

Research Article

Bioacoustic study of a sound produced with forced air by larvae of *Phileurus didymus* (Linnaeus) (Coleoptera: Scarabaeidae: Dynastinae: Phileurini)

Estudio bioacústico de un sonido producido con aire forzado por larvas de *Phileurus didymus* (Linnaeus) (Coleoptera: Scarabaeidae: Dynastinae: Phileurini)

Manuel D. Barria¹ , Dora Isabel Quirós^{1,2}  and Daniel Emmen^{1,2} 

¹Laboratorio de Estudios Biológicos de Artrópodos. Vicerrectoría de Investigación y Postgrado. Universidad de Panamá.

²Departamento de Zoología. Facultad de Ciencias Naturales Exactas y Tecnología. Universidad de Panamá.
✉manuel.barriag@up.ac.pa

ZooBank: urn:lsid:zoobank.org:pub: 21A98CEA-C41E-452D-A8CF-CD0855E5FBE7
<https://doi.org/10.35249/rche.46.3.20.16>

Abstract. The results of the bioacoustic study carried out on larvae of *Phileurus didymus* (L.) which emit a sound by compression and release of air (forced air) when they are disturbed are presented. The average duration and frequency of the acoustic patterns obtained, along with illustrations are presented. The biological functions of these sounds are discussed, as well as the possible applications that techniques used and proposed may have in the behavioral study of the larvae of other Scarabaeoidea. The recording data of the sounds obtained in this work are shared.

Key words: Bioacoustic, coleoptera, frequency, larvae.

Resumen. Se presentan los resultados del estudio bioacústico realizado en larvas de *Phileurus didymus* (L.) las cuales emiten un sonido por compresión y liberación de aire (aire forzado) cuando son perturbadas. Los resultados de la duración promedio y frecuencia de los patrones acústicos obtenidos, así como ilustraciones son presentadas. Se discuten las funciones biológicas de este sonido, así como las posibles aplicaciones que pueden tener las técnicas utilizadas y propuestas en el estudio del comportamiento de las larvas de otros Scarabaeoidea. Se comparten los datos de grabación de los sonidos obtenidos en este trabajo.

Palabras clave: Bioacústica, coleópteros, frecuencia, larva.

Introduction

Many animals, including insects, produce sounds to communicate with others individuals (non-incidental sounds) besides incidental sounds as result of eating, moving, or flying (Chesmore and Ohya 2004). Harvey (2011) conducted a series of non-invasive acoustic monitoring studies on *Lucanus cervus* Linnaeus (Coleoptera: Lucanidae) larvae, and concluded that the pattern obtained from the acoustic records can be used to reveal the presence of larvae, without destroying their habitat. Rosi-Denadai *et al.* (2018) mention that there are many studies on stridulatory sounds produced by different insects, but that

Received 4 August 2020 / Accepted 1 September 2020 / Published online 25 September 2020
Responsible Editor: José Mondaca E.

the production of sound by forced air, although common in vertebrates, is little known in insects. They reported that the caterpillar of *Amphion floridensis* Clark, 1920 (Lepidoptera: Sphingidae) is able to “vocalize” by forcing air in and out of its intestine when disturbed. Ratcliffe comments (personal communication) that there are many Dynastinae that produce stridulation (sound produced by rubbing two surfaces) and vibratory or airborne signals. However, there is rarely anyone listening to them and therefore works on the bioacoustics of Dynastinae and other Scarabaeoidea are scarce. Neita *et al.* (2006) reported a stridulatory sounds produced by *Phileurus didymus* (L.) larvae, but no acoustic data was presented on this, so it is not possible to compare the sounds. In this work we carried out a bioacoustic study on the sounds produced by compression of air and release (forced air) by the larvae of *P. didymus*.

Materials and Methods

Eight second instar larvae of *Phileurus didymus* (Fig. 1) were collected from a standing trunk of decomposing *Inga spectabilis* (Vahl) Willd (Fabaceae) in Cerro Peña Blanca, Altos de la Estancia, Provincia de Coclé, Panamá, 8°35'51.4" N, 80°10'14.2" W; 612 masl. The larvae coexist with fire ants of the genus *Wasmannia* Forel, 1893 (Hymenoptera: Formicidae). The collection site was a fragmented secondary tropical rainforest. The larvae were placed in a plastic container along with substrate material for breeding and study. Recordings were made on third instar larvae (second instar larvae also emitted sound).

Sound recording of the larva. The sounds emitted by the larva under normal conditions (nature or city) is imperceptible to the human ear; therefore, it is necessary to be very close to the larvae and without any noise around. To make the recording, a small microphone modified (from headphones models no specific brand) was used inside the terrarium and on the thorax of the larvae of *P. didymus*, the microphone records omnidirectional. The recording was made at a controlled temperature of 25°C, in the morning (9-11 AM) and afternoon (3-5 PM).



Figure 1. *Phileurus didymus* third instar larvae.

The audio editing program Audacity 2.3.3 version was used to “clean” the audio tracks of possible aberrations caused by other means than the larvae, which would alter the results. The cleaning consisted of eliminating external noises with the “mute section” tool, but maintaining the duration between sounds (interpulses) corresponding to silence between notes. The illustrations were made with the digital program Adobe Illustrator, the photographs were taken with a Canon EOS Rebel T7 camera, and all the images were edited with the digital program Adobe Photoshop for a better quality. The frequencies of the different acoustic patterns were determined and then averaged. The duration of each pulse was determined to know the average range of duration, maximum and minimum. To determine the dominant frequency (average), 80 replicates were made per recording. The adults obtained from breeding were deposited in the personal collection of the first author, kept in Laboratorio de Estudios Biológicos de Artrópodos de la Universidad de Panamá (LEBA-UP: MBPC).

Behavior and responses of the larvae to different stimulations. To determine that sound was not only produced when they were manipulated, they were subjected to different stimulations experiments. The different stimulations were carried out on all the larvae available with 10 replications for each stimulation for each larva. The stimulation experiments were the following:

Disturbance with rod (Fig. 2a). A wooden rod about 15 cm long was used. This was introduced into the plastic container where the larvae were. It was moved from one side to the other to observe the behavior of the larvae (not touching the larvae).

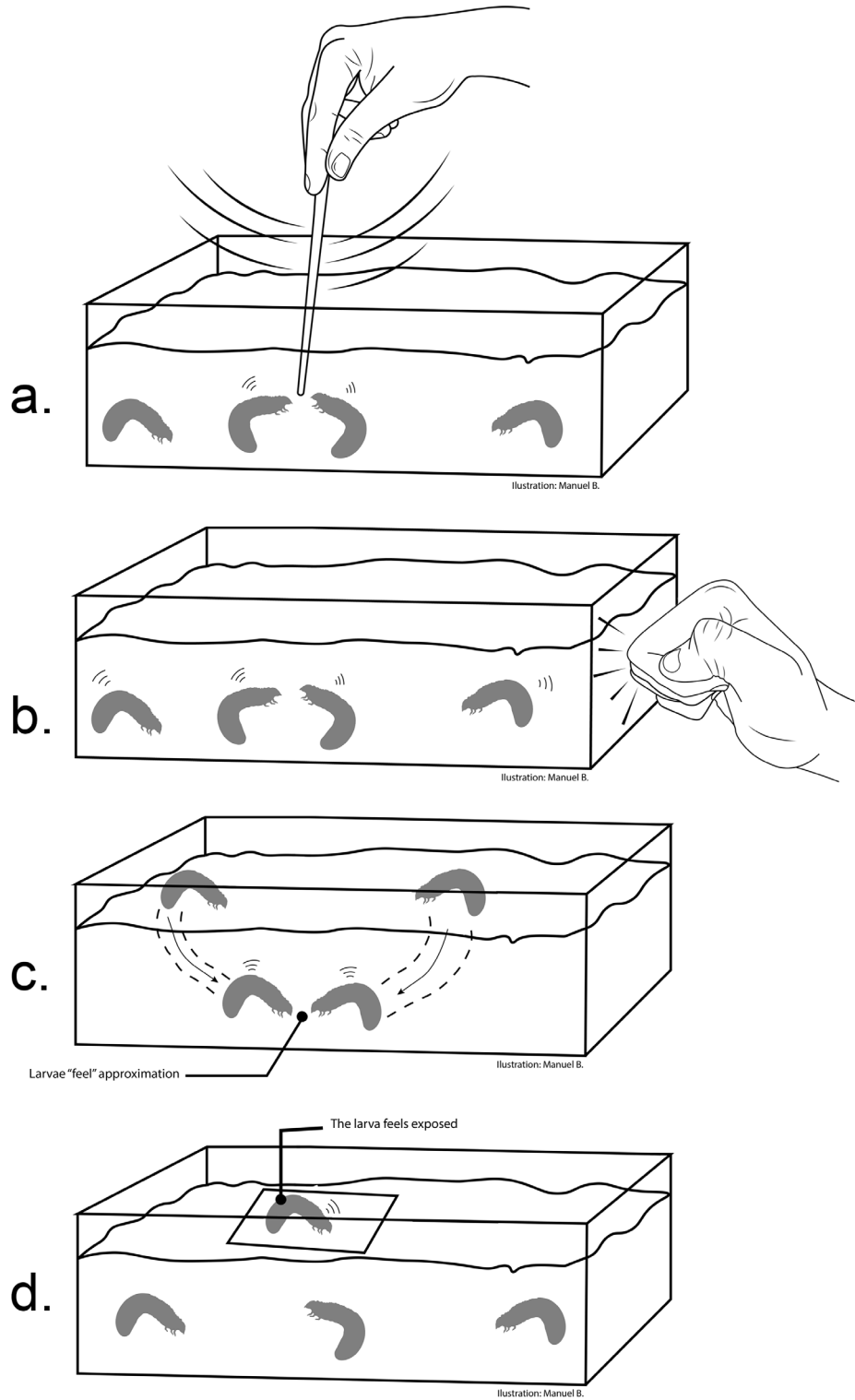
Direct disturbance to the container (Fig. 2b). This test consisted in hitting the surface of the plastic container with the knuckles (similar to knocking on a door), which is intended to replicate what would be the response of the larvae to an animal or external stimulus that would disturb the entire gallery where they are found.

Response to near-larva object (Larva-larva interaction) (Fig. 2c). Interaction and response between them. Two larvae were introduced nearby (10 cm) and allowed to migrate to such an extent that their presence was found or perceived by digging into the substrate.

Open-air exposure of larvae (Fig. 2d). The larva was exposed on a plastic surface about 12 cm long and 12 cm wide, so that the larva could not be buried in the substrate. In this way it was possible to observe its behavior and that of its congeners that were below it in the substrate.

Results and Discussion

We suggest that the sound is produced by means of compression and release of air (forced air). It is likely that the sound has its origin in the larva's intestine. An increase and subsequent relaxation can be noted in the abdominal region of the larva, specifically in the area between the first and third abdominal spiracles (this movement is the result of compression and release of air). Due to the semi-transparent nature of the larvae skin, it was possible to observe an internal movement in the ventral area when it emitted the sound, possibly a muscular compression of the air in the digestive system to make it circulate within it. It does not seem that the air is expelled by the spiracles since they are too small, the moment the air comes out through them the result would be a sound more similar to a “pressure blowing” than a serious sound as it is heard. As was already established by Rosi-Denadai *et al.* (2018), this type of sound produced by compressing the air internally



Figures 2a-d. Techniques for the observation of responses of the larvae to different disturbances. a) Disturbance with a wooden rod. b) Direct disturbance to the container. c) Response to disturbance close to the larva (Larva-larva interaction). d) Open-air exposure of larvae.

to produce low-pitched sounds is something well known in many mammals but hardly in insects. The sampling frequency was 44,100 Hz (44.1 KHz) and a depth of 24 bits. The acoustic patterns obtained in this study present frequency ranges from 84 to 120 Hz, with a dominant frequency (average) of 105.65 Hz. Average duration between pulses (Fig. 3b) was 0.513 seconds. The longest pulse duration was 56 seconds (Fig. 3c), while the shortest pulse duration was 0.040 seconds. The sounds reflect that the acoustic patterns can be of short pulses (Fig. 3a), slightly repetitive with variable sequences (the interpulses varies) (Fig. 3b) and short pulses interspersed with long duration pulses (Fig. 3c). The resulting sound is a series of low frequency sound pulses.

Table 1. Results on different disturbance stimulations on *Phileurus didymus* larvae.

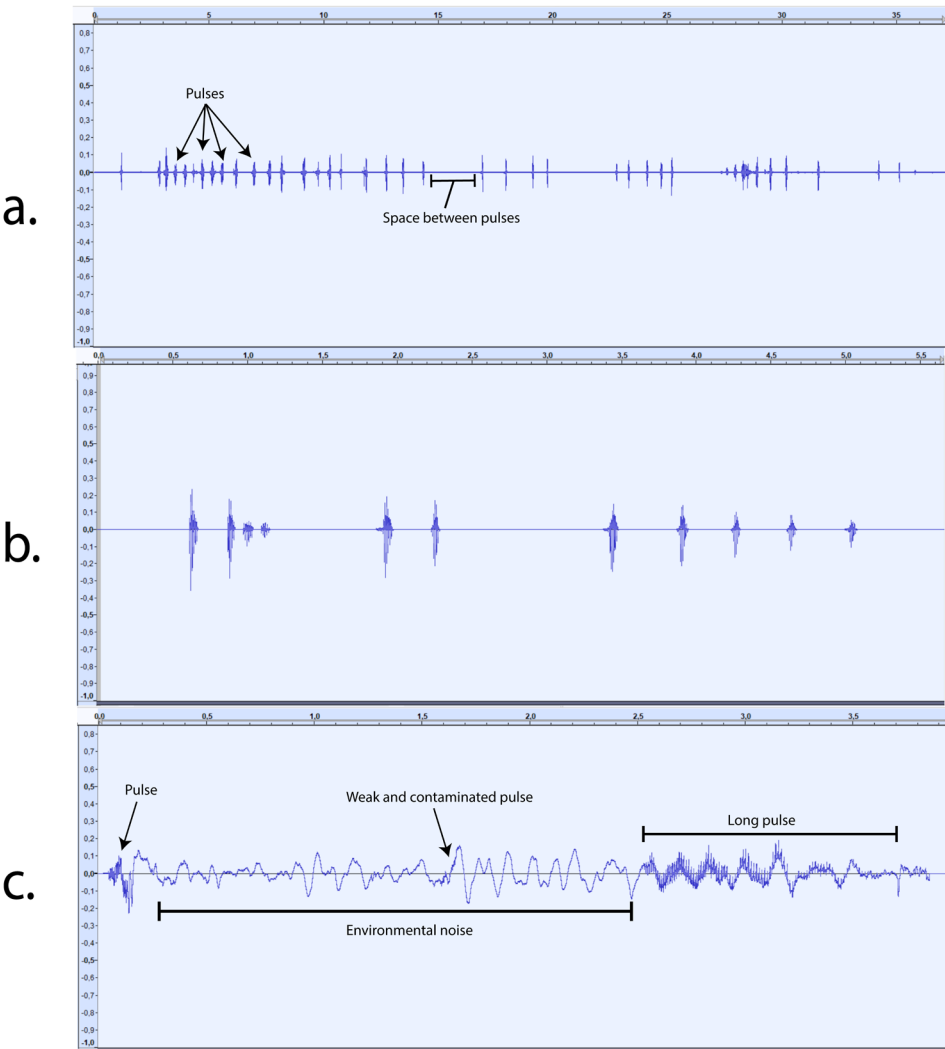
Experiment/Stimulation	Response
Wooden rod disturbance (Fig. 2a)	The larvae closest to the disturbed area began to emit the previously mentioned “distress calls” sounds. However, the larvae farther away from the disturbed area did not emit any distress call. No larvae escaped or migrated from the disturbance.
Direct disturbance to the container (Fig. 2b)	The result was a unified response of all the larvae to such a disturbance. No larvae escaped or migrated from the disturbance.
Response to near-larva object (Larva-larva interaction) (Fig. 2c)	The larvae, upon encountering each other and perceiving an approach, began to emit sounds. No larvae migrating or escaping the disturbance were recorded.
Open-air exposure of larvae (Fig. 2d)	After a few seconds, when it could not hide under a substrate, it began to emit the sound. The larvae underneath had no response to the sound.

The sound and the oscillograms make it possible to dismiss that this sound is stridulatory because stridulatory sounds (in the great majority) presents patterns of interpulses and pulses of constant duration (Fig. 4a) while in the oscillograms obtained from the audios of forced air produced by the larvae, the interpulses are highly variable and the pulses can be short or long (Figs. 3, 4b). In all experiments no larvae migrated or escaped from the site when they emitted sound and/or heard other larvae in the box. Based on the third experiment, the use of this sound to communicate among conspecific larvae is not dismiss, based on the classification of Alexander (1957) it could be considered a “startle cries” or “distress call”. There are no known natural predators of this species, however based on the animals observed at the study area it is possible (due to the low frequency of the sound) that be directed to small animals such as spiders or small lizards. We do not dismiss the possibility that birds of the Momotidae family (which are quite common in the area) may predate larvae, since previously they have been observed feeding on coleopteran larvae which they extract from trunks with their peaks. However, more studies are necessary to probe the effectiveness of these sounds against predators of different sizes. The docile behavior of the larvae, in spite of having strong mandibles, is another characteristic of this gregarious species when are immature. It has been observed that the encounter between larvae and adults of other Dynastinae like *Strategus aloeus* (Linnaeus) (Dynastinae: Oryctini) causes in many cases the mutilation of the appendages of the adults or predation on larvae of its same stage or younger (Personal observation). However, this was not the case with the larvae of *P. didymus* observed during the experiments and the rearing process. It should be noted that due to the myrmecophilic habit of this species as well as many Scarabaeoidea,

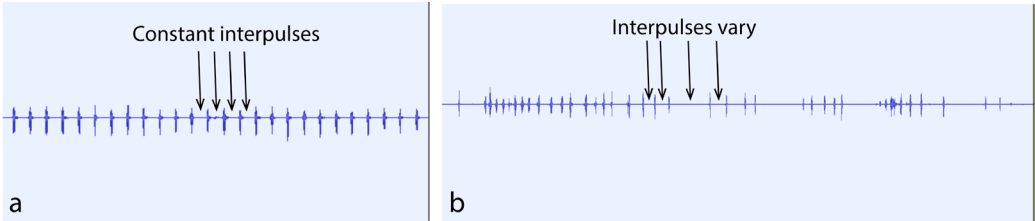
ants may attack animals that try to disturb the gallery, which would be an advantage or defense barrier for the benefit of the larvae.

Phileurus didymus sounds data:

<https://drive.google.com/drive/folders/1iIRcGhY-8SnuOXP008p6api8dGkV7cpP?usp=sharing>



Figures 3a-c. Oscillograms of acoustic patterns of *Phileurus didymus*. (a) Larvae Non-constant, semi-repeating short pulses, note that interpulses (space between pulses) vary. (b) Slightly repetitive pulses with variable sequences (the interpulses varies a little). (c) Short pulses with long duration pulses.



Figures 4a, b. Comparison of oscillograms. (a) Stridulatory sound (stridulatory teeth) of a Dynastinae beetle larvae. (b) Forced air sounds of *P. didymus*.

Conclusion

We suggest that *Phileurus didymus* larvae produce sounds caused by forced air in their digestive system. We did not observe any stridulatory organs apart from the one they have in the maxillo-mandibular region. These stridulatory teeth that produce a mechanical sound, like many Scarabaeoidea larvae (as the observer in Fig. 4a). This means that they are capable of emitting two types of sounds: one stridulatory (reported by Neita *et al.* 2006) and another by compression and release of air (forced air) (reported in this work). Based on the oscillograms obtained from the recordings, we discard that it is a stridulatory sound since in this case the sounds should be notes in a repetitive pattern with constant and non-variable interpulses, and the wave longitude would be constant and determining the frequency (this is not observed in these sounds). The techniques presented in the study may be can help in the advance of understanding of bioacoustics in larvae and adults of Scarabaeoidea and others Coleoptera, since it will allow us to observe the type of response to different stimulations in captivity.

The observations and results show that the sound is producing of the disturbing presence and activities of others animals (including their congeners) or when the larvae feel exposed (see Table. 1). All these types of sounds produced by larvae and other insects were categorized by Alexander (1957) as “alarm cries”, “distress calls” or “protest sounds” which cause to be release by predators, however, when emitting the sounds in the different experiments, no larvae responded to them or migrated away from the site where they were (taking it as a danger signal), a study in captivity of interaction with possible predators is necessary to determine the effectivity of these sounds as anti-predatory methods. It should be noted that the only means of defense of the larvae are their mandibles, which would be really functional only against small predators of similar size as the larva. However, the larvae of *P. didymus* present a quite docile and non-aggressive behavior, which does not make their mandibles so useful as a means of defense. In case of predation, it is likely that the emission of these sounds by the larva causes a negative effect on the predator to want to feed on it. If the gallery is invaded, it is possible that the set of sounds emitted by all the larvae will drive away small predators. While it is likely that eventually a large portion of the larvae will be eaten by the predator, it may result in a substantial survival rate of most of individuals against small predators. All of these possible hypotheses need further study in the habitat where the larvae develop to determine what function these sounds have or how effective they are in their survival in natural environment.

Acknowledgements

We are grateful to Mr. Melquiades Rivas for allowing access to his land to obtain the larvae and his assistance in the field collections. To Professor Jorge García (Universidad de Panamá), for accommodation facilities. To Vielkys Y. Ríos (Universidad de Panamá), for her assistance in the field collection. To Dr. Brett C. Ratcliffe (University of Nebraska, Lincoln, USA), for all the comments and advice that helped to enrich this work. To John C. Neita (Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Colombia), for help to confirm the species. To Vicerrectoría de Investigación y Postgrado de la Universidad de Panamá for the funds awarded to the project: “Biología de *Strategus aloeus* (Coleoptera: Scarabaeidae) plaga de árboles de importancia económica en Panamá”, with code CUFI-2018-CNET-EG-002. To the anonymous reviewers for their comments and observations on the work.

Literature Cited

- Alexander, R.D (1957)** Sound production and associated behaviour in insects. *The Ohio Journal of Science*, 57: 101-113.
- Chesmore, D. and Ohya, E. (2004)** Automated identification of field-recorded songs of four British grasshoppers using bioacoustic signal recognition. *Bulletin of Entomological Research*, 94(4): 319-330. doi: <http://doi.org/10.1079/BER2004306>
- Harvey, D.J., Hawes, C.J., Gange, A.C., Finch, P., Chesmore, D. and Farr, I. (2011)** Development of non-invasive monitoring methods for larvae and adults of the stag beetle, *Lucanus cervus*. *Insect Conservation and Diversity*, 4(1): 4-14.
- Neita M., J.C., Orozco, J. and Ratcliffe, B. (2006)** Escarabajos (Scarabaeidae: Pleurosticti) de la selva baja del bosque pluvial tropical "BP-T", Chocó, Colombia. *Acta Zoológica Mexicana*, 22: 1-36.
- Rosi-Denadai, C.A., Scallion, M.L., Merrett, C.G. and Yack, J.E. (2018)** Vocalization in caterpillars: a novel sound-producing mechanism for insects. *The Journal of Experimental Biology*, 221(4): 1-13. jeb169466. doi: <http://doi.org/10.1242/jeb.169466>