The calling songs of some katydids (Orthoptera, Tettigonioidea) from the tropical forests of Southeast Asia

Ming Kai Tan[1*], Jacob Duncan[2*], Rodzay bin Haji Abdul Wahab[3], Chow-Yang Lee[4], Razy Japir[5], Arthur Y.C. Chung[5], Jessica B. Baroga-Barbecho[6], Sheryl A. Yap[7,8], Fernando Montealegre-Z[2]

1 Department of Biological Sciences, National University of Singapore, 16 Science Drive 4, Singapore 117558, Singapore.
2 School of Life Sciences, Joseph Banks Laboratories, University of Lincoln, Green Lane, Lincoln, LN6 7DL, UK.
3 Institute for Biodiversity and Environmental Research, Universiti Brunei Darussalam, Jalan Universiti, BE1410, Tungku, Brunei.
4 Department of Entomology, University of California, 900 University Avenue, Riverside, CA 92521, USA.
5 Forest Research Centre (Sepilok), Sabah Forestry Department, PO Box 1407, 90715 Sandakan, Sabah, Malaysia.
6 Office of the Vice Chancellor for Research and Extension, University of the Philippines Los Baños, College, Laguna, 4031 Los Baños, Philippines.
7 Institute of Weed Science, Entomology, and Plant Pathology, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, 4031 Los Baños, Philippines.
8 Museum of Natural History, University of the Philippines Los Baños, College, Laguna, 4031 Los Baños, Philippines.

Corresponding authors: Ming Kai Tan (orthoptera.mingkai@gmail.com), Fernando Montealegre-Z (fmontealegrez@lincoln.ac.uk)

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Abstract

Katydids produce sound for signaling and communication by stridulation of the tegmina. Unlike crickets, most katydids are known to sing at ultrasonic frequencies. This has drawn interest in the investigation of the biophysics of ultrasonic sound production, detection, evolution, and ecology (including predator–prey interactions) of these katydids. However, most of these studies are based on species from the Neotropics, while little is known about katydid species from the hyperdiverse region of Southeast Asia. To address this, a concerted effort to document, record, and describe the calling songs of Southeast Asian katydids, especially species that call at ultrasonic frequencies, was made. A study spanning two years (2018–2020) in the Malay Peninsula (Singapore and Malaysia), Borneo (Brunei Darussalam and Sabah), and the Philippines revealed previously unknown calls of 24 katydid species from four subfamilies. The calling songs of Southeast Asian katydid species are highly diversified in terms of time and frequency. Call structure can range from isolated syllables (e.g., *Holochlora*), continuous trills (e.g., *Axylus philippinus*), to short pulse-trains (e.g., *Euanisous teuthroides*) and complex echemes (e.g., *Conocephalus* spp.), with 87.5% of species having ultrasonic peak frequencies and 12.5% being considered extreme ultrasonic callers (peak frequency >40 kHz). The call spectrum ranges from tonal (e.g., spectral entropy is 6.8 in *Casigneta* sp. 2) to resonant (entropy is 8.8 in *Conocephalus cognatus*). Of the 24 species whose calls are described here, we imaged and described the sound-producing structures of 18. This study provides a preliminary overview of the acoustic diversity of katydids in Southeast Asia, and the authors hope to inspire further investigation into the bioacoustics of little-known katydids from these areas. Amassing a database of calling songs and sound-producing organ illustrations from different species is important to address taxonomic impediments while advancing our knowledge about the bioacoustics of Southeast Asian katydids.

Keywords

acoustics, calls, frequency, sound-producing organs, stridulation, Tettigoniidae, ultrasound

Introduction

Katydids are a highly speciose group of insects (Mugleston et al. 2018) known for using acoustic signals for communication. Different species can produce very different calling songs in terms of the temporal (e.g., duration, period, call structure) and frequency (e.g., peak frequency, tonality) domains. Females can use such calling songs to discriminate between conspecific and heterospecific males (Morris et al. 1994, Heller 1995, Morris 1999, Heller and Hemp 2020). For example, some katydids produce songs at frequencies as low as 0.6 kHz (e.g., *Tympanophyllum arcufolium* (Haan, 1843); see Heller 1995), whereas other species can call at as high as 150 kHz (e.g., *Supersonus aequoreus* Sarria-S. et al., 2014; see Sarria-S et al. 2014). Compared to crickets, which generally produce low frequencies (with few exceptions, such as lebinthines; see Robillard et al. 2007 and Tan et al. 2021), such vast frequency variation makes katydids an ideal subject for studying acoustic communication and its evolution.

Katydids generate sounds through stridulation (Morris and Pipher 1967, Bailey 1970, Ewing 1989, Chivers et al. 2014). Typically, the sound is generated when the scraper on the right tegmen makes contact with the teeth on the stridulatory file of the left tegmen within a cycle of wing movement (Walker and Dew 1972,

* These authors contributed equally as co-first authors.

Morris 1999, Bennet-Clark 2003). In some katydids, the velocity at which the scraper passes through the teeth and the density of the teeth dictate the peak frequency of the species calling song, although the mirror area on the right tegmen can also play a role in dictating peak frequency (Bailey 1967, Montealegre-Z 2012, Chivers et al. 2014). However, it is not mechanically possible for katydids to move their tegmina at the velocity needed to produce extreme ultrasound (>40 kHz). Instead, to generate much higher frequency calls than wing movement velocity would allow, the scraper of some katydid species is loaded with resilin and deformable, allowing elastic energy to be stored and released (Morris et al. 1994, Montealegre-Z et al. 2006).

Many katydids emit ultrasonic frequency in their calling songs (e.g., Bailey 1967, 1970, Morris and Pipher 1967), with currently more than 70% documented as singing at ultrasonic frequencies (>20 kHz), with some species reaching extreme ultrasonic frequencies (>40 kHz) (Mason et al. 1991, Montealegre-Z et al. 2017). When compared to low-frequency calls typically produced by crickets, the generation of ultrasonic songs by katydids has clear advantages and disadvantages (Morris et al. 1994, Montealegre-Z et al. 2006). Ultrasound has enhanced directionality and radiation efficiency, allowing males to find mates and be located by females more readily while avoiding detection by predators (Mason and Bailey 1998). For pure-tone callers, another advantage is the ability to avoid eavesdropping by predators, particularly bats (Belwood and Morris 1987). On the other hand, the decay of energy in ultrasound is more rapid, thus reducing the broadcasting distance (Römer and Lewald 1992). Therefore, the ecological and evolutionary consequences of generating ultrasonic songs make these katydids interesting study subjects.

The study of the ecology and evolution of ultrasonic-singing katydids—including the documentation and description of calls (e.g., Montealegre-Z and Morris 1999; ter Hofstede et al. 2020); the systematics (e.g., Siarra-S et al. 2014, 2016, Chamorro-Rengifo et al. 2014, Chamorro-Rengifo and Braun 2016, Chamorro-Rengifo and Olivier 2017); the biomechanics of sound production (e.g., Morris et al. 1994, Montealegre-Z and Mason 2005, Montealegre-Z et al. 2006, 2017), predator–prey interaction between bats and katydids (e.g., Libersat and Hoy 1991; ter Hofstede et al. 2010), and sexual selection (e.g., Bailey and Gwynne 1988, Mason and Bailey 1998)—has traditionally been focused on species from the neotropics, with the acoustic communication of Southeast Asian orthopterans being less well studied (but see e.g., Heller 1995, Ingrisch 1995, 1998, Riede 1996, 1997, Tan 2011, Heller et al. 2017, 2021a; Tan et al. 2019b, 2020a). Despite Southeast Asia being one of the noisiest places due to the high diversity of calling insects, many species are still unknown and require taxonomic description and revision (Tan et al. 2017). Beyond their original descriptions, little is known about the biology of many katydid species in Southeast Asia.

While taxonomy is crucial for accurate identification and cataloging of bioacoustics data for studies on ecology, behavior, and evolution, the use of bioacoustics can also help overcome taxonomic impediment. Recent studies have demonstrated that the calling songs of Southeast Asian katydids can be used to resolve taxonomic problems related to species complexes. Heller et al. (2017) used calling songs to classify cryptic species within the *Ducetia japonica* species group. Previously thought to be a widely distributed species, it has been determined that different regions harbor different cryptic species. Tan et al. (2020a) and Heller et al. (2021a) combined calling songs and stridulatory anatomy to address species delineation in *Lipotactes alienus*-cum-*virescens* and *Mecopoda elongata* species complexes. In the case of *Lipotactes* Brunner von Wattenwyl, 1898, Tan et al. (2020a) provided a foundation for the further taxonomic progress of these little-known katydids from Southeast Asia (Ingrisch 2021, Gorochov 2021). These examples demonstrate the importance of combining bioacoustics and traditional taxonomy to identify species of katydids from Southeast Asia.

This study aimed to initiate a database containing acoustic and morphological data of Southeast Asian katydids. To document the previously unknown calling songs of Southeast Asian katydids, we opportunistically collected 24 species from Singapore and other parts of Southeast Asia, recorded their calling songs under ex-situ conditions, and accurately identified and systematically vouchered the specimens. Given the importance of the morphology of sound-producing organs in dictating key acoustic parameters (e.g., peak frequency and resonance) (Morris and Pipher 1967, Bailey 1970, Montealegre-Z 2009, Montealegre-Z and Postles 2010), we also made images of the sound-producing organs to complement the calling song description. These data can be incorporated into traditional taxonomy and/or used for meta-analysis to overcome taxonomic impediments while advancing our knowledge about the acoustic communication of these katydids.

Materials and methods

Collection and husbandry of katydids.—Katydids were opportunistically collected by sight (mostly at night but occasionally in the day) from six sites in the Malay Peninsula, Borneo, and the Philippines: (1) Singapore from August 2018 to December 2019 and from June to August 2020; (2) Pulau Tioman, Johor, Peninsular Malaysia from 7 to 9 August 2018; (3) Belait and Temburong, Brunei Darussalam from 6 to 18 July 2019; (4) Sandakan, Sabah, East Malaysia from 7 to 12 January 2019 and 30 September to 4 October 2019; and (5) Laguna, Luzon, the Philippines from 11 to 13 May and 6 to 8 September 2019. Whenever possible, in-situ images were taken using a Canon EOS 500D digital SLR camera with a compact macro lens EF 100 mm f/2.8 Macro USM, and a Canon Macro Twin Lite MT-24EX was used for lighting and flash.

The katydids were kept in insect cages. To avoid dehydration, wet cotton balls were provided, cages were covered with a wet cloth, and/or regular spraying was done. The katydids were subjected to light:dark hours corresponding to the locations where they were caught. They were generally fed with Pedigree Adult Chicken and Vegetables (18% protein, 10% fat, 5% fiber, no salt) or SmartHeart Puppy Beef and Milk Flavor (26% protein, 10% fat, 4% fiber, 10% moisture with salt) dog food (sometimes crushed). Fruits were also occasionally provided. Meconematinae were fed with living *Drosophila* fruit flies.

Acoustic recordings and analysis.—Acoustic recording and analysis generally followed that of Tan et al. (2019b, 2020a). All recordings were obtained in laboratory conditions or biological stations in the dark. The calling song of an isolated male placed inside a standardized insect cage (25 cm in diameter and 33 cm tall) with a nylon cover was sampled at a frequency of 256 kHz-samples/s using a Echo Meter Touch or Echo Meter Touch Pro 2 sensor (based on Knowles FG sensor). The recorder was placed horizontally and

at about 2–5 m away from the cage (depending on the loudness of the call to avoid clipping the recording). It should, however, be noted that with this type of microphone, a recording distance of less than 2 m is preferred to minimize distortion of the temporal structure of the signal. The triggered recording was used with the trigger minimum frequency set to 20 kHz. However, this was only a trigger and did not affect the quality of the recording at lower frequencies (i.e., <20 kHz). The recorded signals were saved in 12-bit and 16-bit WAV formats for Echo Meter Touch or Echo Meter Touch Pro 2 sensor, respectively. Ambient temperature was logged using a HOBO 8K Pendant Temperature logger (model: UA-001-08, Onset, Bourne, MA), or a temperature-humidity meter (Smartsensor AR867, Arco Science and Technology Limited, Dongguan, PRC).

The basic katydid song terminology follows Baker and Chesmore (2020):

Calling song = spontaneous song produced by an isolated male to attract a female;
Chirp = a type of echeme consisting of a few definite syllables;
Echeme = a first-order assemblage of syllables;
Echeme sequence = a first-order assemblage of echemes;
Interval = silent interval between calls and/or pulses, or downtime;
Peak frequency = frequency with the highest energy from the mean spectrum;
Period = interval between the start of successive units (e.g., syllable, echeme);
Pulse = a single unbroken wave train, isolated in time, produced by the impact of each tooth;
Pulse train = a series of pulses isolated in time;
Syllable = single complete stridulatory movement (i.e., opening and closing of wings). Since wing movement was not examined, the term syllable is used here as an assemblage of pulses isolated in time and likely to correspond to a single complete stridulatory movement;
Trill = a type of echeme consisting of many syllables.

We also used spectral entropy to estimate signal heterogeneity, in which a low value indicates highly tonal signals and a high value indicates broad-band signals (Chivers et al. 2017a).

Parameters of the temporal domain (e.g., call duration/ period and interval) were measured manually using Raven Lite 2.0.0. For frequency domain parameters, custom-written scripts in MATLAB (R2019a; MathWorks Inc., Natick, MA, United States) were used. This involved determining 2048 Fast Fourier Transformation (FFT) lines, Q_{-3}, and Q_{-10} entropy, spread and flatness.

Specimen curation and identification.—The specimens were preserved in absolute analytical-grade ethanol and later pinned and dry preserved. For future molecular work, a single hind leg from each specimen was also preserved in absolute analytical-grade ethanol. The katydids were identified using taxonomic papers, including Willemse (1959), Jin (1992), Ingrisch (1995, 1998, 2015), Gorochov (1998, 2008, 2011, 2013), Tan and Ingrisch (2014), Tan (2014, 2017), Tan et al. (2015, 2018, 2019a), Tan and Artchawakom (2017), Jin et al. (2020), and by comparing them with photographs of type specimens. Taxonomists, specifically Xing-bao Jin, Sigfrid Ingrisch, and Andrei Gorochov, were also consulted.

Sound-producing structure.—The left and right tegmina were dissected whenever possible. Three-dimensional images of the stridulatory file on the left tegmen and sound-producing organs on the right tegmen were obtained with infinite focus microscopy using an Alicona Infinite Focus (model G5) microscope (OPTIMAX Imaging Inspection and Measurement Limited, Leicestershire, UK).

Depositories.—

FRC	Forest Research Center, Sepilok, Sabah, East Malaysia
UBDM	Universiti Brunei Darussalam Museum, Brunei Darussalam
UPLBMNH	University of the Philippines Los Baños, Museum of Natural History, Philippines
ZRC	Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore

The sound files were deposited in the Orthoptera Species File (OSF) Online Version 5.0/5.0 (Cigliano et al. 2022).

Results

Summary.—In total, 37 individual katydids were collected. Of these, the calling songs of 24 species from 20 genera of the subfamilies Conocephalinae (nine species), Lipotactinae (one species), Meconematinae (seven species), and Phaneropterinae (seven species) were recorded for the first time (Table 1). The peak frequency of each of the 24 katydids species was found to range from as low as 12.6 kHz in *Paragraecia temasek* Tan & Ingrisch, 2014 to as high as 54.2 kHz in an unidentified Meconematini from Sandakan. Twenty-one species (87.5%) were found to have peak frequencies in the ultrasonic range, of which three species (12.5%) can be considered extreme ultrasonic callers (i.e., peak frequency >40 kHz; Table 1). The spectral entropy of the katydids ranges from 6.8 in *Casigneta* sp. 2 to 8.8 in *Conocephalus cognatus* (Redtenbacher, 1891). Of the 24 species whose calls are described here, we imaged and described the sound-producing structures of 18.

Song and sound-producing structure descriptions

***Axylus philippinus* (Hebard, 1922)** (n = 1 male, 10 sound files) (Fig. 1): The calling song is a continuous trill made up of disyllabic echemes (each consisting of two amplitude peaks). At 30.0±0.5°C (28.9–30.3°C), the trill has a echeme repetition rate of 11±1 echeme s^{-1} (9–11 echemes s^{-1}). The echeme period is 92.9±5.5 ms (87.5–104.1 ms). The call spectrum has a peak frequency of 34.7±1.3 kHz (32.5–36.0 kHz) and another peak at 16.4±1.9 kHz (13.5–19.0 kHz) showing energy in the sonic range; the spectral entropy is 8.5±0.1.

Ventrally, the left macropterous tegmen possesses a straight stridulatory file of about 1.556 mm in length with 91 rather broad teeth. The teeth on the stridulatory file of the left tegmen are fairly uniformly distributed and narrowly spaced apart. The inter-tooth distance is nearly constant throughout the file. In the mid-part of the stridulatory file, the teeth density is 48.5 teeth mm^{-1}, and the average tooth width is 105 µm. The file (Cu2) is slightly elevated on a swollen vein buttress. The right tegmen has a rectangular mirror that is longer than broad and a stridulatory file of about 1.203 mm in length with about 59 rather broad teeth and a few indistinct teeth at the anal end.

Table 1. Summary of the species recorded in this study.

	Species	Country of origin	Call structure	Spectral entropy	Peak freq. (kHz)
	Subf. Conocephalinae				
1.	*Axylus philippinus*	Philippines	Continuous trill of disyllabic echeme	8.5±0.1	34.7±1.3
2.	*Conocephalus cognatus*	Singapore	Complex echeme	8.8±0.1	28.7±2.1
3.	*Conocephalus exemptus*	Singapore	Complex echeme	7.8±0.1	15.5±0.2
4.	*Paragraecia temasek*	Singapore	Echeme	7.7±0.1	12.6±0.1
5.	*Peracca macritchiensis*	Singapore	Echeme sequence	7.4	29.3±1.1
6.	*Salomona borneensis*	Malaysia	Echeme sequence	8.4±0.1	13.9±0.4
7.	*Salomona maculifrons*	Philippines	Sequence of isolated echemes	8.5±0.1	30.5±0.7
8.	*Viriacca insularis*	Malaysia	Echeme	8.0±0.2	23.1±1.8
9.	*Viriacca modesta*	Brunei	Echeme sequence	7.8±0.1	26.0±2.4
	Subf. Lipotactinae				
10.	*Lipotactes maculatus*	Singapore	Isolated echemes	8.3	33.1±3.1
	Subf. Meconematinae				
11.	*Alloteratura lamella*	Singapore	Complex echemes or isolated syllables	7.7±0.3	25.5±0.7
12.	*Borneopsis cryptosticta*	Singapore	Sequence of paired syllables or echemes	8.5±0.3	42.3±2.4
13.	*Euanisous teuthroides*	Singapore	Echeme	7.4±0.2	30.3±0.7
14.	*Kuzicus denticulatus*	Singapore	Continuous trill	7.7	39.6±2.4
15.	Meconematini (SDK.19.79)	Malaysia	Continuous trill of paired syllables	7.6	54.2±0.4
16.	*Neophisis siamensis*	Singapore	Sequences of isolated syllables	7.1	36.7±1.8
17.	*Xiphidiopsis* (*Xiphidiopsis*) *dicera*	Singapore	Continuous trill	7.7	40.9±0.4
	Subf. Phaneropterinae				
18.	*Casigneta* sp. 1	Singapore	Pulse train	7.6±0.1	28.7±0.8
19.	*Casigneta* sp. 2	Singapore	Triplet syllables	6.8±0.2	28.2±0.2
20.	*Holochlora* nr. *bilobata*	Singapore	Isolated syllables	8.1±0.4	33.3±1.0
21.	*Phaneroptera brevis*	Singapore	Paired syllables	7.9	21.9±0.8
22.	*Phaulula malayica*	Singapore	Isolated syllables	7.8	23.6±1.2
23.	*Psyrana tigrina*	Malaysia	Pulse train	8.1	35.5±2.1
24.	*Scambophyllum sanguinolentum*	Singapore	Pulse train	7.0±0.1	23.7±0.3

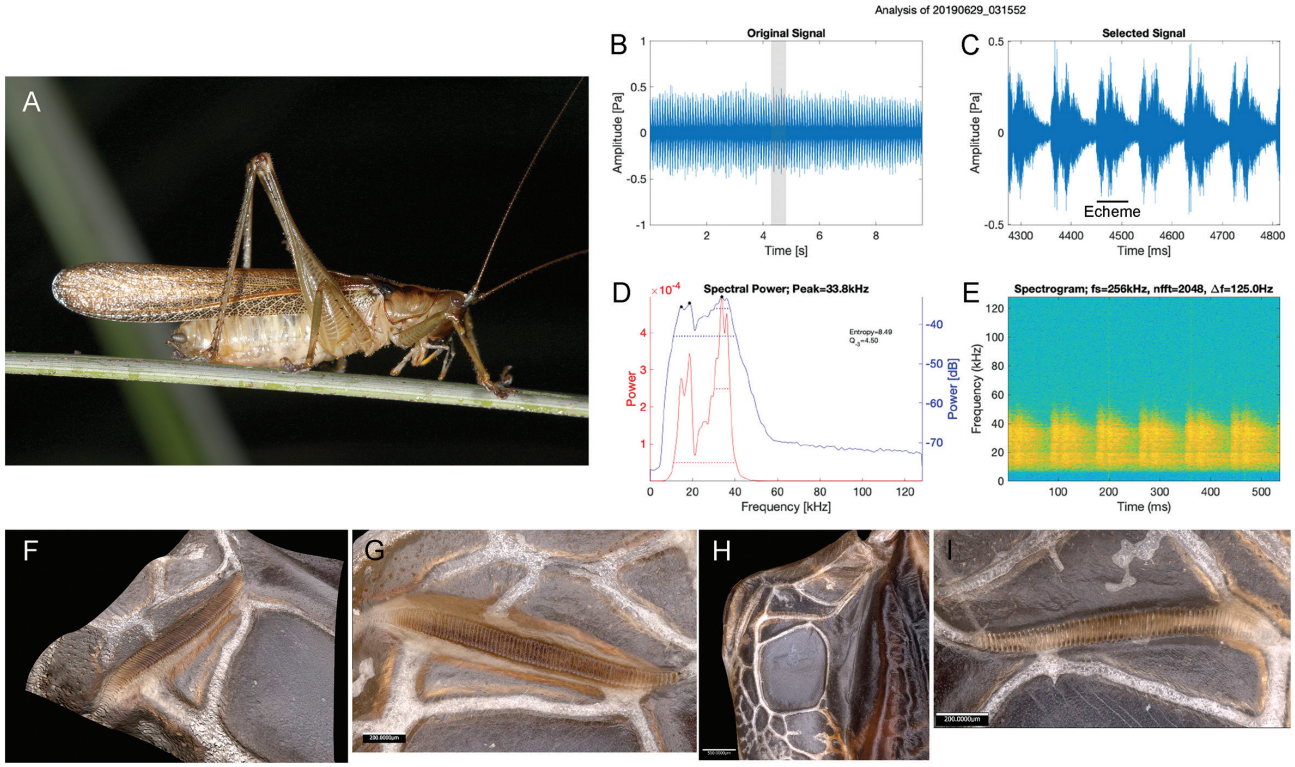

Fig. 1. *Axylus philippinus* male adult in its natural environment in Laguna, the Philippines (**A**). Oscillograms showing a continuous trill (**B**) and a section of the trill consisting of five complete echemes (**C**). Power spectrum (**D**) and spectrogram of the selection (**E**) of the same five complete echemes. Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

***Conocephalus cognatus* (Redtenbacher, 1891)** (n = 5 males, 16 sound files) (Fig. 2): The calling song is an echeme sequence made up of complex echemes. Each echeme consists of two parts. At 29.4±0.6°C (27.8–31.0°C), the first part at the start of an echeme consists of a syllable showing a rapid-decay pulse with a duration of 51.3±10.7 ms (25.0–67.0 ms) and period of 88.1±16.4 ms (62.0–120.0 ms) followed by an interval of 36.9±16.7 ms (6.0–69.0 ms). The second part consists of 2–23 echemes with a echeme repetition rate of 8±1 echemes s^{-1} (8–9 echemes s^{-1}). Each echeme shows 2–4 amplitude peaks; echeme duration is 80.1±16.0 ms (47.0–108.0 ms). Often, only the first part of the calling song is produced. The call spectrum has a peak frequency of 28.7±2.1 kHz (25.6–33.1 kHz), and the spectral entropy is 8.8±0.1.

Ventrally, the left micropterous tegmen possesses a stridulatory file of about 0.988 mm in length with about 56 teeth. The stridulatory file on the left tegmen is primarily straight and strongly curving anteriorly at the basal end. The teeth at the anal end are the smallest (average tooth width is 13.4 µm) and closely packed (average inter-tooth distance is 9.2 µm); the teeth in the mid-part of the file are the largest (average tooth width is 35.7 µm) with an average inter-tooth distance of 26.0 µm. The teeth at the basal end have an average tooth width of 30.5 µm and are most widely spaced apart (average inter-tooth distance is 31.8 µm). The file (Cu2) is only slightly elevated on a swollen vein buttress. The right tegmen has an oblique mirror longer than broad, with the anal margin distinctly shorter than the basal margin. The stridulatory file on the right tegmen is sinusoidal, about 0.802 mm in length, and with about 37 stout teeth.

***Conocephalus exemptus* (Walker, 1869)** (n = 2 males, 9 sound files) (Fig. 3): A sound file was deposited in OSF based on a specimen from Thailand. While the call of the Thailand specimen consists of an echeme made up of four syllables, the calling song of individuals from Singapore appears as a complex echeme consisting of two parts. At 29.4±0.1°C (29.3–29.6°C), the first part, which is not always present, consists of a echeme with a duration of 2.6±1.2 s (1.1–3.9 s). This echeme shows a series of amplitude peaks with increasing amplitude to a maximum. The second part consists of a trill made up of a sequence of syllables. Syllable duration is 0.10±0.01 s (0.09–0.13 s), and syllable period is 0.26±0.05 s (0.19–0.32 s). The syllables have amplitudes similar to the maximum amplitude of the first part of the echeme. Each syllable shows 5±1 (4–6) amplitude peaks. The call spectrum has a peak frequency of 15.5±0.2 kHz (15.2–16.0 kHz), and the spectral entropy is 7.8±0.1.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 1.761 mm in length with about 60 stout teeth and a few indistinct ones at the anal end. The stridulatory file on the left tegmen is faintly curved and strongly curving anteriorly at the basal end. The teeth are smallest (average tooth width is 38.3 µm) and closely packed (average inter-tooth distance is 18.1 µm) at the anal end and largest (average tooth width is 59.9 µm) and most widely spaced (average inter-tooth distance is 35.5 µm) in the middle portion. The file (Cu2) is slightly elevated on a swollen vein buttress. The right tegmen has a distinctly elongated mirror. The stridulatory file on the right tegmen is about 1.217 mm in length with approximately 51 stout teeth.

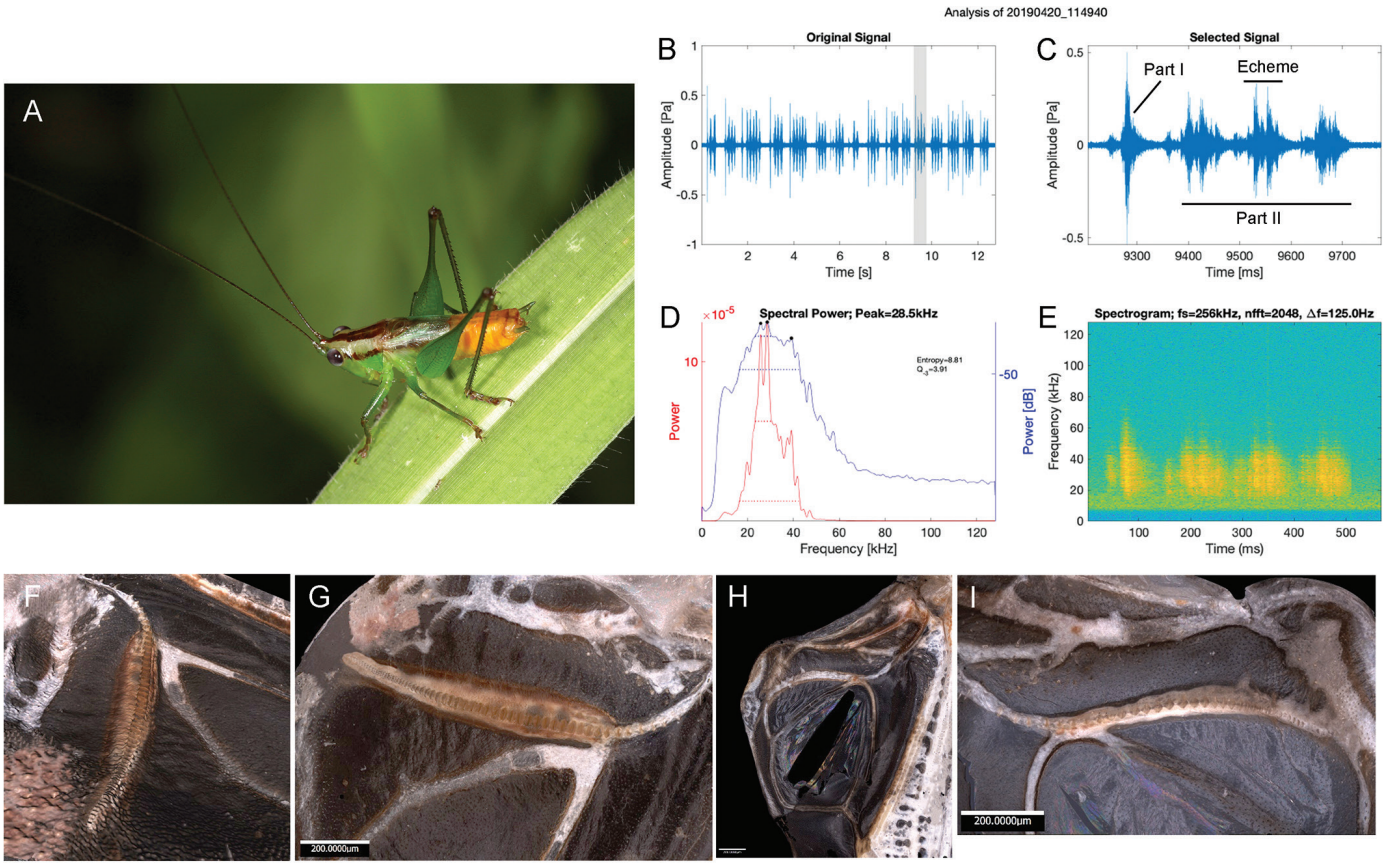

Fig. 2. *Conocephalus cognatus* male adult in its natural environment in Singapore (**A**). Oscillograms showing an echeme sequence (**B**) and a complex echeme with two parts (**C**). Power spectrum (**D**) and spectrogram (**E**) of an echeme made up of two parts. Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

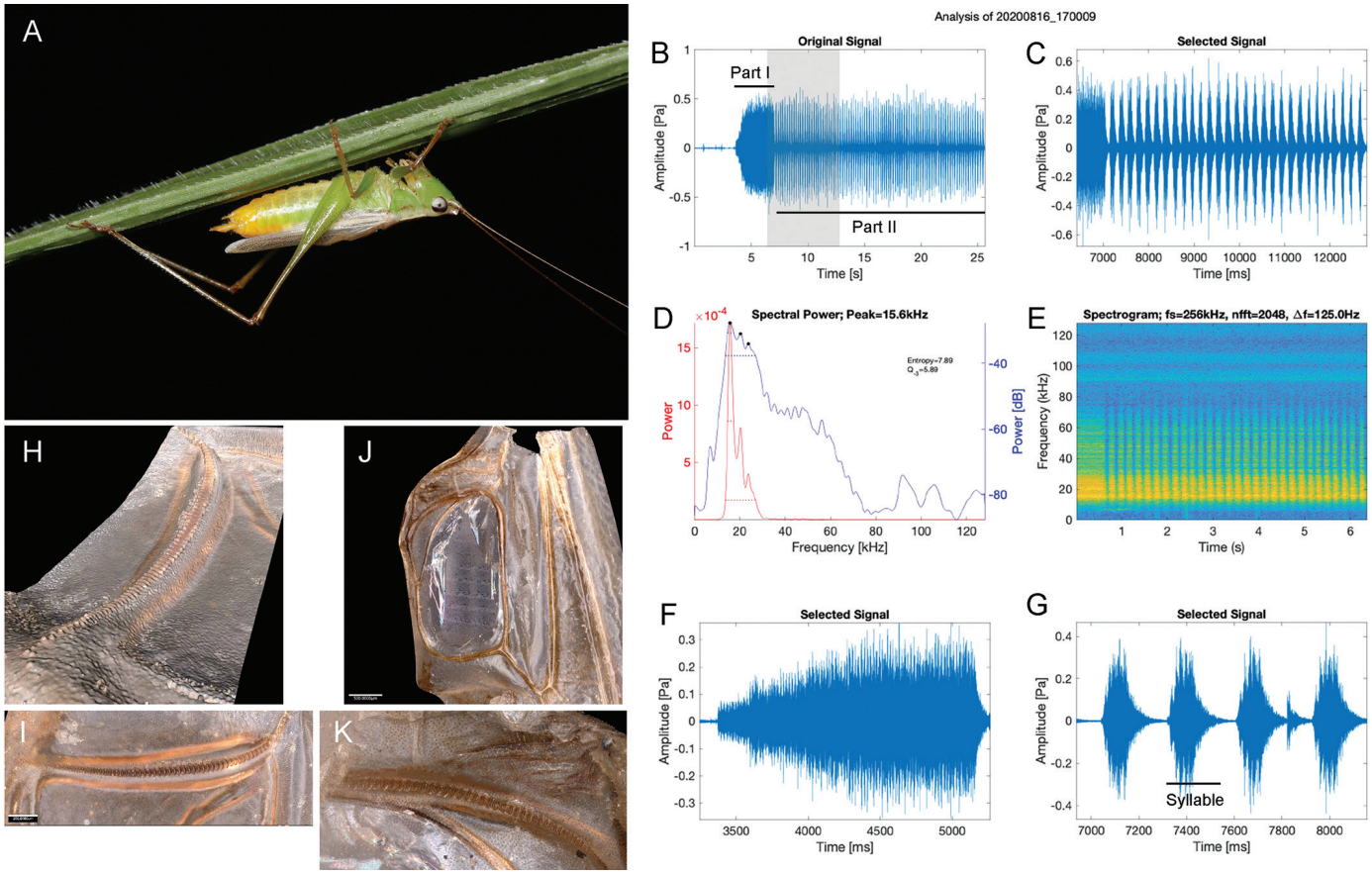

Fig. 3. *Conocephalus exemptus* male adult in its natural environment in Singapore (**A**). Oscillograms showing the start of a complex echeme (**B**) and a section of the echeme at the end of the first part and the beginning of the second part (**C**). Power spectrum (**D**) and spectrogram (**E**) of the echeme at the end of the first part and the beginning of the second part. Oscillograms showing the first (**F**) and second (**G**, showing four syllables) parts of an echeme. Three-dimensional anal view of the left stridulatory file (SF) (**H**), ventral view of the same SF (**I**), ventral view of the right tegmen sound-producing organs (**J**), and ventral view of the right SF (**K**).

Paragraecia temasek **Tan & Ingrisch, 2014** (n = 1 male, 20 sound files) (Fig. 4): The calling song consists of isolated echemes, but sometimes two to four echemes may aggregate together. At 28.9±0.1 °C (28.9–29.1 °C), echeme duration is 0.86±0.25 s (0.59–1.54 s). Each echeme shows a series of syllables with increasing amplitude to a maximum. Each echeme has a syllable repetition rate of ca. 89 syllables s^{-1} (87–91 syllables s^{-1}). The interval between echemes is also variable, ranging from 0.13 s to 2.6 s (0.63±0.58 s). Unlike many of the other katydids discussed here, a harmonic series consisting of three peaks was recorded: fundamental frequency, which is also the peak frequency of 12.6±0.1 kHz (12.4–12.8 kHz) at the sonic range, followed by peaks of decreasing energy at 23.9±0.3 kHz (23.0–24.5 kHz) and 36.2±0.4 kHz (35.5–37.0 kHz) at the ultrasonic range. The call spectrum has a spectral entropy of 7.7±0.1.

Ventrally, the left macropterous tegmen possesses a very straight stridulatory file of about 1.464 mm in length and with more than 250 rather broad teeth. The teeth on the stridulatory file on the left tegmen are fairly uniformly distributed and very narrowly spaced. In the mid-part of the stridulatory file, the teeth density is 10.2 teeth mm^{-1}, and the average tooth width is 103 µm. The teeth are most prominent in the middle portion, and tooth width tapers gently toward the ends. The distance between teeth is nearly constant throughout the file. The file (Cu2) is slightly elevated on a swollen vein buttress. The right tegmen has a rectangular mirror, longer than broad, with curved anal and basal margins, and a stridulatory file of about 1.129 mm in length, with about 130 rather broad teeth.

Peracca macritchiensis **Tan & Ingrisch, 2014** (n = 1 male, 10 sound files) (Fig. 5): The calling song consists of an echeme sequence made up of echemes of highly variable duration. Sometimes, the echeme sequence lasts for a long duration, appearing as a continuous 'trill'. At 28.5±1.1 °C (26.9–29.3 °C), the echeme is made up of closely-spaced syllables with a syllable repetition rate of 81±6 syllables s^{-1} (69–91 syllables s^{-1}). Syllable duration is 9.1±1.5 ms (6.2–11.6 ms). The call spectrum has a peak frequency of 29.3±1.1 kHz (27.8–30.8 kHz), and the spectral entropy is 7.4.

Ventrally, the left micropterous tegmen possesses a very straight stridulatory file of about 0.611 mm in length and with about 117 rather broad teeth. The teeth on the stridulatory file are fairly uniformly distributed and very narrowly spaced. In the mid-part of the stridulatory file, the teeth density is 20.5 teeth mm^{-1}, and the average tooth width is 47.6 µm. The teeth are most prominent in the middle portion, and tooth width tapers gently toward the ends. The distance between teeth is nearly constant throughout the file. The file (Cu2) is faintly elevated on a swollen vein buttress. The right tegmen has a pyriform mirror with a narrower anterior end.

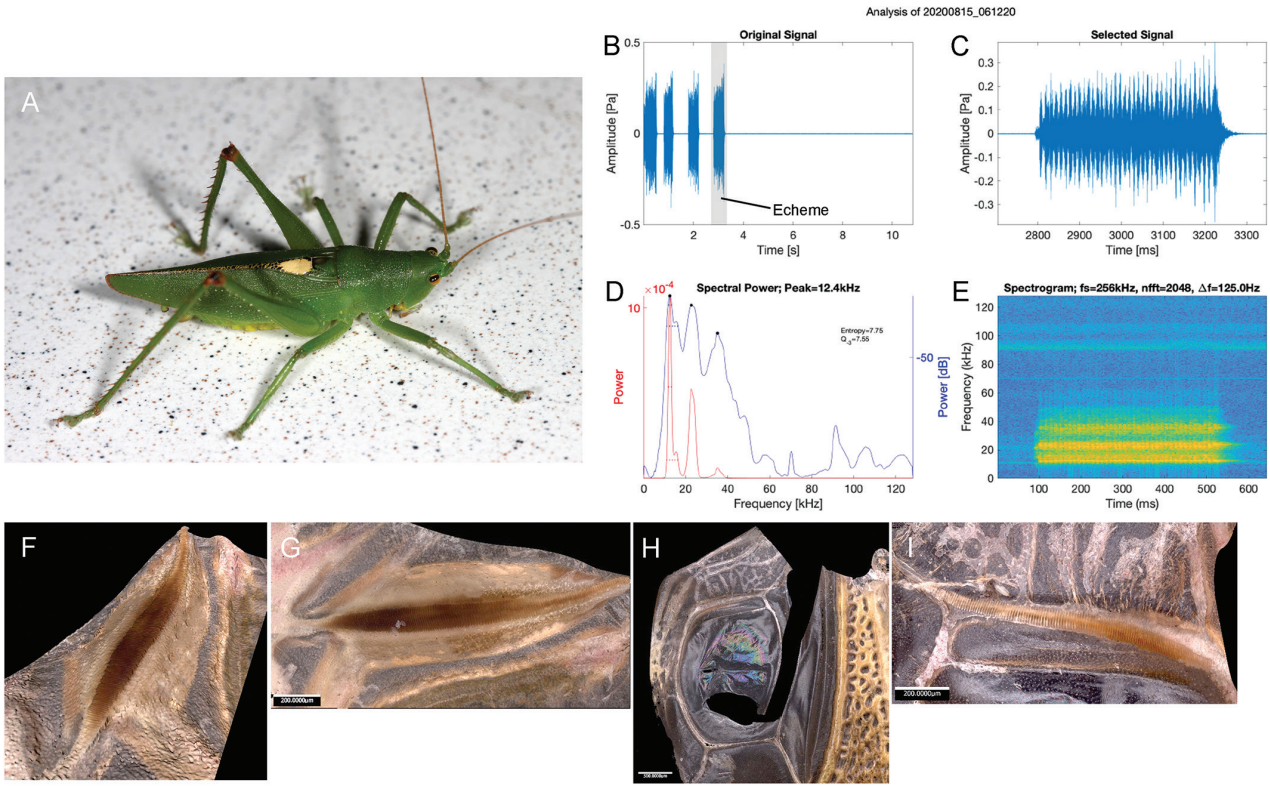

Fig. 4. *Paragraecia temasek* male adult in the lab (**A**). Oscillograms showing four echemes (**B**) and a single echeme (**C**). Power spectrum (**D**) and spectrogram of the same echeme (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

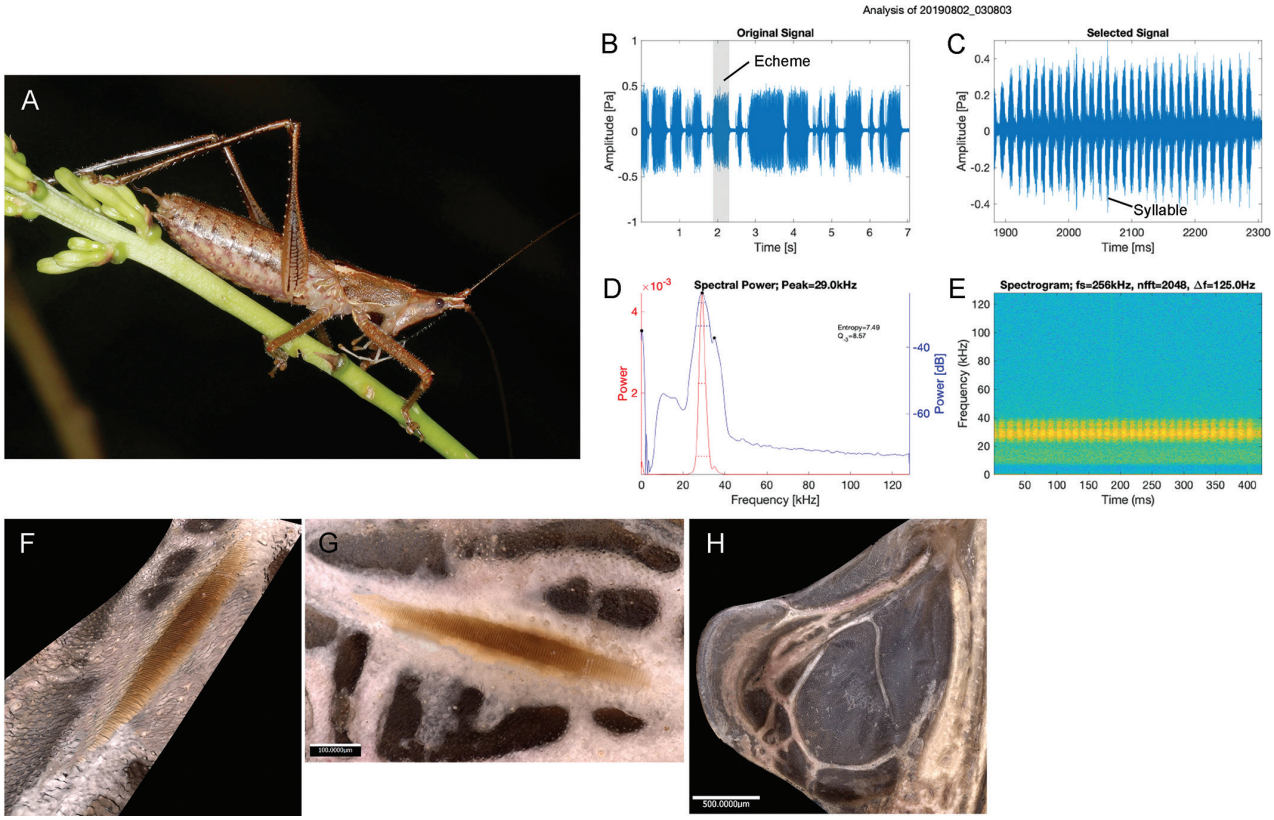

Fig. 5. *Peracca macritchiensis* male adult in its natural environment in Singapore (**A**). Oscillograms showing an echeme sequence (**B**) and a single echeme (**C**). Power spectrum (**D**) and spectrogram of the same echeme (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), and ventral view of the right tegmen sound-producing organs (**H**).

***Salomona borneensis* Willemse, 1959** (n = 1 male, 11 sound files) (Fig. 6): The calling song is an echeme sequence made up of fairly isolated echemes. At 28.7±0.2°C (28.4–29.0°C), the echeme duration is 0.24±0.02 s (0.22–0.28 s), and the interval between echemes is 0.33±0.12 s (0.13–0.52 s). Each echeme consists of two or three closely packed syllables, each lasting about 50 ms. The call spectrum has a peak frequency of 13.9±0.4 kHz (13.0–14.5 kHz) and another peak at 31.2±1.0 kHz (30.0–33.0 kHz) showing energy in the ultrasonic range; the spectral entropy is 8.4±0.1.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 2.872 mm in length with about 79 rather broad teeth. The stridulatory file on the left tegmen is faintly curved and slightly more strongly curving anteriorly at the basal end. The teeth are most prominent in the middle portion, and tooth width tapers gently toward the ends. The distance between teeth is fairly uniform. The file (Cu2) is slightly elevated on a faintly swollen vein buttress. The right tegmen has a squarish mirror. The stridulatory file on the right tegmen is sinusoidal, with a length of about 2.409 mm and with about 65 rather broad teeth.

***Salomona maculifrons* Stål, 1877** (n = 1 male, 15 sound files) (Fig. 7): The calling song is a sequence of distinctly isolated syllables occurring either over a long duration as a trill or a shorter duration as echemes. At 28.2±1.6°C (26.7–30.5°C), the syllable has a duration of ca. 50 ms, and the interval between syllables is 67±47 ms (17–194 ms). The call spectrum has a peak frequency of 30.5±0.7 kHz (30.5–31.5 kHz) at the ultrasonic range and another peak at 14.2±0.2 kHz (13.9–14.5 kHz) showing energy in the sonic range. The two peaks have relatively similar energy, and at times, the non-ultrasonic peak is the dominant frequency. The spectral entropy is 8.5±0.1.

Ventrally, the left macropterous tegmen possesses a very straight stridulatory file of about 2.274 mm in length with about 87 rather broad teeth. The teeth are largest in the middle portion (average tooth width is 202 µm), and tooth width tapers gently toward the ends. The teeth are closely packed, and the distance between teeth is fairly similar. In the mid-part of the stridulatory file, the teeth density is 40 teeth mm^{-1}. The file (Cu2) is slightly elevated on a slightly swollen vein buttress. The right tegmen has a somewhat squarish mirror but slightly broader than long. The stridulatory file on the right tegmen is about 1.615 mm in length with about 69 rather broad teeth.

***Viriacca insularis* Gorochov, 2011** (n = 1 male, 18 sound files) (Fig. 8): The calling song is an isolated echeme made up of syllables of increasing amplitude to a maximum. Sometimes, the calling song can occur over a long duration as a continuous trill. Each echeme has a syllable repetition rate of 22±1 syllables s^{-1} (21–26 syllables s^{-1}) at 28.5±0.4°C (27.7–29.7°C). The call spectrum has a peak frequency of 23.1±1.8 kHz (21.5–28.5 kHz), and the spectral entropy is 8.0±0.2.

Ventrally, the left micropterous tegmen possesses a stridulatory file of about 1.659 mm in length with more than 100 broad teeth. The stridulatory file is very straight and slightly curving anteriorly at the basal end. The teeth are largest in the middle portion (average tooth width is 103 µm), and tooth width tapers gently toward

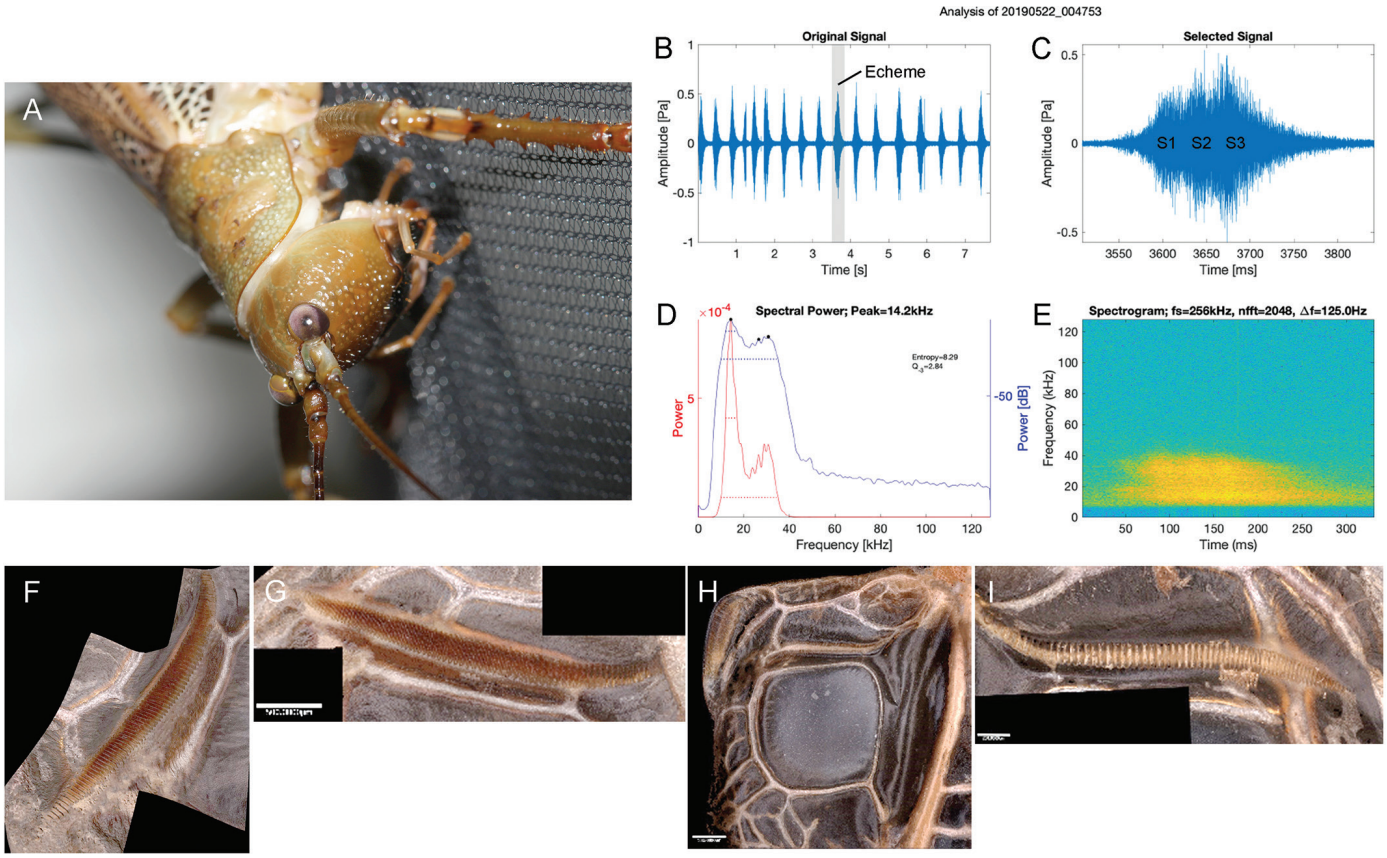

Fig. 6. *Salomona borneensis* male adult in the lab (**A**). Oscillograms showing an echeme sequence with 17 echemes (**B**) and an echeme with three syllables denoted as S1 to S3 (**C**). Power spectrum (**D**) and spectrogram of the same echeme (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

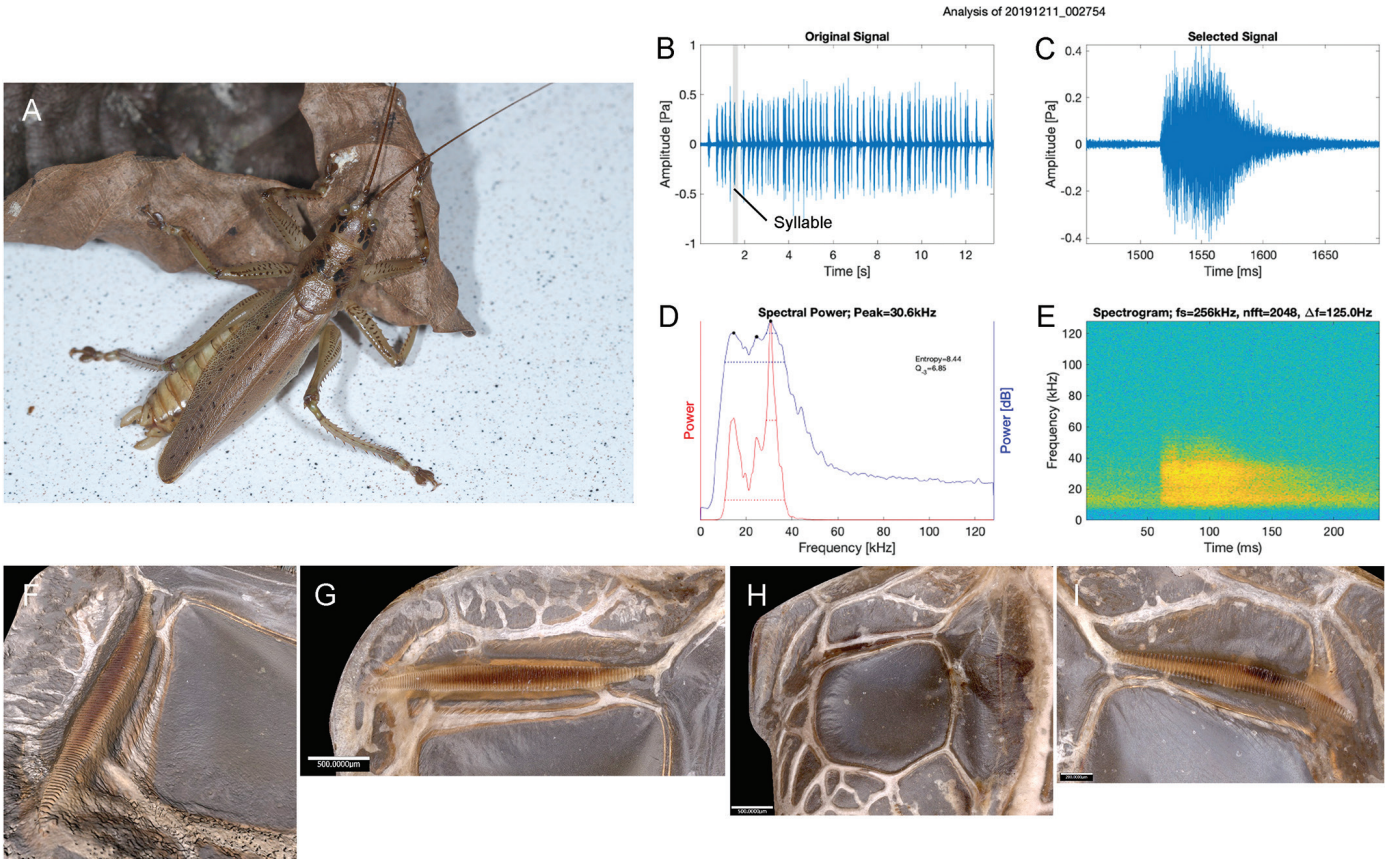

Fig. 7. *Salomona maculifrons* male adult in the lab (**A**). Oscillograms showing a continuous trill (**B**) and a syllable (**C**). Power spectrum (**D**) and spectrogram of the same syllable (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

the ends. The teeth are closely packed, and the distance between teeth is fairly similar. In the mid-part of the stridulatory file, the teeth density is 95 teeth mm^{-1}. The file (Cu2) is slightly elevated on a swollen vein buttress. The right tegmen has a somewhat triangular mirror with anterior margin rounded and posterior end acute, longer than broad. The stridulatory file on the right tegmen is about 0.984 mm in length with about 100 rather broad teeth.

***Viriacca modesta* Gorochov, 2013** (n = 2 males, 13 sound files) (Fig. 9): The calling song is a continuous echeme sequence made up of isolated echemes. Each echeme consists of 1–4 closely packed syllables. At 25.3±3.0 °C (22.9–29.5 °C), the echeme duration is 0.16±0.01 s (0.14–0.19 s), the echeme period is 0.23±0.04 s (016–0.31 s), and the interval between consecutive echemes is 0.07±0.04 s (0.02–0.15). Syllable period is 29.3±5.2 ms (21.0–38.0 ms). The call spectrum has a peak frequency of 26.0±2.4 kHz (21.8–28.6 kHz), and the spectral entropy is 7.8±0.1.

Ventrally, the left micropterous tegmen possesses a stridulatory file of about 1.451 mm in length with about 159 broad teeth. The file is very straight and faintly curving anteriorly at the basal end. The teeth are largest in the middle portion (average tooth width is 100 µm), and tooth width tapers gently toward the ends. The teeth are closely packed, and the distance between teeth is fairly uniform. In the mid-part of the stridulatory file, the teeth density is 10.4 teeth mm^{-1}. The file (Cu2) is slightly elevated on a swollen vein buttress. The right tegmen has a rectangular mirror longer than broad, with anterior margin broader and rounded and with posterior margin truncated and narrower. The stridulatory file on the right tegmen is about 1.072 mm in length, with about 112 rather broad teeth.

***Lipotactes maculatus* Hebard, 1922** (n = 1 male, 16 sound files) (Fig. 10): The calling song was first described from Bukit Timah (Singapore) by Ingrisch (1995) as a trill or as short echemes of 120–190 ms. We recorded another individual from Mandai (also Singapore) using an ultrasound-sensitive recorder to obtain more precise frequency data. The calling song from Mandai consists of an isolated echeme. The echeme duration is 0.14±0.01 s (0.11–0.16 s), the echeme period is 2.48±0.55 s (1.74–3.51 S), and the interval between echemes is 2.34±0.54 s (1.59–3.35 s) at 28.5±1.1 °C (26.9–29.3 °C). Each echeme typically consists of 4 (3–5) closely packed syllables. Syllable period is 23.2±1.8 ms (21.0–26.0 ms). The call spectrum has a peak frequency of 33.1±3.1 kHz (25.9–38.2 kHz), and the spectral entropy is 8.3.

Ventrally, the left micropterous tegmen possesses a stridulatory file of about 1.183 mm in length with about 43 stout teeth. The file is slightly curved. The teeth at the anal end are smallest (average tooth width is 13.4 µm) and closely packed (average inter-tooth distance is 16.6 µm); the teeth in the middle of the file are largest (average tooth width is 38.3 µm) and are most widely spaced apart (average inter-tooth distance is 37.3 µm); the teeth at the basal end have an average tooth width of 20.7 µm and an average inter-tooth distance is 27.8 µm. The file (Cu2) is strongly elevated at the anal end and on a very swollen vein buttress (especially swollen at the anal end). The right tegmen has a triangular mirror. The stridulatory file on the right tegmen is slightly sinusoidal, about 1.176 mm in length, with about 33 stout teeth and a few indistinct teeth at both ends.

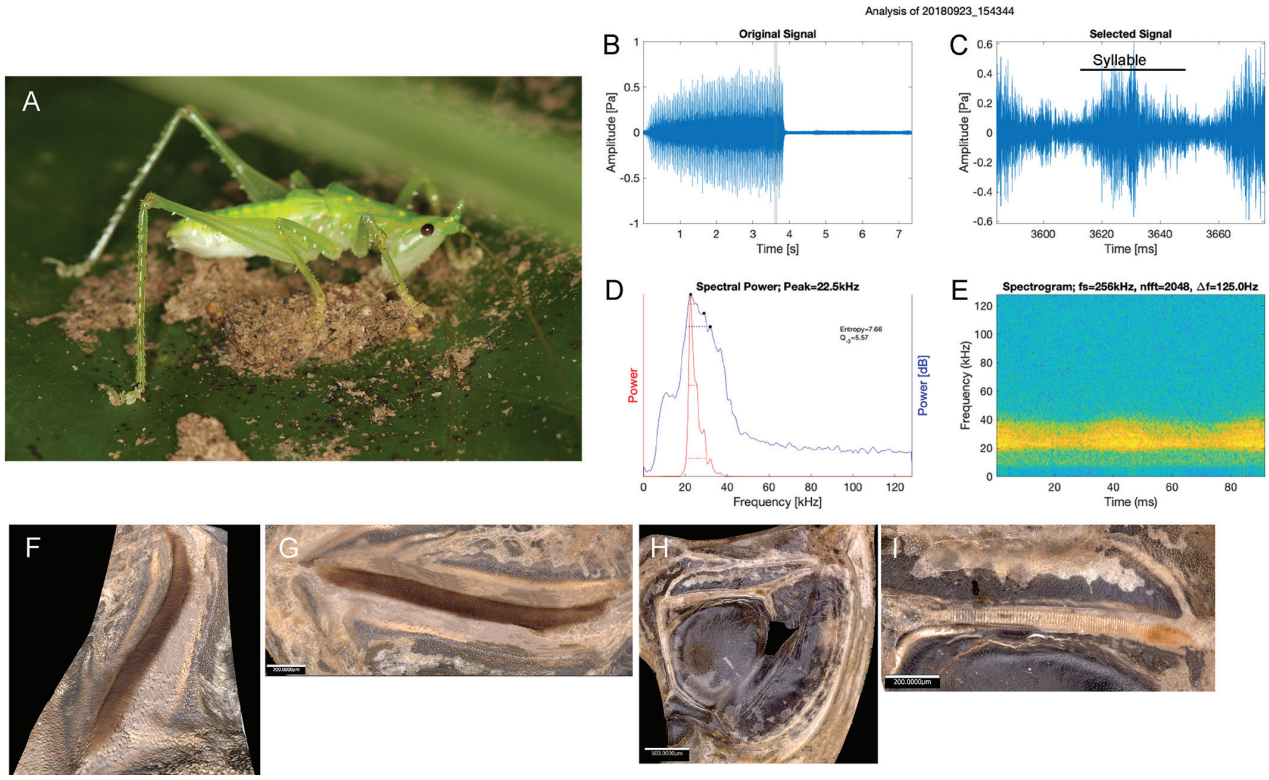

Fig. 8. *Viriacca insularis* male adult in its natural environment in Pulau Tioman, Malaysia (**A**). Oscillograms showing an echeme (**B**) and a syllable (**C**). Power spectrum (**D**) and spectrogram of the same syllable (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

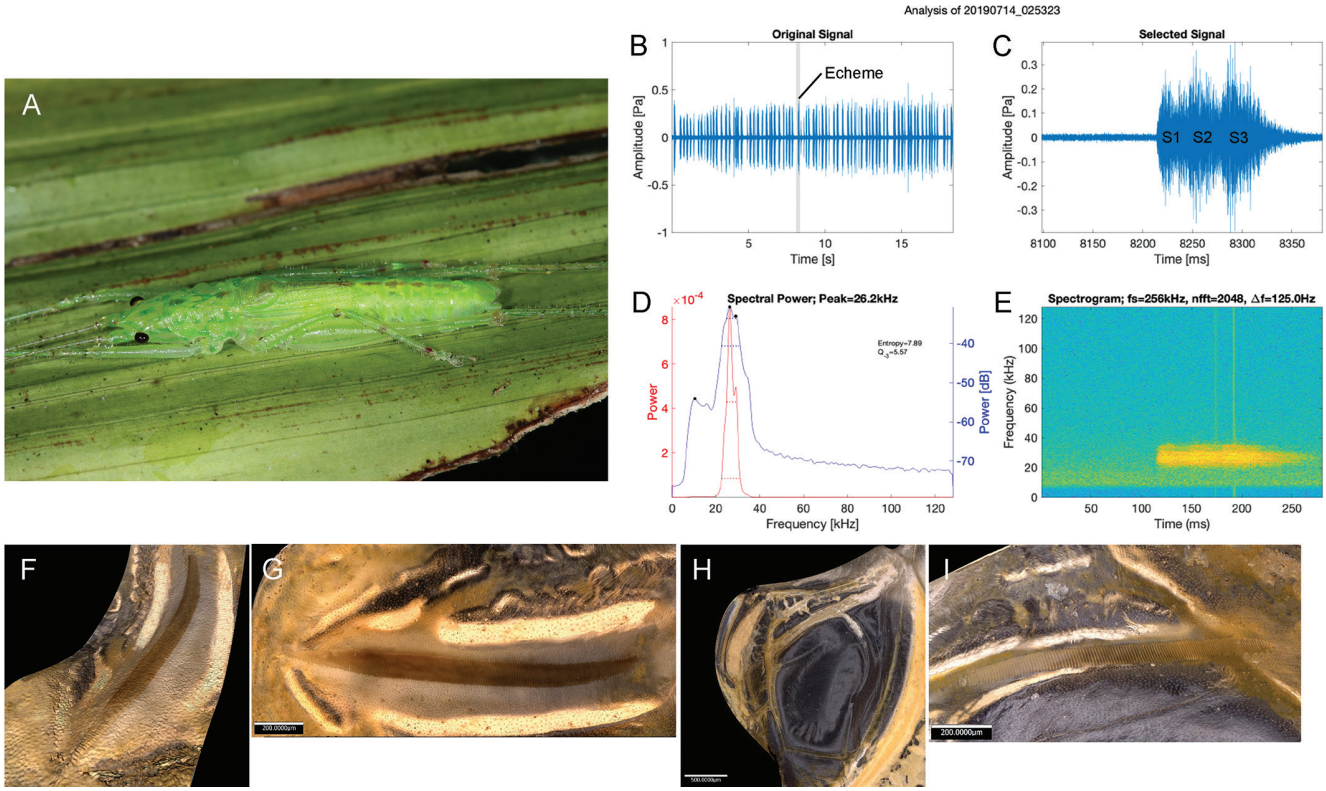

Fig. 9. *Viriacca modesta* male adult in its natural environment in Belait, Brunei Darussalam (**A**). Oscillograms showing a continuous echeme sequence (**B**) and an echeme with three syllables denoted as S1 to S3 (**C**). Power spectrum (**D**) and spectrogram of the same echeme (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

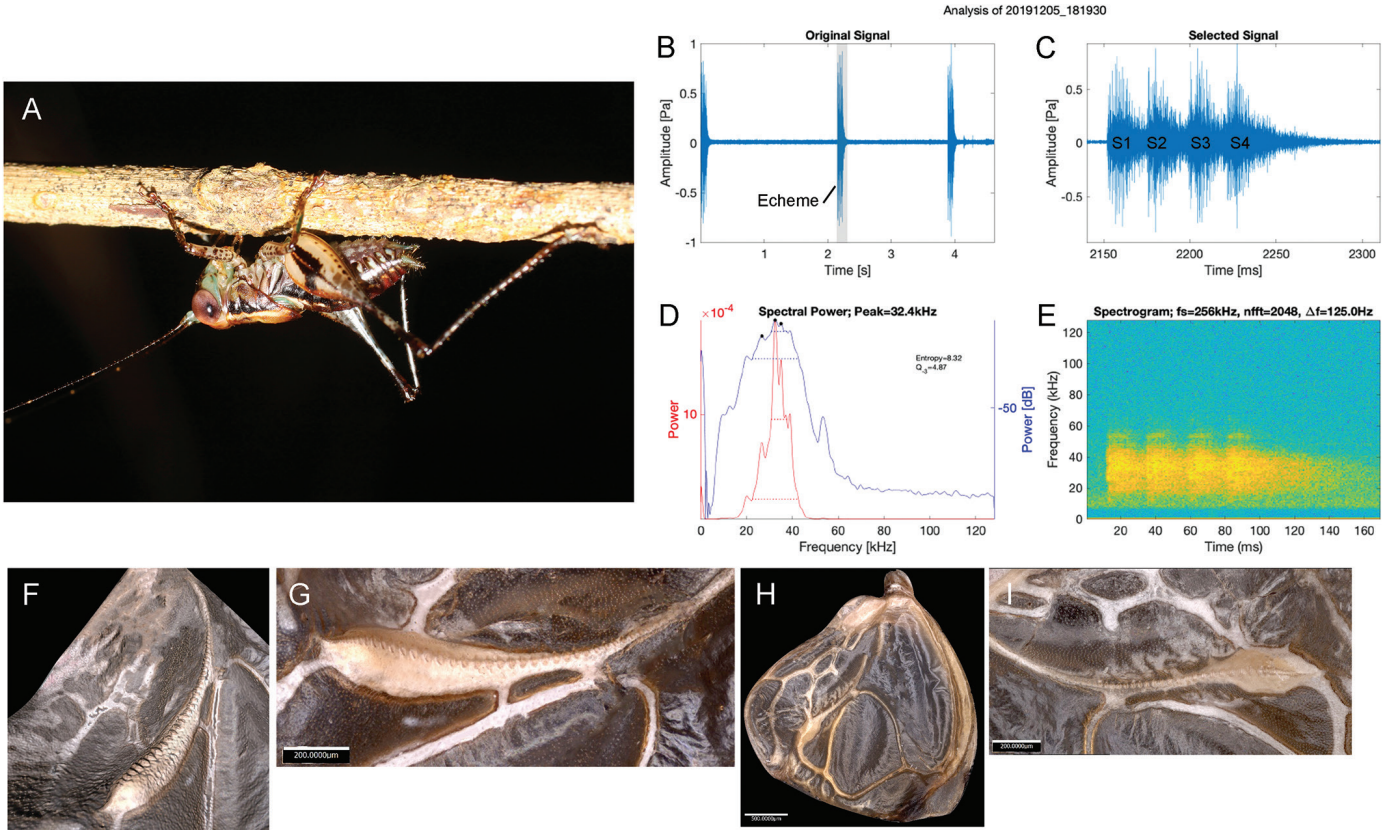

Fig. 10. *Lipotactes maculatus* male adult in its natural environment in Singapore (**A**). Oscillograms showing three isolated echemes (**B**) and an echeme with four syllables denoted as S1 to S4 (**C**). Power spectrum (**D**) and spectrogram of the same echeme (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

***Alloteratura lamella* Jin, 1995** (n = 2 males, 21 sound files) (Fig. 11): This species has two song modes. The first song mode consists of a complex echeme made up of two parts: the first part is comprised of a few isolated syllables, and the second part is a echeme made up of syllables packed closely together. At 29.2±0.7°C (28.6–30.5°C), each syllable of the first part of the calling song has a duration of 0.10±0.01 s (0.08–0.11 s), period of 0.33±0.06 s (0.22–0.46 s), and interval between syllables of 0.24±0.06 s (0.12–0.35 s). The echeme duration is 1.21±0.40 s (0.39–1.75 s), is made of 29±8 (7–35) closely spaced syllables, and the syllable repetition rate is 20±1 syllables s^{-1} (18–22 syllables s^{-1}). The second song mode consists of only isolated syllables. The call spectrum has a peak frequency of 25.5±0.7 kHz (24.6–27.0 kHz), and the spectral entropy is 7.7±0.3.

***Borneopsis cryptosticta* (Hebard, 1922)** (n = 2 males, 17 sound files) (Fig. 12): Two modes of calling songs were recorded. The first and most commonly recorded one consists of syllables occurring in pairs. At 30.2±0.2°C (29.9–30.5°C), each doublet of syllables has a duration of 61.2±6.8 ms (50.1–71.1 ms) and a period of 0.51±0.14 s (0.35–0.82 s), with the interval between consecutive doublets of 0.45±0.14 s (0.29–0.76 s). The second song mode consists of echemes of at least 6–8 syllables closely spaced together, with an echeme duration of 0.15–0.20 s. For both song modes, the call spectrum has a peak frequency of 42.3±2.4 kHz (38.0–46.5 kHz), and the spectral entropy is 8.5±0.3.

***Euanisous teuthroides* (Bolívar, 1905)** (n = 1 male, 13 sound files) (Fig. 13): The calling song consists of echemes that can be highly variable in duration and exhibit frequency modulation. At 29.7±0.3°C (29.4–30.3°C), the syllable period is 5.44±0.35 ms (4.88–6.03 ms). At the start of the echeme, the syllable amplitude increases to a maximum, then decreases slightly and plateaus. The call spectrum has a peak frequency of 30.3±0.7 kHz (29.5–32.0 kHz), and the spectral entropy is 7.4±0.2.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 0.671 mm in length, with about 23 stout and squarish teeth. Unlike the other species reported here, each tooth exhibits an indentation in the middle. The teeth are similar in size (average tooth width in the middle part of the file is 17.8 m), and they are generally widely spaced (average inter-tooth distance is 36.1 µm). The file (Cu2) is elevated on a slightly swollen vein buttress, bent in the middle, with only the basal half possessing the teeth. The right tegmen has a small and rectangular mirror, broader than long, somewhat obsolete. The stridulatory file on the right tegmen is about 0.550 mm in length, with about 18 stout teeth.

***Kuzicus denticulatus* (Karny, 1926)** (n = 2 males, 14 sound files) (Fig. 14): The calling song consists of a continuous trill. At 29.5±0.2°C (29.2–29.9°C), the trill consists of a repetition of syllables at a rate of 187±23 syllables s^{-1} (153–249 syllables s^{-1}). Syllable period is 5.44±0.63 ms (4.01–6.53 ms). The call spectrum has a peak frequency of 39.6±2.4 kHz (33.4–42.2 kHz), and the spectral entropy is 7.7.

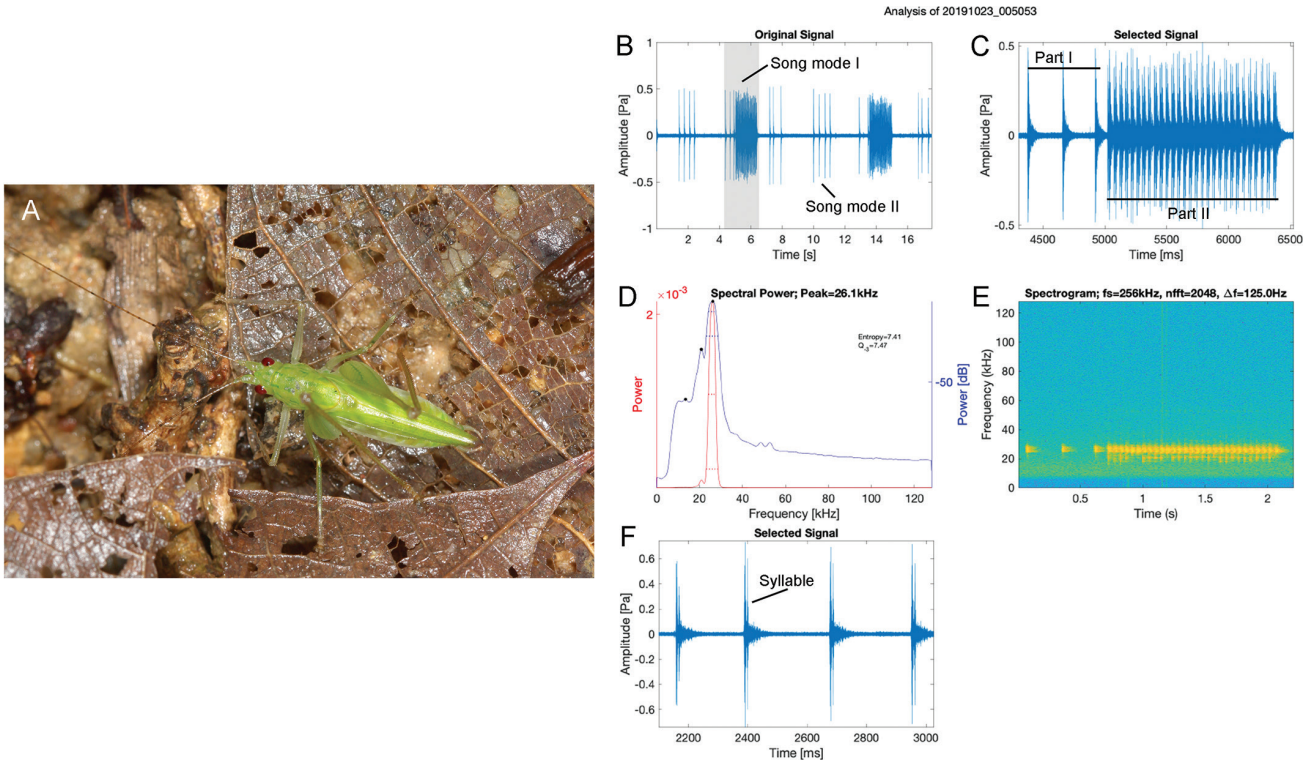

Fig. 11. *Alloteratura lamella* male adult in its natural environment in Singapore (**A**). Oscillograms showing a calling song consisting of both song modes (**B**) and a complex echeme consisting of three isolated syllables and a echeme (**C**). Power spectrum (**D**) and spectrogram of the same complex echeme (**E**). Oscillogram of four isolated syllables representing the second song mode (**F**).

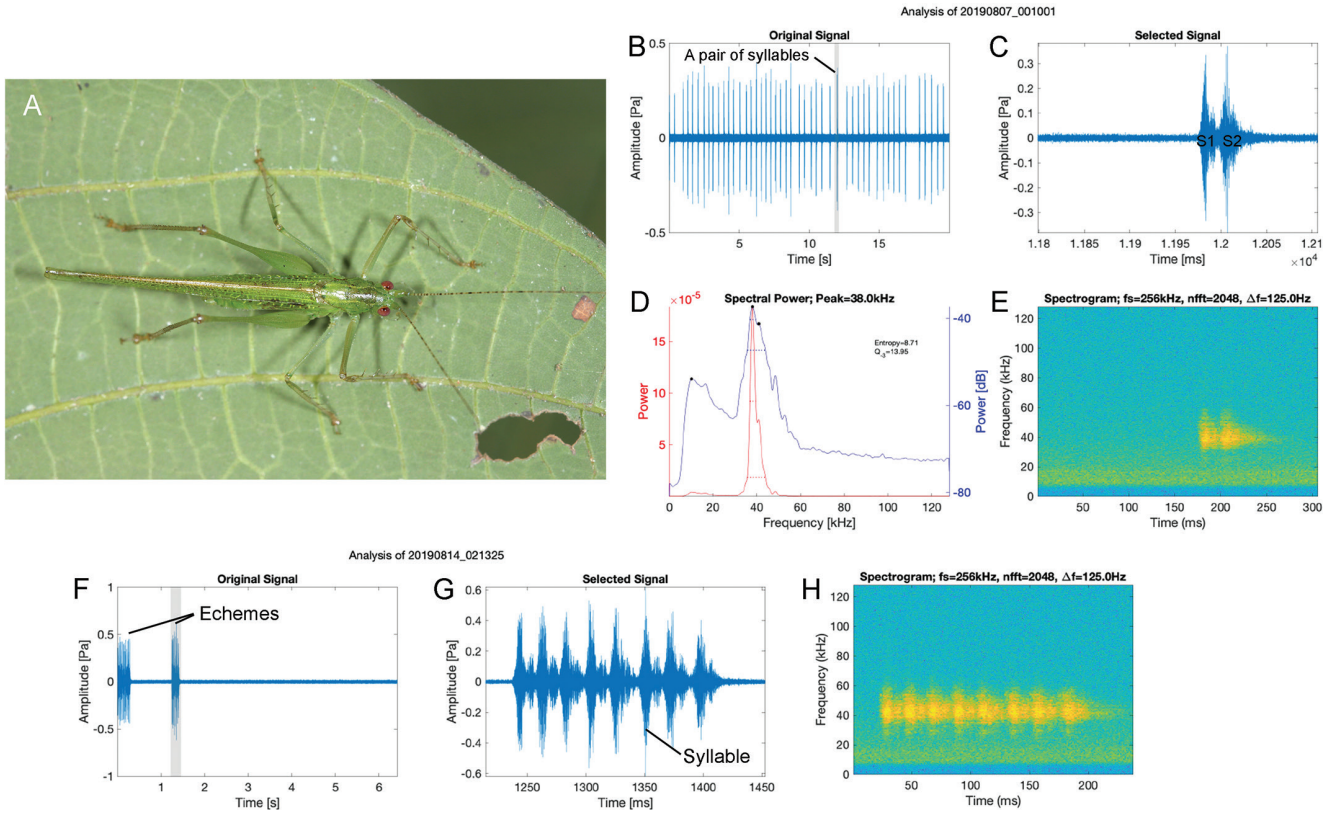

Fig. 12. *Borneopsis cryptosticta* male adult in its natural environment in Singapore (**A**). Oscillograms showing a doublet of syllables (**B**) and a doublet of syllables with the syllables denoted as S1 and S2 (**C**). Power spectrum (**D**) and spectrogram of the doublet of syllables (**E**). Oscillograms showing two echemes (**F**) and an echeme with eight syllables (**G**). Spectrogram of the echeme with eight syllables (**H**).

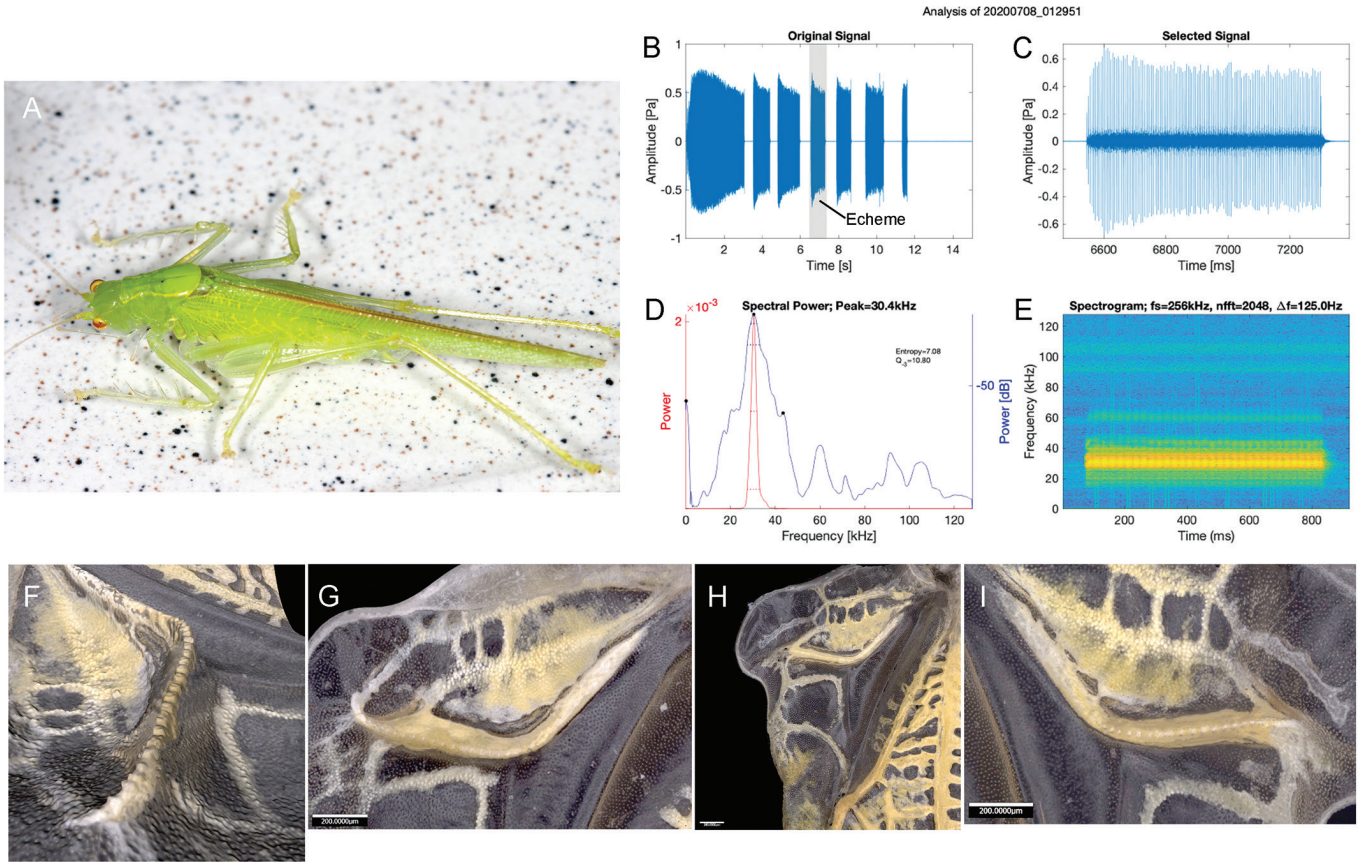

Fig. 13. *Euanisous teuthroides* male adult in the lab (**A**). Oscillograms showing seven echemes of varying duration (**B**) and a single echeme (**C**). Power spectrum (**D**) and spectrogram of the same echeme (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

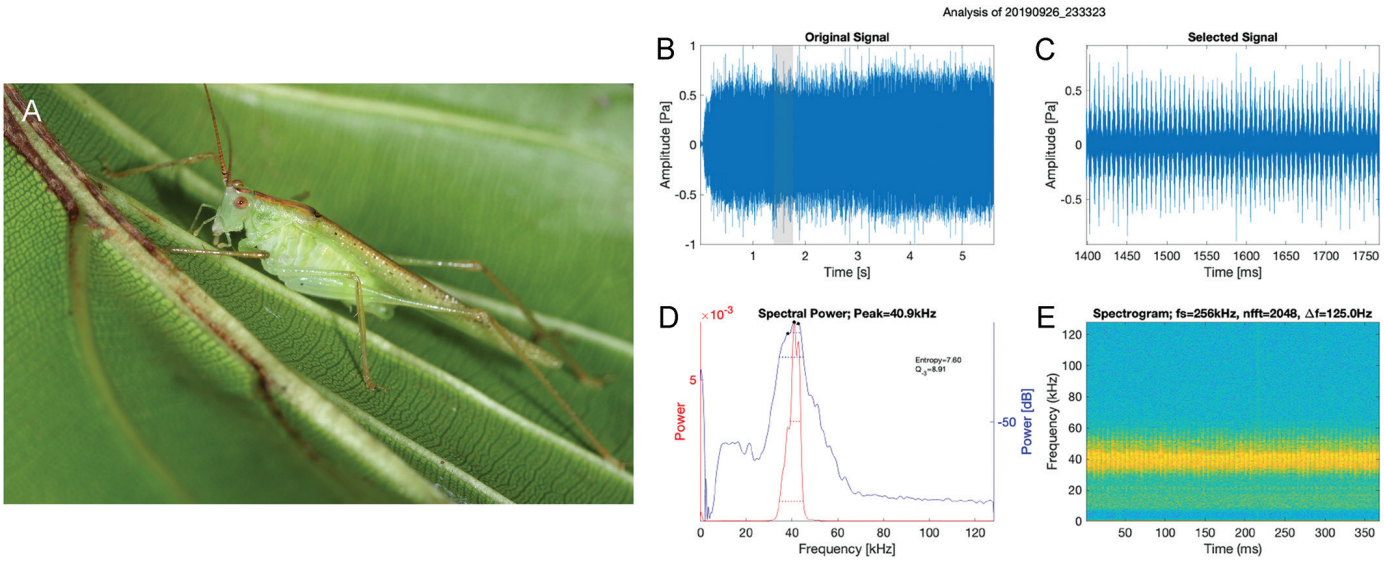

Fig. 14. *Kuzicus denticulatus* male adult in its natural environment in Singapore (**A**). Oscillograms showing a continuous trill (**B**) and closer view of the continuous trill (**C**). Power spectrum (**D**) and spectrogram of the closer view of the continuous trill (**E**).

Meconematini (Sandakan) (n = 1 male, 6 sound files) (Fig. 15): The calling song consists of a continuous trill made up of syllables occurring in pairs. At 29.3 °C, each doublet has a duration of 33.6±3.6 ms (30.0–41.0 ms), period of 116.3±11.7 ms (95.0–146.0 ms) and an interval between consecutive doublets of 82.7±11.6 ms (63.0–114.0 ms). The first syllable has a duration of 7.3±0.6 ms (7.0–9.0 ms), and the second syllable has a duration of 8.0±0.7 ms (7.0–10.0 ms). The call spectrum has a peak frequency of 54.2±0.4 kHz (53.5–54.6 kHz), and the spectral entropy is 7.6.

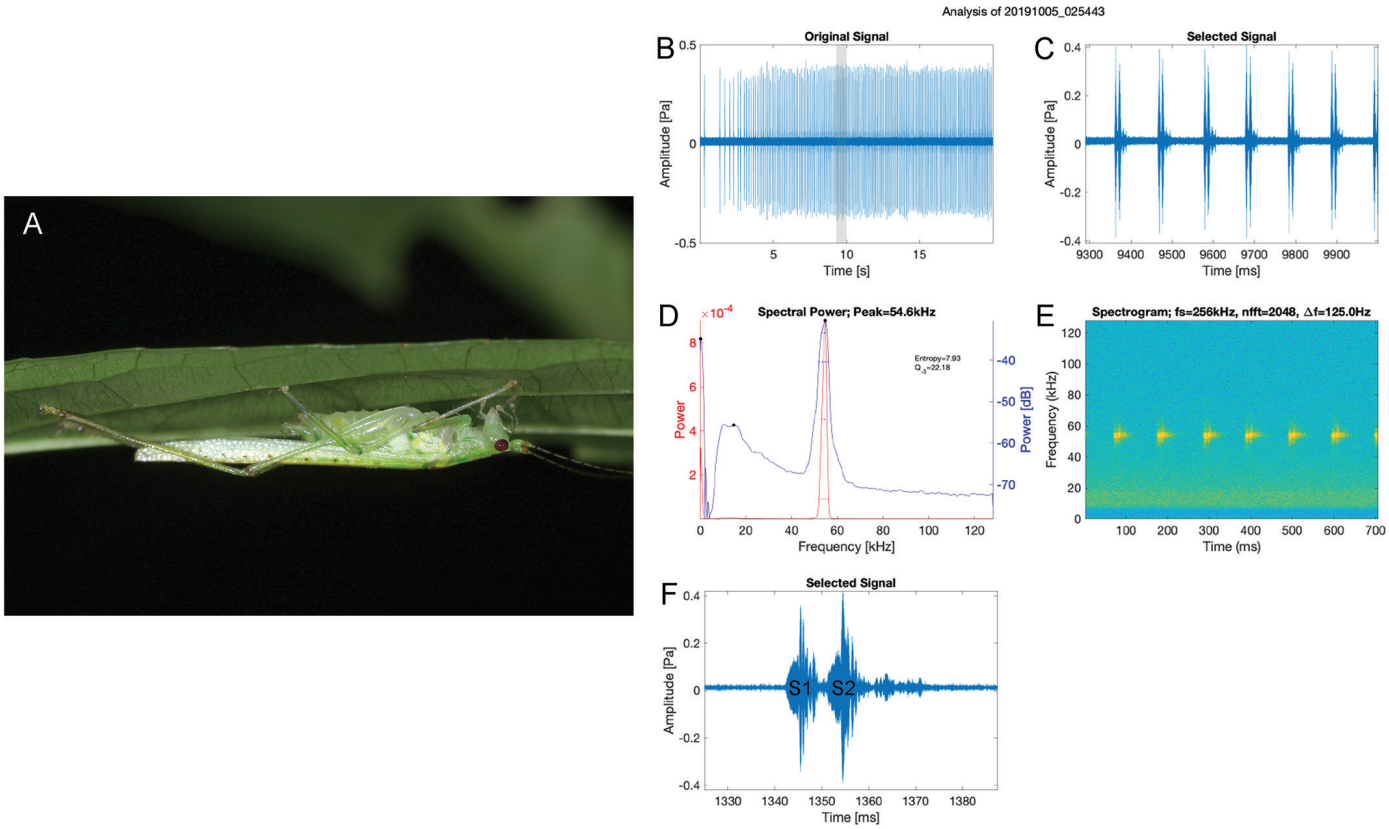

Fig. 15. Meconematini (Sandakan) male adult in its natural environment in Sandakan, Malaysia (**A**). Oscillograms showing a continuous trill (**B**) and a section of the trill with six complete doublets of syllables (**C**). Power spectrum (**D**) and spectrogram of the same six complete doublets of syllables (**E**). Oscillogram showing a doublet of syllables, with the syllables denoted as S1 and S2, in greater details (**F**).

***Neophisis siamensis* Jin, 1992** (n = 3 males, 10 sound files) (Fig. 16): The calling song consists of a sequence of isolated syllables. Each syllable shows two amplitude peaks. At 29.3±0.5°C (28.5–30.4°C), syllable duration is 100.6±17.4 ms (57.0–123.0 ms). The interval between syllables is highly variable, ranging from 42.0 to 270.0 ms (107.4±63.6 ms). The call spectrum has a peak frequency of 36.7±1.8 kHz (32.0–38.2 kHz), and the spectral entropy is 7.1.

***Xiphidiopsis* (*Xiphidiopsis*) *dicera* Hebard, 1922** (n = 1 male, 7 sound files) (Fig. 17): The calling song consists of continuous trill made up of isolated syllables of varying amplitudes. Each syllable is made up of two pulses, with the first pulse typically of lower amplitude than the second pulse. At 29.1±0.2°C (29.0–29.6°C), syllable duration is 56.8±6.3 ms (46.0–70.0 ms) and period is 75.4±17.1 ms (57.0–116.0 ms). The interval between doublets is highly variable, ranging from 3.0 to 62.0 ms (18.6±15.8 ms). The call spectrum has a peak frequency of 40.9±0.4 kHz (40.4–41.4 kHz), and the spectral entropy is 7.7.

***Casigneta* sp. 1** (n = 2 males, 15 sound files) (Fig. 18): The calling song consists of a pulse-train isolated in time. The train may correspond to a long syllable rather than an echeme owing to the presence of frequency modulation. At 29.6±0.6°C (29.0–30.4°C), each pulse train has a duration of 0.36±0.04 s (0.27–0.44 s) and is made up of 24±3 (16–27) pulses of gradually increasing amplitude over time. The call spectrum has a peak frequency of 28.7±0.8 kHz (27.2–30.0 kHz), and the spectral entropy is 7.6±0.1.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 1.338 mm in length with about 110 rather broad teeth. The file is substraight, slightly curved at the basal end. The teeth are largest in the middle portion (average tooth width is 125 µm), and tooth width tapers gently toward the ends. The teeth are most densely packed in the anal end (teeth density is 107 teeth mm^{-1}) then in the middle region of the file (teeth density is 71 teeth mm^{-1}), and least densely packed at the basal end (teeth density is 51 teeth mm^{-1}). The file (Cu2) is barely elevated on a swollen vein buttress. The right tegmen has a trapezoidal mirror. The stridulatory file on the right tegmen is about 1.323 mm in length with relatively stout teeth.

***Casigneta* sp. 2** (n = 1 male, 19 sound files) (Fig. 19): The calling song appears to consist of isolated syllables, each containing three pulses. At 29.8±0.5°C (28.7–30.1°C), each triple of pulses has a duration of 0.15±0.01 s (0.13–0.16 s). The first pulse duration is 12.5±3.5 ms (10.0–25.0 ms), the second pulse duration is 12.1±2.2 ms (10.0–18.0 ms), and the third pulse duration is 12.4±2.6 ms (10.0–19.0 ms). The first pulse is more temporally separated from the second and third pulses. The call spectrum has a peak frequency of 28.2±0.2 kHz (27.8–28.8 kHz), and the spectral entropy is 6.8±0.2.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 1.314 mm in length with about 75 rather broad teeth. The file is substraight, slightly curved at the basal end. The teeth are largest in the middle portion (average tooth width is 95 µm), and tooth width tapers gently toward the ends. The teeth are closely packed, and the distance between teeth is fairly uniform. In the mid-part of the stridulatory file, the teeth density is 56 teeth mm^{-1}. The file (Cu2) is barely elevated on a swollen vein buttress. The right tegmen has an elongated rectangular mirror, distinctly longer than broad. The stridulatory file on the right tegmen is about 0.913 mm in length, with about 52 teeth.

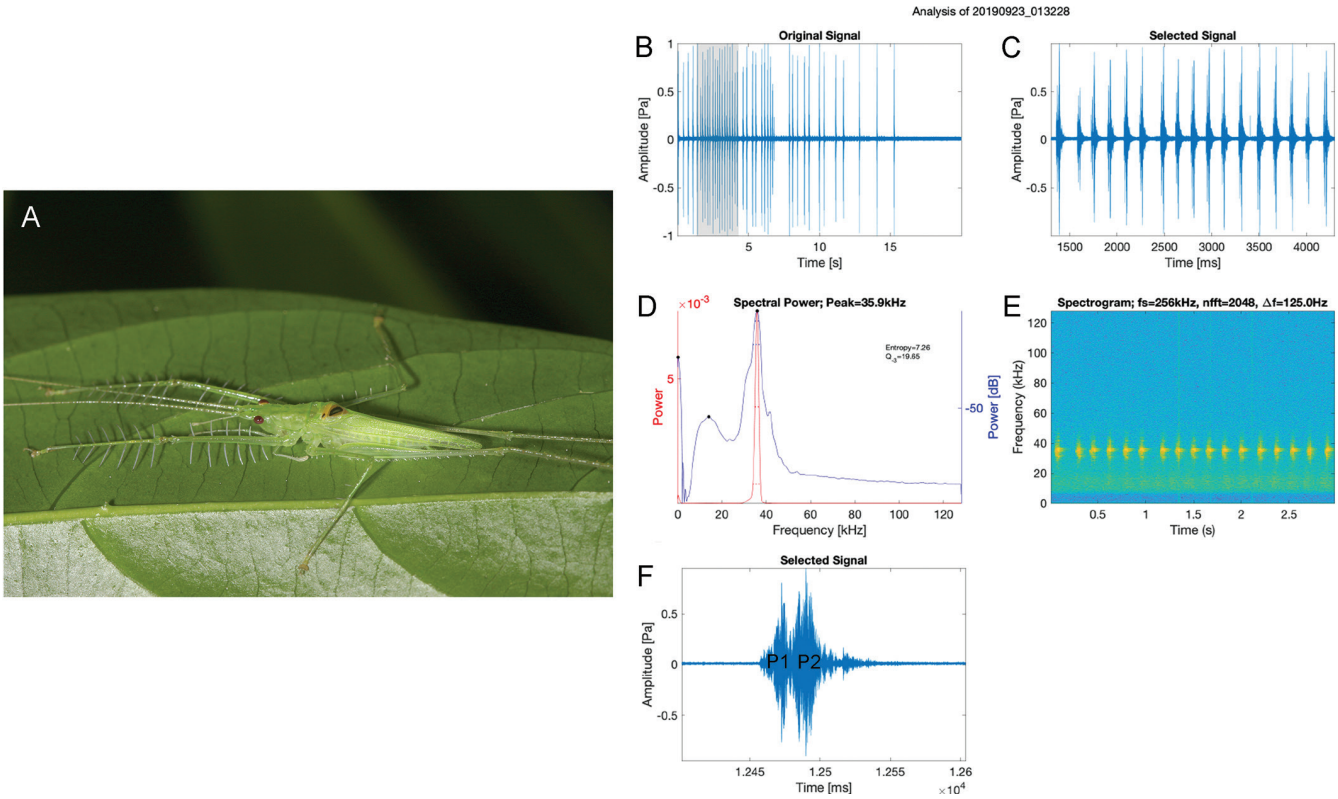

Fig. 16. *Neophisis siamensis* male adult in its natural environment in Singapore (**A**). Oscillograms showing a sequence of syllables (**B**) and a section of the sequence with 17 syllables (**C**). Power spectrum (**D**) and spectrogram of the 17 syllables (**E**). Oscillogram showing a single syllable with two amplitude peaks denoted as P1 and P2 (**F**).

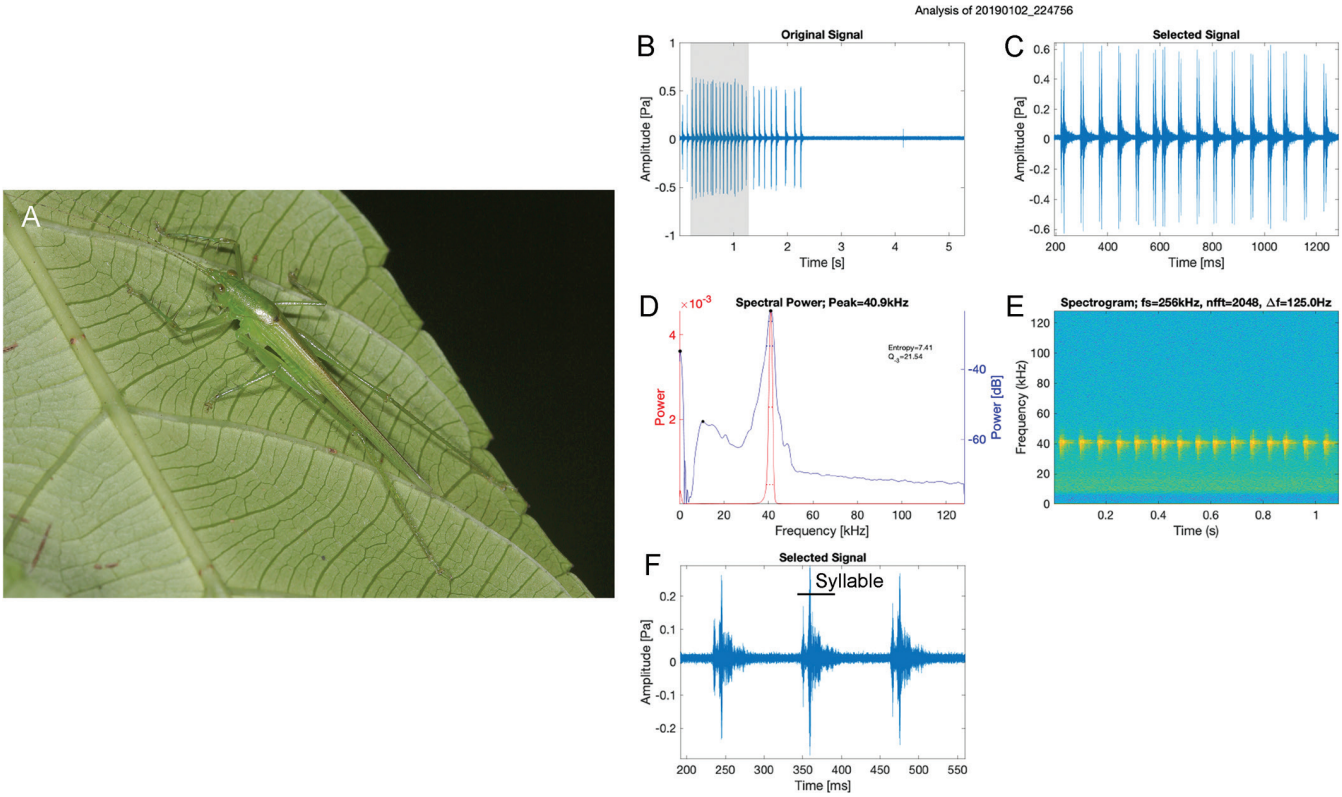

Fig. 17. *Xiphidiopsis* (*Xiphidiopsis*) *dicera* male adult in its natural environment in Singapore (**A**). Oscillograms showing a sequence of syllables (**B**) and a section of the trill with 16 syllables (**C**). Power spectrum (**D**) and spectrogram of the 16 syllables (**E**). Oscillogram showing three syllables, each with two pulses, in greater detail (**F**).

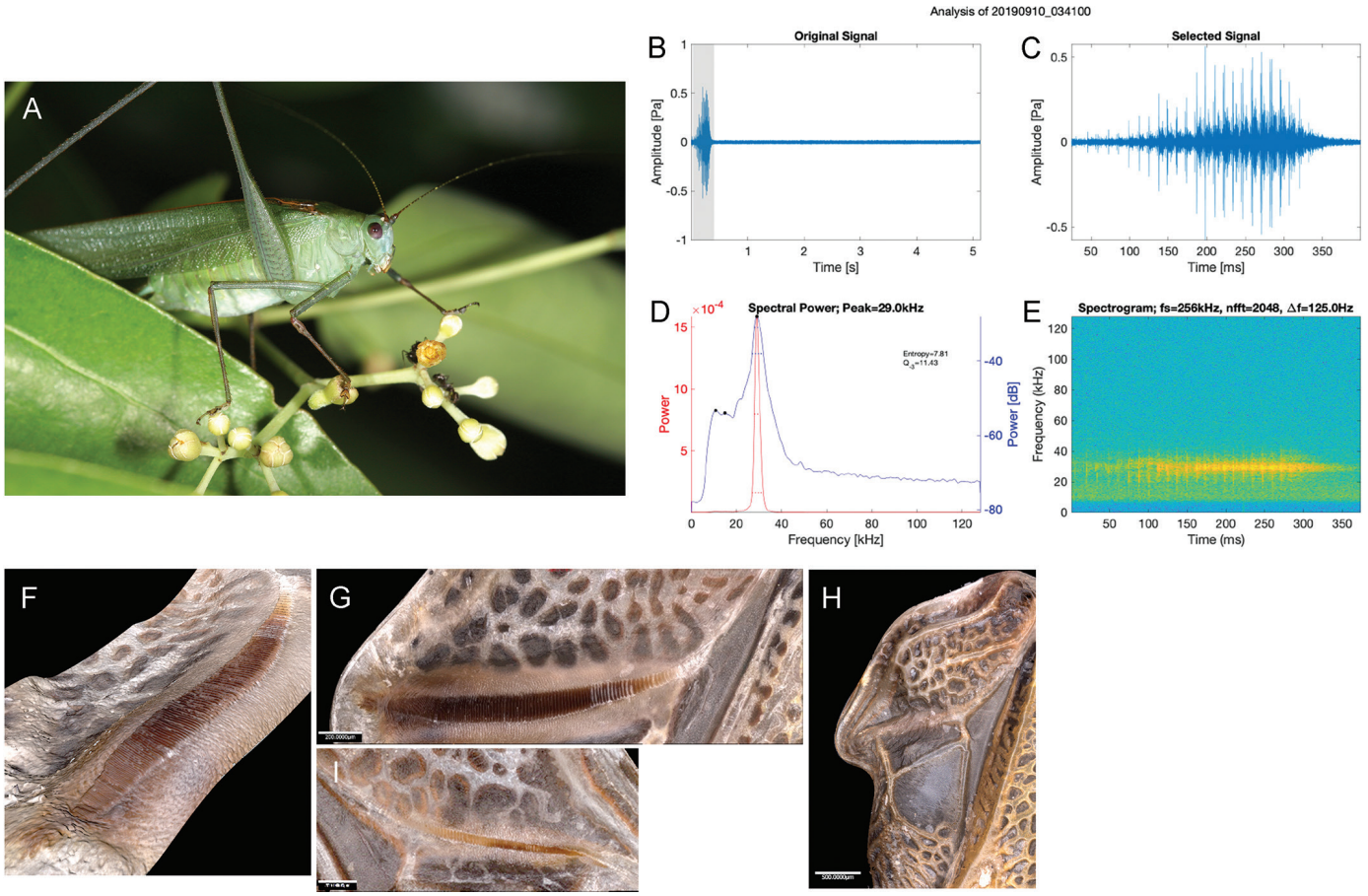

Fig. 18. *Casigneta* sp. 1 male adult in its natural environment in Singapore (**A**). Oscillograms showing a pulse train (**B**) and a closer view of the pulse train (**C**). Power spectrum (**D**) and spectrogram of the same pulse train (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

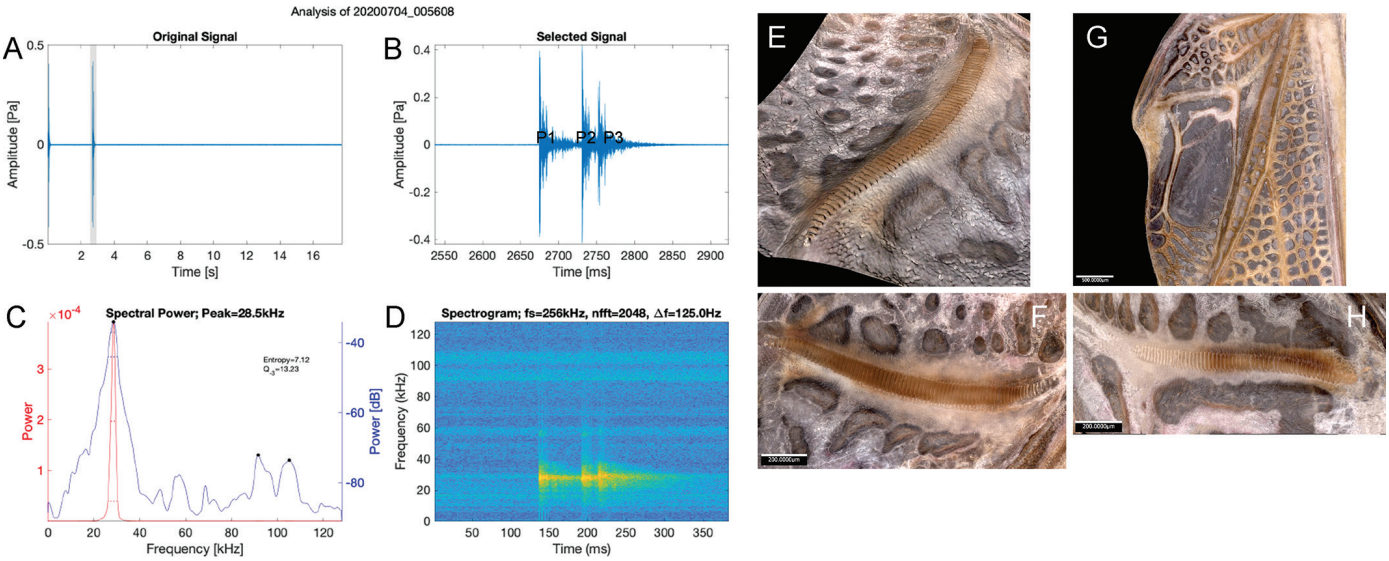

Fig. 19. *Casigneta* sp. 2. Oscillograms showing two triplets of pulses (**A**) and a triplet of pulses denoted as P1 to P3 (**B**). Power spectrum (**C**) and spectrogram of the same triplet of pulses (**D**). Three-dimensional anal view of the left stridulatory file (SF) (**E**), ventral view of the same SF (**F**), ventral view of the right tegmen sound-producing organs (**G**), and ventral view of the right SF (**H**).

***Holochlora* nr. *bilobata* (Karny, 1926)** (n = 2 males, 15 sound files) (Fig. 20): The calling song consists of an isolated syllable. At 29.8±0.4°C (28.4–30.4°C), syllable duration is 37.2±3.5 ms (29.5–45.3 ms). The interval between syllables varies at 4.0±1.7 s (2.3–8.6 s). The call spectrum has a peak frequency of 33.3±1.0 kHz (31.5–34.9 kHz), and the spectral entropy is 8.1±0.4.

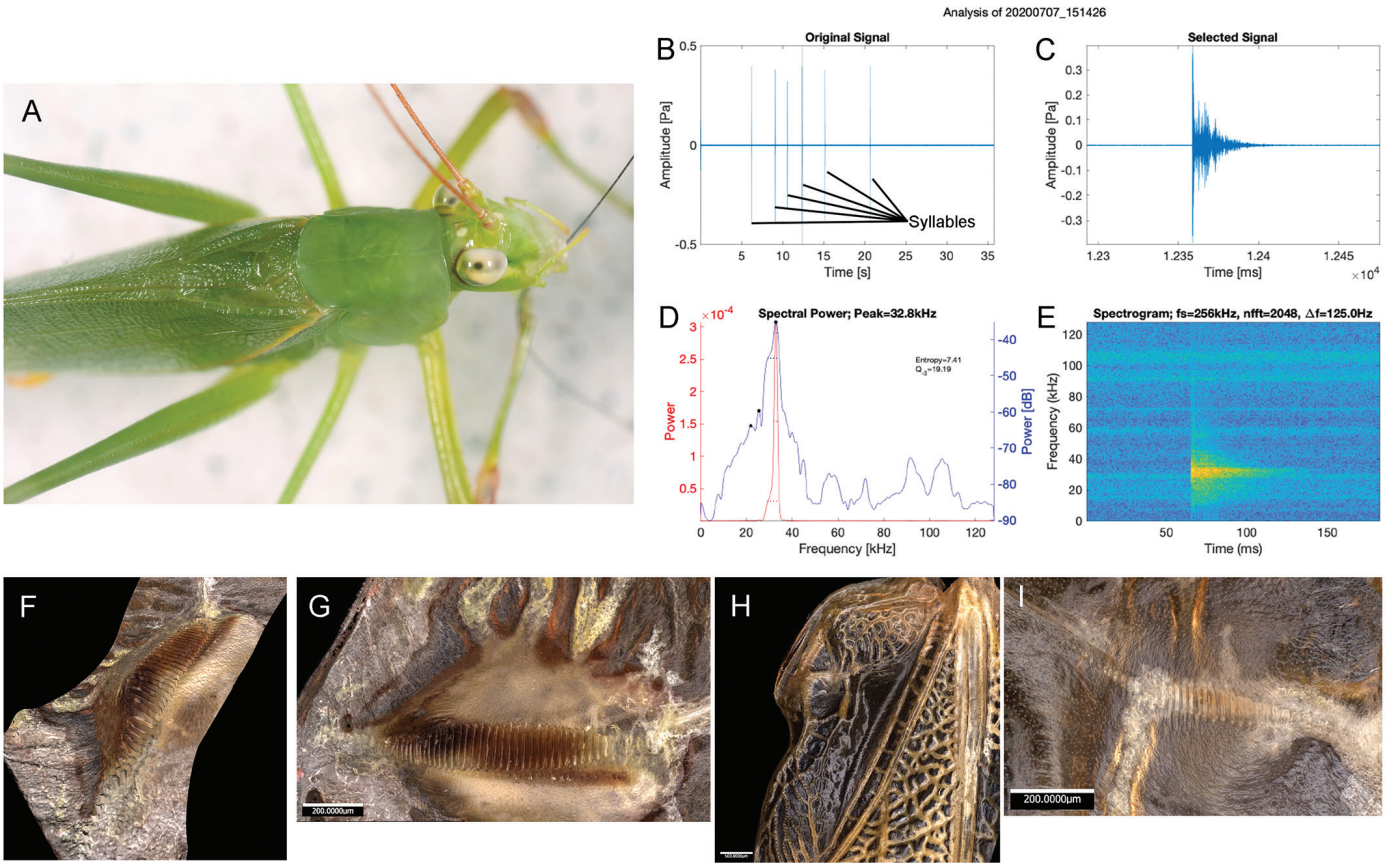

Fig. 20. *Holochlora* nr. *bilobata* male adult in the lab (**A**). Oscillograms showing six isolated syllables (**B**) and a closer view of a syllable (**C**). Power spectrum (**D**) and spectrogram of the same syllable (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

Ventrally, the left macropterous tegmen possesses a stout stridulatory file of about 1.126 mm in length with about 47 broad teeth. The file is straight. The teeth are largest in the middle portion (average tooth width is 95 µm) and distinctly smaller at the ends (average tooth width is 38 µm). The distance between teeth is fairly uniform in the mid-part of the file (teeth density is 44 teeth mm^{-1}), only slightly larger at the ends. The file (Cu2) is elevated in the middle on a very swollen vein buttress. The right tegmen has a small rectangular mirror, somewhat obsolete. The stridulatory file on the right tegmen is about 0.633 mm in length with about 32 indistinct teeth.

***Phaneroptera brevis* Serville, 1838** (n = 1 male, 11 sound files) (Fig. 21): The calling song consists of a pair of syllables. At 29.9±0.2°C (29.6–30.2°C), each pair of syllables has duration of 0.33±0.01 s (0.31–0.35 s). The first syllable has a distinctly lower amplitude and shorter duration of 36.3±8.8 ms (22.0–50.0 ms) than the second syllable (duration is 53.8±10.9 ms [30.0–70.0 ms]). The interval between the two syllables is 0.24±0.02 s (0.21–0.27 s). The call spectrum has a peak frequency of 21.9±0.8 kHz (20.3–22.8 kHz), and the spectral entropy is 7.9.

Ventrally, the left macropterous tegmen possesses a stridulatory file, somewhat split into two parts connected by a perpendicular 'bridge'. The entire stridulatory file on the left tegmen is about 1.753 mm in length. The anal part is short and straight, about 0.335 mm in length with about 24 smaller and stout (of uniform size and spacing) teeth. The average tooth width is 34 µm, and the teeth density is 65 teeth mm^{-1}. The main file is straight, about 1.263 mm in length with about 36 larger teeth. The teeth are largest in the middle portion (average tooth width is 86 µm) and distinctly smaller at the basal end (average tooth width is 52 µm). The teeth are less densely packed in the middle portion (teeth density is 18 teeth mm^{-1}) compared to the basal end (teeth density is 49 teeth mm^{-1}). The file (Cu2) is faintly elevated in the middle on a slightly swollen vein buttress. The right tegmen has a large oblong mirror, distinctly longer than broad.

***Phaulula malayica* (Karny, 1926)** (n = 1 male, 6 sound files) (Fig. 22): The calling song consists of isolated syllables appearing as rapid-decay pulses. At 29.3±0.4°C (29.1–30.1°C), syllable duration is 53.4±7.5 ms (41.0–65.0 ms). The interval between syllables varies at 1.6±0.4 s (1.2–2.7 s). The call spectrum has two peaks in energy, typically with a peak frequency of 23.6±1.2 kHz (22.5–25.2 kHz). In some instances, however, a second peak in the spectrum of 33.5–33.8 kHz can be the dominant frequency. The spectral entropy is 7.8.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 1.364 mm in length with about 45 broad teeth. The file is straight. The teeth are largest in the middle portion (average tooth width is 112 µm), and tooth width tapers towards the ends. The teeth are uniformly packed in the mid-part of the stridulatory file (teeth density is 23 teeth mm^{-1}), less densely packed at the anal end (teeth density is 31 teeth mm^{-1}), and more densely packed at the basal end (teeth density is 50 teeth mm^{-1}). The stridulatory file (Cu2) is faintly elevated in the middle on a swollen vein buttress. The right tegmen has a large mirror, longer than broad.

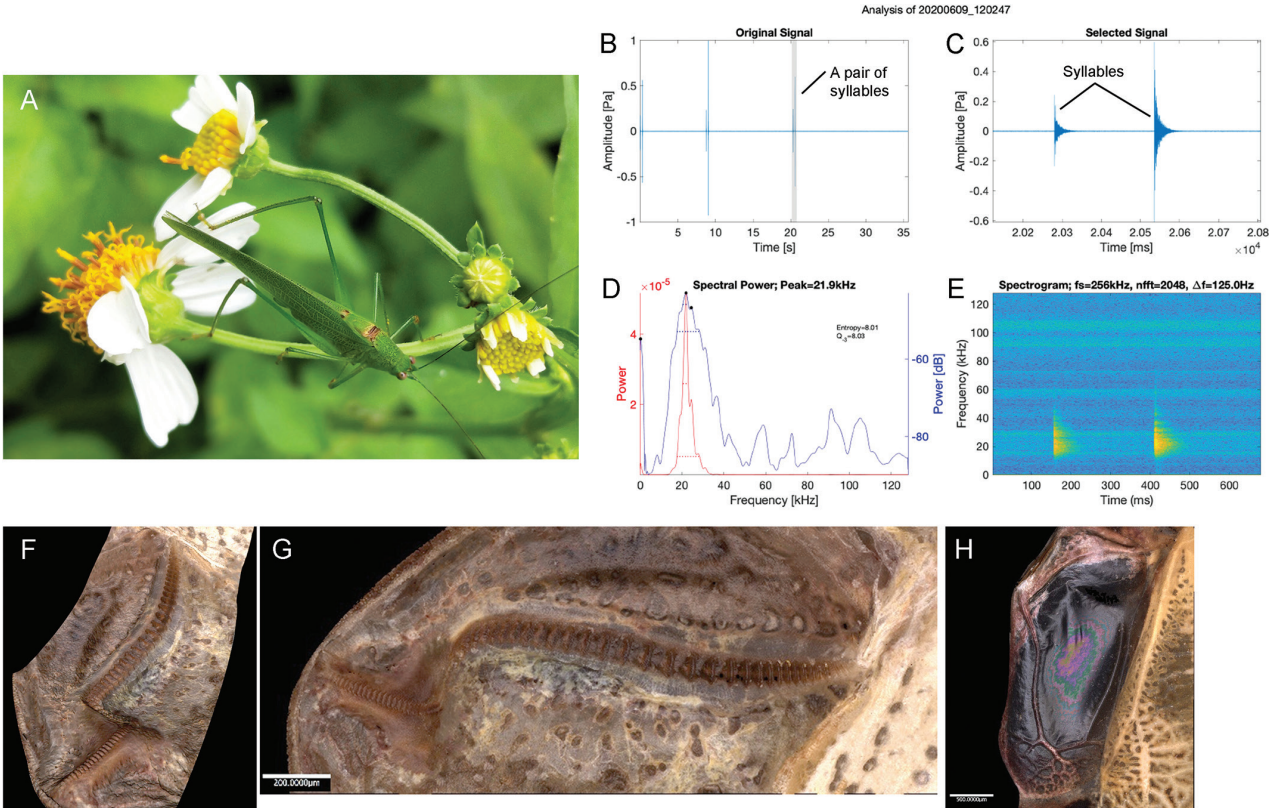

Fig. 21. *Phaneroptera brevis* male adult in its natural environment in Singapore (**A**). Oscillograms showing two complete pairs of syllables (**B**) and a closer view of a pair of syllables (**C**). Power spectrum (**D**) and spectrogram of the pair of syllables (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), and ventral view of the right tegmen sound-producing organs (**H**).

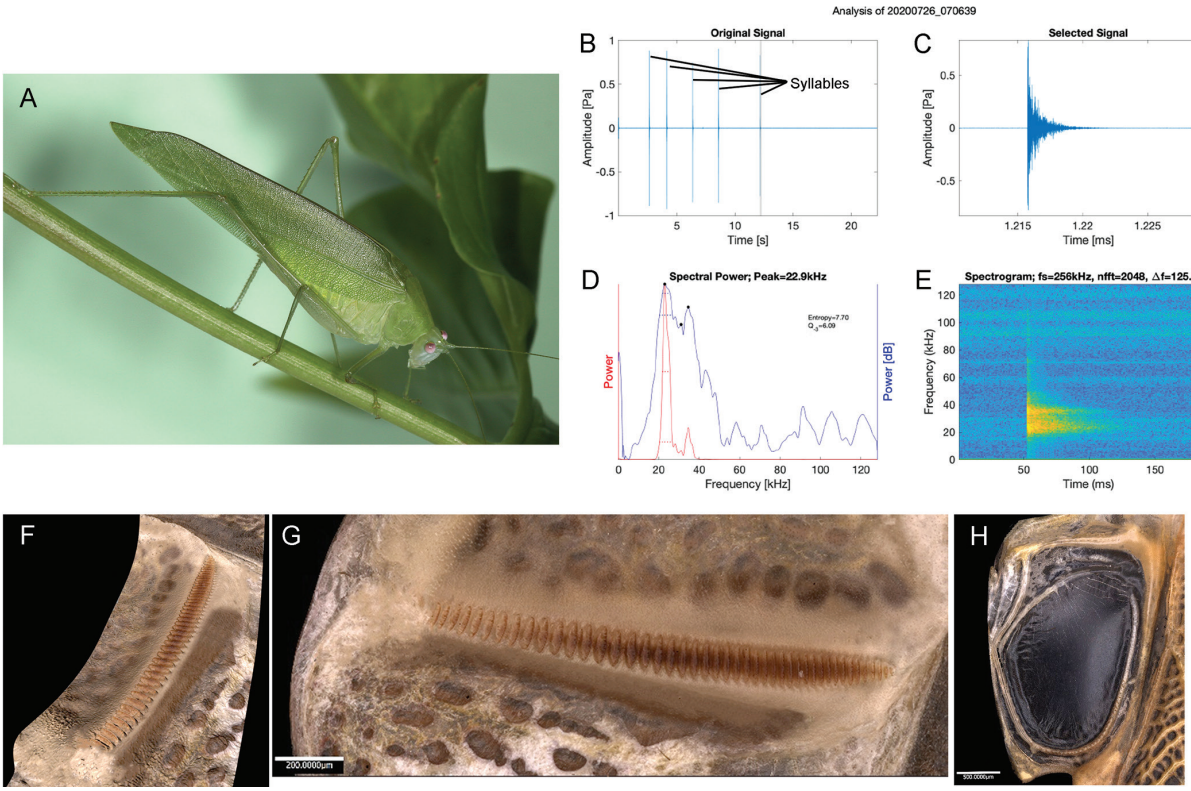

Fig. 22. *Phaulula malayica* male adult in the lab (**A**). Oscillograms showing five isolated syllables (**B**) and a closer view of a syllable (**C**). Power spectrum (**D**) and spectrogram of the syllable (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), and ventral view of the right tegmen sound-producing organs (**H**).

***Psyrana tigrina* (Brunner von Wattenwyl, 1878)** (n = 1 male, 14 sound files) (Fig. 23): The calling song consists of pulse train isolated in time. At 30.2±0.1°C (30.0–30.4°C), each pulse train has duration of 0.26±0.01 s (0.24–0.28 s) and is made up of numerous pulses of varying amplitudes and duration. The pulses steadily increase in amplitude to a maximum in the initial 0.17±0.01 s (0.16–0.19 s) of the pulse train before decreasing rather abruptly in amplitude in the final 0.09±0.01 s (0.06–0.12 s) of the pulse train. The call spectrum has a peak frequency of 35.5±2.1 kHz (31.8–38.0 kHz), and the spectral entropy is 8.1.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 2.236 mm in length with about 83 broad teeth. The file is straight. The teeth are largest in the middle portion (average tooth width is 244 µm), and tooth width tapers at the ends. The teeth are narrowly and uniformly packed in the mid-part of the stridulatory file (teeth density is 34 teeth mm^{-1}). The file (Cu2) is faintly elevated in the middle on a slightly swollen vein buttress. The right tegmen has an elongated rectangular mirror. The stridulatory file on the right tegmen is about 1.851 mm in length with about 38 teeth at the anal half and numerous indistinct teeth at the basal half.

***Scambophyllum sanguinolentum* (Westwood, 1848)** (n = 1 male, 8 sound files) (Fig. 24): The calling song consists of a pulse train isolated in time and very likely a long syllable produced during a single but slow closing wing stroke. Similar syllable patterns have been observed in the genus *Isophya*, e.g., *Isophya costata* (Heller, 1988). The syllable, here recognized as pulse trains, can occur in isolation or in doublets or triplets. At 29.7±0.0°C (29.7–29.8°C), train duration is 0.32±0.01 s (0.30–0.35 s). When occurring in doublets or triplets, train period is 0.97±0.18 s (0.78–1.38 s) and intervals between trains are 0.64±0.18 s (0.43–1.04 s). Each train is made up of 43±2 (39–47) pulses, with pulses increasing in amplitude at the start and remaining relatively consistent. The call spectrum has a peak frequency of 23.7±0.3 kHz (23.2–24.1 kHz), and spectral entropy is 7.0±0.1.

Ventrally, the left macropterous tegmen possesses a stridulatory file of about 1.509 mm in length with about 53 broad teeth. The file is straight and strongly bent at the basal third. The average tooth width in the middle region is 46 µm. Tooth width tapers at the ends. The file (Cu2) is slightly elevated in the middle on a very swollen vein buttress. The right tegmen has a squarish mirror. The stridulatory file on the right tegmen is about 1.208 mm in length with numerous indistinct teeth.

Discussion

Calling songs.—Based on the 24 katydid species recorded in this study (Table 1), we observed that the calling songs of Southeast Asian katydid species are highly diversified in terms of both time and frequency. While some species produce transient calling songs, such as relatively simple and isolated pulses in *Holochlora*, species of *Conocephalus* produce complex echemes with two distinct structures within each echeme. Other species produce continuous trills (e.g., in *Axylus* and *Kuzicus*) and a short sequence of trains (e.g., in *Euanisous* and *Psyrana*). Some species, such as *Alloteratura*

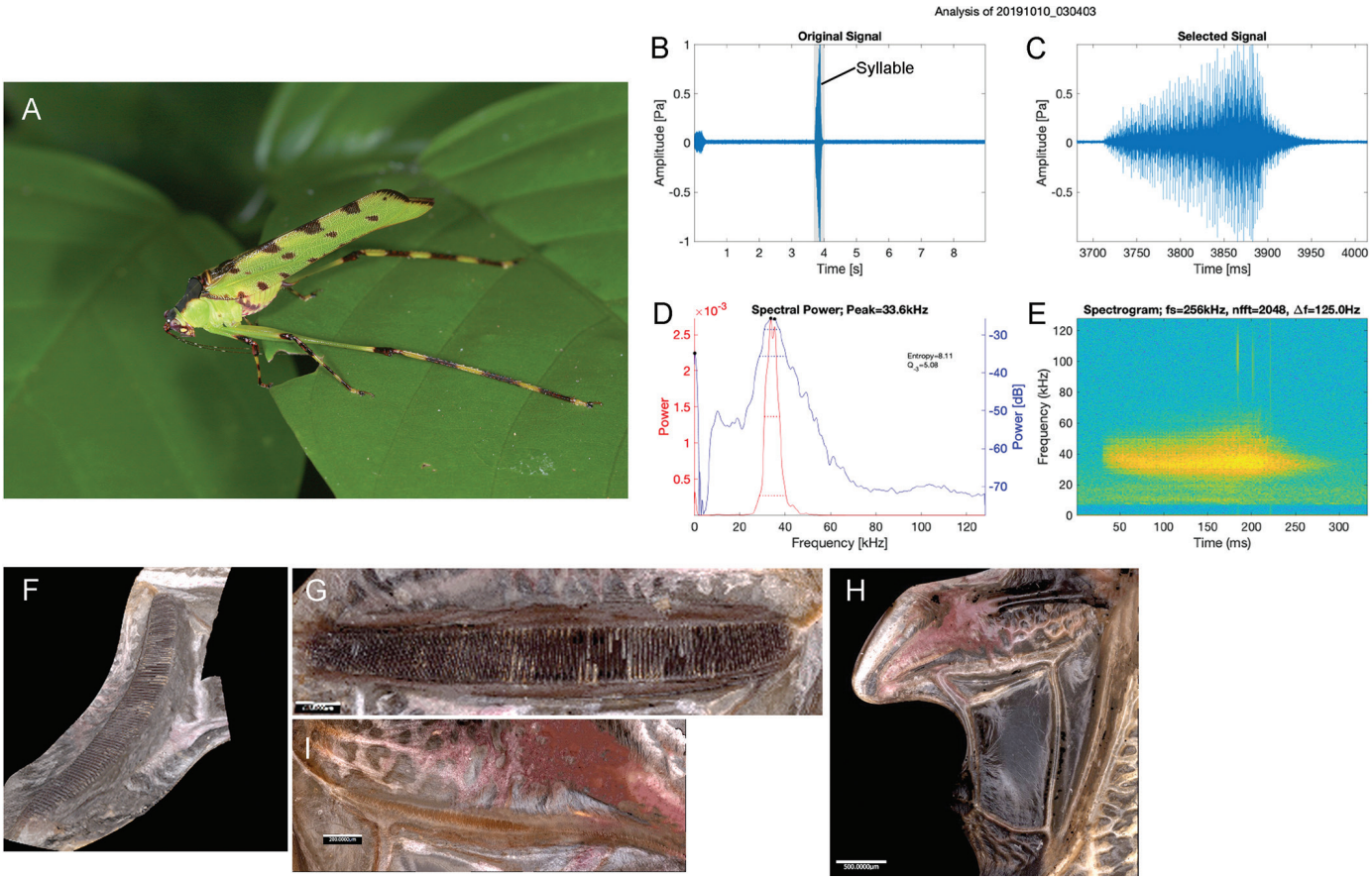

Fig. 23. *Psyrana tigrina* male adult in its natural environment in Sandakan, Malaysia (**A**). Oscillograms showing an isolated pulse train (**B**) and a closer view of the pulse-train (**C**). Power spectrum (**D**) and spectrogram of the pulse train (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**), and ventral view of the right SF (**I**).

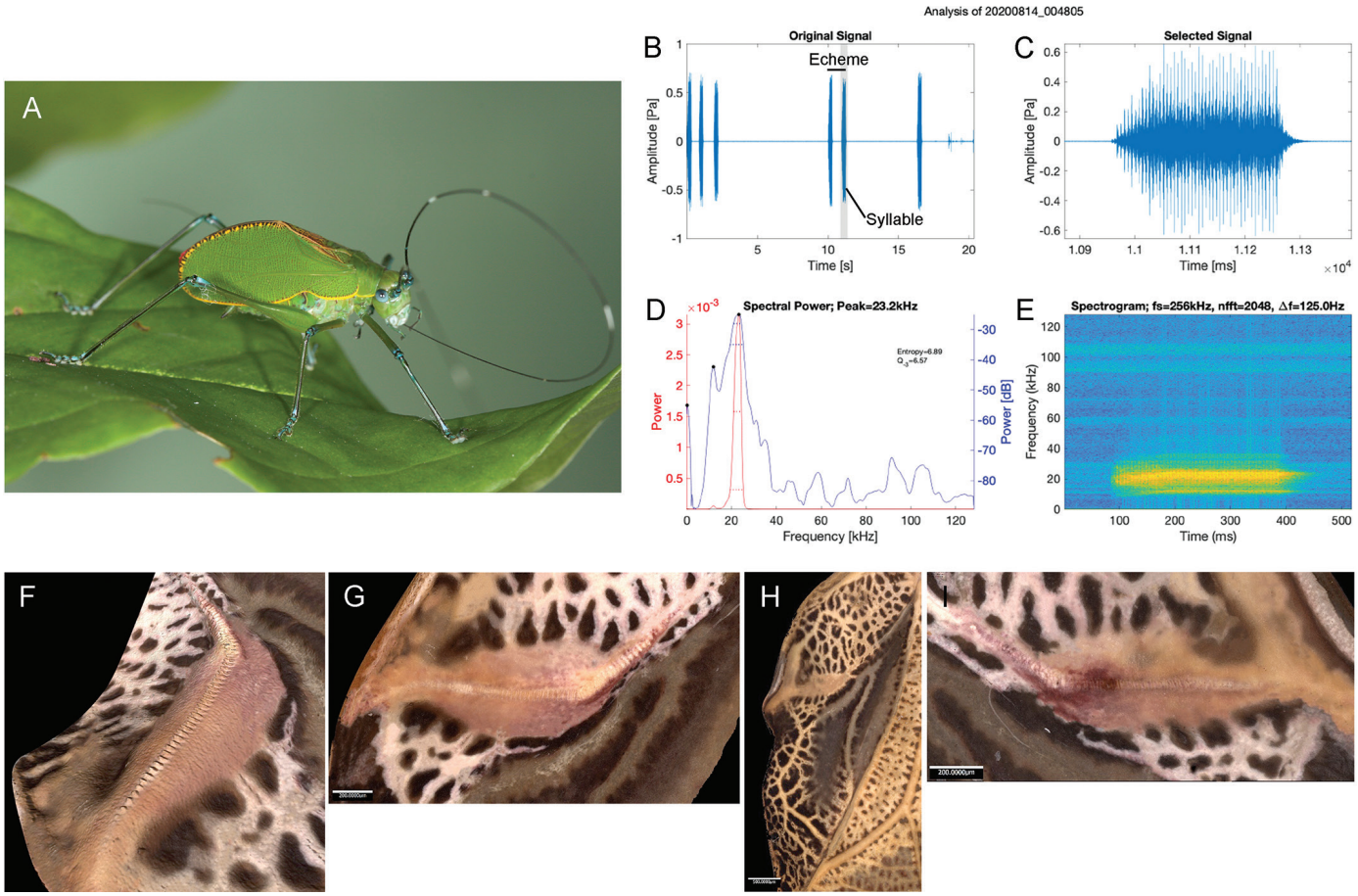

Fig. 24. *Scambophyllum sanguinolentum* male adult in the lab (**A**). Oscillograms showing two echemes (first one with three syllables and second one with two syllables) followed by an isolated syllable (**B**) and a single syllable (**C**). Power spectrum (**D**) and spectrogram of a syllable (**E**). Three-dimensional anal view of the left stridulatory file (SF) (**F**), ventral view of the same SF (**G**), ventral view of the right tegmen sound-producing organs (**H**) and ventral view of the right SF (**I**).

lamella and *Borneopsis cryptosticta*, also had two modes of calling song recorded in the laboratory. The *Conocephalus exemptus* represents a curious case in which the calling songs from Thailand and Singapore differ drastically. Given that the taxonomy of *Conocephalus* is complicated, it could be that the individuals from Thailand and Singapore represent two different cryptic species or that this widely distributed species exhibits population differences in calling songs. Combining the bioacoustic data and further examining the morphology of the 'species' from different areas of its distribution can shed light on its bioacoustics and taxonomy.

The call analysis also provides input on the quality of the signal, and we used the quality factor Q (Q_{-3dB}) to investigate this variable. Although Q assumes that the spectrum is symmetrical (Bennet-Clark 1999), spectral symmetry is rarely the case in the calls of most katydids, especially for broadband singers. For this reason, we present an alternative means of measuring the tendency of a signal to be a random noise rather than the actual signal from the source (i.e., the katydid call). Entropy has been used by various authors to measure this tendency from various perspectives. For example, Sueur et al. (2012) used a normalized form for the calculated value that tends toward 0 for a single pure tone, increases with the number of frequency bands and amplitude modulations, and tends toward 1 for random noise. Chivers et al. (2017a) report entropy values of ~5–9 in neotropical katydids (without normalization). Using the same protocols proposed by Chivers et al. (2017a), here we report entropy values of 6.8–8.8, which suggests that Chivers et al. (2017a) included species with high tonality (common in many neotropical Pseudophyllinae).

The peak frequency of the 24 Southeast Asian katydids ranges from 12.6 to 54.2 kHz, with more than 80% of species having energy peaks in the ultrasonic range (18 species having a peak frequency between 20 and 40 kHz, and 3 species having a peak frequency > 40 kHz) (Fig. 25). This is congruent with what was previously documented: most katydids produce ultrasonic sounds (Montealegre-Z 2009, Montealegre-Z et al. 2017). The three extreme ultrasonic callers (peak frequency >40 kHz) reported here are species from the subfamily Meconematinae. We can expect to find more species of katydid from the region to produce extreme ultrasound as they are collected. These may include species of *Glenophisis* Karny, 1926 from the subfamily Hexacentrinae, a small genus of katydids found in Southeast Asia (Tan 2012). These katydids share superficial morphological resemblance with neotropical *Arachnoscelis* Karny, 1911 species and *Supersonus* Sarria-S et al., 2014 species (both from the subfamily Meconematinae), which can produce calls with frequency peaking at 70 kHz and above 125 kHz, respectively (Chivers et al. 2014, Sarria-S et al. 2014). Unfortunately, we have yet to encounter these rare katydids for such a study. Likewise, some Pseu-

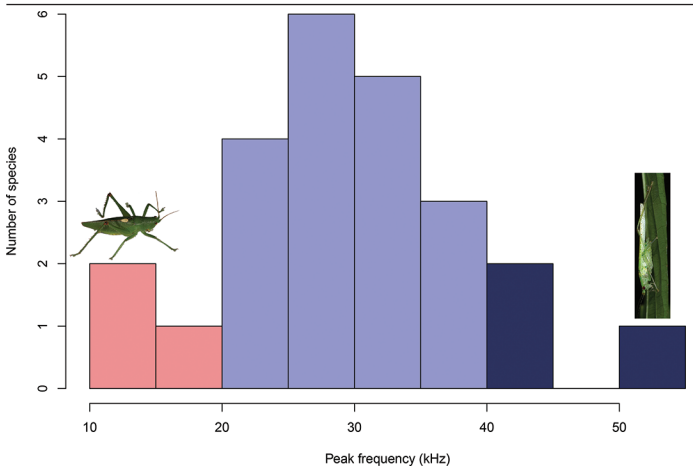

Fig. 25. A histogram showing the number of katydid species and the peak frequency of their calling songs. The red bars represent sonic callers (<20 kHz), light blue bars represent ultrasonic callers, and the dark blue bars represent extreme ultrasonic callers (>40 kHz).

dophyllinae from Southeast Asia can produce exceedingly low frequencies (e.g., 0.6 kHz in *Tympanophyllum arcufolium*) (Heller 1995), and it would not be surprising to find more species that produce such low frequencies.

In this study, all six species of Meconematini were found to produce songs with an entirely ultrasonic spectrum. This is congruent with previous reports of calls of Meconematini from Africa, such as those of *Amytta* Karsch, 1888 species (Hemp and Heller 2017, Hemp 2021). Among the species studied here are also the only group of katydids that produce extreme ultrasound, specifically *Borneopsis cryptosticta*, *Xiphidiopsis* (*Xiphidiopsis*) *dicera* and an unidentified Meconematini from Sandakan (although Tan et al. 2019b also reported extreme-ultrasonic singers among Phlugidini from Southeast Asia). Being highly speciose in Southeast Asia—with at least 104 genera currently known (Cigliano et al. 2022)—this group may hold the key to understanding the evolution of extreme ultrasound production in katydids. However, elucidating the phylogeny of Meconematini is crucial, as the relationships between and among many currently known genera and species are still unknown, and many groups are proabably paraphyletic. The ability to produce calls with entirely ultrasonic spectrum and extreme ultrasound are likely to have evolved multiple times and dependent on other factors instead of merely phylogenetic relatedness. Second, these predatory katydids usually occur in low abundance, and most species were described without having their calls recorded (but see Tan et al. 2020b). With continued effort to document the bioacoustics of these katydids, we can expect to find more species of extreme-ultrasonic singers from more genera, as well as more variations in their call structure and peak frequencies among different clades.

We refrain from classifying each species as either nocturnal or diurnal, even if some species' activity appears rather distinct. For example, the transient calling songs of *Holochlora* and *Psyrana* corroborate field observations suggesting they are most active at night. As the katydids were not always recorded over the entire circadian cycle, and many species only have a few recordings from one or two individuals, we could not model the calling activity found in Tan and Robillard (2021). In that study, the authors recorded eneopterine crickets under standardized conditions, modeled their calling activity over 24 hours, and consequently found that many species exhibit complex circadian rhythms in their calling activity (i.e., multiple peaks in calling activity in both the day and night). Sporadic recordings may give an over-simplified impression about whether a species is strictly nocturnal or diurnal.

Sound-producing organs.—The properties of stridulatory file (length, number of teeth, and teeth density or spacing) and mirror (e.g., stiffness, membrane structure) are important in determining the frequency and resonance of a calling song (Morris and Pipher 1967, Bailey 1970, Montealegre-Z 2009, Montealegre-Z and Postles 2010, Montealegre-Z et al. 2017). Corroborating with previous studies on neotropical katydids (e.g., Montealegre-Z and Morris 1999), we observed vast diversity in the morphology of the Southeast Asian katydids. While the left tegmina of most of the reported species have straight/faintly curved stridulatory files with broad teeth (often closely packed together), a few species exhibit peculiarity. *Euanisous teuthroides* have squarish teeth on the stridulatory file, with an indentation in the middle of each tooth. *Phaneroptera brevis* have two parts to their stridulatory file, with a shorter anal half (with smaller teeth) and longer basal half (with larger teeth), as is typical for the genus *Phaneroptera* (Heller et al. 2017, 2021b). This may contribute to the different call parameters in various parts of the calling songs that have been observed in *Sphagniana sphagnorum* (Walker, 1869) and an eneopterine cricket *Eneoptera guyanensis* Chopard, 1931 (see Morris and Pipher 1972, Robillard et al. 2015).

It has been well established that the mirror area correlates negatively with peak frequency of the calling songs in katydids (Morris et al. 1994, Montealegre-Z 2009, Montealegre-Z et al. 2017). We also found species with mirrors of different sizes relative to tegmina size and shape. Some Phaneropterinae, i.e., *Holochlora* nr. *bilobata* and *Scambophyllum sanguinolentum*, have rather obsolete mirrors. A typical mirror consists of the CuPaβ (and sometimes CuPaα2) and frame surrounding a clear membrane (Chivers et al. 2017b). In *Holochlora* nr. *bilobata*, the mirror membrane is relatively small, whereas in *Scambophyllum sanguinolentum*, the mirror membrane is made up of an interlaced network of veins.

Bioacoustics and integrative taxonomy.—New acoustic data allow us to re-test species hypotheses previously delimited using only morphology. For example, we are able to integrate bioacoustics with traditional taxonomy for the genus *Viriacca* by comparing the calling songs for three of the four known species—*Viriacca insularis* from the Malay Peninsula, *Viriacca modesta* from Borneo, and previously described calls of *Viriacca viridis* Ingrisch, 1998, also from the Malay Peninsula (Ingrisch 1998). Although their sound-producing organs share many similarities, the three species exhibit distinct call structures, frequencies, syllable durations, and intervals between syllables. These differences are congruent with the genitalia differences used to diagnose these congeners (Gorochov 2013). This example also highlights that taxonomy is hypothesis-driven, in which species can be re-evaluated with new and different datasets. In light of this, we also recommend using bioacoustics to validate morphologically similar congeners in other Southeast Asian katydids. These can include the *Peracca subulicerca* species complex consisting of *Peracca subulicerca* (Karny, 1926) from Java, and *Peracca tiomani* Gorochov, 2011 and *Peracca macritchiensis* from Malay Peninsula, in which the species characters remain debatable.

Conclusions

We want to emphasize the preliminary nature of this study, as it is limited by too few species and very few specimens. Nevertheless, by amassing data on the calling songs in understudied katydids from Southeast Asia, this study provides a baseline for building a sound database for Southeast Asian orthopterans. Despite their importance in species recognition, calling songs are not always recorded in taxonomic descriptions. The morphology of the sound-producing organs of katydids (e.g., stridulatory file length, number of teeth, and mirror area) is sometimes overlooked in traditional taxonomy. Incorporating calling songs and/or sound-producing organs into traditional taxonomy can help address the taxonomy impediment while advancing our knowledge about the bioacoustics of Southeast Asian katydids.

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The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus*

Siyumi Mahavidanage[1], Tamara M. Fuciarelli[1], Xiaobing Li[1], C. David Rollo[1]

1 Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON, Canada.

Corresponding author: Tamara M. Fuciarelli (fuciartm@mcmaster.ca)

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Abstract

Alternative food sources have become an important focus of research due to increased food demand coupled with reductions in traditional food productivity. In particular, substitutes for protein sources have been of increasing interest due to the unsustainability of traditional protein sources. Insects have been identified as a sustainable alternative to traditional protein sources, as they are easy to produce and contain essential proteins, fats, and minerals. However, mass-rearing insects requires similar considerations as farming traditional protein sources. To increase productively, growth and survival must be maximized at the highest possible densities while minimizing disease and food requirements. Here, we use the house cricket *Acheta domesticus*, a highly cultivated insect species, to investigate optimal densities for mass rearing at 14 days of age (4th instar). Nymphs were separated into density groups of 0.09, 0.19, 0.47, and 0.93 cricket/cm^2 and monitored for growth and survival. Multiple regression revealed sex ($p < 0.0001$), density ($p < 0.0001$), and sex*density interaction ($p = 0.0345$) as predictors of growth rate. Survival to maturation was significantly reduced in both 0.47 (31%) and 0.93 (45%) cricket/cm^2 groups compared to the controls. A second experiment was then conducted to investigate the starvation resistance of adult crickets reared from 14 days of age at 0.09, 0.19, 0.93, and 1.86 cricket/cm^2. A second multiple regression analysis revealed only density ($p < 0.0001$) and to a lesser extent sex ($p = 0.0005$) to be predictors of starvation resistance. These results indicate that mass-rearing house crickets is most optimal at densities < 0.93 cricket/cm^2, where impacts on survival and starvation are minimal. Although these results have implications for cricket mass rearing, research on other endpoints, including reproduction and the synergistic effects of other environmental factors, such as temperature and humidity, should be conducted.

Keywords

development, growth, insect, life history, resistance, sex differences, stress, survival

Introduction

Accelerating global population growth and consumption has put increasing strain on the world's agricultural system (Godfray et al. 2010, Sorjonen et al. 2019). Our ability to produce enough food for the world's growing population is also diminishing due to factors such as urban expansion, land degradation, climate change, and water scarcity (Sorjonen et al. 2019). As a result, levels of global undernourishment have risen to an all-time high of 9.9%, with between 720 and 811 million people facing food insecurity (United Nations 2020). Food demand worldwide is predicted to increase, with demand rising by 70% by 2070. As traditional food production methods are unlikely to fulfil current and future global food demands, alternative sources of food have been posited (Sorjonen et al. 2019).

One key area of food production that has received much attention due to its current unsustainability is traditional protein sources. Currently, global protein requirements are fulfilled largely by red meat, poultry, and seafood (Thavamani et al. 2020). These foods are a key source of several micronutrients, including iron, zinc, phosphorus, vitamin B6, and B12 (Ajwalia 2020). However, meat production is highly unsustainable, with major concerns surrounding environmental degradation, animal welfare, and the negative effects of excessive meat consumption (Thavamani et al. 2020). Along with plant-based foods, cultured meats, and mycoprotein-based foods, insect-based protein sources are growing in popularity as meat/protein alternatives (Vandeweyer 2018, Thavamani et al. 2020). Insects, like traditional protein sources, provide high volumes of fat, protein, zinc, iron, and several key vitamins (DeFoliart 1991, Schabel 2010, Alexander et al. 2017, Thavamani et al. 2020). The rearing of insects for this purpose is also more efficient and sustainable than for traditional livestock, consuming orders of magnitude less water and producing significantly less greenhouse gas emissions (GHGs) (Lundy and Parrella 2015). Insects are also superior sources of protein when compared to traditional protein products, containing a protein content of 50–82% of the dry weight (Thavamani et al. 2020). In addition to producing significantly less GHGs, insects can utilize organic waste products (low-value diets) as food sources, which provides further prospects for sustainable insect rearing (Sorjonen et al. 2020, Thavamani et al. 2020). Currently, the insect-as-food industry is expected to grow by 47% from 2019 to 2026, with 730,000 tons being produced by 2030 (Savio et al. 2022).

Despite the increasing global production of insect food sources, there is a paucity of information and research on best farming practices, including optimal densities for farmed species (Hanboonsong et al. 2013). Optimizing rearing density is one of the main considerations when seeking to increase production while maintaining food safety. Currently, it is generally understood that the effects of overcrowding include alterations in insect behavior, physiology, and, most importantly, life history (growth and survival) (Maciel-Vergara et al. 2021). However, industrial mass rearing densities are often unnaturally high, and the extent to which these densities affect insect behavior, performance, and well-being is not well understood (Francuski and Beukeboom 2020).

In industrial insect rearing, mortality is a common negative effect of increased density (Peters and Barbosa 1997). In the Mediterranean fruit fly, for example, densities of 1–15 eggs/cm^2 show increased mortality as density increases due to increased food competition (Peters and Barbosa 1997). Insect overcrowding also increases mortality as a result of increased pathogen susceptibility caused by higher temperatures, reduced immune response, and nutrient deficiencies (Vergara et al. 2021, Savio et al. 2022). Viral, fungal, bacterial, and microsporidian pathogens are all frequently found to infect mass-reared insects (Vergara et al. 2021). Viruses such as *Acheta domesticus* densovirus (AdDV) can result in mass mortality and have even been behind the bankruptcy of a few cricket-rearing companies (Weissman et al. 2012). Increases in disease transmission ultimately result in the increased mortality of mass-reared insects.

Insect growth rates are highly dependent on environmental conditions including temperature, predators, humidity, and resource availability (Barragan-Fonseca et al. 2018). Although most of these stimuli can be easily controlled, nutrient deficiencies due to often-overcrowded conditions in mass-rearing facilities can result in delayed maturation, survival, and reduced body size. These are highly negative outcomes when mass-rearing insects where high survival and increased body size are desired (Averill and Prokopy 1987, Agnew et al. 2002, Reiskind et al. 2004, Rivers and Dahlem 2014). In *Anopheles gambiae (*Jannat and Roitberg 2013), larval mortality increased by 36% in high-density groups due to waste products decreasing substrate quality. In both the fruit fly *Bactrocera tryoni* (Morimoto et al. 2019) and Western tarnished plant bugs [*Lygus hesperus* (Brent 2010)], significant reductions in adult emergence, body weight, fecundity, and energetic reserves were evident in crowded treatments. Declines in growth and prolongation of larval developmental periods have also been shown in several species, including *Culex quinquefasciatus* (Ikeshoji and Mullai 1970), *Trogoderma glabrum* (Beck 1973), *Tipula oleracea (*Laughlin 1960), *Endrosis sarcitrella (*Andersen 1956), and *Ptinus tectus (*Peters and Barbosa 1997). Increased population density can also create greater social contact and irritation, increasing competition, aggressive encounters between individuals, fighting, and habitat destruction (Southwick 1971, Weaver and Mcfarlane 1990). Mating can also be affected by overcrowded conditions (Gavrilets 2000). Martin and Hosken (2003) observed that females of the dung fly *Sepsis cynipsea* became less interested in remating when the population density increased.

Interestingly, however, some species have shown a shortening of developmental time in response to increased density (Cohen 1968, Hodjat 1969). In addition, shifts in development time and size have often been shown in both high-density (overcrowding) and low-density (group effects) treatments (Peters and Barbosa 1997). As it is often difficult to determine what rearing densities are indicative of overcrowding in any particular insect, as well as between-species behavioral and physiological differences, research on optimal densities in a variety of species is necessary. Investigating the effects of rearing densities not only on survival and growth but also on resistance to starvation due to these overcrowded conditions (i.e., resource competition) is also of interest.

Although 1900 different species of insects are consumed globally, crickets are currently one of the most highly cultivated insect species, being utilized for both human consumption and as food sources for other species (Lundy and Parrella 2015, Francuski and Beukeboom 2020, Savio et al. 2022). In this study, we investigated optimal rearing densities in the cricket *Acheta domesticus*, considering the negative outcomes of overcrowding on growth, survival, and starvation resistance in groups at densities relevant to mass insect rearing. Densities of 0.09, 0.19, 0.47, 0.93, and 1.86 cricket/cm^2 were reared starting at 14 days of age (4th instar) and maintained until death. Resistance to adult starvation was also investigated at these various densities to understand the impacts of various rearing densities on food competition, as this is likely to occur in high-density mass-rearing facilities.

Methods

Breeding colony.—House crickets *A. domesticus* were housed in a 93 × 64.2 × 46.6 cm acrylic terrarium covered with 1.5-cm thick Durofoam insulation. The colony crickets were from a homogenous stock inbred for > 60 generations. Fans on top of the enclosed structure provided air circulation. Crickets were sustained at a 12 h-day–12 h-night photoperiod and at a constant temperature of 29 °C ± 2 °C using 60-volt UV heat lamps. Ad libitum food (Country Range MultiFowl Grower, 17% protein, Quick Feeds Feed Mill, Copetown, Canada) was provided. Ad libitum distilled water was made available in soaked cellulose sponges, and egg cartons were provided for shelter. Oviposition medium (Vigoro Organic Garden Soil, The Mosaic Co., Lake Forest, IL, U.S.A.) was present in small plastic containers (7 × 7 × 7 cm). Oviposition containers were collected after a 24-hour period and incubated at 29 °C ± 2 °C until eggs hatched (~ 14 days).

Density variation.—*A. domesticus* were taken two weeks (14 days, 4th instar) post-hatch from a single colony oviposition container and randomly separated into four experimental groups. Crickets at this age/molt are approximately 0.25 mm in length. The experimental groups consisted of 50, 100, 250, and 500 crickets, creating densities of approximately 0.09, 0.19, 0.47, and 0.93 cricket/cm^2. respectively. Densities given are approximation. A range of densities below 0.93 cricket/cm2 was chosen to allow for density-dependent observations, as densities above this had been observed in the lab to result in mass die-offs. All groups were given a 2 × 2 egg carton, which lowered the density slightly. The 0.09 cricket/cm^2 group was chosen as the control, as the lowest-density group is expected to maximize resources per cricket and thus have fewer negative interactions. Experimental housing containers were 29 × 18.5 × 12 cm, and the crickets were housed for life and in the same conditions as the colony. Cricket containers were continuously monitored for cleanliness, as extremely dirty rearing environments may negatively impact growth and development. Containers were cleaned (crickets were moved into fresh containers) at a minimum of once a week; however, in the higher-density groups, cleaning occurred approximately every other day due to increased mortality and excrement production. Food and water were replaced daily to ensure the same resource availability in each group.

Life-history measurements.—Maturation in *A. domesticus* is denoted by the adult molt in which wings develop and individuals become sexually mature. Females are easily identifiable by the fully-developed ovipositor. Crickets reach adulthood approximately 5–60 days post-hatch. Sex, maturation mass, and development time (number of days post-hatch) were recorded for all density groups. Sample sizes for growth rate were 0.09 cricket/cm^2 (n = 41), 0.19 cricket/cm^2 (n = 68), 0.47 cricket/cm^2 (n = 142), and 0.93 cricket/cm^2 (n = 224). Sample sizes for the proportion of individuals surviving maturation were 0.09 cricket/cm^2 (n = 50), 0.19 cricket/cm^2 (n = 100), 0.47 cricket/cm^2 (n = 250), and 0.93 cricket/cm^2 (n = 500). Mass was measured using an Accuris analytical balance with a readability of 0.001 g +0.002 g. Maturation mass (g) and development time were employed to calculate the growth rate. The number of crickets that matured was used to determine the proportion of individuals that successfully matured.

Starvation resistance.—After determining the impacts of various rearing densities on growth and survival parameters, a second experiment was conducted to determine the potential responses to adult starvation at various rearing densities. Individuals from a second oviposition container were arranged as described above. Group densities were slightly altered based on our initial results that had suggested that although the proportion matured was reduced at densities ≥ 0.47 cricket/cm^2, growth rate was not affected. The experimental groups were separated and maintained as described above. Experimental groups included 50, 100, 500, and 1000 individuals, representing densities of approximately 0.09, 0.19, 0.93, and 1.86 cricket/cm^2, respectively. Three to four weeks post-maturation, 10 females and 10 males from each density group were weighed and placed in individual containers to prevent cannibalism. The mass of each individual was recorded to determine whether increased body mass is related to starvation resistance. The number of surviving individuals in the 1.86 cricket/cm^2 group consisted of 7 males and 10 females. For the starvation treatment, each individual cricket from each group was placed into a small cylindrical container and covered with plastic wrap secured by a rubber band. Holes were added to the plastic wrap to provide ventilation, and the sex and group of each individual were noted on the container. Ad libitum water was provided by placing a water-soaked cellulose sponge in the container; the sponges were re-soaked daily. Although the crickets consumed the cellulose sponges, they provided insufficient nutrients. Mortality was noted daily and used to determine longevity.

Statistics.—To determine the effect of sex, density, and possible interaction (sex*density) on both growth and starvation resistance, a multiple linear regression was conducted using the most appropriate model. The best-fit model was determined using a step-wise AICc comparison. A D'Agostino–Pearson omnibus normality test was conducted to ensure data was normally distributed. Survival curves were analyzed using a Gehan–Breslow–Wilcoxon survival analysis to determine differences in survivorship among density groups. To analyze differences in the proportion that survived to maturation, chi-square tests were applied. To determine significant differences between the various rearing densities and the control, a Fisher's exact test was applied to each rearing density compared to the control. Finally, significant differences in the mass of starvation groups were determined using a one-way ANOVA followed by Tukey's multiple comparisons test. All statistical analyses were carried out using Prism Graph Pad 9.

Results

Survival to maturation.—Chi-square tests indicated significant differences in proportion matured among the different rearing density groups ($p < 0.0001$). Fisher's exact tests indicated significant differences between the 0.47 cricket/cm^2 ($p = 0.0008$) and 0.93 cricket/cm^2 ($p < 0.0001$) groups compared to the 0.09 cricket/cm^2 density group. This constituted a 31% and 45% decrease in the proportion that matured, respectively (Fig. 1).

Growth rate.—Growth rates were collected for all rearing density groups and are reported as mean growth rate ± SD (Fig. 2). Prior to analysis, a D'Agostino-Pearson omnibus normality test was performed and confirmed normal distribution. We used AICc model selection to determine the best model for describing the relationship between sex, density, and growth. The best-fit model carried 77.46% of the cumulative model weight and included two predictors (sex and density) with interaction effects $F(3, 471) = 70.79$, $p < 0.0001$, $R^2 = 0.3108$; $y = 8.277 + 1.186\beta1 - 1.094\beta2 - 0.5763\beta3$. Results suggest that sex ($p < 0.0001$), density ($p < 0.0001$), and, to a lesser extent, sex*density ($p = 0.0345$) are significant predictors of growth rate. The multiple regression results are outlined in Table 2. A summary of growth and development measurements is outlined in Table 1.

Mass of starvation groups.—The mass (g) of each male and female *A. domesticus* used for starvation-resistant treatment was recorded immediately prior to experimentation and are represented as mean mass ± SD (Fig. 3). A one-way ANOVA indicated significant differences between groups $F(7, 69) = 58.47$, $p < 0.0001$, with a Tukey's multiple comparison test indicating significantly reduced masses ($p < 0.0001$) in both the 0.93 and 1.87 cricket/cm^2 females compared to the 0.09 cricket/cm^2 female controls. Significant reductions in mass were also detected in 0.93 ($p = 0.0018$) and 1.87 ($p < 0.0001$) cricket/cm^2 males compared to the 0.09 cricket/cm^2 male controls. Between-sex differences ($p < 0.0001$) were also detected in the 0.09, 0.19, and 0.93 cricket/cm^2 groups.

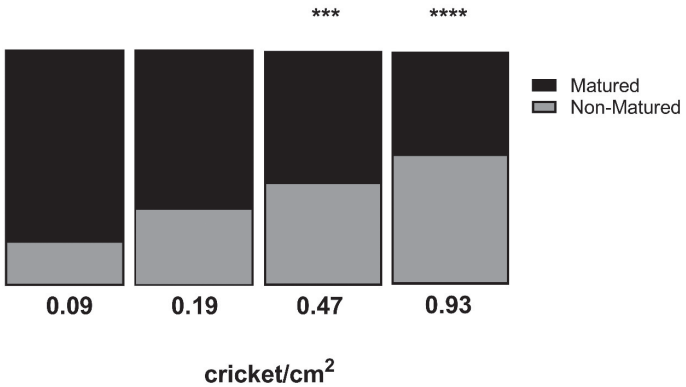

Fig. 1. The proportion of *Acheta domesticus* reaching maturation in each rearing density (0.09, 0.19, 0.47, and 0.93 cricket/cm^2). A chi-square test indicated significant differences in survival among rearing densities ($p < 0.0001$). A Fisher's exact test showed significant differences in proportion matured between 0.19 cricket/cm^2 ($p = 0.0008$) and 0.93 cricket/cm^2 ($p < 0.0001$) compared to the 0.09 cricket/cm^2 density group.

Table 1. Summary of growth parameters of each *Acheta domesticus* rearing density group.

Experimental Group	N	Growth rate (mg/day)	Percentage of controls	Upper 95% CI	Maximal growth rate	Development time (Days)	Mass at maturation (g)
0.09 Male (control)	14	7.629		8.080	8.98	50.79	0.388
0.09 Female (control)	27	8.941		9.280	10.91	48.15	0.431
0.19 Male	34	8.239	8.00%	8.518	10.00	50.00	0.413
0.19 Female	34	9.109	1.88%	9.475	12.04	48.97	0.446
0.47 Male	71	7.830	2.63%	8.026	9.68	49.51	0.387
0.47 Female	71	8.963	0.25%	9.219	11.24	47.32	0.424
0.93 Male	112	7.234	- 5.18%	7.392	9.87	48.21	0.349
0.93 Female	112	7.829	- 12.45%	8.017	10.96	46.71	0.366

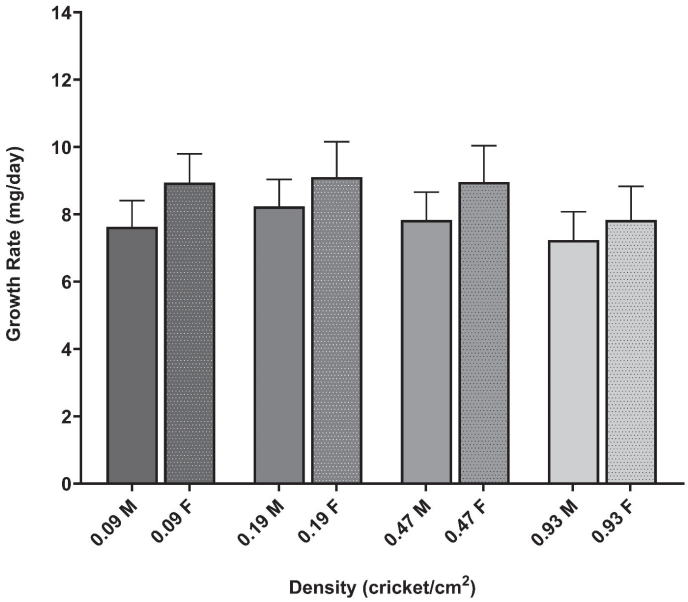

Fig. 2. Density-dependent effects of various rearing densities on the growth rate of male and female *Acheta domesticus*. Values are represented as mean growth rate of each group ± SD. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) of each individual. Multiple regression analysis determined that sex ($p < 0.0001$), density ($p < 0.0001$), and to a lesser extent sex*density ($p = 0.0345$) interaction were strong predictors of growth rate.

Density–starvation.—Starvation resistance was measured as days survived after the removal of sufficient food for all density groups and is reported as mean survival ± SD (Fig. 4). Prior to analysis, a D'Agostino–Pearson omnibus normality test confirmed normal distribution. We used AICc model selection to determine the best model to describe the relationship between sex, density, and starvation resistance. The best-fit model carried 72.65% of the cumulative model weight and included two predictor values: sex and density ($F(2, 74) = 28.05$, $p < 0.0001$, $R^2 = 0.4312$; $y = 10.46 - 3.661\,\beta1 - 2.701\beta2$). The results suggest that both sex ($p = 0.0005$) and density ($p < 0.0001$) are significant predictors of starvation resistance. Results of the multiple regression are summarized in Table 3. The Gehan–Breslow–Wilcoxon test showed significant differences in survivorship between the 0.93 cricket/cm^2 ($p = 0.0030$) and 1.86 cricket/cm^2 ($p = 0.0008$) groups compared to the lowest density (0.09 cricket/cm^2) female group. Variation in survivorship was also evident in the 0.19 ($p = 0.0328$), 0.93 ($p = 0.0063$), and 1.86 ($p = 0.0001$) cricket/cm^2 groups compared to the males in the lowest-density groups.

Table 2. Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting growth rate based on sex and density group. AICc comparison was utilized to select the best model. Our model includes sex, density, and sex*density interactions, which carried 77.46% of the cumulative model weight. Each predictor value had a significant correlation with growth rate: sex ($p < 0.0001$), density ($p < 0.0001$), and sex*density ($p = 0.0345$).

Variable	Coefficient (β)	SE	95% CI	P Value
Intercept	8.277	0.1398	8.002 to 8.552	<0.0001
Sex	1.186	0.1885	0.815 to 1.556	<0.0001
Density	- 1.094	0.1991	- 1.485 to - 0.703	<0.0001
Sex*Density	- 0.576	0.2718	- 1.110 to - 0.042	0.0345

Table 3. Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting survival based on sex and density group. Our model includes both sex and density, with no sex*density interactions, which carried 72.65% of the cumulative model weight. Each predictor value had a significant correlation with growth rate: sex ($p = 0.0005$), density ($p < 0.0001$).

Variable	Coefficient (β)	SE	95% CI	P Value
Intercept	10.46	0.6495	9.164 to 11.750	<0.0001
Sex	2.70	0.7451	1.217 to 4.186	0.0005
Density	- 3.66	0.5405	- 4.738 to - 2.584	<0.0001

Discussion

Insects have been proven to be a valuable alternative source of protein, fat, and essential vitamins and minerals (DeFoliart 1991, Schabel 2010, Zaelor and Kitthawee 2018). Utilizing insects as food may fill the gaps in our ability to meet current and future food demands. Crickets such as *A. domesticus* are one of the most cultivated insect species globally (Lundy and Parrella 2015, Zaelor and Kitthawee 2018, Francuski and Beukeboom 2020). *Acheta domesticus* is considered an excellent candidate for mass-rearing endeavors due to its low food requirements and beneficial nutritional profile (Fernandez-Cassi et al. 2019). To mass rear insects for these purposes, it is vital to understand optimal densities for increasing individual size and survival (output) while reducing disease and stress. In this study, we investigated the impacts of various rearing densities on *A. domesticus* life-history features as well as their ability to survive prolonged starvation. We reared experimental groups for life-history analysis (growth rate and survival to maturation) at 0.09, 0.19, 0.47, and 0.93 cricket/cm^2. We determined that the number of individuals reaching maturation was significantly reduced in the 0.47 and 0.93 cricket/cm^2 density groups (Fig. 1). This represented a decline

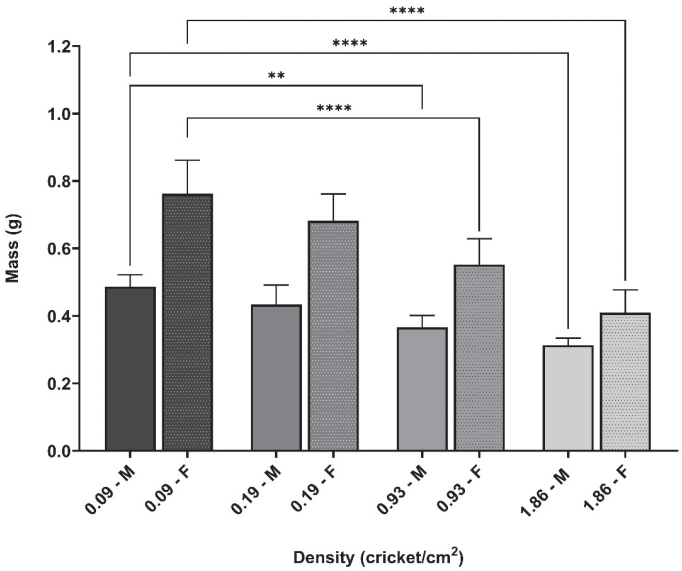

Fig. 3. Mass of adult *Acheta domesticus* used for starvation treatment from each density group. Values represent the mean mass of each group ± SD. The mass of each individual was recorded prior to starvation treatment. A one-way ANOVA indicated significant differences between groups ($F(7, 69) = 58.47$, $p < 0.0001$), with a Tukey's multiple comparison test indicating significantly reduced masses ($p < 0.0001$) in both the 0.93 and 1.87 cricket/cm^2 females compared to the 0.09 cricket/cm^2 female controls. Significant reductions in mass were also detected in the 0.93 ($p = 0.0018$) and 1.87 ($p < 0.0001$) cricket/cm^2 males compared to the 0.09 cricket/cm^2 male controls. Although not represented on the graph, between-sex differences ($p < 0.0001$) were also detected between males and females in the 0.09, 0.19, and 0.93 cricket/cm^2 groups.

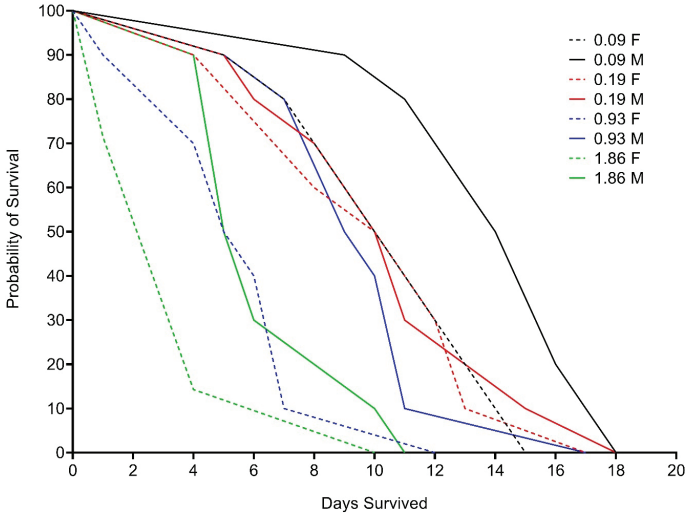

Fig. 4. Starvation resistance of adult *Acheta domesticus* reared at various densities. All density and control groups were separated at 14 days of age (4th instar) and maintained until adulthood. Three to four weeks post-maturation individuals were separated and deprived of sufficient food. The number of days survived was recorded for all crickets and represented as mean ± SD. Multiple regression analysis suggests both sex ($p = 0.0005$) and density ($p < 0.0001$) to be significant predictors of starvation resistance. The Gehan–Breslow–Wilcoxon test showed significant differences in survivorship between the 0.93 cricket/cm^2 ($p = 0.0030$) and 1.86 cricket/cm^2 ($p = 0.0008$) groups compared to the lowest density (0.09 cricket/cm^2) female group. Variation in survivorship was also evident in the 0.19 ($p = 0.0328$), 0.93 ($p = 0.0063$), and 1.86 ($p = 0.0001$) cricket/cm^2 groups compared to the lowest-density males.

of 31% and 45% compared to the lowest density 0.09 cricket/cm^2 group but still resulted in 142 and 224 individuals maturing, respectively. For growth rate, multiple regression analysis found that both sex ($p < 0.0001$) and density ($p < 0.0001$) have highly significant predictive power for growth rate. Interaction between sex and density ($p = 0.0345$) also had a slightly significant impact on growth rate (Fig. 2). A decline in growth rate was evident between the highest density males and females compared to their lowest density conspecifics, constituting a 5.18% and 12.45% decline, respectively.

Although our study did not indicate large declines in growth due to increased density, our results suggest that density is a strong predictor of growth rate. Prior studies have indicated that both increased mortality and decreased growth are expected due to overcrowding (Peters and Barbosa 1997, Zaelor and Kitthawee 2018). These impacts on survival and growth are likely due to increasing population density, which has been shown to increase competition, physical injury, and stress (Parry et al. 2017). In an early study on the rearing densities of larval American cockroach *Periplaneta Americana (*Wharton et al. 1967), increasing density reduced both survival and growth. Other studies show similar trends, although the magnitude of survival and growth reductions seem to vary among even closely related species. A study by Parry et al. (2017) found that both survival and growth were significantly affected by density; the relationship was not linear and was significantly different between the five blow fly species used. Studies on the English grain aphid *Sitobion avenae* (Xing et al. 2021) revealed decreases in both the growth and survival of early-instar nymphs with increased population density. The effects of density also seem to be age dependent, with some species being more resistant to density in later life stages. For example, adult density does not significantly affect survival or reproductive traits in *C. homnivorax* (Berkebile et al. 2006).

Varying results in the literature indicate that population density effects are highly complex and species-specific, with some species being more resistant to the negative effects of overcrowding than others (Xing et al. 2021). Synergistic effects, such as temperature and other environmental factors, should also be considered, as they may influence the effects of density, as was shown in a study using *Sitobion avenae* (Xing et al. 2021). This variability highlights the need for species-specific data on density impacts. It is also likely that a more substantial reduction in growth may have appeared at higher densities than used here. In addition, as shown in Fig. 1, it is likely that the use of even higher densities may not only further impact growth but also survival.

The second key aspect of this study was to investigate the impact of rearing density on starvation resistance. Intermittent food shortages are common in both the wild and in high-density mass rearing environments where competition for food is high (Zhang et al. 2019). Lack of food over extended periods of time can affect the growth, survival, and reproduction of individuals within the population (Zhang et al. 2019). Our chosen regression model suggested that sex ($p = 0.0005$) and to a larger extent density ($p < 0.0001$)—but not the interaction between the two—are significant predictors of starvation resistance (Fig. 4). Analysis of survivorship curves indicated that most groups showed significant differences in survivorship compared to their same-sex lowest-

density group. The survival rate of the males in the 1.86 cricket/cm2 density group was less than the males in the 0.09 cricket/cm2 control group. It is important to note, however, that the mass of individuals used in the starvation resistance experiments was significantly lower in the 1.83 cricket/cm^2 and 0.93 cricket/cm^2 groups for both males and females (Fig. 3). The 1.83 cricket/cm^2 density group was not measured for growth rate, but it is likely that at this extremely high density the growth rate would be reduced given the significant reduction in mass observed before starvation. Our results are in line with to the manner in which insects are able to resist starvation in conditions in which migration is not feasible. Under starvation conditions, insects will undergo physiological modifications that alter their metabolism to help them cope (Zhang et al. 2019). They will first metabolize blood sugar (trehalose) and then lipids (triglycerides) to improve hunger resistance (Zhang et al. 2019). It is therefore likely that the reduction in mass observed in the 1.83 cricket/cm^2 and 0.93 cricket/cm^2 disadvantaged these individuals in terms of their ability to break down sugar and fat stores as effectively as larger individuals.

Although not always considered, sex often plays a key role in stress-related impacts on life-history features. Our results for both growth and starvation resistance showed significant contributions of sex on both variables (Tables 2, 3). Increased density has been shown to not only affect life-history traits but also alter interactions between different sexes (Rull et al. 2012, Parry et al. 2017). This may lead to alterations in fecundity and fertility (Rull et al. 2012). Sex differences are expected, as females of this species are typically larger than males (Lyn et al. 2012). For starvation resistance, sex differences were evident in the 1.86 and 0.93 cricket/cm^2 groups, with females being more sensitive to starvation than males (Fig. 4). However, most studies have found female insects to be generally more resistant to food-related stress, as females are typically larger and therefore have more nutrient stores. For example, Gaskin et al. (2002) found male *Ceratitis capitata* to be more negatively impacted by increases in density than females. The researchers postulated that this was due to increased aggression and behavioral costs to mate successfully in males (Gaskin et al. 2002). A study conducted on five species of blow flies reared at various densities indicated that females survived longer than males across all species used (Parry et al. 2017). Higher mortality in males versus females due to density variation has been recorded in *Lucilia* (Parry et al. 2017) *sericata* and *Ceratitis capitata (*Gaskin et al. 2002), Males of *Cochliomyia homnivorax* (Berkebile et al. 2006, Pitti et al. 2011) tend to show increased mortality under several rearing conditions, including high density, protein rich diets, and high temperature . While it is unclear why females in our study were more sensitive to starvation, trade-offs between cell maintenance and repair, resulting in aging and death, and the energetic costs of egg production have been documented in female insects (De Loof 2011). The females used in this study were sexually mature adults, potentially making them less able to mitigate the impacts of starvation.

These results have profound implications for insect farming in which productivity is often deterred by the increased competition and stress associated with high density (Zaelor and Kitthawee 2018). Our results indicate that optimal densities for the mass rearing of *A. domesticus* are likely to be < 0.93 cricket/cm^2, as this minimizes reductions in growth and maintains adequate resistance to starvation in adulthood. In addition, although survival to maturation is significantly reduced at this density, the number of individuals that do survive, in this case 224, is significantly greater than at densities with < 250 individuals. Thus, as the goal of mass rearing is to produce the largest number of individuals at the maximum body size while reducing stress, we believe that < 0.93 cricket/cm^2 is optimal. These results should guide future mass-rearing endeavors to optimize production while reducing mortality and other negative effects of overcrowding. It is also recommended that future research focus on a diversity of endpoints, such as how density influences reproductive output, immune responses, and survivorship in this species to further inform optimal rearing densities. Synergistic effects between density and other environmental factors such as temperature, humidity, etc., should also be investigated.

Acknowledgments

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Probability of a Central American locust *Schistocerca piceifrons piceifrons* upsurge in the Yucatan Peninsula, Mexico

Mario A. Poot-Pech[1,2]

1 Yucatan State Plant Health, Yucatan, Mexico.
2 Grasshopper-Locust Management in Central America, Yucatan, Mexico.

Corresponding author: Mario A. Poot-Pech (mpootpech@gmail.com)

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Abstract

From ancient times to the present, infestations of the Central American locust (CAL) [*Schistocerca piceifrons piceifrons* (Walker, 1870)] have occurred periodically and with varying intensities in the Yucatan Peninsula (YP), Mexico. Despite efforts to survey the recession zone, an upsurge is still difficult to predict and prevent, and high economic costs are incurred in controlling this pest. For this study, two models were developed to determine the probability of an upsurge in the YP. The first was the Markov chain (MC) with transition probability matrix, which estimates probability by determining the proportion of times that the system moved from one state to another (n_2) over 71, 33, and 24 years in Yucatan, Campeche, and the Quintana Roo States, respectively, divided into different periods; a correlation of the matrix and probability (n_2) of the next period was performed to evaluate the accuracy of the estimation. The other method is the classic probabilistic (CP) model, which uses the number of times the upsurge could happen and the number of possible events. In the MC model, great variation was found in CAL upsurge probabilities between periods, with a similar number of upsurges from the past to the present but with varying intensity. In recent years, the treated area with insecticides has been less than that of the past. The CP model revealed that the locust population reached its maximum peak every four years, with the migration of swarms to neighboring states at the end/start of the year. Validation of the MC and CP models was performed considering information on areas treated in 2019 and 2020, and good accuracy was obtained. Both models provide information on the probability of an upsurge in the YP. This information can be incorporated into economic models to improve management decisions, such as when to announce early warnings, and to implement preventive control strategies.

Keywords

early warning, forecast, preventive management, recession period

Introduction

Locusts are among the most devastating pests of human agriculture (Lockwood 2015), making them a major threat to global food security and the livelihoods of farmers in numerous countries (Simpson and Sword 2008). *Schistocerca piceifrons* (Walker, 1870) (Orthoptera: Acrididae) is the true locust of tropical America (Harvey 1983). This species has two subspecies: the Central American locust (CAL; *S. piceifrons piceifrons*), known in Mexico and Central America, and the Peruvian locust (*S. piceifrons peruviana*), distributed in Peru and Ecuador (Harvey 1983, Barrientos et al. 1992).

CAL damage has been documented from the Mayan culture and colonial period, which reported drought and hunger as results of this pest (Flores 2011, García 2012), to the present (SENASICA-DGSV 2016). According to Trujillo (1975), a CAL swarm can feed on 30 tons of vegetation per day and, based on its food preference and distribution in 10 Mexican states, the species could affect 5.9 million ha (SENASICA-DGSV 2016).

An important CAL breeding zone is located in the Yucatan Peninsula (YP), Mexico (SENASICA-DGSV 2016), which possesses ecological conditions–such as mainly grass vegetation–for high-density locust development (Poot-Pech et al. 2018). According to SENASICA (2019), the YP receives 50% of the national budget for locust control because of the importance of breeding zones. The YP is located in the southeast of Mexico. It is made up of three states–Yucatan, Campeche, and Quintana Roo–that comprise 181,000 km^2 (9.2% of the national territory). The overall climate is tropical, with strong seasonality and dry (November–May) and rainy (June–October) seasons (Barber et al. 2001). Tropical forest is the dominant vegetation, but grassland is increasing (Durán and García 2010). The soil is heterogeneous and mostly stony (Bautista et al. 2011).

Currently, CAL in Mexico are controlled through government regulations and advice from the International Regional Organization for Plant and Animal Health (OIRSA), an intergovernmental organization founded in 1953. OIRSA was created as a cooperative effort of locust control between Central American countries that are part of the insect's migration path (Barrientos et al. 1992).

Locust plagues occur after a series of events that increase the number of locusts. The series normally begins with a calm period of recession followed by localized outbreaks and upsurges from which a plague may develop and eventually decline (Symmons and Cressman 2001). Locusts survive the dry season as adults and

oviposit in bare areas. Young nymphs hatch and feed on adjacent short ephemeral grasses, while older nymphs and young adults survive on taller vegetation (Hunter and Spurgin 1999). An outbreak occurs when multiplication causes a marked increase in locust numbers and densities so that individuals become gregarious and, unless checked, hopper bands and/or swarms are formed (Van Huis et al. 2007). The gregarization process takes several months (Symmons and Cressman 2001). Upsurges are periods after an outbreak that are followed by two or more successive generations of transient-to-gregarious breeding, with populations occupying an expanding breeding area (Van Huis et al. 2007). Periods of one or more years during which upsurges are widespread are considered plagued years (Symmons and Cressman 2001).

The CAL is an old pest in Mexico (Contreras and Galindo 2014) that is still present in breeding areas today. To analyze and forecast the distribution of CAL, Hernández-Zul et al. (2013) built a dynamic simulation to identify suitable conditions for high CAL reproduction rates. Galindo et al. (2014) proposed an early warning system that used multicriteria models and satellite images. Aldama et al. (2014) suggested that outbreak characterization of endemic channels could help in forecasting short-term locust thresholds in zones where outbreaks can occur. The climate, El-Niño Southern Oscillation (ENSO) (Retana 2000, Contreras and Galindo 2014), vegetation, locust density, and predators are additional indicators that could be valuable in predicting a locust outbreak (Henschel 2015).

The strategies for locust control have has recently changed, with attempts focusing on prevention with the use of forecasting tools. Methods for producing Short- and medium-term forecasts have been made indicating potential locust migration and breeding areas (Cressman 1996). Zhang et al. (2019) proposed including monitoring and forecasting as part of current preventive locust management. Presently, biomodels and geographic information systems (GIS) are used to support the monitoring and forecasting of CAL outbreaks (Barrientos et al. 2021), but efficient statistical tools, with or without historical information, are important for the early detection of rapid changes in the breeding and migration of pest species that lead to outbreaks (Naumova and MacNeill 2005). Bayesian statistics and Markov models offer an effective method for long-term population forecasting of pests and can provide locust-control agencies and organizations with the information necessary to implement appropriate management strategies (Xiang-Hui et al. 2010). Knowledge of indirect effects is also a valuable tool for predicting the probability of CAL upsurge, as it is important to be aware of the relationship between areas treated with any pesticide and locust evolution (outbreak, upsurge, or pest) (Prospatin 2012). Van Huis et al. (2007) described the treated areas with any control agent as an answer to the function of locust area and time.

The objective of this research was to develop and compare two models that can produce probability estimates for future upsurges in the YP by analyzing non-weather-related historical CAL data.

Materials and methods

Data acquisition.—To document the occurrence of CAL upsurges, the following sources of historical information on locust were used: Márquez (1963), Trujillo (1975), Pereyra (1991), Chi (2000), newspapers, interviews with locust field officers, and information provided by the National Service of Health, Safety and Agrifood Quality (SENASICA) on the locust control campaign in the YP, Mexico, through its informatics system called the Phytosanitary Campaign System (SICAFI; www.sicafi.gob.mx/DGSV/).

For Yucatan state, CAL data for 71 years (1948–2018) were available (every year had a value). Data for 33 years (1986–2018) were available for Campeche, and 24 years of data (1995–2018) were available for Quintana Roo.

Locust upsurge definition.— Upsurges were used instead of outbreaks because there were two high-density generations–transients-to-gregarious or gregarious-to-gregarious–and migration to other regions (Van Huis et al. 2007); when swarms move beyond breeding areas to other regions, the overall situation exceeds a hazily demarcated threshold from "outbreak" to "upsurge" (Showler 2019). The CAL was present in Yucatan State in a gregarious form in almost years which is reflected in larger treated surface with insecticides (Fig. 1).Upsurges, therefore, allow consideration of the large number of migrant swarms that formed and reached the state of Campeche. As a result of such upsurges, the treated area required for CAL control was high in one year as in 2004, 2006, 2009, 2010, 2014, and 2018 or consecutive years 1952–1955, 1979–1981 and 1986–1989 on gregarious populations (Fig. 1) that reached Campeche during migration. The reaction in this state was the development of highly controlled surface in 2007, 2011, 2015, and 2019 because subsequent swarms (451 in 2006–2007, 245 in 2010–2011, 243 in 2014–2015, and 120 in 2018–2019) migrated from Yucatan (Fig. 2). In contrast, in years without an upsurge, the number of swarms detected in Yucatan was at a minimum (0–60 swarms) and failed to reach Campeche.

Markov chain probabilistic model.—The data were organized by assigning "0" to years with none or low intensity of CAL swarms and "1" to those in which an upsurge occurred. Second, the data were organized into periods of upsurge based on number of years, resulting in 5 periods of 12 years and one of 11 years in Yucatan state; 3 periods of 12 years, except for the first period of 9 years, in Campeche; and 2 periods of 12 years in Quintana Roo.

Classic probabilistic model.—The maximum peak years were obtained using the treated area (ha) in Yucatan state from 2003–2018. Subsequently, a consecutive number was assigned to each of the following years, finalizing in the next peak. A probabilistic analysis of occurrence was performed for each consecutive year. The probability of occurrence in the most recent years (2003–2018) was compared with that of previous periods [1977–1989 (Pereyra 1991) and 1952–1955 (Márquez 1963)].

Model validation.—The probabilistic results of the two models were compared to the results of the treated area in the YP in 2019 and 2020.

Data analysis

Markov chain probabilistic model.—The probability of matrix transition for every period was estimated by determining the proportion of times that the CAL upsurge situation, 0 and 1, moved from one state to another (Kemp 1987). In this context, there were four possible transitions: no upsurge → no upsurge (0→0), no upsurge → upsurge (0→1), upsurge → no upsurge (1→0), and upsurge → upsurge (1→1) (modified from Zimmerman et al. 2004). These estimates were represented by a *p* matrix developed for each year, where

$$P = \begin{pmatrix} P_{00}, P_{01} \\ P_{10}, P_{11} \end{pmatrix}$$

Table 1. Matrix (one-step transition) and n_2, for CAL upsurges in a 71-year period.

Number	Years	Yucatan				Number of years	Upsurge years	Upsurge average	Probability n_2			
		Matrix (One-step transition)										
		0→0	0→1	1→0	1→1				0→0	0→1	1→0	1→1
1	1948–1959	0.5	0.5	0.43	0.57	12	7	1.71	0.46	0.54	0.46	0.53
2	1960–1971	0.5	0.5	0.6	0.4	12	5	2.40	0.55	0.45	0.54	0.46
3	1972–1983	0.71	0.29	0.75	0.25	12	4	3.00	0.72	0.28	0.72	0.28
4	1984–1995	0.2	0.8	0.67	0.33	12	6	2.00	0.57	0.43	0.35	0.65
5	1996–2007	0	1	0.72	0.28	12	7	1.71	0.72	0.28	0.2	0.8
6	2008–2018	0.58	0.42	0.66	0.34	11	4	2.75	0.61	0.39	0.60	0.4
						71 (total)	**33** (total)	**2.15** (mean)				

The elements P_{00} and P_{01} refer to the probability that in X year (present), the following year's CAL population will be classified as low (P_{00}) or high (P_{01}) if X year has low densities. Alternately, if X year has a high locust density, P_{10} is the probability that the following year's populations will be classified as having a low density, and P_{11} is the probability that the state will continue to be classified as having a high density of CAL the next year (Kemp 1987).

The probability of correctly determining the locust density in the next period was obtained using the recursive properties of a two-state Markov chain (Bhat 1972). The matrix P(n–1) was multiplied by the matrix P to generate the matrix p(n). The element-by-element calculations for $P^{(n)}$ were as follows:

$$P_{00}^{(n)} = P_{00}^{(n-1)}P_{00} + P_{01}^{(n-1)}P_{10};$$
$$P_{01}^{(n)} = P_{00}^{(n-1)}P_{01} + P_{01}^{(n-1)})P_{11};$$
$$P_{10}^{(n)} = P_{10}^{(n-1)}P_{00} + P_{11}^{(n-1)}P_{10};$$
$$P_{11}^{(n)} = P_{10}^{(n-1)}P_{01} + P_{11}^{(n-1)}P_{11}$$

The correlation between the probability n_2 of the two-state Markov chain and the probability of the next period (one-step transition)–for example, the probability n_2 of period 1 with the matrix of period 2–was determined using Pearson correlation ($p < 0.05$) in R software version 3.6.0 (R Core Team 2019).

Classic probabilistic model.—We obtained the classic probability using P(1) = #1/M, where P(1) is the probability of an upsurge, #1 is the number of times the upsurge can happen, and #M is the number of possible events (Infante and Zárate 1990). Upsurge years were designated as 1, with years of no upsurge designated as 0.

Results

Markov chain probabilistic model

Yucatan state.—A great deal of variation was found in CAL upsurge probabilities between periods, except in transitions 0→0 and 0→1 of periods 1 and 2. In the first period, transition 1→1 (0.57) was highest, indicating years with contiguous upsurges. The second period was characterized by a reduction in upsurge frequency of 1→1 (0.4). In the third period, this transition had its biggest reduction (0.25), indicating an increase in "recession years," and the transition 0→0 was increased to 0.5 to 0.71 . During periods 4 and 5, the values of the transition matrix were very similar (0→0: 0–0.2, 0→1: 0.8–1, 1→0: 0.67–0.72, and 1→1: 0.28–0.33). Thus, an upsurge appeared in one year, and in the next, it was reduced. The last period was very similar to the second period, where the recession years were increasing 0→0 (0.58) and 1→0 (0.66) and remained high. From 1948 to 2018, there were 33 upsurges at an average of 2.15 per period and with a range of 4 to 7. Periods 1 and 5 had a major upsurge, and periods 3 and 6 had a minor locust presence.

In 4 of 5 cases, the correlation between the matrix and probability n_2 in the next period was negative, and the P-value was not statistically significant ($P > 0.05$). In the period 1972–1983, the correlation was high, positive, and statistically significant ($P \leq 0.01$).

Table 2. Pearson correlation of the matrix and probability n_2 in Yucatan state ($P < 0.05$).

Period	Correlation	P-value
1960–1971	-0.65	0.34
1972–1983	0.98	0.01
1984–1995	-0.26	0.73
1996–2007	-0.75	0.24
2008–2018	-0.64	0.35

Campeche State.—In the matrix, the three periods of CAL upsurge in Campeche State were different. In period 1, which was the shortest, 0→1 (1) and 1→1 (0.72) stood out. Periods 2 and 3 showed identical values–1→0 (1) and 1→1 (0)–and had similar 0→0 (0.57–0.75) and 0→1 values (0.43–0.25).

The number of upsurges per year decreased; the first period had the highest number of upsurges (7), followed by periods 2 (4) and 3 (3).

Table 3. Matrix (one-step transition) and n_2, for CAL upsurges in a 33-year period.

Number	Period	Campeche				Years	Upsurge years	Upsurge overage	Probability n_2			
		Matrix (one-step transition)										
		0→0	0→1	1→0	1→1				0→0	0→1	1→0	1→1
1	1986–1994	0	1	0.28	0.72	9	7	1.29	0.28	0.72	0.2	0.8
2	1995–2006	0.57	0.43	1	0	12	4	3	0.75	0.25	0.57	0.43
3	2007–2018	0.75	0.25	1	0	12	3	4	0.81	0.19	0.75	0.25
						33 (total)	14 (total)	2.3 (mean)				

Table 4. Pearson correlation of the matrix and probability n_2 in Campeche (P > 0.05).

Period	Correlation	P-value
1986–1994	-0.88	0.11
1995–2006	0.67	0.32

The Pearson correlation was different in the two periods. In 1986–1994, it was negative, and in 1995–2006, there was a positive association. However, the differences were not statistically significant (P > 0.05).

Quintana Roo State.—In the 24 years of data (1995–2018), only one year had a CAL upsurge: 2006. Therefore, the probability for 1→0 and 1→1, equal to 1 and n_2 (Table 5), could not be obtained. According to data from the Quintana Roo Plant Health locust control from Yucatan migrated and caused damage to corn and bean crops and cultivated grasslands and caused defoliation in a nature reserve and on urban trees from August–September. In 2006, locust control operations were undertaken in 1,381 ha.

These swarms were able to oviposit in Quintana Roo and complete the second generation. They also returned to Yucatan at the end of 2006 as swarms. In that generation, 173 ha required control operations. In subsequent years, the locust population was present at a low density in the solitarious phase.

Table 5. Matrix (one-step transition) for CAL upsurges over a 24-year period.

Period	Quintana Roo				Years	Upsurge years	Upsurge average
	Matrix (one-step transition)						
	0→0	0→1	1→0	1→1			
1995–2006	0.9	0.1	0	0	12	1	1
2007–2018	1	0	0	0	12	0	0
Total					24	1	1

Classic probabilistic model

Fig. 1 shows a tendency for upsurges to peak every four years (2006, 2010, 2014, and 2018), except for an additional two peaks in 2004 and 2009, from 2010 to 2018, this tendency was clearer than from 2003 to 2009.Therefore and consequently it was assigned values from years 1 to 4, starting with the maximum peak, e.g: year 2006 (year value 1), 2007 (2), 2008 (3), 2009 (4), 2010 (1), 2011 (2) so on (Table 6); from 1952–1955 and 1977–1989, the tendency of the maximum peaks was less clear, and the treated area was larger than in recent years.

This information was used to identify the upsurge years (Table 6). Then, the probability of an upsurge in the past and current periods was determined (Table 7).

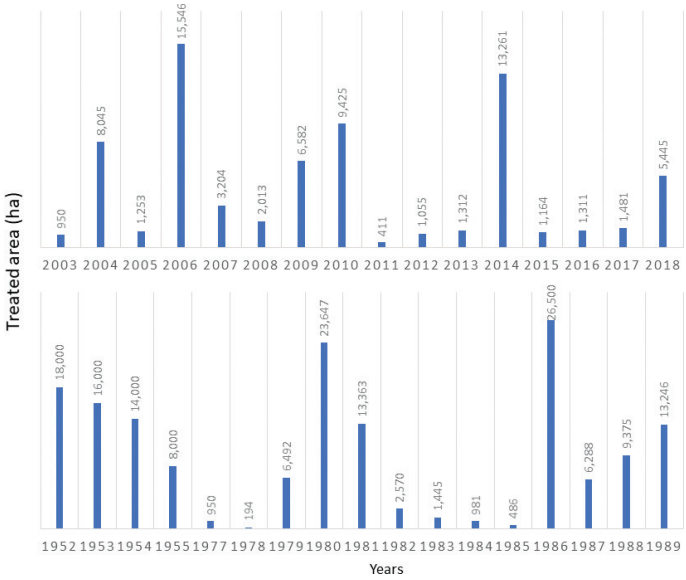

Fig. 1. Treated area in Yucatan State from 2003–2018 (above) and 1952–1955/1977–1989 (below).

For the period 2003–2018 in Yucatan, the highest probability of upsurge was in year 4 (P: 1). There was no probability of an upsurge (P: 0) in year 1 and a minimal probability (P: 0.25) in years 2 and 3. These results were different from the data from the past (1952–1955 and 1977–1988), where years 1 and 2 were similar (P: 0.5) and years 3 and 4 were similar (P: 0.75). For Campeche, the P(1) was 1 in year 1, and years 2–4 had no values. In Quintana Roo, there was only a remote probability of an upsurge, but the probability increased if Yucatan had an upsurge so intense (similar to 2006) that it resulted in locust invasion.

Validation.—According to SENASICA (Table 8), the area treated for locust control was 2,224 ha in 2019 and 3,714 ha in 2020. This information was used to validate the model.

Validation of Markov chain probabilistic model

In Yucatan state starting in 2018, which had a value of 1, the transition 1→0 (one step: 0.66, n_2:0.6) had the highest value. Therefore, the following year, 2019, an upsurge value of 0 would be expected and starting in 2019 as upsurge value 0, the transition (0→0: 0.58, 0.61) was the highest value, so it would also be expected an upsurge value 0 in 2020, both results obtained of the probabilistic model correspond to the results of the low treated area (Table 8). In Campeche in 2018, value = 0, the transition 0→0 (0.75; 0.81) had the highest value, a year with value 0 was expected, but it was not fulfilled, in 2019 according to the treated area the value was 1. The value 1→0 (1, 0.75) was highest for 2020 and

Table 6. Upsurge value for 2003–2018 in the YP and 1952–1955/1977–1989 in Yucatan state.

States	Upsurge value per year															
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Yucatan	0	1	0	1	0	0	1	1	0	0	0	1	0	0	0	1
Campeche	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
Q. Roo	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Yucatan	1952	1953	1954	1955	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988
	1	1	1	1	0	0	1	1	1	0	0	0	0	1	1	1
Year value	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4

it was the upsurge value, through the treated surface (Table 8). In Quintana Roo, despite good results in the probability of 0→0 for 2019 and 2020, no application was made in transition n_2 with an origin of 1 (1→1 or 1→0) because it is an invasion area and not a locust breeding zone.

Validation of Classic probabilistic model

The comparison of the probability of an upsurge P(1), with previous information 2003–2018, with values 2019–2020 indicate that in Yucatan it was according to the model in 2019 and very close to 2020 (P(1)=0.25, value=0), in Campeche and Quintana Roo as predicted.

Table 7. Probability of an upsurge, P(1), from 1952–1955/1977–1988 and 2003–2018.

Year value	1952–1955 and 1977–1988				2003–2018			
	Yucatan				Campeche		Q. Roo	
	Upsurges	P(1)	Upsurges	P(1)	Upsurges	P(1)	Upsurges	P(1)
1	1,0,1,0	0.5	0,0,0,0	0	1,1,1,1	**1**	0,0,0,0	0
2	1,0,0,1	0.5	1,0,0,0	0.25	0,0,0,0	0	0,0,0,0	0
3	1,1,0,1	0.75	0,1,0,0	0.25	0,0,0,0	0	0,0,0,0	0
4	1,1,0,1	0.75	1,1,1,1	**1**	0,0,0,0	0	1,0,0,0	**0.25**

Table 8. Treated area (ha) in the Yucatan Peninsula in 2019 and 2020.

State	Years	
	2019	2020
Yucatan	813	3,420
Campeche	1411	294
Quintana Roo	0	0
Total	2224	3714

Discussion

Markov Chain Model

Yucatan State.—The CAL upsurge from 1948 to 2018 was a sign that the frequency of occurrence had changed, as there were no equal periods. The transition 1→1 had the highest value in the first period (1948–1959); therefore, it was called a "plague period" in which "*favorable breeding conditions are present and control operations fail… two or more regions are affected simultaneously*" (Symmons and Cressman 2001). Upsurge had a higher value in the past than in recent years because there was no organization in charge of a permanent locust control program. Control measures were more recently undertaken over gregarious populations, mainly swarms (Marquez 1963). From 1941–1942, CAL swarms invaded Mexico and Central America,originating from some area of this same region. In 1947, the International Committee for Locust Control (CICLA) was formed; in 1955, the name was changed to OIRSA to include locust surveillance (Marquez 1963, Trujillo 1975). In 1948, the Mexican General Direction of Agricultural Protection was created, an institution that consolidated technical cooperation with CICLA. The first period (1948–1959) in Mexico was characterized by the creation of national and international institutions for locust management (Ortíz and Zuleta 2020).

In the second period (1960–1971), the transition 1→1 decreased, perhaps as an effect of more organized locust control and unfavorable weather conditions, and the number of upsurges per period was reduced from 7 to 5. In period 3, 1972–1983, the transition 0→0 was the highest (0.71). Consequently, because of the fewer number of upsurges (4), the locust was declared to be in its recession period, i.e., a period of several years when the locust population is low (Symmons and Cressman 2001).

In periods 4 (1984–1995) and 5 (1996–2007), the values of the transition matrix were very similar, the transition 0→1 and 1→0 were high, that is the upsurges occurred approximately every 2 years. When starting with a solitarious population, CAL needs three generations to reach the gregarious phase (Barrientos et al. 1992). The CAL presents two generations per year (Astacio 1966, Trujillo 1975); therefore, a third generation is achieved within two years, which concurs with the calculations of Marquez (1963), who indicated that "*the locust does not form suddenly; it takes two or more years to develop*." In periods 4 and 5, transition 1→1 was reduced because locust management was strengthened with the creation of state-run locust control programs. For example, in 1988, the Ministry of Agriculture and Water Resources of Mexico granted greater autonomy to phytosanitary programs in each state, including the locust control program. The Federal Plant Health Law (Ley Federal de Sanidad Vegetal 1994) created plant health committees to develop multiple crop protection programs with government funding. These programs encompassed the locust control campaign. In Yucatan, such a committee was created in 1997 and is currently responsible for the permanent monitoring and control of this pest.

Although Yucatan periods 1 and 5 had similar upsurge years (7), the severity differed. The overall area treated with insecticides in period 1 (1952–1955) was 56,000 ha (Márquez 1963), while it was 26,363 ha in period 5 (2003–2006), which was 47% less than in the first period.

Only 1 out of 5 correlations between the probability n_2 and the matrix was positive and significant. This may be because the outcome of the Markov chain depends on the outcome of previous events, meaning that the next state of the system depends on the present state, and locust outbreaks are erratic events (Symmons 1992). Showler (2002) outlined broad strategic approaches to locust outbreaks based on intervention timing (prevention or reaction) and tactics (survey, control, economic effects, insecure areas, and contingency planning). Management, weather, climate change [World Meteorological Organization-Food and Agriculture Organization (WMO-FAO) 2016], and political decisions differ in different periods, making it difficult to obtain reliable probability.

Markov models have limitations, are problematic with short time intervals, cannot be derived rigorously from deterministic, dynamic models, and rarely provide the range of time for which the model is appropriate (DelSole 2000). However, they permit knowledge of the outbreak population in each period without the necessity of searching for causation through correlative procedures (Kemp 1987). Weather conditions that could have an influence on outbreaks include the amount of rain in the year (Steinbauer 2011), temperature, relative humidity (Al-Ajlan 2007), wind for migration (WMO-FAO 2016), ENSO (Contreras and Galindo 2014), and precipitation in previous years (Skinner and Child 2000, Chiconela et al. 2003).

In some species of Orthoptera, there has been a decrease in outbreaks; for example, outbreaks of rangeland grasshoppers in Wyoming are highly erratic events, with instances of infestations persisting for multiple years being quite low, so there is little basis for prorating the benefits of control beyond the year of treatment (Zimmerman et al. 2004).

Campeche state.—Historically, the Campeche state has been invaded by swarms from Yucatan (Márquez 1963). The entry and migration routes are found in the north, but these swarms can quickly move to the south of the state (Chi 2000). This movement is made by the second generation and at the beginning of the year (Cullen et al. 2017). In the second period (1995–2006), there was a reduction in the number of upsurges per year, probably because the locust campaign of Yucatan Plant Health established control operations in 1997, reducing the possibility of migration to Campeche. Before this period, the largest number of upsurges occurred, with the transition 1→1 indicating several continuous years with an upsurge or a reduced recession period (0→0). In the third period, there was an increase in the recession period 0→0 because the upsurge was dependent on outbreak and migration from Yucatan every 4 years (2006, 2010, and 2014). The probability of upsurge in 2019 was expected because of the migration of swarms from Yucatan in 2018.

With the information obtained on the control and migration of swarms, it was possible to construct the migration route of CAL to Campeche. At the beginning of the year, Campeche is an invasion zone (Fig. 2) for swarms originating from places in Yucatan, where it is difficult to undertake survey and control operations. Therefore, it is important to carry out locust management tasks in Yucatan state with new methods to reduce migration to Campeche. Despite the large number of swarms formed in Yucatan in the upsurge year, the damage was minimal for two reasons: a) in the maximum period of migration that occurs at the end-beginning of the year, there is less cultivated area, as most of the agriculture occurs in June–September when there is a greater amount of precipitation to help rainfed crops, and b) the size of the swarms, according to the classification of Cressman (2001), corresponds to very small swarms (<1 km^2) due to preventive management of first stage nymphs.

Quintana Roo State.—Vegetation is very important for locust development (Sword et al. 2010). Its distribution (Despland et al. 2000), coverage, and status (green or dry) influence locust gregarization (Cisse et al. 2013). Models could be enhanced by using associations between plant communities and insects to predict risk areas (Van Der Werf et al. 2005). The CAL is highly associated with the grass *Panicum maximum* (Poot-Pech et al. 2018); however, in Quintana Roo, the vegetation is 90% tropical forest, 3.3% agriculture, 3.2% grasses, and 3.5% other uses (INEGI 2011). According to survey information from the locust campaign, *P. maximum* is not the dominant grass in Quintana Roo.

Classic probabilistic model

In Yucatan in 1952–1955 and 1977–1988, there were 10 upsurges and 6 recession years, while in 2003–2018, there were 6 upsurges and 10 recession years. This may be because the structure of the locust program was modernized during the latter period, with greater autonomy and economic resources for developing the program and prevention strategies (Rodríguez 2000). The locust program in Mexico receives technical support from OIRSA (Ortíz and Zuleta 2020), and in general, biological and ecological knowledge about locusts has increased, which has helped create better management practices for this pest (Lecoq 2021).

Periods of 4 years (Table 7) were formed, and a linear regression of areas treated for CAL was obtained (Figs 3, 4), finding a positive relationship. Generally, the first year had the least amount of treated area, and the fourth year had the most amount of treated area. In the fourth year, there was a high migration of swarms to Campeche (Fig. 2), so the gregarious area remained at low infestation for three generations. Two years later, the population became high once again (Márquez 1963, Barrientos et al. 1992). However, this pattern occurs when the swarms are controlled

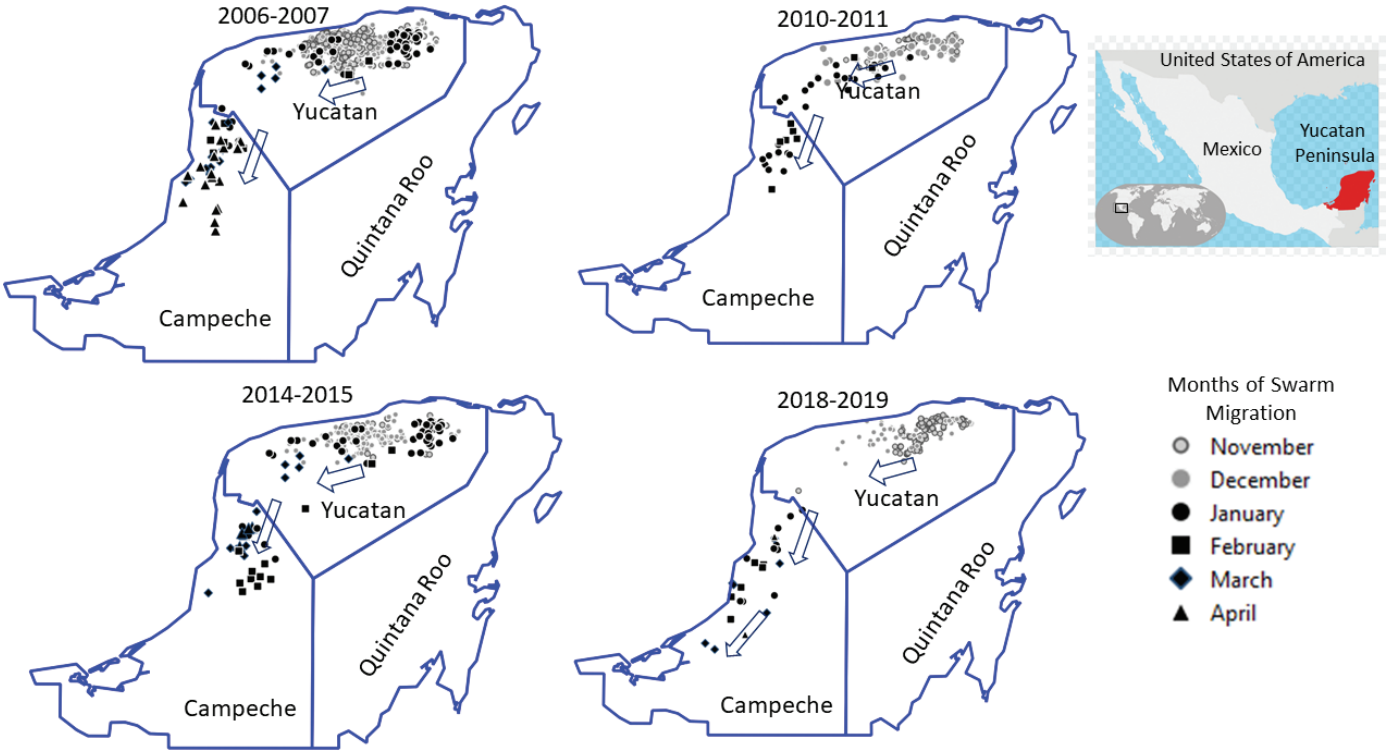

Fig. 2. Swarms detected and treated in the Yucatan Peninsula from November–December (2006, 2010, 2014, and 2018) and January–April (2007, 2011, 2015, and 2019).

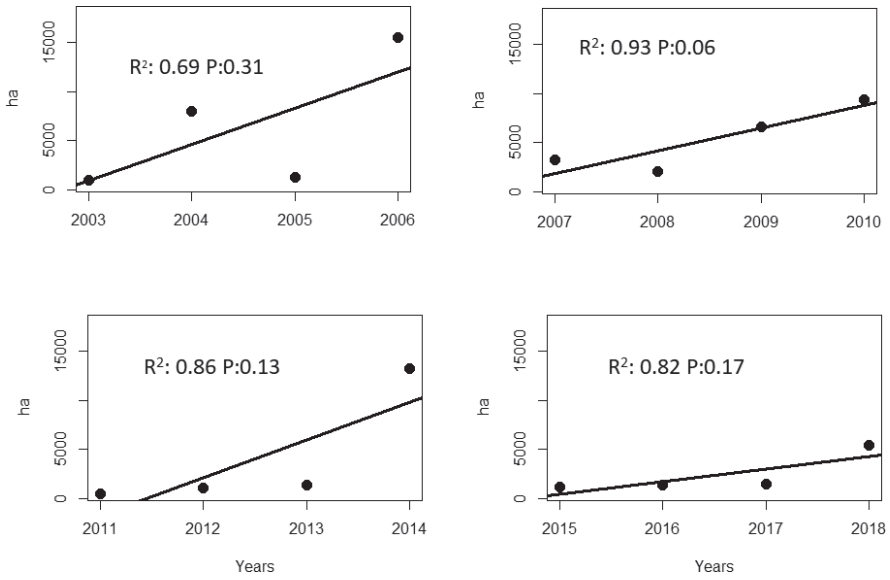

Fig. 3. Linear regression of the treated area in periods of 4 years from 2003–2018 in Yucatán (SENASICA information).

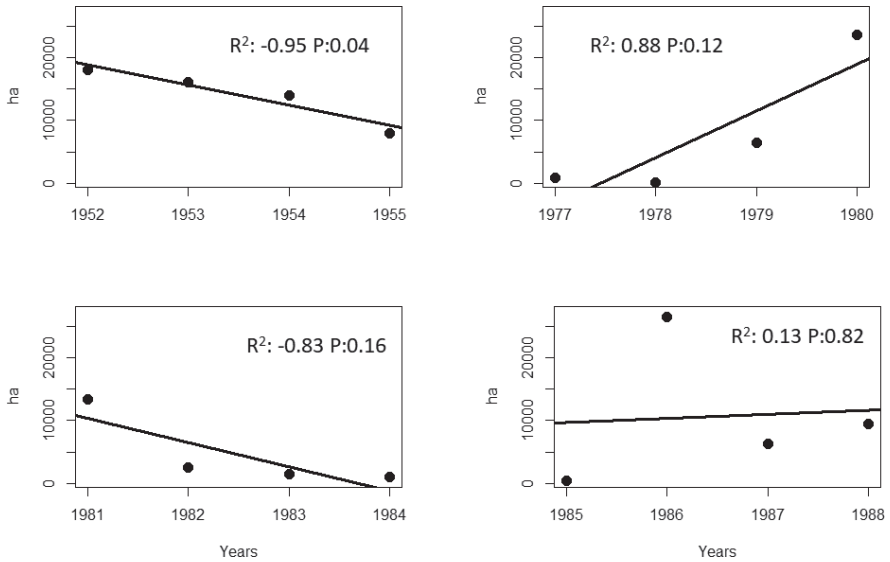

Fig. 4. Linear regression of the treated area in periods of 4 years from 1952–1955 (Marquez 1963) and 1997–1988 (Pereyra 1991) in Yucatán.

along their migration route; if the swarms are not controlled, then year one would have a high infestation when the swarms oviposit and return in the next generation, flying in the opposite direction (Cullen et al. 2017) and repeating the pattern presented in previous periods (1952–1955 and 1977–1988; Tables 6, 7) with at least two years of high infestation. This has been seen recently, such as in the upsurge in 2019 and 2020 of the desert locust in Pakistan (Sultana et al. 2021) and Africa (Peng et al. 2020, Salih et al. 2020).

There were intermediate years–year 2 in 2004 and year 3 in 2009–which were likely the result of suitable weather conditions such as precipitation in the breeding zone. A marked increase in locust numbers on a local scale due to concentration, multiplication, and gregarization can lead to the formation of hopper bands and swarms (Roffey and Popov 1968).

In 1952–1955, there was a "plague" of CAL, requiring 4 years of intense control; gradually, the size of the controlled area was reduced. The opposite situation occurred in 1977–1980 and 2007–2010, with an intermediate rebound in 1979. This situation lasted until 1981, and the treated surface area was reduced until 1984. There was another plague from 1985–1989, with 1986 having the largest controlled area.

From 1952–1955, as shown in Fig. 4, there was a negative regression, indicating a plague of CAL (Symmons and Cressman 2001). Efforts were made to reduce the population each year, but control was aimed at the gregarious phase and mostly swarms (Márquez 1963). International cooperation among countries was still in development (Yam and Zuleta 2020). In 1977–1980, there was a positive regression; an increase in the population occurred in 1979–1980, but in 1981–1984, there was again a negative regression, with a rebound in 1981. Finally, in 1985–1989, the regression value was low because in 1986, there was a higher peak; however, in the period 1986–1987, there was a plague.

In his book, An Account of the Things of Yucatán, written in 1566, Diego de Landa (2003), bishop of Yucatan, wrote about the consequences of locusts and noted the 4-year recession period, saying, "*the locust developed for 5 years, which left no green thing, it was so hungry that people fell dead on the roads* ... with 4 good years after the locust population had improved somewhat."

Table 9. Results of Markov matrix in 2018 compared to results from 2019 and 2020 upsurge values.

State (value year 2018)	Matrix	Values				Upsurge value	
		0→0	0→1	1→0	1→1	2019	2020
Yucatán	One-step transition (2018=1)	**0.58**	0.42	**0.66**	0.34	0	0
	n2	**0.61**	0.39	**0.6**	0.4		
Campeche	One-step transition (2018=0)	**0.75**	0.25	1	0	1	0
	n2	**0.81**	0.19	0.75	0.25		
Q. Roo	One-step transition (2018=0)	**1**	0	0	0	0	0

Table 10. Probability of an upsurge P (1) and results of upsurge values 2019 and 2020 in the YP.

Year value	Years	Yucatan		Campeche		Q. Roo	
		P (1)	Value	P (1)	Value	P (1)	Value
1	2019	0	0	1	1	0	0
2	2020	0.25	0	0	0	0	0
3		0.25		0		0	
4		1		0		0.25	

Infestation of the desert locust, *S. gregaria*, in Africa occurred in four out of five years between 1860 and 1963, and subsequently, in one year out of six (Symmons 1992). In India, from 1863–1962, there were 10 locust infestations, with 5–6 consecutive years of widespread reproduction, swarm production, and damage to crops, followed by 1–8 years of low activity; from 1963 to 2012, there were 18 upsurges (Sharma 2014).

The WMO-FAO (2016) listed the effects of the following factors on the development of a locust outbreak or upsurge: management; the failure to implement a preventive control strategy; inexperienced field survey teams and campaign organizers; insufficient or inappropriate resources; lack of training of the field officers; inaccessibility to important breeding areas; the weather (precipitation, temperature, and wind); and climate change. These factors are likely to have influenced the high occurrence of CAL in the intermediate years (years 2 or 3).

Additionally, limited financial capacity and ongoing armed conflicts could have rendered some of the locust breeding areas inaccessible, and the coronavirus pandemic lockdown has further hampered control efforts (Salih et al. 2020). Political and socio-economic conditions (Meynard et al. 2020) can also greatly influence the likelihood of locust outbreak by affecting management decisions (Gay et al. 2018). In period 2003–2018 there was an important change of government in 2007 in Yucatan State (https://www.yucatan.gob.mx/?p=cronologia) that included a change in locust-control decision-makers. A clear difference between the periods 2003- 2010 and 2011–2018 can be seen: Four upsurges were present in the first period, with only two in the second. In the second period, upsurges only occurred every four years as a result of the preventive actions and the incorporation of new technologies in the locust-control program (Barrientos et al. 2021).

Plagues arise when locusts breed frequently and successfully over a period of one or more years, with repeated and widespread rains in successive, often widely separated, seasonal breeding areas. This allows swarms to form and invade the agricultural zones surrounding the recession area. Pest control, drought, and migration to unsuitable areas can have an effect on ending plagues, although their relative importance is not always clear (Pedgley 1981). The uncertainty of pest outbreaks and associated damage (risk) can be reduced by preventive practices and by the selective use of pesticides based on monitoring and forecasting (Daamen and Rabbinge 1991). It is very important to predict and identify locust recession periods because these populations begin the invasion when ecological conditions become favorable (Lazar et al. 2016), and early prevention strategies have been proven to prevent damage to major agricultural zones in the invasion area (Sharma 2014). However, the response to an alert must be quick; otherwise, swarms will invade several regions, making the forecast pointless (Ceccato 2007).

Curiously, from 2003–2018, the largest treated area against CAL (Fig. 3) coincided with the years in which a soccer championship was held (2006 Germany, 2010 South Africa, 2014 Brazil and 2018 Russia), so in Yucatan these CAL upsurges are known as "soccer world cup locusts".

Both probabilistic models are functional as long as the permanent monitoring system is sustained and allocated resources are maintained or increased. A reduction in budget would risk the development of the pest, as has occurred in the past. The results of the models discussed here provide insights into the probabilities of CAL upsurges in the YP, and this information can be incorporated into ecological models to improve CAL monitoring and aid management decisions.

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Notes on the distribution, ecology, and life history of *Maotoweta virescens* (Orthoptera, Rhaphidophoridae, Macropathinae) and a comparison of two survey methods

James M. H. Tweed[1,2], Michael Wakelin[3], Bruce McKinlay[4], Tara J. Murray[4]

1 Ahikā Consulting, 2 Dowling St, Dunedin 9016, New Zealand.
2 Current address: School of Biological Sciences, Centre for Biodiversity and Conservation Science, The University of Queensland, Brisbane, QLD, Australia.
3 4Sight Consulting, 195 Rattray St, Dunedin 9016, New Zealand.
4 Biodiversity Group, Department of Conservation, Level 1/265 Princes St, Dunedin 9016, New Zealand.

Corresponding author: James M. H. Tweed (jtweed@outlook.com)

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Abstract

When described in 2014, *Maotoweta virescens* was believed to be one of New Zealand's rarest cave wētā (Rhaphidophoridae). Here, we present new information about the distribution, ecology, and life history of the species. *M. virescens* has now been recorded from indigenous forest sites throughout the length of the western South Island, where it can occur in relatively high abundance. *M. virescens* shows a close association with arboreal mosses, particularly *Weymouthia mollis*, roosting within them during the day and feeding on them at night. The wētā has also been documented feeding on lichens and dead insects. The species is hypothesized to have a lifecycle of approximately one year, closely linked to season. Eggs are thought to hatch out relatively quickly after being laid in summer and early autumn, with the species overwintering as nymphs and maturing the following late spring through to early-autumn. Further work is required to fully understand its biology. A comparison was made between active night searching and vegetation beating as two different methods for the detection and monitoring of *M. virescens.* Beating of *W. mollis* and other suitable *M. virescens* microhabitats was found to be significantly more effective than night searching. Our results show that *M. virescens* is widespread and can occur at relatively high densities within South Island temperate forests, with the species' perceived rarity to date largely owing to a lack of survey effort and the past use of ineffective sampling methods.

Keywords

beating, cave wētā, data deficient, New Zealand, moss

Introduction

Orthopteroid insects belonging to the family Rhaphidophoridae are found throughout most temperate regions of the world (Hubbell and Norton 1978). Rhaphidophorids are commonly referred to as cave crickets, camel crickets, or sand-treader crickets in various parts of the world. Many rhaphidophorid species are entirely confined to caves, while many others are typically closely associated with other cavities such as tree hollows, burrows, or leaf litter, sheltering within them during the day and emerging at night to feed (Ingrisch and Rentz 2009).

Within New Zealand (NZ), rhaphidophorids are commonly known as cave wētā. The name 'wētā' is also used for New Zealand Orthoptera in the family Anostostomatidae, including the tree wētā (*Hemideina* spp.) and giant wētā (*Deinacrida* spp.), which are often viewed as national icons. However, the more secretive cave wētā are relatively poorly known. The NZ cave wētā fauna is diverse relative to the country's land area. The 71 known species (Trewick et al. 2016) equate to 8.6% of the world's rhaphidophorid species (Cigliano et al. 2022). Little is known about the ecology and biology of most cave wētā. Previous studies within NZ have focused on cave dwelling species (Richards 1954, 1961, 1962, 1965, 1966, Fea and Holwell 2018), but little has been published on species found in other habitats, such as forests. More recent taxonomic studies have added to our knowledge of the ecology and biology of NZ's cave wētā (e.g., Cook et al. 2010, Johns and Cook 2014, Fitness et al. 2018, Hegg et al. 2019, 2022), but further studies are needed.

None of NZ's cave wētā have yet been assessed for the IUCN Red List (IUCN 2022). However, Trewick et al. (2016) evaluated all 71 species of cave wētā known at that time (56 described, 15 tag named taxa) using the New Zealand Threat Classification System (NZTCS), which assesses a species' risk of extinction based on estimates of population size and trend criteria (Townsend et al. 2008). At that time, 25 cave wētā species were classified as At Risk or Data Deficient (Trewick et al. 2016). The category Data Deficient indicates that there is insufficient information on the population size and trend of a species to properly assess its threat status. Improving our understanding of the distribution, ecology, and biology of Data Deficient species is essential to assigning an appropriate threat classification and enabling effective conservation measures where they are needed.

Despite its distinctive green coloration, which distinguishes it from almost all other NZ cave wētā species, *Maotoweta virescens* Johns & Cook, 2014 escaped scientific attention until recently. At

the time of its description, *M. virescens*, commonly called the moss wētā, was believed to be one of the rarest species of cave wētā in New Zealand as it was known from only seven specimens (Johns and Cook 2014). Five of the seven were collected from the Takitimu Mountains in Southland in 2006, while the remaining two were collected more than 60 years earlier, one each from Fiordland and northwest Nelson (Johns and Cook 2014). The apparent rarity of the species meant that little information existed in relation to its distribution, ecology, and biology and therefore its population size or trend (Johns and Cook 2014), and consequently, the species was classified as Data Deficient within the NZTCS when first assessed (Trewick et al. 2016).

To improve our knowledge of rare or poorly known species such as *M. virescens*, survey methods must be designed to maximize the chance of reliably detecting the target species when it is present (Schori et al. 2020). The use of ineffective survey methods can negatively influence our understanding of species ecology and rarity (Gaston 1994). Forest-dwelling cave wētā are nocturnal (Richards 1961) and so spotlighting desirable habitat at night has traditionally been the main method of detection (e.g., Johns and Cook 2014, Hegg et al. 2019). However, spotlighting has been found to be relatively unsuccessful for the cryptic *M. virescens*, with Johns and Cook (2014) reporting a detection rate of one wētā for every 192 minutes of searching. To resolve this, we attempted to identify the most suitable method for detecting and possibly monitoring *M. virescens* during targeted surveys for Data Deficient invertebrates in the Southland Region.

The objectives of the current study were as follows: (1) to improve our understanding of the distribution, ecology, and life history of *M. virescens*, (2) to identify the most suitable means of detecting and/or monitoring *M. virescens*, and (3) to recommend an appropriate threat classification for *M. virescens* to help determine whether conservation action is required for its protection. The findings from this study may have relevance to other species of forest-dwelling Rhaphidophoridae.

Methods

Field surveys.—Surveys for *M. virescens* were undertaken at 20 South Island sites from January–June 2021 (Table 1). These surveys were part of a wider Department of Conservation (DOC)-led project (Tweed and Wakelin 2021) intended to increase knowledge on the distribution and relative abundance of invertebrate species from the Southland region of NZ classified as Data Deficient within the NZTCS.

Surveys were conducted in a range of habitat types, including mixed temperate forest, open beech forest, subalpine shrubland, and alpine grasslands. However, most surveys were conducted within mixed temperate forest or open beech forest. These are the characteristic forest types of the higher and lower rainfall regions of NZ's South Island, respectively (Table 1).

In the South Island, the canopy of mixed temperate forest comprises a variable mixture of tree species including several species of Podocarpaceae (podocarps), Nothofagaceae (southern beeches), and Myrtaceae (myrtles), among others (Wardle 1991).

Table 1. Locations surveyed for *Maotoweta virescens* during this study arranged from north to south. Site name area codes follow Crosby et al. (1998): FD = Fiordland, OL = Otago Lakes, SI = Stewart Island, SL = Southland, WD = Westland. Annual average rainfall values were extracted from Ministry for the Environment (2015). Survey type codes are as follows: P/A = presence/absence survey (i.e., beating and/or night searches used for detection but not timed); TNS = timed night search; TBS = timed beating survey (during daylight). The total duration for each timed survey is given in minutes.

Site name	Habitat	Average annual rainfall (mm)	Survey date(s)	Latitude (°), Longitude (°)	Survey type	*M. virescens* detected	No. specimens collected
Karangarua Valley, WD	Mixed temperate forest	8,374	08/06/21	-43.707, 169.867	P/A (day + night)	Yes	2
Martins Bay, WD	Mixed temperate forest	5,644	22–23/02/21, 26/02/21	-44.342, 168.009	P/A (day + night)	Yes	6
Hokuri Creek, WD	Mixed temperate forest	6,211	25/02/21	-44.409, 168.059	TBS (225 mins)	Yes	17
George Sound, FD	Mixed temperate forest	7,884	03/02/21	-44.985, 167.438	P/A (day + night)	Yes	2
Henry Pass, FD	Alpine grassland, subalpine shrubland	8,383	01–02/02/21	-45.010, 167.498	P/A (day only)	No	NA
Caswell Sound, FD	Mixed temperate forest	7,386	04/02/21	-45.047, 167.307	TNS (100 mins), TBS (70 mins)	Yes	8
Lake Marchant, FD	Mixed temperate forest	7,386	05/02/21	-45.056, 167.317	P/A (day only)	Yes	1
Junction Burn, FD	Mixed temperate forest	6,306	09–11/02/21	-45.151, 167.498	P/A (day + night)	Yes	4
Lake Mistletoe, OL	Open beech forest	1,311	12/02/21	-45.201, 167.824		No	NA
Hanging Valley Track, Doubtful Sound, FD	Mixed temperate forest	7,782	01/03/21	-45.471, 167.143	TNS (180 mins), TBS (95 mins)	Yes	12
Old Doubtful Track, Doubtful Sound, FD	Mixed temperate forest	7,782	02/03/21	-45.479, 167.171	TBS (90 mins)	Yes	4
Wilmot Pass, FD	Mixed temperate forest and subalpine scrub	7,009	04/03/21	-45.512, 167.197	P/A (day only)	Yes	1
Princhester Creek, Takitimu Mountains, SL	Open beech forest	1,096	06/03/21	-45.600, 167.956	TBS (105 mins)	Yes	1
Grebe Valley, FD	Mixed temperate forest	4,019	26/01/21	-45.659, 167.344	P/A (day only)	Yes	1
Borland Saddle, FD	Open beech forest, alpine grassland	2,278	25/01/21	-45.747, 167.382	P/A (day only)	No	NA
Borland Lodge, FD	Open beech forest	1,318	24/01/21	-45.780, 167.533	P/A (day + night)	No	NA
Rakeahua Valley, SI	Mixed temperate forest	1,751	08–11/03/21	-46.982, 167.881	P/A (day + night)	No	NA
Ulva Island, SI	Mixed temperate forest	1,401	13/03/21	-46.892, 168.099	P/A (day only)	No	NA
Fern Gully, SI	Mixed temperate forest	1,387	14/03/21	-46.929, 168.130	P/A (day only)	No	NA
McLean Falls, SL	Mixed temperate forest	1,339	15/03/21	-46.572, 169.347	TBS (20 mins)	Yes	2

The presence and/or dominance of each of the canopy species varies geographically. The understories are typically dominated by broadleaved trees, shrubs, and ferns. Most surfaces are covered in thick layers of mosses, such as *Weymouthia mollis* (Hedw.) Broth., as well as numerous liverworts and lichens (Fig. 1A). These forests are typical of much of the western South Island, Fiordland, and the Catlins regions (Wardle 1991). The forests of Stewart Island are similar in composition but lack the southern beeches (Wardle 1991). Although numerous subclassifications can be designated (e.g., podocarp-broadleaf, beech-podocarp, etc.), here mixed temperate forest is used to refer collectively to the relatively high-rainfall forests, which are typically more diverse than the open beech forests described below.

The beech forest habitats surveyed were dominated by one or more of the southern beeches, with a relatively open, low-diversity understory comprised largely of divaricating shrubs (Fig. 1B). These open beech forests are typical of the forests of drier areas to the east of the Southern Alps (Wardle 1991). Mosses, lichens, and liverworts were also abundant, though the species composition differed, with a general lack of the hanging mosses seen in the mixed temperate forests.

Surveys for *M. virescens* were undertaken by a) visual spotlight searches at night, and b) beating of understory vegetation during the day. Notes were recorded on the habitat in which each wētā was detected, including the plants on which they were observed, the structure of the surrounding forest, and the elevation. Some wētā encountered at each location were collected for morphological analysis.

Comparison of survey methodologies.—In addition to the presence-absence surveys noted above, timed night searches and daytime beating searches were undertaken at a subset of sites.

Night searches. Timed night searches were undertaken at two Fiordland locations: Caswell Sound and Doubtful Sound (Table 1). Searches involved walking through suitable *M. virescens* habitat and scanning vegetation, particularly hanging and climbing mosses, with head torches. Any *M. virescens* encountered were recorded along with details of location, time of detection, the vegetation they were detected on, weather conditions, and any other notable information. The number of surveyors and the survey duration were recorded to calculate search effort. Night searches occurred between 21:00–23:00 NZDT hours, though the total length of the survey varied. Data on night searches from the type locality of Princhester Creek in the Takitimu Mountains were also extracted from Johns and Cook (2014).

Beating. We used daytime beating as the primary method for detecting *M. virescens* during this study. Preliminary work indicated that *M. virescens* might roost within mosses during the day, so we opted to use daytime beating as a sampling method. The traditional beating method for sampling invertebrates from vegetation uses a flat or slightly convex beating tray. Here, beating for *M. virescens* was undertaken using an entomological net (38 cm diameter) instead of a flat tray, as it ensured wētā were not able to jump away before being recorded. Wētā specimens were primarily collected off the inside walls of the net, although some smaller nymphs were found among the debris in the bottom of the net.

Timed beating surveys were conducted following the same methodology as timed night surveys and were conducted at six locations (Table 1). Hanging and climbing mosses were targeted for beating over an entomological net, as described above. The start and end times of the survey were recorded to allow for calculation of the search effort. Beating surveys typically occurred between 10:00 and 19:00 NZDT hours, although the length of the survey varied between sites.

Fig. 1. Forest types surveyed during this study. **A.** Typical mixed temperate forest photographed at Hokuri Creek in the lower Hollyford Valley, FD (note the extensive covering of *Weymouthia mollis* and other mosses and liverworts on the trunks and limbs of most trees and shrubs, as well as the forest floor); **B.** Typical open beech forest photographed within the Takitimu Mountains, SL (photo credit: N Harbison-Price).

Statistical analysis.—A Wilcoxon rank-sum test was used to compare the mean search time per *M. virescens* specimen observed for night searching and beating surveys. Analysis was conducted using the wilcox.test() function of the statistical software R (R Core Team 2021). Data were plotted using the package ggplot2 (Wickham 2016).

Additional distribution records.—The curators of NZ's largest entomological collections were contacted to determine whether they held any *M. virescens* specimens: New Zealand Arthropod Collection (NZAC), Auckland Museum (AMNZ), Te Papa (MONZ), Canterbury Museum (CMNZ), Lincoln University Entomology Research Collection (LUNZ), and Otago Museum (OMNZ). A single specimen was reported from the AMNZ collection, while CMNZ holds those specimens reported by Johns and Cook (2014). No other specimens were reported. Records were also obtained from T Jewell (pers. comm.), R Morris (pers. comm.), and from iNaturalist (2022).

Distribution and environmental variables.—All available records of *M. virescens* were plotted using QGIS v3.16.0. The elevation of each record was assessed using NZTopo50 map series. Values for the annual rainfall at the detection locations were extracted from the Ministry for the Environment's annual average rainfall data (Ministry for the Environment 2015). Area codes used throughout represent biogeographical regions identified by Crosby et al. (1998) and are routinely used when documenting the collection localities of invertebrate specimens within NZ.

Observations of captive wētā.—A total of five adult female and two adult male *M. virescens* were kept in captivity for up to one month to observe their behavior and diet. The incidentally biased sex ratio among captive specimens available meant that up to four females were kept together with a single male. Wētā were kept in a plastic tank lined with paper towel. The tank was misted once per day to keep it moist. Twigs were placed in the tank for the wētā to climb and roost on. Mosses (*Weymouthia mollis (Hedw.) Broth.*, Orthotrichaceae sp.), lichens (*Cladonia ?confusa* R. Sant., *?Lobaria* sp., *Pseudocyphellaria* sp., *Yarrumia ?colensoi* (C.Bab.) D.J.Galloway), liverworts (*?Trichocolea ?mollissima* (Hook.f. & Taylor) Gottsche, *?Lepicolea* sp.), and the leaves of some vascular plants (*Coprosma rhamnoides* A.Cunn., *Metrosideros* sp., *Fuscospora* sp., *Weinmannia racemosa* (L.f.) Pillon et H.C.Hopkins) were collected from the same localities as the wētā and placed within the tank. Insect carcasses were also offered to the captive wētā in the form of freshly caught hoverflies (*Melanostoma fasciatum* (Macquart, 1850) (Diptera, Syrphidae), which were swept from rank grass and killed by freezing.

Behavioral observations were made opportunistically both during the day and at night, and given the small sample size available, no attempt was made to undertake a full quantitative analysis of behavior. At night, the wētā were observed using a red light, as this is known to cause less disturbance to cave wētā than white or yellow lights (Butts 1983). The relative locations of individuals within the tank, whether they were feeding, and interactions between individuals were all recorded. Lichens and hoverfly bodies were inspected for signs of feeding each morning.

Morphological characteristics.—Both nymphs and adults were collected to study the growth and development of *M. virescens*. A total of 10 adults and 51 nymphs were collected from various locations during the surveys. Specimens were preserved in 70% ethanol and have been deposited within the Phoenix Collection housed at Massey University, Palmerston North, NZ. The length of the hind femur, hind tibiae, body, and ovipositor for the females were measured using digital calipers for all collected specimens. The presence or absence of an ovipositor was used to sex the specimens, which may have led to some early instar female nymphs being falsely identified as males. Due to this uncertainty, no statistical comparison was made between the body sizes of male and female nymphs. Morphology measurements were plotted using the package ggplot2 (Wickham 2016) within the statistical software R (R Core Team 2021).

Results

Distribution.—*Maotoweta virescens* has now been recorded throughout the length of the South Island of NZ, with most records occurring to the west of the Southern Alps (Fig. 2). Records extend from the Cobb Valley (NN) in the northwest to McLean Falls in the Catlins Conservation Park (SL) in the southeast (Fig. 2). The latter record (-46.57157, 169.34744) marks the only confirmation of the species from the east coast of the South Island, extending the known range by 150 km eastwards, with the nearest known population occurring in the Takitimu Mountains, SL. *Maotoweta virescens* was detected at most sites surveyed during this study, with the exceptions of Borland Lodge (FD), Borland Saddle (FD), Henry Pass (FD), and Lake Mistletoe (OL) (Fig. 2, Table 1). *Maotoweta virescens* was also not detected on Stewart Island despite extensive searching (Fig. 2, Table 1).

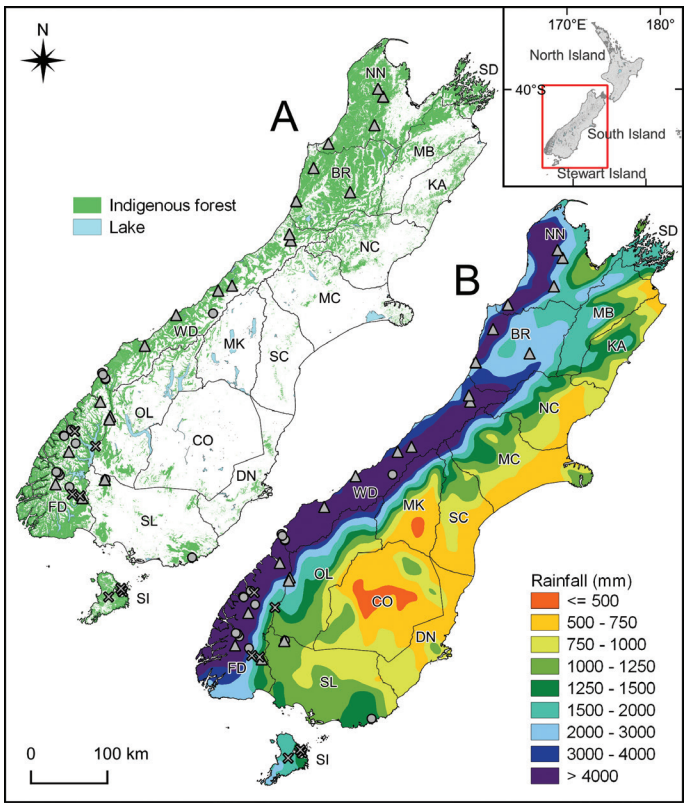

Fig. 2. Known records of *Maotoweta virescens*. **A.** Records overlain on the current extent of indigenous forest; **B.** Records overlain on mean annual rainfall. Circles represent records of *M. virescens* obtained during this study, triangles are confirmed records from other sources, and crosses indicate sites surveyed during this survey at which *M. virescens* was not detected. The extent of indigenous forest was taken from Landcare Research (2020). Rainfall data is from the Ministry for the Environment (2015) Area codes and boundaries follow Crosby et al. (1998).

Habitat.—*Maotoweta virescens* is now known from a wide elevation range, having been recorded from sea level at Caswell Sound, FD during this study, and at altitudes of up to 1,200 masl at Mt Arthur, NN, in the earlier study of Johns and Cook (2014). The species appears to be strongly associated with moderate-to-high rainfall forests with all records occurring in areas averaging 1,096–8,374 mm per year (Fig. 2). Princhester Creek in the Takitimu Mountains, SL, and the Karangarua Valley, WD, represent the lowest and highest precipitation sites, respectively.

Records from this study and additional distribution records indicate that *M. virescens* is primarily associated with mature forest habitats (Fig. 2). It has been recorded from both mixed temperate forests and beech forests but was not detected in the subalpine shrublands or grasslands surveyed during this study.

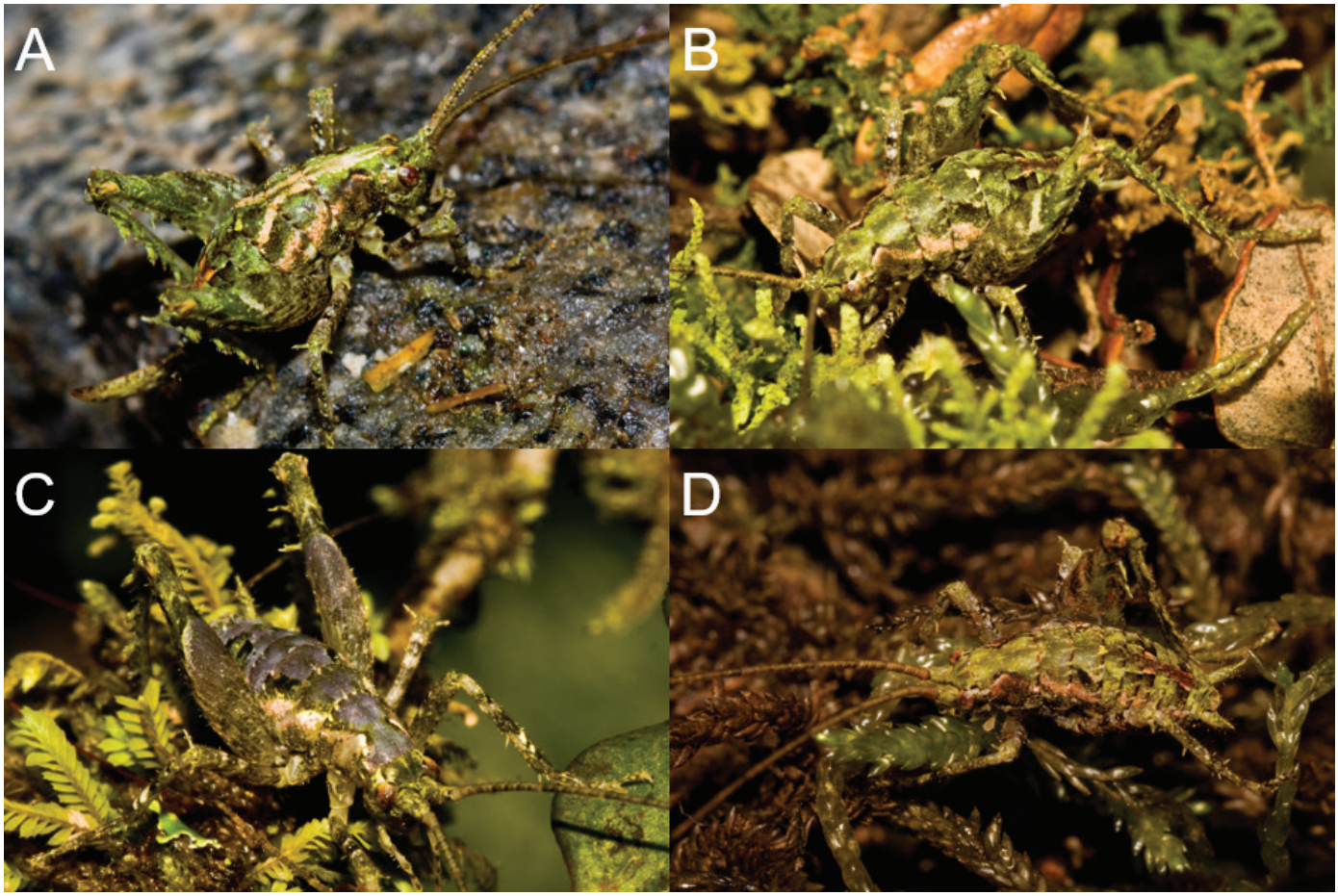

Fig. 3. Photos of adult *Maotoweta virescens*. **A.** Female from Milford Sound, FD; **B.** Female from Lake Gunn, FD; **C.** Male from Milford Sound, FD; **D.** Male from Runanga, WD. Photo credit: Tony Jewell (www.flickr.com/photos/rocknvole/).

Maotoweta virescens was detected in all mixed temperate forest locations surveyed in Fiordland, Westland, and the Catlins (Table 1, Fig. 2). Within this forest type, both nymphs and adults were found to be particularly closely associated with arboreal moss and liverwort communities. Most *M. virescens* detected within this forest type were beaten from the hanging moss *Weymouthia mollis*, though some were also beaten from other moss species, lichens, and leafy liverworts (Marchantiophyta: Jungermanniopsida), while others detected at night were found climbing tree trunks. The plant species on which the moss was growing seemed to have little influence on the presence or absence of *M. virescens,* as individuals were beaten from moss growing on a diverse range of trees, shrubs, and tree ferns, as well as dead trees and limbs. Fig. 3 shows photos of live specimens in their natural habitat.

Maotoweta virescens was also found within open beech forest. A single nymph was beaten from a *Coprosma rhamnoides* shrub in the forest understory at Princhester Creek in the Takitimu Mountains, SL, the type locality for *M. virescens*. This shrub had a thin layer of an unidentified species of moss on its stem. Although mats of mosses and liverworts were present on most tree trunks, the open beech forest habitat lacked the curtains of hanging moss characteristic of *M. virescens* habitat within the mixed temperate forests surveyed. *Maotoweta virescens* was not detected from open beech forest searched near Borland Lodge, FD, or at Lake Mistletoe, OL; however, the species has been confirmed from Monowai Flats, ~1 km from the site surveyed at Borland Lodge (T Jewell pers. comm.; Fig. 2).

Although no adults of any other cave wētā species were encountered while beating moss for *M. virescens*, the nymphs of other species were occasionally caught, including *Talitropsis chopardi* (Karny, 1937) and *Notoplectron brewsterense* (Richards, 1972). Several species of cave wētā were also routinely encountered on the trunks of trees or on the forest floor during night searches for *M virescens*, including *Miotopus richardsae* Fitness, Morgan-Richards, Hegg & Trewick, 2018, *Talitropsis sedilloti* Bolívar, 1882, and several *Isoplectron* species.

Morphological characteristics.—Based on body measurements and the development of female genitalia, all specimens collected appeared to be either adults or early-instar nymphs (Fig. 4). Only a single late-instar nymph was observed during the surveys: a penultimate instar female that underwent its final molt in captivity less than 24 hours after capture and was therefore measured as an adult (depicted in Fig. 5H).

The lengths of the bodies (2.3–5.1 mm), hind femur (2.1–4.2 mm), and hind tibia (2.2–4.5 mm) of the nymphs measured (n = 51) displayed an almost continuous range, with no discrete size classes evident that could be used to separate instars. The two nymphs collected from the Karangarua Valley, WD, had marginally longer hind tibiae and femora than any other early-instar nymphs measured, but their body sizes overlapped with those of the other collected nymphs (Fig. 4A, B). The female nymph from Karangarua also had a longer ovipositor than all other female nymphs (Fig. 4C).

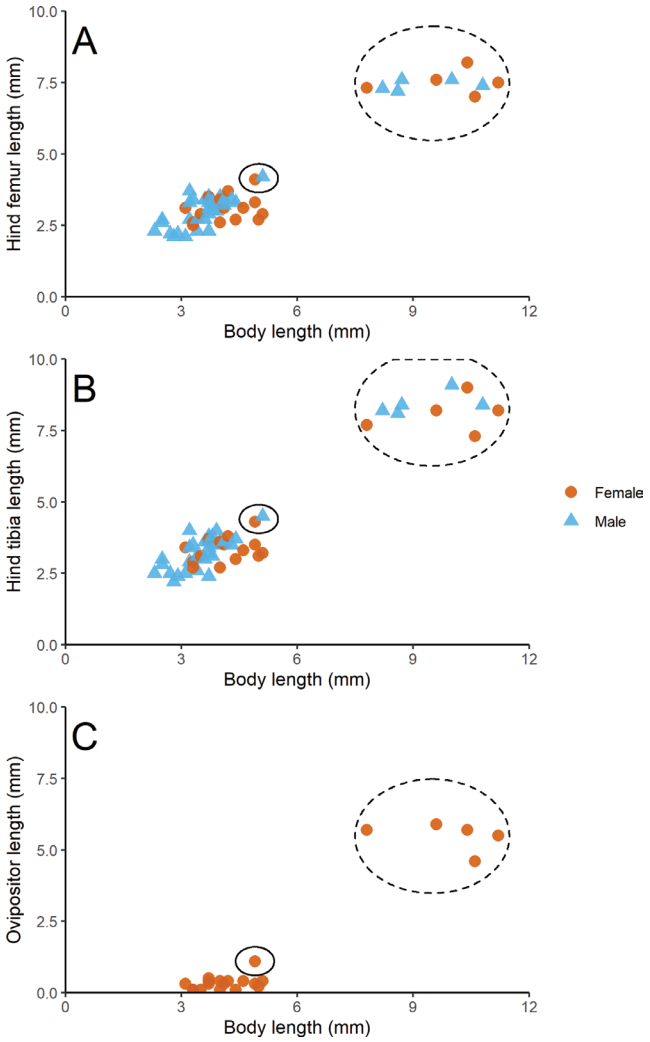

Fig. 4. Hind femur (**A**), hind tibia (**B**), and ovipositor (**C**) length of *Maotoweta virescens* specimens collected in this study plotted against body length. The observations within the solid oval on each plot are the specimens collected from the Karangarua Valley, WD, in June; those within the dashed oval are adult specimens collected during this study; those not circled are other early-instar nymphs collected during this study.

Among adult specimens (n = 10), the length of the body, hind femur, and hind tibia ranged from 8.6–10.8 mm, 7.2–7.6 mm, and 8.1–9.1 mm, respectively, for males (n = 5), and from 7.8–11.2 mm, 7.0–8.2 mm, and 7.3–9.0 mm for females (n = 5). Ovipositor length ranged from 4.6–5.9 mm.

Color variation.—The coloration of *M. virescens* was found to be highly variable (Fig. 5), but all color morphs observed to date resemble the habitat of mosses, liverworts, and lichens in which the wētā lives (Figs 3, 5, 6). Individuals of distinctly different color morphs were found cohabiting on several occasions. Fig. 5 shows some of the color and pattern variations noted during this study, although the full range of variation is even greater than depicted here (also see Fig. 3 for further examples).

Behavioral observations.—In captivity, *M. virescens* was found to be entirely nocturnal. During the day, the wētā would roost on twigs, camouflaged among clumps of moss. When at rest, the wētā would fold their antenna beneath their body and then fold them again, approximately beneath the mesocoxa, so that the tips lay flat along the branch on which they were sitting (i.e., they were folded into a collapsed S-shape when viewed laterally; Fig. 6). Individuals were observed roosting both separately and near one or more other wētā. In the field, the number of wētā beaten from a clump of moss containing *M. virescens* varied between one and five individuals. Nymphs and adults were usually detected separately, although on some occasions, nymphs were beaten from the same clumps of moss as adults.

At night, captive *M. virescens* would roam around their enclosure, climbing along twigs as well as the walls and ceiling of the enclosure. Roaming wētā waved their antenna in front of them constantly, stopping only when they encountered a food source. When two individuals met each other, they would pause briefly, inspect each other with their antenna, and then move around one another, continuing their path. No aggression was observed between individuals. Captive individuals that died overnight were removed the following day. No feeding damage was observed on any of the dead wētā.

No mating was observed in captivity or in the field, but males and females were found in proximity on numerous occasions. Oviposition was also not observed but the swollen abdomen of a female caught at Martins Bay (26 February 2021) suggested at least some females were gravid at the time the surveys were conducted.

A single captive wētā was observed undergoing ecdysis from the penultimate instar to an adult on the 04 March 2021. The female (depicted pre-molt in Fig. 5H) clung to the underside of a twig with its head facing downwards. At the final stages of the process, the wētā hung from its exuviae, attached only by the tips of its antenna and ovipositor, which were the last structures to be withdrawn. The entire process took approximately 30 minutes. The pigmentation of the female was somewhat paler following ecdysis, although this darkened quickly. The patterning of the individual remained unchanged.

Diet.—*Maotoweta virescens* were observed feeding on the moss *Weymouthia mollis* in the field during night searches. *Weymouthia mollis* was actively fed on by captive *M. virescens* but appeared to lose its palatability as it dried out. Some feeding was observed on a second moss offered (Orthotrichaceae sp.) but not for sustained periods, suggesting it was not a preferred food source.

Captive wētā actively fed on the foliose lichens offered, particularly *Yarrumia ?colensoi*. The wētā ate holes through the middle of the sheets of *Yarrumia* but were only observed feeding on the edges of *Pseudocyphellaria* and *?Lobaria* lichens. No feeding was observed on the fruticose *Cladonia confusa*. No browsing was observed on either the liverworts or the leaves of any of the vascular plants offered.

All insect carcasses offered to the captive wētā were consumed on the first night; however, several captive wētā that died overnight were not fed on by others prior to their removal the following day.

Comparison of survey methodologies.—The mean search time per *M. virescens* specimen detected using beating was significantly lower than that of active night searching (Wilcoxon's test, $p = 0.038$, Fig. 7). When searching suitable habitat, *M. virescens* was encountered on average once every 28.1 minutes while beating and every 157.3 minutes while active night searching (Fig. 7). For beating, this dropped to one individual every 12.7 minutes if the Princhester Creek survey (105 minutes per individual) was excluded. Princhester Creek was the only open beech forest habitat included in the timed surveys, with the remaining sites all being mixed temperate forest (see Table 1).

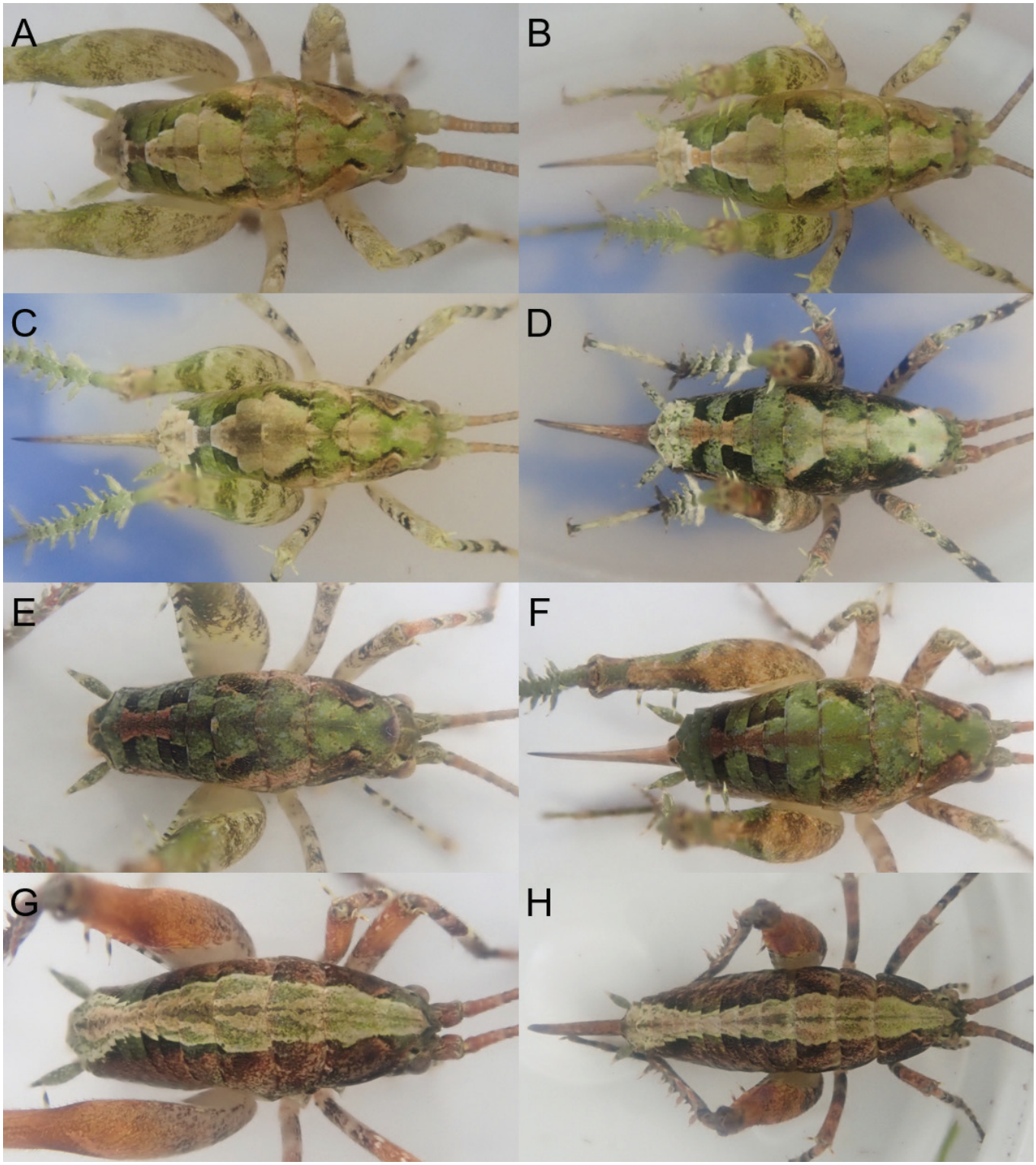

Fig. 5. Examples of phenotypic variation displayed by *Maotoweta virescens*. **A.** Male, Martins Bay, WD; **B.** Female, Martins Bay, WD; **C.** Female, Doubtful Sound, FD; **D.** Female, Wilmot Pass, FD; **E.** Male, Martins Bay, WD; **F.** Female, Martins Bay, WD; **G.** Male, Hokuri Creek, WD; **H.** Penultimate instar female, Doubtful Sound, FD. Note: images are not to scale. Body length of all individuals was between 7.8 mm (**H**) and 11.2 mm (**B**).

Discussion

This study has shown that *M. virescens* is widely distributed throughout NZ's South Island. Current records suggest that the species is most closely associated with the mixed temperate forests of the western and southern South Island. However, records from the Takitimu Mountains, SL (Johns and Cook 2014, R Morris pers. comm., and this study) and Monowai Flats, SL (T Jewell pers. comm.) indicate that the species is also capable of inhabiting open beech forest habitat. Given that the species has been detected

across a wide latitudinal and elevational range, as well as from several different forest ecotypes, it seems likely that the species will be recorded from other locations in the South Island following further survey efforts. We did not detect *M. virescens* on Stewart Island during this study, but given that ours was the first targeted search effort for the species on the island, more survey effort is needed. Several other cave wētā species are known from both the South Island and Stewart Island, including *Talitropsis sedilloti* (Michel et al. 2008) and *Miotopus richardsae* (Fitness et al. 2018). Similarly, although the species has not yet been recorded from the North Island, its presence cannot be discounted, as several of NZ's cave wētā species are known to inhabit both the North and South Islands, including both species mentioned above. Suitable forest habitat for *M. virescens*, including *Weymouthia mollis*, is present on both Stewart Island and the North Island.

Although some species of Rhaphidophoridae are known to be closely associated with plants (e.g., the North American species *Gammarotettix bilobatus* (Thomas, 1872) (Stidham 2005)), most described NZ cave wētā species are typically cavity dwellers, sheltering in locations such as caves, tree cavities, or under and among debris on the ground, coming out only at night to feed (Richards 1962, 1966, Hegg et al. 2019). *Maotoweta virescens* may be unique among the described cave wētā species within NZ and globally, as it appears to spend most, if not all, of its life cycle within arboreal mosses and lichens. The cryptic coloration of the species further supports its moss-dwelling specialization, as it undoubtedly functions to conceal *M. virescens* from native visual predators, such as insectivorous birds, while roosting within mosses during the day and possibly from nocturnal predators such as ruru/morepork (*Ninox novaeseelandiae*) while foraging at night. The diversity of color morphs (see Fig. 5) suggests that *M. virescens* may have evolved to inhabit and blend into a wide range of different micro-habitats. The cavity-dwelling behavior of most other NZ cave wētā during the day means they have less need for such cryptic coloration and may explain why most are various shades of brown and gray. The antennal folding behavior and exaggerated hind tibia spines of *M. virescens* (see Johns and Cook (2014) for morphological description) further enhance the camouflage of this species. The double-folding of the antenna possibly serves to conceal the species further among its moss habitat by reducing its overall length while also protecting them from damage. Tettigonids that rely on plant mimicry are also known to conceal their antennae, with different species displaying methods such as folding them along their bodies, laying them flat along twigs, or in some cases deliberately extending them in unusual positions to mimic the plants among which they are hidden (Nickle and Castner 1995). The spines on the hind tibia of *M. virescens* (clearly visible in Fig. 5C, D) closely resemble the phyllids of the mosses within which they live. Evidence for the effectiveness of this adaptation is shown by the presence of similar spines in the unrelated *Pleioplectron crystallae* Hegg, Morgan-Richards & Trewick, 2019, a ground dwelling species of NZ cave wētā known to be a moss specialist (Hegg et al. 2019). Many tettigoniids also possess anatomical and coloration adaptations to mimic mosses and lichens (e.g., *Lichenodraculus matti* Braun, 2011), traits that Nickle and Castner (1995) suggest evolved primarily to protect the insects against diurnal predators. Further use of beating as a method to search for cave wētā may reveal currently unknown species with similar habits, both within NZ and in other parts of the world.

Mosses, lichens, and dead insects were documented here as part of the diet of *M. virescens* and are also known to be consumed by other cave wētā species (Richards 1962, Butts 1983). However,

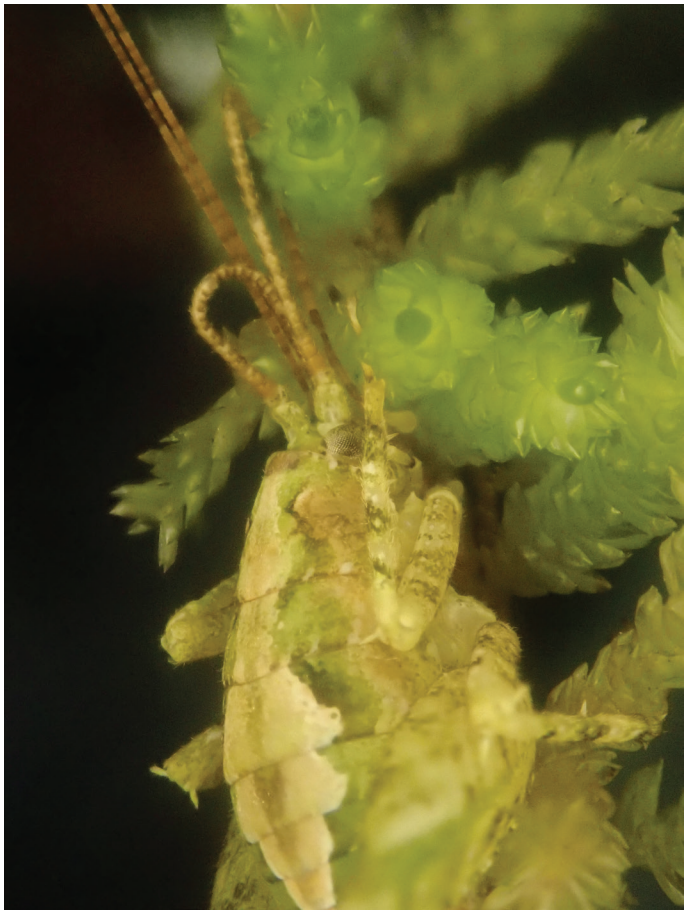

Fig. 6. Adult female *Maotoweta virescens* exhibiting the double antennal folding behavior displayed by this species when roosting within their moss habitat during the day.

M. virescens was not observed feeding on vascular plants, even though ferns and angiosperms are a major dietary component of other cave wētā such as *Insulanoplectron spinosum* Richards, 1970b (Butts 1983), *Macropathus filifer* Walker, 1869 (Richards 1954), *Pachyrhamma waitomoensis* Richards, 1958 (Richards 1962), and *Pallidoplectron turneri* Richards, 1958 (Richards 1962). Apparent diet specialization on moss and lichen has been documented for the ground-dwelling *P. crystallae* (Hegg et al. 2019), and the same may be the case for *M. virescens*, although they are likely to be opportunistic feeders given that they were documented feeding on dead insects in this study. Further work is required to understand the full dietary range of *M. virescens*.

Rearing NZ cave wētā species from eggs and/or nymphs to adulthood has been found to be challenging (Richards 1961, Butts 1983) and was not attempted here. However, based on our observations and knowledge of other cave wētā species, we suggest several hypotheses about the life history of the species for future testing. Adult *M. virescens* have been recorded from December to March, and as one of the females collected here was observed undergoing its final molt in early March, it appears maturation can occur from at least early summer to early autumn. Although it was not observed during this study, mating has been observed in both December (Johns and Cook 2014) and in March (https://inaturalist.nz/observations/21440116). Oviposition therefore likely occurs throughout summer into early autumn, and eggs may be laid in the rotting wood of trees, as has been observed for the other arbo-

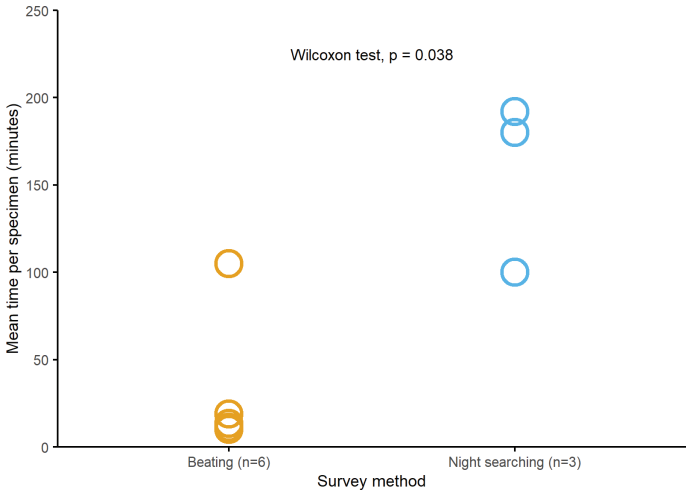

Fig. 7. Mean number of minutes searched per *Maotoweta virescens* individual detected during timed beating and active night searching surveys. The results of the Wilcoxon rank sum test are given.

real cave wētā including *Isoplectron armatum* (e.g., https://inaturalist.nz/observations/91211038) and *Talitropsis sedilloti* (e.g., https://inaturalist.nz/observations/2693757).

Among Orthopteran families such as Tettigoniidae (Hartley and Warne 1972) and Anostostomatidae (Stringer 2001), the duration of the egg stage is known to be highly variable between species and sometimes within species or even egg clutches. Similarly, the eggs of some species undergo notable diapause while others do not (Hartley and Warne 1972). Relatively little is known about the duration of the egg stage in Rhaphidophoridae, with estimates varying greatly between species, ranging from one week to four months for the North American *Daihinibaenetes giganteus* Tinkham, 1962 (Weissmann 1997) and up to eleven to twelve months for the Australian *Pallidotettix nullarborensis* Richards, 1968 (Richards 1970a). As such, one theory is that the eggs of *M. virescens* may overwinter and hatch relatively synchronously the following spring, with adults maturing approximately one year later. A seasonal lifecycle with a prolonged egg stage has been documented for the much larger *Pachyrhamma waitomoensis* with the egg stage lasting six to seven months (Richards 1961). *Pallidotettix nullarborensis* also has a prolonged egg stage, which Richards (1970a) proposed was likely to ensure eggs hatched during optimal climatic conditions.

An alternative to the above theory is that *M. virescens* has a short egg development period. This would mean that egg hatching occurs relatively quickly after mating and oviposition in summer/autumn. Nymphs would then overwinter in their early- to mid-instars before reaching maturity the following year in late spring to early autumn. Similar seasonal lifecycles have been documented for other rhaphidophorids including *Pachyrhamma edwardsii* (Scudder, 1869) from NZ (Richards 1961) and *Hadenoecus subterraneus* (Scudder, 1861) from North America (Hubbell and Norton 1978). Conversely, in *P. turneri*, instars of all ages can be found cohabiting with adults at any given time of year, suggesting an aseasonal lifecycle (Richards 1961). De Pasquale et al. (1995) found that *Dolichopoda* spp. exhibit aseasonal or seasonal development depending on whether they occupied natural or artificial cave sites, respectively, suggesting that the trait may be flexible for at least some rhaphidophorid species. For *M. virescens*, a univoltine cycle with reasonably strong seasonal synchrony in egg hatching is supported in the current study by the detection of only young nymphs and adults between January and March (mid to late summer). If *M. virescens* displayed an aseasonal lifecycle, it would be expected that nymphs of all ages would have been recorded during the present study, similar to what Richards (1961) observed for *P. turneri*. Although based on evidence of only a single specimen, the relatively long ovipositor of the female nymph collected in June (winter) from the Karangarua Valley compared to all other measured nymphs further supports this, as ovipositor length is known to be one of the best indicators of nymphal development among NZ cave wētā (Richards 1961). Further sampling effort is required across all months and seasons to confirm this proposed theory.

The lack of mid-instar nymphs observed during this study may alternatively be explained by an ontogenetic niche shift in *M. virescens*. For example, *M. virescens* may move up higher into the forest canopy during the mid-instar phases of its lifecycle. Cherrill and Brown (1992) documented ontogenetic shifts in microhabitat preference in the bush cricket *Decticus verrucivorus* (Linnaeus, 1758). Within the Rhaphidophoridae, ontogenetic niche shifts have been documented for *Hadenoecus subterraneus*, which only begins to leave its cave habitat to forage at night once it reaches the fifth instar (Hubbell and Norton 1978). Interestingly, *Dolichopoda* spp. also display variation in feeding habits and diet with age; however, it is the subadults that differ from other life stages as they tend to feed outside of caves while adults and early-instar nymphs feed almost exclusively on resources within the cave (de Pasquale et al. 1995).

As has been found for other cave wētā species (e.g., Richards 1961, Butts 1983), the sizes of *M. virescens* nymphs measured here did not fall into discrete size classes corresponding to instar. The number of instars for other rhaphidophorids varies between, and possibly within, species and sexes (Hubbell and Norton 1978). For example, *Pachyrhamma edwardsii* has ten nymphal instars in the male and nine in the female (Richards 1961), while *Insulanoplectron spinosum* has nine in the male and ten in the female (Butts 1983). Because no mid- to late-instar nymphs were found, we are not able to give an accurate estimate of the number of nymphal instars for males or females of *M. virescens*. Clearly, further work is required to fully understand the life history of *M. virescens* and to test the various theories posed here.

When Johns and Cook (2014) described *M. virescens*, they believed it to be one of NZ's rarest cave wētā species. The authors presumably based this assumption on the difficulties that they encountered detecting the species while undertaking targeted night searching in the Takitimu Mountains, as well as the relative absence of *M. virescens* specimens within NZ's entomological collections. However, prior to the current study, no large-scale survey had ever been conducted for *M. virescens* specifically. The evidence presented here suggests that *M. virescens* may not be rare. By compiling all known records, as well as conducting targeted surveys using an ecologically appropriate method (i.e., beating), *M. virescens* has been shown to be geographically widespread within temperate South Island forest habitat and, in some cases, relatively abundant. Although beating can be an effective means of collecting some arboreal Orthoptera (Sperber et al. 2021), including the arboreal Nearctic rhaphidophorid *Gammarotettix bilobatus* (Stidham 2005), it has not typically been employed as a collecting method for NZ cave wētā (e.g., Johns and Cook 2014, Hegg et al. 2019, 2022). Our findings suggest that active night searching, the traditional method of cave wētā detection, is less effective for the detection of *M. virescens* than beating in mixed temperate forest, likely explaining why the species has not been detected regularly in the past. This may explain why the first two known specimens of the species were detected by the late Dr. R. R. Forster (see Johns

and Cook 2014), an esteemed arachnologist who routinely employed beating to target spiders. Future studies of rhaphidophorid diversity should consider employing foliage and moss beating as part of their collection methods.

Within the open beech forest habitat of Princhester Creek in the Takitimu Mountains, beating proved only marginally more effective at detecting *M. virescens* than night searching. This result may indicate that open beech forest represents sub-optimal habitat for *M. virescens*, meaning densities are naturally low. It could also reflect the relative lack of hanging mosses within this habitat type, which made beating more difficult. Habitat structure is known to affect the efficacy of some Orthopteran survey methods, including sweeping (Gardiner et al. 2005), hand collection (Bailey et al. 2003), and pitfall trapping (Schirmel et al. 2010). Targeted surveys employing both beating and night searching may be required to fully understand the relative abundance, ecology, and distribution of *M. virescens*. It should be noted that the results of the beating and active night searching surveys presented in this study are a preliminary comparison in locations already confirmed as suitable *M. virescens* habitat and are based on relatively small sample sizes. It is likely that the total amount of survey time taken to detect the species will vary from those documented here, particularly in surveying sites in which the species occurs at naturally low densities, as may be the case for open beech forest.

The field work reported here was completed as part of a wider project to investigate the distribution of Data Deficient invertebrates in southern NZ (Tweed and Wakelin 2021). Such work is essential for many of NZ's cave wētā species, as well as numerous other poorly known invertebrates, as a prerequisite to enable effective threat classification and to inform whether conservation management is required. This study has greatly improved our knowledge of the cave wētā *M. virescens* and provided a more effective method to detect the species. The evidence presented indicates that the wētā has a much wider distribution than previously thought, indicating a reassessment of the species threat status was required. The evidence presented was assessed by the NZTCS panel and contributed to the reclassification of the species as Not Threatened (Trewick et al. 2022).

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Towards a better understanding of the genus *Scelimena* (Orthoptera, Tetrigidae, Scelimeninae): New insights and notes on the taxonomy, ecology, and physiology of the genus in Peninsular Malaysia

Amira Aqilah Muhammad[1,2,3], Maks Deranja[3,4], Karmela Adžić[3,4], Nurul Ashikin Abdullah[1]

1 Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia.
2 IUCN/SSC Grasshopper Specialist Group, Kuala Lumpur, Malaysia.
3 SIGTET—Special Interest Group Tetrigidae, Bonn, Germany.
4 IUCN/SSC Grasshopper Specialist Group, Zagreb, Croatia.

Corresponding author: Amira Aqilah Muhammad (miamedulla@gmail.com)

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Abstract

One of the two *Scelimena* Serville, 1838 species described from Peninsular Malaysia, *Scelimena gombakensis* Muhammad, Tan & Skejo, 2018 occupies a wide distributional range across the country, contrary to the range described in the original description of the species. Extended research has shown that the species occurs in many localities in Peninsular Malaysia, which is interesting given that such findings are uncommon in the study of Tetrigidae. This paper provides new distribution localities, some ecological and physiological notes, and photographs of living specimens of *Scelimena gombakensis*. Other species synonymized here include *Scelimena razalii* Mahmood, Idris, & Salmah, 2007 **syn. nov.** of species *Falconius dubius* Günther, 1938.

Keywords

citizen science, distribution, iNaturalist, pygmy grasshoppers, Scelimenini, Southeast Asia, synonymy

Introduction

Malaysian Tetrigidae still remains an understudied group of animals since papers on them mostly highlight taxonomy, i.e., species description and occurrence. Examples of such papers from Peninsular Malaysia are Mahmood et al. (2007), Tan and Kamaruddin (2014), Tan and Kamaruddin (2016), Skejo and Bertner (2017), Storozhenko and Pushkar (2017), and Muhammad et al. (2018). In Sabah and Sarawak, Ito and Mohamed (2001), Storozhenko (2012), Kočárek et al. (2015), and Skejo et al. (2019) also focused on taxonomy, with the exception of a paper by Kuřavová et al. (2017) that described Bornean Tetrigidae feeding patterns, offering an ecological insight. Other papers that briefly mention Malaysian Tetrigidae include Zha et al. (2016), Xin and Deng (2019), and Adžić et al. (2020). One of the first papers to cover this family in Peninsular Malaysia was Mahmood et al. (2007). However, some misidentifications from the paper were reported in later publications (Storozhenko and Dawwrueng 2015, Muhammad et al. 2018), and certain species identifications from this paper require future revision.

So far, only two *Scelimena* Serville, 1838 species have been found in Peninsular Malaysia (Cigliano et al. 2022): *Scelimena razalii* Mahmood, Idris & Salmah, 2007 and *Scelimena gombakensis* Muhammad, Tan & Skejo, 2018. While *S. gombakensis* belongs to the *Scelimena discalis* Hancock 1915 species group, *S. razalii* has not been assigned to any due to insufficient evidence and vague descriptions and drawings from Mahmood et al. (2007). Moreover, it is only described in Kuala Lompat, Pahang, Malaysia.

In this study, we synonymized *Scelimena razalii* Mahmood, Idris & Salmah, 2007 **syn. nov.** with *Falconius dubius* Günther, 1938 after close examination of the type specimens of *S. razalii*. We also looked into the distribution of *S. gombakensis* by reporting on all known specimen observation localities and linking this updated distribution of the species with its ecological (i.e., habitat) and physiological (i.e., swimming ability) characteristics. Photos of both living and pinned specimens of *S. gombakensis* and *F. dubius*, detailed scanning electron microscopy (SEM) photos of morphological characters of hind leg tarsi and tibiae of *S. gombakensis*, and a distribution map of *S. gombakensis* are provided.

Material and methods

Institutional abbreviations.—

CIS (UKM)	Center for Insect Systematics, Universiti Kebangsaan Malaysia, Selangor, Malaysia;
FRIM	Forest Research Institute Malaysia, Peninsular Malaysia;
MZUM (UM)	Museum of Zoology, Universiti Malaya, Kuala Lumpur, Malaysia;
ZRC	Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore.

Both physical records (specimen) and virtual records (online database) were assessed. *Falconius dubius* specimens were examined at MZUM (UM) and CIS (UKM), whereas *Scelimena gombakensis* specimens were examined at MZUM (UM), ZRC, and FRIM. Photographs used in this manuscript were taken using an Olympus Compact Stereo Microscope SZ61 with an Olympus Microscope Digital Camera DP22 connected to a desktop computer during museum examination. A Canon EOS 600D camera with 100 mm f/2,8 macro lens attached was used for *in situ* photography. Records of type specimens described in Muhammad et al. (2018) deposited in ZRC, MZUM, and FRIM were assessed by Amira Aqilah Muhammad. Data screening on the iNaturalist website was done using the keywords "*Scelimena gombakensis*," in which the online observations were additionally confirmed by the authors. Supplementary information (i.e., dates of publishing, location names, GPS coordinates, and website identification numbers) are listed in Table 1. Only iNaturalist observation data available prior to the date of this paper's submission (29 August 2022) were included in this paper. The tabulated coordinates were input into QGIS version 3.16.13 to visualize the distribution on a map.

Table 1. Observations of *Scelimena gombakensis* Muhammad, Tan & Skejo, 2018 specimens in Peninsular Malaysia found on iNaturalist platform (www.inaturalist.org) (the last column refers to iNaturalist observation ID).

Observer	Date	Locality	Coordinates	Observation ID
Fabio Cianferoni	Jul. 4, 2008	Hulu Perak, Perak, Malaysia	5°30'10"N, 101°26'11"E	45968367
CheongWeei Gan	Dec. 5, 2009	Hulu Yam, Selangor, Malaysia	3°24'11"N, 101°41'4"E	127199050
CheongWeei Gan	Dec. 5, 2009	Hulu Yam, Selangor, Malaysia	3°19'55"N, 101°42'6"E	127199054
Phil Benstead	Aug. 26, 2013	Hulu Langat, Selangor, Malaysia	3°7'48"N, 101°53'17"E	71201238
Erland Refling Nielsen	Aug. 30, 2013	Gombak, Selangor, Malaysia	3°19'27"N, 101°44'54"E	63194111
Kees van Reenen	Jan. 29, 2019	Seremban, Negeri Sembilan, Malaysia	2°47'55"N, 101°48'3"E	35679667
Yiquan Chin	Feb. 23, 2019	Timur Laut, Penang, Malaysia	5°26'2"N, 100°17'46"E	20687721
Alexius L.Z.L	Dec. 19, 2019	Timur Laut, Penang, Malaysia	5°25'54"N, 100°17'55"E	36773269
Vatcharavee Sriprasertsil	June 2021	Waeng, Narathiwat, Thailand	5°47'43"N, 101°49'42"E	84546541
Chloe Alison	Dec. 14, 2021	Genting Highlands, Pahang, Malaysia	3°24'40"N, 101°47'40"E	103086028
Alexius L.Z.L	Dec. 11, 2021	Mukim 17, Penang, Malaysia	5°21'35"N, 100°29'32"E	102908617
Puteri Nuraida Syuhada Binti Abdullah	Dec. 11, 2021	Hulu Langat, Selangor, Malaysia	3°12'37"N, 101°50'33"E	102847175
Albert Kang	Mar. 1, 2022	Tanjung Bungah, Penang, Malaysia	5°27'54"N, 100°16'55"E	107814195
Aiman Azmi	May 13, 2022	Pasir Puteh, Kelantan, Malaysia	5°45'49"N, 102°24'32"E	116921973

Field observation was done at the type locality—Ulu Gombak Field Study Center, Ulu Gombak, Selangor, Malaysia—where the authors focused on several aspects of tetrigid behavior during the daytime, including food preference and locomotion (utilization of legs and hindwings). Several individuals were collected and preserved in 95% ethanol to observe the leg structure under Hitachi's Table Top Scanning Electron Microscope (SEM) TM3030. The specimens' legs were mounted on a conductive double-sided tape on a specimen stub that was then set on a holder before the height level was adjusted by controlling the height gauge. The stage was then carefully placed in the chamber before closing, and the imaging process followed the instructions given by the user's manual. Scanned images were edited using TM3030 software installed on a desktop computer connected to the SEM machine.

Results

Taxonomy

Family Tetrigidae Rambur, 1838
Subfamily Scelimeninae Bolívar, 1887
Tribe Scelimenini Bolívar, 1887

***Falconius dubius* Günther, 1938**

Falconius dubius Günther, 1938: 399, 404, 419.
Scelimena razalii Mahmood, Idris & Salmah, 2007, syn. nov. – Mahmood, Idris and Salmah 2007: 1279; Muhammad et al. 2018: 53; Maitlo and Panhwar 2021: 34.

Material examined.—MALAYSIA, **Selangor** • 2 ♂; holotype and paratype of *Scelimena razalii*; 24 July 2004; Rezwana Rezali leg.; CIS (UKM) • 2 ♂; Ulu Langat; 16 Mar. 1964; AAM leg.; MZUM IOt 002323, 002324 • 1 ♂; Ulu Langat; 7 Mar. 1964; AAM leg.; MZUM IOt 002353 • 1 ♂; Ulu Gombak Bt 16; 7 Mar. 1964; AAM leg.; MZUM IOt 002370 • 1 ♀; same data as for preceding; MZUM IOt 002377 • 1 ♂; Ulu Gombak; 15 July 2019; Adzic, K. leg.; MZUM • 1 ♂; Ulu Gombak; 15 July 2019; Deranja, M. leg.; MZUM • 1 ♂; Ulu Gombak, 24 July 2019; Muhammad, A. A. leg.; MZUM • 1 ♂; Ulu Gombak; 29 July 2019; Adzic, K. leg.; MZUM. • 1 ♀; same data as for preceding. **Negeri Sembilan** • 1 ♀; Negeri Sembilan, Pasoh; 26 June 1997; Rina Silviana leg.; MZUM IOt 002383. **Pahang** • 1 ♂; Eko Rimba Lentang; 22 July 2019; Adzic, K. leg.; MZUM • 1 ♀; Eko Rimba Lentang; 22 July 2019; Deranja, M. leg.; MZUM.

Justification of the synonymy.—After examination of the *Scelimena razalii* Mahmood, Idris & Salmah, 2007 type material, we consider it to be a synonym of *Falconius dubius* Günther, 1938. We found many *Falconius* Bolívar, 1898 specimens in the UKM collection, but none were identified as belonging to the genus *Falconius* as described in Mahmood et al. (2007). The genus can easily be distinguished from other Scelimenini genera by the swollen hind tarsi in females (Fig. 1D, indicated by an arrow. Note that this character is present in *Eufalconius* Günther, 1938 as well), a relatively stout head with the most prominent feature being the frontal costa forking relatively close to the fastigium, the anterior margin of the eyes raised slightly above the fastigium, and a relatively high position of the paired ocelli and the antennal grooves in relation to the compound eyes (Bolivar 1898, Muhammad et al. 2018). Upon further comparison with photographs of the type material of *F. dubius* available on Orthoptera Species File (OSF) database, we found no differences between these two species. We cannot be certain that the *S. razalii* specimens reported from Pakistan (Maitlo and Panhwar 2021) also belong to this species because we did not

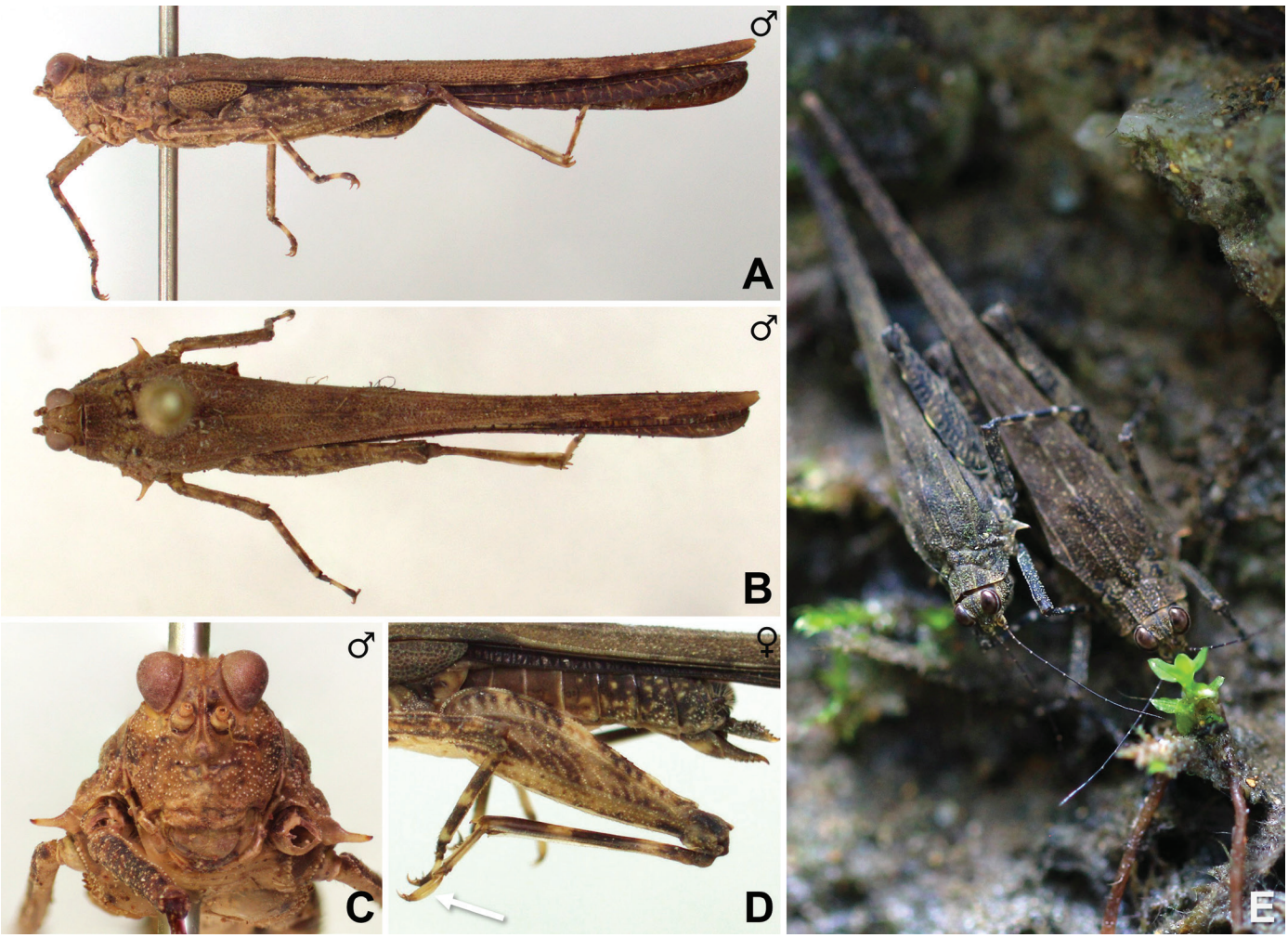

Fig. 1. Specimens of *Falconius dubius* Günther, 1938 from Peninsular Malaysia. **A–C.** Holotype of *Scelimena razalii* Mahmood, Idris & Salmah, 2007 **syn. nov.**; **D.** A female specimen from Universiti Malaya collection; **E.** The morphology of living specimens in their natural habitat in Ulu Gombak, Selangor, Peninsular Malaysia, both male and female.

have the opportunity to examine this material. Hence, those specimens should be re-examined in the future.

This paper is the first official record for the species from Peninsular Malaysia (following data from Adžić (2021); note that the type material specimens are listed incorrectly in the thesis, which is corrected in this paper) (Fig. 1).

***Scelimena gombakensis* Muhammad, Tan & Skejo, 2018**

Scelimena producta (Serville, 1838) - Mahmood et al. 2007: 1279; Storozhenko and Dawwrueng 2015: 543).
Scelimena gombakensis Muhammad, Tan & Skejo, 2018: 6, 46.

Examined material.—**Holotype:** PENINSULAR MALAYSIA, **Selangor** • 1 ♂; Ulu Gombak Field Studies Centre; 24 Sept. 2017; Muhammad, A. A., Muhammad Hafiz Mohd Amin & Afyza Maisarah Azizan leg.; ZRC. **Paratypes:** PENINSULAR MALAYSIA, **Selangor** • 1 ♀; Ulu Gombak Field Study Centre; 9 Mar. 2018; Muhammad, A. A. & Abdullah, N. A. leg.; ZRC • 1 ♂; same data as of preceding; FRIM • 1 ♀; same data as of preceding; FRIM • 2 ♂; same data as of preceding; MZUM • 1 ♀; Ulu Gombak Field Study Centre; 24 Sept. 2017; Muhammad, A. A., Muhammad Hafiz Mohd Amin & Afyza Maisarah Azizan leg.; FRIM • 1 ♀; same data as of preceding; MZUM.

Other material: PENINSULAR MALAYSIA, **Selangor** • 8 ♂; Ulu Gombak; 7 Mar. 1964; AAM leg.; MZUM IOt 002350, 002351, 002355, 002361, 002362, 002366, 002367, 002369 • 3 ♀; same data as of preceding; MZUM IOt 002357, 002363, 002379 • 1 ♂; Ulu Gombak 16ms. [miles]; 27 May 1964; AAM leg.; MZUM IOt 002347 • 2 ♂; Ulu Gombak, 16ms. [miles]; 15 Apr. 1964; AAM leg.; MZUM IOt 002371, 002373 • 1 ♂; Ulu Gombak, 12ms; 15 Apr. 1964, AAM leg.; MZUM IOt 002372 • 3 ♂; Ulu Gombak, Bt [Batu] 16; 7 Mar. 1964; AAM leg.; MZUM IOt 002348, 002349, 002356 • 4 ♀; same data as of preceding; MZUM IOt 002358 to 002360, 002365 • 7 ♂; Ulu Langat; 16 Mar. 1964; AAM leg.; MZUM IOt 002333, 002334, 002340, 002342 to 002345 • 2 ♀; same data as of preceding; MZUM IOt 002339, 002341 • 1 ♀; Ulu Gombak; 15 Jul. 1969; JAB leg.; MZUM IOt 002428 • 1 ♂; Ulu Gombak; 15 Sept. 1974; Chua Eng Lok leg.; MZUM IOt 002407 • 1 ♂; same data as of preceding; L. Teo leg.; MZUM IOt 002408 • 1 ♂; same data as of preceding; B. H.Voon leg.; MZUM IOt 002415 • 1 ♀; same data as of preceding; Aru leg.; MZUM IOt 002413 • 1 ♀; same data as of preceding; K. C. Tung leg.; MZUM IOt 002414 • 1 ♀; Ulu Langat; 27 Oct. 1974; Wong Yow Sin leg.; MZUM IOt 002411, • 1 ♀; same data as of preceding; P. F. K. leg.; MZUM IOt 002412 • 1 ♀; Pansoon; 27 Oct. 1974; Heng. L. P. leg.; MZUM IOt 002409 • 1 ♀; same data as of preceding; Hoo Ah Teng leg.;

MZUM IOt 002416 • 1 ♂; Sg. [Sungai] Tua; 27 July 1979; C. L. leg.; MZUM IOt 002406 • 1 ♀; Ulu Gombak; 1 Dec. 1982, Zuraidah Mian leg.; MZUM IOt 002388 • 1 ♀; Ulu Gombak; 21 Oct. 1984; Daiqah leg.; MZUM IOt 002410 • 1 ♀; Ulu Gombak; 18 Sept. 1995; Hasleyza leg.; MZUM IOt 002386 • 1 ♂; Ulu Gombak; 7 July 1997; Rosliza leg.; MZUM IOt 002430 • 1 ♂; Ulu Gombak; MZUM IOt 002391. **Negeri Sembilan** • 1 ♂; Ulu Bendul; 12 Aug. 1989; ZZ leg.; MZUM IOt 002427. **Pahang** • 1 ♂; Ketari; 7 June 1961; MZUM IOt 002385 • 1 ♀; same data as of preceding; MZUM IOt 002387 • 1 ♂; Nenasi; 17 Nov. 1974; W. C. Kang leg.; MZUM IOt 002404 • 1 ♀; Lentang; 2 Dec. 1995; Rohaya leg.; MZUM IOt 002426. **Perak** • 1 ♀; Grik; 17 Feb. 1991, McGyver leg.; MZUM IOt 002418 • 1 ♂; Perlok; 20 May 1997, Khaironizam Md. Zain leg.; MZUM IOt 002399. **Johor** • 1 ♂; B[atu] Pahat; 20 Nov. 1974; M. Ali-S leg.; MZUM IOt 002403 • 1 ♂; Duhsun; 17 Nov. 1974; Salleh leg.; MZUM IOt 002417. **Terengganu** • 1 ♂; Jerangau; 2 Mar. 1974; Baki leg.; MZUM IOt 002405. **Kelantan** • 1 ♂; Kg Senyul; 15 June 1962; KJK leg.; MZUM IOt 002375 • 1 ♀; same data as of preceding; MZUM IOt 002374 • 1 ♀; F[ort] Brooke; 15 Apr.1962; JAB leg.; MZUM IOt 002381.

iNaturalist observation.—PENINSULAR MALAYSIA, **Perak** • Hulu Perak; 5°30'10"N, 101°26'11"E; 4 July 2008; Fabio Cianferoni leg.; iNaturalist.org: https://www.inaturalist.org/observations/45968367. **Selangor** • Hulu Yam; 3°24'1"N, 101°41'4"E; 5 Dec. 2009; CheongWeei Gan leg.; iNaturalist.org: https://www.inaturalist.org/observations/127199050 • Hulu Yam; 3°19'55"N, 101°42'6"E; 5 Dec. 2009; CheongWeei Gan leg.; iNaturalist.org: https://www.inaturalist.org/observations/127199054 • Hulu Langat; 3°7'48"N, 101°53'17"E; 26 Aug. 2013; Phil Benstead leg.; iNaturalist.org: https://www.inaturalist.org/observations/71201238 • Gombak; 3°19'27"N, 101°44'54"E; 30 Aug. 2013; Erland Refling Nielsen leg.; iNaturalist.org: https://www.inaturalist.org/observations/63194111 • Hulu Langat; 3°12'37"N, 101°50'33"E; 11 Dec. 2021; Puteri Nuraida Syuhada Binti Abdullah leg.; iNaturalist.org: https://www.inaturalist.org/observations/102847175. **Negeri Sembilan** • Seremban; 2°47'55"N, 101°48'3"E; 29 Jan. 2019; Kees van Reenen leg.; iNaturalist.org: https://www.inaturalist.org/observations/35679667. **Penang** • Timur Laut; 5°26'2"N, 100°17'46"E; 23 Feb. 2019; Yiquan Chin leg.; iNaturalist.org: https://www.inaturalist.org/observations/20687721 • Timur Laut; 5°25'54"N, 100°17'55"E; 19 Dec. 2019; Alexius L.Z.L leg.; iNaturalist.org: https://www.inaturalist.org/observations/36773269 • Mukim 17; 5°21'35"N, 100°29'32"E; 11 Dec. 2021; Alexius L.Z.L leg.; iNaturalist.org: https://www.inaturalist.org/observations/102908617 • Tanjung Bungah; 5°27'54"N, 100°16'55"E; 1 Mar. 2022; Albert Kang leg.; iNaturalist.org: https://www.inaturalist.org/observations/107814195. **Pahang** • Genting Highlands; 3°24'40"N, 101°47'40"E; 14 Dec. 2021; Chloe Alison leg.; iNaturalist.org: https://www.inaturalist.org/observations/103086028. **Kelantan** • Pasir Puteh; 5°45'49"N, 102°24'32"E; 13 May 2022; Aiman Azmi leg.; iNaturalist.org: https://www.inaturalist.org/observations/116921973. THAILAND, **Narathiwat** • Waeng; 5°47'43"N, 101°49'42"E; June 2021; Vatcharavee Sriprasertsil leg.; iNaturalist.org: https://www.inaturalist.org/observations/84546541.

Examination of the specimens deposited at FRIM, MZUM, and ZRC revealed that the species was previously known from the region but was not recognized as a separate species. *S. gombakensis* had been wrongly identified by researchers as *Scelimena producta* (Serville, 1838) (Mahmood et al. 2007, D.K. McE. Kevan's notes on specimen labels in the UM collection). *S. producta producta* has been described from Java and has a similar body coloration to *S. gombakensis* (see specimen photographs available on OSF). However, it can be morphologically differentiated by two characteristics: pointed tubercles at humeral angles to the pronotum (rounded in *S. gombakensis*) and numerous clearly visible teeth on the ventral side of its hind femora (while *S. gombakensis* has only two small teeth) (Muhammad et al. 2018). Thus, we correct previous identifications and consider all listed specimens to belong to *S. gombakensis*, making these identifications new locality records for the species.

Variability.—Thanks to the large number of specimens that we were able to examine, we found *S. gombakensis* to be a rather variable species, especially in terms of body size (♂: 18.0–24.0 mm; ♀: 24.0–31.0 mm). Nymphs do not differ much from adults, having similar coloration and general appearance, although they do have significantly shorter pronotum, making them easily distinguished from other Tetrigidae in Peninsular Malaysia. During the fieldwork, we observed one specimen belonging to *S. gombakensis* with unique coloration—the specimen's general body coloration was brown (opposed to the common dark green coloration) with orange markings (opposed to yellow markings; Fig. 2E, indicated by an arrow). This coloration could be recessive given its rarity, or it could be caused by some kind of infection.

A change of coloration was also observed in pinned specimens (Fig. 2A–D), which is a very interesting but rarely mentioned observation in Tetrigidae (an example of such observation was described by Mathieu et al. (2021)). In the MZUM collection, which holds a large number of *S. gombakensis* specimens, we observed color degradation in the older specimens. The colors of the older specimens were generally faded more than in recent ones, which gradually lost their dark green coloration with yellow markings and became generally brown in coloration. However, it is important to note that not all specimens had lost their color to the same extent, as some specimens from the 1960s are not as faded as the one represented in this paper (Fig. 2A). Since all the specimens were collected around the same time and were all stored in the same box under the same conditions, it is possible that pre-pinning conditions play a vital role in coloration preservation in *S. gombakensis* specimens. Such factors might be influenced by the sampling and euthanization methods, duration of pinning of the specimens, preservation techniques, etc. Since we did not have information on the pre-pinning conditions older specimens were exposed to, this hypothesis requires consideration in the future.

Distribution of S. gombakensis.—At first, it was hypothesized that this species would have general ecological traits similar to those of other Tetrigidae, including the fact that Tetrigidae are, in general, highly confined to water bodies, as they are often found in close proximity to fast-flowing rocky rivers (Tan et al. 2017). Even so, we have also observed specimens flying long distances and maneuvering well during flight. It is likely that this species, like many other Tetrigidae, is tightly linked to large flowing bodies of water and disperses passively by water during diving as an escape behaviour.

With recently described species such as *S. gombakensis*, additional knowledge on species distribution, behavior, and other aspects of the species' ecology are frequently understudied and incomplete. Revisiting old specimens deposited in museums or private collections is thus an important next step in understanding a species, as these repositories might hold historical specimens belonging to the species. Muhammad et al. (2018) stated that *S. gombakensis* is restricted to Sungai Gombak of the Gombak Catchment in Selangor, Peninsular Malaysia. Revision of specimens deposited

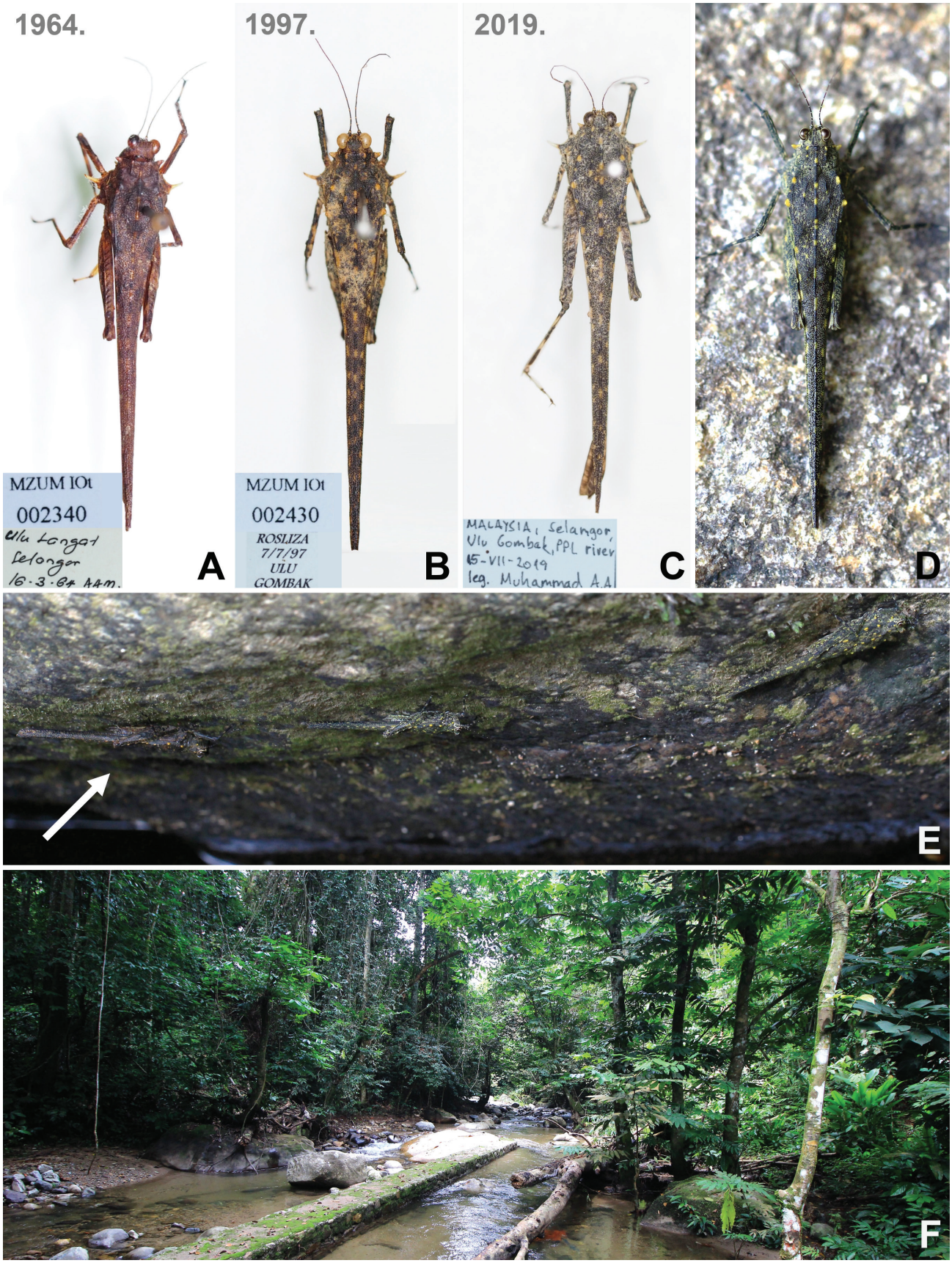

Fig. 2. Change of coloration in pinned specimens of *Scelimena gombakensis* Muhammad, Tan & Skejo, 2018 compared to the living specimen. **A–C.** Pinned *S. gombakensis* specimens; **D.** Living *S. gombakensis* specimens. **E.** Coloration variability and camouflage of three living *S. gombakensis* specimens; **F.** Natural habitat of *S. gombakensis* at the type locality Ulu Gombak, Selangor, Malaysia.

in museum collections (UKM, UM, FRIM, and ZRC) and photos on online databases have shown that the species is, in fact, widely distributed across the country, especially along the West coast (Fig. 3). Social media observations on iNaturalist have greatly contributed to understanding species distribution (Table 1), once again demonstrating how useful citizen science can be (for other examples of the contributions of citizen science to the study of Tetrigidae, see Skejo et al. 2019, Kasalo et al. 2021, and Pavlović et al. 2022).

S. gombakensis is an example of a species that is easily recognized through photographs and highly unlikely to be misidentified in Peninsular Malaysia, making identification using iNaturalist very reliable. Additionally, thanks to iNaturalist, we were able to discover an observation of *S. gombakensis* in the Waeng district of Narathiwat, Thailand, very close to the border of Peninsular Malaysia; this represents the first official record of the species outside of Peninsular Malaysia, making this the first record for the country (Fig. 3, Table 1). Further clarification of the species' distribution is possible by comparing this species with *Scelimena discalis* Hancock, 1915 specimens from Thailand (Storozhenko and Dawwrueng 2015), which may include some misidentified specimens. For now, we refrain from comparing *S. gombakensis* to *S. discalis* due to the inaccessibility of physical specimens and the lack of molecular and online data.

Ecology and habitat of Scelimena gombakensis.—Muhammad et al. (2018) noted that *S. gombakensis* can be found perched on river rocks in lowland secondary rainforest where it is cool and very humid, especially in areas shadowed by tree canopies. Further field observation revealed that high moisture and indirect sunlight may be key to their survival, in tandem with the abundance of lichens and mosses that are their food source (Zha et al. 2017). On the rocks, *S. gombakensis* tends to crowd near the water surface, where the high turbidity of the flowing stream creates a suitable humid biota. Juveniles are more likely to be observed close to the water surface than adults, as they are more susceptible to desiccation due to their immature exoskeletons. The juveniles are also less agile and unable to fly; hence, staying close to the water surface might increase their

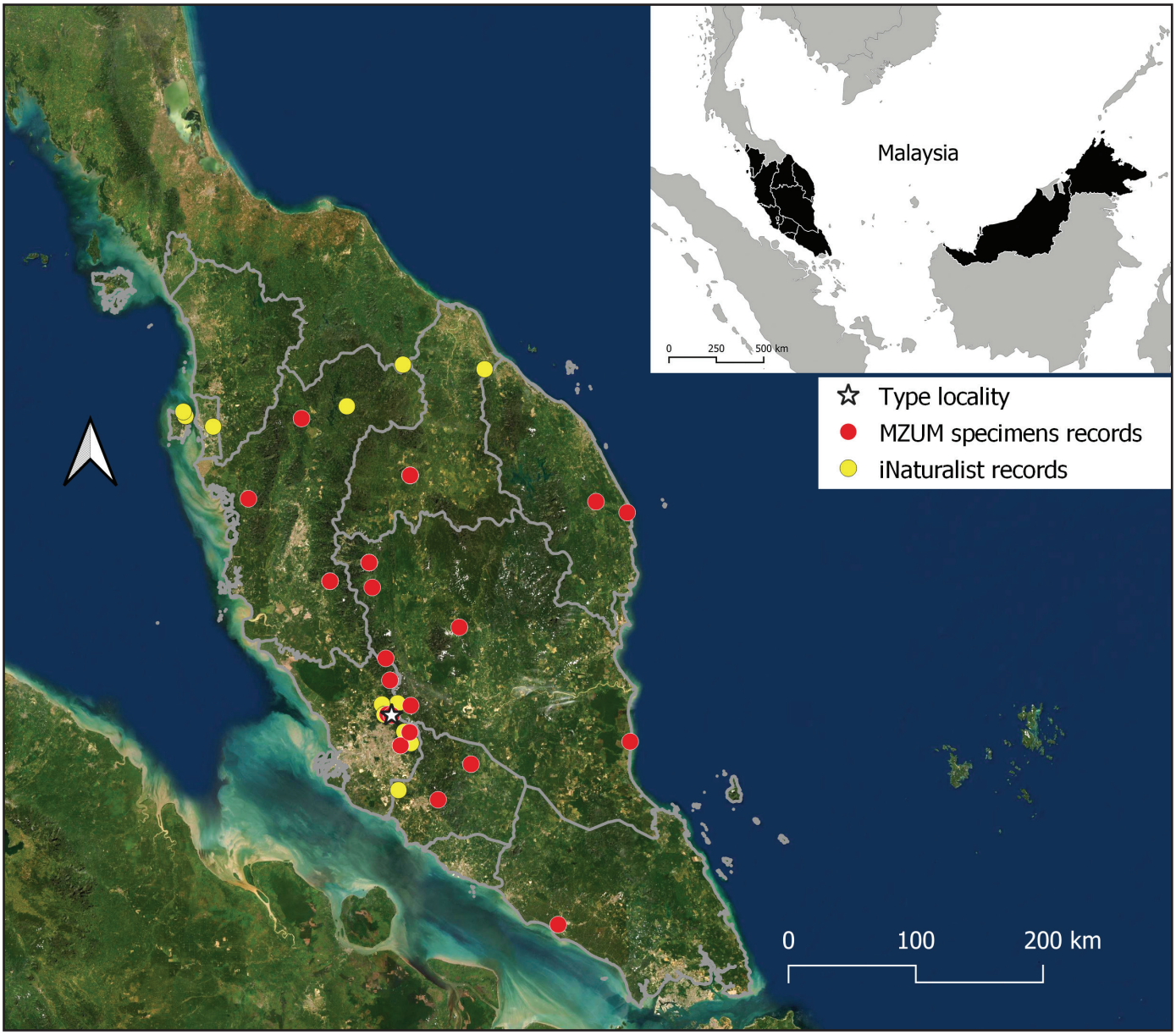

Fig. 3. Distribution map of species *Scelimena gombakensis* Muhammad, Tan & Skejo, 2018 based on museum collection and iNaturalist data.

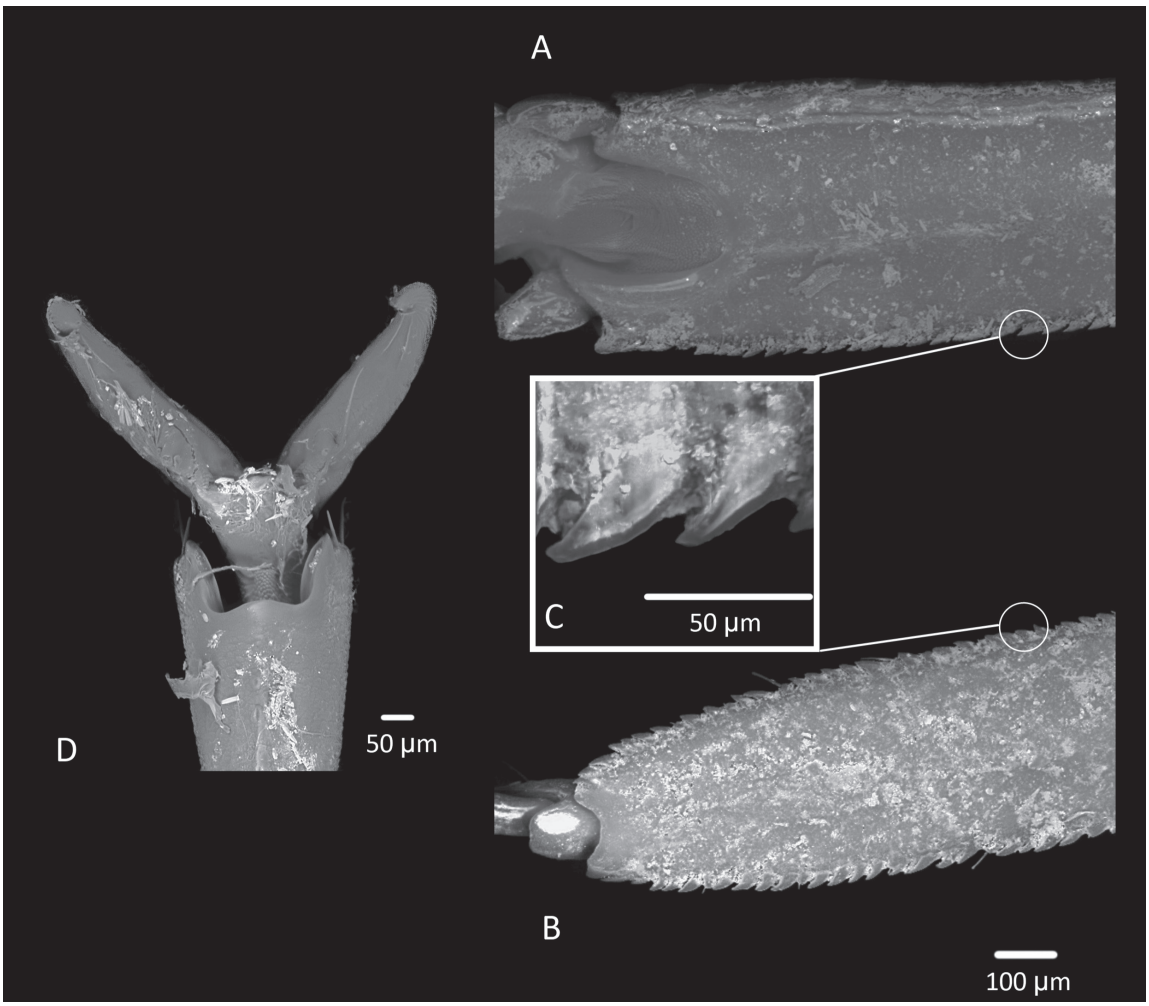

Fig. 4. Scanning electron micrographs of the hind leg of *S. gombakensis* in ventral view. **A.** Hind tibia; **B.** First tarsal segment; **C.** Magnified view of the serration lining the edges of both tibia and tarsal segment; **D.** Tarsal claw. Scale bars: 100 µm (**A, B**); 50 µm (**C, D**).

success rate when escaping from predators by diving underwater. Although individuals of various life stages can be found on the same rock, the place where *S. gombakensis* lays their eggs remains a mystery. For some *Scelimena* species, it is speculated that the eggs are laid elsewhere, such as in the sand along the riverbanks, and are perhaps well adapted to waterlogged conditions (Zha et al. 2017).

Physiology of Scelimena gombakensis *as key to adaptation to a semi-aquatic environment.*—Closer observation of the characteristics of the hind legs of *S. gombakensis* elucidates their swimming ability (Fig. 4). A close-up of the insect's hind tibia and its first tarsal segment under a scanning electron microscope (SEM) reveals the following hydrodynamic characters: the first tarsal segment is laterally compressed along the whole length, and serration exists along the lateral edges of both the first tarsal segments and the hind tibiae (Fig. 4A, B, C). The lateral compression provides a wider surface area for paddling underwater and likely aids in better propulsion during swimming. The serration along the lateral edges could serve multiple functions, but the tarsal serration most likely enhances the legs' grip on wet and slippery rock surfaces since the arolium is absent underneath the claws (Fig. 4D). Thus, the presence of microscopic serrations means porous rock surfaces offer more grip to the species' limbs. Morphologically similar to riblet-like shark skin, another possible function of the serrations is to improve hydrodynamics by reducing the water drag acting upon its serrated tibiae when propelling underwater, reducing the formation of vortices and making swimming more energy efficient. The orientation of both microscopic structures is also parallel to the flow of water, hinting at functional homology (Han et al. 2008, Zhang et al. 2011).

Our observations suggest that *S. gombakensis* utilizes both its hind legs and hindwings for navigation and survival, unlike *Scelimena songkrana* Zha & Wen, 2017 from Thailand, which seldom flies but often jumps, as described by Zha et al. (2017). On the other hand, *Scelimena melli* Günther, 1938 from China behaves similarly to *S. gombakensis* except for the fact that *S. melli* leaps further (for 10 meters or more). The mating behavior of *S. gombakensis* is consistent with that of other *Scelimena* (Zha et al. 2017), in which the male securely positions itself on top of the female.

Conclusions

Scelimeninae of Peninsular Malaysia is poorly known because few specimens have been collected in the region, and comprehensive taxonomic research of pygmy grasshoppers in the region is lacking. The short discussion on *Scelimena razalii* given in this paper has resulted in its synonymisation with *Falconius dubius*. The authors recognize only one valid species of the genus *Scelimena* as inhabiting the region, although further research is likely to result in the dis-

covery of new species. Likewise, *Scelimena gombakensis* was found to have a wider distribution range than originally considered, and it is expected that new localities will be discovered with further research. In this paper, we summarized a wide array of information concerning the ecology and physiology of *S. gombakensis* and provided comments and hypotheses that should serve as inspiration and motivation for further research of this genus in Peninsular Malaysia.

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Aryalidonta itishreea, a new genus and species of Thoradontini (Orthoptera, Tetrigidae) from Nepal honors the Emperor of Laughter

Madan Subedi[1,2*], Niko Kasalo[2,3*]

1 Agriculture and Forestry University, Directorate of Research and Extension, Agriculture Science Center, Ghyalchok, Gorkha, Nepal.
2 SIGTET-Special Interest Group Tetrigidae, Bonn, Germany.
3 University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, Evolution Lab, Rooseveltov trg 6, HR-10000 Zagreb.

Corresponding authors: Madan Subedi (madansubedi13@gmail.com); Niko Kasalo (niko.kasalo5@gmail.com)

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Abstract

Aryal's Ten Avatar Groundhopper, *Aryalidonta itishreea* **gen. et sp. nov.**, named in honor of the late Bhairav Aryal, an iconic Nepali satirist, is a new genus and species of Tetrigidae described as a part of the tribe Thoradontini. The species is native to Nepal, a country with a rich tetrigid fauna in need of taxonomic revisions. This monotypic genus can be easily separated from other Thoradontini genera by its enlarged proximal halves of middle femora, a peculiar lateral lobe morphology (small caudal protrusion in its caudal part and a sharp lateral tip), a triangular, anteriorly narrowing vertex, and by its unique head morphology. The species was observed in its natural habitat. It was found to harbor many color variations that are cryptic in nature. It feeds on detritus, algae, lichen, and moss. Specimens heavily infested by mites were found, as well as those in interaction with wasps (possibly Eulophidae), but the nature of the latter could not be determined.

Keywords

Bhairav Aryal, ecology, Eulophidae, Gorkha, groundhopper, Himalayas, Scelimeninae

Introduction

Nepal is a country of rich geography and biodiversity owing to its unique position at the junction of the Palaearctic and Palaeotropical biogeographic realms extending from an altitude of 59 m.a.s.l to the highest point on the Earth, Mount Sagarmatha (8848.86 m.a.s.l) in a mere distance of under 200 km (Udvardy 1975, Paudel et al. 2012). Unfortunately, the fauna of pygmy grasshoppers (Orthoptera: Tetrigidae) is still not well documented. Only a handful of works have been published regarding these beautiful insects, mainly by Chopard and Dreux (1966), Bey-Bienko (1968), Martens (1987), Balderson and Yin (1987), Ingrisch (1987, 2001a, b, 2006), Ingrisch and Garai (2001), Tumbrinck (2015), Subedi (2022), and Subedi (in press). Sigfrid Ingrisch, a father figure to Nepali Orthoptera, published some phenomenal works describing many species new to science, as well as a checklist of Nepali Tetrigidae (along with other Orthoptera families) (Ingrisch 1987, 2001a, b, 2006). The checklist was later updated by Tumbrinck (2015) with the addition of an identification key to the species level. The fauna of Nepali Tetrigidae currently numbers 70 species belonging to 25 genera (Tumbrinck 2015, Cigliano et al. 2022, Subedi in press; this paper) and, as is evident from this paper, more are yet to be described.

The tribe Thoradontini Kevan, 1966 used to belong to the subfamily Scelimeninae, which has been a target of many studies (Skejo 2017, Skejo and Bertner 2017, Muhammad et al. 2018, Adžić et al. 2020). Following work by Adžić et al. (2020), who concluded that Thoradontini do not form a monophyletic clade with Criotettigini Kevan, 1966 and Scelimenini Bolívar, 1887, Thoradontini were left without subfamilial placement. Thoradontini currently consists of the genera *Thoradonta* Hancock, 1909, *Loxilobus* Hancock, 1904, and *Eucriotettix* Hebard, 1930, with the addition of *Aryalidonta* **gen. nov.** in this paper.

The aim of this paper is to describe a new genus and species of Tetrigidae from Nepal and offer insights into its ecology.

Materials and methods

Museum abbreviations.—
ANHM Annapurna Natural History Museum, Pokhara, Nepal

Study area.—The study was conducted in the vicinity of the village of Ghyalchok, Gorkha District, Nepal. The village is situated in a subtropical climate zone that is under the influence of a south-

* Both authors contributed equally to the manuscript.

easterly monsoon that provides most of the area's precipitation during the summer months. The temperature averages between 0 and 25°C, with a warm period between April and September (Shrestha and Aryal 2011). The species was studied for a whole year (October 2021 to September 2022) in the denoted area. A precise description of its typical habitat is presented under the appropriate section in the Results.

Identification, taxonomy, and nomenclature.—The whole type series, along with several individual adults and nymphs, were photographed, and videos were recorded in situ with a Canon EOS 80D with a Canon EF 100mm f/2.8 USM macro lens. Specimens were also collected for examination. The videos were uploaded to YouTube (channel: Nepali Grasshoppers), and links are provided in the appropriate parts of the text.

The type series was pinned using Phusis stainless steel insect pins (size #1) and deposited in ANHM, Pokhara, Nepal. Measurements were made with ImageJ by calibrating the images with millimeter paper. Species descriptions are based on images taken using the macro lens.

The newly described species was compared to the others present in the region using the material available on the Orthoptera Species File (OSF; Cigliano et al. 2022) and by consulting the original descriptions of the relevant taxa. Morphological terminology follows Tumbrinck (2014), and measurements follow Tumbrinck (2014) and Tan and Artchawakom (2015). Taxonomy follows the OSF (Cigliano et al. 2022). Nomenclature is in accordance with the 4th edition of the International Code of the Zoological Nomenclature (ICZN 1999).

Rearing in captivity.—Four adult individuals (2 males and 2 females) were hand-picked from the wild and reared in a plastic jar (1 L). They were fed with moss and detritus and were examined for their excreta.

Results

Taxonomy

Family Tetrigidae Rambur, 1838
Tribe Thoradontini Kevan, 1966

Genus *Aryalidonta* gen. nov.
https://zoobank.org/B8D80222-B260-404F-9E5E-12B8492BCD81

Justification of the tribal assignment and the genus description.—The herein described species shares many similarities with members of Thoradontini, the most notable of which are as follows: (i) the u- or v-shaped carinae of the vertex visible in frontal view, (ii) high-placed frontal costa bifurcation and usually low-placed antennal grooves, (iii) a vertex that is usually triangular and narrowing anteriorly, (iv) eyes adjacent to the anterior margin of the pronotum, (v) bilobate lateral lobes, (vi) wings approximately equally long as the pronotum, and (vii) nodulate surface of the pronotum. Although an overview of the material showed that the tribe requires a thorough revision, we place the new genus and species within it to allow for easier revision in the future.

The new species does not fit with the type species of any of the relevant genera and is thus described under its own genus. It is possible that the subsequent revisions will recognize more species that belong in this genus, so it is important that the genus be defined by a well-documented species.

Etymology.—Patronymic. The genus is named in honor of the late Bhairav Aryal (Nepali: भैरव अर्याल), an iconic satirist of Nepali literature popularly known as the Emperor of Laughter (Nepali: हाँस्य सम्राट). The second part of the name, -donta, derives from the Greek word "ὀδών", meaning "tooth," and is a reference to Bhairav Aryal's iconic smile. The genus name is of feminine gender.

Type species.—*Aryalidonta itishreea* **sp. nov.** by original monotypy.

Species included.—Thus far, only the type species is known.

Distribution.—Currently known only from Nepal, from the type locality (Fig. 1).

Generic diagnosis.—The generic diagnosis is provided as a comparison of the type species of the tribe Thoradontini to better represent the true definitions of each genus.

From *Eucriotettix tricarinatus* (Bolívar, 1887), the type species of the genus *Eucriotettix*, this species is differentiated by the following characters: (i) the flat vertex with a low medial carina instead of well-expressed medial carina as in *E. tricarinatus*; (ii) bifurcation of the frontal costa in the upper quarter of the compound eye height is higher in *E. tricarinatus*; (iii) the prozonal carinae converging caudally instead of running parallel as in *E. tricarinatus*; (iv) rectangular lateral lobes provided with small protrusions instead of with long and sharp protrusions as in *E. tricarinatus*; and (v) the proximal halves of the middle femora are enlarged while the femora are slim throughout in *E. tricarinatus*.

From *Loxilobus acutus* Hancock, 1904, the type species of the genus *Loxilobus*, the new species is differentiated by the following characters: (i) the bifurcation of the frontal costa in the upper quarter of the compound eye height is a little higher in *L. acutus*; (ii) the paired ocelli in *A. itishreea* **sp. nov.** are placed a little below half of the compound eye height while in the upper quarter in *L. acutus*; (iii) the middle-level of the antennal grooves are at the level of the bottom of the compound eyes in the new species while in the bottom third of the compound eye height in *L. acutus*; (iv) the vertex is triangular and gently narrowing in the new species while triangular and more sharply narrowing in *L. acutus*; (v) the surface of the vertex flat with low carinae but convex in *L. acutus*; (vi) rectangular lateral lobes with small protrusions in the new species while with long and sharp protrusions in *L. acutus*; and (vii) the proximal halves of the middle femora are enlarged instead of slim as in the femora in *L. acutus*.

From *Thoradonta dentata* Hancock, 1909, the type species of the genus *Thoradonta*, *A. itishreea* **sp. nov.** is differentiated by the following characters: (i) the paired ocelli are placed a little below half of the compound eye height instead of in the lower third as in *T. dentata*; (ii) the middle-level of the antennal grooves at the level of the bottom of the compound eyes instead of antennal grooves below the level of the compound eyes; (iii) narrow instead of wide vertex; (iv) external carinae straight caudally of the humeral angles instead of incurved; (v) the lateral lobes projected laterally, rectangular with a small protrusion caudally and a sharp tip laterally instead of simple sharp lateral lobes; and (vi) the proximal halves of the middle femora are enlarged instead of slim throughout.

Fig. 1. Map of Nepal with the type locality marked. Gorkha District is shown in red, and the precise place of the type locality is indicated by the blue dot. The map is adapted after Sagarjkhatri, own work, CC BY-SA 4.0, Wikimedia commons.

***Aryalidonta itishreea* sp. nov.**

https://zoobank.org/62BD925B-D71C-4C92-BDF8-43C5956B95BF

Figs 2–6

Etymology.—The specific epithet is derived from the Nepali word "itishree", which is the title of one of Bhairav Aryal's books and translates to "The End". The name is also a reference to the tragic end of Bhairav Aryal's life, as well as to his unyielding belief that an end is an invitation to a new beginning. The name is Latinized with the suffix "-a" to form a noun in the nominative case and is feminine in gender.

Common name.—Aryal's Ten Avatar Groundhopper (Nepali: अर्यालको दश औतारी भुइँफड्के).

Common name etymology.—Named after one of Bhairav Aryal's masterpieces, Dash Autar (Nepali: दश औतार; English transl. Ten Avatars). The name symbolically refers to the many color forms observed among individuals of this species.

Type locality.—Amaldarchaur, Ghyalchok, Gorkha, Nepal (Nepali: अमलदारचौर, घ्याल्चोक, गोरखा, नेपाल) situated at an altitude of 465 ± 10 m asl (approximate) with GPS coordinates 27.809511°N, 84.718849°E. Shown in Fig. 1.

Material examined.—Type material. ***Holotype***: (Fig. 3) NEPAL • ♀; Gandaki Province, Gorkha District, Gandaki Rural Municipality, Ghyalchok, Amaldarchaur; 27.809511°N, 84.718849°E; 465 ± 10 m a.s.l; 30 Aug. 2022; M. Subedi leg.; sub-tropical forest with freshwater stream and agricultural lands, collected by hand; ANHM. ***Paratypes***: (Figs 4–6) NEPAL • 2 ♂, 1 ♀; Gandaki Province, Gorkha District, Gandaki Rural Municipality, Ghyalchok, Amaldarchaur; 27.809511°N, 84.718849°E; 465 ± 10 m.a.s.l; 30 Aug. 2022; M. Subedi leg.; sub-tropical forest with fresh water stream and agricultural lands, collected by hand; ANHM.

Additional material.—Numerous photographs of the individuals in their natural habitat, taken by the first author.

Photographic and video material.—The specimens of the type series in their natural habitat can be seen in Fig. 2 and in the video at https://youtu.be/iQi8iAH_DSQ.

Distribution.—Known only from the type locality and the surrounding areas.

Diagnosis.—This species is differentiated from all other Thoradontini species by the following combination of characters: (i) the bifurcation of the frontal costa in the upper quarter of the compound eye height; (ii) the paired ocelli placed a little below half of the compound eye height; (iii) vertex triangular, narrowing anteriorly, narrower than a compound eye in its anterior part; (iv) surface of the vertex flat with low carinae; (v) lateral lobes projecting laterally, rectangular with a small protrusion caudally and a sharp tip laterally; (vi) low and barely distinct carinae of the pronotum; (vii) prozonal carinae converging caudally; and (viii) proximal halves of middle femora enlarged.

Description.—(Fig. 3) Head: Eyes oval. Top margin of eyes above vertex. Vertex bulging between the carinae of vertex; small areas close to the medial carina are lowest. Frontal costa bifurcates in the upper quarter of the eye height. Facial carinae slightly divergent, forming a narrow scutellum a little wider on bottom. Lateral carinae of vertex follow outline of eye anteriorly, curving at level of frontal costa bifurcation and joining the scutellum a little below that level. Paired ocelli placed a little below half of compound eye height. Top margin of antennal groove above bottom margin of eyes, bottom margin below. Caudal margin of eye not in contact with anterior margin of pronotum. Vertex not visible above eyes. Facial carinae protruding in front of anterior level of eyes in lateral view. Head exserted above level of pronotal surface. Vertex at base of eyes same width as eye; slightly narrowing anteriorly; wider than half a compound eye at its apex. Anterior margin of vertex does not reach anterior margin of eyes; frontal costa at level of the anterior margin of eyes. Medial carina of vertex present in anterior half between eyes. Lateral carinae of vertex

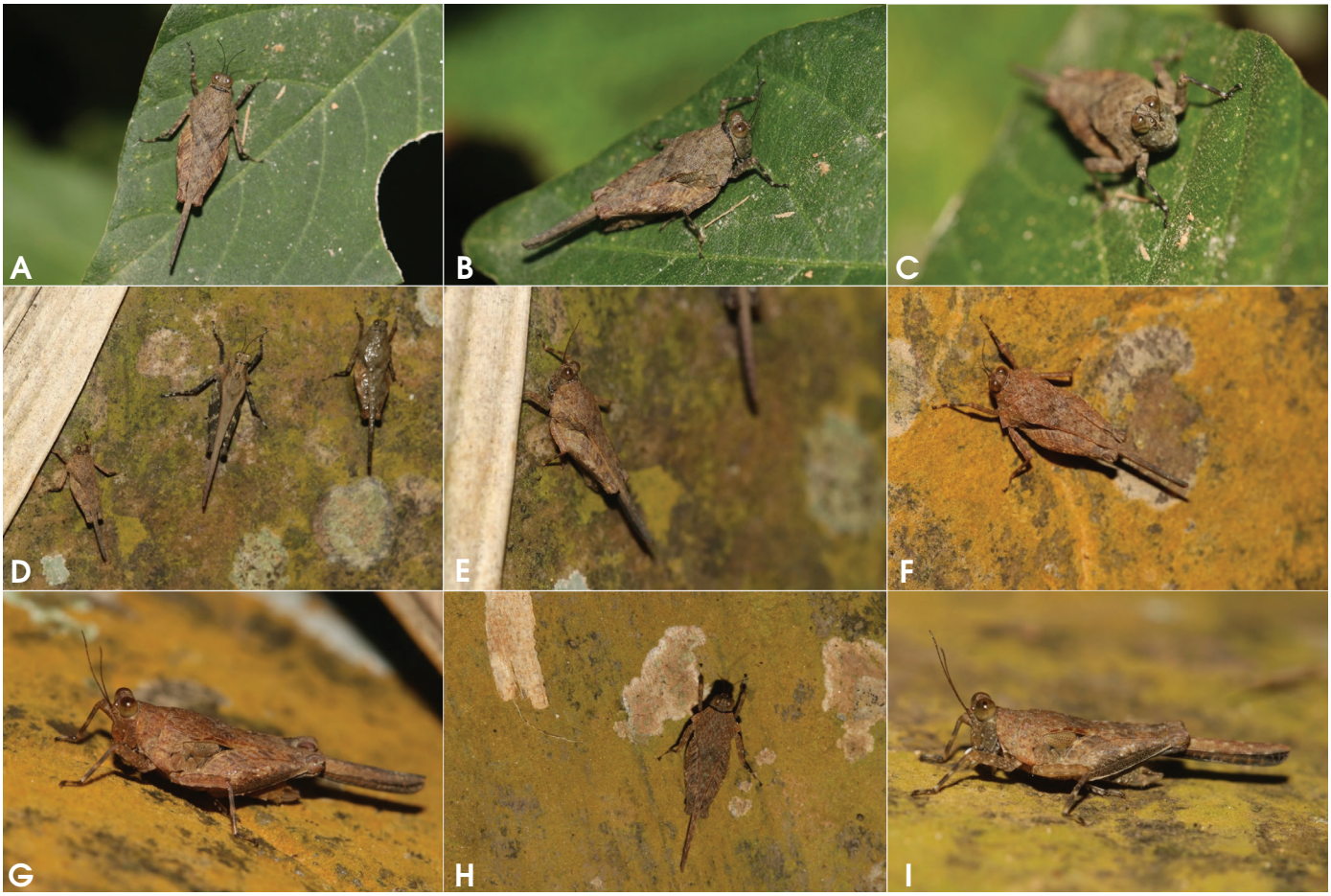

Fig. 2. Type specimens of *Aryalidonta itishreea* **gen. et sp. nov.** in their natural habitat. **A–C**. Holotype (♀); **D.** Paratype 1 (♂) (left) with *Criotettix* sp. (middle) and an individual of *Aryalidonta itishreea* **gen. et sp. nov.** (right); **E.** Paratype 1 (♂) in dorsolateral view; **F–G**. Paratype 2 (♀); **H–I**. Paratype 3 (♂).

present in anterior third between the eyes. Fossulae shallow, elongated, and present in anterior half of vertex between eyes.

Antennae: Filiform. As long as length between anterior margin of head and humeral angles. 14 antennomeres, apical one consisting of fused segments, possibly 2 or 3.

Pronotum: Macropronotal. Lateral surfaces of pronotum moderately converge dorsally. Pronotum widest at humeral angles. Dorsal surface mostly flat. Prozonal carina weakly elevated, slightly visible. Prozona sulcated with sulci of irregular shape. Apex of lateral lobe rectangular with slight protrusion in caudal part. Ventral and tegminal sinus in shape of a right angle. Humero-apical carina moderately visible. Infrascapular area subrectangular, a little narrower in anterior half. Lateral area progressively widening caudally. Median carina slightly elevated at transition between prozona and metazona, otherwise flat. Tubercles present throughout surface of pronotum. Entire surface covered with small nodules and larger tubercules. Anterior margin of pronotum truncated. Prozonal carinae composed of small nodules, weakly visible, converging caudally. Median carina continuous, reaching the apex of the pronotum, weakly visible in some areas. Lateral lobes projected laterally, rectangular with small protrusion caudally and sharp tip laterally. Humeral angles blunt. Last third of pronotum strongly narrowing. Before the narrowing, internal lateral carinae barely concave, revealing very narrow lateral area. Caudally of the narrowing, internal lateral carinae progressively converging towards apex. Apex of pronotum bluntly rounded.

Wings: Alae reaching apex of pronotum. Tegmina oval, entirely visible.

Legs: Front legs: Femora long and slim. Dorsal margin of femora slightly convex; ventral margin straight. Tibiae smooth. Middle legs: Femora long and slim; expanded in the proximal half, narrowing distally. Tibiae smooth. Hind legs: Femora smooth. Dorsal external area with slight parallel elevations. Antegenicular teeth moderately sized, triangular. Genicular teeth moderately sized, rectangular, parallel to bottom margin of femur. Tibiae smooth with several small spines. First tarsal segment longer than third. Pulvilli triangular, sharp; distal one two times larger than proximal two.

Sexual dimorphism.—No dimorphism observed between sexes except for the more expanded proximal parts of mid femora in males, and different terminalia. Female: Ovipositor valves elongated. Bottom valve narrow and serrated. Top valve expanded distally, serrated. Apices of valves acute, hook-like. Male: Elongated subgenital plate enclosing reproductive organs. Blunt apex.

Notes on variability.—Due to the position of the head during the fixation process of the holotype and the way it was pinned, its eyes do not reach the anterior margin of the pronotum. In other observed specimens, the eyes reach (or nearly reach) the anterior margin of the pronotum, which is the way this character appears when the animal is in a resting state.

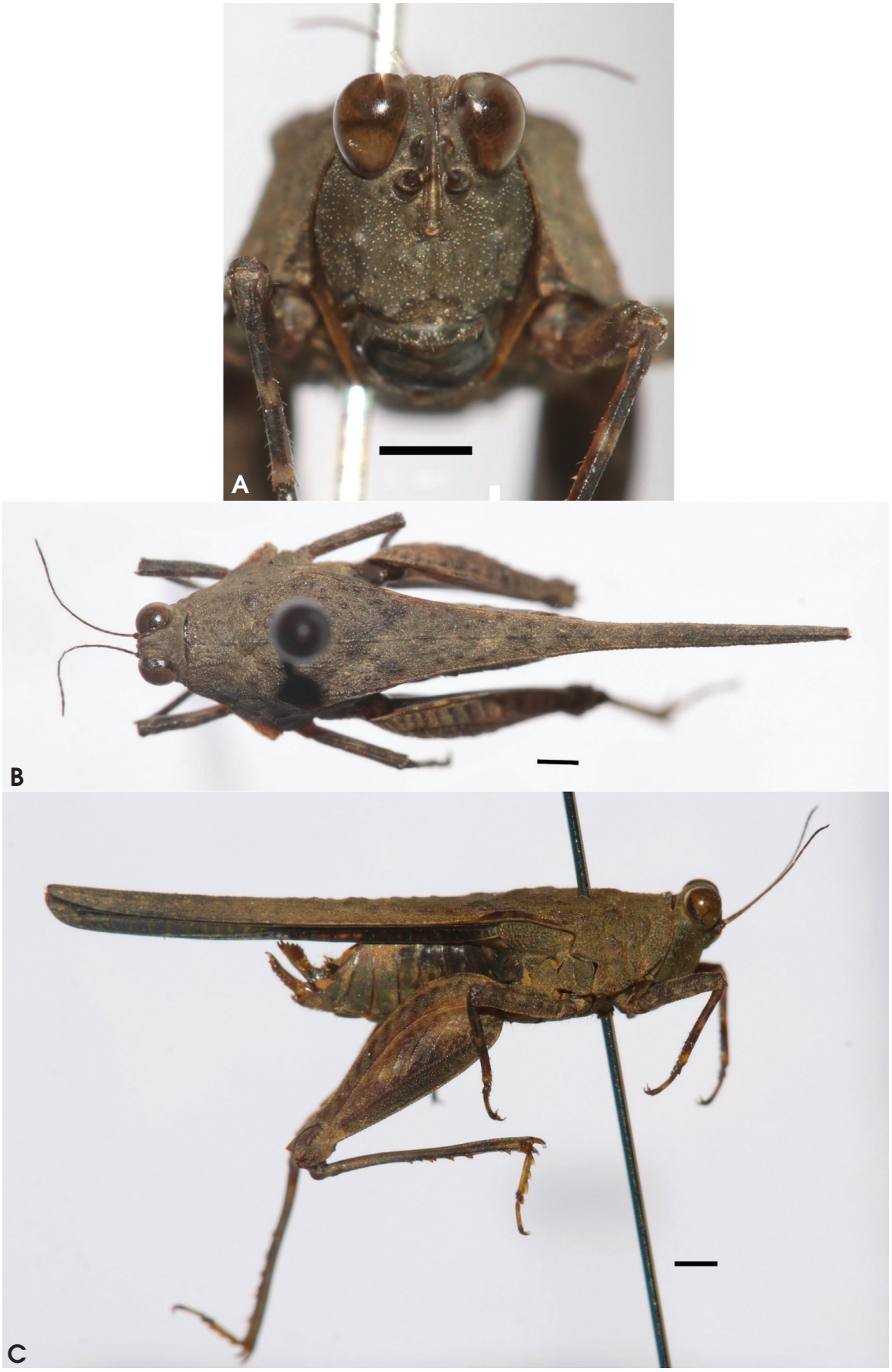

Fig. 3. Holotype (♀) of *Aryalidonta itishreea* **gen. et sp. nov. A.** Frontal view; **B.** Dorsal view; **C.** Lateral view. Scale bars: 1 mm.

Fig. 4. Paratype 1 (♂) of *Aryalidonta itishreea* **gen. et sp. nov. A.** Frontal view; **B.** Dorsal view; **C.** Lateral view. Scale bars: 1 mm.

Fig. 5. Paratype 2 (♀) of *Aryalidonta itishreea* **gen. et sp. nov. A.** Frontal view; **B.** Dorsal view; **C.** Lateral view. Scale bars: 1 mm.

Fig. 6. Paratype 3 (♂) of *Aryalidonta itishreea* **gen. et sp. nov. A.** Frontal view; **B.** Dorsal view; **C.** Lateral view. Scale bars: 1 mm.

The shape of the lateral carinae of the vertex is variable. These carinae usually form a u- or v-shaped structure in the anterior view but the parts of the carinae that are closer to the medial carina can be variably developed, i.e., the length of that part is variable.

The proximal part of the midfemora is expanded in all specimens, but this character is much more apparent in males than in females and can be considered to represent sexual dimorphism.

The basic shape of the lateral lobes is rectangular with more- or less-expressed protrusions laterally and caudally. In some cases, the lateral protrusion can form a short tooth or spine. The variability of this character is presented in Fig. 7.

Nymphs.—For the most part, the nymphs resemble the adults, with the obvious exception of the nymphs being brachypronotal and lacking wings and antegenicular teeth. All carinae in nymphs are better expressed than in adults. The lateral lobes in all the observed nymphs are of a basic shape, lacking the finer structures present in adults. The colors of nymphs are more saturated than those of adults. Nymphs of this species can be seen in Fig. 8.

Coloration.—Many different patterns of coloration have been observed and can be seen in Fig. 9. The coloration is cryptic, with patterns of coloration similar to that of the surrounding surfaces. The individuals of this species can be mostly uniformly colored (Fig. 9D, F, G, H) or have a more complex pattern in the form of a differently colored anterior part of the pronotum (Fig. 9B, C, E, I) or differently colored legs (Fig. 9A).

Measurements.—The key measurements of the holotype and the paratypes are presented in Table 1.

Note.—All measurements follow Tumbrinck (2014) and Tan and Artchawakom (2015), except the vertex width and eye width measured in frontal view (Fig. 10). The measurements of pronotum length were taken from the anterior margin of the pronotum to its tip, which is mistakenly shown from the tip of the head by Tumbrinck (2014).

Habitat description.—(Fig. 11) The habitat is a blend of agricultural land (Fig. 11E, F) and subtropical forest (Fig. 11B) dominated by Sal (*Shorea robusta*) trees with a freshwater stream, Tirtire khola (Nepali: तरितरि खोला) (Fig. 11D). The species is commonly found along the banks of the stream and desires paths—with plenty of algal and moss growth during the rainy season—made through the forest. The area experiences hot and humid spring/rainy seasons (March to September) followed by cool and dry autumn/winter seasons (October to February). The species is found in abundance during the hot and humid seasons that favor the growth of moss and algae (a food source of the species). The habitat stands on the steep slopes of red soil with ground vegetation dominated by *Chromolaena odorata*, *Oplismenus undulatifolius*, *Urena lobata*, *Murraya koenigii*, *Ageratum* sp., *Phyllanthus* sp., *Justicia adhatoda*, *Clerodendrum infortunatum*, *Lygodium microphyllum* under the covers of *Shorea robusta*, *Schima wallichii*, *Castanopsis indica*, and *Bambusa bambos*.

Species found in close proximity.—(Fig. 12) Tetrigids such as *Coptotettix annandalei* Hancock, 1915, *Criotettix* sp., *Hebarditettix quadratus* (Hancock, 1915), *Teredorus carmichaeli* Hancock, 1915,

Table 1. Measurements (in mm) of the holotype (HT) and the paratypes (PT) of *Ayalidonta itishreea* **gen. et sp. nov.**

Body parts	HT(♀)	PT1 (♂)	PT2 (♀)	PT3 (♂)
Body length	10.75	8.39	10.59	7.97
Vertex width	0.50	0.44	0.50	0.38
Eye width	0.80	0.64	0.70	0.63
Scutellum width	0.19	0.14	0.15	0.10
Pronotum length	16.73	14.62	16.24	12.06
Pronotum lobe width	5.00	4.38	5.16	3.63
Pronotum height	2.54	1.86	2.24	1.66
Tegmen length	1.84	1.39	1.89	1.42
Tegmen width	0.69	0.57	0.74	0.57
Alae length	12.44	10.57	13.34	10.62
Fore femur length	2.09	1.58	2.18	1.74
Fore femur width	0.56	0.51	0.55	0.44
Mid femur length	2.30	2.14	2.44	1.78
Mid femur width	0.66	0.50	0.62	0.44
Post femur length	6.15	5.29	6.64	5.56
Post femur width	2.16	1.77	2.24	1.76
Hind tibia length	5.40	4.61	5.59	4.45
First tarsal segment (basal) length	1.10	0.94	`1.07	0.85
Third tarsal segment (apical) length (without claws)	0.73	0.67	0.74	0.60
Subgenital plate length	–	0.80	–	0.84
Subgenital plate width	–	0.47	–	0.50
Ovipositor dorsal valve length	1.68	–	1.32	–
Ovipositor dorsal valve width	0.34	–	0.39	–
Ovipositor ventral valve length	1.43	–	1.21	–
Ovipositor ventral valve width	0.24	–	0.24	–

Fig. 7. The lateral lobe variability of *Aryalidonta itishreea* **gen. et sp. nov.** The basic shape is rectangular with more- or less-expressed protrusions laterally and caudally.

Fig. 8. Different nymphal instars of *Aryalidonta itishreea* **gen. et sp. nov.** (Note: the images are not on the same scale).

Thoradonta sp., *Xistra angusta* Ingrisch, 2001a share the habitat with *Aryalidonta itishreea* **gen. et sp. nov.**

Food source.—(Fig. 13) The species generally feeds on moss (adults feeding: https://youtu.be/rW_f3n_Yhf8, nymphs feeding: https://youtu.be/U7kM0Gme8ms), algae, lichens, and detritus. The individuals of this species can frequently be found on "desire paths"—paths mostly devoid of vegetation, formed by the frequent passage of animals or humans (Fig. 11A)—which have plenty of moss and algal growth during the monsoon period, and on the stones around a source of water, overgrown with moss, algae, and lichens (Fig. 11C).

Fig. 9. Variability of coloration in *Aryalidonta itishreea* **gen. et sp. nov.**

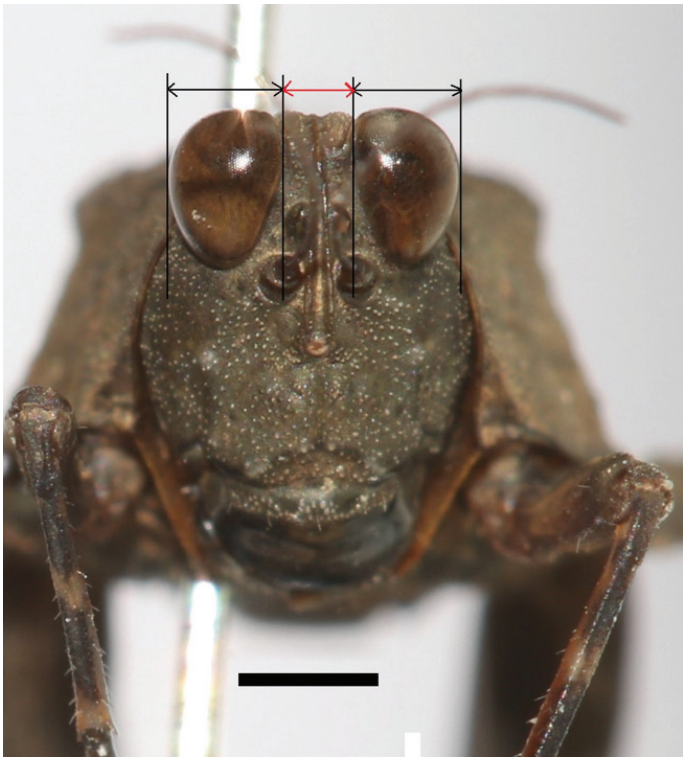

Fig. 10. Frontal view of *Aryalidonta itishreea* **gen. et sp. nov.** holotype showing the measurements of the vertex width (indicated by the red two-headed arrow) and eye width (indicated by the black two-headed arrow). The eye width is the average of the two eyes of the tetrigid. Scale bar: 1 mm.

Feces.—(Fig. 14) The feces are excreted in the form of pellets. The pellets appear as pyriform to oval or elongated balls of mud, suggesting that detritus is a major component of food intake. There may be remnants of undigested fibers in feces (indicated by the black arrow in Fig. 14), which are fragments of mosses or algae.

Interactions with other animals.—Wasps: (Fig. 15) Some individuals of the species were observed carrying adult wasps on their body surfaces. The wasps were not identified, but they possibly belong to the family Eulophidae as individuals belonging to this family have been found on Tetrigidae (Skejo 2017). This interaction was observed only among the individuals found along a desire path inside the Sal forest around 150 m east of the type locality (Fig. 15A). The wasps were observed either single (Fig. 15D) or in groups (Fig. 15B, C), mostly on the pronotum. The wasps were seen tightly holding onto the integument surface and were unmoved even when the groundhopper jumped or flew away. They appeared to be feeding on a substance on the surface of the pronotum, but the substance could not be seen using a macro lens. Video link to the observation: https://youtu.be/PO4gUwlDQbk

Mites: (Fig. 16) Several individuals of *Aryalidonta itishreea* **gen. et sp. nov.** were observed to be heavily infested with mites (Fig. 16B). These observations were made only on the bunds of rice fields in the type locality (Fig. 16A). However, the mites appear not to be species-specific and were also observed on *Thoradonta* sp. (Fig. 16C), which is the only other species observed on the rice bunds alongside the species of interest. The presence of mites created difficulty in the movement of the individuals, and the infested individuals were not as agile as other normal individuals. Interestingly, the groundhopper made kicking movements with its forelegs, presumably in an attempt to remove the mites from the body. Video link: https://youtu.be/i0t36jpxrdM (kicking movements 20 seconds into the video).

Discussion

Ecology.—Tetrigidae are well known for their cryptic coloration (Skejo and Caballero 2016, Skejo et al. 2020), and the new species is no exception. Its color forms are plentiful and reflect the different types of surfaces it inhabits. The nymphs are similar to adults in both coloration and general morphology and are thus easy to separate from other species. The importance of recognizing the nymphal morphology must be stressed, as there have been numerous cases of nymphs being described as separate species (Skejo et al. 2018). Studies on the ecology of Tetrigidae are not common, but some remarkable publications on that front have been produced, with ever-increasing innovations in data gathering and reporting (Paranjape and Bhalerao 1985, Paranjape and Bhalerao 1994, Pushkar 2009, Kočárek et al. 2011).

The gathered data on feeding habits (Fig. 13) conform to the known fact that Tetrigidae use detritus as their main source of food, supplemented by algae, mosses, lichens, and some other minor substances (Kuřavová et al. 2017). The excrement often contains strands of undigested plant matter (Fig. 14), but it is unclear what percentage of the total plant intake it represents, i.e., how well Tetrigidae digest cellulose and hemicellulose. Such research has been conducted on some Caelifera (Wang et al. 2020) and should be conducted on Tetrigidae as well to expand on the work by Kuřavová et al. (2017).

The wasps, tentatively identified as Eulophidae members, interact with *Aryalidonta itishreea* **gen. et sp. nov.** (Fig. 15) in a way that currently cannot be conclusively explained. Eulophidae consist mostly of parasitoids of holometabolous insects, but many other life cycles are represented within that family (Gauthier et al. 2000). There are many species capable of penetrating tree bark to lay eggs in their hosts (Beaver 1966, Abell et al. 2012), so it is entirely possible that a groundhopper can be oviposited in through the hard surface of the pronotum. However, the wasps seemed to be feeding on a substance attached to the pronotum or drinking fluids trapped on it, making the possibility of parasitoidism unlikely. It is likely that the wasps' feeding is opportunistic, but the possibility of them being drawn to a specific symbiotic organism should also be examined.

Mites are commonly spotted on tetrigids, but the taxonomy of the group associated with Tetrigidae is still young (Seeman et al. 2018). The observed specimens of mites (Fig. 16) were not identified, so nothing can be confidently said about host specificity or habitat preference, although it seems that the bund on the edge of a rice field houses an unusual number of mites.

The state of Thoradontini.—The tribe Thoradontini currently stands alone, as it was recently excluded from Scelimeninae due to mounting evidence that it does not form a monophyletic clade with Scelimenini (Adžić et al. 2020). The relationships within the tribe are unclear, and the currently available molecular data are scarce, encompass a small number of genes, and are not representative of the tribe as a whole (Chen et al. 2018, Adžić et al. 2020). Our own examination of the species within this tribe showed that many of its species do not fit completely with the genera under which they are classified. For example, *Loxilobus willemsei* Günther, 1938a, *Loxilobus insularis* (Günther, 1935),

Fig. 11. Type locality and habitat of *Aryalidonta itishreea* **gen. et sp. nov.** (Amaldarchaur, Ghyalchok, Gorkha, Nepal). **A.** Desire path through the type locality (Note: the algal growth is indicated by the arrow); **B.** Subtropical forest; **C.** Stones with moss, lichen, and algal growth (Note: several *Aryalidonta itishreea* **gen. et sp. nov.** individuals on the stone); **D.** Tirtire khola, a freshwater stream in the heart of the locality; **E.** Rice fields with ample greenery during the rainy season; **F.** Rice fields during the fall season.

and *Loxilobus insidiosus* (Bolívar, 1887) have lower-placed antennal grooves and paired ocelli, a distinct convexity in the middle part of the vertex, narrower vertex, narrower space between the prozonal carinae, and simpler lateral lobes than in the type species, *L. acutus*. They could represent a single genus or multiple genera separate from *Loxilobus* and of still undetermined taxo-

Fig. 12. Different Tetrigidae species found in close proximity to *Aryalidonta itishreea* **gen. et sp. nov.** in their natural habitat. **A.** *Coptotettix annandalei*; **B.** *Criotettix* sp. (bottom left) with *Aryalidonta itishreea* **gen. et sp. nov.** (top right); **C.** *Hebarditettix quadratus*; **D.** *Teredorus carmichaeli* (right) with *Aryalidonta itishreea* **gen. et sp. nov.** (left); **E.** *Thoradonta* sp. (bottom right) with *Aryalidonta itishreea* **gen. et sp. nov.** (top left); **F.** *Xistra angusta*.

nomic placement. Furthermore, *Eucriotettix molestus* Günther, 1938b, *Eucriotettix aequalis* (Hancock, 1912), *Eucriotettix spinilobus* (Hancock, 1904), and *Eucriotettix hainanensis* Günther, 1938a each differ from the type species, *E. tricarinatus*, by different combinations of antennal groove placement, shape of the vertex in frontal and dorsal view, and shape of the lateral lobes. It is un-

Fig. 13. Individuals of *Aryalidonta itishreea* **gen. et sp. nov.** on different food sources. **A.** Lichen growing on the stones; **B.** Detritus on a desire path; **C.** Algal growth on the banks of a freshwater stream (Note: soft algal growth on the body surface indicated by greenish–yellow coloration); **D.** Moss growth on the desire path.

Fig. 14. Feces of *Aryalidonta itishreea* **gen. et sp. nov.** (Note: The feces were collected from four individuals (2 males and 2 females) raised in captivity by feeding moss and detritus). The black arrow points to an undigested plant fiber.

Fig. 15. *Aryalidonta itishreea* **gen. et sp. nov.** interaction with a wasp (likely family Eulophidae). **A.** Desire path (indicated by black arrow) inside the Sal forest with moss and algal growth; **B.** Multiple wasps resting on the pronotum of the individual tetrigid; **C.** Closeup of lateral view of wasps; **D.** Closeup of dorsal view of an individual wasp.

clear whether these represent species-level characters or imply that the genus should be split into more genera. The situation is further complicated by the existence of the genus *Criotettix* Bolívar, 1887, assigned under a separate tribe, Criotettigini. Within that genus, there are species such as *Criotettix pallitarsis* (Walker, 1871), *Criotettix armigera* (Walker, 1871), and even the type species *Criotettix bispinosus* (Dalman, 1818) that, with their convex and triangular vertices and their facial morphologies, seem to belong to *Eucriotettix*, which signifies some future nomenclatural acts and further complications within *Eucriotettix* and Thoradontini. It is difficult to determine an extensive set of valuable diagnostic characters for the genera within Thoradontini and Criotettigini, which is why nomenclatural acts are not proposed in this study. A comprehensive revision of these taxa is urgently needed to resolve more than a hundred years of previous taxonomic work (Hancock 1904, Hancock 1909, Günther 1937, Günther 1938a, b, Blackith 1992, Tumbrinck 2018).

The herein described genus and species, *Aryalidonta itishreea* **gen. et sp. nov.**, does not fit with the diagnoses of the other genera of Thoradontini but shares many similarities with them. In this study, we do not transfer any species between genera because this would, in essence, require us to resolve a myriad of problems with the definition of Thoradontini taxa, which is an undertaking that should be approached carefully and thoroughly. We place *Aryalidonta* **gen. nov.** within this tribe, as the morphology of the new species is very similar to many of the recognized species within the tribe but is still distinct enough to warrant a clear separation from them. Additionally, due to the previously mentioned problems, we believe it is important to describe a genus with clear diagnostic characters and with ample material, as this will assist the subsequent revisions and possibly offer a taxon under which some of the existing, more weakly defined species can be classified.

Considering the state of Thoradontini, it is impossible to comment on the way the new genus phylogenetically relates to the other genera within it. A passing comment can be made on the shape of the lateral lobes. These protrusions are well developed in some species of Thoradontini, notably in most *Thoradonta* species. This character in *Aryalidonta* **gen. nov.** is simpler and closer to that of most other Tetrigidae species. This suggests that the shape of lateral lobes in *Aryalidonta* **gen. nov.** represents an ancestral form of that character within Thoradontini and that the complex morphologies of lateral lobes within Thoradontini could have descended from such a shape.

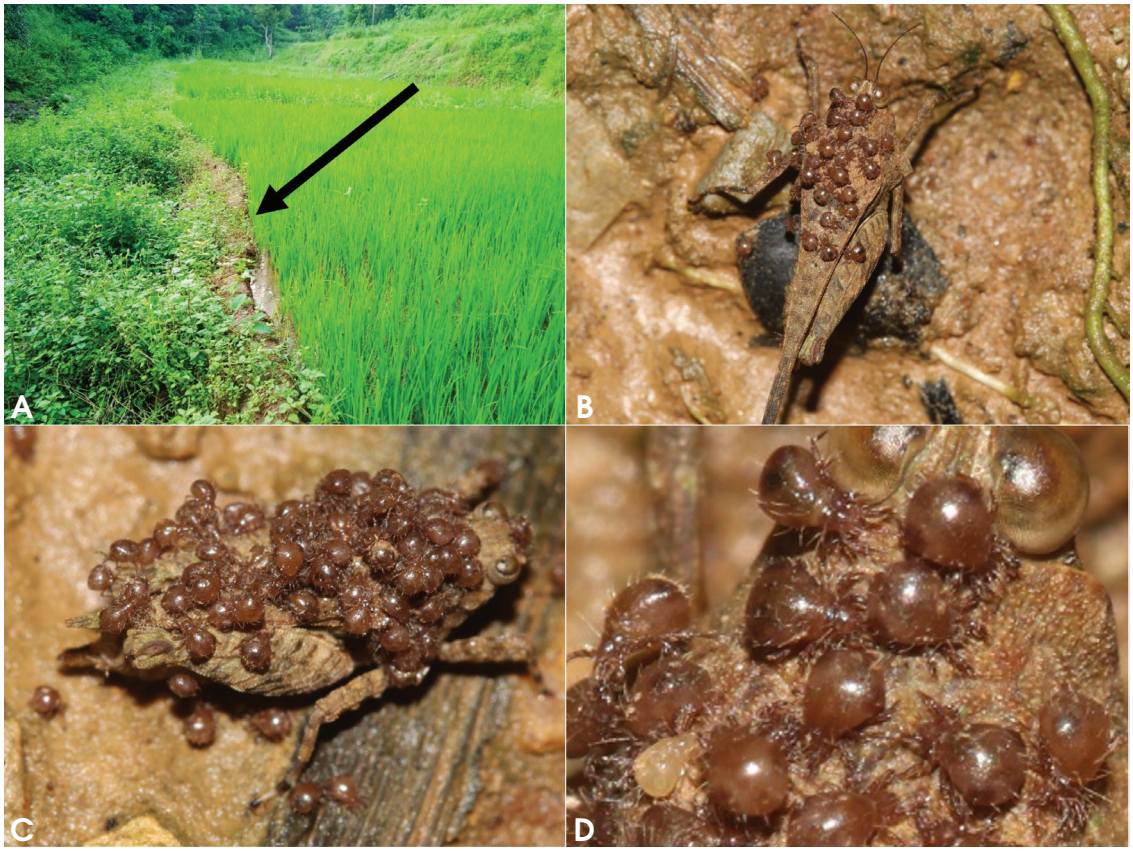

Fig. 16. *Aryalidonta itishreea* **gen. et sp. nov.** interaction with mites: **A.** Bund on the edge of a rice field (indicated by the black arrow); **B.** An individual *Aryalidonta itishreea* **gen. et sp. nov.** infested with mites; **C.** An individual *Thoradonta* sp. infested with mites; **D.** Closeup of mites on the body surface.

Conclusions

In this paper, we presented a detailed account of observations pertaining to *Aryalidonta itishreea* **gen. et sp. nov.** but it is clear that many unknowns remain, within the new species and without it. Thoradontini and Criotettigini are tribes of uncertain placement and in certain need of revision. The conclusion of this study is just a starting point for many to follow, or in the words of Bhairav Aryal, "Itishree is not an approval of the end; just the end of a chapter" (Aryal, 1971). Itishree.

Authors' contributions

MS conducted the fieldwork. MS and NK analyzed the data, wrote the manuscript, and created the figures. Both authors are equal in contribution.

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A new species of the genus *Skejotettix* (Orthoptera, Tetrigidae) from Nepal

Madan Subedi[1,2]

1 Agriculture Science Center, Directorate of Research and Extension, Agriculture and Forestry University, Ghyalchok, Gorkha, Nepal.
2 SIGTET-Special Interest Group Tetrigidae, Bonn, Germany.

Corresponding author: Madan Subedi (madansubedi13@gmail.com)

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Abstract

This paper describes a new species of *Skejotetix* Subedi, 2022, *S. kasalo* **sp. nov.,** from the temperate forests of Bajung, Parbat, Nepal. The genus was known only from the subtropical regions of Nepal. The new species and its different life stages were observed in the natural habitat. It was found to have many colors that match perfectly with the surroundings. The genus *Skejotettix* was considered brachypronotal until now, but a macropronotal form was also found alongside the typical brachypronotal form in *S. kasalo* **sp. nov.** The macropronotal form is an important piece of the puzzle in determining the relationship between *Skejotettix* and *Ergatetttix* Kirby, 1914.

Keywords

Gandaki Province, habitat, Parbat District, pygmy grasshoppers, sloping grassland, taxonomy, temperate forests, Tetriginae

Introduction

Nepal is a biogeographical and geomorphological pearl of Asia (Bhuju 2007), immensely rich in biodiversity, comprising 69 species of tetrigids (Subedi 2022, this paper). This number is remarkable given the size of the country. However, the fauna of Tetrigidae is still an understudied group of Orthoptera in Nepal (Subedi 2022, Subedi and Kasalo in press). This is mainly attributed to the lack of local researchers, as the sparse information available on the group was studied and collected entirely by foreign researchers (Chopard and Dreux 1966, Bey-Bienko 1968, Balderson and Yin 1987, Martens 1987, Ingrisch 1987, 2001a, b, 2006, Ingrisch and Garai 2001, Tumbrinck 2015). Now, with the advent of local researchers and collaborators, several species new to science have been discovered, and more information is available on these insects (Subedi 2022, Subedi and Kasalo in press).

Skejotettix Subedi, 2022 is a genus endemic to Nepal, hitherto comprising two small and brachypronotal subtropical species, *S. netrajyoti* Subedi, 2022 and *S. muglingi* (Ingrisch, 2001b) (Ingrisch 2001b, Subedi 2022). With the discovery of a new species of the genus from temperate regions, this paper aims to describe this species, give insights into its ecology, and present some hypotheses on the evolutionary history of the genus.

Materials and methods

Museum abbreviations.—

ICAG Insect collection of Agriculture Science Center, Ghyalchok, Gorkha, Nepal.

Fieldwork.—The new species was spotted for the first time while photographing a *Criotettix* cf. *bispinosus* on a lichen-covered stone amid Crofton weed bushes (Fig. 2A) near the freshwater stream of Panikhola, Bajung, Parbat on 28th September 2022. Only a single individual female was spotted at that locality. Later on the same day, an adult male and a nymph were spotted on a clifftop of Basnee Gaun. More individuals were found in Kafleko Pakho, a location around 500 meters north of Basnee Gaun, when investigated two days later. As an entire population of the species was found in Kafleko Pakho, it was selected as the type locality. The holotype and several paratypes were photographed in the wild, collected by hand, killed using an ethyl acetate killing jar, and pinned with Phusis stainless steel pins (size #0) for storage in a collection box. Additional material is represented by photographs taken in the field. The localities were visited on a regular basis over a period of a month (September–October 2022) to gain more insights into the life stages and ecology of the species. The vegetation in the type locality were identified first by their vernacular names with the help of local people, which were then checked for the appropriate binomial names following Jnawali and Neupane (2021). This was again cross-checked with the help of images available on the internet.

Fieldwork localities.—(Figs 1, 2). The localities investigated by the author are listed in Table 1.

Table 1. Investigated localities in Bajung, Parbat, Nepal, with local Nepali names, coordinates, elevations, brief habitat type descriptions, and video links to the locality. Kafleko Pakho is the type locality.

Locality information	Localities		
	Kafleko Pakho	**Basnee Gaun**	**Pani Khola**
Nepali	काफ्लेको पाखो	बस्नी गाउँ	पानी खोला
Coordinates	28.27626°N, 83.69860°E	28.27321°N, 83.69778°E	28.27569°N, 83.70610°E
Elevation (m a.s.l.)	1920 (approx.)	1880 (approx.)	1665 (approx.)
Habitat type	Countryside road amid sloping grassland and forest	Sloping grassland on the edge of a cliff	Stream bank
Video link	https://youtu.be/JtwmYAKEayo	https://youtu.be/_B9wkh7LBRc	https://youtube.com/shorts/PNub1-S_b80?feature=share

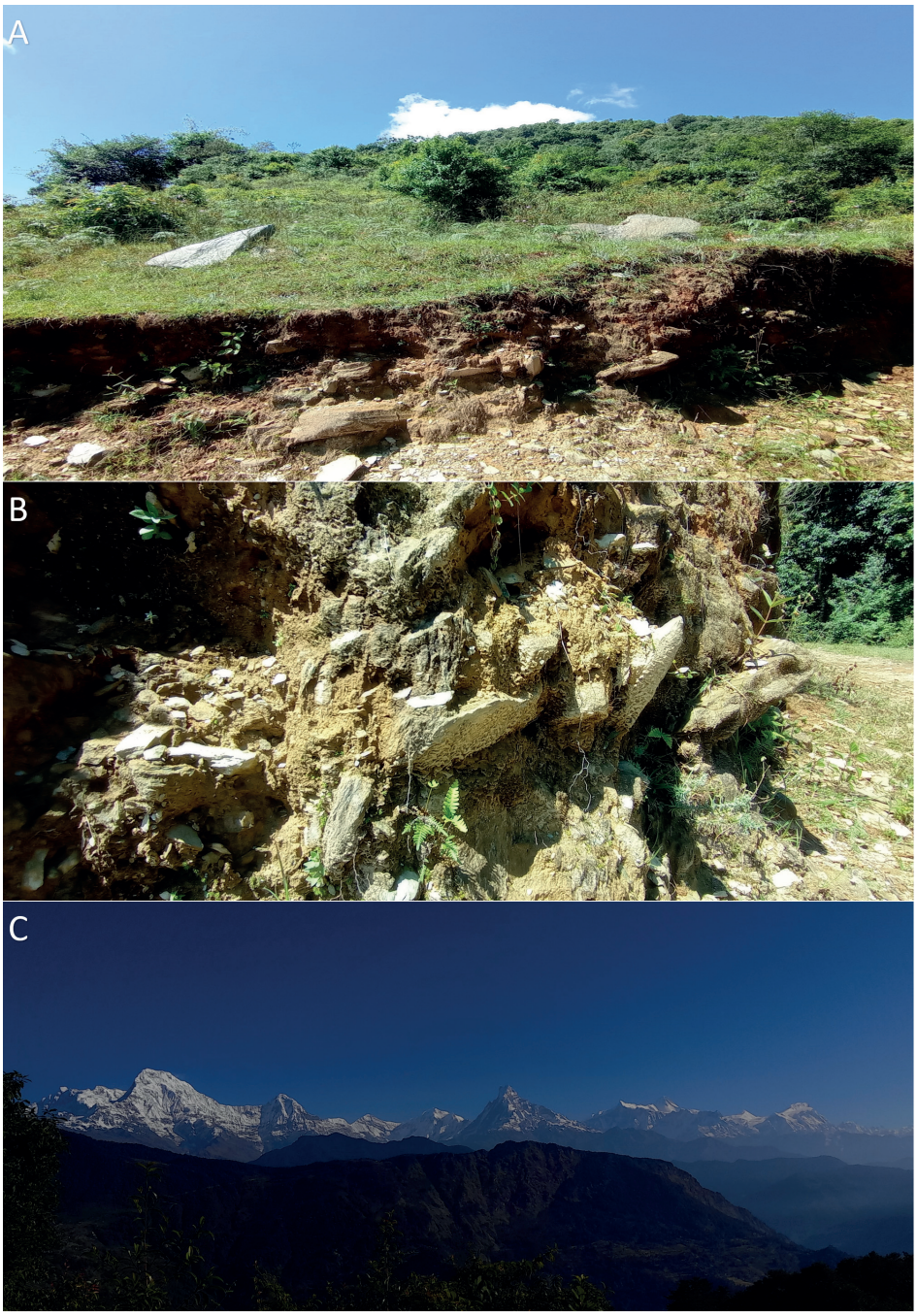

Fig. 1. Type locality of *Skejotettix kasalo* **sp. nov.**, Kafleko Pakho, Bajung, Parbat. **A.** Sloping forest and grassland; **B.** Muddy walls alongside the dirt road through the type locality; **C.** Beautiful Annapurna and Mansiri Himal range seen from the type locality.

Fig. 2. Investigated localities from Bajung, Parbat, Nepal. **A–C.** Panikhola. **A.** Lichen-covered stone (indicated by a white arrow) amid Crofton weed bushes; **B.** Fresh water stream; **C.** Forest and bushes; **D–E.** Basnee Gaun. **D.** Pond and surroundings; **E.** Sloping grasslands.

Identification, taxonomy, and nomenclature.—The morphological characters of the new species were compared with the original descriptions of the aforementioned species of the genus *Skejotettix* Subedi, 2022 (Ingrisch 2001b, Subedi 2022). Taxonomy follows the international database of Orthoptera, the Orthoptera Species File (OSF), version 5.0/5.0 (Cigliano et al. 2022). Nomenclature is in accordance with the 4th edition of the International Code of Zoological Nomenclature (ICZN 1999).

Photography.—A Canon EOS 80D field camera, with a Canon macro lens 100 mm 1:2.8 USM, was used for photographing the species in their natural habitat and for type digitalization. Photos and videos of the localities and habitats were taken by mobile phone cameras (Xiaomi Redmi Note 9 and Xiaomi Poco C3). Images of the pinned type series were post-processed with the software Photoshop 2022 v40.0. The scale was added after calibration with millimeter paper with ImageJ v1.53k software (Rueden et al. 2017).

Morphological terminology and measurements.—Morphological terminologies follow Tumbrinck (2014), and measurements used follow Tumbrinck (2014) and Tan and Artchawakom (2015). The eye width and vertex width are measured as per Subedi and Kasalo (in press).

Results

Taxonomy

Family Tetrigidae Rambur, 1838
Subfamily Tetriginae Rambur, 1838

Genus *Skejotettix* Subedi, 2022

Updated generic diagnosis.—Small-sized, macropronotal or brachypronotal genus. Antenna inserted between or lower than the ventral margins of compound eyes. Scapus of antenna with a large notch in lateral view. Frontal costa visible in front of the compound eyes in lateral view. Lateral carinae curved inward to the frontal costa in a more or less straight manner. Vertex narrower than the width of a compound eye. Median carina entire; raised in the anterior part of the pronotum. Wings extended beyond the pronotum, reduced, hidden, or absent. Femora robust.

Type species.—*Skejotettix netrajyoti* Subedi, 2022.

Composition.—Three species. *S. netrajyoti*, *S. muglingi*, and *S. kasalo* **sp. nov.** are currently known.

Distribution.—Endemic to Nepal, the genus (with its three species) is distributed in subtropical and temperate regions. *Skejotettix netrajyoti* is known only from the subtropical Sal forest of Churlingtar, Ghyalchok, Gorkha (Subedi 2022). *S. muglingi* is known from the Trishuli River near the Mugling Bazar riverbed (Ingrisch 2001b); the stream banks of Buldi khola, Vyas Municipality, Tanahun; in the vicinity of the manmade ponds of Ghadiyal Breeding Center, Kasara amid the subtropical Sal forest; and in the vicinity of the manmade ponds of Umari, Kapilvastu (Subedi 2022). *Skejotettix kasalo* **sp. nov.** is known from different localities of Bajung, Parbat, Nepal: Kafleko pakho (type locality), Basnee Gaun, and Panikhola. The locations are shown in Fig. 3.

***Skejotettix kasalo* sp. nov.**
https://zoobank.org/EF029366-B317-40FB-912B-DAA2C09F10C7

Justification of genus assignment.—The herein-described species conforms to the typical characters of the genus *Skejotettix* as mentioned above in the diagnosis section.

Etymology.—The species is named after Niko Kasalo, an emerging tetrigidologist from Croatia and a good friend of mine. The

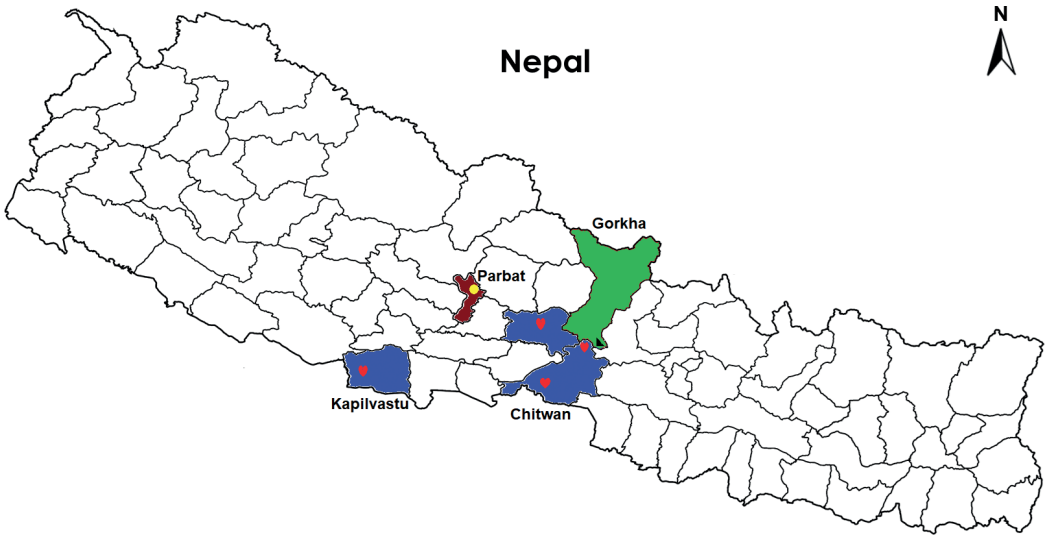

Fig. 3. Map of Nepal showing the distribution of *Skejotettix*. The district in green denotes the distribution of *S. netrajyoti*, with the precise locality denoted by a black triangle. The districts in blue denote the distribution of *S. muglingi*, with the precise localities denoted by red hearts. The district in dark red denotes the distribution of *S. kasalo*, with the precise locality denoted by a yellow dot. The map is adapted after Sagarjkhatri, own work, CC BY-SA 4.0, wikimedia commons. The localities for *S. muglingi and S. netrajyoti* are based on the findings of Ingrisch (2001b) and Subedi (2022). (Note: The names in the map refer to the respective adjacent districts in color.)

specific epitheton is a Latinized noun in apposition referring to the surname of Niko Kasalo (patronymic).

Common name.—Bajung's cliffhopper.

Common name etymology.—The first part of the name is after the village of Bajung where the species was discovered. The second part of the name denotes the habitat type of the species.

Type locality.—(Fig. 1) Kafleko Pakho, Bajung, Parbat, Nepal (Nepali: काफ्लेको पाखो, बाजुङ, पर्वत, नेपाल) situated at an altitude of 1920 m a.s.l. with GPS coordinates, 28.27626°N, 83.69860°E. The type locality is situated at the top decile of the hill of Bajung village, which has a cool temperate climate. The area is a part of the Dhiku-Maidan community forest with sloping terrain composed of grassland and forest. The ground vegetation includes *Ageratina adenophora* (Spreng.) King & H. Rob., *Anaphilis* sp., *Nephrolepis cordifolia* (L.) C. Presl, *Gnaphalium affine* D. Don, *Rubus ellipticus* Sm under the cover of *Symplocos theifolia* D. Don, *Daphniphyllum himalayense* (K. Rosenthal), *Rhododendron arboreum* Sm., *Schima wallichii* (DC.) Korth, *Prunus cerasoides* D. Don., *Eurya acuminata* DC., *Semecarpus anacardium* L.f., *Myrsine semiserrata* Wall., *Elaeagnus parvifolia* Wall. ex Royale, and *Myrica esculenta* Buch.-Ham. ex D. Don. A dirt road (constructed in 2019) now runs through the locality; the road has mud walls, supporting a plethora of moss and algal growth. The holotype and several paratypes, as well as additional materials, were collected and photographed on these walls.

Material examined.—**Type material. Holotype** (Fig. 5A–C): NEPAL • ♀; Gandaki Province, Parbat District, Modi Rural Municipality, Bajung, Kafleko Pakho; 28.27626°N, 83.69860°E; 1920 m a.s.l; 30.IX.2022; M. Subedi leg.; countryside road amid sloping grassland and forest, collected by hand, ICAG; ICAG-ORT-TETR1.

Paratypes.—(Figs 5D–L, 6) NEPAL • 1♂; Gandaki Province, Parbat District, Modi Rural Municipality, Bajung, Basnee Gaun; 28.27321°N, 83.69778°E; 1880 m a.s.l; 28.IX.2022; M. Subedi leg., sloping grassland on the edge of a cliff, collected by hand, ICAG; ICAG-ORT-TETR2 • 1♀; Gandaki Province, Parbat District, Modi Rural Municipality, Bajung, Panikhola; 28.27569°N, 83.70610°E; 1665 m a.s.l; 28.IX.2022; M. Subedi leg.; stream bank; collected by hand, ICAG; ICAG-ORT-TETR3 • 1♂; Gandaki Province, Parbat District, Modi Rural Municipality, Bajung, Kafleko Pakho; 28.27626°N, 83.69860°E; 1920 m a.s.l; 30.IX.2022; M. Subedi leg.; countryside road amid sloping grassland and forest; collected by hand, ICAG; ICAG-ORT-TETR4 • 1♀; Gandaki Province, Parbat District, Modi Rural Municipality, Bajung, Kafleko Pakho; 28.27626°N, 83.69860°E; 1920 m a.s.l; 11.X.2022; M. Subedi leg.; countryside road amids sloping grassland and forest, collected by hand, ICAG; ICAG-ORT-TETR5.

Additional material.—Several adults and nymphs were photographed in the wild to study the characters and variations among individuals. The videos recorded are uploaded to the social media platform YouTube (YouTube channel: Nepali Grasshoppers, https://www.youtube.com/channel/UCp9QdbRCPSy19KZbnsPw_JQ).

Photographic material.—The specimens of the type series in their natural habitat are shown in Fig. 4.

Diagnosis.—*Skejotettix kasalo* differs from the type species of *Ergatettix* Kirby, 1914, *E. dorsiferus* (Walker, 1871) in having a wider vertex with parallel lateral borders instead of a narrow vertex with lateral borders usually converging towards the front, with convex vertex (in frontal view) without horns instead of being concave with the presence of horns, and with the middle femora being robust instead of being slender. However, the species also share similarities: (i) presence of a notch on the scapus of the antenna; (ii) wings extending beyond the pronotum (valid in macropronotal form of *S. kasalo*); (iii) antennal grooves distinctly below the ventral margins of compound eyes; and (iv) median carina (in lateral view) undulated.

Skejotettix kasalo **sp. nov.** can be differentiated from other species of the genus by the set of characters listed in Table 2.

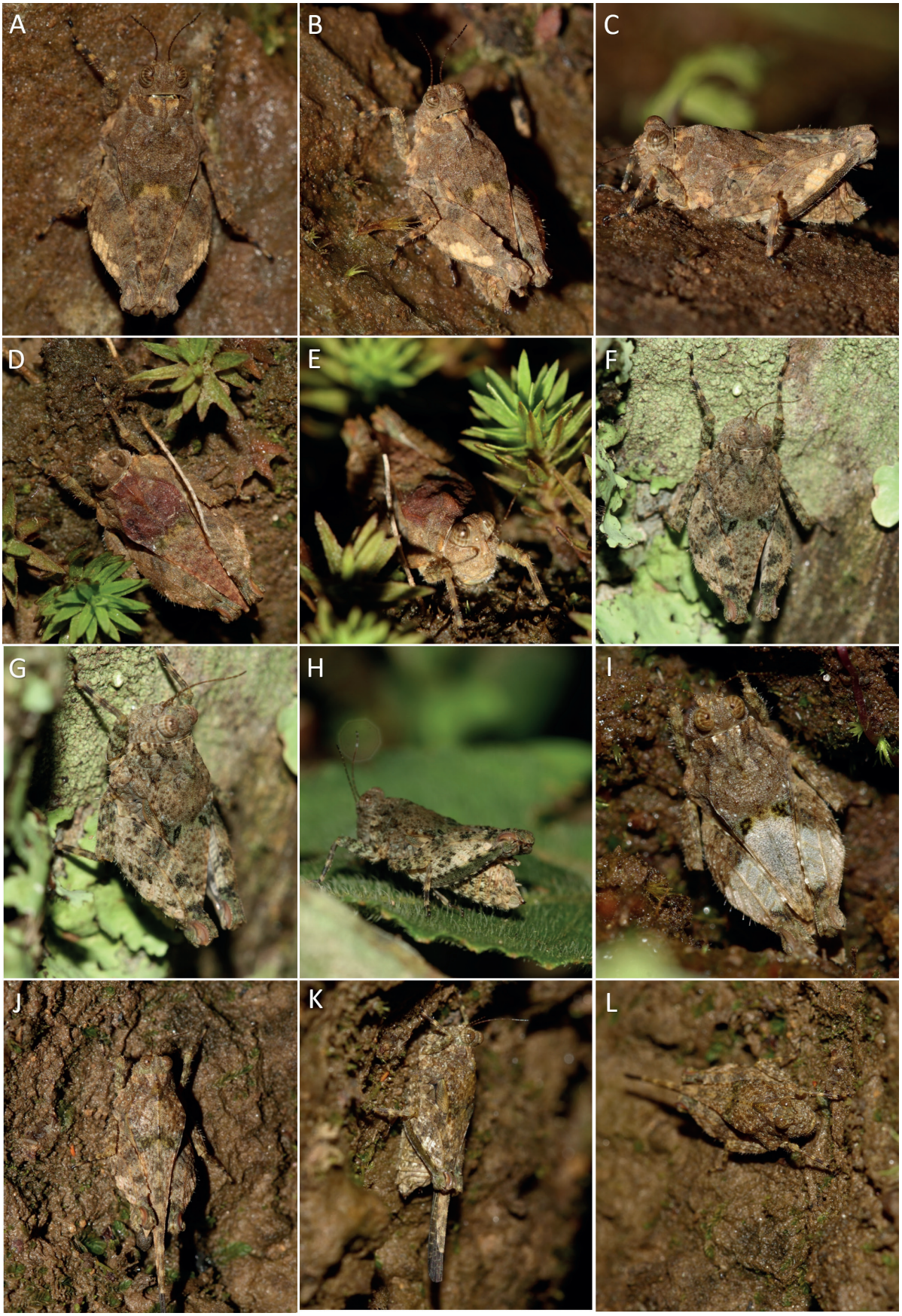

Fig. 4. The type specimens of *Skejotettix kasalo* **sp. nov.** in their natural habitat. **A–C.** Holotype (♀); **D–E.** Paratype 1 (♂); **F–H.** Paratype 2 (♀); **I.** Paratype 3 (♂); **J–L.** Paratype 4 (♀).

Table 2. Comparison of the diagnostic characteristics of the three currently known *Skejotettix* species.

Character	***S. kasalo*** **sp. nov.**	***S. netrajyoti***	***S. muglingi***
Upper point of insertion of the antennal grooves	Below the lower margin of the compound eyes	In line with the lower margin of the compound eyes	In line with the lower margin of the compound eyes
Pronotum	Brachypronotal or macropronotal; apex bluntly rounded	Brachypronotal, cuneate with apex emarginate	Brachypronotal, obtuse–angularly rounded
Alae	Produced distinctly beyond or slightly shy of the pronotum	Reduced	Reduced, absent, or hidden
Median carina (in lateral view)	Distinctly undulated	Undulated	Relatively uniform
Claws	Larger	Shorter	Shorter
Infrascapular area	Intermediate in width	Narrower	Widened
Scutellum (in relation to scapus)	Wider	Almost as same width	Narrower
Habitat	Temperate forests	Subtropical Sal forests	Near the water sources of the subtropical regions

*Description.—**Head***: Eyes reniform. Top margin of eyes higher than vertex. Vertex low and flat between lateral carinae of vertex. Frontal costa bifurcates at around half length of compound eye from top. Facial carinae divergent, forming scutellum as wide as scapus before widening distinctly at bottom. Lateral carinae of vertex following outline of eye anteriorly; curved inward, almost straight at half-length of frontal costa from the top. Nodules distinct from top of vertex to halfway of compound eye. Paired ocelli placed at about bottom third. Top margin of antennal groove below lower margin of compound eyes. Minute hairs distributed all over face. Caudal margin of eye not in contact with anterior margin of pronotum. Vertex below top margin of eyes. Frontal costa visible as straight in front of anterior level of eyes, incurves halfway from top of compound eye, diverges into two facial carinae and runs straight down to lower margin of compound eye. Facial carinae curve outwards, forming a convex protrusion. Head exserted above general surface of pronotum, vertex approximately at level of elevation in anterior part of pronotum. Hairs present in lower half of face. Vertex at base of eyes wider than an eye, slightly narrowing anteriorly, as wide as an eye at its apex. Anterior margin of vertex in line with anterior margin of the eyes; frontal costa produced slightly ahead of anterior margin of eyes. Medial carina of vertex extending slightly beyond anterior half of vertex between eyes. Lateral carinae of vertex present in anterior half of vertex between eyes. Fossulae shallow, elongated, and present in anterior two-thirds of vertex between eyes. Posterior margin of eyes distinctly in front of anterior margin of pronotum.

Antennae. Filiform. As long as length between anterior margin of head and lateral lobes of pronotum. Scapus with a large notch in lateral view. The number of antennomeres is 16, with apical one consisting of fused segments, possibly 2 segments.

Pronotum: Brachypronotal, tip of the pronotum slightly shy of tip of abdomen. Sub rectangular. Median carina raised in anterior part of pronotum above vertex giving a tectiform appearance. Median carina sinuate, with most prominent elevation at transition between prozona and metazona; undulation gradually decreases in elevation afterwards. Prozonal carinae elevated, clearly visible. Prozona sulcated with sulci of an irregular shape. Lateral lobe sub-rectangular, sub-rounded. Ventral and tegminal sinus in shape of an obtuse angle. Humero-apical carina distinctly visible. Infrascapular area long; subrectangular in anterior half, gradually narrowing toward end. Lateral area progressively widening caudally. Small nodules present throughout the surface of pronotum. Small nodules distributed over entire surface. Anterior margin of pronotum truncated. Prozonal carinae distinct, converging caudally. Median carina continuous, reaching apex of pronotum. Lateral lobes projected downward; apex sub-rounded. Humeral angles oblique. Pronotum progressively narrowing caudally, ending in bluntly rounded apex. External lateral carina undulating. Internal lateral carina converges internally in last fifth, thereafter runs straight to tip, exposing wide lateral area. Two dark humeroapical spots present.

Wings: Alae not reaching apex of pronotum. Tegmina oval, entirely visible.

Legs: Fore legs setulose over entire length. Fore femora about 3.36 times as long as wide; dorsal margin denticulate; ventral margin erose. Fore tibia maculate; dorsal and ventral margins crenulate. Fore tarsus with first segment short (dark brown to black in color); second segment maculate, elongated, and with claws. Mid femora about 3.36 times as long as wide; dorsal margin denticulate; ventral margin erose; dorso-external and ventro-external carinae distinct; area enclosed between the ventro-external carina and ventral margin concave. Mid tibia Setose; dorsal and ventral margins erose; ventral margin with large spines. Mid tarsus with first segment short (dark brown to black in color); second segment maculate, elongated, and with claws. Hind femora robust; about 2.41 times as long as wide; its dorsal margin serrate, serrations uniform throughout length of dorsal margin before being raised into a group of closely placed 5 dents just before antegenicular tooth; ventral margin erose; antegenicular teeth arc-shaped, tip pointed. Hind tibia Smooth; ventral margin with large spines. Hind tarsus with first segment long (dark brown to black in color), having pulvilli with spinose tips; second segment small, third elongated and with claws.

Sexual dimorphism.—No dimorphism observed between sexes except body size and terminalia. Female: Ovipositor valves widened; serrated with pointed apex. Male: Subgenital plate widened; curving upwards with blunt apex.

Notes on variability.—Skejotettix kasalo **sp. nov.** is known to have two forms: macropronotal and long-winged (Figs 4J–L, 6, video: https://youtu.be/boJsRQK9LSU), and brachypronotal and short-winged (Fig. 5). The undulation of the median carina in lateral view is more distinct and higher in the brachypronotal form than in the macropronotal form.

Fig. 5. Holotype (♀) of *Skejotettix kasalo* **sp. nov. A.** Lateral view; **B.** Frontal view; **C.** Dorsal view. Paratype 2 (♀) of *Skejotettix kasalo* **sp. nov. D.** Lateral view; **E.** Frontal view; **F.** Dorsal view. Paratype 1 (♂) of *Skejotettix kasalo* **sp. nov. G.** Lateral view; **H.** Frontal view; **I.** Dorsal view. Paratype 3 (♂) of *Skejotettix kasalo* **sp. nov. J.** Lateral view; **K.** Frontal view; **L.** Dorsal view. Scale bars: 1 mm.

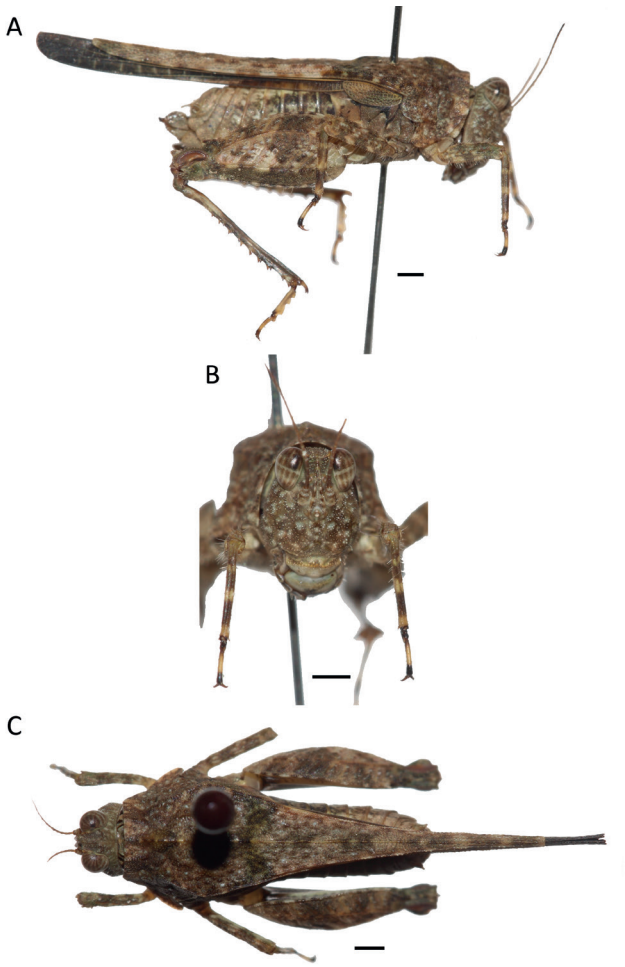

Fig. 6. Paratype 4 (♀) of *Skejotettix kasalo* **sp. nov. A.** Lateral view; **B.** Frontal view; **C.** Dorsal view. Scale bars: 1 mm.

The posterior margin of the compound eyes in most of the pinned specimens, including the holotype and those photographed in nature, do not reach the anterior margin of the pronotum. However, in some cases, such as paratype 2, the eyes reach the anterior margin of the pronotum due to the position of the head during the fixation process and the way it was pinned.

Coloration.—The coloration is cryptic, enabling the individuals to perfectly blend in with their surroundings (Fig. 7). For example, individuals found on the stones with lichen growths are entirely (Fig. 7A) or partially (Fig. 7E) blue-green in coloration, while individuals found on the muddy walls are light to rusty brown in coloration (Fig. 7B–D, F–I). The coloration can be uniform throughout the body (Fig. 7A, B, D, F, I), mottled, or different in the anterior and posterior halves of the body (Fig. 7C, E, G, H).

Nymphs.—(Fig. 8) The nymphs resemble the adults (Fig. 8) in coloration and the majority of the traits. However, the nymphs differ from adult individuals in having shorter pronotum (pronotum nearly reaches the hind knees in brachypronotal and extends beyond the hind knees in macropronotal adults), absence of wings, lack of antegenicular teeth on hind femora, poorly developed external genitalia, and the carinae being comparatively more distinctly expressed. The nymphs develop from one instar to the other by casting off the exoskeleton in the form of exuviae (Fig. 9) in the process of molting. Typically, the parts from the tip of the vertex to the tip of the pronotum are opened dorsally (Fig. 9B) to let the individual tetrigid out while the other parts remain intact. The intactness of the exuvia might make them useful for some morphological studies.

Measurements.—The key measurements of the holotype and paratypes are presented in Table 3.

Closely found species.—Two tetrigids, *Criotettix* cf. *bispinosus* (Fig. 10A) and *Hedotettix* sp. (Fig. 10B), were found alongside *Skejotettix kasalo* **sp. nov.** in the localities investigated.

Table 3. Measurements (in mm) of the holotype (HT) and the paratypes (PT) of *Skejotettix kasalo* **sp. nov.**

Body parts	HT(♀)	PT1(♂)	PT2(♀)	PT3(♂)	PT4(♀)	Avg. (♀)	Avg. (♂)
Body length	11.77	9.70	10.44	9.85	13.27	11.83	9.78
Vertex width	0.62	0.61	0.65	0.58	0.67	0.65	0.60
Eye width	0.69	0.68	0.70	0.65	0.71	0.70	0.67
Scutellum width	0.31	0.31	0.32	0.31	0.33	0.32	0.31
Pronotum length	9.11	8.65	8.67	6.74	14.19	10.66	7.70
Pronotum lobe width	4.54	4.08	4.47	3.66	4.79	4.60	3.87
Pronotum height	3.04	2.93	2.92	2.47	3.10	3.02	2.70
Tegmen length	1.71	1.75	1.80	1.34	2.14	1.88	1.55
Tegmen width	0.71	0.69	0.68	0.52	0.81	0.73	0.61
Alae length	6.05	6.61	5.58	4.43	12.58	8.07	5.52
Fore femur length	2.52	2.26	2.38	2.06	2.54	2.48	2.16
Fore femur width	0.75	0.72	0.70	0.61	0.68	0.71	0.67
Mid femur length	3.10	2.45	2.58	2.33	3.17	2.95	2.39
Mid femur width	0.99	0.82	0.69	0.67	0.89	0.86	0.75
Post femur length	6.81	6.51	6.63	5.41	6.45	6.63	5.96
Post femur width	2.82	2.73	2.76	2.39	2.65	2.74	2.56
Hind tibia length	6.42	5.72	6.09	4.98	6.59	6.37	5.35
First tarsal segment (basal) length	0.98	0.99	1.13	0.93	1.33	1.15	0.96
Third tarsal segment (apical) length (without claws)	0.78	0.77	0.84	0.77	0.94	0.85	0.77
Subgenital plate length	–	0.89	–	0.72	–	–	0.81
Subgenital plate width	–	0.51	–	0.50	–	–	0.51
Ovipositor dorsal valve length	1.22	–	1.08	–	1.34	1.21	–
Ovipositor dorsal valve width	0.58	–	0.47	–	0.64	0.56	–
Ovipositor ventral valve length	1.04	–	1.01	–	1.19	1.08	–
Ovipositor ventral valve width	0.36	–	0.35	–	0.39	0.37	–

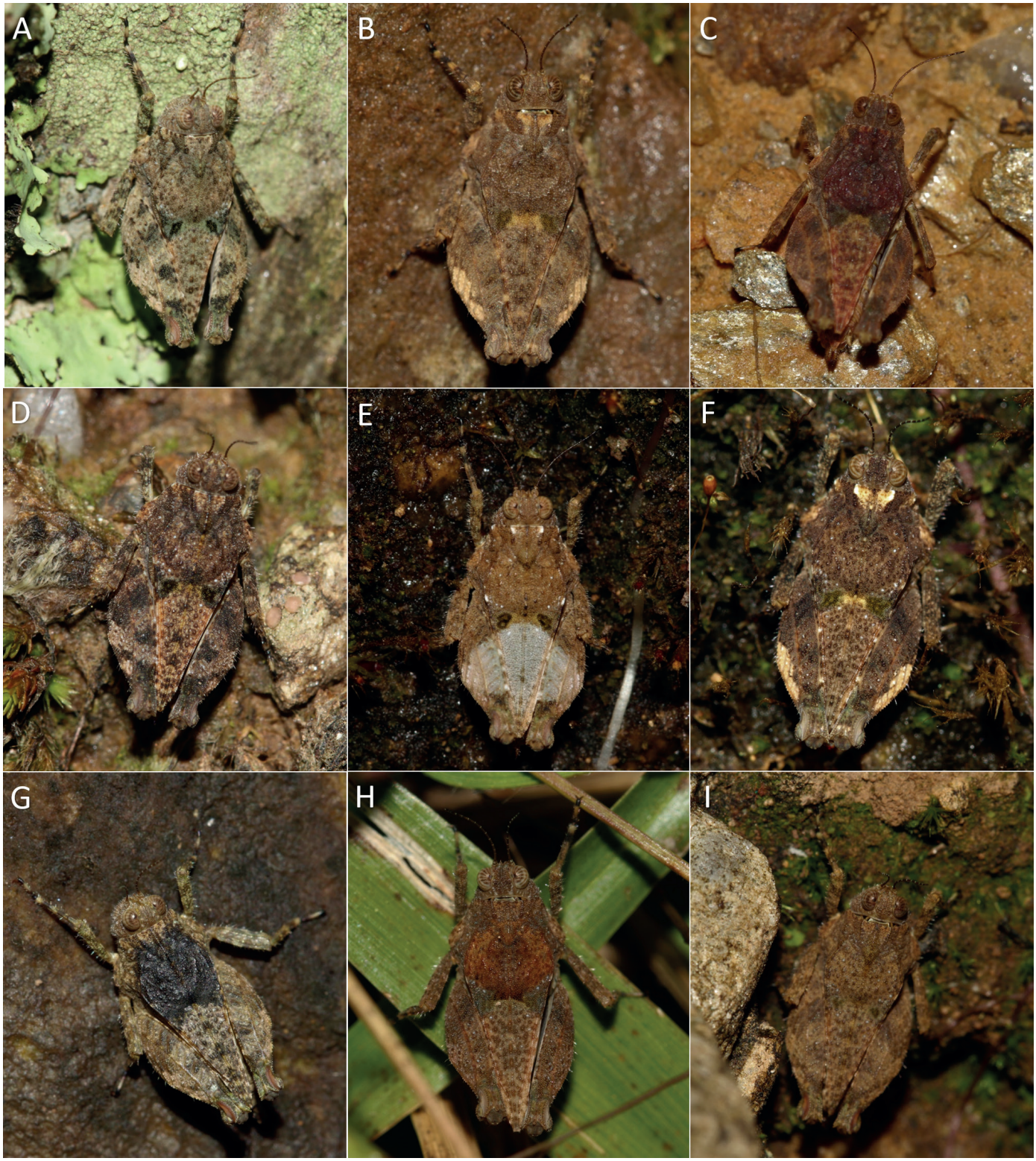

Fig. 7. Variability of coloration in *Skejotettix kasalo* **sp. nov.**

Food source.—The individuals of *Skejotettix kasalo* **sp. nov.** were observed feeding on food sources such as detritus (Fig. 11A), moss (Fig. 11B), lichens (Fig. 11C), and algae (Fig. 11D). Video links: A nymph feeding in the wild: https://youtu.be/6CeSekOTe4w; An adult feeding in the wild: https://youtu.be/PWYw-7Rvt3U.

Discussion

Hitherto, the genus *Skejotettix* comprised species with distributions restricted to the subtropical regions of Nepal (27.55362°N–27.98745°N; 155–460 m a.s.l.) (Subedi 2022). With the discovery of *S. kasalo* **sp. nov.** from the temperate regions (28.27569°N–

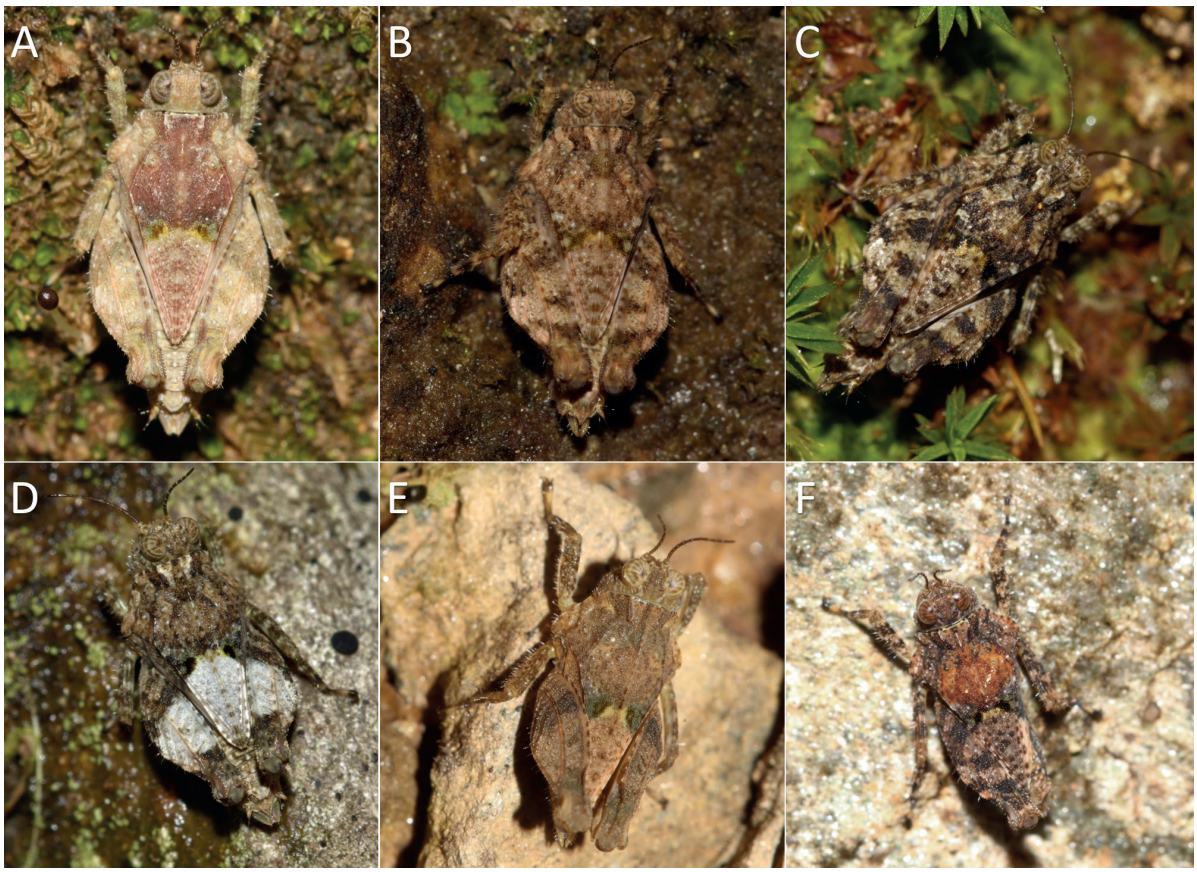

Fig. 8. Different nymphal instars of *Skejotettix kasalo* **sp. nov.** in natural habitat. (Note: the images are not on the same scale.)

Fig. 9. Exuviae of *S. kasalo* **sp. nov.** in natural habitat. (Note: **A–C** are the images of different views of the same exuvia.)

28.27626°N; 1665–1920 m a.s.l.), the distribution range of this genus has expanded along the altitudinal and climate gradient. The new species has a forest-type habitat similar to that of *S. netrajyoti*. However, the flora composition and climatic conditions of the forests are entirely different. There is not much known about the seasonality of all species of the genus. However, *S. netrajyoti* is observed only during the hot and humid summer months, with the nymphal stage as the probable overwintering stage (Subedi 2022). The observation of nymphs as the most abundant life stage during the last weeks of September (coinciding with the onset of winter) hints at nymphs as the probable overwintering stages and, thereby, the presence of seasonality in *S. kasalo* **sp. nov.** However, further studies and research should be done to verify this. Only two Tetrigidae species were found alongside *S. kasalo* **sp. nov.** One reason for the low number of species could be attributed to the time of the year (beginning of winter) of the study period. Taylor and Kerkut (1958) showed that poikilotherm insects show decreased activities with a decrease in the temperature of the surroundings. Furthermore, this was the first time the above-mentioned localities were studied for Tetrigidae, and the study was limited to the outskirts of temperate forests. Therefore, future studies penetrating the unexplored areas during the summer months are certain to provide more information on the species composition and more insights into the biology, seasonality, and ecology of *S. kasalo* **sp. nov.**

Until now, *Skejotettix* was known to be a brachypronotal and short-winged species (Ingrisch 2001b, Subedi 2022); in *S. netrajyoti*, the wings are reduced while hidden and absent in *S. muglingi*. However, a macropronotal and long-winged form co-exists

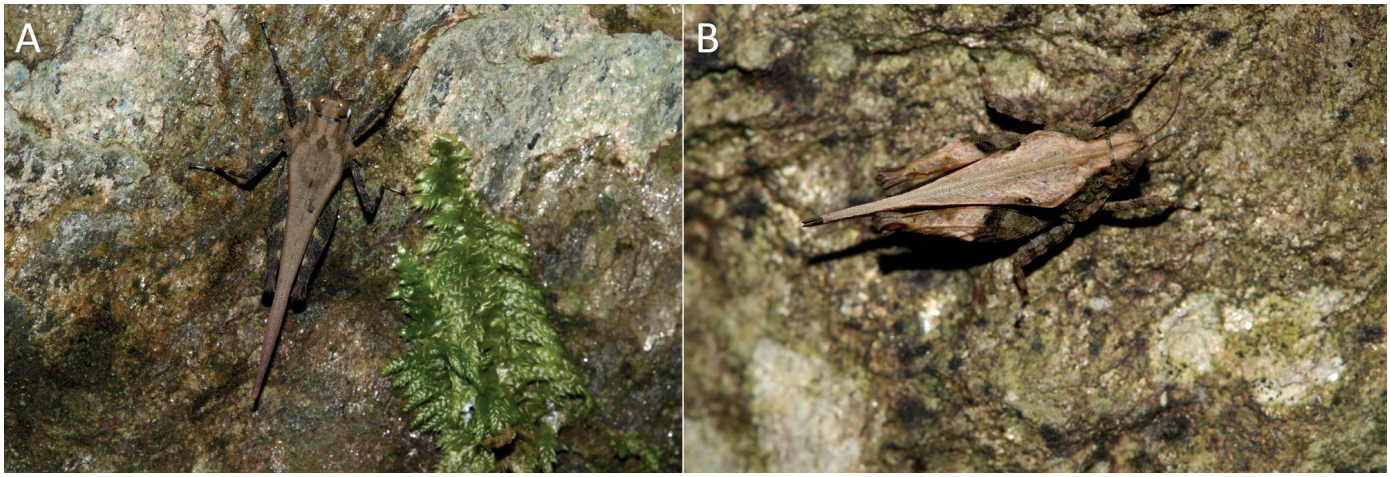

Fig. 10. Tetrigids closely found with *Skejotettix kasalo* **sp. nov. A.** *Criotettix* cf. *bispinosus;* **B.** *Hedotettix* sp.

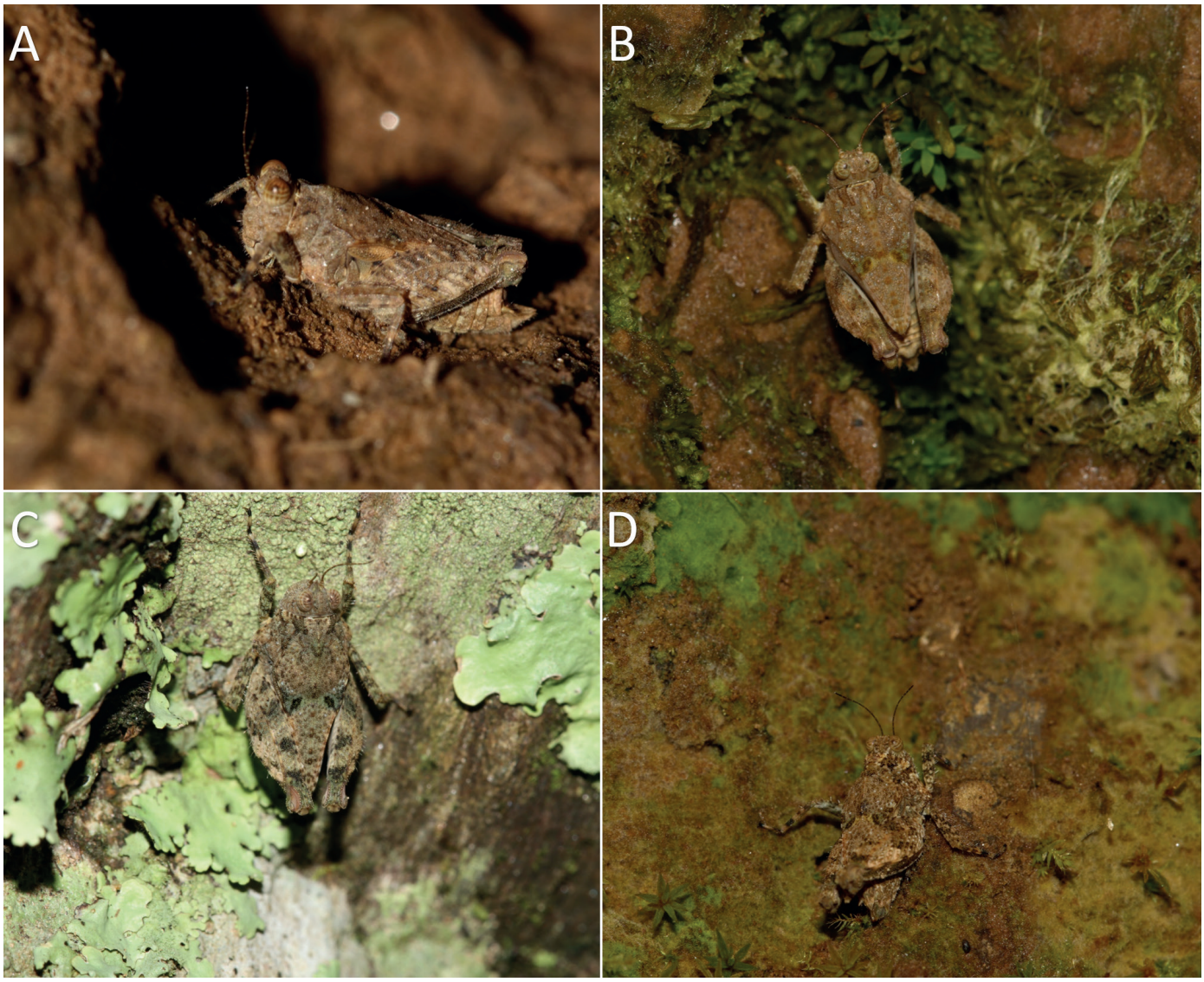

Fig. 11. Individuals of *Skejotettix kasalo* **sp. nov.** on different food sources. **A.** Detritus on the muddy walls; **B.** Moss growth on the muddy walls; **C.** Lichen growth on the stones; **D.** Algal growth on the dirt road through the type locality (Note: **A, C.** Adults; **B, D.** Nymphs)

alongside the typical brachypronotal and short-winged form in *S. kasalo* **sp. nov.** This phenomenon is also known in other Tetriginae, such as some species of *Paratettix* (Rehn and Grant 1957, Tumbrinck 2015). The presence of both the macro- and brachypronotal forms in *S. kasalo* **sp. nov.** suggests that it might be a basal species on the evolutionary tree of the genus Skejotettix. It has a variable morphology from which the morphologies of the rest of the species in the genus could have been derived. It is also possible that apterousness appeared several times independently within *Skejotettix*. However, the evolutionary idea is just a hypothesis provided to direct future studies. Considering the variability of forms within *S. kasalo* and the similarities between *Skejotettix* and *Ergatettix* (denoted under Diagnosis), the hypotheses that the two genera are closely related and that there is an evolutionary relationship between the species of *Skejotettix* are important to mention but should be carefully explored through detailed morphological and molecular studies, which is beyond the scope of this paper.

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Biology of *Patanga japonica* (Orthoptera, Acrididae): Nymphal growth, host plants, reproductive activity, hatching behavior, and adult morphology

Seiji Tanaka[1]

1 Matsushiro 1-20-19, Tsukuba, Ibaraki 305-0035, Japan

Corresponding author: Seiji Tanaka (stanaka117@yahoo.co.jp)

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Abstract

The biology of *Patanga japonica* (Bolívar, 1898), including seasonal nymphal growth, host plants, mating, oviposition, hatching, and adult morphology, was studied under outdoor and indoor conditions in central Japan. A field census showed that this grasshopper had a univoltine life cycle and overwintered in the adult stage. Body size was found to increase with a delay in the time of adult emergence in females but not in males, and protandry was observed. The insects were mainly associated with a few plant species. Feeding tests showed that at least one of the 5 test nymphs molted to the second stadium on 37 plant species, and more than 50% did so on 23 plant species. Mating was frequently observed in April and May under outdoor conditions, and the daily maximum number of copulating pairs was positively correlated with air temperature. Copulatory behavior, including stridulation, is described in detail. Oviposition was frequently observed in May and June under outdoor conditions. Larger females produced more eggs per pod, and a negative relationship was observed between egg lengths and the number of eggs per pod, showing a trade-off. On average, female adults had 124 ovarioles. Egg hatching occurred at different times during the daytime, but the eggs from each pod hatched synchronously. Synchronous hatching was also observed in eggs kept in groups of 2, 4, and 10, but hatching occurred earlier in larger group sizes. Eggs achieved synchronous hatching by either delaying or advancing hatching time. Two eggs separated by several millimeters hatched less synchronously than those kept in contact with one another. However, similarly separated eggs restored hatching synchrony when connected by thin wire, suggesting the involvement of vibrational signals in embryo–embryo communication. Morphometric analysis suggested that *P. japonica* adults change some morphometric ratios in response to crowding. Variation in pronotum shape was not significantly affected by crowding.

Keywords

copulatory behavior, hatching synchrony, mounting, phase polyphenism, protandry, stridulation

Introduction

The grasshopper, *Patanga japonica* (Bolívar, 1898) (also known as *Nomadacris*), is widely distributed in Asia, including Japan, Korea, Taiwan, China, Vietnam, and India (Cigliano et al. 2022). In subtropical regions, including Okinawa Prefecture, it has a univoltine life cycle and overwinters in the adult stage (Tanaka and Okuda 1996). This and another large grasshopper, *Patanga succincta* (Johannson, 1763), are often found in the same sugarcane fields and adjacent areas in subtropical regions. In the temperate region of Japan, *P. japonica* is the only grasshopper known to overwinter as an adult (Ichikawa et al. 2006, Murai and Ito 2011). Nymphs and adults have been observed feeding on the Kudzu, *Pueraria montana* var. *lobata* (Willd.) Sanjappa and Pradeep, and the Japanese hop, *Humulus scandens* (Lour.) Merr., as well as species of Poaceae (Ichikawa et al. 2006). However, the range of host plants is poorly known. Although it is a common species, it is not an economically important grasshopper and has received little attention from researchers, except for some morphological and physiological studies (Okuda et al. 1996, Tanaka and Okuda 1996, Nakamori and Sadoyama 2001). Having obtained various information in the laboratory on *P. japonica* (Tanaka and Okuda 1996, Okuda et al. 1996), I became interested in studying this species in the field. To understand the significance of morphological, behavioral, and physiological variations in an insect, it is important to obtain basic knowledge about its biology, particularly its seasonal life cycle and behavior in the field.

The purpose of the present study was to describe the biology of *P. japonica*, including seasonal development, host plants, mating, oviposition, and hatching behavior, in central Japan. I recorded the plants on which nymphs and adults stayed during the growing season to determine whether there was a difference in the plants

used by the two stages. I also conducted indoor feeding tests of 51 species of plants found in the habitats of this grasshopper to determine the host range. In mating behavior, the copulating posture and stridulating behavior were observed in detail. By rearing adults under outdoor conditions, the number of egg pods laid by females and the length of the oviposition season were determined. I also measured egg pod size and examined its relation to the number of eggs contained as well as the relationships between the number of eggs, egg size, number of ovarioles, and body size of the female parent. I confirmed that nymphs of this grasshopper hatched in synchrony and focused on the mechanism controlling hatching synchrony, with particular attention paid to embryo–embryo communications. Finally, I tested whether this grasshopper exhibited density-dependent variation in adult morphometric traits as often observed in other grasshoppers and locusts (Uvarov 1966, 1977, Pener 1991, Pener and Yerushalmi 1998, Pener and Simpson 2009). In this paper, I describe the results of these observations and discuss the characteristics of this grasshopper.

Materials and methods

Field census.—The number of *P. japonica* individuals was recorded in a grassy area in Tsukuba, Ibaraki prefecture (36.1°N, 140.1°E), in Japan every week from July to November, except for the week of September 2 in 2021. The grasses were cut short on June 29 and October 30 by the government of the city of Tsukuba. The study site was adjacent to a pedestrian road and measured approximately 2 m by 200 m. As I walked slowly along this area, I visually counted grasshoppers and identified their developmental stages. It usually took 30–60 minutes to finish each census. For the first four stadia, personal experience made visual identification of nymphal stadia relatively easy: nymphal body length and head width increased as nymphs grew bigger (Figs 1, 2). In this paper, I use 'stadium' to count the nymphal stages from hatching and 'instar' to describe the penultimate and last nymphal stages that can be identified by the characteristic wing pads. The process for measuring the body lengths of nymphs was as follows: Nymphs were reared singly on cut leaves of *Echinochloa crus-galli* (L.) P. Beauv. in plastic cups (9 cm in diameter, 4.5 cm in height) set near a window and kept at room temperature. A few days after each molt, each nymph was put in a transparent plastic bag, and the body length from the head to the tip was measured with a digital caliper to the nearest 0.1 mm (Digipa pro; Mitsutoyo Co., Kanagawa, Japan). After the fourth stadium, most fifth-stadium nymphs developed wing pads on the dorsal body surface by which they could easily be identified as penultimate instars (Fig. 1E). They molted to the last instar with larger wing pads (Fig. 1F). Adult stage was attained after the sixth stadium. Preliminary observations indicated that some female fourth-stadium nymphs went through an additional stadium and reached the penultimate instar at the sixth stadium (Suppl. material 1). Thus, this grasshopper passes 6 or 7 nymphal stadia before the adult stage. In the field, however, it was not possible to identify the nymphal stadium of the last two stadia with certainty. Therefore, in this census, nymphs at the last two stadia were identified as the penultimate and last instars, respectively, based on their wing pad sizes. Thus, it was likely that individuals without penultimate wing pads at the fifth stadium were included in the category of fourth-stadium nymphs.

Host plants.—During the above census, I recorded the plant species on which nymphs and adults were feeding or sitting. Because feeding activities are not easily observed in the field, these plants do not necessarily represent the host plants of this grasshopper. To solve this problem, I collected 51 species of plants found in

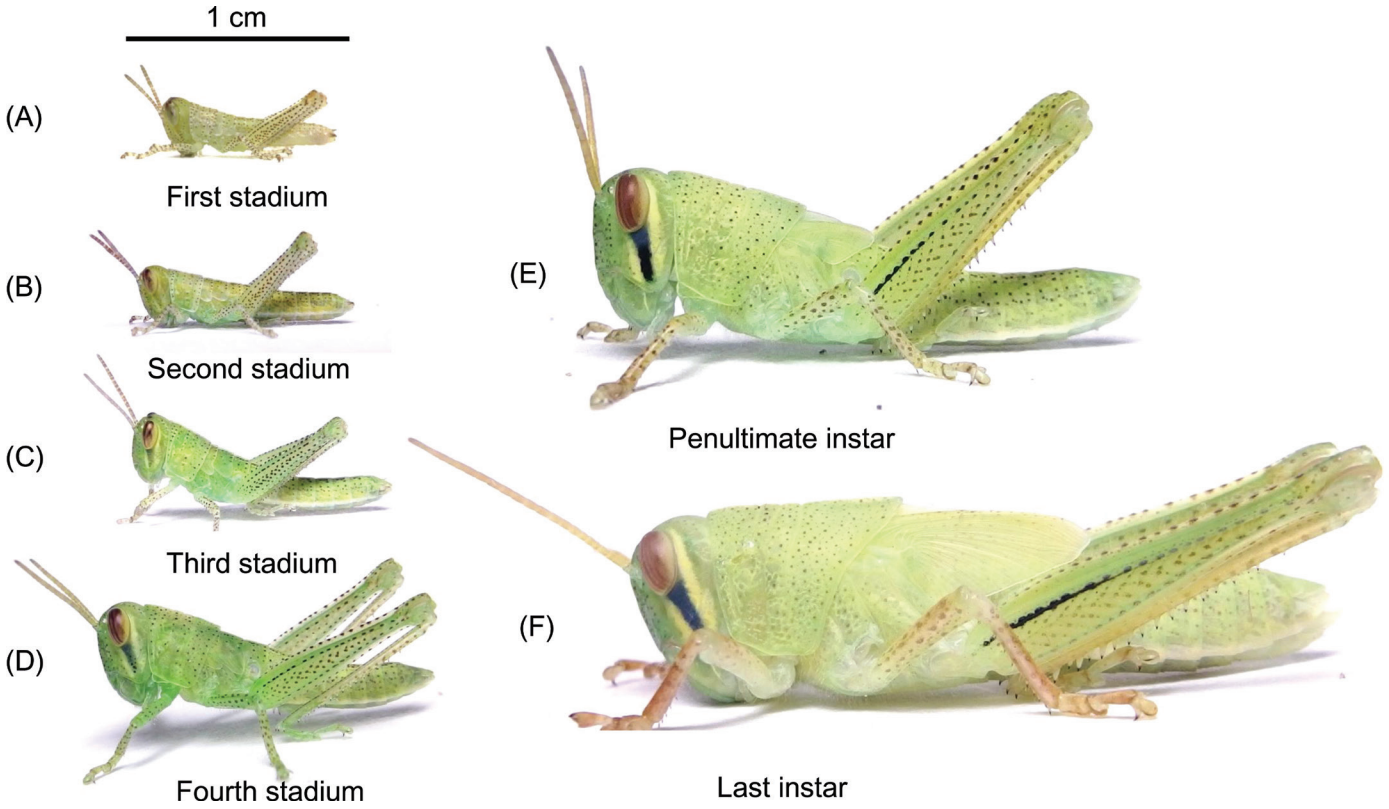

Fig. 1. Nymphal growth of *Patanga japonica*. First to fourth stadia (**A–D**). Penultimate (**E**) and last (**F**) instars are characterized by wing pads.

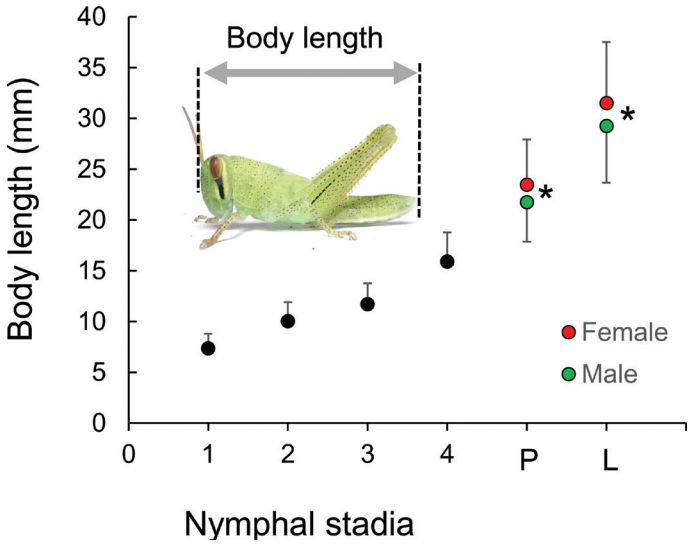

Fig. 2. Changes in body length (mean + or - SD, N = 20 each) during nymphal development in *Patanga japonica*. Asterisks indicate a significant difference between sexes by *t*-test ($p < 0.001$). P, penultimate instar; L, last instar.

their habitats, including the study site, and determined if nymphs would feed and grow while eating them. Five newly hatched nymphs were kept with pieces of leaves from each plant in a plastic cup (9 cm in diameter, 4.5 cm in height) covered with a perforated lid. Plant leaves were replaced by fresh ones every day. The nymphs kept with appropriate host plants molted to the second stadium in 5–7 days at room temperature, whereas those kept with a moist cotton ball alone died on the third day without molting. No cannibalism was observed.

Mating.—To determine when mating begins in the spring and to observe the mating behavior of *P. japonica*, 10 female and 10 male adults collected in the field were housed in a wood-framed enclosure (50 × 80 × 30 cm, Suppl. material 2A) on the litter ground in Tsukuba on February 4, 2022 and fed with cut leaves of *Bromus catharticus* Vahl held in a jar of water. The walls of the enclosure were covered with nylon mesh, and the top was covered with a glass plate during the day for observation and with nylon mesh during the night for natural ventilation and rainfall. All adults were marked on the thorax and forewing with different paint colors for identification and usually checked for copulation every 1 or 2 h from 08:00 to 18:00. Most mounting males were copulating. Temperature on the litter floor was recorded every hour with a thermo recorder (TR-52i, Ondotori Jr., T & D Co. Nagano, Japan).

Grasshoppers generally copulate in a characteristic fashion (Uvarov 1966). Typically, the male sits on the back of the female and copulates by curving down his abdomen to reach her genitalia. In this case, the male bends his abdomen either from the right or left side of the female body. In *P. japonica*, I examined this phenomenon by observing adults in cages mainly during the day. On some nights, I observed their behavior using a red LED lamp. To examine whether this behavior was controlled by males or females, I changed their partner.

Oviposition.—Oviposition activity was observed for 20 pairs of a female and a male housed in small cages (28 × 12 × 28 cm; Suppl. material 2B) and a group of 10 females and 10 males housed in a large cage (42 × 22 × 42 cm) in 2021. The air temperatures recorded at Tsuchiura, Ibaraki (36.06N, 140.13E), during the observation period were obtained from the Japan Meteorological Agency (2022). A plastic cup (10 cm in diameter, 5.5 cm in height) filled with moist sand (approximately 15% water by volume) was placed in each cage and checked for egg pods every day. The cups containing egg pods were kept at 30 ± 1 °C in an incubator (CN-40A; Mitsubishi Electric Engineering Co., Tokyo, Japan) for 1 or 2 days, and the egg pods were washed in chlorinated tap water to remove sand. The maximum width and length of the egg pods were then measured with a digital caliper and held in moist sand in plastic cups (100 ml in volume) for incubation (30 ± 1 °C). The eggs of 5 egg pods incubated at this temperature took 30.0 days to hatch (SD = 0.7 days; N = 271). Four or five days before the expected day of hatching, the number of eggs contained in each pod was counted, and the lengths of 10 eggs taken from each pod were measured with a digital caliper under a binocular microscope. The number of ovarioles was counted for 16 female adults collected in the fall by dissecting one ovary from each female under a binocular microscope. The number multiplied by two was regarded as the number of ovarioles for each female.

Hatching.—Hatching time was determined under semi-outdoor conditions in June 2021. Egg pods that were expected to hatch within 4–5 days at 30 °C were buried at a depth of 2–5 cm in soil held in a plastic washtub (50 cm in diameter, 25 cm in height). They were covered with a transparent perforated cylinder (8.5 cm in diameter, 17 cm in height) covered with nylon mesh. The washtub was placed outdoors and photographed every 30 minutes with a digital camera to determine the hatching time (Suppl. material 2C). The number of hatched nymphs was calculated later on a computer. As described below, once hatching started, most eggs hatched within the same 30-minute period. Similar phenomena have been observed in the Eastern lubber grasshopper, *Romalea microptera* (Palisot de Beauvois, 1817) (Smith et al. 2013) and the Western lubber grasshopper, *Taeniopoda eques* (Burmeister, 1838) (Whitman and Orsak 1985). Therefore, in this study, the start of hatching from an egg pod was regarded as the hatching time of each egg pod.

To confirm that synchronized hatching from the egg pods occurred at a constant temperature, the eggs were removed from each pod 10 days before the expected time of hatching, soaked in 6% sodium hypochlorite (Haiter, Kao Co., Tokyo) for 1 min to remove the pod material, and rinsed with tap water three times. Preliminary observations showed that this handling caused no detrimental influence on the hatching rate and time (Tanaka S, unpublished data). The eggs of each pod were then divided into two batches. The eggs in one batch were buried in a group of 10 or 20 eggs in a sand pit in a Petri dish (9 cm in diameter, 2 cm in height), and 10 or 20 eggs in the other batch were individually buried in sand pits in another dish, as previously described for the migratory locust, *Locusta migratoria* (Linnaeus, 1758) (Tanaka 2017), except that dark-colored river sand was used because the hatchlings were light green in color. The eggs were incompletely covered with sand, allowing them to receive light. They were incubated at 30 ± 1 °C under continuous illumination and photographed from above every 30 minutes with a digital camera. The hatching time was determined as described above.

To examine the effect of group size on hatching, eggs from the same pod were divided into two batches of similar sizes that were then kept in groups of 2 versus 4 eggs or 4 versus 10 eggs at 30 ± 1 °C under continuous illumination. Their hatching times were determined as described above. The effects of group size on the hatch-

ing time were compared by calculating the mean hatching times of different group sizes relative to the value for group size 10 that was designated as 1 h. Hatching intervals from the first to the last hatchings were also calculated. Only those egg groups with 100% hatching were used. Because photographs were taken at 30-minute intervals, 30 minutes was added to the differences in hatching times of eggs; therefore, 30 min was the minimum hatching interval.

The mechanism controlling hatching synchrony in *P. japonica* was examined by carrying out three experiments at 30 ± 1 °C under continuous illumination using pairs of eggs according to the methods described for other grasshoppers (Tanaka 2017, 2021a, b).

In experiment 1, the effect of the presence of hatched nymphs on the hatching time of late-hatching eggs was tested by treating pairs of eggs in three different ways: 1) two eggs kept in contact with one another on sand in a well of a 24-well plate (Thermo Fisher Scientific KK, Tokyo, Japan), 2) two eggs kept separately with a distance of 2–5 mm, and 3) two eggs separated by a stainless wire screen (12 mm in length, 15.7 mm in width) that physically prevented the hatched nymph from touching the unhatched egg in the same well.

In experiment 2, whether vibrational stimuli are involved in inducing hatching synchrony or not was tested by placing pairs of eggs from the same egg pod horizontally on moist sand in wells of a 24-well plate in three different ways: 1) eggs kept in contact with one another, 2) eggs kept separated by 2–5 mm, and 3) eggs similarly kept separated but connected by two pieces of stainless wire (7 mm in length).

In experiment 3, I examined how eggs achieved hatching synchrony. In other words, does the hatching time of eggs advance or delay in response to stimuli from adjacent eggs? To answer this question, eggs from the same pod were divided into two batches 10 days before the expected date of hatching. One batch was incubated at 12–14 °C for various lengths of time to suppress embryonic development and returned to 30 °C, while the other batch was continuously kept at 30 °C. Five days before hatching was expected to start, eggs from the respective batches were paired and placed together vertically in sand pits in wells of a 24-well plate as mixed pairs (12–14 pairs). In this case, care was taken to ensure that the two eggs were kept in contact with one another. Other eggs from the two batches were used as controls, each consisting of 6 or 7 pairs of eggs. The eggs in each control pair were buried individually in sand pits but kept in the same well. One control hatched earlier than the other, which are here called controls 1 and 2, respectively. The pits were incompletely covered with sand, allowing the eggs to receive light but preventing the nymph that hatched first from touching the unhatched egg. The hatching times were determined as described above, and the hatching intervals for the mixed and control pairs were calculated. The early and late hatching eggs in each mixed pair are referred to here as mixed eggs 1 and 2, respectively. Only those pairs in which both eggs hatched successfully were used for the analyses. In the three experiments, the hatching times were determined as described above, and the hatching intervals of eggs in pairs were calculated.

Morphometrics of adults grown under different conditions.—Adults of *P. japonica* were collected at the study site in October 2020 and March 2021. The density of individuals at this site was not measured precisely, but it is probably safe to say that it was lower than 1 per m^2. By following the method of Dirsh (1951, 1953), the maximum head width (C), hind femur length (F), and forewing length (E) of adults were measured using a digital caliper to the nearest 0.1 mm. My main interest was to determine if this grasshopper would change its morphometric characters in response to crowding, as observed in locusts (Uvarov 1966, 1977, Pener 1991, Pener and Simpson 2009). According to previous studies on phase polyphenism in morphometric ratios in several locust species, such as *Schistocerca gregaria* (Forskål, 1775) and *Locusta migratoria* (Dirsh 1953, Farrow and Colless 1980, Sugahara et al. 2015, 2016), the F/C, E/F, and E/C ratios were adopted to assess the presence of density-dependent morphometric changes. Two experiments were carried out. One experiment compared the above parameters in field-collected adults (low density) and adults reared outdoors in a group (high density) in Tsukuba. Two groups of approximately 200 and 100 nymphs that hatched on July 5 and July 25, respectively, were reared in large cages. They fed on the leaves of *E. crus-galli*. In the other experiment, the parameters of adults reared individually in plastic containers (10 cm in diameter, 4.5 cm in height) and those reared in a group of 5 individuals per container or in a group of 50 individuals per large cage (high density) were compared. All insects were reared indoors (at room temperature and semi-natural daylengths) from June to August. Body dimensions were measured for the adults as described above. Additionally, the shape of pronotum, which is known to be another density-dependent character in *L. migratoria* (Uvarov 1966, Tanaka et al. 2002), was recorded for the indoor-reared adults. The ridge of the pronotum was recorded as arched, flat, or concaved according to the method of Tanaka (2022) for *L. migratoria*.

Statistical analyses.—The body size of *P. japonica* nymphs was compared between treatments using a *t*-test. The proportions of plant species were analyzed using a chi-squared test. Pearson's correlation coefficient and linear regression were used to analyze the relationships between egg number, egg length, and head width of female parents. Hatching rates were compared using a Mann–Whitney U test. Hatching intervals were compared using a Steel–Dwass test and a chi-squared test'. Hatching time was compared with Tukey's multiple comparison test. Adult dimensions and morphometric ratios were analyzed with Tukey's multiple comparison, a *t*-test, and a Steel–Dwass test. These analyses were performed using a statistics service available at http://www.gen-info.osaka-u.ac.jp/MEPHAS/kaiseki.html, Descriptive Statistics (Excel, Microsoft Office 365), or StatView (SAS Institute Inc., NC, USA). Differences were judged as significant when $p < 0.05$.

Results

Seasonal development.—*P. japonica* nymphs of different sizes, ranging from first stadium to penultimate instar, were observed on July 29, 2021, when the census was begun (Fig. 3). My observations suggested that hatching started in early July, which was confirmed in 2022, when 88.2% of the nymphs observed at the site were at the first stadium and 11.8% and 4.1% were second and third stadium nymphs, respectively, on July 7 (N = 43, Tanaka, S. unpublished observation). Nymphs grew gradually as the season advanced, and adults began to be seen on September 16, with only adults observed on November 24. On October 30, the grasses at the study site were cut and removed, which caused most grasshoppers to move to adjacent bushes or to be killed.

To observe the pattern of adult emergence, nymphs that hatched on July 5 were reared in a cage under outdoor conditions. Adults started appearing on September 5, and the mean duration of nymphal development was 76.0 days (SD = 6.1, N = 46) and 81.6 days after hatching (SD = 5.9, N = 67) in males and females, respectively, which corresponded to September 19 and 25 (Fig. 4).

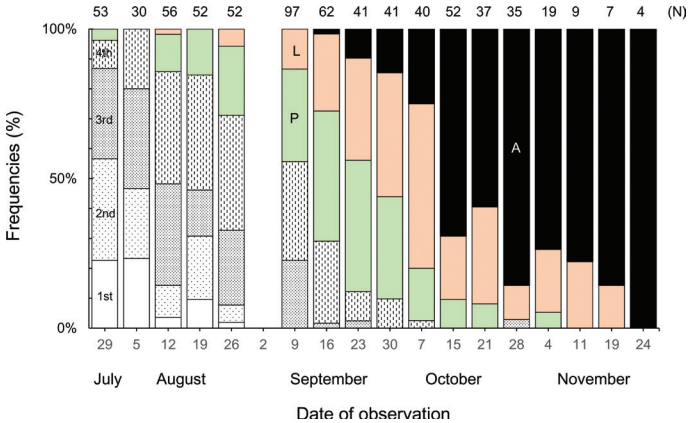

Fig. 3. Seasonal changes in nymphal development in *Patanga japonica* in Tsukuba in 2021. For nymphal stadia and instars, see Fig. 1. 1st–4th, stadia; P, penultimate instar; L, last instar; A, adult.

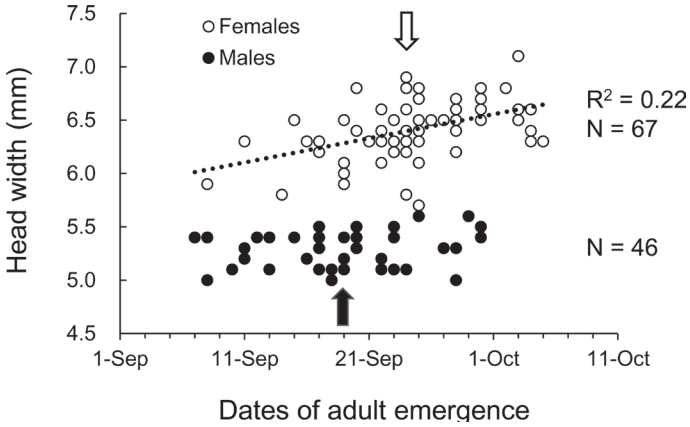

Fig. 4. Seasonal changes in adult head width in *Patanga japonica* individuals that hatched on July 5 in 2021 and were reared outdoors in a group. Arrows indicate the mean time of adult emergence.

No significant sex difference was observed in variance (*F*-test, $p = 0.41$). On average, males emerged as adults 5.6 days earlier than females ($t = 4.86$; DF = 111, $p < 0.001$), indicating the presence of protandry. Female adults showed a significant increase in body size with delay in the time of adult emergence ($r = 0.46$, $p < 0.001$, Fig. 4), while no significant correlation was observed between the two variables in males ($r = 0.22$, $p = 0.14$).

Plants used by P. japonica.—Fig. 5 compares the plants on which grasshoppers were observed. Nymphs mainly stayed on the leaves of three plant species—(92.7%) *Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida ex Sanjappa & Pradeep, *Miscanthus sinensis* Andersson, and *Imperata cylindrica* (L.) P. Beauv. *a*—representing 66.3%, 12.0%, and 14.4%, respectively (N = 409). The corresponding figures for adults were 56.2%, 10.0%, and 9.2% (N = 130). The remaining 24.6% of adults were observed on various other plants. The composition of the four categories in Fig. 5 was significantly different between nymphs and adults ($\chi^2 = 29.5$, DF = 1, $p < 0.01$). Both nymphs and adults of this grasshopper were frequently observed making holes in the leaves of *P. montana var. lobata* rather than feeding on them from the edges (Suppl. material 2D).

The above results do not necessarily indicate that grasshoppers feed on those plants. To examine the range of host plants of *P. japonica*, newly hatched nymphs were held with leaves of various plant species and observed until they molted to the 2nd stadium or died without molting. All nymphs kept with only a moist cotton ball died on the third day. Out of the 51 plant species tested, 37 (72.5%) belonging to 16 families led at least one *P. japonica* nymph to attain the following stadium (Table 1). All nymphs reached the 2nd stadium on 7 plant species, and more than 50% of nymphs (3–5 nymphs) did so on 23 plant species belonging to 10 different families. The results suggest that *P. japonica* could use a wide range of plant species for development.

Mating.—In 2021, the first mating pair among adults kept in an outdoor cage was observed on March 14. In 2022, the first mating was observed in an outdoor cage on March 19, and the second one was observed on April 2 (data not shown). Mating was frequently observed in April and May (Fig. 6A) in 2022 and was continuously observed until late July, when a few adults were still alive (data not shown). In the field, the last male was collected on July 7, 2021. In 2022, the last male and female were captured at the study site on July 7 and August 10, respectively. The latter laid an egg pod 3 days later and then died.

From April 2 to May 13, copulation was observed on a total of 26 days (Fig. 6A), and the mean number of copulating pairs was positively correlated with the time of day within the observed range (Fig. 6B). The number of copulating pairs gradually increased from 08:00 to 14:00 and leveled off thereafter. The daily maximum number of copulating pairs showed a higher correlation with the mean temperature of the day ($r = 0.39$, N = 41, $p < 0.01$) than with that from 08:00 to 18:00 ($r = 0.32$, N = 41; $p < 0.05$) or from 10:00 to 16:00, during which the temperature tended to be high due to sunshine ($r = 0.27$, N = 41, $p = 0.09$, Fig. 6C). Few observations were made between 18:00 and 8:00, during which the mean number of copulating pairs decreased by 41.9% from 2.96 to 1.72 (Fig. 6B, $t = -2.50$, DF = 44, $p < 0.05$).

The length of copulation was not precisely determined in this study. Some adults were found copulating at 8:00 when observations started each day, and mating ended 1–14 h later (Suppl. material 3). However, many pairs (42 pairs, 71.2%) remained without separating at the end of the daily observation (18:00–22:00, Suppl. material 3), and some continuously remained copulating for a few days, although the possibility that they separated during the night and mated again in the morning could not be ruled out completely.

In *P. japonica*, the male copulated with the female by bending his abdomen either from the right or left side of her body to reach her genitalia (Suppl. material 2E). The frequencies of males copulating from the right and left sides were 31 and 35, respectively, and their proportions did not significantly deviate from unity ($\chi^2 = 0.12$, DF = 1, $p > 0.05$). Some males were moved to another cage to allow them to mate with a second female to determine whether the bending direction was fixed or changeable depending on the partner. Of a total of 26 males tested during the period from May 19 to June 18 in 2022, 16 were observed copulating with two different females from the same side, although some of these females (N = 11) had previously copulated with a male bending his abdomen from the opposite side. These results suggest that the side of the female that was used for copulation depended on the male.

P. japonica adults were seen to stridulate by rubbing their abdomens or forewings with the hind tarsi. The stridulation was usually repeated 3 or 4 times each time using one leg (Suppl. material 4). This behavior was displayed almost exclusively by males, with one exception: one female stridulated by moving both legs alternately on the floor of a cage. In males, this behavior was observed only during copulation, suggesting that it was not for calling a female. In group-rearing, a copulating pair was often surrounded by other males, but

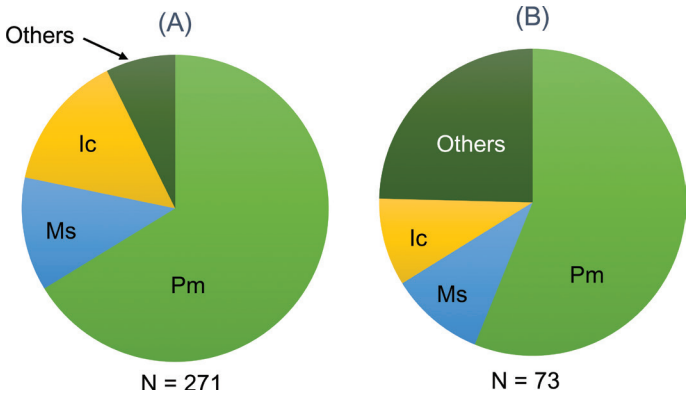

Fig. 5. Composition of plant species on which *Patanga japonica* nymphs (**A**) and adults (**B**) were observed. Pm, *Pueraria montana var. lobata;* Ms, *Miscanthus sinensis;* Ic, *Imperata cylindrica*.

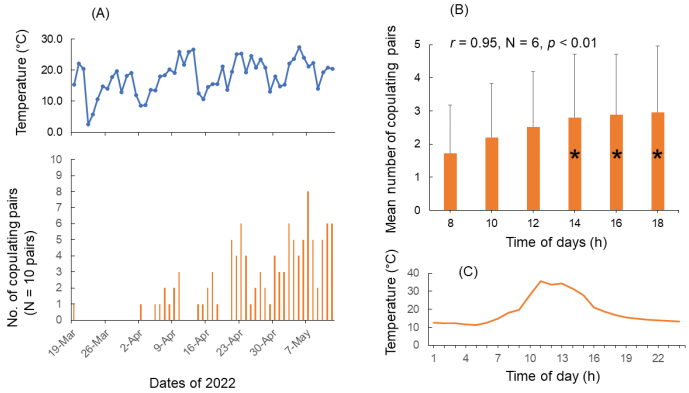

Fig. 6. Mating activity of *Patanga japonica* observed outdoors in a wood-framed enclosure in 2022. **A.** Daily mean air temperatures and number of copulating pairs; **B.** Mean number (+ SD) of copulating pairs at different times of day; C. Mean temperature on the litter floor.

male–male physical competition for a female appeared to be rare. I did not encounter a situation in which a male took over and copulated with the female by physically fighting the mounting male.

It was also noticed that a male reaching the female genitalia from the left (right) side used his right (or left) leg to stridulate. To determine whether such a relationship was a rule, copulating pairs were checked once a day from April 10 to May 2 in 2022. As expected, 62 males (93.9%) followed the above rule, while 4 males used both legs to stridulate. In the latter mating pairs, the females tilted their body by approximately 30 degrees to orient it toward the sun (Suppl. material 5). As a result, it was possible that the males had difficulty in stridulating using their leg on the appropriate side. After bathing ended, these males stridulated using the 'appropriate' leg as observed in the other males.

In another observation carried out from April 17 to May 4 in 2022, 8 males with a missing hindleg were paired with an intact female to test the hypothesis that these males would copulate by bending their abdomen from the same side as that of the missing leg. The female partner was changed every day. The above hypothesis was supported by all but one case (N = 20).

Oviposition.—Oviposition started on April 27 and continued until August 1 in 2021 under outdoor conditions (Fig. 7). Oviposition intervals were 10.4 days on average (range = 4–22, N = 98, Suppl. material 6A). High oviposition activities were observed on warm

Table 1. Number of hatchlings that molted to the 2nd stadium when five hatchlings were kept at room temperature with various plant species taken from habitats of *Patanga japonica*.

No.	Plant species	Family	No. of nymphs that molted to 2nd stadium
1	*Chenopodium album* L. var. *centrorubrum* Makino	Amaranthaceae	5
2	*Humulus scandens* (Lour.) Merr.	Cannnabaceae	5
3	*Trifolium repens* L.	Fabaceae	5
4	*Trifolium pratense* L.	Fabaceae	5
5	*Bromus catharticus* Vahl (1791)	Poaceae	5
6	*Digitaria ciliaris* (Retz.) Koel	Poaceae	5
7	*Echinochloa crus-galli* (L.) P. Beauv	Poaceae	5
8	*Achyranthes bidentata* Blume var. *japonica* Miq.	Amaranthaceae	4
9	*Cyperus microiria* Steud.	Cyperaceae	4
10	*Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida ex Sanjappa & Pradeep	Fabaceae	4
11	*Perilla frutescens* (L.) Britton var. *acuta* (Thunb.) Kudô	Lamiales	4
12	*Eleusine indica* (L.) Gaertn.	Poaceae	4
13	*Dactylis glomerata* L.	Poaceae	4
14	*Setaria viridis* L.) P. Beauv.	Poaceae	4
15	*Miscanthus sinensis* Andersson (1855)	Poaceae	4
16	*Imperata cylindrica* (L.) P. Beauv.	Poaceae	4
17	*Rumex acetosa L.*	Polygonaceae	4
18	*Erigeron annuus* (L.) Pers.	Asteraceae	3
19	*Taraxacum officinale* Weber ex F.H. Wigg. (1780)	Asteraceae	3
20	*Commelina communis* L.	Commelinaceae	3
21	*Equisetum arvense* L.	Equisetaceae	3
22	*Lespedeza bicolor* Turcz. (1840)	Fabaceae	3
23	*Lolium multiflorum* Lam.)	Poaceae	3
24	*Lactuca indica* L.	Asteraceae	2
25	*Artemisia indica* Willd Willd. var. *maximowiczii* (Nakai) H.Hara	Asteraceae	2
26	*Galinsoga quadriradiata* Ruiz et Pav.	Asteraceae	2
27	*Trichosanthes cucumeroides* (Ser.) Maxim. ex Franch. et Sav.	Cucurbitaceae	2
28	*Aeschynomene indica* L.	Fabaceae	2
29	*Desmodium paniculatum* L.	Fabaceae	2
30	*Cocculus orbiculatus* L.	Menispermaceae	2
31	*Plantago asiatica* L.	Plantaginaceae	2
32	*Persicaria longiseta* (Bruijn) Kitag.	Polygonaceae	2
33	*Houttuynia cordata* Thunb. (1783)	Saururaceae	2
34	*Solanum nigrum* L.	Solanaceae	2
35	*Erigeron annuus* L.	Asteraceae	1
36	*Cirsium japonicum* Fiscb. Ex DC.	Asteraceae	1
37	*Rumex japonicus* Houtt.	Polygonaceae	1
38	*Rhus javanica* L.	Anacardiaceae	0
39	*Erigeron annuus* (L.) Pers.	Asteraceae	0
40	*Bidens biternata* (Lour.) Merr. et Sherff	Asteraceae	0
41	*Solidago canadensis* var. *scabra* L.	Asteraceae	0
42	*Conyza canadensis* (L.) Cronquist	Asteraceae	0
43	*Dioscorea japonica* Thunb. (1784)	Dioscoreaceae	0
44	*Lespedeza juncea* (L.fil.) Pers. var. *subsessilis* Miq.	Fabaceae	0
45	*Oenothera biennis* L.	Onagraceae	0
46	*Oenothera rosea* L'Hér. ex Aiton	Onagraceae	0
47	*Xanthoxalis corniculata* (L.) Small	Oxalidaceae	0
48	*Phytolacca americana* L.	Phytolaccaceae	0
49	*Paederia scandens* (Lour.) Merr.	Rubiaceae	0
50	*Smilax china* L.	Smilacaceae	0
51	*Cayratia japonica* Gagn.	Vitaceae	0
52	Cotton ball moistened with tap water		0

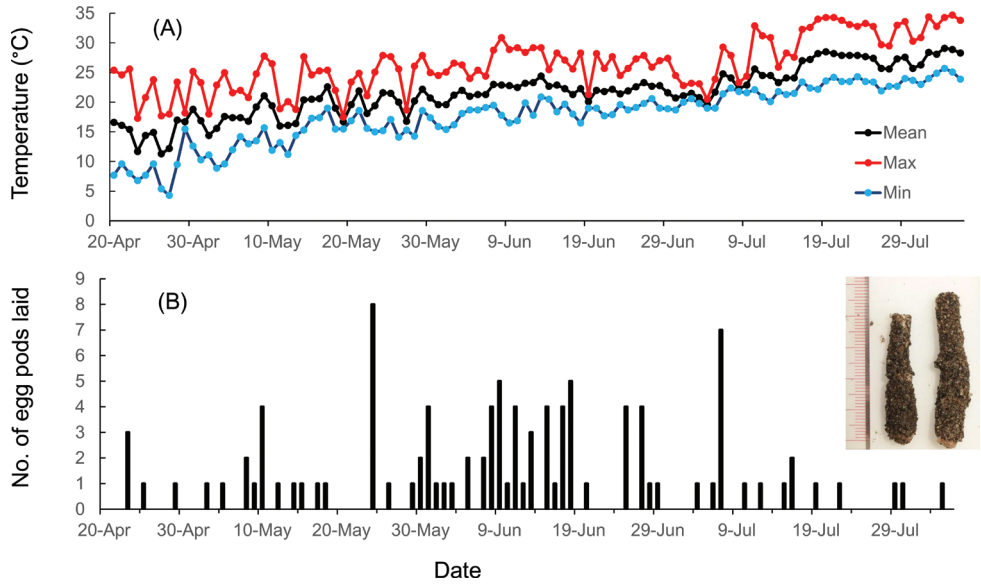

Fig. 7. Air temperatures (**A**) and daily number of egg pods laid by *Patanga japonica* in outdoor cages in 2021 (**B**). Inset shows egg pods. Air temperatures are derived from the Japan Meteorological Agency (2022).

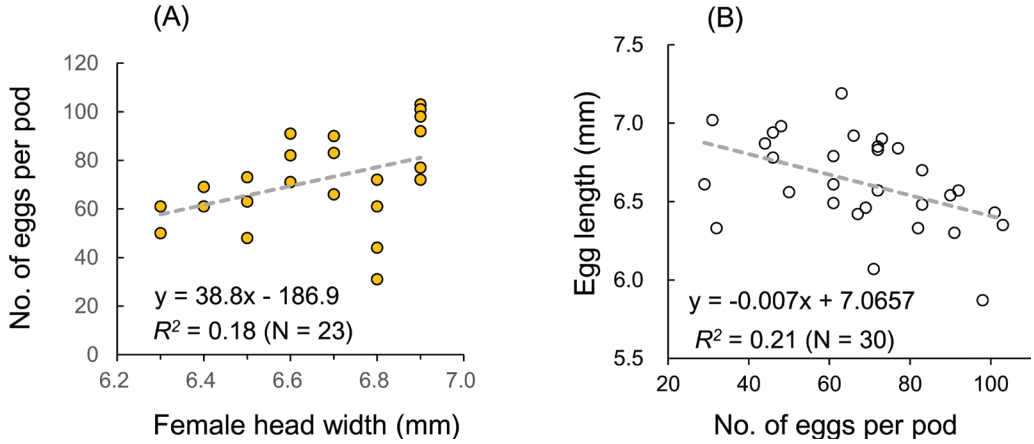

Fig. 8. Relationships between female head widths and numbers of eggs per pod (**A**) and between number of eggs per pod and egg lengths (**B**) in *Patanga japonica*.

days. Half of the females deposited their last egg pod by early July, and most females ceased oviposition by the end of July (Suppl. material 6B). As mentioned earlier, the female collected on August 10 in the field in 2022 laid her last egg pod on August 13 and died the following day. Females laid 5.4 egg pods on average (SD = 2.7 pods, range = 0–9 pods, N = 20). Egg pods were 9.1 mm wide (SD = 0.8 mm, range = 1.2–0.7, N = 99) and 60.7 mm long on average (SD = 16.5 mm, range = 91.0–30.3, N = 74).

The average number of ovarioles was 123.5 (SD = 14.7, N = 16) and showed no significant correlation with head width (r = 0.09, $p > 0.05$). The average number of eggs per pod was 72.1 (SD = 18.9, range = 44–103, N = 23). The lifetime fecundity, which was estimated by multiplying the number of egg pods produced by the number of eggs per pod, was 446.8 on average (range = 144–904 eggs, N = 11).

A significant positive correlation was observed between the numbers of eggs per pod and the head widths of the female parents (Fig. 8A; r = 0.42, N = 23, $p < 0.05$), suggesting that larger females tended to produce more eggs per pod. A significant negative correlation was observed between the number of eggs per pod and egg length (Fig. 8B; r = –0.46, N = 30, $p < 0.05$), suggesting a trade-off between the two traits. In contrast, the correlation between adult head width and egg length was statistically insignificant (r = –0.11, N = 11), probably because the sample size was small. Lifetime fecundity was not significantly correlated with adult head width (r = 0.12, N = 11, p = 0.71).

Egg pods produced by adults that were reared in a group of 5 females and 5 males after collection in March contained significantly fewer eggs (mean = 53.6, SD = 20.7, N = 7) than those produced by females kept with a single male (mean = 72.16, SD = 18.1, N = 23, t = 2.23, DF = 28, $p < 0.05$). However, there was no significant difference in egg length between egg pods produced by the two groups of females [mean = 6.6 mm, SD = 0.2 mm, N = 7 for eggs laid by group-reared females; mean = 6.6 mm, SD = 0.3 mm, N = 23 for egg pods laid by females kept with a male (t-test, $p > 0.05$)].

Hatching under outdoor conditions.—Once hatching started under outdoor conditions, most nymphs hatched from the egg pod within 30 minutes (Fig. 9A). Because hatching from the egg pod occurred simultaneously, the start of hatching identified by interval photographing was regarded as the time of hatching for each egg pod. Egg hatching occurred at different times of day between 10:45 and 18:30 (Fig. 9B). No eggs hatched during the night.

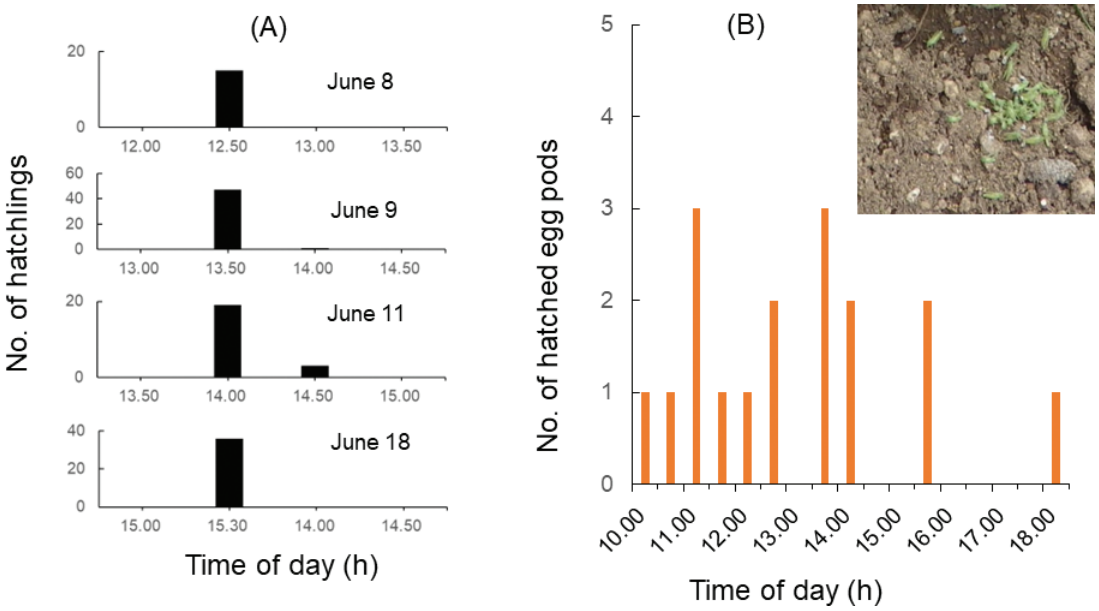

Fig. 9. *Patanga japonica* egg hatching. **A.** Hatching time from 4 egg pods; **B.** Number of egg pods that hatched at different times of the day. Inset in (**B**) shows simultaneous hatching from the egg pod.

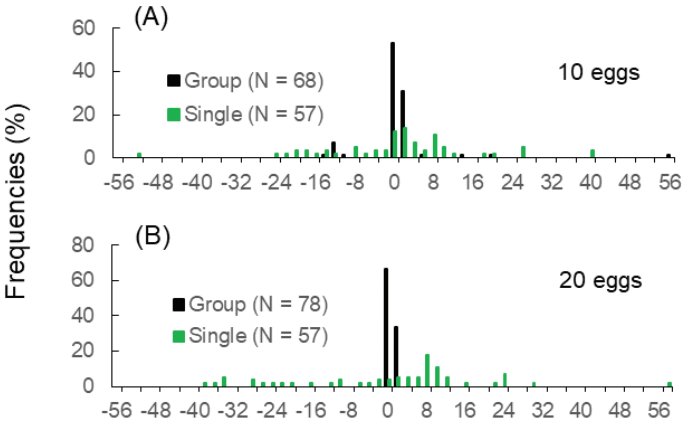

Fig. 10. Hatching distribution of *Patanga japonica* eggs kept singly and those kept in a group of 10 (**A**) and 20 (**B**) at 30°C when the mean hatching time was adjusted to 0 h. *F*-test shows that the variances of two treatments are significantly different from one another ($p < 0.05$).

Mechanism controlling hatching synchrony.—To confirm the presence of synchronous hatching under controlled conditions, the pattern of hatching was compared between eggs kept in a group and those kept singly. Fig. 10 shows that most eggs kept in a group of 10 or 20 eggs hatched within 1 h of the mean hatching time (0 h), with the SD being 0.8 h and 0.2 h, respectively, whereas those kept singly hatched over a wider range of time, with the SD being 15.3 h and 19.0 h. The mean hatchability of pooled data for the eggs kept in a group and those kept singly was 97.1% and 78.1%, respectively, and the difference was statistically significant (Mann–Whitney *U*-test, $z = 3.15$, N = 11 each, $p < 0.05$).

Hatching tended to occur earlier in eggs kept in a larger group in a sand pit (Fig. 11A). The mean hatching interval between the first and last hatching was similar among the three groups ($p > 0.05$, Steel–Dwass test, Fig. 11B). The proportion of pairs with hatching intervals ≤ 1h also showed no significant difference between the three treatments ($\chi^2 = 0.56$, DF = 2, $p > 0.05$).

Two eggs kept in contact with one another hatched more synchronously than those kept separated by 2–5 mm on sand (Fig. 12A; Steel–Dwass test, $p < 0.05$). The hatching interval of the two eggs was further prolonged if an early hatched nymph was prevented from touching the other egg by a mesh separator. Hatching time was significantly delayed when two eggs were isolated by a mesh separator (Fig. 12B; Tukey's multiple test, $p < 0.05$).

Two eggs separated by 2–5 mm showed a significantly wider mean hatching interval (6.9 h) than those kept in contact with one another (1.6 h; Fig. 13A, B; Steel–Dwass test, $p < 0.05$). In contrast, separated eggs hatched as synchronously as those kept in contact with one another when they were connected by two pieces of wire (2.4 h; Fig. 13A, C; $p > 0.05$), suggesting that some physical stimuli transmitted via the wire facilitated synchronous hatching.

To determine how hatching synchrony was achieved between eggs, the contents of 14 egg pods were divided into two batches of eggs, and one batch was chilled in a refrigerator for various lengths of time, and the other batch was continuously kept at 30°C. One egg was taken from each of the two batches and put together as mixed pairs in a sand pit (mixed pair) or buried individually as controls at 30°C (Fig. 14A). It was assumed that if there was no interaction between the two eggs in the mixed pairs, their hatching time would be similar to the difference in the mean hatching time between the two controls (dotted line in Fig. 14A). It was reasonable to expect small hatching intervals when the two eggs in the mixed pairs had similar hatching times, as shown in Fig. 14A. However, their hatching intervals remained short (1–3 h), even when the two controls hatched 18 h apart or less, suggesting that the hatching timings were adjusted for synchronization. Hatching intervals tended to increase as the difference in mean hatching time between the two controls increased ($r = 0.94$, N = 14, $p < 0.001$), but the hatching intervals for the mixed eggs remained below the dotted line, suggesting that the hatching time of the mixed eggs modified even when the difference in development between the two controls exceeded 18 h (but less than 40 h). In this experiment, the time of the first hatching in each egg pod was designated as 1 h, and the mean hatching intervals of control 1 (15.3 h, N = 14) and 2 (17.7 h, N = 14) did not differ significantly from one another (Mann–Whitney's *U*-test; $p = 0.42$).

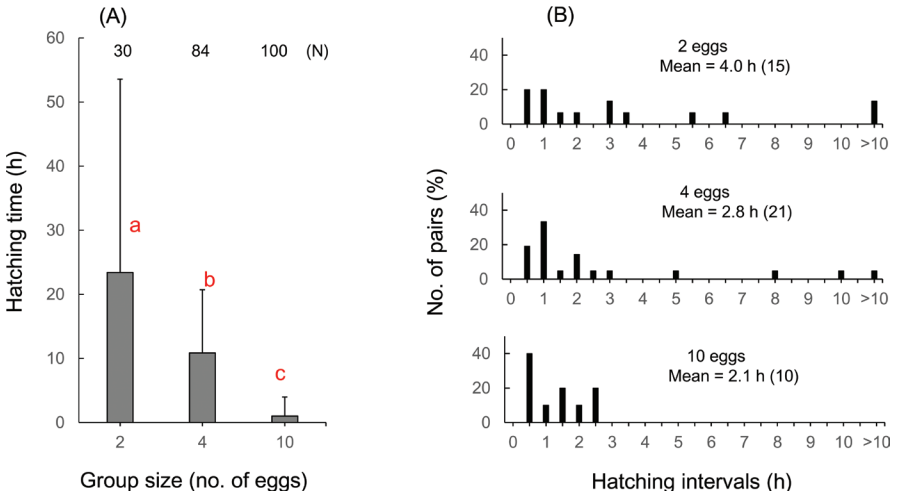

Fig. 11. Hatching times (**A**) and hatching intervals (**B**) of *Patanga japonica* eggs in different group sizes at 30 °C. Different letters in (**A**) indicate significant differences at the 5% level by Tukey's multiple test. In (**B**), the numbers in parentheses indicate N, and no significant difference was observed in mean value between treatments by Steel-Dwass test at the 5% level.

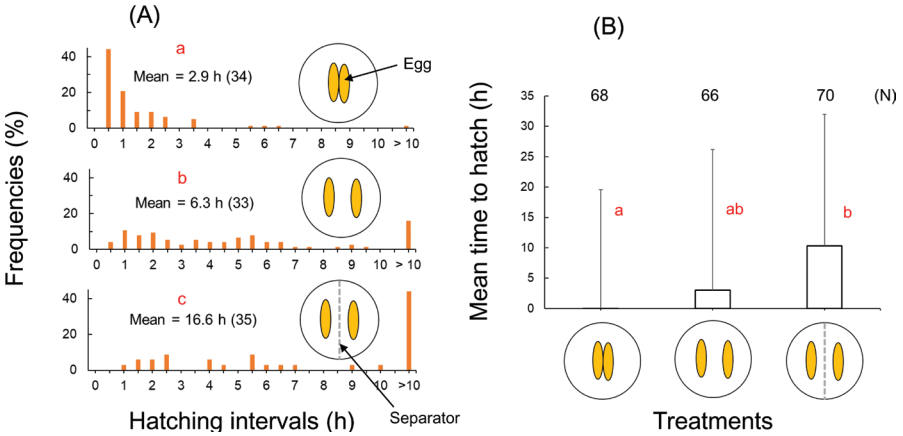

Fig. 12. Hatching intervals (**A**) and hatching times (mean + SD, B) in pairs of *Patanga japonica* eggs treated in different ways at 30 °C as shown by diagrams. Different letters indicate significant differences at the 5% level by Steel-Dwass test in (**A**) and by Tukey's multiple test in (**B**). In (**A**), the numbers in parentheses indicate N.

For synchronous hatching to be achieved, egg hatching times must be adjusted. Fig. 14B gives an example showing that the early hatching eggs in the mixed pairs (mixed 1) delayed the hatching time for synchronization by 13.4 h, on average, compared with control 1 (t = –2.27, DF = 20, p < 0.05). In another experiment (Fig. 14C), the late hatching eggs in the mixed pairs (mixed 2) advanced the hatching time by 18.2 h, on average, compared with control 2 (t = –2.19, DF = 18, p < 0.05).

Adult morphology and crowding.—*P. japonica* adults collected before and after winter showed no significant difference in all body size dimensions measured for both sexes (Table 2), suggesting no significant size-dependent winter mortality. Therefore, the two datasets were combined in the following analyses.

Adults collected in the field (low density) and crowd-reared adults (high density) under outdoor conditions showed no significant difference in head width in either sex (Fig. 15A). The F/C ratio showed a tendency to increase in crowd-reared adults compared with those collected in the field (Fig. 15B), the direction of change being opposite to that observed in other locust species (Uvarov 1966). The E/F ratios showed an opposite tendency: crowding caused similar or reduced E/F ratios in both sexes (Fig. 15C). In

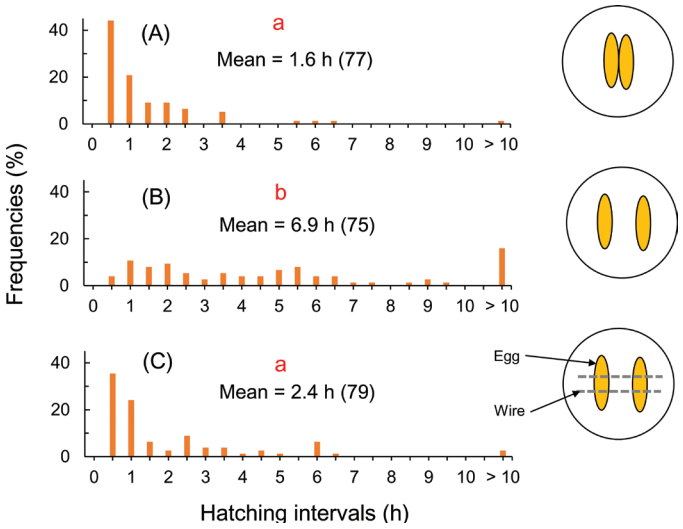

Fig. 13. Hatching intervals in pairs of *Patanga japonica* eggs treated in different ways at 30 °C as shown by diagrams. Different letters indicate significant differences at the 5% level by Steel-Dwass test. The numbers in parentheses indicate N.

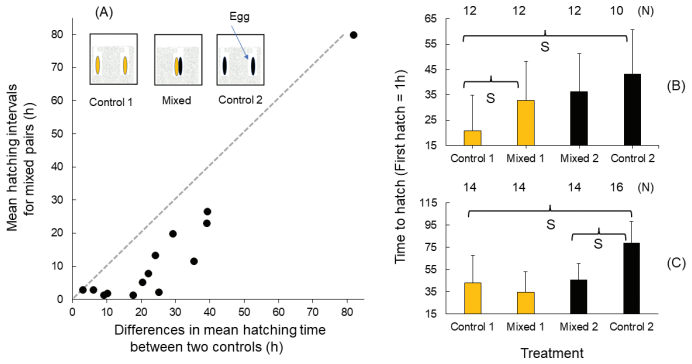

Fig. 14. Hatching intervals (**A**) and hatching times (**B, C**) of two *Patanga japonica* eggs buried individually (controls 1 and 2) or together (mixed) in sand at 30°C. In (**A**), diagram illustrates the experimental setup, and the dotted line indicates that two eggs in the mixed pairs had similar hatching times. The mean hatching time of mixed pairs was significantly accelerated (**A**) or delayed (**B**) compared with control eggs (s, $p < 0.05$; *t*-test).

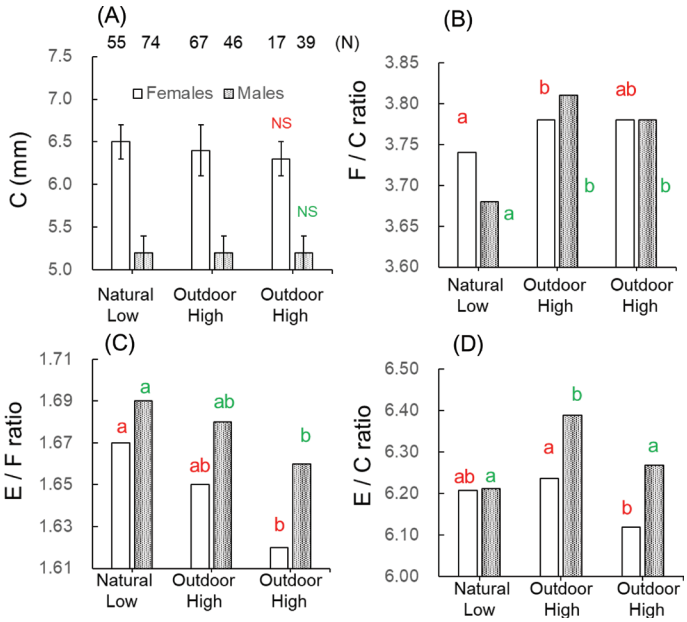

Fig. 15. Effects of growing density on (**A**) adult head width (mean ± SD), (**B**) femer length/head width, (**C**) forewing length/femur length and (**D**) forewing length/head width in *Patanga japonica* grown under natural (low density) or outdoor conditions (high density). Mean values were compared by Tukey's multiple comparison test in (**A**) and by Steel-Dwass test in (**B**), (**C**), and (**D**). Different letters indicate significant differences at the 5% level. NS indicates no significant difference. C, head width; F, femur length; E, forewing length. Adults in the two high-density groups hatched on July 5 and July 25, 2021 and were reared in a large cage in groups of approximately 200 and 100 individuals, respectively.

regards to E/C ratio, the males that hatched on July 5 had a higher ratio than those that hatched on July 25 or those collected in the field (Fig. 15D). In females, this ratio in crowd-reared adults remained similar to that of the field-collected adults.

In adults reared indoors, head width was similar among males in different treatments irrespective of the rearing densities, whereas female adults reared singly in containers were significantly larger than those reared in a group in containers or cages (Tukey's multiple comparison, $p < 0.05$; Fig. 16A). The F/C ratio was similar among the three treatments, except for the males reared in a group of 50 individuals in cages, which showed a significantly larger value (Fig. 16B). The E/F ratio was significantly larger in males reared in a group of 5 than in the other treatments, and crowding caused no significant difference in the ratio in females (Fig. 16C). No significant difference was observed in E/C ratio in females, but the crowd-reared males showed a significantly larger E/C ratio than the males reared singly (Fig. 16D).

The proportions of adults with different shapes of pronotum in each sex were similar irrespective of rearing conditions (outdoors or indoors) or densities ($\chi^2 = 6.00$, DF = 4. $p > 0.05$ in females; $\chi^2 = 4.40$, DF = 4, $p > 0.05$ in males; Fig. 17). In males reared indoors in a group of 5 individuals per cup, there was only one individual with an arched-shaped pronotum. Therefore, the proportions of males with arched and flat pronota were combined to perform the above χ^2 tests. More concaved pronota and fewer arched pronota were observed in males than in females, and a significant sex difference was observed in the proportions of individuals with different shapes of pronotum in the three treatments (Fig. 17). However, neither rearing conditions (indoor or outdoor) nor density appeared to consistently explain the pattern observed.

Discussion

Seasonal life cycle and development.—The present study confirmed that *P. japonica* in central Japan has a univoltine life cycle. Nymphs started appearing early in the summer and emerged as adults in the fall. No adults reproduced before winter, and no new nymphs were observed in the field until the following July. Field observations, together with indoor rearing results, suggest that this species has 6 or 7 nymphal stadia. Preliminary observations suggest that significant sexual dimorphism in body size occurs at the third stadium, and the two molting groups started showing a difference in body size at the fifth stadium: the mean head width was significantly smaller in the nymphs that underwent an extra molt than in the penultimate females but similar to the value in the penultimate males, including singly reared individuals (Suppl. material 1B). The former molted to larger last-instar nymphs than the latter. Only females underwent 7 nymphal stadia in the present observations, and this included the individuals reared singly.

New adults started appearing in mid-September, and most individuals emerged as adults by November. This was confirmed by outdoor rearing in a cage (Fig. 4). Interestingly, body size tended to increase as the time of adult emergence was delayed in females, whereas it remained similar in males. It is likely that this difference is partly a reflection of the sex differences in the number of nymphal stadia.

Males emerged as adults earlier than females, indicating protandry. Because this grasshopper does not reproduce before winter, the protandry may confer no advantage to males in terms of mating. Furthermore, adults of this grasshopper mate more than once during the adult stage. Thus, it is likely that the protandry in this grasshopper is a result of the body size difference between sexes. A similar situation might occur in solitarious *L. migratoria* in which protandry is also observed (H. Tanaka 1982). *L. migratoria* also shows polyandry, and the proportion of offspring sired by the last male to mate tends to be high (Tanaka and Zhu 2003). Therefore, the early adult emergence of males may not be so advantageous in terms of reproduction.

During the census, predatory animals were frequently encountered. They included mantises (*Tenodera aridifolia* (Stoll, 1813) and

Table 2. Morphological measurements of *Patanga japonica* adults collected in the field or reared under various conditions (A) and comparisons of body dimensions and morphometric ratios of adults collected before and after winter (B).

(A)

Sex	Growing conditions	Time of collection or rearing conditions	Females N	Mean ± SD (range) mm C	F	E	TBL	F / C	E / F	E / C	Hatching date
Females											
	Natural	2020 Fall	38	6.5 ± 0.2 (6.0 – 7.1)	24.3 ± 0.8 (22.5 – 26.0)	40.7 ± 2.1 (35.7- 44.1)	51.6 ± 2.3 (45.3 – 55.5)	3.73	1.67	6.25	Unknown
	Natural	2021 Spring	17	6.6 ± 0.2 (6.2 – 7.0)	24.7 ± 1.2 (22.4 – 26.9)	40.9 ± 2.3 (37.2 – 44.9)	52.4 ± 2.8 (47.5 – 57.0)	3.76	1.65	6.18	Unknown
	Natural	Fall + Spring	55	6.5 ± 0.2 (6.0 – 7.0)	24.5 ± 0.9 (22.4 – 26.9)	40.7 ± 2.2 (35.7 – 44.9)	51.9 ± 2.6 (45.3 – 57.0)	3.74	1.67	6.21	Unknown
	Outdoor	Group-reared 1	67	6.4 ± 0.3 (5.7 – 7.1)	24.2 ± 1.1 (21.8 – 26.8)	40.0 ± 2.0 (35.7 – 44.9)	51.5 ± 2.7 (45.9 – 60.4)	3.78	1.65	6.24	5-Jul
	Outdoor	Group-reared 2	17	6.3 ± 0.2 (5.9 – 6.6)	23.8 ± 1.1 (20.8 – 25.6)	38.5 ± 1.8 (35.1 – 40.3)	49.6 ± 1.8 (46.0 – 51.6)	3.78	1.62	6.12	25-Jul
	Indoor	Group-reared 3	50	6.2 ± 0.3 (5.7 – 7.1)	23.2 ± 1.3 (21.2 – 263)	38.4 ± 1.8 (35.3 – 41.9)	49.2 ± 2.3 (45.6 – 54.5)	3.75	1.66	6.20	Jun 1 – Aug 14
	Indoor	Group-reared 4	45	6.2 ± 0.2 (5.8 – 6.6)	23.3 ± 0.8 (20.9 – 25.1)	38.7 ± 1.7 (35.5 – 42.0)	49.4 ± 1.9 (45.3 – 52.7)	3.77	1.66	6.26	May 31 – Jun 4
	Indoor	Isolation-reared	40	6.4 ± 0.2 (6.0 – 6.9)	24.0 ± 1.3 (21.4 – 27.8)	40.0 ± 1.8 (36.4 – 43.4)	50.8 ± 2.5 (42.3 – 55.9)	3.76	1.66	6.22	May 27 – June 4
Males											
	Natural	2020 Fall	50	5.2 ± 0.2 (4.7 – 5.5)	19.1 ± 0.7 (17.2 – 20.3)	32.4 ± 0.9 (29.6 – 34.2)	41.7 ± 0.1 (38.3 – 43.8)	3.67	1.70	6.22	Unknown
	Natural	2021 Spring	24	5.2 ± 0.2 (4.8 – 5.5)	19.2 ± 0.8 (17.4 – 21.0)	32.1 ± 1.4 (29.1 -34.5)	41.3 ± 1.7 (37.6 – 44.0)	3.69	1.67	6.17	Unknown
	Natural	Fall + Spring	74	5.2 ± 0.2 (4.7 – 5.5)	19.1 ± 0.7 (17.2 – 21.0)	32.3 ± 1.1 (29.1 -34.5)	41.6 ± 1.4 (37.6 – 44.0)	3.68	1.69	6.21	Unknown
	Outdoor	Group-reared 1	46	5.2 ± 0.2 (5.0 -5.6)	20.1 ± 1.1 (18.5 – 25.0)	33.7 ± 1.5 (31.1 – 37.0)	43.5 ± 1.7 (39.9 – 47.2)	3.81	1.68	6.39	5-Jul
	Outdoor	Group-reared 2	39	5.3 ± 0.2 (5.0 – 5.8)	20.1 ± 0.6 (18.9 – 21.6)	33.5 ± 1.0 (31.4 – 35.6)	43.2 ± 1.3 (40.9 – 46.4)	3.78	1.66	6.30	25-Jul
	Indoor	Group-reared 3	49	5.2 ± 0.2 (4.9 – 5.6)	19.7 ± 0.9 18.1 – 22.3)	33.6 ± 1.4 (30.4 – 37.7)	43.0 ± 2.2 (39.3 – 52.5)	3.78	1.70	6.43	Jun 1 – Aug 14
	Indoor	Group-reared 4	52	5.2 ± 0.2 (4.9 – 5.8)	20.1 ± 0.9 (18.2 – 23.3)	33.6 ± 1.5 (30.7 – 36.8)	43.2 ± 1.8 (39.0 – 47.0)	3.84	1.67	6.41	May 31 – Jun 4
	Indoor	Isolation-reared	32	5.2 ± 0.2 (4.9 – 5.6)	20.0 ± 0.7 (18.7 – 21.3)	32.3 ± 2.3 (22.6 – 35.8)	42.5 ± 2.2 (39.3 – 51.5)	3.76	1.64	6.18	May 27 – June 4

(B)

Sex	Test	mm C	F	E	TBL	F / C	E / F	E / C
Females	t-test or Mann-Whitney's *U*-test	*t* = -1.49	t = -1.8	t = -0.33	t = -1.02	*z* = -0.11	*z* = -1.70	*z* = -1.04
	p value	0.14	0.07	0.75	0.31	0.913	0.089	0.299
Males	t-test or Mann-Whitney's *U*-test	t = 0.52	t = -0.37	t = 0.91	t = 0.96	*z* = -1.11	*z* = -1.60	*z* = -0.525
	p value	0.60	0.72	0.37	0.35	0.268	0.110	0.525

T. angustipennis (Saussure, 1869)), dragonflies (*Orthetrum albistylum speciosum* (Uhler, 1858)), ants, paper wasps, spiders (*Trichonephila clavata* (L. Koch, 1878)), lizards (*Plestiodon japonicus* (Peters, 1864) and *Takydromus tachydromoides* (Schlegel, 1838)), tree frogs (*Dryophytes japonica* (Günther, 1859)), and birds. However, I only witnessed one *P. japonica* nymph caught by a *T. aridifolia* nymph and one nymph and one adult trapped by the webs of *T. clavata*.

Host plants.—*P. japonica* has been known to feed on various plants, including *P. montana var. lobata*, *Humulus scandens*, and grasses (Ichikawa et al. 2006). However, no quantitative data on the host plants of this grasshopper have been reported for this grasshopper. In the present study, nymphs and adults were observed on *P. montana var. lobata*, *M. sinensis*, *I. cylindrica*, and other plants. While most individuals were observed on the first three plant species, the proportion of adult individuals on the other plants was larger than the proportion of nymphs. This difference was probably not due to the difference in the plants they fed on but rather to behavioral differences: adults often stayed on non-feeding plants, such as *Solidago canadensis* var. *scabra* (Muhl. ex Willd.) Torr. & A. Gray and *Conyza canadensis* (L.) Cronquist, whereas nymphs primarily stayed on feeding plants.

Out of the 51 plant species that were collected in the habitats of *P. japonica*, 37 (73%) were consumed by nymphs, and at least one individual (out of 5) molted to the second stadium. The three plants—*P. montana var. lobata*, *M. sinensis*, and *I. cylindrica*—which were most frequently observed with *P. japonica* at the study site, supported the development of most or all nymphs to the second stadium. In the present study, the test nymphs were given only single plant species, and the test was discontinued upon ecdysis to the second stadium. Therefore, how many plant species can support the full development of this grasshopper remains unknown. In nature, *P. japonica* is likely to use a limited number of host plants because of their preference. The range of host plants would be different in different flora habitats. During the present study, I visited another habitat of *P. japonica* where nymphs were mainly observed on *E. crus-galli*, *H. scandens*, and *Setaria viridis* (L.) P. Beauv., which are the dominant plant species in the area. It is

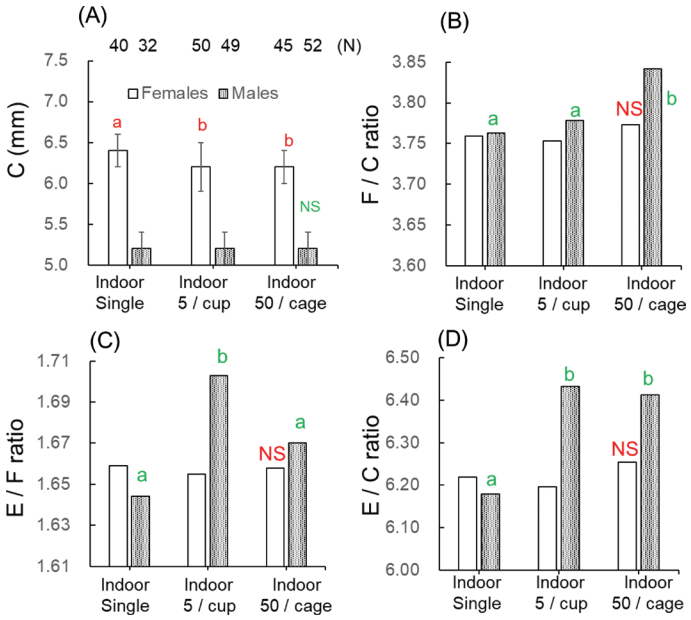

Fig. 16. Effects of growing density on (**A**) adult head width, (**B**) femur length/head width, (**C**) forewing length/femur length, and (**D**) forewing length/head width in *Patanga japonica* grown under indoor conditions. Nymphs were reared singly, in a group of 5 per cup, or in a group of 50 per cage. Mean values were compared by Tukey's multiple comparison test in (**A**) and by Steel-Dwass test in (**B**), (**C**), and (**D**). Different letters indicate significant differences at the 5% level. NS indicates no significant difference. C, head width; F, femur length; E, forewing length.

also likely that they use different plant species in different seasons, even in the same habitat. Nevertheless, the present study documented that this grasshopper could use a wide variety of plant species to develop.

Mating behavior.—Overwintered *P. japonica* adults started mating in the spring. In outdoor cages, mating was observed frequently in April and May. As mentioned earlier, most mounting pairs were copulating, suggesting that pre-copulatory mounting was very short in this grasshopper, unlike other species (Parker 1970, Parker and Smith 1975, Zhu and Tanaka 2002, Tanaka and Zhu 2003). Adults became very rare in the field in July.

Under outdoor conditions, the number of copulating pairs tended to increase during the day, and the daily maximum number of copulating pairs correlated with the mean daily temperature. In this study, the length of copulation was not determined precisely, but it varied greatly from less than 1 h to 14 h from 08:00 to 22:00. Some marked pairs were observed copulating during 2 or 3 consecutive observation periods (08:00–18:00), suggesting that copulation might last for 2 or 3 days if copulation continued without interruption during the night.

As observed in other grasshoppers (Uvarov 1966), males of *P. japonica* mount a female and copulate by reaching their penis to her genitalia. In this case, the males reach the females' genitalia from either side. The number of males reaching from the right or left was similar. In the mantis *T. aridifolia*, males all reach the female genitalia from her right side (Ando 2021). If a right-reaching (or left-reaching) male of *P. japonica* was removed from the copulating female and introduced to a second female, he reached her genitalia from the same side, even when the previous male that had mated with her was left-reaching (or right-reaching).

However, the male's behavior in this is probably not fixed but flexible. Males with one hindleg missing copulated with a female from the side of the missing leg. This may be related to the male's stridulation behavior that is observed during copulation. Males rubbed a hind tarsus against their forewing or abdomen 3 or 4 times consecutively and repeated this behavior while copulating (Suppl. material 4). Therefore, this stridulation may be categorized as a copulation song (Uvarov 1977). It seems that the function of this stridulation is to calm down the partner while copulating. The male with a missing hindleg had to use the remaining leg for stridulation, which may explain why such a male copulated with a female from the side of the missing hindleg: because he needed to be able to balance for stridulation. In one case, I observed a male that lacked the tarsus of his right hindleg. He copulated with a female as if the whole right hindleg had been missing: he reached her from the right side and stridulated using his left hindleg. Future studies should confirm the existence of this behavior by removing one hindleg or tarsus from a male and observing how he copulates. In the present study, no pre-copulatory behavior was investigated.

Oviposition.—In a survey of 163 grasshopper species, 85% laid their eggs in soil, 7% laid them on plants, 5% laid them in plants, and 4% laid them in detritus (Stauffer and Whitman 1997). *P. japonica* females deposit their egg pods in soil. Judging from the sites where hatchlings appeared in the field, eggs were laid in vegetation areas where the ground was partially exposed. Under outdoor conditions, oviposition began in late April and continued until the beginning of August. Peak oviposition activity occurred in June. Egg pods contained 72.1 eggs on average. Because female adults had an average of 124 ovarioles, they use only 58.1% of their ovarioles for egg production, which is similar to the value for solitarious females of migratory locust (62.8%, N = 470, Tanaka, S. unpublished observation).

Egg size is influenced by various physical and biological factors (Stauffer and Whitman 1997, Whitman 2008). As often observed in other insects (Honěk 1993, Davidowitz 2008, Yanagi and Tuda 2012), larger females of *P. japonica* appeared to have more ovarioles and lay more eggs per pod. However, unlike other insects showing a positive correlation (Atkinson and Begon 1987, 1988, Davidowitz 2008), *P. japonica* showed no significant correlation between female body size and egg size. A negative correlation or trade-off between egg size and number is common in insects (Branson 2008, Whitman 2008). A similar relationship was observed between the number of eggs per pod and egg length in *P. japonica*. In the present study, no attempt was made to investigate the evolutionary significance of egg size variations.

Hatching and the mechanism controlling hatching synchrony.—Under outdoor conditions, egg hatching in *P. japonica* occurred during the daytime, but the eggs of each pod hatched simultaneously. Hatching during the daytime is common in grasshoppers (Tanaka 2021b). In the desert locust, however, eggs kept under 12-h photocycles or thermocycles hatch during the dark or cryoperiod (low temperature period) rather than during the light or thermoperiod (Padgham 1981, Nishide et al. 2015a, b). In the field, they hatch over a brief period from shortly before dawn to the first 4 h after sunrise (Ellis & Ashall, 1957). In *L. migratoria* under laboratory conditions, eggs also hatch in response to temperature and photoperiod (Nishide et al. 2015b). In the field, however, eggs are buried in soil and rely on changes in temperature alone to control hatching time (Nishide et al. 2015b, 2017a). It would be interesting to explore on a molecular basis why and how this locust perceives and uses light to control hatching time. In *L. migratoria*, the eggs hatched between 09:00 and

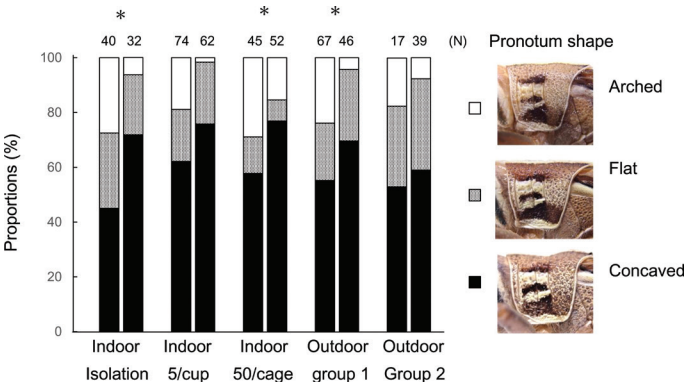

Fig. 17. Variation in adult pronotum shape in *Patanga japonica* reared singly or in groups under indoor or outdoor conditions. Photographs on the right show different shapes of pronotum. Individuals in group 1 and 2 hatched on July 5 and July 25 in 2021 and were reared in a group of approximately 200 and 100 in a large cage, respectively. Asterisks indicate a significant difference between sexes by χ^2 test ($p < 0.05$).

16:00 in the field (Chen 1999, Nishide et al. 2017b). *P. japonica* eggs also hatched during the day under outdoor conditions. However, no detailed analysis was carried out on the hatching time of this grasshopper in relation to temperature or other factors.

Hatching synchrony of *P. japonica* was confirmed to occur at a constant temperature of 30°C. In this case, only eggs buried as a group in sand hatched in synchrony, while those individually buried in sand hatched sporadically over a longer period. Hatching synchrony was achieved even in pairs of eggs kept in contact with one another. Greater increases in group size did not bring about significant changes in the hatching interval. However, hatching became earlier as group size increased. Two explanations for this phenomenon, which are not mutually exclusive, have been suggested: 1) A larger egg group is more likely to contain an early-hatching egg and 2) there are group-size dependent embryo–embryo interactions that might control hatching time (Tanaka 2021a). Two eggs separated by several millimeters on sand hatched less synchronously than those kept in contact with one another, but hatching synchrony occurred when the similarly separated eggs were connected by a steel wire, suggesting that physical stimuli transmitted via the wire induced hatching synchrony.

A similar phenomenon is known in other grasshoppers and locusts, and embryo–embryo interactions are involved in controlling hatching synchrony (Nishide and Tanaka 2016, Tanaka 2017, 2021a, b, Tanaka et al. 2018, Sakamoto et al. 2019). In *L. migratoria*, two eggs that would have otherwise hatched more than one day apart hatched together when kept in contact with one another (Tanaka 2017). To achieve synchronous hatching, egg hatching time had to be adjusted. In *L. migratoria* and the Bombay locust, *Patanga succincta* (Johannson, 1763), the hatching time of eggs is either delayed or advanced depending on the development of adjacent eggs. In contrast, the hatching time of the eggs of *S. gregaria* only advance to achieve synchronous hatching (Tanaka 2021a). The present study suggests that *P. japonica* is similar to the former.

In the field, newly hatched nymphs of *P. japonica* were observed climbing up *P. montana var. lobata* quickly and forming small aggregations of 3–7 individuals on the leaves (Suppl. material 2F). They appeared to be quite sensitive to vibrational or visual stimuli because they quickly scattered in all directions when I came close to them for photographing. Similar behaviors were observed in other grasshoppers. In *R. microptera* and *T. eques,* newly hatched nymphs quickly aggregate, sometimes forming a tight, touching ball of 10–40 nymphs. In *T. eques*, these tight aggregations break apart by the second stadium, but in *R. microptera*, they may continue into the third stadium. When such a ball of touching nymphs is approached, the nymphs jump wildly in every direction (Whitman DW, pers. comm.). This kind of behavior suggests that one of the functions of synchronous hatching is to form an aggregation to escape from approaching predators, as suggested for *P. succincta* (Tanaka 2021b). Synchronous hatching is also likely to reduce the probability of detection by predators by limiting the duration of hatching from the pod. Eggs buried separately in sand showed a lower rate of hatchability than those buried in a group. These observations suggest the possibility that group hatching facilitates successful egg hatching and might have influenced the evolution of egg deposition in an egg pod in grasshoppers.

Adult body size and crowding effects.—In the temperate region of Japan, *P. japonica* is the only grasshopper species known to overwinter as an adult (Ichikawa et al. 2006). A comparison of adults collected before and after winter suggested that there was no size-dependent mortality during winter. This grasshopper also overwinters in the adult stage in the subtropical region and maintains a univoltine life cycle (Ichikawa et al. 2006, Murai and Ito 2011). However, there is a large difference in adult body size between temperate and subtropical populations. For example, the female and male mean head widths of an Okinawa population are 9.0 cm (range, 9.5–8.5 mm; N = 31) and 6.9 mm (7.2–6.3 mm; N = 41), respectively (Tanaka and Okuda 1996), whereas the corresponding figures for the Tsukuba population are 6.5 cm (6.0–7.0 mm; N = 55) and 5.2 cm (4.7–5.5 mm; N = 74) (Table 1). Despite the large difference in body size, large females from a subtropical population copulated with small males from a temperate population in a small cage (Tanaka and Okuda 1996). Whether such pairing produces viable eggs, however, is unknown. In a small cage, a *P. japonica* female and a male of another species, *P. succincta,* collected in Okinawa, were observed copulating (Suppl. material 7), although no such heterospecific mating pair was observed when more than 40 mating pairs were examined at a sugarcane field in 1990 where the two species occurred sympatrically (Tanaka S, unpublished observation). In contrast, the incubation period of eggs at 30°C is approximately 7 days longer in the Okinawa population than in the Tsukuba population of *P. japonica* (Tanaka and Okuda 1996, and this study). It would, therefore, be worthwhile to confirm the species status of the temperate and subtropical populations.

Density-dependent changes in morphometric ratios are one of the most important characteristics of phase polyphenism (Uvarov 1966, 1977, Pener 1991, Pener and Yerushalmi 1998, Pener and Simpson 2009). Two species of locusts, *S. gregaria* and *L. migratoria*, are typical examples showing such changes, and the F/C and E/F ratios of these species are frequently used to separate individuals in different phases (Dirsh 1951, 1953, Farrow and Colless 1980, H. Tanaka 1982, Yerushalmi et al. 2001, Hoste et al. 2002 2003, Tanaka et al. 2002, Yamagishi and Tanaka 2009, Sugahara et al. 2015, 2016). In the above two locusts, crowding causes the F/C ratio to decrease and the E/F ratio to increase. Similar changes have been observed in *P. succincta* in Okinawa (Yasuda 1986). In *P. japonica*, these ratios also tended to be influenced by crowding, but the direction of the changes was opposite to what was expected.

The E/C ratio showed an interesting response to crowding, tending to increase in males but not in females. This ratio is higher in long-winged individuals than in short-winged ones in *L. migratoria* (Tanaka and Nishide 2013, Nishide and Tanaka

2013) and *S. gregaria* (Sugahara et al. 2017). If the ratio reflects flight capability, it might follow that *P. japonica* males emerge as better fliers under high population density than under low population density. Under outdoor conditions, this ratio increased in one of the crowd-reared treatments in which most adults emerged in September when the temperature was still relatively high and might be favorable for flight activity. To understand the significance of this response, further research should investigate the other factor(s) that are responsible for the changes in E/C ratio and dispersal activity in this grasshopper.

The pronotum shape is another character known to change density dependently in *L. migratoria* (Uvarov 1966, Tanaka et al. 2002). In *P. japonica*, a significant difference was observed in the proportions of arched, flat, and concave pronota between sexes, but there was no evidence indicating the presence of a density-dependent change in this trait.

As is often observed in locusts (Pener 1991), *P. japonica* nymphs become heavily melanized under crowded conditions (Tanaka and Okuda 1996), and this phenomenon was also observed during the present study (details to be published). Unlike locusts, however, nymphs of *P. japonica* were considerably inactive, even under crowded conditions, and no increased locomotor activity was seen, although no attempts were made to quantify their activities. Crowding is also known to induce a difference in egg size in locusts (Hunter-Jones 1958, Uvarov 1966, Nishide and Tanaka 2019). In *P. japonica*, however, crowding caused a reduction in egg number but not in egg size. In the temperate area, no records indicate the formation of aggregation or swarming in this species, except for small aggregations of hatchlings observed at low-population density. However, the available information suggests that *P. japonica* exhibits density-dependent polyphenism in some characters, as observed in locusts.

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Supplementary material 1

Author: Seiji Tanaka
Data type: JPEG file
Explanation note: Nymphal growth (head width, mean ± SD mm) of *Patanga japonica* under outdoor conditions. Nymphs were reared in a group from June to August (A) and from August to September (B). In (B) males, females and females that underwent an extra molt and their mean head widths (± SD) are shown separately as black, red, and green bars or letters, respectively. A few days after each molt, nymphs were measured, marked with white paint on a hind femur, and transferred to another cage. Roman numerals indicate the stadia. Numbers in parentheses indicate N. Significant differences were observed in mean head width among the three groups of last instar nymphs by Tukey's multiple test ($p < 0.05$). F, female; M, male; O, penultimate instar: L, last instar.

Link: https://doi.org/10.3897/jor.32.95753.suppl1

Supplementary material 2

Author: Seiji Tanaka
Data type: JPEG file
Explanation note: Wood-framed enclosure for grasshopper behavior observations; B. Small cages; C. Setup for observing hatching time; D. Feeding mark; E. Mating postures; F. Small aggregation of hatchlings.

Link: https://doi.org/10.3897/jor.32.95753.suppl2

Supplementary material 3

Author: Seiji Tanaka
Data type: JPEG file
Explanation note: Lengths of copulation under outdoor conditions in *Patanga japonica*. Observations were made from April 21 to April 25 in 2022. X-axis indicates the lengths of copulation recorded during the daily observation period (08:00–18:00 or 22:00). Pairs that were still mating at the end of daily observation are labeled 'copulation continued'.

Link: https://doi.org/10.3897/jor.32.95753.suppl3

Supplementary material 4

Author: Seiji Tanaka
Data type: movie
Explanation note: Video showing a typical mating and stridulation by a *Patanga japonica* male.

Link: https://doi.org/10.3897/jor.32.95753.suppl4

Supplementary material 5

Author: Seiji Tanaka
Data type: movie
Explanation note: Slow-motion video showing stridulation behavior by a male on a female of *Patanga japonica*. The female body was tilted by approximately 30 degrees to orient their body towards the sun and the male used both legs for stridulation.

Link: https://doi.org/10.3897/jor.32.95753.suppl5

Supplementary material 6

Author: Seiji Tanaka
Data type: JPEG file
Explanation note: Oviposition intervals in *Patanga japonica* in outdoor cages (A) and cumulative number of females that deposited the last egg pod on the indicated date (B) in 2021. In (A), mean ± SD is given.

Link: https://doi.org/10.3897/jor.32.95753.suppl6

Supplementary material 7

Author: Seiji Tanaka
Data type: JPEG file
Explanation note: Heterospecfic mating of *Patanga japonica* female and Bombay locust *Patanga succincta* male (A). Note that the eye stripes are conspicuous only in *P. succincta* (B).

Link: https://doi.org/10.3897/jor.32.95753.suppl7