 mm macro lens (20X zoom lens used for the terminalia and stridulatory apparatus) and Macro Twin Lite MT-24EX flash on a Cognisys Stackshot 3X system. Images were stacked using Zerene Stacker v.1.04 (Zerene Systems LLC, Richland, WA). All images were processed using Adobe Lightroom CC 2015.8 to crop and adjust white balance. Composite figures were created using Adobe Photoshop CC, v.14.2.1. Maps were created with SimpleMappr (Shorthouse 2010). GPS data were acquired using Google Maps.

Depositories.—

ABS   Archbold Biological Station Arthropod Collection, Venus, FL, USA;
ANSP   Academy of Natural Sciences, Philadelphia, PA, USA;
CUIC   Cornell University Insect Collection, Ithaca, NY, USA;
FSCA   Florida State Collection of Arthropods, Gainesville, FL, USA;
UMMZ   University of Michigan Museum of Zoology, Ann Arbor, MI, USA.

Results

Key to the species of Tridactylidae found in Florida (modified from Günther 1975 and Günther 1977).

1	Larger, body length 3.8–10 mm; hind tarsus present.....*Neotridactylus* 2
–	Smaller, length 3–5 mm; hind tarsus reduced to a tiny flap concealed between the hind tibial spurs .... *Ellipes* 3
2	Larger, length 5.5–10 mm; fully winged; swimming plates on hind tibia present; color variable; usually found in sandy or muddy areas along water..*N. apicialis*
–	Smaller, length 3.8–4.9 mm; tegmina abbreviated, hind wings absent; swimming plates absent; uniformly dark colored; always found in xeric scrub habitats.......................................*N. archboldi*
3	Fully winged; color variable but never uniformly pale; usually found in sandy or muddy areas along water.. 4
–	Tegmina abbreviated, hind wings absent; coloration pale white and brown; always found in xeric scrub or sandhill habitats................... 5
4	Antennae in both sexes 10–segmented ....*E. minuta*
–	Antennae in the male 11-segmented, in the female 12-segmented.. *E. gurneyi*
5	Smaller, length 3.3–3.5 mm; head blackish brown with narrow cream lines along inner orbits and coronal and frontal sutures; found in open sandhill habitat on the Brooksville Ridge.....*E. eisneri*
–	Larger, length 3.5–4.1 mm; head white with irregular blackish brown band at its base and an irregular line of the same color between the compound eyes; found in yellow sand scrub habitat on the northern Lake Wales Ridge ....*E. deyrupi* sp. nov.

Taxonomy

Family Tridactylidae Brullé, 1835
Subfamily Tridactylinae Brullé, 1835

Genus *Ellipes* Scudder, 1902

Ellipes Scudder, 1902: 309; Günther 1977: 47.

Type species.—*Tridactylus minutus* Scudder, 1862 (= *Ellipes minuta*), by original monotypy.

Ellipes deyrupi sp. nov.

http://zoobank.org/6F2B3CE8-7073-4EAE-91C9-2BB208CE113F
(Figs 1–5)

Material examined.—**Holotype:** USA • ♂; Florida: Polk County: Sarasota Court, east of Lake Marion; 28.084972, -81.514889; 07 Aug. 2019; red type label, "Holotype *Ellipes deyrupi* Woo"; B. Woo leg.; CUIC. **Allotype:** USA • ♀; Florida: Polk County, same information as holotype; specimen in ethanol; CUIC. **Paratypes:** USA • 2 ♂, 2 ♀, Florida: Polk County: same information as holotype; specimens in ethanol; CUIC • 1 nymph; Homosassa Lane, east of Lake Marion; 28.089583, -81.519583; 07 Aug. 2019; specimen in ethanol; B. Woo leg.; CUIC • 6 ♀, 2 nymphs; Tiger Creek Preserve, yellow sand scrub on preserve road just east of office; 27.809, -81.483; 05 Aug. 2018; specimens in ethanol, B. Woo leg.; 2 ♀, FSCA; 2 ♀, UMMZ; 2 ♀, 2 nymphs, CUIC • 1 ♀, 1 nymph ; same locality as previous; 03 Aug. 2018; dry pinned specimens; B. Woo leg.; CUIC • 1 ♂; same locality as previous; 27.82158, -81.47717; 07 Jul. 2009; yellow bowl trap, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 2 ♂, 2 ♀; same locality as previous; 27.82158, -81.47717; 08 Jul. 2009; flight trap with pan, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 2 ♂, 2 ♀; same locality as previous; 27.82158, -81.47717; 10 Jul. 2009; yellow bowl trap, Florida scrub; M. Deyrup, H. Otte, A. May leg.; ABS • 1 ♀; same locality as previous; 27.83383, -81.46682; 09 Jul. 2009; yellow bowl trap, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 1 ♂; same locality as previous; 27.83383, -81.46682; 10 Jul. 2009; flight trap with pan, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 1 ♀; same locality as previous; 27.83383, -81.46682; 13 Jul. 2009; flight trap with pan, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 2 ♂; same locality as previous; 27.82795, -81.46720; 10 Jul. 2009; Townes trap with pan, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 5 ♂, 6 ♀; Allen David Broussard Catfish Creek State Park; 27.98503, -81.49542; 05 Jun. 2009; yellow bowl trap after rain; M. Deyrup, H. Otte, A. May leg.; ABS • 8 ♂, 5 ♀; same locality as previous; 27.98598, -81.49663; 05 Jun. 2009;; flight trap with pan, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 3 ♂, 3 ♀; same locality as previous; 27.98542, -81.49517; 05 Jun. 2009; flight trap with pan, scrub habitat; M. Deyrup, H. Otte, A. May leg.; ABS • 1 ♂, 3 ♀; same locality as previous; 27.98457, -81.49606; 05 Jun. 2009; Townes trap, scrub habitat; M. Deyrup, A. May, H. Otte leg.; ABS • 2 ♂, 10 ♀; same locality as previous; 27.98503, -81.49542; 05 Jun. 2009; specimens in ethanol; M. Deyrup leg.; ABS • 2 ♂, 5 ♀; same locality as previous; 27.98531, -81.49645; 06 May 2009; specimens in ethanol; M. Deyrup leg.; ABS.

Diagnosis.—Differs from all other known species of *Ellipes*, except *E. eisneri* in its flightless condition, pale white and brown coloration, lack of swimming plates, and occurrence in xeric habitat. Easily distinguished from *E. eisneri* by its larger size, distinctive coloration, and disjunct range.

Description.—**Male (holotype).** (Fig. 3). Coloration in life (Fig. 2): Background color pale white; head white with interrupted blackish brown band basally and an irregular line of the same color between eyes; antennae cream, with terminal segments tinged with brown; pronotum entirely white with cream tinge on dorsum; forelegs white, with tibial spines and tarsus tinged with pale brown; middle legs white with dark markings (Fig. 3A, B); hind femora white, with small basal brown tinge, a median transverse brown band, a brown dorsal subapical chevron, apical crescents of hind femora pale salmon, hind tibiae and tarsi cream; tegmina blackish brown basally, color fading to white apically, anal area white; abdomen white, with tergites 5–8 bearing rounded blackish brown median spots; basal segment of cercus black, apical segment dark cream; ventral cercus-like organ (paraproctfortsatz of Günther [1977]) cream.

Structural characters: antennae 10-segmented, front tibia with 4 teeth; swimming plates of hind tibiae absent; forewing abbreviated, hind wings absent; scraper present on the underside of the forewing (Fig. 3C).

Terminalia as in Fig. 3D: Epiproct not longer than wide; hooks of paraprocts pale brown, with long distal part that curves upwards and tapers to end.

Female. (Fig. 4). Coloration as in male, except brown band on head not interrupted medially, median spots on tergites 5–8 more elongate than rounded. Structural characters similar to male, except stridulatory apparatus absent on underside of forewing. Subgenital plate more tapered than in *E. eisneri* (Fig. 4C, D).

Measurements (in mm).—**Male holotype.** length of head and body: 3.50; length of pronotum: 0.75; width of pronotum: 1.2; width of head across eyes: 0.75; length of hind femur: 2; length of hind tibia: 1.50; length of forewing: 0.4.

Female allotype. length of head and body: 4.10; length of pronotum: 1.00; width of pronotum: 1.20; width of head across eyes: 0.70; length of hind femur: 2.10; length of hind tibia: 1.60; length of forewing: 0.75.

Etymology.—This species is named in honor of Dr. Mark Deyrup, Emeritus Research Biologist in Entomology at the Archbold Biological Station, who first collected the species and recognized it as distinct, and who has contributed the bulk of what is known about scrub tridactylid biology. Dr. Deyrup encouraged the author to describe this new species and look for other species across the southeastern U.S. and has been enormously supportive and generous with his knowledge of Florida natural history.

Common name.—Deyrup and Carrel (2012) gave this species a provisional common name of "Broussard Pygmy Mole Cricket" due to its occurrence at the Allen David Broussard Catfish Creek Preserve, but given that the species is now known to occur at other localities, I propose the common name of "Deyrup's Pygmy Mole Cricket" to recognize Deyrup's contributions to the biology and conservation of Florida scrub arthropods.

Distribution.—Restricted to the north-central Lake Wales Ridge in Polk County, FL (Fig. 5).

Habitat.—All specimens were collected in bare sand patches within yellow sand scrub habitat (Fig. 1A, B, E). These habitats have grasses and forbs scattered across the sand, along with various lichens, pines, and oaks. Tumuli and raised feeding burrows were
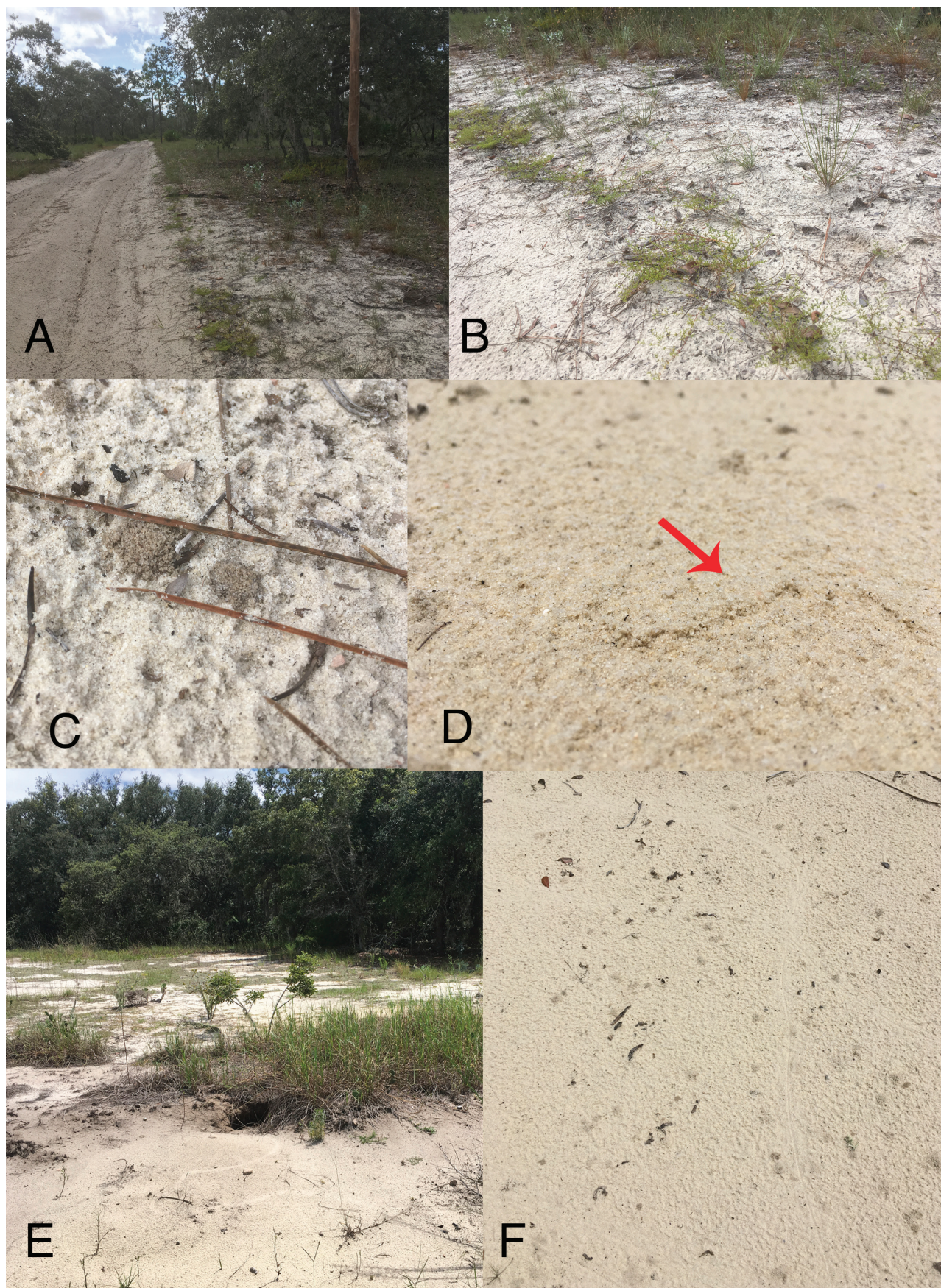
Fig. 1. Habitat and traces of *Ellipes deyrupi* **sp. nov. A.** Habitat at Tiger Creek Preserve; **B.** Detail of same; **C.** Tumuli at Tiger Creek; **D.** Feeding burrow at Sarasota Court; **E.** Habitat at same locality. Note gopher tortoise burrow entrance at center; **F.** Tumuli at same locality.
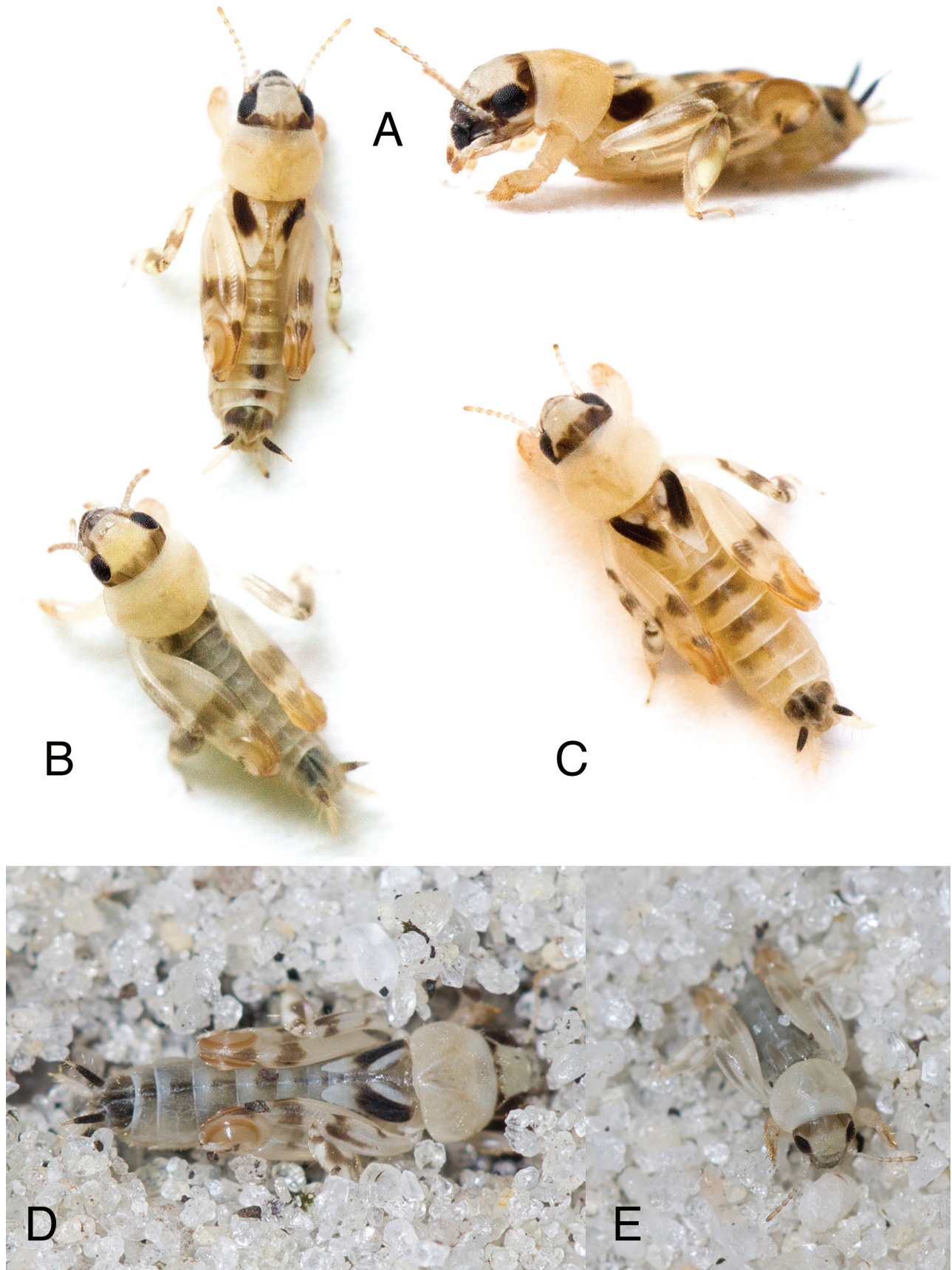
Fig. 2. Live nymph and adult images of *Ellipes deyrupi* **sp. nov. A.** Dorsal and lateral view of adult from Tiger Creek Preserve; **B.** Nymph from same locality; **C.** Adult from Sarasota Court; **D.** Adult burrowing in sand at Tiger Creek; **E.** Nymph burrowing at same locality.
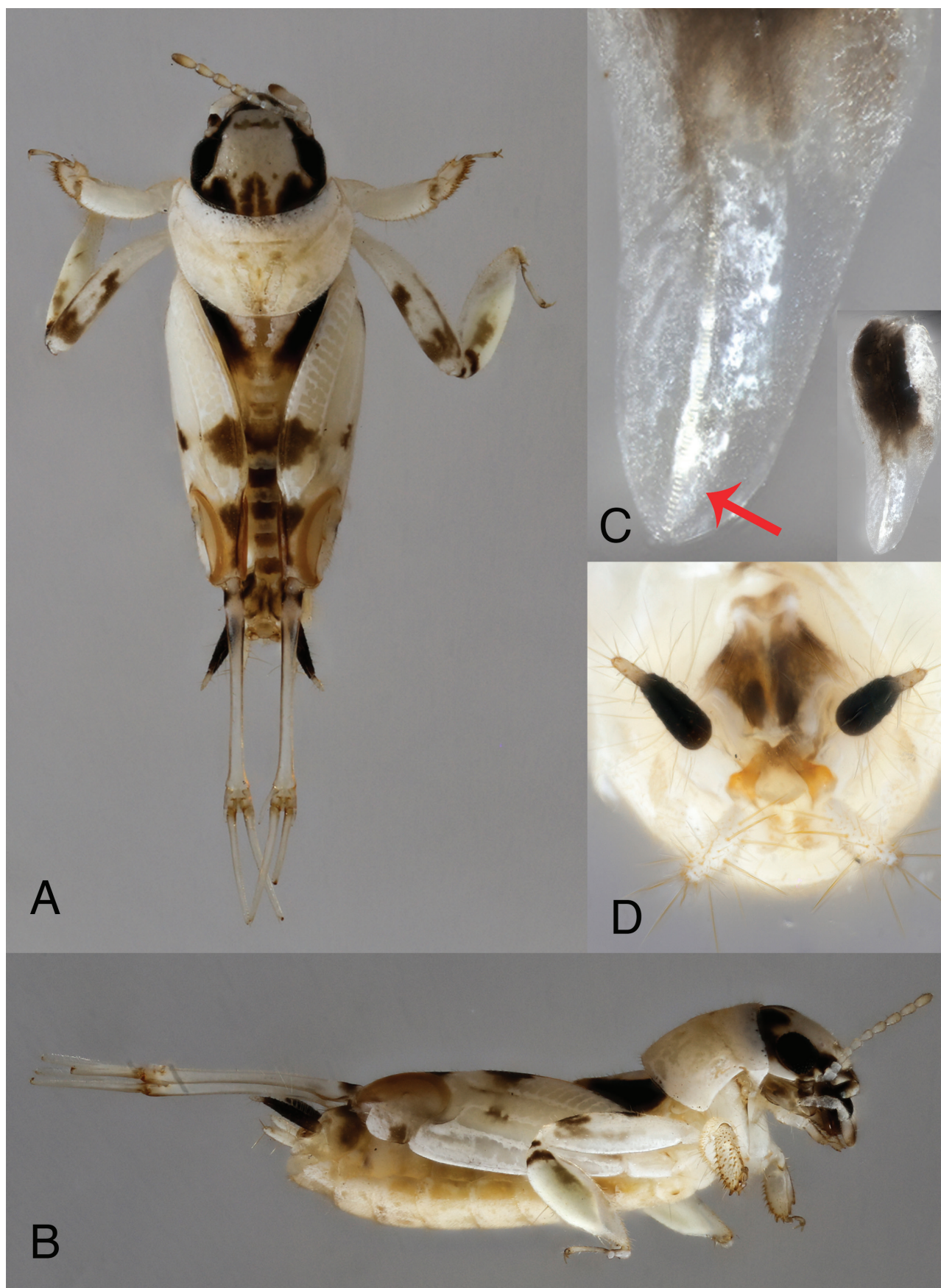
Fig. 3. Male *Ellipes deyrupi* **sp. nov. A.** Dorsal view of holotype; **B.** Lateral view of same; **C.** Underside of right tegmen showing stridulatory file. Inset shows entire tegmen; **D.** Terminalia.
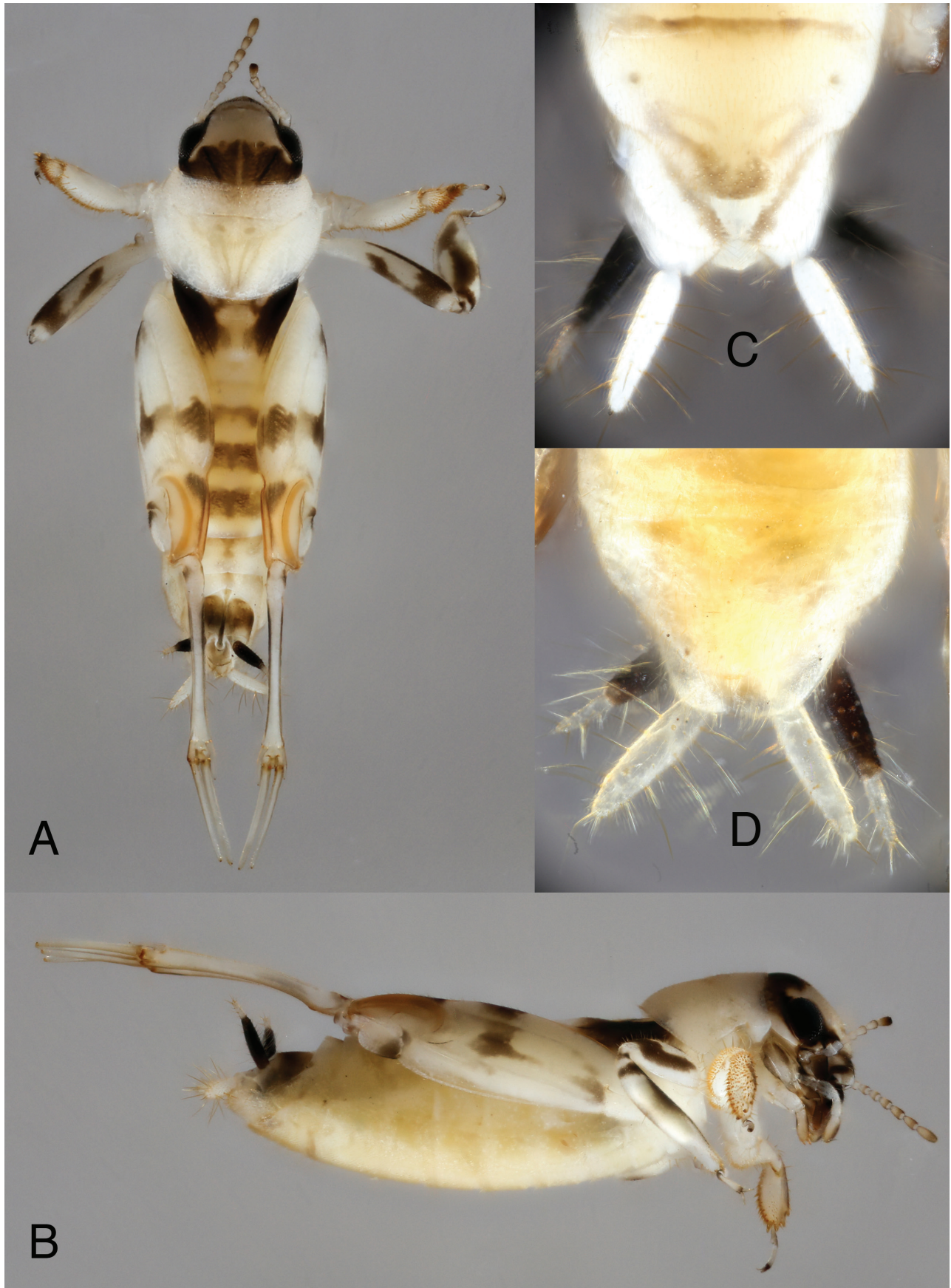
Fig. 4. Females of upland *Ellipes* spp. **A.** Dorsal view of allotype *E. deyrupi* **sp. nov.**; **B.** Lateral view of same; **C.** Subgenital plate of same; **D.** Subgenital plate of female *Ellipes eisneri* from Floral City.

common wherever tridactylids were found (Fig. 1C, D, F). At the Tiger Creek Preserve, typical plants in the habitat included *Aristida* sp. grasses, Ware's hairsedge (*Bulbostylis warei* (Torr.) C.B.Clarke), paper nailwort (*Paronychia chartacea* Fernald), and sandhill sky-blue lupine (*Lupinus cumulicola* Small). At the roadside sites east of Lake Marion, typical plants included paper nailwort (*Paronychia chartacea* Fernald), sandhill sky-blue lupine (*Lupinus cumulicola* Small), scrub Stylisma (*Stylisma abdita* Myint), prickly pear cactus (*Opuntia* sp.), and *Panicum* sp. grasses along the road's edge. Lichens present in this habitat included *Cladonia leporina* Fr., *C. evansii* Abbayes, and *C. subtenuis* (Abbayes) Mattick.

Photographs of nymphs and adults of *E. deyrupi* are posted by the author to BugGuide.net for public viewing, at https://bugguide.net/node/view/1575492/bgimage

Ellipes eisneri Deyrup, 2005
Figs 6–8

Ellipes eisneri Deyrup, 2005: 142.

Type material.—**Holotype:** USA • ♂; Florida: Citrus County: near Inverness, Withlacoochee Forest, Citrus Area, Forest Road 13, 1.3 mi. south State Road 44; 03 Apr. 1995; sandhill habitat with bare sand, dug from vertical burrow; M. Deyrup leg.; FSCA [not examined]. **Paratypes:** USA • 4 ♂, 4 ♀; same information as holotype; dry pinned specimens; 1 ♂, 1 ♀, FSCA; 1 ♂, 1 ♀, ANSP • 2 ♂, 10 ♀; "Pine Oak Estates", State Road 488, 3.7 mi. south of junction with U.S. 41; 04 Ap. 1996; sandhill area with bare sand; specimens in ethanol; M. Deyrup leg.; ABS [examined].

Material examined.—USA, Florida • 2 ♀;Citrus County: Floral City, side of road on Bedford Dr.; 28.746, -82.299; 28 Jul. 2018; specimens in ethanol; B. Woo leg.; CUIC • 1 ♀;Citrus County, Lecanto, side of road on 490; 28.832, -82.519; 28 Jul. 2018; photograph; B. Woo leg.; CUIC • 1 ♀, 5 nymphs, Marion County, Dunnellon, side of road on SW Rainbow Lakes Blvd; 29.140, -82.493; 29 Jul. 2018; specimens in ethanol; B. Woo leg.; CUIC • 4 ♀, 4 nymphs;; Marion County, Dunnellon, round dune off Indian Hill Dr.; 29.169, -82.531; 29 Jul. 2018; specimens in ethanol; B. Woo leg.; CUIC • 1 nymph; Levy County, Bronson, side of road on NE 75th St.; 29.443, -82.539; 30 Jul. 2018; specimen in ethanol; B. Woo leg.; CUIC.

Remark.—These are the only two sites from which the species has been known prior to this work. Herein, I present the following specimen data and localities. For photographs of living and preserved specimens of *E. eisneri*, see Figs 7 and 8.

Distribution.—Found on the Brooksville Ridge in Levy, Marion, and Citrus Counties, FL (Fig. 5). Probably also in adjacent Hernando County.

Habitat.—Specimens were collected in bare sand patches within open sandhill habitats (Fig. 6A, C, E). These areas have scattered lichens and forbs and are usually surrounded by oaks and pines. Tumuli and raised feeding burrows were observed at all sites (Fig. 6B, D). Typical plants present included longleaf pine (*Pinus palustris* Mill.), chapman oak (*Quercus chapmanii* Sarg.), turkey oak (*Quercus laevis* Walter), oak ridge lupine (*Lupinus diffusus* Nutt.), and dogfennel (*Eupatorium capillifolium* (Lam.) Small) along the road's edge. Lichens present in these habitats included *Cladonia leporina* Fr., *C. evansii* Abbayes, and *C. subtenuis* (Abbayes) Mattick.
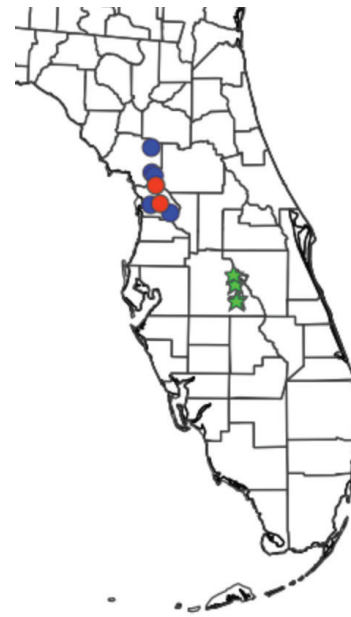

Fig. 5. Distribution of Florida upland *Ellipes* spp. Green stars indicate collection localities of *Ellipes deyrupi* **sp. nov.**; red dots indicate previous (2005) known collection localities for *Ellipes eisneri*; blue dots indicate newly reported collection localities for *E. eisneri*.

Discussion

Ellipes deyrupi represents the third species of Tridactylidae known to be restricted to sandy Florida uplands. Its discovery vindicates Deyrup's 1996 and 2005 statements that more species were likely to be found in such habitats and suggests that there are still more undiscovered species hiding in similar disjunct xeric habitats of the southeastern U.S. The new species can be collected using yellow bowl traps after a rain or by sifting in the sand where tumuli and feeding burrows are present. In the right conditions, it can be quite abundant, but only those knowing what to look for are likely to come across it. The genus *Ellipes* is now known to contain two rather distinct upland species with restricted ranges in Florida, whereas populations of scrub *Neotridactylus* are more ambiguous, perhaps consisting of only one wide-ranging, variable scrub species in a similar situation to the Florida endemic cockroach *Arenivaga floridensis* Caudell, which is also restricted to scrub and sandhill (Lamb et al. 2018). The status of scrub *Neotridactylus* will be explored in a forthcoming paper.

The situation of the two species of scrub *Ellipes*, in which there is one species (*E. eisneri*) on the Brooksville Ridge and another species (*E. deyrupi*) on the northern LWR, shares several similarities with that of Florida *Geopsammodius* Gordon & Pittino, 1992 scarab beetles. These blind, flightless beetles also live just below the surface of the sand in scrub and sandhill habitats, and the author often found and collected them while searching for tridactylids (specimens are in the CUIC). *Geopsammodius withlacoochee* Skelley, 2006 is a Brooksville Ridge endemic like *E. eisneri*, and *G. morrisi* Skelley, 2006 is only known from the ridge habitat around Lake Marion (including the Catfish Creek Preserve), where *E. deyrupi* was found (Skelley 2006, Deyrup and Carrel 2012). The circumstances are somewhat different, however, as *E. deyrupi* also occurs at the Tiger Creek Preserve, which is inhabited by the more widespread southern LWR scrub *G. relictillus* (Deyrup & Wood-
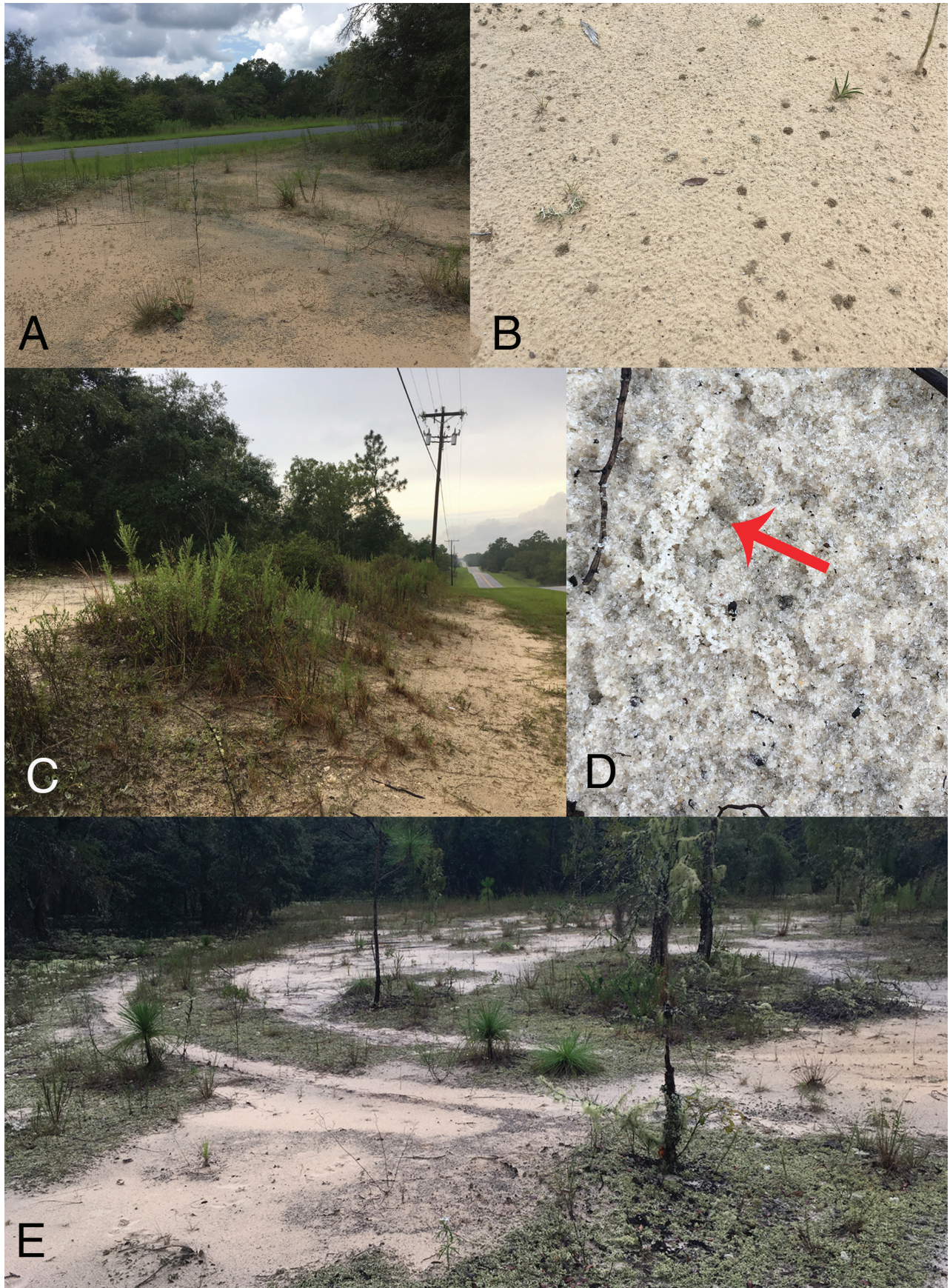
Fig. 6. Habitat and traces of *Ellipes eisneri*. **A.** Habitat at Floral City; **B.** Tumuli at same locality; **C.** Habitat at SW Rainbow Lakes Blvd; **D.** Feeding burrow at same locality; **E.** Habitat at Indian Hill Dr.

Fig. 7. Live nymph and adult images of *Ellipes eisneri*. **A.** Dorsal view of adult from Lecanto; **B.** Dorsal and lateral views of adult from Floral City; **C.** Lateral view of adult from Floral City, showing lack of tarsus; **D.** Nymph from Floral City; **E.** Nymph burrowing in sand at SW Rainbow Lakes Blvd.

Fig. 8. Dorsal and lateral view of female *Ellipes eisneri* from Floral City.

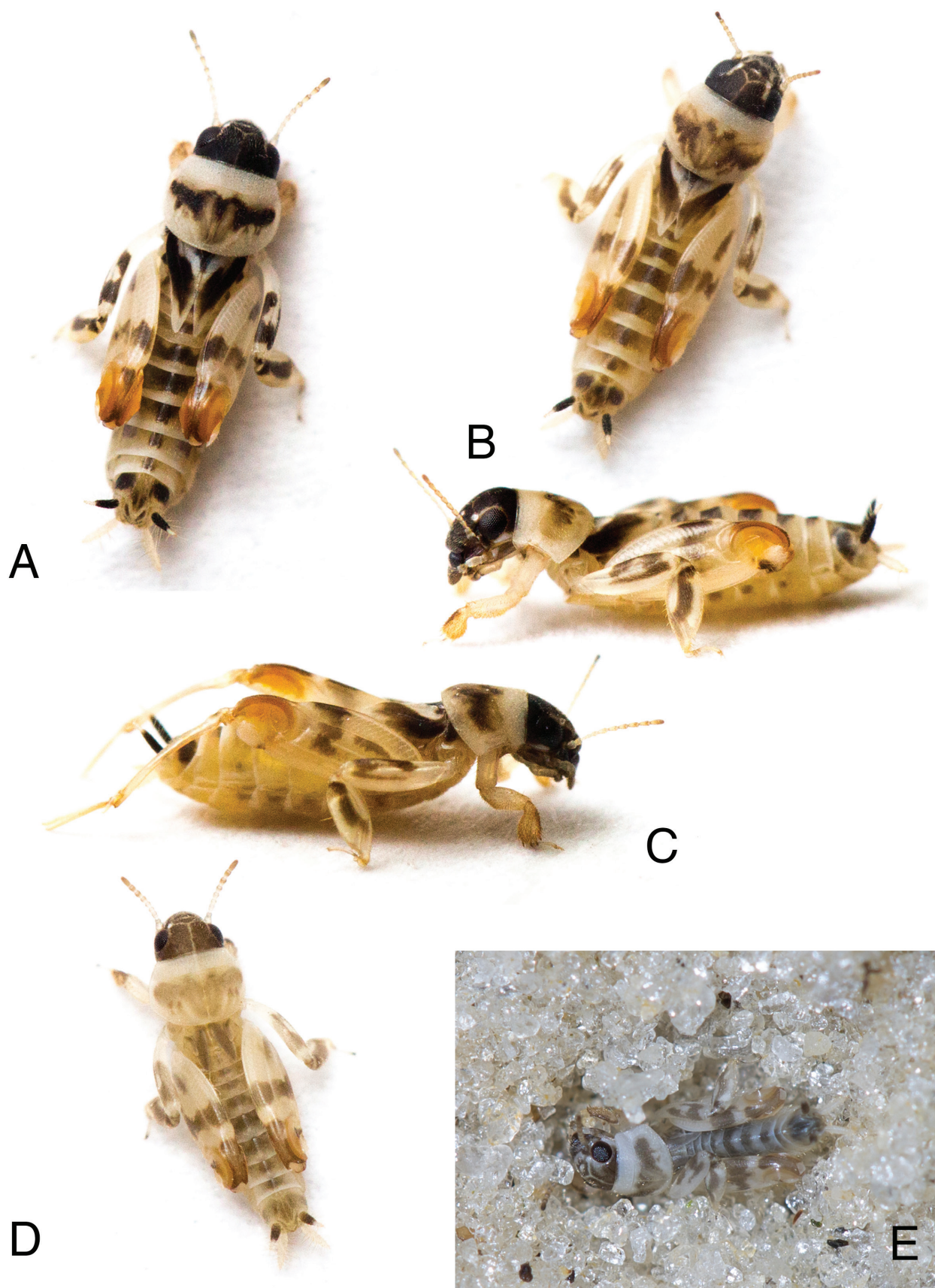ruff, 1991). More extensive surveys of both scrub tridactylids and *Geopsammodius* scarab beetles may reveal more about the biogeographic patterns underlying their distributions in the state.

The ecology and behavior of scrub tridactylids is largely unknown. The only species that has been studied in any detail at all is *Neotridactylus archboldi*, which feeds on a layer of subsurface algae (Deyrup and Eisner 1996). Adults and nymphs of *Ellipes deyrupi* have been collected from May to August and in December; they likely occur year round but would be rather difficult to locate during periods of little to no rain. The males of *E. deyrupi*, like many other tridactylids, have a stridulatory apparatus comprised of a scraper on the underside of the tegmen and a file on the fourth abdominal tergite. Deyrup and Eisner (1996) speculated that song may be an important isolating mechanism in tridactylids, but nothing is known of the nature or function of their calls, which are likely transmitted through the sand substrate. *E. deyrupi* co-occurs with *N. archboldi* at all locations where it has been found, often in the same patches of sand. In other areas of Florida that have been surveyed for scrub tridactylids, only flightless *Neotridactylus* have been found, except on the Brooksville Ridge, where apparently only *E. eisneri* occurs. The fully winged *Ellipes minuta* sometimes occurs in scrub and sandhill (pers. obs), but this generalist species can fly and is likely present in these habitats only temporarily, when wet conditions prevail. Thus, the northern LWR appears to be the only known habitat where two scrub-restricted tridactylid species occur in sympatry. This brings up questions about interspecific competition for limited space and food resources. A vial containing live adults and nymphs of *E. deyrupi* and *N. archboldi* in sand substrate from the same locality was found to only contain *N. archboldi* after two months. This is, of course, anecdotal evidence, but it suggests that *N. archboldi* may be a better competitor in situations where space and food are limited. *Geopsammodius* scarab beetles, which likely feed on detritus in the sand (Skelley 2006), were often encountered in the same patches of sand, representing another potential competitor in these limited habitats.

Ellipes deyrupi may be a species of conservation concern. If the species occurred further south on the LWR, Deyrup and Carrel (2012) would most likely have detected it. The majority of its habitat on the northern LWR has been converted to housing or agriculture, and roadside sites where it still occurs are at risk of being developed. A thorough Google Maps search of this part of Polk County turned up no obvious unsurveyed areas that might contain *E. deyrupi*. In addition, the author could find no suitable *E. deyrupi* habitat while driving around in the intervening land between the three collection localities. At the Hickory Lake Scrub, only about 16.4 km south of the Tiger Creek Preserve, an hourlong search in December 2019 by the author turned up many *N. archboldi* but no *E. deyrupi*. At present, there are only two protected localities for the species, although fortunately these two preserves host large populations of *E. deyrupi*. Threats as described by Deyrup and Carrel (2012) include its restriction to open bare sand patches within Florida scrub and specifically within the unique yellow sand scrub found only on the LWR. This habitat can be quickly overgrown by vegetation without prescribed burns, but too frequent fires can convert it to sandhill habitat. Thus, yellow sand scrub management requires a careful balance to maintain its distinctive qualities and organisms.

The new records for *E. eisneri* reported here extend its range to the Northern Brooksville Ridge, and my suspicion is that it likely occurs across the entire ridge in suitable habitat. Its occurrence on the northern part of the ridge is interesting because the other Brooksville Ridge endemic Orthopteran, the flightless grasshopper *Melanoplus withlacoocheensis* Squitier et al., 1998, is restricted to the southern part of the ridge, with rigorous collecting efforts failing to locate it on the northern Brooksville ridge (Squitier et al. 1998). This was hypothesized to be due to the bisection of these two areas by the Withlacoochee River, which would serve as an effective barrier to flightless insects tied to specific habitats. Yet, *E. eisneri* occurs on both the Northern and Southern Brooksville Ridge, implying either that *E. eisneri* was historically present on the Ridge before the formation of the river or that it can navigate aquatic habitats. It might also have been possible for a population of tridactylids to be transported to the Northern Brooksville Ridge in bulk sand, perhaps due to human activity. Phylogenetic analysis of *E. eisneri* populations from both the northern and southern parts of the Brooksville Ridge might shed light on this situation.

Ellipes eisneri was relatively abundant wherever found, judging by the number of tumuli and burrows present at all sites, so it is most likely not a rare species within its known range. While its distribution appears to be wider than previously known, the fact remains that it has not been found on any other of Florida's sand ridges, and that most of the open sandhill habitat on the Brooksville ridge is under threat from human development. The Withlacoochee State Forest (Citrus Tract) is still the only known protected site for *E. eisneri*, as the new localities reported here are all roadside sandhill habitats. The Bronson site, however, is adjacent to the Watermelon Pond Wildlife and Environmental Area, owned by the Florida Fish and Wildlife Conservation Commission. It is almost certain that there are populations of *E. eisneri* present in the extensive sandhills of this protected area. The nearby Goethe State Forest and Goethe Wildlife Management Area are also likely to hold populations of *E. eisneri*. It would be prudent to sample within these localities during rainy weather to determine whether *E. eisneri* occurs there.

Acknowledgements

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The 2019–2020 upsurge of the desert locust and its impact in Pakistan

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Abstract

The recent upsurge of the desert locust *Schistocerca gregaria* (Forskål, 1775) has had an impact on East Africa and the Middle East as far as India. It has affected and slowed down many aspects of the Pakistani economy. Swarms of locusts have infested many areas and caused immense damage to all types of crops. Both farmers and economists are concerned and are trying to get the most up-to-date information on the best strategy to manage this pest. This paper is an attempt to (i) provide insight into the dynamics of this upsurge internationally as well as in the various regions of Pakistan, (ii) briefly assess its local impact and locust control measures, and (iii) clarify the role of the various stakeholders in the management, both nationally and internationally, suggesting various improvements for the future.

Keywords

control strategies, crop damage, desert locust, outbreak, pest, *Schistocerca gregaria*

Introduction

In 2019 and 2020, large swarms of desert locusts again threatened parts of East Africa and large areas as far as India and Pakistan via the Arabian Peninsula. The Food and Agriculture Organization (FAO) of the United Nations has described this locust situation as the most serious in decades (FAO 2019, 2020a). The swarms reached Kenya, Uganda, and Tanzania, which had not faced a threat of this magnitude for 70 years. Although we have already seen classic images of these devastating swarms in the past, their impact is still impressive. Trees can twist and branches can break under the weight of locusts. Without adequate means of control, farmers are made desperate by the loss of their crops. Equipped with manual sprayers and often poorly protected against insecticides, technicians try to fight against these insects where only aerial means would be effective. Fortunately, even if detected too late, this upsurge quickly became the subject of major control operations, with the assistance of various donors and under the co-ordination of the FAO. However, after two years of intensive fighting, the situation is still not under control. In early 2021, calm returned to Southwest Asia, and in particular Pakistan, but these swarms have yet to be contained in the Horn of Africa (Dowlatchahi et al. 2020b).

The desert locust *Schistocerca gregaria* (Forskål, 1775) (Insecta: Orthoptera: Acrididae) is considered a serious agricultural pest in West and North Africa, the Middle East, and Southwest Asia (Steedman 1990, Cressman 2016, Lecoq 2019), and regular invasions of this insect pose a real threat to agricultural production and have devastating consequences for food security in more than 50 countries (Lecoq 2003, 2004, 2005, Brader et al. 2006). The social impact of an invasion can be visible in the long term, even after 20 years (De Vreyer et al. 2014). Like other locust species, the desert locust exhibits phase polyphenism, a plastic response to population density associated with several changes in behavioral, morphological, anatomical, and physiological traits. Isolated, harmless, and hidden solitary locusts transform into huge hopper bands and devastating swarms of the gregarious form under conditions of overpopulation (Uvarov 1921, 1966, Pener and Simpson 2009, Piou et al. 2017). Of the 31 million km² that can be invaded by the desert locust (the invasion area), the remission area (where low-density solitary-phase populations exist during calm periods) covers only 15 million km². In this zone, the outbreak areas (where the first outbreaks that could lead to invasions occur due to appropriate ecological characteristics) occupy an even smaller area of about 1.7 million km² (Sword et al. 2010, Gay P.E. p.c.).

The lifespan of a locust generation, under optimal conditions, is 40–50 days, and the annual number of generations varies between two and three. Young adults may remain immature (quiescent) for several months until they find moist conditions favorable for egg laying, with 20–25 mm of rainfall being normally sufficient (Duranton and Lecoq 1990, Symmons and Cressman 2001). As rainfall is seasonally distributed throughout the habitat area, this results in the existence of three main breeding seasons—spring, summer, and winter—between which the imagos undertake seasonal migrations to benefit from favorable breeding conditions (COPR 1982,

Duranton and Lecoq 1990, Steedman 1990, Symmons and Cressman 2001). During periods of remission, solitary populations are dispersed in desertic areas. There, gregarization (transformation of locusts from solitary to gregarious) begins in the outbreak areas, thanks to rainy sequences favorable to reproduction in grouped vegetation and on mainly sandy or sandy-clay soils (Collett et al. 1998, Despland et al. 2000, Cissé et al. 2013). Outbreaks develop and then, if good conditions persist, follow increasingly severe phases of upsurge and plague (classic terminology defined by FAO 2009a) as populations and the number of occupied sites increase. The invasions develop intermittently and, in the past, have frequently persisted for 5, 10, or more years (Sword et al. 2010).

Since the 1960s, a preventive control strategy has been recommended by the FAO based on the monitoring of outbreak areas and ecological conditions (Showler et al. 2021, Lecoq 2003, 2004, Sword et al. 2010), followed, if necessary, by early intervention and thus limited use of pesticides. The implementation of this strategy helps to maintain low densities and to stop any outbreak as soon as possible (Duranton and Lecoq 1990, Martini et al. 1998, Magor et al. 2008, Sword et al. 2010). Consequently, and with 60 years of hindsight, it is clear that invasions are now less frequent, smaller in scale and, if they cannot be stopped at an early stage, shorter and better managed (Magor et al. 2008, Sword et al. 2010). However, financial and political uncertainties, as well as recurrent insecurity in many areas of desert locust distribution, continue to maintain the threat, and some outbreaks cannot be stopped at an early stage, as was observed again recently (Meynard et al. 2020, Showler and Lecoq 2021, Showler et al. 2021).

Pakistan has historically been subject to periodic swarm invasions. The country also contains outbreak areas, where particularly ecological conditions can favor, when suitable rains occur, the concentration, reproduction, and intensive multiplication of locusts and give rise to outbreaks and plagues. In recent years, the greatest outbreaks were noted in 1993 and 1997. These invasions have caused incalculable damage to crops, sometimes leading to severe famines. The recent upsurge in 2019–2020 seriously affected the country (Dowlatchahi 2020a). Here, we present a summary of these two years of upsurge by focusing on its impact in Pakistan, the damage caused in this country, and the surveillance and control operations undertaken. Furthermore, we try to clarify, both nationally and internationally, the role of the various stakeholders in the management of this pest, suggesting some improvements for the future.

Materials and methods

The general pattern of the current global upsurge was taken from the Desert Locust Bulletin produced monthly by FAO-DLIS (Desert Locust Information Service) in Rome based on information from all the countries within the desert locust habitat area (FAO 2019, 2020a). These bulletins also provide information about the likely migration of swarms based on the study of the meteorological situation and the use of migration trajectory models. Meteorological data and remote sensing imagery are used to help estimate rainfall, detect green vegetation, and identify areas where ecological conditions may be favorable for locust breeding (Cressman 2008). Regarding Pakistan, close contacts were maintained with the Department of Plant Protection (DPP)—the lead institution tasked with monitoring and managing the desert locust threat in Pakistan—to obtain information on the ongoing invasion and damage in various regions of the country. Desert locust field information was collected by DPP survey teams according to a standardized procedure recommended by FAO-DLIS and

was used to produce monthly locust situation maps (Cressman 2001). Contacts were also maintained with inhabitants, farmers, and local entomologists to obtain more daily information on the presence and migration of locusts. In addition, field surveys were carried out by the authors in different locust-affected localities, mainly in Sindh province; locust samples were collected, and photos were taken in different affected areas of Sindh to document the situation on the ground.

Results

General course of the desert locust upsurge in 2019–2020

The last major desert locust plague ended in 1962 (Magor et al. 2008, Sword et al. 2010). In recent years, for Pakistan, the greatest outbreaks were in 1993 and 1997 (FAO 1993, 1997). Globally, the last major upsurge was in 2004–2005 (FAO 2004, 2005). At the end of 2018, the situation was calm throughout the desert locust habitat area (FAO 2018). The FAO situation bulletins contained almost no observations; in particular, no gregarious formations were reported, and there was a single report of a group of hoppers in northern Somalia during September. Then suddenly, in December 2018, laying swarms were reported on the coast of the Red Sea, in Sudan, and in Eritrea. In January 2019, such laying swarms were also seen on both sides of the Red Sea, and immature swarms were seen in Saudi Arabia (FAO 2019). Iran and Pakistan were warned of the possible migration of swarms. The situation quickly escalated in the following months (Figs 1, 2). Southern Iran was affected in February, Pakistan in March, and India in May. The desert locust situation continued to worsen across the Arabian Peninsula. In June, the Horn of Africa (Sudan, Eritrea, Djibouti, and Somalia) was affected by swarms originating from Yemen. In this region, the cyclonic rains and floods of October and November 2019 created good conditions in which the desert locust could continue to multiply. Kenya was infested as of December 2019. From June to December, the locust situation remained serious in southwest Asia, particularly in Pakistan and western India. In 2020, the desert locust situation continued to worsen (FAO 2020a). Strong spring breeding occurred in April, May, and June. The locust situation remained critical in the eastern region in Iran, Pakistan, and India until August and was only brought under control beginning in September. This was largely a result of the capacity and experience of these countries to monitor and control desert locusts in their outbreak areas. On the other hand, the desert locust situation did not improve on both sides of the Red Sea and the Horn of Africa, where, in December 2020, it was deemed very critical. In the end, and for the moment, only West Africa has been spared.

Although not seen until January 2019, the development of this upsurge was the result of favorable conditions for the desert locust, which were occurring as early as 2018. Two cyclones brought heavy rains in the Rub al Khali, or Empty Quarter of the Arabic peninsula, in May and October 2018 (FAO 2020b). Rains were very heavy in Yemen, Oman, Djibouti, northern Somalia, eastern Ethiopia, and southern Saudi Arabia (Meynard et al. 2020). Favorable conditions for desert locust breeding were maintained for many consecutive months, allowing at least three successive generations to develop. However, locusts went undetected and unchecked for a significant amount of time, mainly due to the insecure conditions in the areas of origin, particularly in Yemen (Showler and Lecoq 2021). Had the initial outbreak in Saudi Arabia's Rub al Khali been detected in the early stage and controlled in the summer of 2018, swarms may not have reached Yemen, the
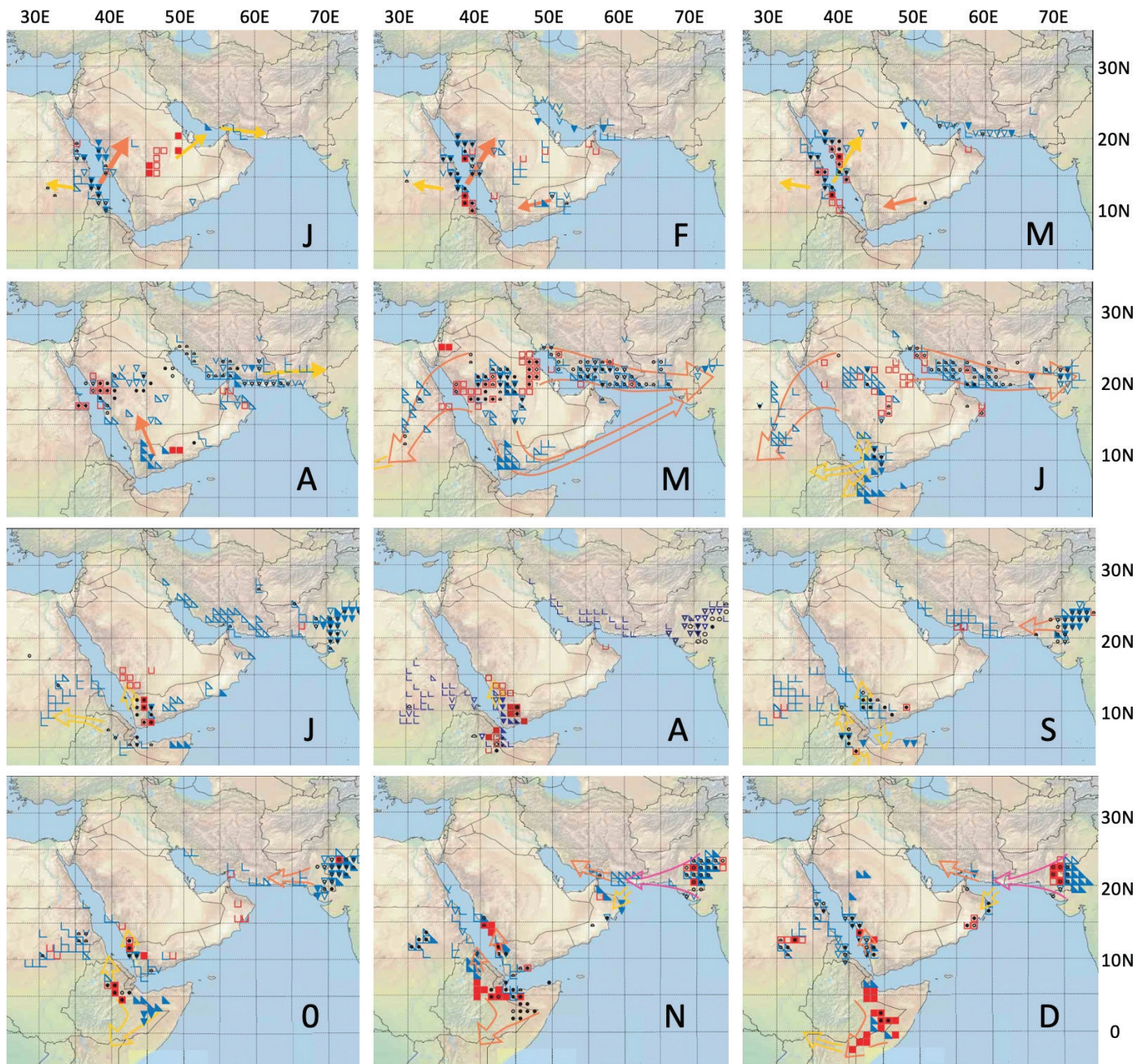
Fig. 1. General situation of the desert locust from January to December 2019 (modified from FAO 2019). Swarms or hopper bands: immature adults, red square; mature adults, blue triangle; maturity unknown, black triangle up; egg laying or eggs, black triangle down; hoppers, black circle; hoppers and adults, combined symbols. Groups of adults or hoppers: same symbols but hollowed out. Density unknown: same symbols, but partial.

African Red Sea coast, and parts of Iran. In addition, an initially weak response in Iran (where very heavy flooding in the southwest of the country allowed two generations of breeding) allowed swarms to move to Pakistan and India (FAO 2020c, d), where an unusually long summer monsoon resulted in three generations of breeding along both sides of the Indo-Pakistan border.

The 2019–2020 upsurge dynamic in Pakistan

In early 2019, no locusts were reported in Pakistan. It was not until March that isolated solitarious adults first appeared on the Baluchistan coast in the Uthal region west of Karachi, pre-sumably coming from Iran, which had been invaded in previous months. On the 16th March, a mature swarm and groups of mating and laying adults were seen on the coast at Pasni, on the Iranian border, and in the Kulanch valley region, west of Pasni (Fig. 3). These arrivals continued in April, and the migrant populations continued to lay eggs. In April, the first larvae of the spring reproduction appeared in Balochistan in the coastal areas of Pasni. Hoppers continued to emerge and develop in May between Turbat and Gwadar, near Uthal, and in the interior near Kharan. Groups of gregarious hoppers of all stages were then found, mixed with scattered adults. The spring breeding ended in early June in Balochistan, with a last report of a laying swarm
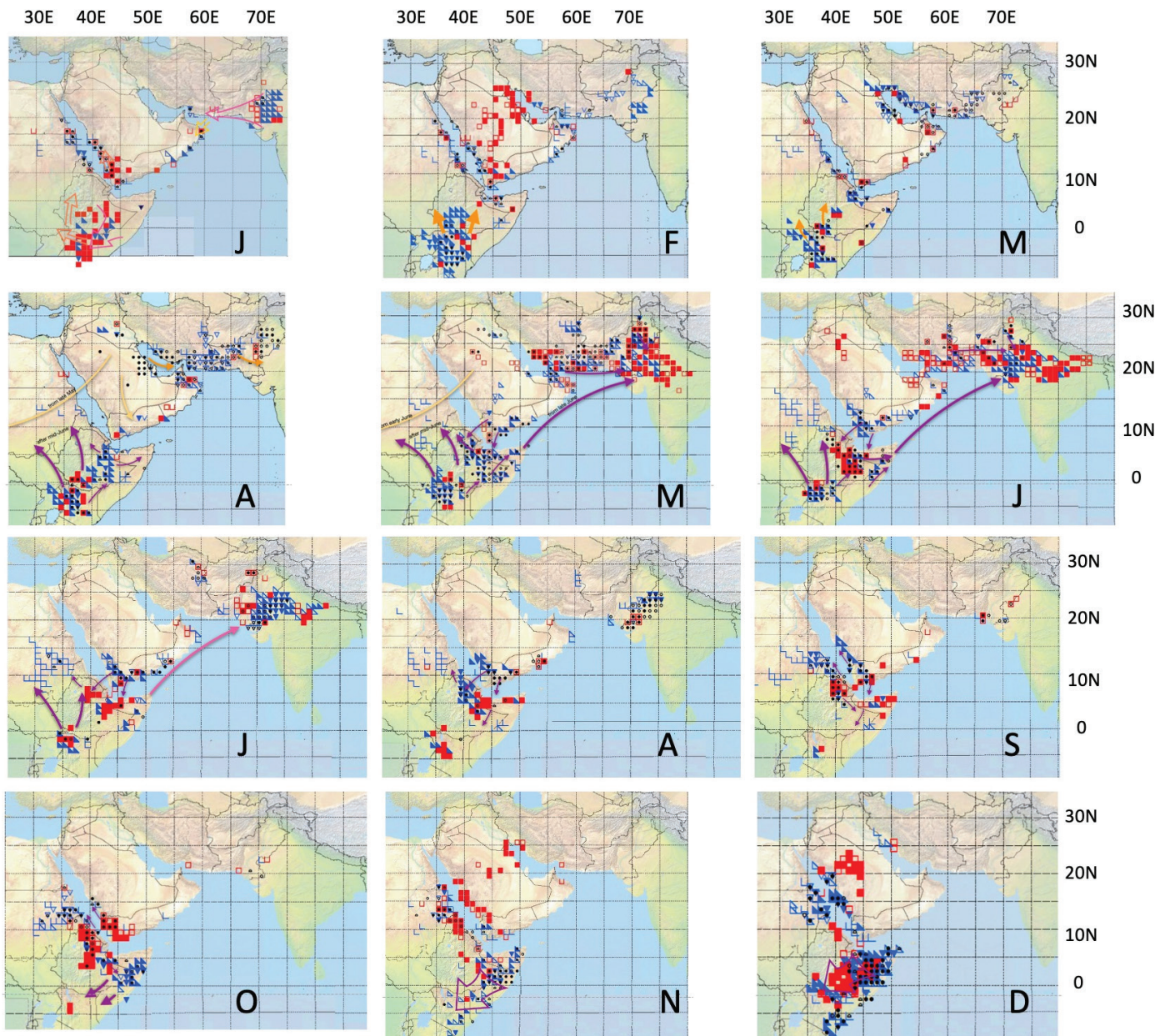
Fig. 2. General situation of the desert locust from January to December 2020 (modified from FAO 2020a). Swarms or hopper bands: immature adults, red square; mature adults, blue triangle; maturity unknown, black triangle up; egg laying or eggs, black triangle down; hoppers, black circle; hoppers and adults, combined symbols. Groups of adults or hoppers: same symbols but hollowed out. Density unknown: same symbols, but partial.

on 1st June near Lasbela, while hoppers and hopper groups persisted near Lasbela, Turbat, Gwadar, and in the northern interior near Dalbandin.

The adults from the spring breeding gradually migrated to the summer breeding area (June–November) on the Indo-Pakistan border. Some swarms may have originated from the Horn of Africa after migrating over the Indian Ocean. This summer breeding started at the end of May with scattered gregarious adults that appeared during the last week of May near the Indian border southeast of Chaman starting to lay. It developed mainly in June, July, and August in the Nara, Cholistan, and Thar deserts east of the Indus Valley. In Cholistan, egg laying continues until August. Thus, from mid-August, outbreaks of a second generation caused locust numbers to further increase. This second generation de-

veloped mainly in September, October, and November. Widespread breeding was then observed in the deserts of Cholistan, Nara, and Thar, where numerous hopper bands were forming, giving rise to numerous swarms. During November and December, a third generation of breeding occurred in the Thar, Nara, and Cholistan deserts, where numerous hopper groups formed, resulting in numerous adult groups and immature swarms (FAO 2019) (Fig. 4).

The swarms then began to move westward to the winter-spring breeding areas (February–June). Cross-border movements of swarms from the summer breeding areas of Rajasthan in India occurred. On 11 November, an immature westward swarm was seen flying over Karachi. In southern Balochistan, immature swarms from the summer breeding areas started to arrive in December.

Fig. 3. Desert locust breeding areas in Southern Pakistan during invasions (adapted from Symmons and Cressman 2001). Arrows: main orientation of migrating swarms in March–July (blue) and August–October (red).

In January 2020, a few nymphs of the 3rd generation continued to molt. Groups of immature adults persisted in the Thar, Nara, and Cholistan deserts. Cross-border movements of immature swarms continued westward. On 21 February, three swarms reportedly arrived in the Afghan province of Khost from adjacent areas in northwest Pakistan.

A new spring breeding started in March 2020 and went until May. During March, breeding took place mainly in Balochistan (Khuzdar, Nushki, Washuk, Kharan and Dalbandin, Chagai, Panjgur, Turbat, and Pasni) and in the Indus valley (Rajanpur, Kashmore, Sukkur, Dera Ismail Khan, and Rohri), as well as in the plains of Punjab. Breeding continued into April and May, and a second generation of laying began in mid-April in the north near Dalbandin in Balochistan. As a result of this breeding, an increasing number of adult groups and immature swarms formed and began to mature during May.

During June, as conditions dried out, these swarms moved from the spring breeding areas eastward to the summer breeding areas of the Cholistan, Nara, and Thar deserts in Punjab and Sindh provinces. Some continued to India due to the too-dry conditions. Summer breeding started in late June and continued into July and August. Numerous first-generation hopper groups and bands formed, especially in the Thar desert up to the Indian border in the extreme southeast of Sindh. The imaginal molts began during the first week of August, causing groups of immature adults to form on the Indian border.

Then, in September, the situation improved dramatically. In Sindh, a very limited second-generation breeding occurred in September west of Hyderabad and in Tharparkar. Improvement continued in October, and no locusts were seen in November and December (Dowlatchahi et al. 2020b, FAO 2020a, d).

Damage and control measures in Pakistan

Damage.—The desert locust can consume most plant species and crops (COPR 1982). Only a few plants are not eaten, such as the neem *Azadirachta indica* A. Juss., Genista sp. (broom bush), and

Euphorbia hirta L. (asthma plant). Indeed, during this upsurge, a great deal of damage was caused to all types of crops, including wheat, cotton, rice, sugarcane, tobacco, corn, chickpea (gram) sunflower, sorghum, pearl millet, mung bean (*Vigna radiata* (L.) R. Wilczek), muth bean (*Vigna aconitifolia* Jacq.), sesame, cluster bean (guar), potato, tomato, cabbage, cauliflower, carrot, peas, onion, melon, cucumber, water-melon, chilies, eggplant (brinjal), okra (lady finger), mango, citrus, apple, grapes, strawberry, peaches, banana, and guava.

This upsurge has been devastating for a country where agriculture represents around 20% of the GDP and where 61% of the population lives and works in agricultural areas (FAO 2016). About 52 districts were reported to have suffered locust damage. According to FAO estimates in May 2020 and assuming that the damage accounts for about 25% of growing crops, losses could reach 353 billion Pakistani rupees (2.19 billion US$) for "rabi crops" (sown in winter and harvested in spring) and about 464 billion Pakistani rupees (2.88 billion US$) for "kharif crops" (summer sown crops) (FAO 2020e). The final balance has yet to be established and, in the end, the damage from this upsurge will undoubtedly be much higher. In 2020, the Government of Pakistan's preliminary estimate of monetary losses due to desert locusts over the two coming agricultural seasons in 2020 and 2021 may range from 3.4 billion US$ to 10.21 billion US$. More than 3 million people in Pakistan are facing severe acute food insecurity, with the situation particularly precarious in Balochistan. It is estimated that approximately 34,000 households will need emergency livelihood and food security assistance due to crop losses. Many more people may be indirectly affected by crop losses, leading to price rises in key commodities (FAO 2020).

Control measures.—To better coordinate control operations, the Government of Pakistan declared the locust invasions to be an emergency. Many anticipatory measures have been taken in collaboration with the FAO, in coordination with neighboring countries, and with the support of international partners to face the threat and be ready to respond quickly and effectively (FAO 2019, 2020a). In addition, the Space and Upper Atmosphere Research
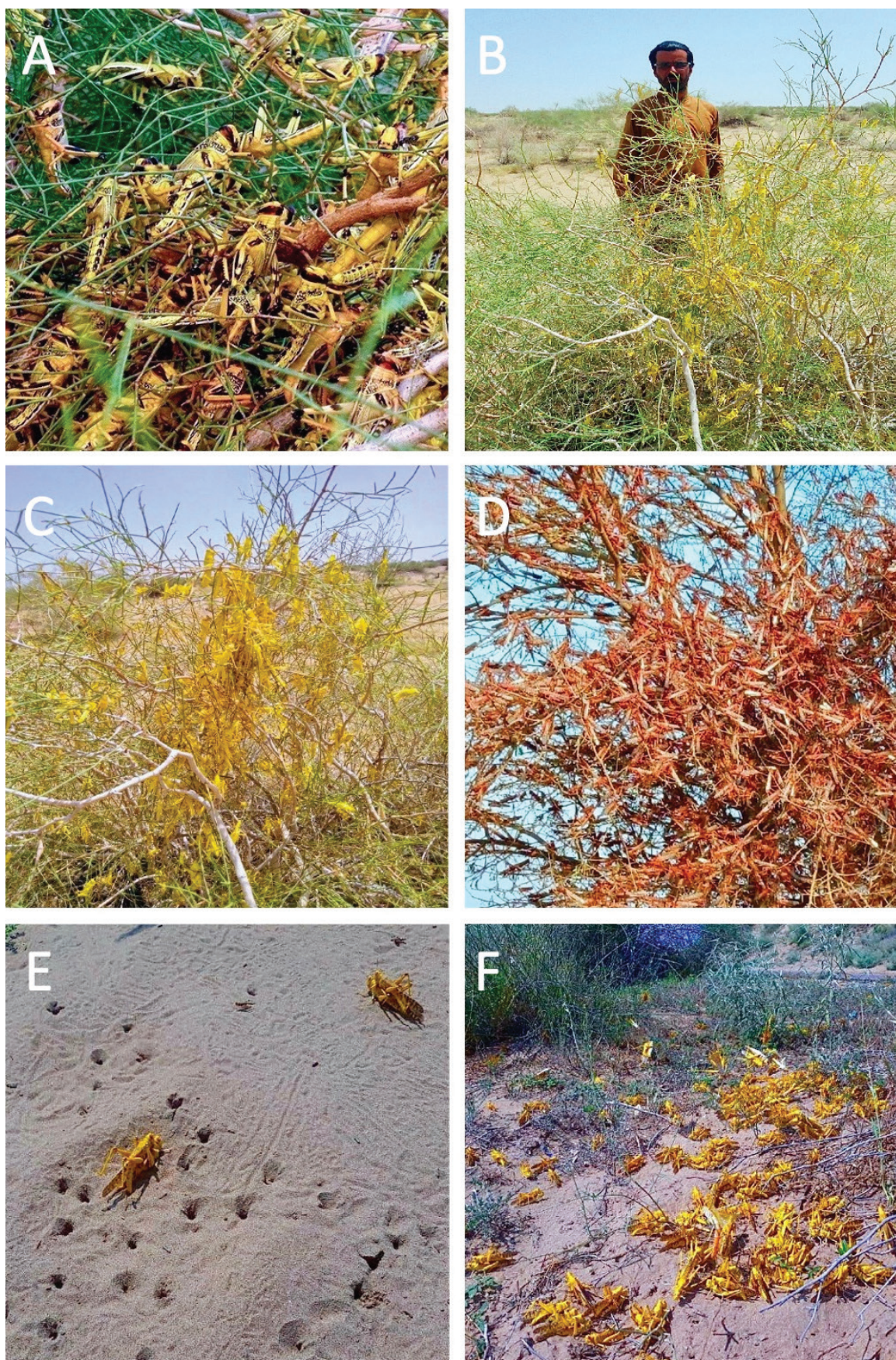
Fig. 4. Photos of the desert locust in Pakistan. **A–D.** Outbreaks in various localities during the 2019–2020 upsurge; **E, F.** Individual and group mating in the Thar desert (photos from the authors).
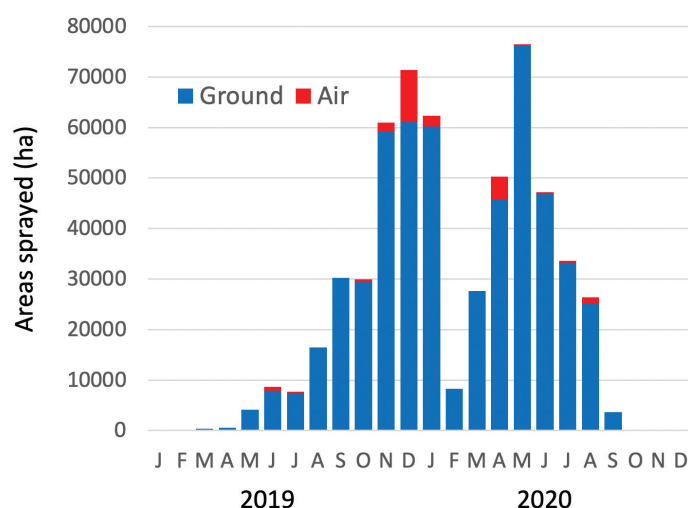
Fig. 5. Areas sprayed with pesticides in Pakistan to control the desert locust upsurge in 2019–2020 (source FAO 2019, 2020a).

Table 1. Areas sprayed with pesticides to control the desert locust upsurge in 2019–2020 over all affected regions (source FAO 2019, 2020a).

Countries	ha sprayed	Countries	ha sprayed	Countries	ha sprayed
Afghanistan	2969	Jordania	2900	Saudi Arabia	505829
Algeria	1138	Kenya	168484	Somalia	170495
Bahrain	3	Kuwait	15841	Sudan	331368
Egypt	24206	Libya	70	South Sudan	250
Eritrea	113794	Mali	40	UAE	6102
Ethiopia	1177607	Mauritania	1056	Uganda	7154
India	682790	Niger	3897	Yemen	58709
Iran	1036510	Oman	13907		
Iraq	2610	Pakistan	566390	TOTAL	4891150

Commission (SUPARCO) has helped through the use of remote sensing used to delimit the areas more vulnerable to locust attacks in the various affected districts of Pakistan based on vegetation, soil type, and other factors.

Large areas, about 65 M ha, were surveyed by the DPP, and in 2 years, based on data collected by the FAO (2019, 2020a), a total of 566,390 ha was treated (FAO 2019, 2020b). Most of the treatment was done using specialized ultra-low volume (ULV) vehicle-mounted sprayers, mainly with malathion (ULV formulation) in desert areas and lambda-cyhalothrin (EC formulation) for protection against desert locust attacks in crop production areas; 543,370 ha were treated by land (Fig. 5). Only a small part was treated by air (23,020 hectares). According to DPP, aerial spraying was carried out on locust hoppers when large areas were involved; otherwise, ground spraying was considered more effective. As with other neighboring South Asian countries, Pakistan was unprepared for the scale of the upsurge (Balakrishnan 2020). One challenge DPP had to deal with was obsolete or non-functional equipment for control operations, as the last serious desert locust outbreak was over 25 years ago (Dowlatchahi et al. 2020b). Small aircrafts for pesticide spraying were unavailable or not operational. Some also expressed regret that control operations started too late and that the federal government did not take the threat seriously enough from the start in 2019, when locusts were confined to Balochistan (Ellis-Petersen and Baloch 2020, Nawaz 2020). When operations finally started, despite the efforts of government authorities, DPP, local authorities and local inhabitants/farmers, it took 16 months, from March 2019 to September 2020, for the upsurge to be brought under control in Pakistan and in the whole eastern region, including India and Iran. Ultimately, the control of this upsurge was a success, which was the result of extensive, strategically planned, and technically well-executed control operations in the country. Strong coordination at federal and provincial levels and with all relevant actors carried out under the National Locust Control Centre set up in Islamabad increased the effectiveness of the response (Dowlatchahi et al. 2020b). For all the countries affected by this upsurge, 4,891,150 ha were treated for the years 2019 and 2020 (Table 1). However, at the start of 2021, the situation remained very worrying in the Horn of Africa and in the Arabian Peninsula. It is not yet time to put down our guard, and vigorous monitoring to detect any signs of breeding desert locust is necessary (Dowlatchahi et al. 2020b).

In some areas, local governments have announced compensation measures for farmers who have suffered from locust attacks. Amid the current COVID-19 pandemic, farmers have found it difficult to control locusts on their own due to restrictions on transport and communication. The supply of reliable, affordable pesticides and spraying equipment has been insufficient. There are no crop insurance programs in the country, and in some areas, farmers have had to plant crops twice, as the first crops were completely eaten up by locusts. Locusts were not only attacking crops, but also damaging rangelands and other vegetation. Thus, livestock keepers and nomadic communities were also suffering. Such damage was most visible in arid regions like Balochistan, where rangelands were already in poor condition. Many affected areas were not treated due to a lack of small airplanes that can be used for spraying pesticides.

Farmers have been known to adopt different ways to protect their crops besides insecticide treatments. For instance, one measure taken by many was beating drums at high volume to scare the locusts. In some areas, farmers also used smoke from burning bushes and vegetation to repel them. According to some local people, since the last major attack was 58 years ago, the current generation has no direct experience of handling locusts using local knowledge (Nawaz 2020). They may not be fully aware of methods to catch locusts or about how to use them as a food source, a compensatory measure that can reduce the number of locusts locally and provide a food supplement to poor and undernourished rural populations (Samejo et al. 2021).

Discussion

The way forward—Institutional aspects and preventative actions for the future

The situation Pakistan faced in 2019–2020 was the most serious in many years. Nevertheless, desert locust invasions are now better controlled, being less frequent, less important, and of shorter duration than in the past (Sword et al. 2010, Zhang et al. 2019). The large invasions that followed one another with a high frequency ended at the beginning of the 1960s with the establishment of a proactive/preventive strategy and thanks to increasingly effective surveillance and continuously improving control methods (Magor et al. 2008, Sword et al. 2010, Lecoq 2019).

Clearly, the problem remains. These invasions are, as always, the result of exceptionally big rains that occurred in the past and that are certain to continue to occur. Presently, climate change cannot be blamed for the ongoing upsurge, even though it will undoubtedly have consequences for outbreaks of this insect in the future (Meynard et al. 2020). Thus, these desert locust invasions will continue to occur. Upsurges over the past 50 years that were

not stopped at an early stage were the result of gaps or a lack of vigilance in the prevention system implemented at the international level (Lecoq 2001, 2005). Most often, insecurity in key areas or the too-late provision of emergency funds are the cause of these upsurges. Then, control operations start too late, often in countries that are still poorly prepared, and the swarms disseminate rapidly (Showler and Lecoq 2021). Therefore, the countries concerned, and Pakistan in particular, must remain mobilised to improve the prevention system. Addressing these challenges requires investment into making the country capable of handling the menace (Dowlatchahi et al. 2020a, b). For more information on prevention system failures, see Showler (2019) and Showler et al. (2021).

Like other front-line countries, Pakistan contains certain desert locust outbreak areas, located in desertic areas on the Indo-Pakistan border and in the Makran region on the border with Iran (Symmons and Cressman 2001, Cressman 2016). Pakistan, therefore, plays a key role in the prevention strategy by conducting regular surveillance of these areas. However, the country can also be invaded by swarms that originate outside its borders, as was the case in 2019. International cooperation is essential to better control these migratory insects. As mandated by its Member States, the FAO ensures the coordination of monitoring and control activities of the desert locust on an international scale (Lecoq 2003). Via its Desert Locust Information Service (DLIS), it issues a monthly locust situation and forecast bulletin (FAO 2009b). This bulletin is based on reports from the affected countries, as well as on the analysis of the ecological conditions in the habitats of the locust (using satellite remote sensing data, weather reports related to rains and direction of the winds, etc.) (Cressman 2008, 2013). In addition, the FAO provides a forum for the meetings of the Desert Locust Control Committee (DLCC), formed by representatives from all the countries affected by desert locust as well as those that take part in locust control campaigns.

Since 1955, Pakistan has been a member of both the DLCC and the South West Asia Commission (SWAC), established in 1964 under Article XIV of the FAO Constitution. SWAC has four member states: Afghanistan, India, Iran, and Pakistan. All activities of SWAC contribute to the strengthening of the national capacities of its member countries in desert locust survey, control operations, reporting, training, preparedness, contingency planning, emergency response, biopesticides, and health and safety (FAO 2021a). SWAC also promotes cooperation among its member countries and, in particular, the conduct of regular joint surveys for desert locust surveillance and early warning, as well as the exchange of information on the locust situation. It supports training and capacity building activities and the promotion of new technologies (FAO 2021a). SWAC is also collaborating with the other two FAO commissions—CRC for the central region and CLCPRO for the western region (FAO 2021b, c)—with regard to the use of biopesticides, the development of risk management plans, setting up inventory systems, and environmental monitoring. Obviously, all of these measures should be developed at the level of each member state, and it is in Pakistan's interest to strengthen its cooperation with SWAC.

As a result, monitoring and preventative organization against desert locust invasions is a leading example in the field of crop protection (Hamouny 2021). However, while this prevention system is genuinely effective, it also has its flaws, as the current situation unfortunately reminds us. Various reasons for failure have been given: inexperience of field survey teams and campaign organizers, insufficient or inappropriate resources, inaccessibility of some important breeding areas for security reasons, deterioration of survey, and control capacities during recession periods (WMO & FAO 2016). Some issues, such as security concerns, are beyond Pakistan's control. Others may find solutions locally. We give details on three main points below.

1. Desert locust require concerted monitoring and on-the-ground control effort across borders, along with the resources, expertise, and infrastructure to support those actions. Moreover, these efforts must be kept in place over the long term to build resilience, despite the apparent lack of imminent threats (Lecoq 1991, Gay et al. 2018). The lack of such coordinated and sustained efforts is likely to put human populations at higher risk. Pakistan had to fight the recent upsurge with insufficient funding, operational resources, obsolete equipment, and an eroded expertise that left the DPP with only a few high-level experts (Dowlatchahi et al. 2020a, b). The highest priorities should therefore be (a) to ensure that the political and socio-economic conditions are in place so that vulnerable human populations can adapt to new large-scale threats and (b) to maintain a long-term risk assessment culture with ongoing financial, material, and expertise support (Meynard et al. 2020). Perhaps maintaining funding mechanisms that provide sustainable support during periods of recession, when priorities are elsewhere, is one of the most difficult but key points to be solved. Yet, it has been shown that funding institutions (governments, donors) could considerably improve the effectiveness of the prevention system by increasing their support by only a few percent (Gay et al. 2018).

2. Pakistan, as with all countries concerned with the desert locust, must remain ready and develop compensatory measures for the local populations in the event of an invasion that is not controlled early on. Farmers are most often helpless in the face of the threat from locusts. Prevention remains the best rampart, but if this fails, local populations must have access to information, advice, and support, both technical and financial. It is advisable, for instance, to develop desert locust control material for the education of farmers and agriculture extension staff and organize farmers' schools for desert locust control.

3. Finally, current treatments are based almost exclusively on traditional chemical insecticides that pose various risks to both human health and the environment (Everts and Ba 1997, Samways and Lockwood 1998, van der Valk 1998, Peveling 2001, FAO 2014). The end result of these quick control measures is still massive damage, both to crops and, perhaps worse, to the ecosystem, from the enormous amounts of pesticides sprayed (Balakrishnan 2020). Alternative products such as mycopesticides, which have been used for some time by countries such as Australia and China, should be able to find a larger audience globally (Lomer et al. 2001, Hunter 2004, 2010, Zhang and Hunter 2005, Zhang 2011). These products are currently commercially available, and the ongoing upsurge has given rise to their use in various countries (Zhang et al. 2019). In 2020, mycopesticides were successfully applied to at least 10,845 ha in Somalia against desert locusts (FAO 2020g, h). In collaboration with the FAO, trials focused on the introduction of mycopesticides in Pakistan have been done, which should obviously be encouraged.

Acknowledgments

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Limited evidence for learning in a shuttle box paradigm in crickets (*Acheta domesticus*)

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Abstract

Aversive learning has been studied in a variety of species, such as honey bees, mice, and non-human primates. Since aversive learning has been found in some invertebrates and mammals, it will be interesting to know if this ability is shared with crickets. This paper provides data on aversive learning in male and female house crickets (*Acheta domesticus*) using a shuttle box apparatus. Crickets are an ideal subject for these experiments due to their well-documented learning abilities in other contexts and their readily quantifiable behaviors. The shuttle box involves a two-compartment shock grid in which a 'master' cricket can learn to avoid the shock by moving to specific designated locations, while a paired yoked cricket is shocked regardless of its location and therefore cannot learn. Baseline control crickets were placed in the same device as the experimental crickets but did not receive a shock. Male and female master crickets demonstrated some aversive learning, as indicated by spending more time than expected by chance in the correct (no shock) location during some parts of the experiment, although there was high variability in performance. These results suggest that there is limited evidence that the house crickets in this experiment learned how to avoid the shock. Further research with additional stimuli and other cricket species should be conducted to determine if house crickets and other species of crickets exhibit aversive learning.

Keywords

Introduction

Aversive learning is crucial to an individual's survival. One example of aversive learning is taste aversion, which is an important defense against potential poisoning (Logue 1985, Bernstein 1999). We would expect many species to develop aversive learning since it is crucial to learn whether something is aversive rather than make the potentially fatal error of not learning from previous experience(s). As a result, aversive learning is found across many species, such as goldfish, terrestrial mollusks, mice, coyotes, non-human primates, and humans (Gelperin 1975, Garcia et al. 1985, Logue 1985, Manteifel and Karelina 1996, Wright et al. 2010, Goltseker and Barak 2018).

Insects also demonstrate aversive learning (Abramson et al. 1977, Dethier 1980, Abramson 1986). Specifically, researchers have studied insect taste aversion related to foraging choices (Dethier 1980, Bernays 1993). Dethier (1980) first studied food aversion in polyphagous insects using two wooly bear caterpillars. These caterpillars exhibited aversive learning to petunias after recovering from acute illness linked to the consumption of the plant (Dethier 1980). Additionally, the grasshopper *Schistocerca americana* (Drury, 1773) exhibited taste aversion depending on the palatability of the food (Bernays and Lee 1988), although individuals of this species did not exhibit aversive learning following nicotine hydrogen tartrate poisoning when the food was highly palatable broccoli (Bernays and Lee 1988). In comparison, when presented with a less palatable food such as spinach, grasshoppers exhibited aversive learning (Bernays and Lee 1988). Honey bees also exhibit aversive learning, as demonstrated in escape, punishment, and avoidance paradigms (Abramson 1986), and harvester ants can learn to go to a specific area to terminate and passively avoid vibration (Abramson et al. 1977).

Shock is commonly used as an aversive stimulus in learning experiments (Garcia and Koelling 1966). Researchers have used shock as a stimulus in aversive learning experiments with rats (Garcia and Koelling 1966), humans (Lovibond et al. 2008), honey bees (Abramson 1986, Nuñez et al. 1997, Agarwal et al. 2011), and fruit flies (Tully and Quinn 1985). Nuñez and colleagues (1997) used shock to stimulate the stinging response in honey bees following previous injection of isopentyl acetate, which is the main component of the honey bee alarm pheromone. In a different experiment, Tully and Quinn (1985) used electrical shock pulses paired with a conditioned odor to determine whether trained fruit flies could learn to avoid the shock based on the paired odor stimulus.

Another way that shock is administered in experiments is through the use of an apparatus called the shuttle box—one of the oldest and most widely used apparatuses for the study of learning and memory (Warner 1932). A shuttle box is a chamber in which an organism can move back and forth ('shuttle') to avoid an aversive stimulus (Abramson 1986). A shuttle box can also be used to train an organism to move towards an attractive stimulus that is delivered to one side of the chamber (Abramson 1986). One of the earliest uses of the shuttle box was by Warner (1932), who studied avoidance learning in rodents. Over the years, shuttle boxes have been built for many organisms, including aquatic animals (Horner et al. 1961) and insects such as cockroaches, ants, and honey bees (Abramson et al. 1982, Abramson 1986). The shuttle box is designed primarily to study aversive conditioning such as escape, punishment, and both unsignaled and signaled avoidance (Abramson 1986). However, a shuttle box can also be used to study time allocation (DeCarlo and Abramson 1989), place learning (Agarwal et al. 2011), learned helplessness (Dinges et al. 2017), and caste differences in learning using social species like honey bees (Dinges et al. 2013). Moreover, with slight modifications, the shuttle box can be used to deliver appetitive stimuli such as food and odors (Abramson et al. 1982, DeCarlo and Abramson 1989).

Crickets are ideal for studying aversive learning because this group of species exhibits a variety of learned behaviors, ranging from associative learning of olfactory cues (Matsumoto and Mizunami 2000, 2002), to spatial learning (Wessnitzer et al. 2008, Doria et al. 2019), and even social learning (Coolen et al. 2005, Ebina and Mizunami 2020). For instance, the Mediterranean field cricket (*Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae)) has been shown to learn the association between specific odors with paired rewards and punishments (Matsumoto and Mizunami 2000). *G. bimaculatus* has also been shown to have good olfactory memory, with some associations being remembered for at least six weeks (Matsumoto and Mizunami 2002). Other work on memory in *G. bimaculatus* has shown that caffeine can improve long-term memory in this species (Sugimachi et al. 2016).

Crickets also exhibit spatial learning and memory. *G. bimaculatus* were placed in a stadium similar to a Morris water maze in which the traditional water and the hidden platform were replaced with a hot metal surface possessing a cool area on the platform's surface (Wessnitzer et al. 2008). The time that it took for *G. bimaculatus* to find the cool spot decreased with experience, indicating spatial learning (Wessnitzer et al. 2008). Texas field crickets (*Gryllus texensis* Cade & Otte, 2000) have also been tested in radial-arm mazes, where they had to remember which arm contained a food reward (Doria et al. 2019). The Texas field cricket's ability to learn has been linked to thigmotaxis (crickets' movement towards or away from a physical stimulus) (Doria et al. 2019). *G. bimaculatus* has also been used in prediction error theory experiments using visual and olfactory stimuli (Terao et al. 2015). The crickets were trained on either an olfactory or visual stimulus; after the training, they were given a combined visual/olfactory stimulus before being tested on the stimulus that they were not initially tested on. The crickets that initially learned by a visual pattern were less capable of finding the reward when only olfactory stimuli were available, even though they ran several trials with both stimuli combined (Terao et al. 2015).

There is also some evidence for social learning in crickets, although this has been less explored. One social learning experiment involved naive *Nemobius sylvestris* (Bosc, 1792) learning anti-predator behaviors from more experienced conspecifics (Coolen et al. 2005). This was done by placing naive crickets with demonstrators and placing an odor that demonstrators had learned to associate with a predator in a container. The demonstrators would burrow into the leaf litter when exposed to that odor, followed by the naive crickets, whereas on their own the naive crickets did not display any anti-predator behavior (Coolen et al. 2005). *G. bimaculatus* is also capable of associating the presence of conspecifics with rewards (Ebina and Mizunami 2020).

No studies of cricket learning have employed a shuttle box. The shuttle box has two major advantages that make it worth exploring as a test paradigm in crickets: it is automated, and it can be used to test a wide range of learning behaviors. The automation of the shuttle box is a major advantage since it allows for the apparatus to be used consistently and repeatedly with a variety of species and experimental designs. Furthermore, as there have been many shuttle box experiments with a wide variety of organisms, it will be interesting to compare cricket behavior in the shuttle box to that of other species to gain insight into species differences in learning. It would also open the door to the 'psychological' study of cricket behavior, as many interesting psychological phenomena such as social learning and spatial memory can be explored (see above).

In the present study, we tested the suitability of using the shuttle box for behavioral studies of learning using house crickets *Acheta domesticus* (Linnaeus, 1758) (Orthoptera: Gryllidae). Crickets have many benefits as model organisms for behavioral studies. They are usually easy to maintain and exhibit a wide range of interesting behaviors, including social behaviors and learning. In addition, crickets are short-lived and have clear developmental markers (e.g., wing development, chirping, and development of an ovipositor). These traits allow researchers to use individuals that are all at the same life stage, identify the males and females, and help to minimize differences between subjects.

Methods

Subjects.—Subjects consisted of 130 house crickets [females n = 78, males n = 52 (*Acheta domesticus*)] collected from colonies maintained for laboratory purposes sourced from Fluker Farms, Louisiana. Crickets were sorted into two different communal containers based on sex. In the containers, crickets had a source of cover (piece of egg carton or cardboard), ground up chicken feed in petri dishes, and distilled water in a Falcon tube closed with a cotton ball. The food and water were refilled every 48 hours. Crickets were housed in this manner until they were needed for the experiment. Only mature crickets (crickets with fully developed wings) were tested.

Apparatus.—The present experiment made use of a modified shuttle box apparatus (see Fig. 1 and Suppl. material 1: Cricket Shuttle box Video), similar to that used by Dinges et al. (2013). The apparatus consisted of two separate compartments with external dimensions of 200 mm × 60 mm ×25 mm and internal dimensions of 140 mm × 20 mm × 10 mm. Each compartment contained a series of 55 shock grid pins and a set of two infrared LED and receptor pairs. Pins were 1 mm in diameter and placed 2.5 mm apart. Pins were placed so that when subjects contacted consecutive pins—completing the circuit—shock was applied to the grid, which would be felt by the animal. Once an animal was introduced to a compartment, a piece of clear Plexiglas measuring 145 mm × 25 mm × 5 mm was placed on top of the compartment to ensure that the crickets remained in contact with the grid. When each cricket was in its compartment, it was unable to see or communicate with the cricket in the other compartment.

Fig. 1. The shuttle box apparatus with propeller controller in the upper left-hand corner. In this image, one of the plexiglass covers (**A**) has been removed so that the shock grid can be seen more easily. In the experiments, one cricket was placed into the compartment labeled **A** and one cricket was placed into the compartment labeled **B**. A piece of filter paper was placed under the left-hand side of the shuttle box to illustrate the midpoint for the two halves of the compartment. Once the cricket was inside the compartment, the plexiglass was placed over the compartment to ensure that the cricket maintained contact with the grid.

Each compartment was then connected to a control box containing a Propeller Experiment Controller (Parallax Inc., Rocklin, CA; Varnon and Abramson 2013, 2018) and an external variable DC power supply for the shock grid. Experimental parameters (location of the shock based on the position of the master cricket: left half near, left half away, right half near, and right half away) were set using the control panel and automatically implemented through the Propeller controller. When master crickets (see below) crossed into the side of the compartment paired with shock, electricity was applied to the grids of both compartments. This ensured that the yoked subjects experienced shock at the same time and duration as the master subjects, regardless of their position in the compartment. Shock terminated upon the master subject entering the safe side of the apparatus.

Behavioral assay.—Preliminary experiments were conducted to determine the appropriate voltage and amplitude. During the preliminary experiments, individual crickets were shocked at various voltages and amplitudes. Crickets were observed for behaviors

(such as digging) that indicate the presence of a noxious stimulus (i.e., shock). Observations were made about the ability of the individuals to enact behaviors to escape the shock.

For the formal experiment, same-sex cricket pairs were captured from the container using a plastic shot glass and a notecard. Each individual was placed in a separate compartment. Once they were in the shuttle box, a three-minute habituation phase started where no shock was administered. The habituation phase provided crickets with time to recover from any stress associated with the transfer from the container to the apparatus. After the habituation phase, and once both crickets crossed the midline in either direction, the experimental trial began. During the experimental trial, shock was delivered at 12 VDC and 0.5 A.

The formal trial duration was 9 minutes. Results of previous research using this setup with honey bees (*Apis mellifera* Linnaeus, 1758) found that experimental fatigue occurs after 5 minutes (Black et al. 2018), so we tested for longer than this to see if crickets exhibit a similar behavioral pattern to the honey bees. After each cricket pair completed the trials, the shuttle box was cleaned with 90% EtOH and a Kimwipe (Kimtech Science) to remove chemical cues. Crickets were only used once. A Propeller Experiment Controller (Varnon and Abramson 2013, 2018) automatically recorded cricket position, time of movement between the two sides, and onset or offset of the shock.

Crickets were randomly assigned to the role of 'master,' 'yoked,' or 'baseline.' Crickets were placed in the apparatus with one cricket in each compartment. The baseline cricket pair served as an experimental control in which no cricket received a shock. The master and yoked cricket pairs were the experimental pairs where one cricket's behavior (master) determined whether both crickets were shocked. For these pairs, the shuttle box was assigned to administer shock on one of the two sides of the grid in each compartment (i.e., either on the half away from or towards the researcher). The side that was associated with the shock was randomly chosen and did not change during the trial. If the master cricket moved to the side that was shocked, then both crickets received the shock. Once the master cricket left the side corresponding to the shock, both crickets stopped receiving the shock. The yoked cricket was unable to observe or communicate with the master cricket and therefore received the shock regardless of what side of the compartment it was on.

The reason for the pairing between the master and the yoked cricket was to set up a situation where one cricket had the opportunity to learn (the master cricket) and the other (yoked cricket) was essentially a control that experienced the same conditions, including the experience of electric shock, but could not learn because there was no consistent association between its behavior and the shock. Master crickets could learn to avoid the side associated with the shock, while yoked crickets would not be able to associate either side with a shock. As a result, the side preferences of the yoked crickets should resemble those of the baseline crickets: neither of these crickets should show a bias towards one side or the other. In contrast, if the master crickets are able to learn the association, they should spend less time on the side associated with the shock than either the baseline or the yoked cricket.

The data came from a 9-minute trial that was divided into 60-second intervals for data management purposes. The amount of time that was spent on the side that was not linked to shock (hereafter 'correct side') was calculated for nine 60-second intervals starting at 0 s and ending at 540 s. The proportion of time spent on the correct side was calculated in 60-second intervals by dividing the actual amount of time spent on the correct side by 60 s.

All the data were analyzed using R version 4.1.0 (R Core Team 2021) and R Studio version 1.4 (R Core Team 2021). A suite of one sample *t*-tests with Bonferroni corrections were used to determine whether the performances (proportion of time spent on the correct side for each 60 s interval) of the experimental groups for the male and female crickets differed from chance (0.50). The requirements for the parametric tests were satisfied. A Bonferroni adjusted α value of 0.003 was used. Then, a linear mixed model (LMM) was conducted using the lmer function in lme4 version 1.1-27 package (Bates et al. 2015) to determine the effects of time point (i.e., 60-second intervals) and the interaction between experimental role and sex on the amount of time spent on the correct side.

Results

The first two sets of *t*-tests were used to compare the percentage of time the male and female shock-free control (e.g., baseline) crickets spent on the correct side compared to random chance (50%) (Table 1; Figs 2, 3). Neither the male nor female baseline crickets exhibited significant differences from chance in the amount of time spent on the correct side (Table 1; Figs 2, 3). These results indicate that the baseline crickets are representative of normal behavior when in the apparatus without aversive conditions (Black et al. 2021).

Two additional sets of *t*-tests were conducted on the male and female yoked crickets to see if the percentage of time they spent on the correct side differed from chance (Table 2; Figs 2, 3). Like the baseline crickets, the yoked crickets did not exhibit significant differences from chance (Table 1; Figs 2, 3). In comparison, the master crickets exhibited statistically significant differences at the 60 s time

point for the males and the 480 s time point for the females (Table 3; Figs 2, 3). These results indicate that at the 60 s time point and at the 480 s time point, the males and the females, respectively, spent significantly more time on the correct side compared to chance.

The results from the LMM showed that time point was a significant predictor of amount of time spent on the correct side, with crickets spending more time on the correct side as the experiment progressed (Table 4). The interactions between experimental role and sex for master and yoked were not significant (Table 4). Overall, these results suggest that the amount of time that crickets spent on the correct side differed depending on the time point.

The findings from the first LMM suggest that cricket learning occurred at different time points, as seen by the difference in male (beginning of trial) and female (end of trial) learning (Fig. 3). The second LMM with only the master crickets did not show that time point or sex were significant predictors of amount of time spent on the correct side (Table 5). Additionally, the interaction between time point and sex was not significant. These findings suggest that the amount of time the male and female crickets spent on each side did not differ over time.

Simultaneous pairwise comparisons using Tukey's HSD test indicated that the difference between the master group and the yoked control was statistically significant (Table 6). Tukey's HSD test did not indicate significant differences between the master group and the shock-free baseline control group (Table 6). There was also no significant difference between the yoked control and the shock-free control group (Table 6). These results suggest that learning occurred for the master crickets when they were compared to the yoked crickets, but not when they were compared to the baseline crickets.

Table 1. One sample *t*-test results for male and female no shock control (baseline) crickets compared to random chance (0.5). *M* = proportion of time spent on the correct side; females df = 17; males df = 9; Bonferroni adjusted α value of 0.003.

Time Point (s)	Females		t	p	Males		t	p
	M	*SD*			*M*	*SD*		
60	0.55	0.33	0.63	0.538	0.75	0.25	3.13	0.012
120	0.56	0.35	0.67	0.513	0.57	0.30	0.76	0.464
180	0.35	0.32	-2.03	0.058	0.50	0.29	-0.00	1.000
240	0.43	0.37	-0.74	0.471	0.59	0.38	0.77	0.461
300	0.51	0.41	0.11	0.916	0.44	0.40	-0.50	0.629
360	0.44	0.40	-0.62	0.544	0.39	0.40	-0.83	0.429
420	0.59	0.45	0.85	0.407	0.35	0.35	-1.31	0.224
480	0.67	0.42	1.74	0.100	0.49	0.38	-0.04	0.966
540	0.54	0.40	0.43	0.672	0.54	0.46	0.31	0.766

Table 2. One sample *t*-test results for male and female yoked control crickets compared to random chance (0.5). *M* = proportion of time spent on the correct side; females df = 29; males df = 20; Bonferroni adjusted α value of 0.003.

Time Point (s)	Females		t	p	Males		t	p
	M	*SD*			*M*	*SD*		
60	0.45	0.37	-0.81	0.427	0.46	0.27	-0.71	0.488
120	0.53	0.41	0.40	0.692	0.42	0.32	-1.18	0.251
180	0.41	0.37	-1.33	0.194	0.44	0.36	-0.79	0.439
240	0.53	0.40	0.46	0.652	0.51	0.45	0.06	0.950
300	0.60	0.42	1.35	0.188	0.42	0.42	-0.91	0.373
360	0.55	0.41	0.70	0.489	0.44	0.44	-0.61	0.551
420	0.46	0.43	-0.54	0.591	0.51	0.47	0.13	0.897
480	0.60	0.42	1.25	0.222	0.54	0.45	0.41	0.688
540	0.60	0.44	1.24	0.226	0.53	0.41	0.29	0.771

Fig. 2. Proportion of mean time spent on correct side of the apparatus for female crickets during each 60 s interval. Female N values: Baseline = 18, Yoked = 30, and Master = 30. The bars on the data points represent the standard errors. The solid line at 0.5 indicates chance.
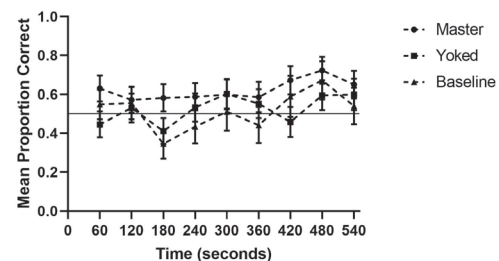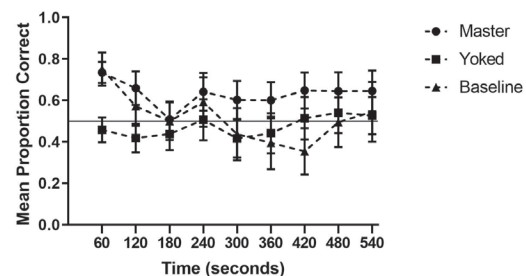

Fig. 3. Proportion of mean time spent on correct side of the apparatus for male crickets during each 60 s interval. Male N values: Baseline = 10, Yoked = 21, Master = 21. The bars on the data points represent the standard errors. The solid line at 0.5 indicates chance.

Table 3. One sample *t*-test results for male and female behavioral (master) crickets compared to random chance (0.5). M = proportion of time spent on the correct side; females df = 29; males df = 20; Bonferroni adjusted α value of 0.003.

Time Point (s)	Females		t	p	Males		t	p
	M	SD			M	SD		
60	0.63	0.37	1.94	0.062	0.73	0.23	4.620	0.000*
120	0.57	0.36	1.10	0.280	0.66	0.37	1.972	0.063
180	0.58	0.38	1.17	0.250	0.51	0.40	0.089	0.930
240	0.59	0.39	1.24	0.224	0.64	0.42	1.555	0.136
300	0.60	0.42	1.29	0.206	0.60	0.42	1.108	0.281
360	0.59	0.43	1.10	0.282	0.60	0.40	1.142	0.267
420	0.67	0.40	2.37	0.025	0.65	0.40	1.690	0.106
480	0.72	0.38	3.22	0.003*	0.64	0.42	1.596	0.126
540	0.65	0.39	2.13	0.042	0.65	0.45	1.474	0.156

Table 4. Results of the LMM model to test the effects of time point, experimental role, and sex on time spent on the correct side. Significant predictors: * $p <0.05$ and ** $p < 0.001$.

Independent	Predictor	Estimate	SE	t-value	95% CI		p
Percent of time spent on correct side	Intercept	29.17	3.73	7.83	21.87	36.48	<0.001**
	Time Point	0.01	0.00	2.04	0.00	0.01	0.042*
	Exp. Role [Master]	6.67	4.56	1.45	-2.33	15.66	0.146
	Exp. Role [Yoked]	0.72	4.56	0.16	-8.27	9.71	0.875
	Sex [Male]	1.28	7.25	0.18	-12.93	15.50	0.860
	Exp. Role [Master] *Sex [Male]	-1.12	8.41	-0.13	-17.61	15.37	0.894
	Exp. Role [Yoked] *Sex [Male]	-5.14	8.4	-0.61	-21.63	11.35	0.541

Table 5. Results of the master cricket LMM model to test the effects of time point, experimental role, and sex on time spent on the correct side. Significant predictors: * $p < 0.05$ and ** $p < 0.001$.

Independent	Predictor	Estimate	SE	t-value	95% CI		p
Percent of time spent on correct side	Intercept	35.02	3.35	10.45	28.45	41.59	<0.001**
	Time Point	0.01	0.01	1.50	-0.00	0.02	0.134
	Sex [Male]	3.36	5.09	0.66	-6.62	13.33	0.510
	Time Point *Sex [Male]	-0.01	0.01	-1.23	-0.03	0.01	0.220

Table 6. Results of the LMM model Tukey post-hoc pairwise comparisons for experimental role. * $p < 0.05$, df =126.

Exp. Role 1	Exp. Role 2	Estimate	SE	t-value	p
Baseline	Master	-6.11	4.21	-1.45	0.32
Baseline	Yoked	1.85	4.21	0.44	0.90
Master	Yoked	7.95	3.02	2.64	0.03*

Discussion

The series of experiments presented in this study had two goals. One goal was to investigate whether house crickets, *A. domesticus*, exhibit aversive learning. The other goal was to determine if the shuttle box is a suitable apparatus for studying aversive learning with crickets. The results show that the male master crickets' behavior exhibited learning at the beginning of the experimental trials, while the female master crickets' behavior exhibited learning towards the end of the experimental trials (Table 1). As in experi-

ments with other species of crickets (Matsumoto and Mizunami 2000, 2002, Doria et al. 2019, Wessnitzer et al. 2008), both the male and female house crickets in our experiments exhibited behavior indicative of learning (Tables 3, 4).

The master male crickets exhibited learning early in the experiment because they spent significantly more time than chance on the correct side. In comparison, the master female crickets exhibited learning later in the experiment, as they spent significantly more time than chance on the correct side. For both males and females, the yoked control pairs and no shock control pairs all performed similarly and were no different from the chance expectation of 50% (Fig. 2). While the amount of time spent on the correct side for both the male master and female master, yoked controls, and no-shock baseline control crickets all appear to be similar, the results from the LMM indicated that there was a significant difference between the master and yoked experimental roles but not between the master and the baseline crickets (Fig. 3). The performance of the controls was as expected: their behavior did not differ from chance. The master crickets, however, were expected to perform better than chance, but, at most, they performed better than chance for only small portions of the experiment.

Generally, honey bees hit around 60–75% on performance in this assay and maintained that performance over time, which has been taken as evidence for aversive learning (Dinges et al. 2013). The crickets from this experiment demonstrated similar behavioral patterns, with the average proportion of time spent on the correct side by the male and female master crickets ranging from 0.56 to 0.75 (Table 3; Figs 2, 3). The reason for the statistical differences between the crickets and the bees might be due to greater variance in performance by the crickets. Although on average the master male and female crickets spent around 60–75% of their time on the correct side throughout the trials (Table 3), the standard errors of the mean proportion of time on the correct side for the master crickets are quite large, indicating substantial variance within the data (Figs 2, 3).

Unlike the honey bees (Black et al. 2018), the crickets' behavior does not suggest that they experienced experimental fatigue or a decrease in their performances. The average proportion of time spent on the correct side for the master crickets in the experiment decreased somewhat for the males at 180 s and then increased at 240 s and remained relatively stable (Fig. 3). The master female crickets' proportion of time spent on the correct side stayed relatively stable and increased at 420 and 480 s. Overall, despite the slight decrease for the males, the proportion of time spent on the correct side for both males and females appeared to be on average better than chance (50%); however, these findings were not statistically significant (Table 3), suggesting no evidence for learning when considering performance across the entire duration of the experiment.

Crickets in the shuttle box responded to the shock by exhibiting digging behavior (Suppl. material 2: Cricket Digging Behavior Video). We observed the crickets digging with their front legs in response to the shock. Anecdotal observations of this behavior indicated that crickets displayed differences in digging behavior during the experimental trials when they were shocked. An experiment by Coolen et al. (2005) found that crickets exhibited burrowing behaviors such as digging when certain odors and cues associated with wolf spiders were put in their containers. We believe that the crickets in our experiment exhibited this digging response in the presence of the shock due to it being an aversive stimulus.

One improvement to the design that may enhance the ability of crickets to learn would be the addition of visual or olfac-

tory stimuli. Previous research showed that individuals of other species are able to orient and can learn that if they are getting shocked, the shock will cease when they move to the other side of the arena. The addition of other cues could enhance learning but are not necessary for learning to occur (Dinges et al. 2013). Findings from previous experiments demonstrate that crickets can use visual and olfactory stimuli in learning experiments (Doria et al. 2019, Matsumoto and Mizunami 2000, Terao et al. 2015). Additional stimuli might, therefore, provide the crickets with information that would allow them to learn the association with the shock more easily.

Another possible improvement would be to replicate this experiment using a cricket species other than *A. domesticus*. Previous learning experiments in crickets have focused on species in the genus *Gryllus,* e.g., *Gryllus bimaculatus* (Doria et al. 2019; Matsumoto and Mizunami 2000) and *Gryllus texensis* (Terao et al. 2015), and there may be species differences in learning ability. The house crickets that we used were purchased from a cricket farm and have likely been in captivity for many generations. As a result, they may have been selected for fast growth, aggression in foraging, and disease resistance, which could affect performance relative to natural populations of other species.

The use of the shuttle box as described here is promising. We were able to demonstrate the predicted avoidance behavior in a majority of our animals in the master group. However, there are still some unanswered questions that must be addressed before the apparatus can gain wide applicability. These questions include appropriate spacing between the shock bars and variations in shock intensity. We believe that these are relatively minor issues and easily addressed in future studies. For example, consider a lever press situation for crabs. Abramson and Feinman (1990) found that restraining them with clamps produced poor results, but enclosing the crabs in a small box produced effective lever pressing. A similar situation was found with the proboscis conditioning of stingless bees. Restraining stingless bees in tubes did not produce any proboscis conditioning, but putting them in small bottles where they made contact with the stimuli through a screen produced rapid learning (Amaya-Márquez et al. 2019). A similar modification may be needed if the shuttle box is to be useful. One potential idea is to create a 'one-way' or 'circular' shuttle box where the cricket is always going in the same direction and therefore avoids entering a compartment where it just received a shock.

This study provided important information about the learning abilities of house crickets and the suitability of using a shuttle box. Our experiment tested the house crickets' ability to learn through aversive stimuli (i.e., shock). The behavior of both the female and male master cricket demonstrated limited aversive learning. Previous research has provided evidence of the learning abilities of crickets in other contexts. Further investigation into the learning abilities of house crickets and other cricket species though modifications of this aversive learning paradigm might provide more evidence on whether house crickets and other cricket species can learn through aversive conditioning using a shuttle box.

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Establishing the nutritional landscape and macronutrient preferences of a major United States rangeland pest, *Melanoplus sanguinipes*, in field and lab populations

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Abstract

When given a choice, most animals will self-select an optimal blend of nutrients that maximizes growth and reproduction (termed "intake target" or IT). For example, several grasshopper and locust species select a carbohydrate-biased IT, consuming up to double the amount of carbohydrate relative to protein, thereby increasing growth, survival, and migratory capacity. ITs are not static, and there is some evidence they can change through ontogeny, with activity, and in response to environmental factors. However, little research has investigated how these factors influence the relative need for different nutrients and how subsequent shifts in ITs affect the capacity of animals to acquire an optimal diet in nature. In this study, we determined the ITs of 5th instar (final juvenile stage) *Melanoplus sanguinipes* (Fabricius, 1798), a prevalent crop and rangeland grasshopper pest in the United States, using two wild populations and one lab colony. We simultaneously collected host plants to determine the nutritional landscapes available to the wild populations and measured the performance of the lab colony on restricted diets. Overall, we found that the diet of the wild populations was more carbohydrate-biased than their lab counterparts, as has been found in other grasshopper species, and that their ITs closely matched their nutritional landscape. However, we also found that *M. sanguinipes* had the lowest performance metrics when feeding on the highest carbohydrate diets, whereas more balanced diets or protein-rich diets had higher performance metrics. This research may open avenues for studying how management strategies coincide with nutritional physiology to develop low-dose treatments specific to the nutritional landscape for the pest of interest.

Keywords

Introduction

Rangelands in the western United States are important agricultural and environmental resources, serving not only as grazing lands for livestock, but also as habitats for wildlife (Havstad et al. 2007). While the U.S. has not been home to a locust species since the 1870s [the extinct Rocky Mountain locust, *Melanoplus spretus* (Walsh, 1866) (†), originally described within *Caloptenus* by Walsh in 1866, see Lockwood 2004], the rangelands are sometimes plagued by large grasshopper outbreaks (Capinera and Sechrist 1982, Joern and Gaines 1990, Schell and Lockwood 1997). Grasshoppers represent a significant problem for ranchers and farmers across the United States, but especially in the western rangelands (Hewitt and Onsager 1983). Grasshopper outbreaks can represent a significant economic hardship to ranchers, with conservative estimates placing the economic damage of grasshoppers at $393 million in rangeland forage lost annually (Hewitt and Onsager 1983). There is support for the use of fungal management for rangeland grasshoppers (Streett 1996–2000, Bidochka and Roberts 2000, Branson et al. 2006), and nutritional ecology can be helpful when applying these management strategies. However, few studies have bridged lab and field research to understand how the nutritional requirements of grasshoppers relate to the host plants available to them in the field.

In rangelands, the migratory grasshopper *M. sanguinipes* (Fabricius) is the most destructive grasshopper, causing more forage loss than any other grasshopper species in the United States (Pfadt 2002). This grasshopper is not exceptionally large, with a body length of approximately 16–23 mm for 5th instars, 20–26 mm for adult males, and 20–29 mm for adult females (Pfadt 2002).

Management of this species is particularly challenging because populations that emerge in rangelands can then migrate to croplands miles away. Due to their mixed (grasses and forbs) feeding behavior, they will consume vegetables in addition to cereal crops and grasses (Pfadt 2002, Murray 2016). Indeed, as described in COPR (1982), this species is "decidedly ambivorous" and will "devour and thrive on almost any available plant," including plants from grasslands and crops to hedge mustard and moss. This grasshopper is especially problematic because of its large outbreak potential, with outbreak populations able to reach 80 individuals/yd[2] (Murray 2016). Due to its economic impact, *M. sanguinipes* has often been the subject of population management research (Pickford and Mukerji 1974, Hewitt 1977, Hewitt and Onsager 1983, Pfadt 2002), as well as nutritional and life history studies (Behmer and Joern 2008, Fielding and Defoliart 2008). However, there is still much unknown about this major rangeland pest species' nutrient preferences in field populations and how those compare with long-term lab colonies.

When given a choice, most animals will self-select the blend of nutrients that maximizes growth and reproduction (termed "intake target" or IT), which arises from the Geometric Framework for Nutrition, or GFN (Raubenheimer and Simpson 1997, Raubenheimer et al. 2009, Simpson and Raubenheimer 2012). GFN research spans many taxa and has demonstrated that numerous insect populations, particularly lab colonies, exhibit a consistent IT. For example, *Plutella xylostella* (Linnaeus, 1758) caterpillars select a macronutrient ratio similar to ancestral colony conditions (3.25 mg protein: 3.00 mg carbohydrates), and that ratio corresponds with a narrow and high peak in performance (Warbrick-Smith et al. 2009). Several migratory grasshopper and locust species select a carbohydrate-biased IT, consuming up to double the amount of carbohydrate relative to protein, which increases growth, survival, and migratory capacity (Behmer and Joern 2008, Cease et al. 2012, 2017, Le Gall et al. 2019, Le Gall et al. 2020a, b, Talal et al. 2020).

ITs are dynamic, and there is some evidence they can shift through ontogeny, with activity, and in response to environmental factors (Raubenheimer and Simpson 1997, Simpson and Raubenheimer 2012, Lawton et al. 2020). There is also evidence that meeting an IT not only maximizes growth performance under optimal conditions, but also aids in survival when faced with toxins and pathogens. *Helicoverpa armiger* (Hübner, 1808) and *Helicoverpa punctigera* (Wallengren, 1860) caterpillars fed on diets that match their IT have lower susceptibility to several *Bt* toxins (Tessnow et al. 2018). *Chortoicetes terminifera* (Walker, 1870), the Australian plague locust, adjusts its IT when faced with a pathogen challenge, and the adjusted IT reduces the grasshopper's susceptibility to the pathogen (Graham et al. 2014). Given how important external factors are on ITs, little research has investigated how these factors influence the relative need for different nutrients and how subsequent shifts in ITs affect the capacity of animals to acquire an optimal diet in nature.

The GFN has been used to analyze long-term and first-generation lab colonies of *M. sanguinipes*. For example, one lab study on 5th instar first-generation lab-reared grasshoppers collected from Arapaho Prairie (Arthur Co., Nebraska) showed that *M. sanguinipes* had a 1:0.96 preferred dietary ratio of protein to carbohydrate (p:c) (Behmer and Joern 2008). Results from another study on two 2nd generation lab populations of grasshoppers from Alaska (1p:0.90c) and Idaho (1p:0.95c) suggested that the ITs of both populations remained similar, though the Alaska population regulated more tightly than the Idaho grasshoppers (Fielding and Defoliart 2008). However, no studies have examined macronutrient preferences of *M. sanguinipes* collected directly from field populations. Understanding

how an organism's nutritional requirements compare to their habitat's macronutrient composition can aid in developing management strategies based on nutrition (e.g., Cease et al. 2015, Le Gall and Tooker 2017, Word et al. 2019, Le Gall et al. 2020a).

The primary goals of this study were to 1) compare the IT of two field populations of *M. sanguinipes* to their given nutritional landscape, 2) compare the IT of these two field populations to the IT of a long-term lab colony, and 3) determine if the lab colony IT maximized performance by restricting grasshoppers to one of five diets varying in p:c ratio. Our null prediction was that a given field population of grasshoppers would have an IT that roughly matched the protein and carbohydrate ratios of plants available to them. We predicted that, relative to the long-term lab colony, the field populations would be more carbohydrate-biased, similar to other field populations of migratory acridids (Cease et al. 2012, Le Gall et al. 2020b, Lawton et al. 2021), perhaps due to increased activity or stressors. Finally, we hypothesized that grasshoppers select a diet that provides optimal performance and, thus, predicted that the p:c ratio of the diet on which the lab colony performed the best in restricted diet experiments would be similar to the lab colony IT.

Methods

Field population studies

Studied species and studied area.—*M. sanguinipes* is an abundant rangeland grasshopper with a range that extends throughout most of the United States and into Canada (Pfadt 2002, Otte 2013). This grasshopper has 5–6 nymphal instars, and nymphal development takes 35–55 days. The 5th instar is easily recognized by the wing buds shifting from small buds on the side to larger buds along the dorsal side of the grasshopper (Pfadt 2002). We consulted with USDA surveyors in Idaho to determine locations with sufficient populations of *M. sanguinipes* for this study. Based on their surveys, we selected two locations. Location 1 was a 3.9 ha plot of Bureau of Land Management (BLM) cattle grazing land in Bliss, Idaho (see Table 1 and Suppl. material 1: Fig. S6 for specific locations) and was mostly dry rangeland with forbs, some light woody vegetation, and an abundance of grasses. Location 2 was a 1.2 ha plot of private non-grazed property in Boise, Idaho (see Table 1 and Suppl. material 1: Fig. S7 for specific locations). The vegetation was not irrigated and had a similar plant community composition to Bliss. Grasshoppers were collected from the field in 2018 on June 26 for Bliss and July 2 for Boise.

Plant collection.—We sampled plants from each location concurrent with the sampling of grasshoppers. We randomly selected five collection plots per site using a random number generator. Plots were 5 m × 5 m, and we mapped the plots for each location using Google Maps (2021a, b) (Suppl. material 1: Figs S6–S7). In each plot, we visually estimated the percent ground cover at ground level for grasses, forbs, and shrubs using the relevé method (Poore 1955, Minnesota Department of Natural Resources 2013). We measured humidity, wind speed, and temperature at each plot with a digital anemometer (Ambient weather WM-4). To broadly assess the nutrient contents of the plots, we collected living leaf material (the part generally eaten by grasshoppers) eaten by the most abundant species from each functional group. Some plant species were completely dead in a plot and were not collected. Plants were stored in paper bags, air-dried for three days, and then further dried for another 24 hours in a 60°C oven.

Grasshopper collection.—We collected grasshoppers throughout the field locations using sweep nets. All specimens were identified to species by KCR, who has over 40 years of experience with the identification of rangeland grasshopper species. We recorded the sex and developmental stage of grasshoppers upon capture, and early 5th (final) instar grasshoppers were kept for the experiment. We separated grasshoppers by sex and kept them in separate cages with a selection of plants wrapped in wet paper towels from the collection site for 24 hours prior to starting experiments. All collected specimens were then brought to a private ranch southeast of Boise for the experiments.

Intake targets.—We started IT experiments for field populations on June 27 for Bliss and July 3 for Boise. To determine self-selected ITs, we used a restricted diet choice experiment that gave grasshoppers a choice between two complementary diets. We had two treatment groups where one diet was kept constant between the two treatments and the other diet had a variation in the protein and carbohydrate ratio so we could ensure the ITs were not a result of random eating. Twelve male and 12 female grasshoppers were placed into each treatment group for a total of 48 grasshoppers from each population. Both treatments received two complementary (high protein (p): low carbohydrate (c) and low p: high c) isocaloric diets. By percentage of dry mass, Treatment A contained 7p:35c and 28p:14c, while Treatment B contained 7p:35c and 35p:7c. We selected these two different diet pairings so we could determine if grasshoppers were regulating to a specific p:c ratio; if so, grasshoppers from both treatment A and B would end up selecting the same p:c ratio, regardless of their diet pairings. This range of dietary p:c pairings encompassed all but a couple of the most carbohydrate-biased plants, meaning that grasshoppers could reach the same IT on the artificial diets as they could eating field plants. Diets were made based on Dadd (1961) and as modified by Simpson and Abisgold (1985). The protein was a 3:1:1 mix of casein, peptone, and albumen; the digestible carbohydrate (hereafter, carbohydrates) was a 1:1 mix of sucrose and dextrin. All diets contained similar amounts of Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%), and vitamin mix (0.2%).

At the start of the experiments, we weighed the grasshoppers and placed them into individual plastic cages (17.5 × 11.8 × 4.3 cm), perforated for airflow and with a water tube and the two diet dishes. Five extra cages without grasshoppers were set up containing a dish of each of the three diets and a water tube to record water mass gained by the diet during the experiment. The grasshoppers were in their treatment for 48 hours in Bliss (ended early due to high mortality) and 72 hours in Boise. We checked the cages daily and recorded any mortality or molting, and additional water was added as needed. Grasshoppers that died during the experiment were not included in our final analyses. At the conclusion of the experiment, we recorded grasshopper mass. We weighed the diet dishes before and after the experiment and calculated the amount of protein and carbohydrate consumed by each grasshopper, accounting for any water mass gain in the diets by adjusting the initial weights of the diets based on the average proportion change found in the diets kept in the extra five cages.

We recorded temperature and relative humidity in the cages using iButtons (Thermochron, Maxim Integrated). Cages were kept inside a garage on, approximately, a 15h/9h light/dark cycle directly correlating to the natural light/dark cycle at the time and at ambient shade temperature. For the Bliss experiments, the average daytime (6:10 am–9:30 pm) temperature and humidity

+/- SEM were 24.18 +/- 0.26°C and 27.18% +/- 0.53% and average nighttime (9:31 pm–6:09 am) values were 24.50 +/- 0.21°C and 27.64% +/- 0.40%. For the Boise experiments, daytime averages were 25.87 +/- 0.22°C and 22.94% +/- 0.43%; nighttime averages were 24.86 +/- 0.22°C and 28.37% +/- 0.66%.

Chemical analyses.—For each vegetation survey 5 m × 5 m plot, we mixed leaves from plants of the same functional group (grasses and forbs) together and ground them into a fine powder using a ball mill (30 s at 30 Hz using a Retsch MM 400 ball mill) for a total of 5 samples per functional group per field site. The carbohydrate content of each sample was determined using the phenol-sulfuric acid carbohydrate assay (DuBois et al. 1956), and the protein content was determined using the Bradford protein assay (Bradford 1976). Shrubs were excluded from the analyses, as they do not typically make up the natural diet of this grasshopper (Pfadt 2002).

Lab studies

Lab colony.—The Arizona State University *M. sanguinipes* lab colony used in these experiments originally came from eggs from a USDA ARS lab colony based in Sidney, MT. The USDA colony was established in approximately 1970 from non-diapausing *M. sanguinipes* from Arizona and maintained as such over the decades mostly on an artificial diet, supplemented with head lettuce. Between 2000 and 2005, the colony was hybridized with individuals from the Agriculture Agrifood colony in Saskatoon, Canada. In approximately 2005 and 2013, genetic material was added to the colony by mating with field-collected female non-diapausing *M. sanguinipes* collected from Arizona. Starting in 2017, the colony was moved to Arizona State University with funding from the USDA's nearby Science and Technology Phoenix Laboratory for the purpose of local lab experiments. The colony has been kept at 32.2°C during the day and 25°C at night, and the humidity fluctuates from 20–50% RH with a 14h:10h light/dark cycle. The colony is reared on a combination of organic romaine lettuce, wheatgrass, and wheat bran. Overall, the lab colony had access to a wide range of protein and carbohydrates: two food sources were carbohydrate-biased and the third was protein-biased. The mature wheat grass available to the colony was analyzed using a phenol-sulfuric acid carbohydrate assay (DuBois et al. 1956), and the protein content was determined using the Bradford protein assay (Bradford 1976) and found to be 27.62% ± 6.467 protein (Mean % ± SEM) and 14.24% ± 1.78 carbohydrate (Brosemann et al. unpubl. data). Reviews of USDA databases show that the romaine lettuce is approximately 1.24% protein and 3.24% carbohydrate, and the wheat bran is approximately 15.6% protein and 64.5% carbohydrate (FoodData Central 2021b).

Self-selected IT and performance curves.—We determined the self-selected IT and performance of the lab colony using choice and no-choice diet experiments split into three consecutive blocks using three consecutive cohorts of fifth instar nymphs. Each block contained all treatment groups for the choice and no-choice experiments, with eight grasshoppers per treatment group (four males and four females) for a total of 56 grasshoppers in each block and a total of 168 grasshoppers for the full experiment (24 grasshoppers in each treatment). Grasshoppers were removed from colony cages during the 4th instar stage and provided the same food from the colony cages until they molted into 5th instars. On the first day of the 5th (final) instar, grasshoppers were placed into the experiment.

The experiments were run in an environmental chamber kept at 32.2°C during the day and 25°C at night, and the humidity fluctuated from 20–50% RH with a 14h:10h light/dark cycle.

For the lab choice diet experiments, we had two treatments: Treatment A: 7p:35c and 28p:14c, and Treatment B: 7p:35c and 35p:7c. For the lab no-choice diet experiments, we restricted individual grasshoppers to one of the five isocaloric diets (7p:35c, 14p:28c, 21p:21c, 28p:14c, and 35p:7c). We weighed grasshoppers and placed them in individual perforated plastic cages (18.891 cm × 13.494 cm × 9.525 cm) with a similar set-up to that used to measure the field population IT. The rest of the methods are identical to the field population IT, with the exception that the experiments ran for 14 days, and food was changed every 3 days.

For the performance analyses, the specific growth rate (μ) was calculated for each grasshopper using the following formula: $\mu = \ln (M_1/M_2)/dt$, where M_1 is the initial mass of the grasshopper, M_2 is the final mass of the grasshopper, and dt is the days between weight measurements. Total days survived and total days spent in the 5th instar prior to molting to an adult were calculated. The proportion of grasshoppers surviving or molted was calculated for each day of the experiment.

Statistical analyses.—We tested all data for assumptions of normality and homoscedasticity implicit in parametric tests. We transformed any of the data that did not meet these requirements prior to analyses, or non-parametric analyses were used. Outliers were removed from the analyses. We performed analyses for ITs using IBM SPSS Statistics 24 (2017), with all other analyses performed using R 3.5.1 (2020). To determine IT differences among different populations, we used generalized additive models to detect nonlinear trends as discussed by Lawton et al. (2021), which ultimately resulted in a generalized linear model (family: multivariate normal distribution, link: identity).

Results

Field population studies

Plant collection.—The Bliss location was primarily composed of dead vegetation cover, with some live vegetation in each sample plot. The average percent ground covered by dead and living grasses was 57.4 ± 12.137 (Mean % ± SD), the average percent ground covered by dead and living forbs was 0.6 ± 0.548, and 2–15% ground covered by dead and living shrubs when shrubs were present (Table 1). The Boise location consisted of more live

vegetation than the Bliss location. The average percent ground covered by dead and living grass was 39 ± 12.942, the average percent ground covered by dead and living forbs was 25 ± 11.726 (Mean % ± SD), and there were no shrubs present at this site (Table 1).

Intake targets.—Both field populations (Bliss and Boise, ID) of grasshoppers ate non-randomly from the two diet dishes. There was a significant interactive effect of sex and treatment on carbohydrate and protein consumption for the Bliss population, but no significant effect on the Boise population. The Boise, population tended to regulate its IT more tightly (Fig. 1, Table 2). The ITs of both field populations were carbohydrate-biased, with population 1 consuming 1p:3.5c and population 2 consuming 1p:2.1c (Fig. 2A, B).
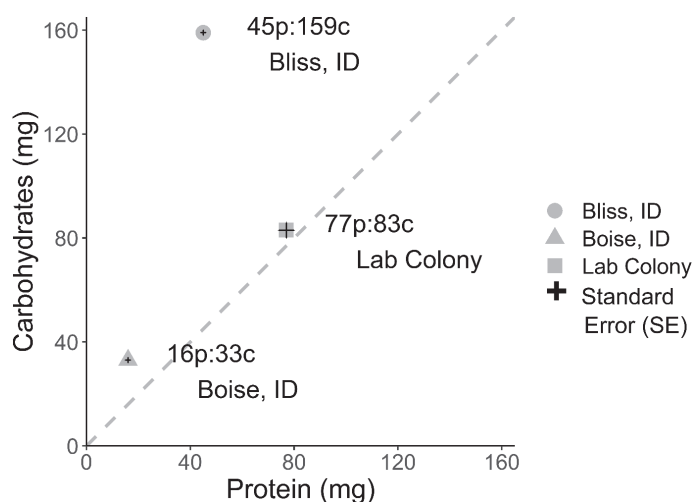

Fig. 1. Field populations and lab population ITs. Average intake target (+/- SEM) for two field populations, Bliss and Boise, ID, and the lab colony. The dashed line represents a 1:1 ratio of protein and carbohydrates, and the crosses on the data points represent SE.

Chemical analyses.—The macronutrient ratios of the sampled plants in the Bliss and Boise locations were close to the self-selected ITs for both populations, as indicated by the small Euclidean distances between the sampled plants' p:c ratio and the IT (Average Euclidean distances of combined plants ± SE: Population 1 = 0.021 ± 0.006; Population 2 = 0.010 ± 0.002). Using a Wilcox rank-sum test, we found there was no significant difference in the average Euclidean distances, calculated between the sampled

Table 1. Field sites. Habitat and environmental data from field plots in Idaho (see Suppl. material 1: Figs S6–S17 for maps and habitat plots). Plot location coordinates are based on WGS84.

| Plot	Date	Time (PM)	Latitude, Longitude	Temp °C	RH %	Wind m/s	Live Veg %	Total Veg %	Rock %	Litter %	Dung %	Grasses % Ground Cover	Forbs % ground cover	Shrubs % ground cover
Bliss, ID 1–1	26-Jun	12:00	42.981492, -114.9288965	26.1	26.1	4.8	<5	75–100	0	0	<5	74	1	0
Bliss, ID 1–2	26-Jun	12:25	42.9813708, -114.929146	26.3	22.9	5.5	<5	50–75	<5	0	<5	59	1	0
Bliss, ID 1–3	26-Jun	12:50	42.9814282, -114.9310661	27.1	18.8	6.0	<5	50–75	<5	0	0	55	0	2 (dead)
Bliss, ID 1–4	26-Jun	1:05	42.9811249, -114.9307657	28.5	18.1	4.6	5–25	50–75	0	0	<5	40	0	15
Bliss, ID 1–5	26-Jun	1:30	42.9799997, -114.9294353	28	17.8	5.2	<5	50–75	0	0	<5	59	1	0
Boise, ID 2–1	2-Jul	1:00	43.3943539, -115.9510955	22.7	28.7	4	<5	50–75	0	0	0	55	5	0
Boise, ID 2–2	2-Jul	1:20	43.394394, -115.9512032	24	28.6	4.6	25–50	75–100	0	0	0	50	30	0
Boise, ID 2–3	2-Jul	1:40	43.3944951, -115.9512009	23.5	28.7	4.5	25–50	50–75	0	<5	0	30	30	0
Boise, ID 2–4	2-Jul	2:00	43.3946555, -115.9525402	23.4	26.7	6	25–50	50–75	0	<5	0	25	35	0
Boise, ID 2–5	2-Jul	2:15	43.3940407, -115.9512009	24.2	23.4	5.3	25–50	50–75	0	<5	0	35	25	0
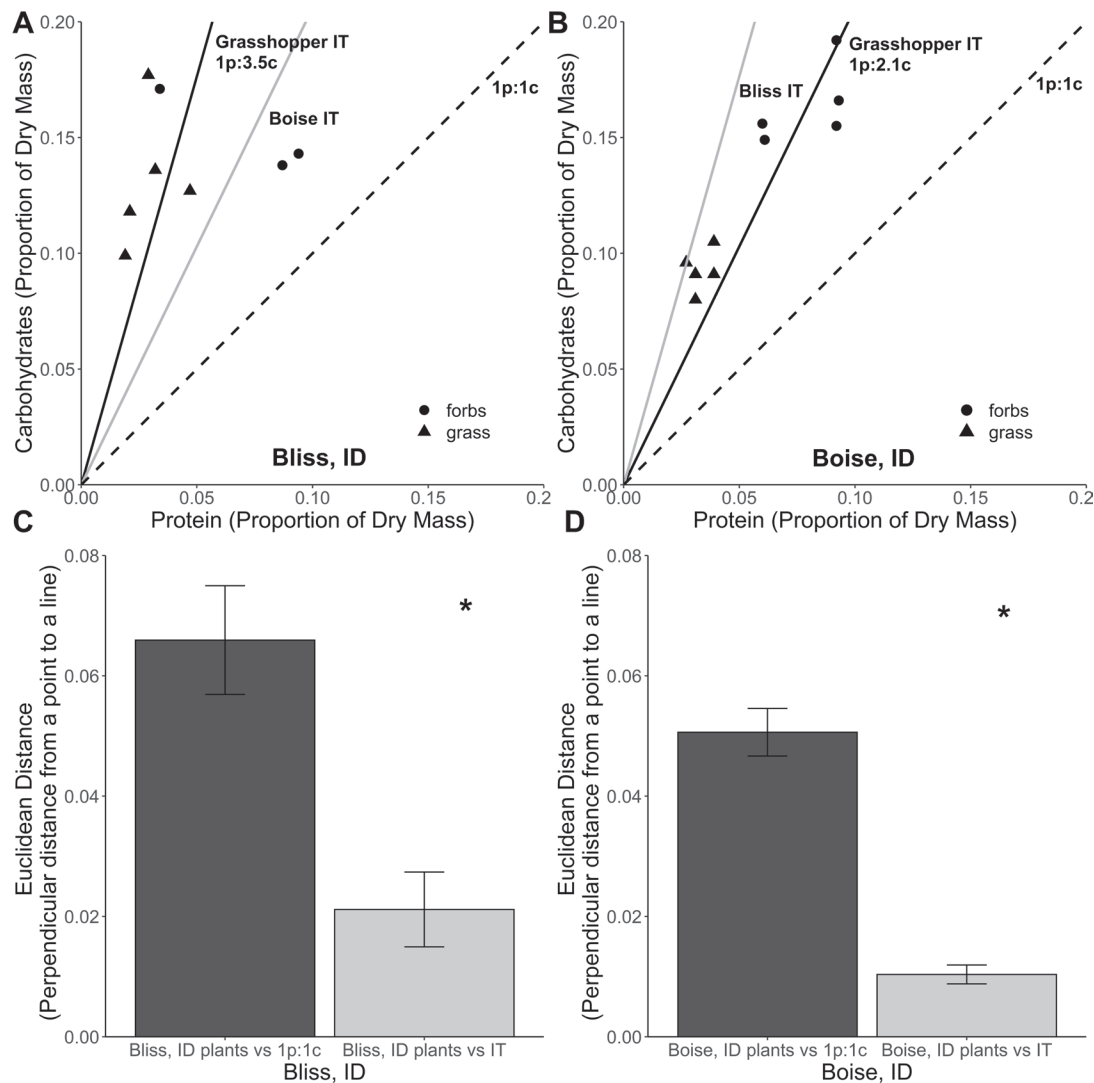
Fig. 2. Field IT compared to nutritional landscape. **A, B.** Grasshopper intake targets of the field populations (black solid line) alongside the nutrient contents of grasses (triangles) and forbs (circles) collected from the same fields. The grey solid line represents the intake target from the other field population. The dotted line represents a 1p:1c ratio. **C, D.** The average Euclidean distance between the plants (triangles and circles in A and B) and either the grasshopper IT from each location or the 1p:1c line. * denotes a significant difference between the Euclidean distances calculated from the IT and the 1p:1c line.

plants and the IT (Fig. 2A, B), between populations 1 and 2 (w = 53 p = 0.274). We found that when comparing the Euclidean distance of the plants calculated with the population IT versus calculations using the 1p:1c ratio, there was a significant difference in the Euclidean distances. In this case, the Euclidean distance of the plants was lower when calculated to the population IT than to a 1p:1c ratio for both Bliss (t = -4.0827 df = 14 p = 0.001) and Boise (t = -9.464 df = 11.773 p = 7.536$^{e\,-07}$) field sites (Fig. 2C, D), suggesting that the sampled plants macronutrient rations were closer to the IT than to a balanced ratio.

Lab studies

Lab self-selected intake targets.—We calculated ITs for each of the three blocks of the experiment, and t-tests were used to determine if both treatment groups were regulating consumption or eating randomly and were compared to each other. Grasshoppers given Treatment A ate significantly different portions from the high carbohydrate and high protein dishes, overall consuming slightly more from the high protein dish, and appeared to regulate their consumption (Table 3). Grasshoppers given Treatment B ate equally from both dishes, but grasshoppers from both treatments arrived at similar p:c ratios (Table 3). We used a full MANCOVA with sex and diet pairing as independent variables and total carbohydrates and protein eaten as separate dependent variables. We included block as a random factor. There was a main effect of diet treatment (group) and block (Table 2). For simplicity, we report the overall ITs for males and females combined in Fig. 1, which was 0.77 mg (+/-3) protein to 83 mg (+/-3) carbohydrates. We also used generalized linear model methods to determine if the ITs of our lab colony were significantly different from ITs of the Bliss and Boise populations. Using the first three days of the lab colony IT experiment, we found that our lab colony's carbohydrate and protein consumption was significantly different from the Bliss population, but not the Boise population (Suppl. material 1: Table S1). We found that the Bliss population was consuming more carbohydrates and protein than both the Boise population and our lab colony (Suppl. material 1: Figs S1–S4).

Table 2. IT Statistics. MANCOVA statistics for field and lab intake target (IT) studies testing the effects of sex, diet treatments, and cohort (lab only) on the total amount of protein and carbohydrates consumed.

Population	Effect	Pillai's trace Value	F	Error df	Sig.
	Intercept	0.972	364.1	21.000	0.000
	Initial mass	0.146	1.792	21.000	0.191
Bliss, ID	Sex	0.255	3.599	21.000	0.045
	Diet pair	0.662	20.580	21.000	0.000
	Sex * Diet pair	0.008	0.086	21.000	0.918
	Intercept	0.037	0.774	40.000	0.468
	Initial mass	0.341	10.330	40.000	0.000
Boise, ID	Sex	0.008	0.155	40.000	0.857
	Diet pair	0.126	2.890	40.000	0.067
	Sex * Diet pair	0.005	0.108	40.000	0.898
	Intercept	0.474	13.070	29.000	0.000
	Initial mass (covariate)	0.029	0.438	29.000	0.649
	Sex	0.152	2.606	29.000	0.091
	Cohort	0.510	5.141	60.000	0.001
Lab Colony	Diet pair	0.215	3.964	29.000	0.030
	Sex * Cohort	0.029	0.220	60.000	0.926
	Sex * Diet pair	0.033	0.502	29.000	0.610
	Cohort * Diet pair	0.074	0.578	60.000	0.680
	Sex * Cohort * Diet Pair	0.212	1.783	60.000	0.144

Table 3. IT paired t-tests to determine if there was equal consumption from both diets in each treatment group.

Population	Paired t test	t	p	df
Bliss, ID	a 7p:35c + 28p:14c	112.520	<0.001	12
Bliss, ID	b 7p:35c+ 35p:7c	123.330	<0.001	13
Boise, ID	a 7p:35c + 28p:14c	-20.384	<0.001	22
Boise, ID	b 7p:35c+ 35p:7c	-51.120	<0.001	22
Lab	a 7p:35c + 28p:14c	-4.008	0.006	21
Lab	b 7p:35c+ 35p:7c	-0.008	0.994	20

Lab performance.—Using the no-choice experiments, we determined the specific growth rate (Fig. 3A) and absolute growth rate (Suppl. material 1: Fig. S5), as well as the proportion of individuals surviving and successfully molting on diets that differed in p:c ratio. Because the data did not meet the assumptions for an ANOVA, we used the non-parametric Kruskal-Wallis test to determine if there was a significant effect of diet on specific growth rate (χ^2 = 32.41, df = 4, p = 1.576, e $^{-06}$). We used the pairwise Mann-Whitney non-parametric post hoc tests to determine that there were no significant differences between all the treatment groups, except 7p:35c, which was significantly lower than all other treatments (Fig. 3A).

We analyzed the final proportion molted and proportion survived using Fisher's exact test of independence since grasshoppers were removed from the experiment after they had either molted or died. There was no significant difference in survival among the treatment groups (p = 0.4298). However, there was a significant difference among treatment groups regarding the final proportion of grasshoppers successfully molted (p = 0.003), with the 7p:35c treatment group being significantly different from all other diet treatments. Overall, diet treatment 7p:35c had the lowest proportion of grasshoppers survive and molt (Fig. 3B–C). All diet treatments except 7p:35c had a large increase in molts by day 6 or 7, whereas diet treatment 7p:35c delayed molting (Fig. 3C).

Discussion

Our long-term lab colony selected a balanced 1p:1c IT, which is similar to previous studies using first (1p:0.96c; Behmer and Joern 2008) and second (1p:0.90c and 1p:0.95c; Fielding and Defoliart 2008) generation lab-reared *M. sanguinipes*. Our lab colony performance experiments supported our hypothesis that *M. sanguinipes* selects an IT range that aligns with high performance, similar to Behmer and Joern (2008) and Fielding and Defoliart (2008). In contrast with the lab populations, we found that *M. sanguinipes*

collected directly from field populations had carbohydrate-biased ITs (1p:2.1c and 1p:3.5c). The field populations may have shifted their ITs to better match their nutritional landscape and/or in response to disease, elevated activity, or other environmental factors (Fig. 2). For example, the Boise landscape had a higher representation of forbs than Bliss, ID, which may have contributed to the slightly less carbohydrate-biased IT of that population (Table 1); further studies are needed to disentangle these potential hypotheses. Understanding how these factors impact ITs will be key to predicting the nutritional physiology of these organisms in different environments, which, in turn, may assist in the development of novel management methods.

There is some evidence from lab-based experiments that populations either adapt or acclimate to their nutritional environment by matching their IT and performance to their ancestral diet (Raubenheimer et al. 2012). For instance, as mentioned briefly earlier, *P. xylostella* moths reared on a single homogenous food with a fixed nutrient composition for about 350 generations exhibited a strong selection for the same nutrient balance as their ancestral colony diet and had a sharp decline in performance when they deviated from that macronutrient balance, showing an extreme food specialization (Warbrick-Smith et al. 2009). A prior study reared the same species for multiple generations on either carbohydrate-rich or poor foods. The selected lines developed the capacity to minimize or maximize body fat accumulation to improve fitness when confined to their treatment diet (Warbrick-Smith et al. 2006). The diets did impact host plant preference for egg laying, but the authors did not test whether the selection experiment shifted ITs.

Results from field-based research, on the other hand, suggest that aligning ITs and performance to match the nutritional landscape is uncommon in the absence of long-term specialization and that physiological status is a better predictor of IT than ambient plant nutrient contents. For example, in West Africa, *Oedaleus senegalensis* (Krauss, 1877), the Senegalese grasshopper, did not shift its IT to match seasonal shifts in plant p:c; instead, ITs correlated poorly with plant nutrients and varied with age and sex (Le Gall et al. 2021). In Australia, a comparison of a non-migratory and two migratory grasshopper species through space and time revealed that populations of the non-migratory grasshopper had different ITs through space, but it was likely to redress nutrient imbalances from the local environment rather than to match them (Lawton et al. 2021). The migratory species largely maintained the same IT, even when there was a mismatch between their IT and their nutritional landscape, similar to Senegalese grasshoppers (also a migratory species).

In Paraguay, field populations of *Schistocerca cancellata* (Serville, 1838), the South American locust, maintained a carbohydrate-biased IT and only gained mass when fed the most carbohydrate-biased plants, despite being in a quite protein-biased landscape (Talal et al. 2020). This result corroborates earlier re-
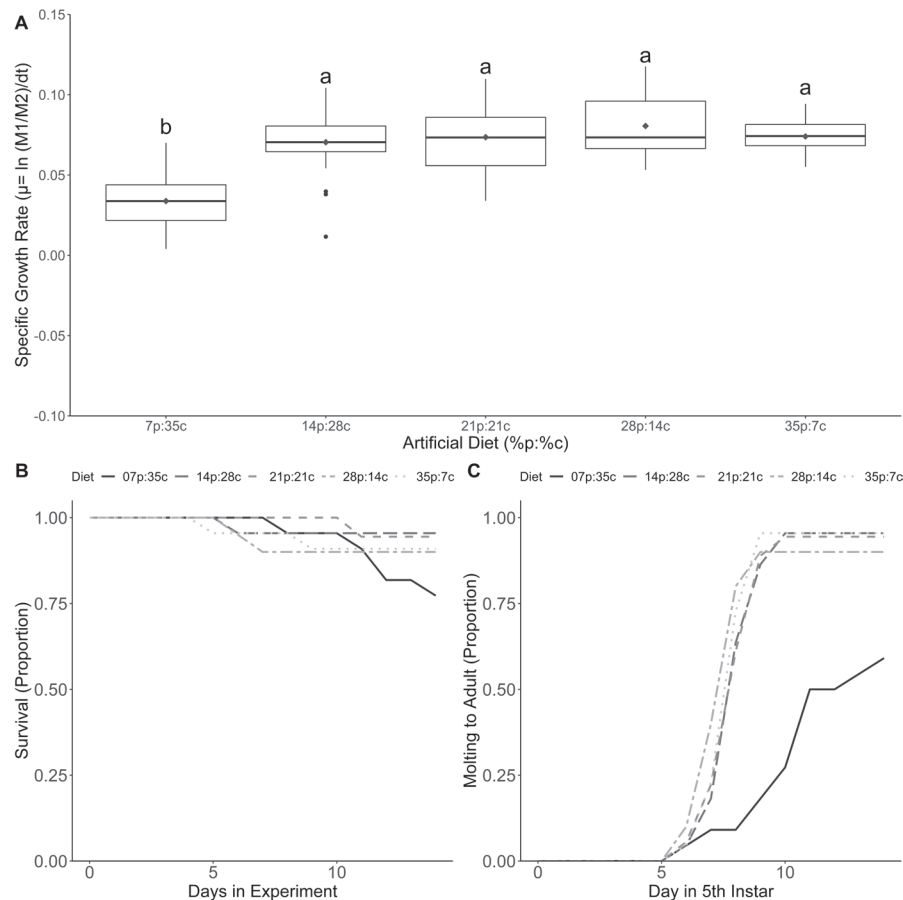
Fig. 3. Performance experiments. Survival and specific growth rates of grasshoppers from the long-term lab colony no-choice diet experiments. **A.** The specific growth rates for each diet treatment. Diamonds indicate the mean and bolded lines indicate the median. Boxes are +/- 25%, lines represent minimum and maximum values excluding extreme values, and dots indicate data points > 1.5 farther from the box edge than the interquartile range. Lower case letters indicate differences from Mann-Whitney post-hoc analyses. **B.** The proportion of grasshoppers surviving through time on each diet treatment. Most diet treatments did not have individuals die until the 5th day of the experiment, and most treatments except 7p:35c had minimal deaths (although there were no significant differences among treatments). **C.** Proportion of grasshoppers molting to adults over time. Most of the diets saw increases in molting from days 5–7, except diet treatment 7p:35c, which was delayed and had the least number of grasshoppers successfully molt (significantly different from all other treatments).

search indicating that locusts and migratory grasshoppers require a carbohydrate-biased diet to undergo long-distance migration (Cease et al. 2017) and that low p: high c environments support population growth (Cease et al. 2012, Word et al. 2019, Le Gall et al. 2020a, b). In the face of shifting environments, organisms and populations will acclimate, evolve, move, or perish. Collectively, these studies suggest that migratory species may rely on migration or post-ingestive mechanisms to regulate nutrient balance in the face of environmental change and that physiological constraints in nature limit matching a population's IT to its nutritional landscape except when under strong selection.

The current study using the migratory grasshopper provides some support for both non-mutually exclusive hypotheses: that population IT is shaped by local nutritional landscape and by physiological status. Under standard rearing conditions, the colony has *ad libitum* access to foods encompassing a broad macronutrient range: wheat seedlings (1.9p:1c), romaine lettuce (1p:2.6c), and wheat bran (1p:4.1c) (Brosemann et al. unpubl.; FoodData Central 2021a). Therefore, it is unlikely that there was strong selective pressure to develop a narrow IT based on the lab diet, in con-

trast to the long-term moth colony reared on a single food choice (Warbrick-Smith et al. 2009). The lab colony likely arrived at a 1p:1c IT (Fig. 1) because it maximizes performance in a lab colony. While there was not a narrow performance peak, the IT was within the range where the lab colony maintained a high growth rate and fast development time across the 1:1, 1:2, 2:1, and 5:1 p:c diets (Fig. 3).

The field populations both had carbohydrate-biased ITs that matched their local environments. Although the 1p:2c Boise population IT could have been reached based on the plants available in both field environments, the 1p:3c IT that the Bliss population selected could only have readily been reached in the Bliss location (Fig. 2). Furthermore, neither field environment would have supported populations to select for the 1p:1c IT that the lab population selected. The Boise location had an average of 25% forb ground cover, and the Bliss location had <1% (Table 1), indicating that the few forbs closer to 1p:1c were sparse in the landscape and that the Bliss population was in an extremely carbohydrate-biased landscape. Therefore, it is possible that the grasshopper field populations, particularly for Bliss, were under some selective pres-

sure to have carbohydrate-biased ITs to align with their nutritional landscape. However, all tests of lab colony ITs of *M. sanguinipes* nymphs performed to date resulted in a narrow range (1p:0.90c to 1p:1c) regardless of being first or second generation, being in a colony since 1970, or being originally collected from Alaska, Idaho, Nebraska, or Arizona (Behmer and Joern 2008, Fielding and Defoliart 2008; Fig. 1). Therefore, it is unlikely that the alignment between IT and local plant nutrients that we measured in this study represents local adaptation, though it could be evidence of an evolved ability to plastically respond to a restricted diet.

Many environmental factors can influence herbivore physiology and result in shifting the IT, such as activity level and pathogens. For example, *Locust migratoria* (Linnaeus, 1758), the migratory locust, increased carbohydrate, but not protein, consumption following 120 min of tethered flight (Raubenheimer and Simpson 1999). Nutrient balance affects insect immune function, and thus their ability to survive sickness and infections (Ponton et al. 2011a, b; Graham et al. 2014, Deans et al. 2017). Different immune components may be heightened by diets with different macronutrient contents, as was the case for *Spodoptera littoralis* (Boisduval, 1833) caterpillars (Cotter et al. 2011). Furthermore, Graham et al. (2014) found that Australian plague locusts that selected more carbohydrate-biased diets were better able to fight infections from the fungal pathogen *Metarhizium*. ITs that are highly carbohydrate-biased, such as we observed in our study's Bliss, ID population, could be indicative of grasshoppers selecting this diet to fight sickness or infection. Indeed, we found that the Bliss population suffered significant mortality during the experiment and prior to the start of the experiment. When we completed the setup of the Bliss population experiment, approximately 30% of the grasshoppers we had collected the previous day were dead. The Bliss population also had more cases of grasshoppers losing mass than the Boise population. While not conclusive, these signs indicate that the population may have been suffering from pathogens or parasites. Further studies on field populations are needed to determine if the selected ITs maximize performance in those conditions, as well as the potential mechanisms driving variation in population ITs. However, our data suggest that, at least for these populations, they could achieve their preferred p:c ratio locally.

Understanding the nutritional requirements of rangeland grasshoppers is important not only for understanding what types of vegetation grasshoppers will be most likely to eat but also for developing novel management strategies. For example, the balance of macronutrients is important for immune function in insects and may be important to consider when biopesticides are used for management (Lee et al. 2008, Ponton et al. 2011a, b, Deans et al. 2017, Tessnow et al. 2018). Insects that can meet their ITs in their nutritional landscapes are likely to be less susceptible to biological control strategies—either less susceptible to pathogens or less susceptible to toxins produced by the biological control agent (Graham et al. 2014, Deans et al. 2017, Tessnow et al. 2018). There is evidence that for some insects, nutritional physiology differs between populations. Research on *Spodoptera frugiperda* (J.E. Smith, 1797), studying its susceptibility to *Bt* toxins, showed that for one population, meeting the IT actually increased that population's susceptibility to the toxins. In the other two populations, eating at the IT did not affect individuals' susceptibility to the toxins (Tessnow et al. 2021). This suggests that not only will there be differences in how IT relates to performance and survival between species, but there could also be differences among populations, so analyzing populations' nutritional physiology and ecology is critical to any management strategy. Understanding how the nutritional landscape interacts

with an organism's IT and macronutrient requirements is going to be important, especially with more farmers and ranchers turning to biopesticides as means of managing grasshopper outbreaks (Gardner and Thomas 2002). Similar to how there are recommended temperature ranges across which biopesticides are most effective (McNeill and Hurst 2008, Rai et al. 2014, Kim et al. 2019), there should be guidance as to what types of nutritional landscapes will make pests most susceptible to biopesticides.

Another aspect to consider is how biopesticide treatment might affect pest host plant preference, as it could cause the target pests to consume crops and other plants it might not normally otherwise. Biopesticides aside, knowing how pests respond to nutritional landscapes can open pathways for population suppression through agricultural practices. For example, for locusts and migratory grasshoppers that thrive in low nitrogen environments (Cease et al. 2012, Word et al. 2019), the nutritional landscape could be altered through soil amendments, crop rotations, or other practices that increase soil organic matter and nitrogen availability; this would, in turn, increase the plant protein: carbohydrate ratio and suppress pest populations (Cease et al. 2015, Word et al. 2019). To support the development of sustainable management options, future research should study how biopesticide challenges affect the nutritional demands of *M. sanguinipes* and if this species can alter its diet to decrease its susceptibility. As with the Australian plague locust, such research would add greater understanding to the potential relationship between the nutritional physiology of grasshoppers and biopesticide efficacy, leading to more diverse, sustainable, and efficient management options.

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Supplementary material 1

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Data type: Environmental data

Explanation note: **Table S1.** Field sites. Habitat and environmental data from grasshopper field study plots in Idaho. Plot location coordinates are based on WGS84. **Field Diet Choice Data Set.** Diet choice experimental data from Field locations in Boise and Bliss Idaho. **Lab Diet Experiment Data Set.** Lab Diet choice and restricted diet experimental data sets. **Plant Macronutrient Data.** Data set with the carbohydrate and protein content of plants sampled from the field sites in Boise and Bliss Idaho. **Supplementary figures and tables covering additional data.**

Link: https://doi.org/10.3897/jor.26.61605.suppl1

Discovering insect species based on photographs only: The case of a nameless species of the genus *Scaria* (Orthoptera: Tetrigidae)

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Abstract

A heated debate on whether a new species should be described without a physical specimen, i.e., by designating a photographed specimen to serve as a holotype, has been ongoing for a long time. Herewith, without nomenclatural actions, a new species of the Batrachidein pygmy grasshoppers belonging to the genus *Scaria* Bolívar, 1887 is identified from the Andean rainforest in Peru. This species is clearly different from all its congeners by morphology and coloration. Two individuals of this peculiar species are known only from the photographs found on iNaturalist. The species has not been observed since 2008 when the photographs were taken. A short historical overview of the topic is given, illustrating the pros and cons of photograph-based species description. The concepts of names, holotypes, research effort, and conservation are discussed and related to the problem at hand. The current state of the taxonomic community's beliefs regarding this issue is reflected by the authors' three unsuccessful attempts to name this new species.

Keywords

Introduction

Describing species from photographs or illustrations is not a common practice, but it has been done a number of times (Fricke and Kacher 1982, Welch et al. 1986, Jonkers and Roersma 1990, Wallach and Jones 1992, Jones et al. 2005, Sinha et al. 2005, Robb et al. 2013, Cheng et al. 2015, Marshall and Evenhuis 2015, Ingrisch et al. 2016, Lonsdale and Marshall 2016, Nardelli 2016). Most of the names based on photographs are valid, but exceptions do exist (for an extensive historical overview of this practice, see Krell and Marshall (2017)). Some of these publications have sparked a debate about the validity of describing species in this way. The following paragraphs provide a brief overview of the current state of the debate. Some authors (Timm and Ramey 2005,

Santos et al. 2016) have implied that the International Code of Zoological Nomenclature (ICZN 1999) prohibits naming a species without a physical holotype, which is not correct (see Discussion).

While Marshall and Evenhuis (2015) agree that collecting specimens is "highly desirable" as it allows for consideration of their internal morphology, microscopic and genetic characters and preservation of data for future access, they argue that, in certain cases, the specimens cannot be preserved and are getting harder to come by due to rigorous restrictions on collecting and transporting biological material, finally concluding that "collecting specimens is highly desirable, but it is indeed no longer required" because there is a growing number of high-quality photographs of taxa made by "digital collectors." A prediction is made that "this situation will inevitably force the biodiversity community to adapt to growing numbers of new taxa recognized without benefit of dead, preserved type specimens" (Marshall and Evenhuis 2015). Löbl et al. (2016) consider such a practice dangerous, as it promotes quick capturing of visual data instead of careful observation. They fear that works describing taxa based on an incomplete set of characters (i.e., photographs) may encourage non-experts to try to publish their works, harming the whole field of taxonomy as a result. These fears are considered unfounded by Shatalkin and Galinskaya (2017), as there are indeed very rigid requirements (ICZN 1999) for describing species from photographic evidence. Marshall and Evenhuis (2015) cite Minteer et al. (2014) in claiming that "collecting specimens is no longer required to describe a species..." The work in question, though, presents a limited set of examples (birds, amphibians, and plants) where scientists played a role in the extinction of those animals and suggests using alternative means of sampling endangered species through high-resolution photography, audio recording, and nonlethal sampling. The case has never been made for insects, and Marshall and Evenhuis (2015) themselves agree that such a case would be difficult to make. Whether the cases illustrated by Minteer et al. (2014) even represent the issue was called into question by Krell and Wheeler (2014), but this is beyond the scope of our paper. Löbl et

al. (2016) suggested publishing information about the existence of a new species without naming it. This is certainly possible but effectively discouraged due to the difficulty of finding a journal that would publish such a finding (Amorim et al. 2016). Having exhausted all the alternatives, we do exactly that.

Amorim et al. (2016) also suggest that, while being a fine addition to the process of describing a species, photographic evidence cannot replace the usual process of "collecting, preparing, comparing, describing, and delimiting species, that allows identifications (which are always hypotheses) to be double-checked." We agree with this view but also feel the need to claim that there are and should be certain exceptions where blindly following this protocol could rob the scientific community of valuable knowledge. The immense administrative complexity of establishing a new species based on photographs has prevented us from doing so. An example to illustrate how photography-based taxonomy may introduce chaos is *Presbytis johnaspinalli* Nardelli, 2015, a new monkey species in the subfamily Colobinae, described from pictures of caged animals found on the Internet (Nardelli 2015). The morphological characters provided were not sufficient to clearly differentiate it from similar species, and some (Nijman 2015) have even suggested that the monkeys were bleached by the traders who were trying to make them more visually appealing. Nardelli (2016) responded to the criticism, defending his claims, but the status of *Presbytis johnaspinalli* remains unclear. In the case of *Scaria* sp., it is clear that this is a hitherto unknown species.

In this paper, we describe an unnamed species of a pygmy grasshopper (family Tetrigidae, subfamily Batrachideinae Bolívar, 1887), belonging to the tribe Batrachideini and genus *Scaria* Bolívar, 1887 (according to Cigliano et al. 2020) based on two photographs posted on the iNaturalist website by one of the authors (Roberto Sindaco; https://www.inaturalist.org/observations/9968031) and three previously unpublished photographs taken by Roberto Sindaco at the same time as the original two. The photographs, taken in August 2008 in Peru (Fig. 1), were posted in 2018, and the specimens photographed were identified by the senior author as a new species belonging to the subfamily Batrachideinae. Since the photographs were posted, no one has described the species, nor has there been another recorded sighting of it. Differences in morphology observed from the five photographs may be sufficient to propose a new species within the genus *Scaria* Bolívar, 1887. Despite that, we decide not to establish one. The taxonomic community does not accept photography-based taxonomy for reasons developed above. This is proven by rejections of this very manuscript when the naming of the species was included. Thus, this study aims to report an interesting unknown *Scaria* without nomenclatural actions. The specimens of the unnamed species were found in Peru (Bongará Province, Department of Amazonas, Peru), which is a part of the Neotropical biogeographic region. This area is home to an astonishing number of animal and plant species (Elton 1973), owing such diversity to favorable environmental factors and long evolutionary history (Condon 2008). Considering these facts, it is clear that there is a wealth of species
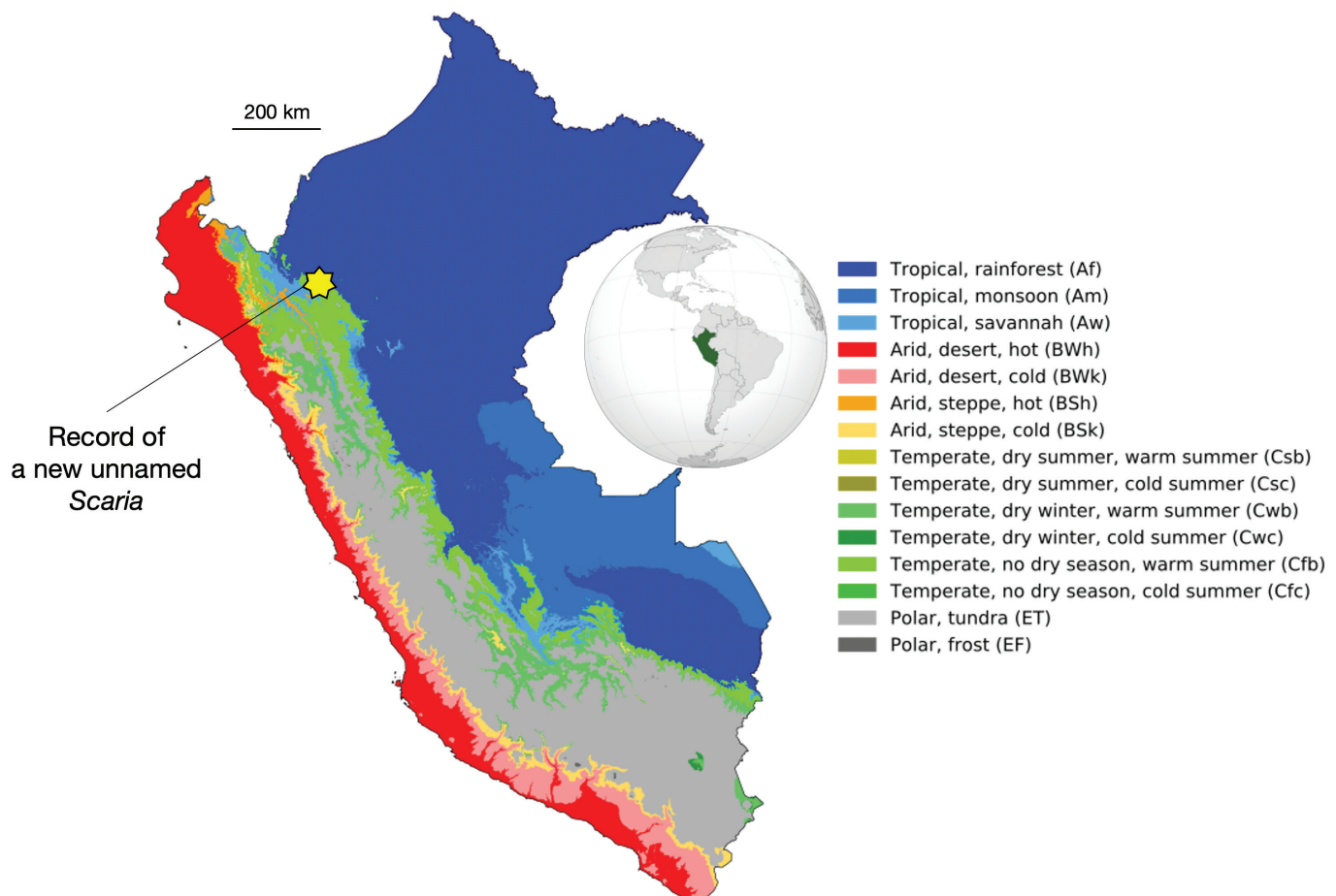

Fig. 1. Position of the only known locality of *Scaria* sp. Peroles near Yambrasbamba, marked with a star on the map of Peru with annotated Köppen–Geiger climate classification. Adapted from Beck et al. (2018).

unknown to science that are waiting to be described. Unfortunately, this impossible task (due to its sheer volume) is becoming even more discouraging due to the ongoing destruction of the entire region (Kehoe 2019). Drastic measures should be taken to reduce our negative impact on the environment because stable ecology is not only the playground of taxonomists, but also the cornerstone of the sustainability of life itself (Brown 1997, Kehoe 2019). The problem of declining biodiversity (Leather 2017, Forister et al. 2019) is only growing more significant, and cases such as this will keep happening. The unwillingness of the taxonomic community to discuss this issue and arrive at a set of rules that will apply to everybody equally is a major obstacle for scientists who find themselves in possession of publishable material in this field. With this paper, we hope to take a step towards an eventual solution.

Materials and methods

Locality information.—The unnamed *Scaria* species is, for now, known only from a single locality in Peru. Peroles, near Yambrasbamba and 1905 m above sea level [5.670300°S, 77.918900°W], is one of the northernmost rainforests of the Peruvian Yungas and connects Amazon and Andes, a peculiar region where mosaics of unique rainforests reach extremely high altitudes. This ecoregion is considered to be in an almost critically endangered state (Beck 2018, WWF 2020). Although the locality is very close to two protected areas—the Cordillera Colan Natural Sanctuary and the Alto Mayo protected forest—the area is severely deforested and looks like a mosaic of patches of forest alternating with deforested areas for grazing cattle. This forest is known for hosting the critically endangered yellow-tailed woolly monkey (*Oreonax flavicauda* (Humboldt, 1812)), one of "The World's 25 Most Endangered Primates" (Shanee et al. 2019).

Taxonomy.—Taxonomy follows Orthoptera Species File (OSF; Cigliano et al. 2020), while nomenclature is in accordance with the International Code of the Zoological Nomenclature (ICZN 1999). Systematics of the genus *Scaria* follows Cadena-Castañeda et al. (2019), who divided it by means of a cladistic analysis into three species groups— groups *S. hamata* (De Geer, 1773), *S. lineata* Bolívar 1887, *S. producta* Hancock, 1907—and *S. laeta* Günther, 1940 without assignment to any species group.

Comparative material examined.—Materials of *Scaria* species identified by Cadena-Castañeda et al. (2019) for which photographs were available and which were used by us for comparison with the new species are included in Table 1. Museum collections acronyms used are as follows: **ANSP**—The Academy of Natural Sciences of Drexel University, Philadelphia, Pennsylvania, USA; **BYUC**—Monte L. Bean Life Science Museum, Brigham Young University, Provo, Utah, USA; **CAUD**—Colección de Artrópodos y otros Invertebrados de la Universidad Distrital Francisco José de Caldas, Bogotá, Colombia; **INPA**—Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil; **JSC**—Collection Josip Skejo, Zagreb, Croatia; **MNCN**—Museo Natural de Ciencias Naturales, Madrid, Spain; **MRSNT**—Museo Regionale di Scienze Naturali di Torino, Torino, Italy; **NMW**—Naturhistorisches Museum Wien, Vienna, Austria; **SMDT**—Staatliches Museum für Tierkunde, Dresden, Germany; **UFAM**—Coleção Zoológica Prof. Paulo Bührnheim, Universidade Federal do Amazonas, Manaus, Brasil.

Morphological terminology.—Terminology of morphological characters follows Rehn (1904), Grant (1962), and Tumbrinck

(2014). Batrachideinae is a subfamily with well-defined synapomorphies: 1) antennae usually more than 20 antennomeres, 2) anterior and mid femora with dorsal furrow, 3) female's spermatheca with two diverticula, 4) square-shaped paranota, 5) frontomedial projection in the form of a spine, 6) fastigium of the vertex continuously and uniformly curved and marginally projected above the compound eyes, and 7) (usually) maculated tegmina. These characters are taxonomically reliable and have already been thoroughly discussed by several authors who have also listed exceptions (Bolívar 1887, Rehn 1904, Grant 1962, Tumbrinck 2014, Tumbrinck and Skejo 2017). Division of the genus *Scaria* into four species groups was supported by differences in the I) morphology of pronotum, II) male and female terminalia, III) head morphology, IV) general appearance of the anterior spine of pronotum, and V) body coloration. Key for identification of the species groups of genera *Scaria* was given in Cadena-Castañeda et al. (2019). Cadena-Castañeda et al. (2019) state that characters such as postocular stripe and tegmina maculation might represent homoplasic characters but are still useful in the identification key.

Artificial intelligence (AI) enhancement of photographs.—An AI service (letsenhance.io) was used to enhance the photographs from iNaturalist. We used the "smart enhance" setting with 2× upscaling. These enhanced photographs were used to discern small details, in combination with the original photographs, in order to avoid any potential visual artifacts in the upscaled photographs (Fig. 2). AI photography enhancement has proven to be a very useful tool in our case. We would like to note that it should be used with a measure of precaution, as the tool is not perfect, and visual artifacts in the upscaled photographs may appear. These problems are easily noted and avoided by people with experience with, in this case, animal morphology and anatomy.

Results

Taxonomy

Family Tetrigidae Rambur, 1838
Subfamily Batrachideinae Bolívar, 1887
Tribe Batrachideini Bolívar, 1887

Genus *Scaria* Bolívar, 1887

Type species.—*Scaria hamata* (De Geer, 1773).

Composition and distribution.—With this unnamed species, genus *Scaria* currently includes 13 species. All the species inhabit South America, with only one species (*S. fasciata*) reaching central America (e.g., Panama and Nicaragua) (Hancock 1907, Cadena-Castañeda et al. 2019). The genus is currently divided into four species groups (see Table 1). Members of *Scaria* inhabit the Amazon rainforest and its vicinity.

Generic identification of the unnamed species.—Grant (1956) listed three characters as differentiable between *Scaria* and newly established *Rehnidium* Grant, 1956: 1) morphology of female subgenital plate, 2) smaller body size of *Rehnidium* members, and 3) frontal costa more protrusive in *Rehnidium* than in *Scaria*. Cadena-Castañeda et al. (2019) added a few more characters, among them 4) *Scaria* is generally slenderer than *Rehnidium*; 5) tegmina of *Scaria* members are black, while they are brown in all other

Table 1. Material of *Scaria* species examined and organized by species groups by Cadena-Castañeda et al. (2019) containing brief information on the distribution.

Species	Material examined	Distribution
***Scaria* (*hamata*) group**		
S. boliviana Bruner, 1920	(1) **NT**: 1♀ Bolivia: La Paz: Nor Yungas, Yolosa (S16.24, W67.74) 1260 m a.s.l., 13.XII.2008. leg. S.M. Clark (BYUC); (2) 1♂ Bolivia: La Paz: Nor Yungas, Pacallo, (S16.21, W 67.79) 29.IV. 2005. leg. S.M. Clark & R.L. Johnson (BYUC)	Bolivia (La Paz, Santa Cruz)
S. granti Cadena-Castañeda, Mendes & Silva, 2019	**HT**: 1♂ Brasil: Acre: Bujari, Floresta Estadual Antimary (S9.33, W68.32) 27.VII. 2016. leg. J.A. Rafael (INPA)	Brazil (Acre state: Bujari, Floresta Estadual Antimary)
S. hamata (De Geer, 1773)	(1) **NT**: 1♂ Brasil: Amazonas: Universidade Federal do Amazonas (UFAM) (S3.09, W59.97) 03.II.1979. leg. J.A. Rafael (INPA); (2) 1♀ Ecuador: Zamora-Chinchipe, Río Zamora valley, El Pangui, Maralí (S3.71, W78.55) 900 m a.s.l. 29.XII. 2009. leg. H. Braun, det. J. Skejo (JSC)	Brazil (Amazonas state); Ecuador
S. jonasi Cadena-Castañeda, Mendes & Silva, 2019	**HT**: 1♂ Brasil: Amazonas: Tefé (S3.33, W64.69) 01.–05.XI.2016. leg. J.A. Oliveira & D.M.M. Mendes (INPA)	Brazil (Amazonas state, Tefé)
S. rafaeli Cadena-Castañeda, Mendes & Silva, 2019	(1) 1♂ Brasil (Cadena-Castañeda et al. 2019, fig. 24); (2) 1♀ Brasil (Cadena-Castañeda et al. 2019, fig. 25)	Brazil (Amazonas state and Rondônia state)
***Scaria* (*lineata*) group**		
S. fasciata Hancock, 1907	(1) 1♀ Ecuador: Cachabi, leg. Rosenberg (ANSP) (photographic record (Cadena-Castañeda et al. 2019, fig. 13)); (2) 1♀ Colombia: Chocó: Bahia Solano (N6.21, W77.40) (photographic record (Cadena-Castañeda et al. 2019, fig. 33)), (3) 1♂ (photographic record (Cadena-Castañeda et al. 2019, fig. 14))	Colombia; Ecuador; Panama; Nicaragua
S. ferruginea Hancock, 1909	(1) 1♀ Brasil: Rondônia: Candeias do Jamari, Usina Hidrelétrica de Samuel (S8.95, W63.18) 17.VIII.2016. leg. D.M.M. Mendes, F.F. Xavier F°, A.A. Agudelo, & J.A. Rafael (INPA); (2) 1♀ Bolivia: La Paz: Parque Nacional Madidi, (photographic record (Cadena-Castañeda et al. 2019)); (3) 1♀ Colombia: Vaupés: Mitu (N1.27, W70.22) (photographic record (Cadena-Castañeda et al. 2019))	Brazil (Rondônia state); Colombia
S. lineata Bolívar, 1887	(1) **LT**: 1♂ Peru: Alto Amazonas (S5.56, W76.00) (MNCN); (2) **PLT**: 1♀ Peru: Alto Amazonas (S5.56, W76.00) (MNCN) (photographic record (Cadena-Castañeda et al. 2019, figs 15, 16))	Ecuador (Pastaza Province); Peru (Department of Loreto)
S. veruta (Grant, 1956)	(1) **HT**: 1♀ Peru: Junín, Puerto Bermudez, Río Pichis, 12–19.VII.1920 (ANSP) (photographic record: Cadena-Castañeda et al. 2019, fig. 17) (wrongly cited 'male' in OSF); (2) 1♀ Bolivia: PN Madidi (photographic record (Cadena-Castañeda et al. 2019, fig. 34)); (3) 1♀ Peru, P.N. Manu, Pantiacolla rainforest (photographic record: http://orthoptera.speciesfile.org/Common/basic/ShowImage.aspx?TaxonNameID=1100469&ImageID=203222)	Peru (Río Pichis, Puerto Bermúdez)
***Scaria* (*producta*) group**		
S. maculata Giglio-Tos, 1898	**LT**: 1♂ Ecuador: Valle de Santiago (S3.53, W78.46) (MRSNT)	Ecuador (Valle de Santiago)
S. producta Hancock, 1907	(1) 1♂: Peru: Loreto, Picuroyacu, 2013, (photographic record (Cadena-Castañeda et al. 2019, fig. 32)); (2) 1♀: Colombia: Putumayo: Mocoa (N1.15, W76.64) 500 m a.s.l. 2017. (Photographic record (Cadena-Castañeda et al. 2019, fig. 32))	Colombia; Ecuador; Peru
***Scaria* (*laeta*) group**		
S. laeta Günther, 1940	(1) **LT**: 1♂ Brasil: Amazonas: São Paulo de Olivença, southern banks of upper Amazonas, leg. S. & I. Waehner (SMTD); (2) **PLT**: 1♀ Brazil, Amazonas: São Paulo de Olivença, mouth of Rio Javary (S3.76, W69.09) leg. S. & I. Waehner (SMTD); (3) **PLT**: 1♂ Brasil: Amazonas: São Paulo de Olivença, southern banks of upper Amazonas, leg. S. & I. Waehner (SMTD); (4) **PLT**: 1♀ Brasil: Amazonas: São Paulo de Olivença (S3.38, W69.07) 65 m a.s.l., leg. S. Waehner (NMW); (5) 1♀ Brasil: Amazonas: Tefé (photographic record (Cadena-Castañeda et al. 2019, fig. 31)); (6) 1♂ (photographic record (Cadena-Castañeda et al. 2019, fig. 8)); (7) 1♀ (photographic record (Cadena-Castañeda et al. 2019, fig. 9))	Colombia; Ecuador; Peru

genera of Batrachideinae; and 6) hind femora sulcated in *Scaria*, unlike carinated in *Rehnidium*. We identify this species as *Scaria* Bolivar, 1887 because it shows the following characters typical of the genus: 1) median carina of the pronotum projected above the head anteriorly in the form of a well-developed spine (similar to *Rehnidium* Grant, 1956 in which the spine is less pronounced); 2) slender (slim) and elongated body (differing it from robust members of the genus *Rehnidium* Grant, 1956); 3) black basal color of

tegmina (not brown as in *Rehnidium*); and 4) flat pronotum (not roof-like as in *Rehnidium*).

The two individuals of the newly reported species are macropterous and macropronotal, pronotum being longer than hind femora. Characters that are present in all *Scaria* species known so far, but at first seem to be absent in the unnamed one, are 1) absence of clearly visible pale colored spot at posterior part of tegmen and 2) a lack of dark lateral stripe on the pronotum. The

tegminal spot might be present in the reported specimens, but it is simply covered by a wide yellow-colored stripe that lies in the middle part of tegmen. This is the first *Scaria* species with medial stripe on tegmina (for comparison, *S. laeta* Günther, 1940 has the yellow stripe in the ventral portion of the tegmen, while *S. jonasi* Cadena-Castañeda et al. 2019 has it on the dorsal portion of tegmina). Arguments for not identifying the species as *Rehnidium* are 1) members of *Rehnidium* always have a brown base color of tegmina and 2) members of the genus *Rehnidium* have short and wide ovipositor valves (Cadena-Castañeda and Cardona 2015, Cadena-Castañeda et al. 2019).

Scaria sp.

Material examined.—Peru •1 M, 1 F; Department of Amazonas: Bongará Province: Peroles near Yambrasbamba, mountain rainforest belonging to the Peruvian Yungas biogeographic ecoregion; 5.67°S, 77.92°W; 1905 m a.s.l.; 19 August 2008; R. Sindaco leg.; photographs only, available on iNaturalist (https://www.inaturalist.org/observations/9968031), supplemented by Figures in this publication.

Habitat.—The specimens were observed and photographed on the ground inside a well-preserved patch of forest with muddy base covered by abundant leaf litter; tree trunks were covered by mosses, rich epiphytic vegetation (many Bromeliaceae), and arboreal ferns.

Specific traits.—The main differences between *Scaria* sp. and the five other morphologically similar species that occur in the region are listed in Table 2. The unnamed species is easily distinguished from other *Scaria* species by the following set of characters: 1) vertex wider than in any other known *Scaria* species, 2) small apical teeth on mid femora (in almost all the other species, teeth are larger), 3) longer fore and mid femora (length/width ratio of 6 or more) than in any other species, 4) generally stouter appearance than any other *Scaria* species, 5) yellow stripe placed medially on tegmina, and 6) distinctive coloration pattern of pronotum. Concerning the key to *Scaria* species by Cadena-Castañeda et al. (2019), our unnamed species shows a unique combination of characters not present in any other known species, namely yellowish face, eyes

projected above the dorsalmost level of tegmina, absence of dorsal midline (coloration), lightly colored stripe covering the median part of tegmina, and absence of stripe on the upper half of the lateral margin of pronotum. The only *Scaria* species similar to this unnamed one is *Scaria veruta* (Grant, 1956), which also has longer fore and mid femora.

Morphological description.—**General characters and coloration:** Relatively slender body, slightly stouter than other *Scaria* species described so far. Body smooth, without warts or dorsal projections. Coloration pattern of the entire body homogeneous, with interchanging black and yellow coloration. Yellow coloration varying from dark to paler yellow (close to white coloration) in some parts of the body. Antennae black, sometimes with a paler tip. Head exhibiting similar coloration and texture to that of the rest of the body, with black stripe behind the eyes. Compound eyes pale at the top, otherwise black. Carinae of pronotum (interhumeral carinae, external lateral carinae, internal lateral carinae, prozonal carinae, and median carina) mostly yellow to light yellow. Fore and mid femora black with all carinae yellow; fore and mid tibiae following the same pattern. Hind femora bearing a yellow to light yellowish stripe in the mid part. Front and mid tarsi dark; hind tarsi lighter but with dark coloration on the first segment ventrally. Tegmen black with a yellow longitudinal stripe in the middle covering more than three quarters of the area of the tegmen.

Head: (Fig. 2A, E) Antennae with 20 antennomeres. Scapus oval in cross-section. Short axis of the scapus 1.5 times wider than second antennomere (pedicel); long axis 2 times wider than second antennomere. Second antennomere 1.5 times wider than the third and all other antennomeres. Basal antennomeres from 3rd to 7th, central antennomeres from 8th to 14th, preapical antennomeres from 15th to 17th, and apical antennomeres final three segments (18th to 20th) reduced. Pale rings in joints visible in central segments. In fronto-lateral view: Frontal costa bifurcates above the middle of the compound eyes (Fig. 5). Head below level of pronotum (head in contact with the underside of the anterior side of pronotum). Lateral ocelli barely above mid-level of a compound eye. Fastigium verticis barely below level of dorsal margin of a compound eye. Frontal costa bulging for half the length of

Table 2. Tabular comparison of *Scaria* sp. to five other species that are morphologically similar or found in the same area.

	Scaria sp.	*S. hamata*	*S. lineata*	*S. maculata*	*S. ferruginea*	*S. veruta*
fore femur length/width (height) ratio	6	3.9–4.5	4.7–5.1	5.1	4.65–4.85	6.25
relation between lateral and humero-apical carinae	parallel	almost touch	almost touch	parallel	almost touch	almost touch
convexity in fronto-lateral area of pronotum	noticeable	slight	absent	absent	absent or very weak	absent
tarsal pulvilli	rounded, bulbous	pointed, triangular	rounded, bulbous	pointed, triangular	rounded, triangular	bulbous, rounded
apical teeth of mid femora	small	large	medium-large	small-medium	medium-large	medium
width of vertex to compound eye length (dorsal view)	1 (male), 1.7 (female)	1.3	1.3	1 (male)	1.4	1.2 (female)
PM elevation	present	present	absent	present	sometimes present	slight
humeral angles	rounded	rounded	rounded	slightly angled	slightly angled	rounded
rising of pronotum after PM towards anterior spine	very slight	slight	pronounced	pronounced	pronounced	pronounced
ovipositor valve length	long	medium	medium-long	N/A	short	medium-long
pronotum length in comparison with hind knee	surpassing	surpassing	surpassing	surpassing	surpassing	barely surpassing
post ocular stripe	present	present	present	absent	absent or very weak	present
tegminal coloration	wide band	spot	no coloration	no coloration	spot	spot

Fig. 2. Example of AI enhancement of *Scaria* sp. body details compared to original photography in the background. **A.** Upper part of the head, showing an eye, a scapus, and a pedicel; **B.** Ovipositor; **C.** Hind tarsus; **D.** Ventral and tegminal sinuses; **E.** Bottom part of the head, showing the mouthparts. Photo credit: Roberto Sindaco.

scapus. Pedipalps white. Transverse carinae concave. Fossulae deep and pronounced. Median carina absent. In dorsal view: Vertex the same width as compound eye (male) or 1.7 times as wide as compound eye (female). Eyes of a bulbous kidney shape. Frontal costa not straight after bifurcation (see Fig. 4A).

Pronotum: Frontal view is not seen in the photographs. In lateral view: Pointy short frontomedial (anterior spine) present in the anterior margin. Prozonal carina visible, yellow in coloration. Sulci visible, dark in coloration. Humeroapical carina connected with external lateral carina, both yellow in coloration. Posterior margin of the lateral lobe yellow/pale with ventral sinus more obtuse than tegminal sinus. Pronotal disc flat; median carina flat except on the places of promedial and first metamedial projections. Pronotum reaching far beyond hind knees. Extralateral carina not visible, but a yellow spot in its place. Infrascapular area virtually non-existent. In dorsal view: Pronotum covering the whole abdomen. Pronotal apex surpassing hind femora. Coloration of pronotum similar to rest of body. promedial projection with characteristic yellow coloration. Prozonal carinae parallel. Humeral angles slender, oblique. Pronotal process bearing yellow to pale yellowish x-shaped mark. Median carina present, clearly visible thanks to the contrast in coloration, but rather flat. Internal lateral carina yellow to pale yellow and clearly visible. Posterior margin of pronotum truncated. Interhumeral carina absent. Characteris-

tic yellow line visible in the area where an interhumeral carina is usually present.

Wings: Macropterous specimen. Wings (alae) well developed, visibly longer than pronotum, black with white anterior edge. Tegmina present; reaching coxa of hind legs; black with thick yellow medial stripe from anterior to posterior part of tegmen.

Legs: Fore legs: Femora and tibiae smooth, without teeth. Yellow and black stripes following visually unperceivable carinae on femora. Fore femora 6 times longer than wide. Yellow and black stripes present on tibiae as well. Tarsi two segmented. Proximal segment much shorter than distal. Mid legs: Femora and tibiae smooth except for the apical (genicular) teeth present and clearly visible in distal part of femora. Yellow and black stripes, which are 6 times longer than wide, following virtually non-existent carinae on femora. Yellow and black stripes present on tibiae. Tarsi two segmented, proximal segment much shorter than distal. Hind legs: Femora 3.6 times longer than wide. Dorsal margin with minuscule teeth along dorsal margin. Genicular and antigenicular teeth clearly visible, but small. Ventral margin smooth. Inner external area of hind femora with a few transverse ridges of yellow color. In the mid length of femur, a transverse yellow to pale yellow band is present. Tibiae yellow in dorsal, black in ventral part, with recognizable miniscule teeth on dorsal margins with few larger, but still tiny, teeth. Third segment of

Fig. 3. The picture of the habitat taken by R. Sindaco during the trip to Peru during which the pictures of *Scaria* sp. were taken (*Homo sapiens* (Alberto Venchi) for scale).

Fig. 4. Living female of *Scaria* sp. in **A.** Dorsal view; **B.** Lateral view. Photo credit: Roberto Sindaco.

arsus (Fig. 2C) 1.4 times longer than proximal segment. Tarsal pulvilli orbicular, first and second of same length, third (= distal) 1.4 times longer than first two. Proximal segment of tarsus in dorsal part yellow to light yellowish; in ventral part black, but tarsal pulvillus pale.

Sexual dimorphism.—This unnamed species exhibits marked sexual dimorphism in general appearance of sexes, vertex width, pronotum stature, and coloration. Some of the observed differences could be due to the limited sample, i.e., we have examined photographs of only one male and one female, but we nonetheless decide to discuss them as they could prove to be useful in the future. Vertex: Ratio of vertex width and width of compound eye in dorsal view much higher in female (1.7) than in male (1). Pronotum: Ratio of width between prozonal carinas and the width between humoral angles are equal in both sexes. However, the ratio of the length of the pronotum and the widest width between the humeral angles is much less in the female (5.15) than in the male (5.7). Coloration: Observed pattern of coloration is almost identical in both sexes, with varying degrees of color saturation in certain areas. Legs in male appear more saturated than in female. Pronotum of female appears more saturated than that of male.

Limits of the description from photographs.—Since some angles are missing from the pictures, certain characters were described incompletely or were not described at all. We must stress, once again, that those characters do not limit us in concluding that this is a new species, but could limit the comparison with future specimens or photographs. The following is a list of characters seen only in frontal and ventral views, which should, for detailed description, be examined in a laboratory environment when a physical specimen is collected. Frontal view: level of bifurcation of the frontal costa; scutellum width; position of the antennal grooves; distance between the antennal grooves; position of lateral ocellus; shape of the vertex. Ventral view: shape of thoracal and abdominal sternites (incl. sternomentum and subgenital plate).

Measurements.—No specific measurements can be given due to lack of physical specimens, but some specific proportions can be calculated from photographs (see Table 2).

Discussion

On the taxonomy of *Scaria*

Specimens of *Scaria* sp. that we report here are somewhat similar to the holotype of *Scaria veruta* (originally placed in *Rehnidium*

Fig. 5. Living female of *Scaria* sp. in frontolateral view. Photo credit: Roberto Sindaco.

Grant, 1956) (Grant 1956, Cadena-Castañeda et al. 2019, Silva et al. 2021). The lack of a lateral stripe on the pronotum of *Scaria* sp. might be caused by the fact that our species has a multicolored pronotum on which the stripe might simply be difficult to notice and distinguish. A similar case has been observed in a living specimen of *S. hamata* (De Geer, 1773) reported by Cadena-Castañeda et al. (2019: fig. 36). In the key given by Cadena-Castañeda et al. (2019), *Scaria veruta* can be distinguished from other species by tegmen with an ovoid subapical spot. However, the key then forwards a reader to the previously mentioned figure 17, a specimen of *S. veruta* with yellowish ventral line on the tegmina. Also, figure 34 shows a living specimen of *S. veruta* with tegminal sinus and tarsal pulvilli different from the ones on specimen from figure 17. This brings us to question whether all these specimens belong to the same species.

Describing species from photographs

On the nature of the name

The nature of the name is an important point deserving to be separately discussed. Despite the existing rules of nomenclature provided by the ICZN, we did not manage to "legally" name this new species. The name is one of the first steps in investigating any species, learning about its behavior, habitat, and distribution. Article 72.5.6. of the ICZN states, "*In the case of a nominal species-group taxon based on an illustration or description, or on a bibliographic reference to an illustration or description, the name-bearing type is the specimen or specimens illustrated or described (and not the*

Fig. 6. Living male of *Scaria* sp. in dorsal view. Photo credit: Roberto Sindaco.

illustration or description itself)." Article 73.1.4. states, "*Designation of an illustration of a single specimen as a holotype is to be treated as designation of the specimen illustrated; the fact that the specimen no longer exists or cannot be traced does not of itself invalidate the designation.*" Recommendation 73B of the ICZN states, "*An author should designate as holotype a specimen actually studied by him or her, not a specimen known to the author only from descriptions or illustrations in the literature*" (ICZN 1999). It is clear that, despite the recommendation that new species should be based on physical specimens, it is not forbidden to describe a species from a photograph, i.e., from the specimen a photograph represents, if there is reason to do so. As 73B is 'only' a recommendation, it has provided room for well-documented exceptions from physically collected species (Marshall and Evenhuis 2015, Ingrisch et al. 2016). If the goal of the taxonomic community was to disallow photo-based descriptions entirely, Recommendation 73B should have been made into an Article. Since it is not an Article, there obviously are exceptions where photography-based description should be permitted. Thus, according to the ICZN (1999), it is not legal to reject a photography-based new species description unless the provided data does not support a new species alongside being useful for its identification in the future.

The ICZN anticipated descriptions of typeless species. Recommendation 73G asks the authors to provide an explanation for why they lack physical types, and this is asked in order to avoid

"immediate science," i.e., fast typeless descriptions of taxa found in online photographs but for which physical types could easily be provided. The ICZN asks as follows: *"An author should provide detailed reasoning why at least one preserved specimen, whether a complete individual organism or a part of such an individual, was not used as the name-bearing type for the new taxon and why the formal naming of the taxon is needed at a point in time when no preserved name-bearing type will be available"* (ICZN 1999). Marshall and Evenhuis (2015) raise a good point to complement the above-mentioned technical aspects of publishing photo-based descriptions. It is not practical or realistic to speak about a distinct new species with a code, such as *"undescribed species #nnn,"* only because the physical holotype was not collected, when there are perfectly valid rules to provide it with a name.

A name of any kind would make referring to this species easier in subsequent publications. A proper binomial name would carry taxonomic information. A nomenclatural valid name would allow species systematization in databases of all kinds.

On the nature of the holotype

The ICZN states that *"a holotype is the single specimen upon which a new nominal species-group taxon is based in the original publication"* (ICZN 1999). The holotype is only one because numerous problems existed with the syntypes, such as more than one species being contained within a single type series (Baur and Coray 2004). Hence, there is only one holotype, only one lectotype, and only one neotype. The exact nature of the holotype has been sporadically discussed, but the intuitiveness of the practice of depositing and studying holotypes makes this discussion unimportant in the general case. Schopf (1960) pointed out that the word itself is partly misleading, as the holotype is not and cannot possibly be "typical" of any taxon, as one specimen cannot include all the characteristics present in the population. The holotype is the name-bearer, a voucher upon which a taxon's name rests. If a holotype is atypical, it does not invalidate it at its function as a name-bearer. Although the primary role of the holotype is to bear the name, it is immensely valuable as a reference for any possible future research concerning the species in question and has the quality of retaining information that the original author might have missed or deemed unimportant. The value of the holotype has already been pointed out (Amorim et al. 2016) and can be further solidified by the example of a 145-year-old holotype from which DNA was successfully extracted and which helped resolve a cryptic species problem (McGuire et al. 2018). If this were not possible, the status of the original specimen in relation to the closely related species would be unsolvable, but differentiation of the species would still be quite possible, as only the status of the holotype as the name-bearer would be called into question. McGuire et al. (2018) also provide commentary on fixation methods that damage the specimen. Alongside improper conservation, improper curation can lead to damaging of the stored types and loss of information (Borczyk 2013). While an obvious effort to preserve the types as much as possible is apparent, there are no explicit standards for fixation and curation. Curation is especially problematic as it is dependent on the individuals who curate the collections and on the institutions that oversee their efforts; both can show blatant disregard for their inventory, which can also be damaged by unpredictable circumstances such as fires or earthquakes.

Another problem is the inaccessibility of certain holotypes, either in museums (Tang et al. 2020) or in private collections (Korb 2011, Pacheco 2017, Kral et al. 2021). Recommendation 16C of the ICZN states: *"Recognizing that name-bearing types are international standards of reference (see Article 72.10) authors should deposit type specimens in an institution that maintains a research collection, with proper facilities for preserving them and making them accessible for study (i.e. one which meets the criteria in Recommendation 72F)"* (ICZN 1999), which further illustrates the double standards in interpreting recommendations. As can be seen in the cited papers, new species are published with holotypes that are not deposited according to the ICZN's recommendations, which is allowable if the rules are consistently interpreted. However, a holotype that is buried in an inaccessible private collection or in an uncurated basement of a museum is of equal value as an undeposited holotype, the difference being that one is allowed without explanation and the other according to the personal preferences of journals' editors. It is not unheard of for holotypes to disappear. In some cases, the only remaining records of the name-bearing type are illustrations that depict taxonomically important characteristics (Woodman 2009). Even in the absence of illustrations, the taxon is valid, and a neotype can be designated only if there is some doubt about the definition of the taxon (ICZN 1999).

The taxonomic community seems content with the idea that the holotype merely exists somewhere but does not clearly mandate the ways of their preservation, accessibility, or eventual replacement due to inevitable degradation. This is excusable if the function of the holotype is to provide the basis for a name (which it is) but is equivalent to institutionalized malpractice if the holotype is supposed to be accessible at any moment (which it is not). If the designated holotype or the depiction of it represents sufficient evidence to differentiate the named taxon from the others, its function is fulfilled. The missing data can be acquired in subsequent research and published separately. Publishing a species name does not entail providing complete and non-editable information about the species.

On the nature of fruitless research

The decision to try to describe a new species from photographs (iNaturalist) was made after we found no similar pygmy grasshopper in the literature. This nameless *Scaria* was recorded only once in 2008 in a severely deforested area. The fact that we made no attempt to physically find it, either by ourselves or with help from potential colleagues in Peru, is one of the criticisms we received, and it certainly warrants an explanation. After examining the photographs of the specimens and concluding that they represent a currently unknown species, we felt obliged to add this species to the globally accessible fund of knowledge, which entails naming it according to the accepted rules. As this was all we set out to do and the data available to us allowed for delimiting the new species from its congeners, there is no need for additional research at this stage.

Plenty of papers that present only basic information, i.e., without DNA sequences (Hemp 2017, Zha et al. 2020, Zhu et al. 2020) or with DNA sequences only (Sharkey et al. 2021), are being published regularly, the only difference being that they had access to physical specimens. This demonstrates that it is not the lack of information that is problematic, but the fact that no physical holotype exists even though the results of those publications and our own point to the same thing: a scientific name with accompanying characteristics by which the animal bearing the name can be recognized. All the photographs are publicly available and can be independently checked by other researchers to reaffirm or deny our hypothesis.

Field work is a necessary part of research, and we will, in time, take steps to either visit the locality or have it visited by somebody else. Considering the cost of this endeavor, the time it takes to forge a robust relationship with local researchers, and the restrictions imposed by the pandemic, this will happen later rather than sooner.

Incidentally, research concerning the genus *Scaria* in an area that encompasses the area in which the nameless *Scaria* lives was recently conducted by Cadena-Castañeda et al. (2019). They found three new species, none of which is the herein identified one. The absence of the nameless *Scaria* in this research shows that the species cannot be easily found and the efforts to do so would prove to be inexcusably great considering the scope of this paper.

The existence of protected areas where collecting specimens is prohibited and even touristic visits are heavily regulated is very positive, and governments around the world should be urged to designate more such areas for obvious reasons. However, those strict limitations can impede research to the point that many expeditions of a smaller scope will simply not be undertaken. It is impossible to acquire permits for those areas, as the process is arduous and (understandably) discouraging (SEARRP 2021). Ingrisch et al. (2016) faced this exact problem. They decided to describe a new katydid species based on photographs taken in a protected area. They correctly concluded the following: *"Therefore, we opted for a timely description of both species, hoping that our publication will lead to further photographic and/or acoustic detections, that it will convince authorities to grant permits for collection of type material, and that it may stimulate habitat protection measures for these enigmatic species."* By describing a new species, Ingrisch et al. (2016) illustrated the problem and made steps not only to inspire further research but also to grasp the elusive limits of modern species research. The paper was published in this very journal, proving that exceptions do exist. The photographed katydid could, compared to the nameless *Scaria*, be collected with relative ease, requiring just a few months of effort (SEARRP 2021). This was not asked of the authors while we are being urged to undertake unjustifiable collection.

On conservation

Even though the name carries a certain significance by itself, it also has one strictly practical value. Laws are written in such a way as to direct conservation efforts towards entities bearing a certain name, and not towards the species itself. Changing the name can impact the conservation status of a species regardless of its actual status (IUCN SSC 2012, ICZN 2021).

From the available data, it is impossible to make less than speculative statements about the status of the nameless *Scaria*, and we will refrain from doing so. It does not have to be extinct or even endangered for these arguments to have weight. Every species should be named if the naming can be done legitimately. This allows the law to recognize it and take appropriate action. The name can be changed in cases of fresh discoveries, so there is no apparent reason to circumvent rules in order to *disallow* a name and, in doing so, make further research on the species more difficult while robbing the area's checklist of a new species. What is discussed here is principle, not the significance of a single species.

Concluding thoughts

There is a nameless new *Scaria* species from Peru, clearly distinguishable from other species of the genus. All of the species of that genus are clearly differentiable by their morphology and col-

oration, which are visible in photographs. The ICZN allows naming species from photographs if there is reason to do so; however, we did not manage to name this nameless species. We do not propose that photograph-based descriptions become the norm, only that in certain cases and for certain taxa this can be beneficial. We understand how important the physical holotype is, but because of numerous issues, we did not manage to collect one.

By the time a scientist collects, examines, and describes one new species, several more have gone extinct (Stork 2009). Every new species' description serves as a reminder of those that could not be studied in time and are now irretrievably lost. We have thus decided to describe this nameless species, celebrating the role that taxonomy has on species preservation (O'Brien and Mayr 1991, Johnson et al. 2018). A charismatic name would have allowed it to reach a wider audience, but the nameless *Scaria* will nonetheless serve as a symbol of the species we wait for, hoping that it will not be only Godot.

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Substrate-borne vibrations used during acoustic communication and the existence of courtship songs in some species of the genus *Anaxipha* (Saussure) (Orthoptera: Trigonidiidae: Trigonidiinae)

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Abstract

Anaxipha (Saussure, 1874) are small, swordtail crickets found in much of eastern North America. Many species within the genus *Anaxipha* were only recently described and their calling songs characterized. However, little is known about their courtship songs or use of substrate-borne communication (drumming). This study is the first documentation of the existence of courtship songs and substrate-borne vibrational communication in the genus. Courtship songs and substrate-borne vibrational communication were first detected in the following species: *Anaxipha exigua* (Say, 1825), *A. tinnulacita* Walker & Funk, 2014, *A. tinnulenta* Walker & Funk, 2014, and *A. thomasi* Walker & Funk, 2014. When in the presence of a conspecific female, males of all four species perform courtship songs that are distinctly different in pattern of echeme delivery and syllable details compared to their respective calling songs. Additionally, males of all four species exhibited drumming behavior during courtship singing and variably during calling songs. Examination of video recordings of males drumming during courtship singing showed that they are apparently using the sclerotized portion of their mandibles to impact the substrate on which they are perched to create vibrations. Courtship song and drumming bout characteristics were statistically different among the four species studied here, although *A. tinnulacita* and *A. tinnulenta* were similar in some measurements. Drumming during calling songs was common only in *A. tinnulacita*, where drumming occurs predominately during the first forty percent and last twenty percent of the long echemes of calling songs. Additional study is needed to further explore the use of substrate-borne vibrational communication in this genus.

Keywords

communication, courtship, courtship song, drumming, echeme, substrate-borne vibrations, vibration

Introduction

Large numbers of species of small insects use substrate-borne vibrations for near-field communication with conspecifics (Caldwell 2014, Yack 2016). Many of these species inhabit herbaceous and woody plants and use the plant's stems and leaves to transmit these vibrational signals (Michelsen et al. 1982, Eriksson et al. 2011). Small, plant-inhabiting insects that produce acoustic signals to attract mates most likely face challenges when trying to locate a singing male once the female is within a meter or so of the singer (Cocroft et al. 2000, Cokl and Virant-Doberlet 2003). Within the tangles of vegetation that these insects inhabit, the use of substrate-borne vibrations, in addition to acoustic signals, would be beneficial. This would allow for orientation toward and then finding of potential mates once the searcher is on, or close to, the stem or leaf from which the male is communicating (Hill 2001).

All the species of *Anaxipha* (Saussure, 1874) in this study are tiny insects, less than 8 mm in length, and occur on a wide variety of plants and plant structures in North America (Walker and Funk 2014). Males often sing from concealed perches, typically on the underside of leaves or stems, and are difficult to locate visually. Males sing calling songs, both day and night, which are loud and easily heard at distances of several meters (Walker and Funk 2014). Only at night can males and females be found foraging on the top side of vegetation or wandering along stems. In this study, the existence of courtship songs and drumming in the four *Anaxipha* species found in West Virginia are documented.

Materials and methods

Males and females of *A. exigua* (Say, 1825), *A. tinnulacita* Walker & Funk, 2014, *A. tinnulenta* Walker & Funk, 2014, and *A. thomasi* Walker & Funk, 2014 were collected in August and September during the years of 2019 and 2020 from appropriate habitats (shrubs, coarse weeds, and grasses at the edges or the understory of deciduous woods) in Berkeley and Jefferson Counties in West Virginia, USA. As in Hershberger (2021), all specimens were maintained in plastic containers that were modified by removing most of the plastic from the sides and lid. The open areas were then covered with no-see-um netting held in place with high-temperature hot glue. These singing cages are practically transparent to sound. The crickets were fed iceberg lettuce, Fluker's High-calcium Cricket Diet (Port Allen, LA), and water *ad libitum*. Audio recordings of calling songs were made with caged, individual males placed in an anechoic room, with dim or no light. Temperatures were taken
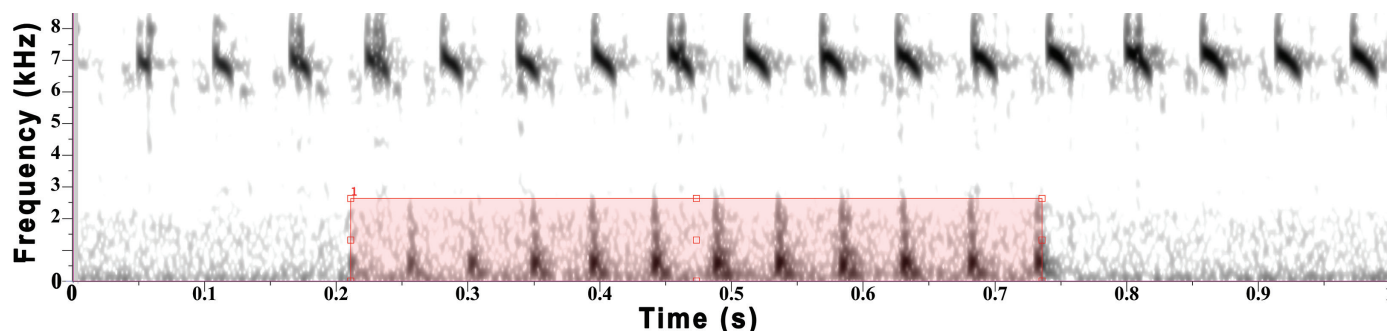
Fig. 1. A typical drumming bout showing the low-frequency and brief nature of these sounds. The figure is the selection of a drumming bout from a courtship song of *A. thomasi* showing the selection window spanning from the middle of the first tap to the middle of the last tap. This tapping bout consists of 12 taps.

with either a Cooper Atkins DFP450W digital pocket thermometer (Middlefield, CT, USA) or a Digi-Sense ZM-94460-78 receiver and ZM-90205-10 transmitter (Vernon Hills, IL, USA). Recordings were made with a Sound Devices 702, Sound Devices MixPre-6 (Reedsburg, WI, USA), or a Zoom F8n digital recorder (Hauppauge, NY, USA) at a sampling rate of 96 kHz at 24-bit depth. The microphone, a Sennheiser MKH 8020 RF condenser (Solrød Stand, Denmark), was placed within 8 cm of the singing males. Audio recordings were processed using Adobe Audition CC 2020 (San Jose, CA, USA) and examined using Raven Pro v 1.6.1 (Cornell Lab of Ornithology). Data analysis was performed using Microsoft Excel (Redmond, WA, USA) and DataGraph software (Chapel Hill, NC, USA).

Acoustic terminology follows Baker and Chesmore (2020): a unit of sound produced by one closing stroke of the cricket tegmina is called a syllable; syllables are grouped into short echemes that are organized into calling songs or courtship songs.

Courtship songs were recorded by placing a female into the cage with the male, placing the cage in a dimly lit anechoic room, and allowing the insects to interact. A minimum of three different males were used for capturing courtship songs for each species. Three to four females were used for different courtship sessions. The recordings were made using the same equipment and techniques described for recording calling songs.

Videos of courtship behavior were made by placing a male and female of the same species into a clear, plastic container with a removable lid. A hole was cut into the lid and covered with no-see-um netting. Video was captured through the side of the clear enclosure using a Canon 5DSR digital camera with a Canon 180 mm macro lens with or without a 22 mm extension tube (Huntington, NY, USA). Lighting was provided by a table lamp with a 40-watt-equivalent LED bulb (to reduce heat). Audio was simultaneously recorded using the Sound Devices 702 digital audio recorder and a Sennheiser ME62 electret-condenser microphone (Solrød Stand, Denmark) placed within a few millimeters of the netting-covered hole of the enclosure. Video was edited in Final Cut Pro X (Apple Corp., Cupertino, CA, USA). All audio and video files are archived at the Macaulay Library, Cornell Laboratory of Ornithology (audio files are ML numbers 305982-306011. Video files are ML number 488780-488784).

Calling songs and courtship songs were processed in Adobe Audition to amplify the frequency band from 0–2 kHz by 10–30 dB to make it easier to detect the drumming bouts in Raven Pro 1.6.1. Drumming bouts were selected by hand from the middle of the first tap's signature through the middle of the last tap's signature in each tapping bout (Fig. 1). The tapping rate (taps/sec)

was determined by dividing the number of taps in the bout, minus one, by the duration of the tapping bout in seconds. The syllable rate for the calling and courtship songs was determined at several haphazardly chosen times during the song. Syllables were selected from the beginning of one syllable through to the beginning of the eleventh syllable and dividing ten by the measured time (in seconds). Several measurements were averaged, and standard deviations were calculated for each recording. To show that taps within an individual drumming bout increase in amplitude, I selected the first two taps and the last two taps from each drumming bout in several courtship songs for each species. This created an average power (dBFS) for each selection. The means for the first two and the last two taps were compared using a *t*-test of the means. To determine the peak carrier frequency of the tapping bouts, I selected all drumming bouts from several courtship songs for each species, allowing Raven Pro v 1.6.1 to determine the peak frequency of the sounds within the selections. These were averaged and standard deviations calculated in DataGraph. The data were not heavily skewed, so means were compared using *t*-tests to calculate *p*-values using SciStat.com comparison of means calculator (https://www.scistat.com/statisticaltests/comparison_of_means.php).

Results

All four species produced calling songs consistent with the descriptions in Walker and Funk (2014). Analysis of audio recordings of calling songs revealed brief, low-frequency sounds, from 2 kHz down, that occurred in groups of varying numbers (detail of a drumming bout from *A. thomasi*, Fig. 1). Three of the species in this study were found to perform these drumming events during calling songs (*A. tinnulacita*, *A. tinnulenta*, and *A. thomasi*) (Table 1). No drumming events were detected for *A. exigua* in the nine major echemes of calling songs analyzed for this species (these represent hours of recorded calling songs).

Table 1. Occurrence of drumming within calling songs of *Anaxipha* species (average ± SD).

Species	Taps/bout	Taps/s	Syllables/s	Temp. (°C)	n
A. exigua	ND	ND	39.35 ± 0.834	22.6	9*
A. tinnulacita	4.27 ± 1.500	18.00 ± 1.525[a]	11.20 ± 0.233	24.1	530
A. tinnulenta	2.83 ± 0.578	17.36 ± 2.829[ab]	4.82 ± 0.045	22.7	12
A. thomasi	7.28 ± 0.813	17.39 ± 0.504[b]	17.90 ± 0.071	22.1	34

ND = not detected.
[a] *p* = 0.161.
[b] *p* = 0.952.
* song segments analyzed, otherwise n = number of drumming bouts measured.

Drumming bouts within calling songs were rare except for *A. tinnulacita*, which produced drumming bouts throughout long periods of calling songs. The rate of tapping (taps/s) in these calling song drumming bouts was somewhat similar across the three species at approximately 18 taps/s (range 14.53–19.53 taps/s, n = 576), and the syllable rate of the calling songs varied across these species in a typical, species-specific pattern (Walker and Funk 2014) (Table 1). The range of the number of taps per drumming bout during calling songs was different across the three species, with *A. tinnulacita* giving 2–8 taps/bout, *A. tinnulenta* giving 2–4 taps/bout, and *A. thomasi* giving 6–9 taps/bout (Table 2).

Table 2. Range of the number of taps per drumming bout by song type. ND = not detected.

Species	Song Type	
	Calling	Courtship
A. exigua	ND	4–10 (33)
A. tinnulacita	2–8 (1502)	3–9 (21)
A. tinnulenta	2–4 (12)	4–9 (98)
A. thomasi	6–9 (31)	3–14 (92)

Number in parentheses = number of drumming bouts in the analysis.
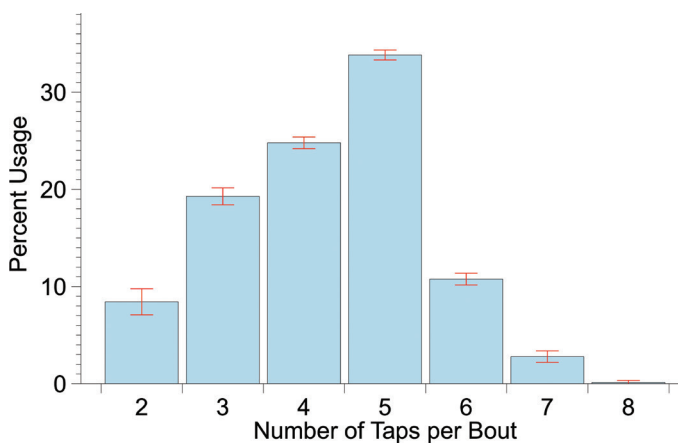

Fig. 2. Percent usage of different length drumming bouts during calling songs in *A. tinnulacita*. (n = 24 songs; 1,503 tapping bouts; error bars are ±SD of the means).
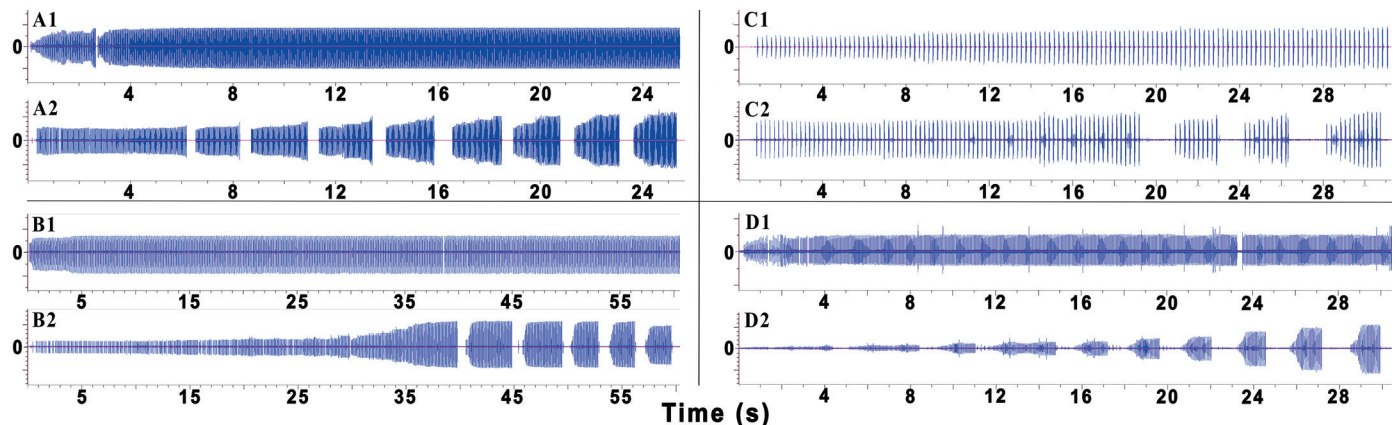
A. tinnulacita produced numerous drumming bouts during calling songs. Analysis of the different drumming bout types (number of taps per bout) used during calling songs showed that *A. tinnulacita* uses the three, four, and five taps per bout tap-type most of the time (Fig. 2). From one major echeme of calling song to the next, the use of drumming bouts was variable, but showed a pattern of more drumming bouts occurring during the first 40 percent and the last 20 percent of these calling songs (Fig. 3).

When males were placed in the same container with a conspecific female, all four species performed courtship songs. The males for the species in this study held their wings up, nearly perpendicular to their bodies, during calling and courtship singing. The courtship songs were different from calling songs in their overall amplitude and the amplitude envelope over time. Courtships songs were also broken into smaller echemes, particularly after the first several seconds of song during any particular courtship song bout (Fig. 4). Courtship songs also differed from calling songs in the shape and duration of the individual syllables that make up the echemes, with an increase in lower amplitude sounds between the louder syllables. These weaker sounds must be associated with
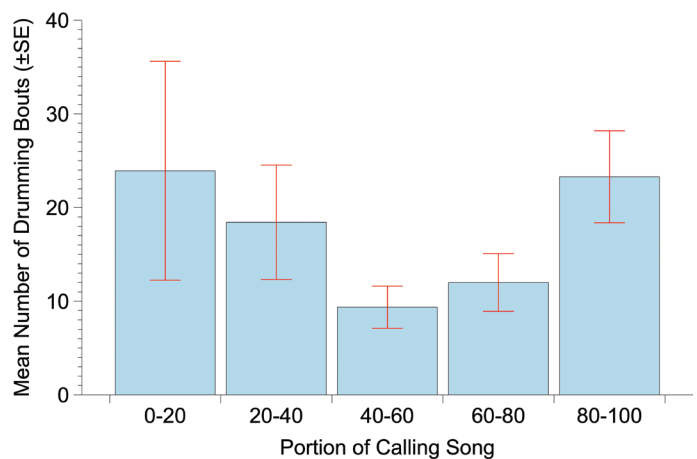

Fig. 3. Mean number of drumming bouts performed during different portions of calling songs in *A. tinnulacita* showing standard error of the means. The occurrence of drumming was variable from one calling song to the next. Consistently, more drumming bouts were given during the first 40 percent and the last 20 percent of the major echemes of calling song (n = 14 songs, 1218 drumming bouts total).
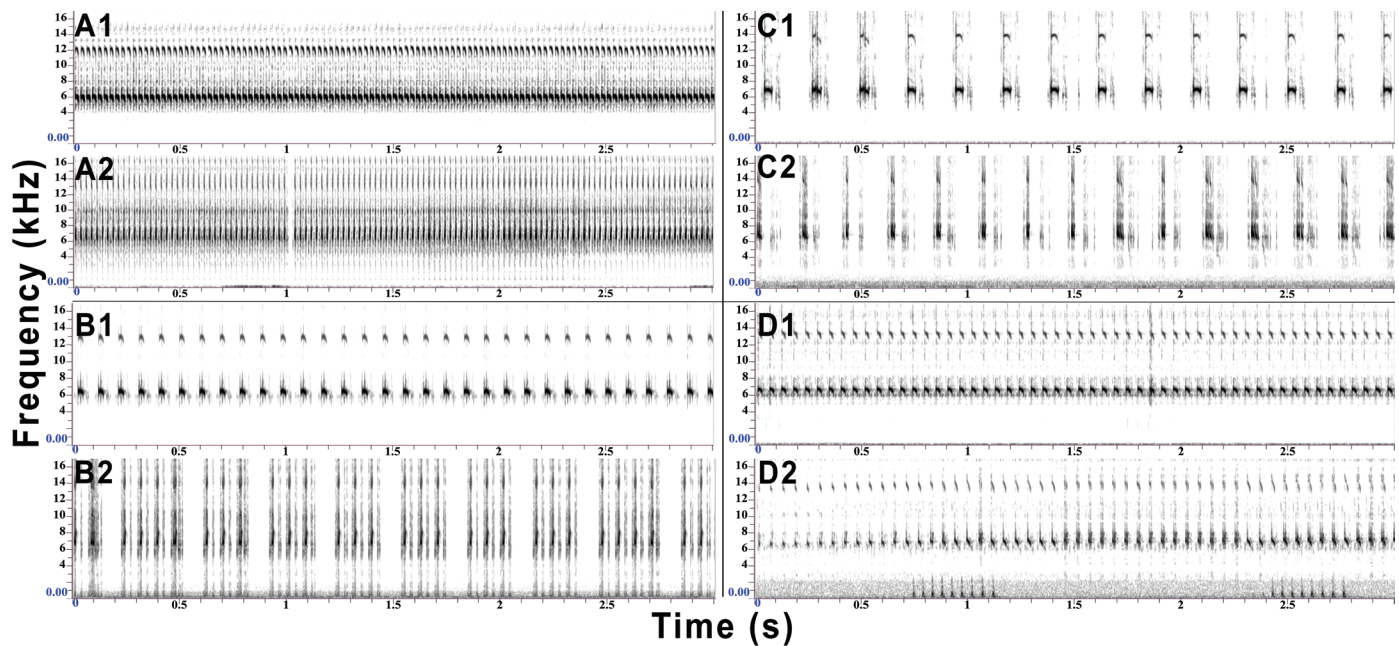

Fig. 4. Oscillograms of the calling and courtship songs of the four species of *Anaxipha*. **A1.** *A. exigua* calling song; **A2.** *A. exigua* courtship song; **B1.** *A. tinnulacita* calling song; **B2.** *A. tinnulacita* courtship song; **C1.** *A. tinnulenta* calling song; **C2.** *A. tinnulenta* courtship song; **D1.** *A. thomasi* calling song; **D2.** *A. thomasi* courtship song. All audio files were normalized to -3 dBFS for comparison. Y-axis represents amplitude and is analogous to dBFS (full scale).

Fig. 5. Audio spectrograms of three seconds of calling and courtship songs of the four species of *Anaxipha*. **A1.** *A. exigua* calling song; **A2.** *A. exigua* courtship song; **B1.** *A. tinnulacita* calling song; **B2.** *A. tinnulacita* courtship song; **C1.** *A. tinnulenta* calling song; **C2.** *A. tinnulenta* courtship song; **D1.** *A. thomasi* calling song; **D2.** *A. thomasi* courtship song. Compared to calling song, the individual syllables of courtship songs are shorter, patterned differently, with more sounds associated with wing-opening movements (wing-dragging).

wing-opening movements (wing-dragging) (Fig. 5). The syllable duration within courtship songs was always significantly shorter when compared to the syllable duration of the calling songs for each species (Table 3). Due to the high skewness of the untransformed data, *p*-values were calculated from *t*-tests of Log10 transformed data comparing the sample means.

Table 3. Syllable duration comparison between song types (average ± SD). The syllable duration is statistically significantly shorter in courtship songs across the four species in this study. Numbers in parentheses are the number of syllables analyzed from each song type. Due to the significant skewness of the raw data, *p*-values were calculated from *t*-tests of Log10 transformed data comparing the sample means.

Species	Syllable Duration (ms)		p-value
	Calling	Courtship	
A. exigua	17.61 ± 2.662 (961)	2.83 ± 0.468 (102)	< 0.00001
A. tinnulacita	28.06 ± 0.875 (480)	12.63 ± 4.764 (480)	< 0.00001
A. tinnulenta	36.11 ± 1.967 (583)	5.34 ± 1.805 (103)	< 0.00001
A. thomasi	16.85 ± 1.625 (540)	13.61 ± 5.655 (362)	< 0.00001

All four species in this study also produced numerous drumming bouts during courtship songs. The range of the number of taps per drumming bout was higher in courtship songs than in calling songs, with *A. exigua* giving 4–10 taps/bout, *A. tinnulacita* giving 3–9 taps/bout, *A. tinnulenta* giving 4–9 taps/bout, and *A. thomasi* giving 3–14 taps/bout (Table 2). The average number of taps/second in courtship song drumming bouts showed that the rates were statistically different between all species (*p*-value ≤ 0.0022); the syllable rate of the courtship songs varied across species, similar to their individual calling song rates (Table 4). While drumming bouts were given throughout courtship singing for *A. exigua* and *A. thomasi*, it was found that drumming bouts were

Table 4. Average taps/bout and taps/s within courtship songs of the four *Anaxipha* species compared to the average syllable rate of their respective courtship song during these tapping bouts (± SD).

Species	Taps/bout	Taps/s	Pulses/s	Temp. (°C)	n
A. exigua	6.61 ± 1.340[a]	19.91 ± 0.941	39.15 ± 0.952	22.6	33
A. tinnulacita	6.19 ± 1.540[a]	20.67 ± 0.667	13.21 ± 0.067	23	21
A. tinnulenta	6.93 ± 0.865[a]	17.58 ± 1.130	4.63 ± 0.053	22.1	98
A. thomasi	8.41 ± 3.210	23.15 ± 0.715	20.04 ± 0.221	23.5	92

n = the number of drumming bouts analyzed.
[a] *p*-values from *t*-test comparisons of means shows these means to be statistically similar. All other comparisons were statistically different at the 95% level.

typically given later in courtship songs for *A. tinnulacita* and *A. tinnulenta* (Fig. 6). The peak audio frequency of the taps of drumming bouts during calling and courtship songs were similar within each species and were no doubt related to the substrate that the males were tapping on (plastic containers). However, the taps created by *A. exigua* were consistently of a significantly lower peak carrier frequency than those produced by the other species in this study (*p* < 0.0001), with the carrier frequencies of *A. tinnulacita* and *A. tinnulenta* (*p* = 0.21) and *A. tinnulacita* and *A. thomasi* (*p* = 0.367) being statistically similar (Table 5).

During courtship songs, tapping within any particular drumming bout increases in peak amplitude in all four species, with the ending of a drumming bout being statistically louder than the beginning, with a *p*-value of < 0.0001 for each comparison (Fig. 7).

A. thomasi was the only species in this study that performed jumping motions during the silent periods between echemes within courtship songs. The male would repeatedly jump backwards slightly, with irregular, rapid motions creating sounds when coming to rest on the substrate. These motions created low-frequency sounds similar in carrier frequency to those in typical tapping bouts, but the pattern of these sounds was very different from the tapping bouts given during courtship or calling song echemes
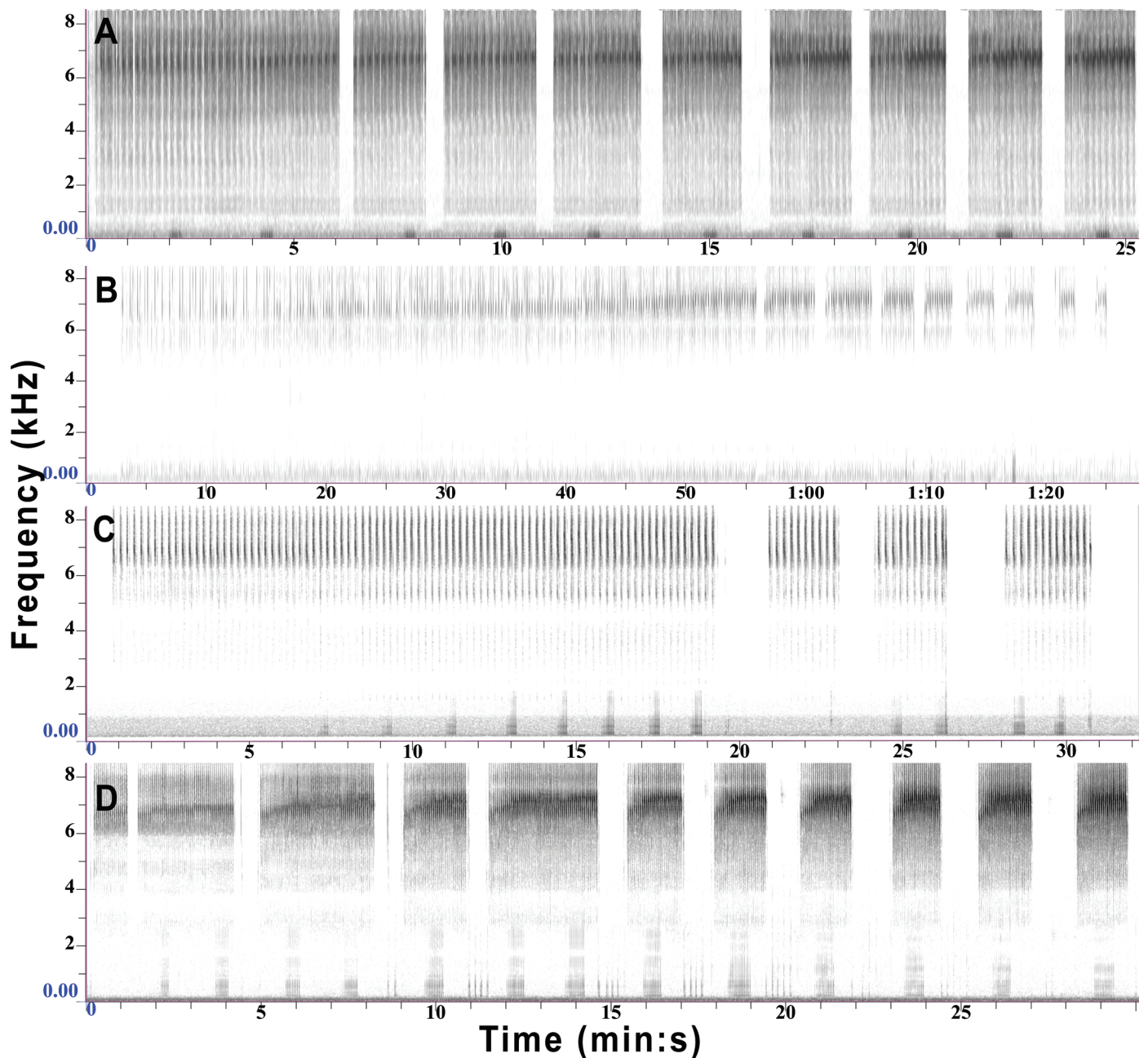
Fig. 6. Audio spectrograms of courtship songs of four species of *Anaxipha* showing drumming bouts (below 2 kHz) and tegminal sounds (above 4 kHz). Low frequencies (below 2 kHz) were enhanced to better show the low-frequency drumming bouts. **A.** *Anaxipha exigua*, dark areas below 1 kHz are drumming bouts; **B.** *Anaxipha tinnulacita*, fine, dark lines after 1:05 (min:sec) are the drumming bouts; **C.** *Anaxipha tinnulenta*, the dark areas after 5 sec and below 2 kHz are the drumming bouts; **D.** *Anaxipha thomasi*, the compact dark areas below 2 kHz are the drumming bouts. The clustered, individual taps between drumming bouts are sounds made by the insect jumping repeatedly and rapidly between echemes during the courtship display. Note that the time scales for each courtship song are different, as each species courtship song length is different and variable.

(Fig. 6D at 9, 11.5, 15, 19.5, 20, 22.5, and 25 sec) (a video file of this behavior is at ML488781). These movements and sounds were not studied further here.

Also noted during close examination of these species' courtship interactions were palp-drumming sessions performed by the males typically during non-singing portions of the courtship interactions when the male and female were in close proximity (video files of this behavior are at ML488780, ML488782, and ML488784). These palp-drumming sounds were of extremely low

amplitude and required significant amplification of the audio to hear them; they were not studied further here.

Discussion

This is the first report showing that members of the genus *Anaxipha* use substrate-borne vibrations as a possible method of communication and the first to demonstrate that these trigs produce courtship songs that are distinctly different from their
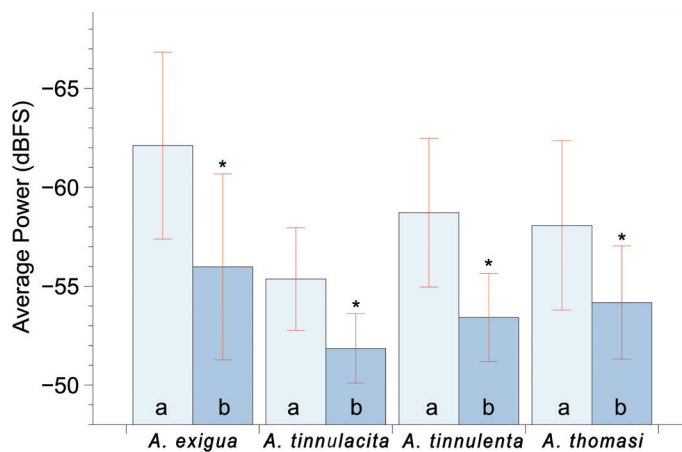
Fig. 7. Examination of the means of the average power of the combined first two and the combined last two taps of drumming bouts in courtship songs ±SD. In nearly all instances, tapping becomes louder during an individual drumming bout within courtship songs across all four species. Numbers closer to the abscissas are louder. a = average of the first two taps, b = average of the last two taps in bouts of four taps or more. * = *t*-tests comparing the means of first two taps to the last two taps, within each species, showed the *p*-values were all < 0.0001, showing that the taps are significantly louder at the end of drumming bouts (*A. exigua* n = 5 songs, 45 drumming bouts; *A. tinnulacita* n = 4, 29; *A. tinnulenta* n = 4, 98; *A. thomasi* n = 5, 75).

Table 5. Average of the peak frequencies in Hz of highest amplitude frequency of taps of drumming bouts for the specific container type in which these insects were kept.

Species	Peak Frequency ± SD	n
A. exigua	89.8 ± 35.59	5, 48
A. tinnulacita	277.0 ± 58.12[ab]	5, 33
A. tinnulenta	266.5 ± 27.84[a]	5, 73
A. thomasi	287.8 ± 55.96[b]	5, 72

[a] *p*-values show no statistically significant difference between these two species (p = 0.21).
[b] *p*-values show no statistically significant difference between these two species (p = 0.367).
p-values for comparisons of *A. exigua* to the other three species were all *p* < 0.0001.
n = number of songs, total number of drumming bouts.

typical calling songs. The use of courtship songs in other Gryllidae is well known (Alexander and Otte 1967), but little is known about the acoustic courtship behavior of the Trigonidiinae, with mating behavior reported in only a few species (Spooner 1972, Ingrisch 1977, Mendelson and Shaw 2002, Fergus et al. 2011, Shaw and Khine 2004, Funk 2016, Centeno and Zefa 2019). Males of all four species in this study produced songs that were statistically different from their calling songs when in the presence of conspecific females, and all four species exhibited drumming during courtship singing. While calling songs consisted of long, rarely broken echemes that crescendo quickly to full volume, courtship song echemes crescendo more slowly and are broken into more discrete intervals, typically in the later portion of the courtship song.

The high amplitude of calling songs would seem to make it harder for females to locate a displaying male in the tangle of vegetation typical of the habitat types used by these insects. The use of substrate-borne vibrations (drumming) certainly would make it easier for searching females to find courting males (Hill 2001, 2009, Eriksson et al. 2011, Caldwell 2014, Yack 2016). Further investigations into this mode of communication within the *Anaxipha* will be required to determine the range and efficacy of these vibrations to facilitate the orientation of searching females. The use of laser-vibrometers or sensitive transducers may be ideal for this work.

Three of the species studied here used drumming during calling songs. While these substrate-borne vibrations would certainly aid searching females looking for a perspective mate, it is unclear why *A. exigua* did not appear to perform drumming during calling song bouts, while drumming bouts were readily detected in the other three species studied here. Also, it is interesting that drumming bouts were easily detected in the courtship songs of *A. exigua*. Further study of *A. exigua*'s calling songs in a different container type would be illuminating to ensure that some aspect of the structure of the singing cages used here was not preventing the insect from producing these particular sounds.

It appears that the combination of calling song syllable rate, courtship song syllable rate, and the tapping rate in drumming bouts would clearly define each of these four species as distinct and separate to listening females. The change in the syllable length and syllable loudness in courtship songs along with the enhanced use of wing-closing sounds, the change in the pattern and amplitude envelope, and the increase in production of longer drumming bouts (more taps/bout) would clearly signal to the female the presence of a receptive male of the correct species.

Additional maneuvers and vibrations were only observed in *A. thomasi*, with males jumping (irregular, rapid, backward motions) during the silent portions of courtship encounters. It appeared that the sounds were created by his feet hitting the substrate as he lands from the jumping motions. These jumping sessions were only performed when he was not creating sounds with his tegmina. Also, males did not appear to drum during these jumping displays. The significance of these additional displays requires further investigation.

In all the species in this study that were videoed during courtship displays, males were observed to perform palp-drumming during quiet portions of the courtship display. These sounds were extremely faint. Future study of palp-drumming will require very sensitive equipment, such as laser-vibrometry, to fully detect what the males and the females are communicating to one another during these easily overlooked courtship interactions.

Acknowledgements

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