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Acoustic Stridulating Responses of Various Tarantula Species in Peninsular Malaysia

(Tindak Balas Stridulasi Akustik Pelbagai Spesies Tarantula di Semenanjung Malaysia)

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ABSTRACT

Stridulation by stridulatory organs has been linked to tarantulas' (Araneae: Theraphosidae) defence or sexual communication, and the morphology of such organ has been extensively used in tarantula systematics. This study was conducted to characterise and compare differences in the acoustic pattern of stridulating sound among seven tarantula species for juveniles and adults of both sexes in Peninsular Malaysia. The species were *Psednocnemis jeremyhuffi*, P. *brachyramosa*, *Selenocosmi*a sp. 'Johor', *Omothymus violaceope*s, *Cyriopagopus robustus*, *Chilobrachys* sp. 'Kedah', and *Coremiocnemis* sp. 'Kelantan'. Five provocation methods were used to record the sound which was by tapping the substrate/enclosure, blowing air, waving a pen, poking with blunt object and shaking the enclosure. The stridulating behaviour was assessed using a set of parameters. The result showed that the stridulating behaviour differed between species. Dwarf species like *P. jeremyhuffi* and *P. brachyramosa* did not make any audible stridulating sound and preferred to remain motionless. Meanwhile, for the other five species only the large adult females stridulated. Adult males of all species did not make any audible sounds, but rather appeared to be more aggressive and frequently bite. Kruskal-Wallis test showed that intensity, pitch frequency, call duration and pulse duration were significantly different between species. Principal component analaysis (PCA) showed the dissimilarity between the adult females from the five species with *Omothymus violaceopes* and *Chilobrachys* sp. 'Kedah' the most distinct in terms of call patterns. This study provides evidence on the acoustics pattern of stridulating sound for the tarantulas in Peninsular Malaysia.

Keywords: Animal behaviour; bioacoustics; sound intensity; stridulation; Theraphosidae

ABSTRAK

Bunyi desingan telah dikaitkan dengan pertahanan atau komunikasi seksual tarantula (Araneae: Theraphosidae), dan morfologi organ tersebut telah digunakan secara meluas dalam sistematik tarantula. Penyelidikan ini dijalankan untuk mengenal pasti ciri dan membandingkan perbezaan corak akustik bunyi desingan antara tujuh spesies tarantula bagi juvenil dan dewasa yang terdiri daripada kedua-dua jantina di Semenanjung Malaysia. Spesies tersebut ialah *Psednocnemis jeremyhuffi*, *P. brachyramosa*, *Selenocosmia* sp. 'Johor', *Omothymus violaceopes*, *Cyriopagopus robustus*, *Chilobrachys* sp. 'Kedah' dan *Coremiocnemis* sp. 'Kelantan'. Enam kaedah provokasi telah digunakan untuk merekod bunyi tersebut iaitu dengan mengetuk tanah/sangkar, menghembus udara, menggerakkan pen, mengusik dengan objek tumpul dan menggoncang sangkar. Perilaku berdesing dinilai menggunakan satu set parameter. Keputusan menunjukkan bahawa perilaku berdesing berbeza antara spesies. Spesies kerdil seperti *P. jeremyhuffi* dan *P. brachyramosa* tidak mengeluarkan sebarang bunyi desingan yang boleh didengari dan lebih memilih untuk tidak bergerak. Sementara itu, untuk lima spesies yang lain hanya betina dewasa sahaja yang dapat berdesing. Jantan dewasa daripada semua spesies tidak mengeluarkan sebarang bunyi yang boleh didengar, sebaliknya kelihatan lebih agresif dan kerap menggigit. Analisis Kruskal-Wallis menunjukkan frekuensi, kekuatan bunyi, tempoh bunyi dan tempoh denyut berbeza secara jelas antara spesies. Analisis komponen prinsipal (PCA) menunjukkan perbezaan antara betina dewasa daripada lima spesies dan mendapati *Omothymus violaceopes* dan *Chilobrachys* sp. 'Kedah' mempunyai jarak yang ketara dari segi pola bunyi. Kajian ini memberikan bukti tentang corak akustik bunyi desingan untuk tarantula di Semenanjung Malaysia.

Kata kunci: Bioakustik; desingan; kekuatan bunyi; sikap haiwan; Theraphosidae

INTRODUCTİON

Tarantulas (Araneae: Theraphosidae) are typically ground dwelling invertebrates, living in silk-lined burrows. They are classified into two groups, the Old World and the New World tarantulas. The presence of urticating hairs is one of the most noticeable physical differences between them. As compared to the New World tarantulas, the Old World tarantulas lack the urticating hairs, and rely solely on a threatening posture to deter predators (Shirey & Rayburn 2013). This posture is assumed when the tarantula rears up and extends its front legs, moving its palpi vertically into the air and opening its chelicerae in this position. When provoked repeatedly, the tarantula will lunge forward and bite, producing a more serious but less painful bite in humans (Gallon 2000).

Meanwhile, the New World tarantulas have tiny (0.2 mm - 1.2 mm long) urticating hairs on their abdomens, which are used as their primary defence against predation. These hairs are armed with spines and barbs designed to penetrate vertebrate skin and other sensitive tissues with which they come into contact, such as the eyes and nasal cavity (Mullen & Vetter 2019). Currently, there are seven types of urticating hairs described based on morphology. They differ in terms of location, shape, size, and orientation of barbs along the shaft, as well as the length/width ratio (Kaderka et al. 2019). Specialised hairs on the proximal segments of tarantula appendages are linked to stridulation, which are used in defence or sexual communication (Perez-Miles et al. 2005). Stridulation is defined as the act of producing sound, usually by rubbing two body parts together or, in the case of tarantulas, by stridulatory organs, which are made up of specialised setae and antagonistic structures on the integument (Jocqué 2005; Lima & Guadanucci 2018).

Several tarantula characteristics have been previously described and classified based on their stridulatory organs. In fact, the stridulatory organs have been used extensively in the tarantula systematics (Pérez-Miles et al. 1996; Pocock 1895; Schmidt 1999; Simon 1903). According to Wood-Mason (1877), the first audible sound reported for a spider was made by the Indian theraphosid (*Chilobrachys stridulans*). An Australian theraphosid is also known to have the ability to make sound by rubbing its spines against bristles on chelicerae, and thus giving them the name 'whistling or barking spiders' (Mascord 1980). The sound produced by *Selenocosmia crassipes* tarantula when it is provoked or irritated is a faint or whistling sound that is audible to the human ears. Another method of stridulation is to rub the trochanters of palp against the leg (Perez-Miles et al. 2005). Marshall, Thoms and Uetz (1995) used the stridulatory setae to study the sound production of a few species and discovered that the audible sound produced by *Theraphosa blondi* was for self-defence. Another species which made an audible hissing sound when threatened is the pink-toed tarantula (*Avicularia avicularia*), which raises two pairs of its legs emitting a clear hiss that could be heard from a few metres away (Stradling 1976).

In addition, a study on the bioacoustics of the African 'baboon spiders' discovered that *Anoploscelus lesserti, Citharischius crawshayi,* and *Pterinochilus murinus* could stridulate at different durations and frequency of a single sound (Henning, Weberz & Schottler 2002). As for the Malaysian tarantulas, the female *Omothymus violaceopes* is known to stridulate when being provoked; it makes a very faint hiss which sounded like a serpent, followed by the act of striking to attack (Abraham 1924). Although tarantulas (Araneae: Theraphosidae) have a wide range of morphological features, little is known regarding the documentation of stridulatory organ in sound or signal production and its role in communication (Marshall, Thoms & Uetz 1995), especially for Malaysia's tarantula species. Therefore, this study was conducted to characterise the acoustic pattern of stridulating sound and compare the differences in acoustic sound among selected tarantula species in Peninsular Malaysia. This is because it was hypothesized that sound characteristics and structure differed between species.

MATERIALS AND METHODS

STUDY AREA

This study covered several genera of Theraphosidae in Peninsular Malaysia, except for the genus Lyrognathus. Different species were collected from different locality, i.e., *Psednocnemis jeremyhuffi* (West & Nunn 2010) were collected from three states (Perak, Selangor and Negeri Sembilan). *Psednocnemis jeremyhuffi* can be found at lowland forest area, mostly below 400 m of elevation. This species prefer to make its burrow on hiking trail and old logging road. *Coremiocnemis* sp. 'Kelantan' was collected from lowland forested area with elevation of 110 m in Kelantan state. *Chilobrachys* sp. 'Kedah' was collected from lowland forested area in Kedah state. The spider is opportunistic and can be found at wide type of habitat such as waterfall, cave area, lowland forest and also on wall crevices. *Cyriopagopus robustus* (Strand 1907) from Selangor can be found in highland areas in Selangor state. This species prefers to make its web on sloped areas with good amount of forest canopy. This spider also utilizes dead leaf on the entrance of its burrow. Three species used in this study were collected from Johor state, which were *Selenocosmia* sp. 'Johor', *Psednocnemis brachyramosa* and *Omothymus violaceopes*. *Selenoocsmia* sp. 'Johor' was collected from a lowland forest area in Johor and was found living primarily on river embankment and waterfall area. *Psednocnemis. brachyramosa* (West & Nunn 2010) can be found at several different habitat types such as old logging road, hiking trails and river bank. It can be found living in forested area below 400 m elevation. Lastly, *Omothymus violaceopes* (Abraham,1924) was collected from a lowland forest area in Johor where the species can be found living inside tree trunks and is arboreal. Subjects of experiment were taken to the laboratory at Universiti Malaysia Terengganu to observe and record the stridulating sound.

DATA COLLECTION

A total of 110 specimens of seven tarantula species from Peninsular Malaysia were used in this study. Tarantulas from this study were lured out from its burrow by using small sticks without destroying their burrow. Collection of tarantulas for this study was done by *ad-hoc* sampling, either unplanned or planned on a case by case basis, as an approach for obtaining the maximum information about tarantulas with a minimum effort. A field protocol named COBRA–Conservation Oriented Biodiversity Rapid Assessment (Cardoso 2009) was followed despite the absent of animal ethics approval. The seven species were (a) *C. robustus* (three adult females, two sub-adults and one juvenile) (b) *Coremiocnemis* sp. 'Kelantan' (one adult female) (c) *Chilobrachys* sp. 'Kedah' (three adult females, two adult males, two sub-adults and four juveniles) (d) *Selenocosmia* sp. 'Johor' (six adult females, one adult male, two sub-adult and four juveniles) (e) *P. jeremyhuffi* (seven adult females, four adult males, five sub-adult and 11 juveniles) (f) *O. violaceopes* (six adult females, three adult males, three sub-adults and four juveniles) (g) *P. brachyramosa* (nine adult female, five adult males, ten sub-adult, and twelve juveniles) (Figure 1). The juveniles, sub-adults, adult males and females from all species were experimented to observe whether all stages have the ability to stridulate. Sexes of the species were determined only for the adult spiders. Adult male spiders can be identified by the presence of pedipalps, have smaller body elongated legs whereas adult females were identified by applying ventral sexing and looking at the spermathecae of the exuvia. All species were identified up to species or genus level by using keys following the World Spider Catalogue (2023).

BEHAVIOURAL OBSERVATION AND SOUND RECORDING TECHNIQUES

The documentation of the stridulating behaviours was done along with sound recording made *ad libitum.* Stridulation occurred when the tarantula was provoked, causing it to turn into its defensive posture. Five methods of provocation were used in this experiment to cause the tarantulas to stridulate. The methods were: tapping the enclosure/substrate, blowing air, waving a pen in front of the spider, poking it with a blunt object and shaking the enclosure. Tapping was done by using a pencil to tap either on the substrate or the enclosure. Blowing air was done by using mouth to blow air directly towards the spiders. Waving a pen is done in front of the tarantula, 5 cm to 10 cm from the tarantula without any physical contact. Shaking of the enclosure was done by shaking the enclosure mildly without hurting the tarantula. To obtain the sound data, a blunt probe was used to trigger the defensive reaction on the acquired specimen, following Marshall, Thoms and Uetz (1995). The specimen sound recording was held in a laboratory room with sound proof material attached on the wall of the room by using thick blanket to minimize sound interference from the outside. Recording of the sound was done at night time between 21:30 and 23:00 hour to minimize sound disturbance coming from the outside of the laboratory. Setup for the experiment was made by using a large-sized aquarium enclosure with some substrate. Recording was done for roughly seven to ten minutes per individual. The recording device was set to 16 bit/44.1 kHz (Hrušková-Martišová, Pekár & Gromov 2008). The induced stridulating sound was recorded with a portable device (Sony ICD-PX470 Black Digital Voice Recorder with USB) connected to a stereo omnidirectional condenser microphone (mic 3.5 mm). Time taken for stridulating that was associated with the rubbing of chelicerae movement was recorded for each recording session.

AUDIO SOUND PROCESSING AND ANALYSIS

The obtained sound data was processed by using Audacity 3.0.5, following Banga et al. (2019). Audacity is software that can perform sound processing operations. To analyse only the stridulation sound made by the tarantulas, unwanted background sound was removed from each recording using the software. Audio sound analysis was done by using Praat Version 6.3.03 to obtain details of the sound. Only five of the seven species observed are capable of stridulation. From the five species, only adult females produce audible stridulating sound. Because of that, only sounds from adult females from the five species were included in this acoustic analysis. Thirty calls from each species were extracted and analysed. Acoustic variations among individuals from each species were not accounted due to limitations of collecting certain specimens. For example, only one adult female from *Coremiocnemis* sp. 'Kelantan' was found and this species is very rare from our observation. Software Past Version 4.0 was used to perform statistical analyses. Since the data were not normally distributed, Kruskal-Wallis test was used to compare intensity, pitch frequency, call duration and the pulse duration. Principal component analysis (PCA) was used to test dissimilarity between species for the intensity, pitch frequency, call duration, pulse duration, note per call and

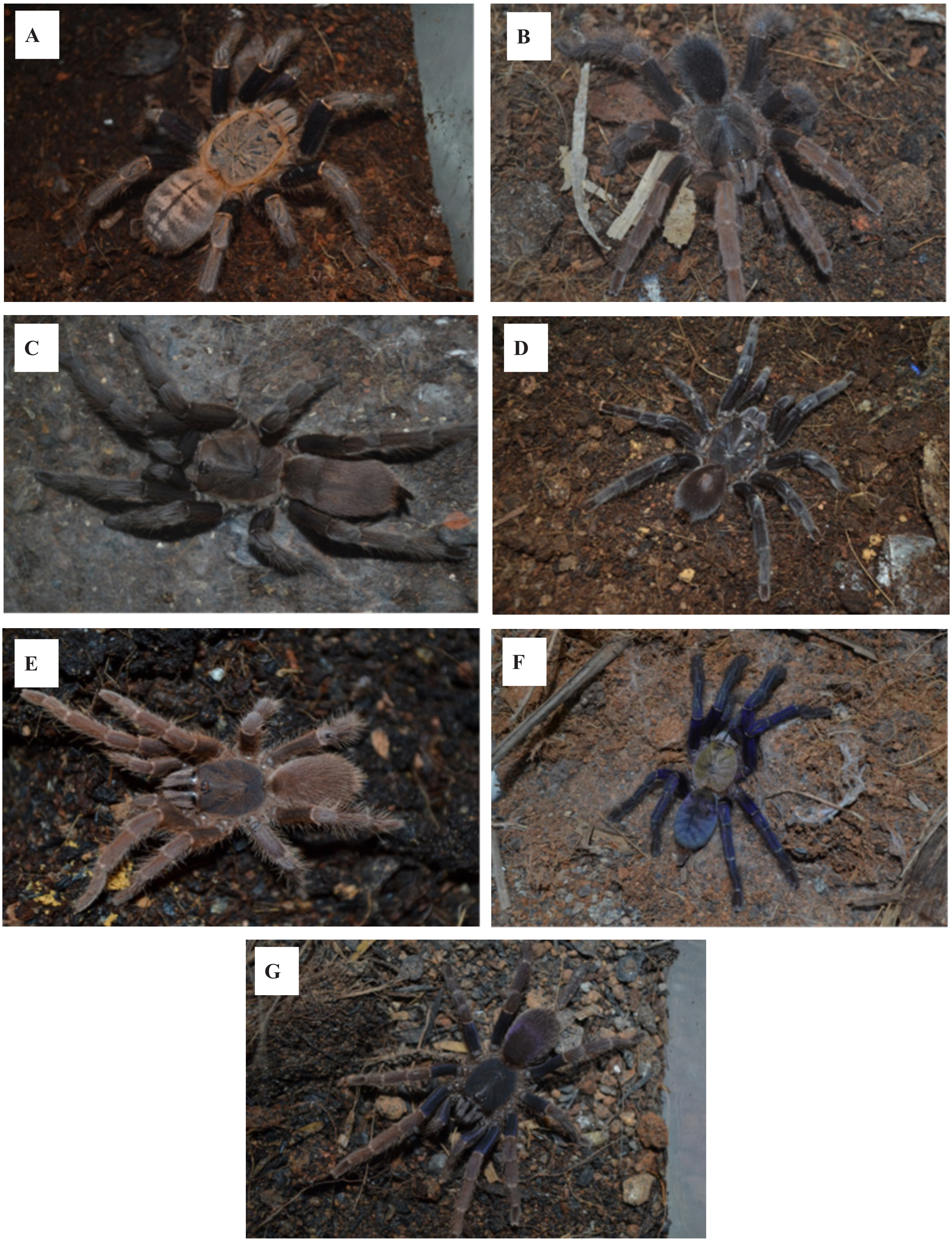

FIGURE 1. The tarantula species used in the experiment: (a) *Cyriopagopus robustus* (b) *Coremiocnemis* sp. '*Kelantan*' (c) *Chilobrachys* sp. '*Kedah*' (d) *Selenocosmia* sp. '*Johor*' (e) *Psednocnemis jeremyhuffi* (f) *Omothymus violaceopes* (g) *Psednocnemis brachyramosa*

note duration. MATLAB R2021a Version 9.10 software was used to generate time domain signal and spectrograms. For the sound signal analysis, vocal parameters measured were based on Köhler et al. (2017) and Thomas et al. (2014), i.e., 1) pitch frequency: sound frequency in Hertz which is closely related with fundamental frequency, F0 (Fermo et al. 2019; Larsen et al. 2022); 2) intensity of the sound in decibel; 3) call duration between the start of the first pulse and end of last pulse of a call; 4) note duration measured from the start of the first pulse in a note and end of last pulse in a note; 5) note per call; and 6) pulse duration measured the start and end of the middle pulse in a call. The call parameters were calculated by using data from 30 calls from 21 adult females. The sound structure in this analysis was evaluated in accordance with Köhler et al. (2017): 1) single note per call or multiple notes per call; 2) simple call (all notes of one type) or a complex call (different note types); 3) pulsatile or non pulsatile notes; 4) continuous or discontinuous call and 5) presence or absence of harmonics in the sound signal.

RESULTS

STRIDULATION BEHAVIOUR

From behavioural observation, it was discovered that stridulation occurred when the tarantula was provoked. Provocation caused the tarantula to turn to its defensive posture. It was observed that not all tarantulas made audible stridulating sounds (Table 1). Out of the tarantula species, *Psednocnemis jeremyhuffi* and *P. brachyramosa* did not make any audible stridulating sound. Female *Chilobrachys* sp. 'Kedah', *Selenocosmia* sp. 'Johor', *O. violaceopes*, *C. robustus*, and *Coremiocnemis* sp. 'Kelantan' were amongst species that made sounds. However, result showed that adult male, sub-adult (unknown sex), and juvenile did not stridulate. All adult females of *Chilobrachys* sp. 'Kedah' and *C. robustus* specimens stridulated. This was not the case for *Selenocsomia* sp. 'Johor', in which only half of the specimens stridulated while the remaining three did not. Figure 2(a) depicts the threat pose made by *Selenocosmia* sp. 'Johor', while Figure 2(b) and Figure 2(c) depicts the side and frontal views of threat pose made by *Chilobrachys* sp. 'Kedah', respectively. The movement of fangs while making the stridulating sound can also be seen in Figure 2(c).

Meanwhile, dwarf specimens, such as those from the genus *Psednocnemis*, did not stridulate. It posed a defensive posture, but there was no movement of brushing between its fangs. In addition, when threatened individuals from this genus preferred to play dead or curl up, rather than respond with a defensive posture. Furthermore, of the five provocation methods used in this experiment, waving a pen in front of the spider, elicited the most reaction in stridulating tarantulas. This method caused the tarantulas to stand still without biting but stridulating greater and longer than normal. However, none of these methods caused *P. jeremyhuffi* or *P. brachyramosa* to stridulate. Other methods for eliciting the stridulating sound included blowing air at the tarantulas and tapping on the substrate/enclosure. It was noticed that when there was physical contact, such as poking it with a blunt object, the tarantula chose to bite rather than to stridulate.

SOUND ANALYSIS AND CHARACTERISTIC OF THE AUDIO SIGNAL

Referring to Table 2, the results showed that *Selenocosmia* sp. 'Johor' produced the highest mean sound intensity (65.74 dB), with a range of 62.2 dB-70.65 dB. Meanwhile, for pitch frequency *Omothymus violaceopes* had the highest mean (13331.18 Hz), with a range of 4588 Hz–17604.27 Hz, while the lowest mean pitch frequency was produced by *Chilobrachys* sp. 'Kedah' (9514.4 Hz), with a range of 7093.01 Hz-1344.03 Hz. *Cyriopagopus robustus* had the longest mean call and note durations, which were 17.95 and 0.65 seconds, respectively. Furthermore, the notes per call were not distinguishable for *Selenocosmia* sp. 'Johor', but the other species it can be distinguished. *C. robustus* had the highest note per call (34.5 s) whereas the longest mean pulse duration was produced by *Coremiocnemis* sp. 'Kelantan' (6.81 ms), with a range of 4 ms-11 ms.

Call description or spectral structure of the sound, as well as general call and spectral structure were found to be differed among species (Table 3). Except for *Selenocosmia* sp. 'Johor' which produced single notes per call, all species produced multiple notes per call which were considered complex because they had different types of notes. The calls of all species also exhibited pulse and pulsatile features. *Chilobrachys* sp. 'Kedah', *C. robustus* and *O. violaceopes* could produce a continuous sound. In contrast, *Selenocosmia* sp. 'Johor' and *Coremiocnemis* sp. 'Kelantan' were considered to have a no continuous call as the sounds that were projected by both species were very short in duration. All species made sounds with visible harmonics. Furthermore, based on time domain of signal, the sound structure was clearly different between species, which visualised the acoustic pattern in a waveform and spectrogram. The signal in time domain waveform and the signal spectrogram derived from each species are presented in Figure 3. The spread of sound intensity differed in terms of its closeness between the peak, and density of sound in the time domain.

TABLE 1. The specimens and categories: ‘Yes’ indicated that sound emitted; while ‘No’ indicated no sound was emitted

Species	Locality	Category				Total
		Adult Female	Adult Male	Sub-adult (unknown sex)	Juvenile (unknown sex)	
P. jeremyhuffi	Perak, Selangor, Negeri Sembilan	(Yes) 0 (No) 7	(Yes) 0 (No) 4	(Yes) 0 (No) 5	(Yes) 0 (No) 11	27
P. brachyramosa	Johor	(Yes) 0 (No) 9	(Yes) 0 (No) 5	(Yes) 0 (No) 10	(Yes) 0 (No) 12	36
Chilobrachys sp. ‘Kedah’	Kedah	(Yes) 3 (No) 0	(Yes) 0 (No) 2	(Yes) 0 (No) 2	(Yes) 0 (No) 4	11
Selencosmia sp. ‘Johor’	Johor	(Yes) 3 (No) 3	(Yes) 0 (No) 1	(Yes) 0 (No) 2	(Yes) 0 (No) 4	13
C. robustus	Selangor	(Yes) 3 (No) 0	(Yes) 0 (No) 0	(Yes) 0 (No) 2	(Yes) 0 (No) 1	6
O. violaceopes	Johor	(Yes) 6 (No) 0	(Yes) 0 (No) 3	(Yes) 0 (No) 3	(Yes) 0 (No) 4	16
Coremiocnemis sp. ‘Kelantan’	Kelantan	(Yes) 1 (No) 0	(Yes) 0 (No) 0	(Yes) 0 (No) 0	(Yes) 0 (No) 0	1
Total of specimens used:						110

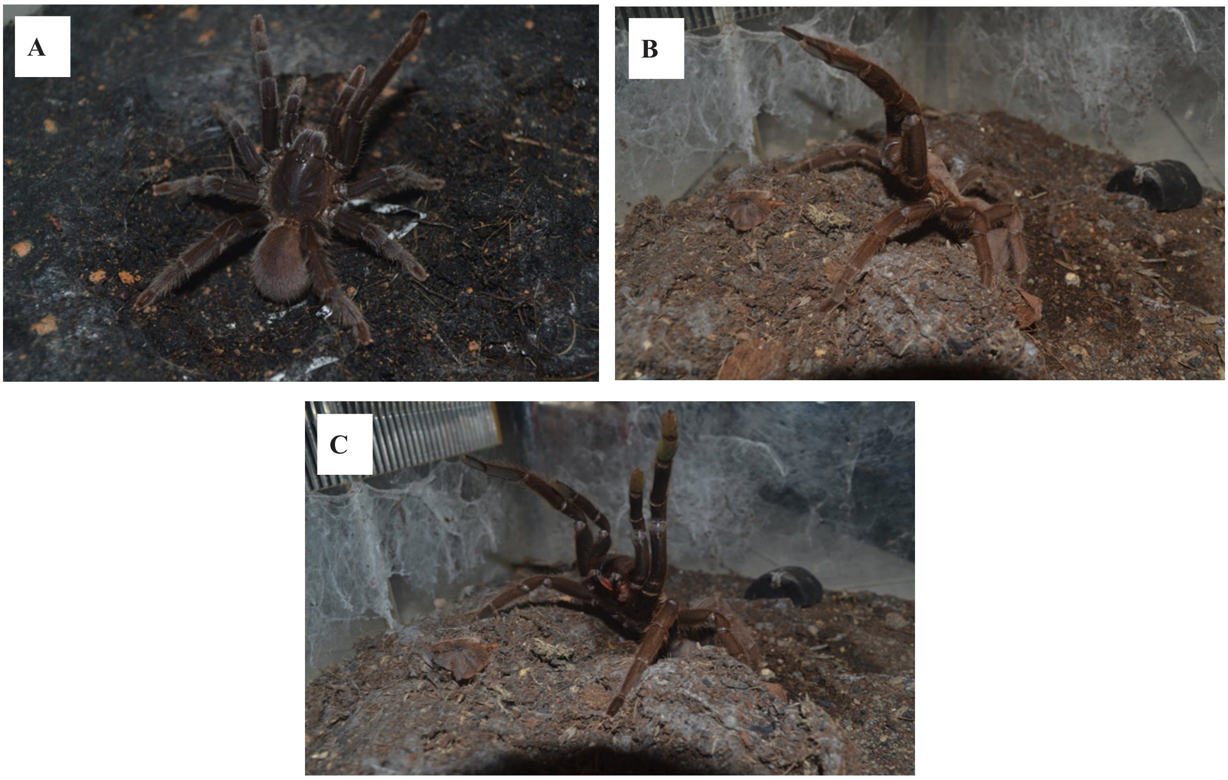

FIGURE 2. The tarantula species (a) *Selenocosmia* sp.: ‘Johor’ making its threat pose. (b) *Chilobrachys* sp. ‘Kedah’ side view of its threat pose (c) Frontal view of *Chilobrachys* sp. ‘Kedah’ threat pose. Noted that fangs observed moving when the stridulation sound was produced

COMPARISON OF THE SOUNDS BETWEEN GENERA

Kruskal-Wallis test was applied to test the comparison between species. For the sound intensity, the result was lower than the significance level ($p = 0.05$), which was 6.141×10^{-16}. It indicated that there was a statistically significant difference between sample medians. This was also the same p-value for pitch frequency (1.06×10^{-4}), call duration (2.28×10^{-19}) and pulse duration (4.53×10^{-17}) (Table 4). All p-values showed that there were significant differences between sample medians for intensity, pitch, call duration and pulse duration.

According to the PCA performed on the bioacoustic parameters, the principal component 1 (PC1) and principal component 2 (PC2) explained the variance at 99.997% and 0.002%, respectively. It was noted that PC1 was highly associated with the pitch frequency of the sound. However, PC2 illustrated that it was strongly associated with intensity of sound. A scatterplot based on principal component 1 (PC1) and principal component 2 (PC2) was generated as shown in Figure 4; it was shown that *Omothymus violaceopes* and *Chilobrachys* sp. ‘Kedah’ are distant with each other when it comes to the call patterns. *Omothymus violaceopes*, *Coremiocnemis* sp. ‘Kelantan’ and *Selenocosmia* sp. ‘Johor’ was close to each other in a group, while *Chilobrachys* sp. ‘Kedah’ and *Cyriopagopus robustus* was closer to each other.

DISCUSSION

DEFENSIVE BEHAVIOUR OF TARANTULAS

In this study, the tarantulas’ defensive response was discovered to be associated with stridulation sound for certain species particularly the females. It was supported by Marshall, Thoms and Uetz (1995), who stated that sound was made when tarantula was in a defensive posture, with elevated front legs. Adult females of some medium- to large-sized species were observed to make audible sounds

TABLE 2. The values of call parameter; n = the number of individuals of each species used

	Mean	SD	Range (Min-Max)	CV (%)
	Selenocosmia sp. 'Johor' (n=3)			
Sound intensity (dB)	65.74	4.59	62.20-70.65	7.00
Pitch (Hz)	11562.67	3192.91	6546.16-17401.52	27.61
Call duration (s)	1.10	0.73	0.30-2.75	66.18
Note duration (s)	1.63×10^{-2}	5.43×10^{-3}	9.00×10^{-2}-2.20×10^{-2}	33.41
Note per call	-	-	-	-
Pulse duration (ms)	4.05	1.61	1.00-8.00	39.75
	Omothymus violaceopes (n=6)			
Sound intensity (dB)	64.17	3.99	55.24-72.66	6.22
Pitch (Hz)	13331.18	1683.60	4588-17604.27	12.63
Call duration (s)	9.18	7.02	1.2-31	76.51
Note duration (s)	0.48	6.53×10^{-2}	0.35-0.57	13.58
Note per call	29.66	12.22	18.00-51.00	41.20
Pulse duration (ms)	6.34	2.11	2.00-10.00	33.28
	Cyriopagopus robustus (n=3)			
Sound intensity (dB)	61.56	4.39	50.55-71.88	7.13
Pitch (Hz)	10665.37	2813.3	4870.83-16952.04	26.37
Call duration (s)	17.95	12.62	3.80-48.10	70.28
Note duration (s)	0.65	0.07	0.51-0.78	10.76
Note per call	34.5	12.52	16.00-57.00	36.29
Pulse duration (ms)	4.46	1.33	2.00-7.00	29.82
	Coremiocnemis sp. 'Kelantan' (n=1)			
Sound intensity (dB)	49.54	2.85	42.83-62.46	5.75
Pitch (Hz)	12493.19	3450.43	83188.89-16072.41	27.61
Call duration (s)	4.78	3.08	1.10-15.50	64.52
Note duration (s)	0.33	0.10	0.21-0.53	4.19
Note per call	13.30	7.60	2.0-24.00	57.14
Pulse duration (ms)	6.81	1.82	4.00-11.00	26.73
	Chilobrachys sp. 'Kedah' (n=3)			
Sound intensity (dB)	64.36	2.54	46.30-72.19	3.94
Pitch (Hz)	9514.40	3737.41	7093.01-1344.03	39.28
Call duration (s)	12.96	5.28	3.50-27.20	40.73
Note duration (s)	0.44	7.71×10^{-2}	0.30-0.57	17.68
Note per call	31.00	12.94	8.00-57.00	41.74
Pulse duration (ms)	1.70	0.65	4.00-11.00	38.29

TABLE 3. Description for call/spectral structure of the sound

Structure of sound	Specimens				
	Selenocosmia sp. 'Johor'	*O. violaceopes*	*C. robustus*	*Coremiocnemis* sp 'Kelantan'	*Chilobrachys* sp 'Kedah'
Single notes per call	Yes	No	No	No	No
Multiple notes per call	No	Yes	Yes	Yes	Yes
Simple call	No	No	No	No	No
Complex call	Yes	Yes	Yes	Yes	Yes
Pulsed	Yes	Yes	Yes	Yes	Yes
Pulsatile	Yes	Yes	Yes	Yes	Yes
Unpulsed	No	No	No	No	No
Continous call	No	Yes	Yes	No	Yes
Noncontinous call	Yes	No	No	Yes	No
Presence of visible harmonics	Yes	Yes	Yes	Yes	Yes
Absence of visible harmonics	No	No	No	No	No

when threatened. *Psednocnemis* sp., a dwarf-sized tarantula, made no audible sound and preferred to remain motionless. According to Evans (1999), a less aggressive small spider will choose to play dead in order to avoid being eaten by a predator. Moreover, in this experiment, the adult males from all species did not make any audible sounds; hence, there was no rubbing movement via the chelicerae and they only made a threat posture when disturbed. The male spiders appeared to be more aggressive and bite more frequently than did the females. In contrast to the findings by Bertani, Fukushima and da Silva Júnior (2008), they discovered that males of *Pamphobeteus crassifemur* species found in Brazil could stridulate while shedding their urticating hairs. It produced a sound that was similar to a whistle while in a resting position. The resting posture of *P. crassifemur* differs from the striding posture of Malaysian tarantula, in which the anterior legs and palps of tarantula are lifted upward and outward, exposing the fangs.

It demonstrated that biting was their primary defence and stimulating sound was their secondary defence. This finding was supported by Akinde (2022) and Gallon (2000), who stated that due to lack of urticating hairs, the Old World tarantulas rely solely on a threatening posture to deter predation as their primary defence and deliver a very painful bite when threatened. Therefore, it is possible that the absence of audible sounds upon provocation of the male tarantulas in this study were due to difference in terms of capability of male species to stridulate between Old World tarantula (Malaysia) and New World tarantula (Brazil). For juvenile tarantulas, they did not produce any audible sound when being provoked. From a study on *Theraphosa blondi* from South America, the stridulating setae first appeared in the fifth instar (Marshall, Thoms & Uetz 1995), with the species becoming adult at the 10th instar (Marshall & Uetz 1993). The method of the Malaysian tarantulas to produce sound was similar to that of the Australian tarantulas (*Selenocosmia crassipes*), which rubbed the bristles between its fangs when irritated (Mascord 1980). Furthermore, when provoked, particularly by waving a pen in front of the spider, the large female tarantulas displayed more aggressive and longer stridulating

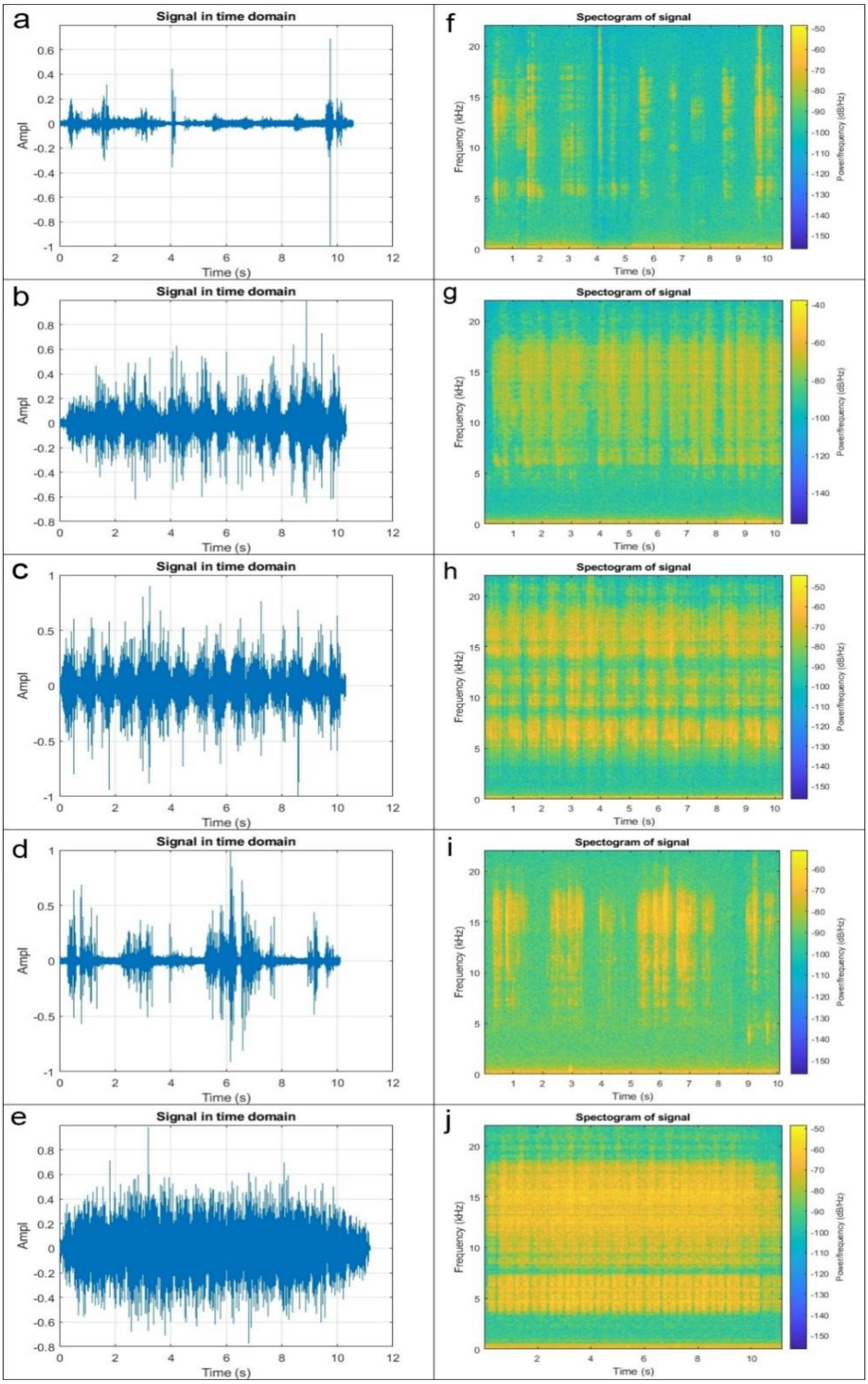

FIGURE 3. The signal in time domain waveform (left) and the signal spectrogram (right). (a) and (f) *Selenocosmia* sp. 'Johor'; (b) and (g) *O. Violaceopes*; (c) and (h) *C. robustus*; (d) and (i) *Coremiocnemis* sp. 'Kelantan' and (e) and (j) *Chilobrachys* sp. 'Kedah'

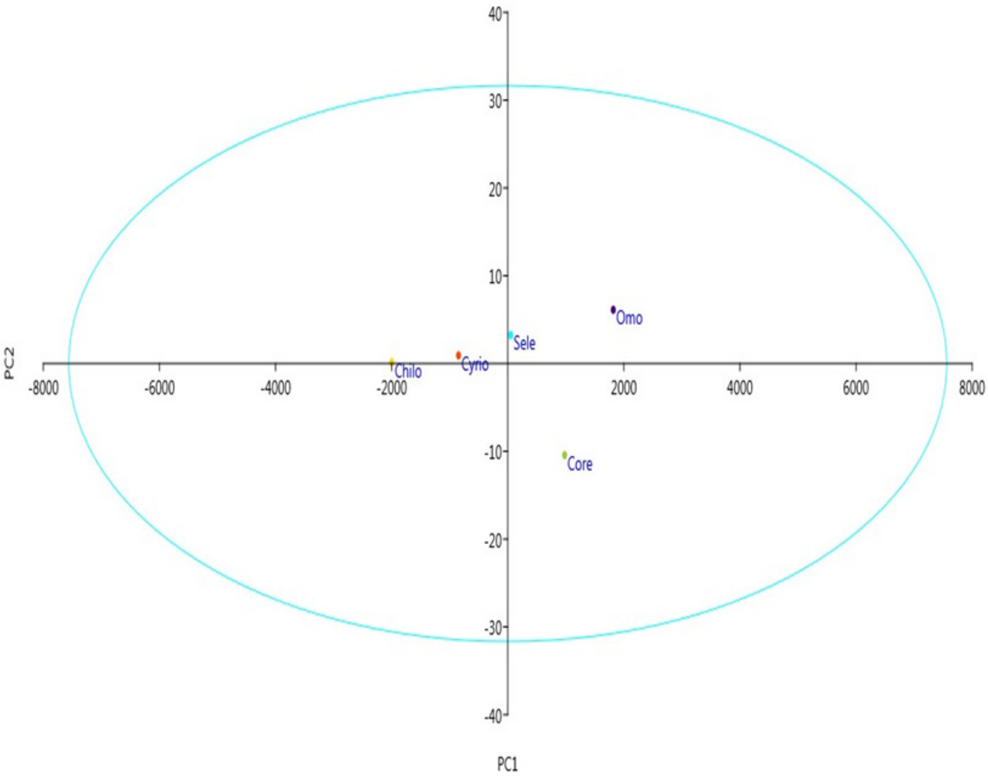

FIGURE 4. PCA scatter plot (PC1 vs PC2) for the acoustic parameters of the studied tarantulas. Chilo = *Chilobrachys sp*. 'Kedah'; Core = *Coremiocnemis sp*. 'Kelantan'; Cyrio = *C. robustus*; Omo = *O. violaceopes*, and Sele = *Selenocosmia sp*. 'Johor'

TABLE 4. Results of the Kruskal-Wallis test performed on the sound perimeters for each Theraphosidae species in this study

Perimeters	Kruskal-Wallis Test	
	H	p
Intensity	77.41	6.141×10^{-16}
Pitch	23.39	1.06×10^{-4}
Call duration	93.57	2.28×10^{-19}
Pulse duration	81.30	4.53×10^{-17}

behaviour. This result was also consistent with a previous report, which stated that a new study in Austria confirmed that spiders were extremely sensitive to vibrations, ranking them second only to cockroaches (Edwards 2011).

SOUND CHARACTERISTICS AND DIFFERENCES BETWEEN SPECIES

Stridulation sound of the tarantulas was assessed from the waveform and spectrogram to evaluate their acoustic patterns. It was observed that the pulse duration and pattern were different between species. This sound pattern was repeated and interspersed irregularly, depending on the tarantula's response, which affected the rubbing intensity of chelicerae. All spectrograms showed that the basal pulse was absent. This demonstrated that the sound only consisted of a stridulation sound and not a compound sound (Barria, Quirós & Emmen 2021). The stridulation was above the basal region, near the mid-frequency range (approximately from 5 kHz to 15 kHz). The pulse varied between species. Some species had a constant pulse, whereas others had a constant pulse that was not clearly differentiated. The Malaysian tarantulas that we studied were capable of producing noise that ranged approximately from 5 kHz to 17 kHz. The sound produced by the Malaysian tarantula was higher than that of South American species (*Thearaphosa blondi*), which was approximately 3 kHz - 5 kHz (Marshall, Thoms & Uetz 1995). However, the sound ranges of Malaysian tarantulas are considered similar to those of an African species (*Citharischius crawshayi*), which could reach approximately 17.4 kHz, but are lower than those of *Pterinochilus murinus* and *Anoploscelus lesserti*, which could reach approximately 21 kHz (Henning, Weberz & Schottler 2002). It was discovered that some Malaysian tarantulas' sound samples could also reach 20 kHz. It was also mentioned that the sound signal of the African tarantulas was used to warn off enemies like small mammals or tarantula wasps. Up until now, there was no study on the Asian tarantulas' stridulation which can be referred to for the tarantula sound studies in Malaysia. More taxonomic and molecular studies need to be done regarding tarantula species in Malaysia. During this study, several species from this study has not been described and can only be identified until its genus.

The sound characteristics and structure analysis among different species showed statistically significant differences in sound intensity, pitch, call duration, and pulse duration between species. Furthermore, the PCA showed that the tarantula call patterns also differed between species. This experiment had proven that the acoustic signal produced by tarantulas varies significantly between species according to Kruskal-Wallis test. Bertani, Fukushima and da Silva Júnior (2008) stated that differences in the structure and position of stridulatory organ amongst tarantula subfamilies demonstrated that the subfamilies were not identical morphologically. Therefore, this explains the different sounds produced by the Malaysian tarantula in this study. The acoustic signal produced by the spiders is believed to be related to the position, structure of the stridulatory organ and type of stridulatory organs. Because of this distinct feature of the acoustic signal between species, it demonstrated that the acoustic signal has potential to be used to identify the tarantula in the country. Adult female tarantulas were known to spend most of their time inside burrow for a long period of time (Fukushima et al. 2019). In relation, stridulating sound can become one of its defence mechanisms when a sudden loud noise coming from inside of the burrow is produced that might scare the approaching predators. It was also noted that all adult male specimens in the study did not produce stridulating sound. Once becoming adult, male tarantula will wander out of its burrow to find females (Fukushima et al. 2019). Thus, adult male tarantula is totally exposed to threats and might rely solely on its bite to deter predators as a quicker defence compared to giving an earlier warning by sound. In conjunction with molecular and morphological data (Turner et al. 2017), tarantulas' acoustic signal can be used as a part of the classification and in understanding their evolution, ecology, and behaviours.

CONCLUSION

Stridulating organs are an important feature used in tarantula taxonomy and morphology classification. The stridulating sound was found to be associated with defensive behaviour and produced in a variety of ways. The majority of adult female tarantula species observe in this study in Peninsular Malaysia can make an audible distress call. This study proved that different tarantula genera have different call patterns. As this was the first study to focus solely on tarantula acoustics in Asia, more research is needed to better understand the role of bioacoustics in tarantulas' life history.

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