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Research Article

Bioacoustic analysis of a compound sound with stridulation and forced air produced by the larva of *Phileurus valgus* (Olivier, 1789) (Coleoptera: Scarabaeidae: Dynastinae: Phileurini)

Análisis bioacústico de un sonido compuesto con estridulación y aire forzado producido por la larva de *Phileurus valgus* (Olivier, 1789) (Coleoptera: Scarabaeidae: Dynastinae: Phileurini)

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Abstract. It was observed that *Phileurus valgus* larvae are capable to emit stridulation and forced air sound when disturbed, being able to emit a "compound sound". Duration, frequencies, oscillograms, spectrograms of the sounds obtained are presented. The results obtained are compared with previous studies and the possible communicative or warning functions they may have in their natural environment when interacting with other larvae (other species) or their congeners (same species). The audios obtained in the study are shared.

Key words: Bioacustic; disturbance; oscilograms; phileurines.

Resumen. Se observó que las larvas de *Phileurus valgus* son capaces de emitir estridulación y sonido por aire forzado cuando son perturbadas, siendo capaces de emitir un "sonido compuesto". Se presentan la duración, frecuencias, oscilogramas y espectrogramas de los sonidos obtenidos. Se comparan los resultados obtenidos con estudios anteriores y las posibles funciones comunicativas o de advertencia que pueden tener en su medio natural cuando interactúan con larvas de otras especies o con las de sus congéneres (misma especie). Los audios obtenidos en el estudio son compartidos.

Palabras clave: Bioacústica; perturbación; oscilogramas; phileurinos.

Introduction

The larvae of *Phileurus didymus* (Linnaeus, 1758), are capable of emitting sounds by forced air and stridulatory sounds when they are disturbed or feel threatened. Forced air sound appears to be produced by the larva's digestive system, while stridulation is caused by stridulatory teeth located in the maxilla. Forced air sounds are characterized by being "bass" and low-frequency sounds, while stridulation is higher-pitched and higher-frequency sounds. These sounds can have antidepredative functions, as well as social functions (Barria *et al*. 2020).

Phileurus valgus (Olivier, 1789) is a Phileurini beetle with a size between 18.0-28.8 mm.

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The adults are nocturnal and are attracted to light and can be found together with the larvae living in wood in a state of decomposition. They are collected at heights from sea level to 1200 meters above sea level (Ratcliffe 2003). The species is widely distributed from the southern United States to Argentina and West Indies (Endrodi 1985; Ratcliffe 2011). The larva was initially described by Ritcher (1966) with the name of *Phileurus castaneus* (Haldeman). Ibarra-Polesel *et al*. (2017), re-described the larva and pupa. However, stridulation, much less forced air sounds, had not been reported in the larvae of this species in the literature. In this work we present a bioacoustic analysis of the sounds produced by *P. valgus* larvae, being the second Scarabaeoidea larva reported to be capable of emitting sounds by forced air.

Materials and Methods

Two male pupae, 11 third instar larva, 1 prepupa, and an adult female were collected from a tree fallen of *Mangifera indica* Linnaeus (Anacardiaceae) in a state of decomposition in the locality of Los Caobos, Chilibre, 9°09'02.9"N - 79°37'00.5"W. The adults obtained from the rearing were deposited in the collection of the first author, kept in Laboratorio de Estudios Biológicos de Artrópodos de la Universidad de Panamá (LEBA-UP), Panamá.

The photographs were taken with a Canon EOS Rebel T7 camera. The illustrations were made with the program Adobe Illustrator 2020, 20.0.2 version. The photographs, oscillogram images, and illustrations were processed with the program Adobe Photoshop CC 2020, 21.0.2 version.

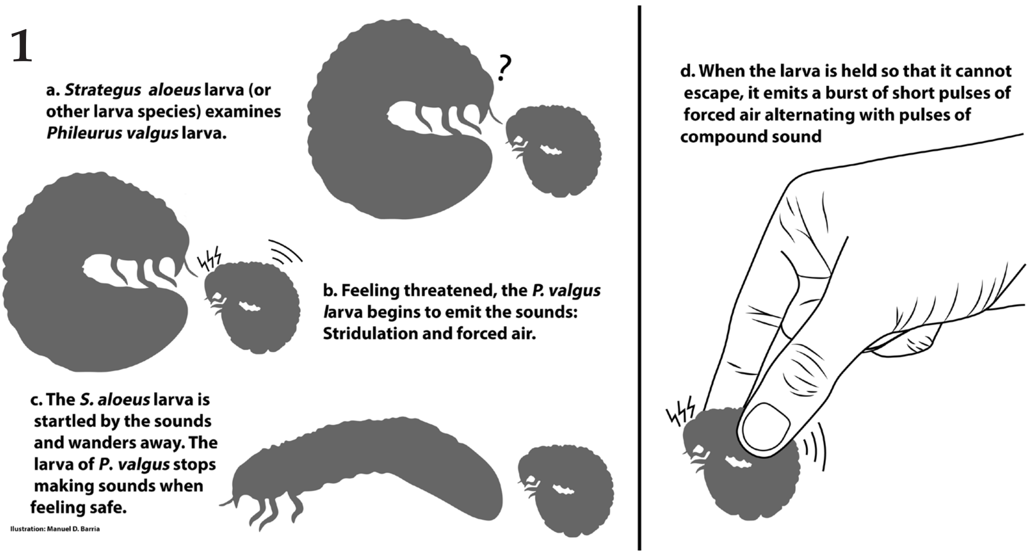
Sound recording. Under normal conditions, the sounds emitted by the larva are imperceptible to the human ear. To record the sound, a modified microphone with omnidirectional recording was used (without a specific mark), which was placed on the larva to obtain the sounds. The recording was made in the morning and night at a temperature of 23-25 °C. The compound sound is formed by two sounds of different origins. Due to this, they were recorded separately and later both together for analysis. The microphone was placed under the maxilla of the larva for recording the stridulation sounds. For the forced air recording, the microphone was placed between the first and second abdominal spirals. For the compound sound, the microphone was placed in the lateral thoracic region of the larva, near to the thoracic spiracle. The stridulation was not clearly appreciated because the microphone was far from the site of the sound production, for this the decibels of the audios were also manipulated (increased: +5 to +12 db) so that they could be visible in oscillograms and spectrograms and audible in audio files. This did not affect the results since they were analyzed separately and then compared together. The sampling frequency was 44,100 Hz (44.1 KHz) and a depth of 24 bits. For the processing of the audios, we use the digital program Adobe Audition CS6 2020, 5.0 version. The external sounds were eliminated, the pulses were extracted, and they were processed as new files in "WAV" audio format to avoid the loss of information. The frequency was calculated by dividing the number of patterns of each pulse by the unit duration following to Rosi-Denada *et al*. (2018).

Behavior and response of the larvae to different stimulations. To obtain the response to different stimulations we used the experiments proposed by Barria *et al*. (2020): Disturbance with a rod; Direct disturbance to the container; Response to near-larva object (Larva-larva interaction) and Open-air exposure of larvae. Additionally, we include three new experiments:

Direct interaction larva-larva (Different species) (Figs. 1a-1c). We placed the *Phileurus valgus* larva with a third stage larva of *Strategus aloeus* Linnaeus, with the idea that these will interact. The results of the *P. valgus* and *P. didymus* sounds are compared in Table 2.

Direct manipulation (Fig. 1d). The larvae are grasped with the fingers so that they cannot move, exerting a little pressure without hurting the larvae**.**

Wound to larva. We sacrificed two larvae to observe if the acoustic pattern changed. For this, we made a small incision behind the cephalic capsule with a scalpel. As happens when the larva is held for a prolonged period of time, the larva emits frenetic sounds. The result of the experiments has been described in Table 1.
Five third instar larvae were used for each experiment and 15 replicates were performed for each larva (except for the "wound to larva" experiment, where 2 third instar larvae were used and one replicate for each).



Figures 1a-1d. Diagrams of the experiments. a-c) Direct interaction (larva-larva) and response experiment. d) Direct manipulation experiment. / Diagramas de los experimentos. a-c) Experimento de interacción directa (larva-larva) y respuesta. d) Experimento de manipulación directa.

Results and Discussion

Table 1. Results on different disturbance stimulations on *Phileurus valgus* larvae. / Resultados de diferentes estímulos de perturbación en larvas de *Phileurus valgus*.

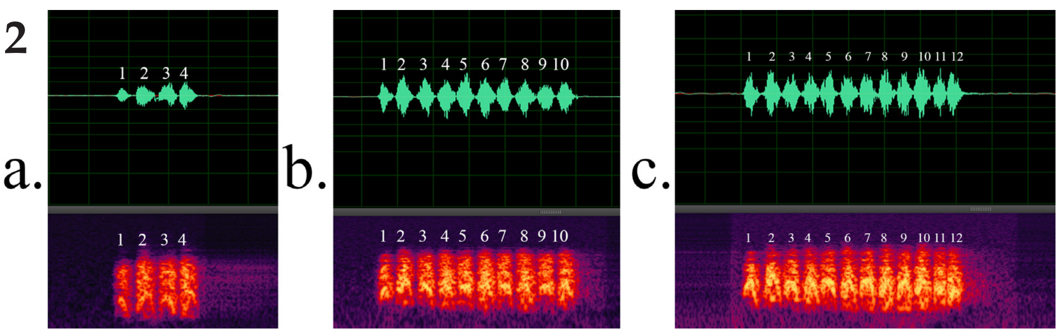
Experiment/Stimulation	Response
Wooden rod disturbance	The larva emits the sounds in response to the disturbance.
Direct disturbance to the container	The larvae respond to sound, but not all of them respond at the same time.
Response to near-larva object (Larva-larva interaction). Same species	When the larvae interact with each other they emit the sounds and move away from each other.
Direct interaction larva-larva. Different species	When the larva of *S. aloeus* interacts with the larva of *P. valgus*, this last emits the sounds. The larva of *S. aloeus* is startled and moves away from it, losing interest. When the larva feels safe, it stops emitting the sounds.
Open-air exposure of larvae	The larvae emit the sounds when they feel exposed.

Direct manipulation / Wound to larva	When is held with the fingers or is injured it feels highly threatened. The larva begins to emit the sounds in a more frenetic way emitting a greater than normal forced air pulse burst. The larva may resort to defensive regurgitation and defecation to make the predator release it (audio also available in attached audio data).

***Phileurus valgus* sounds data.** We suggest to not have the maximum volume while listening with headphones. The short pulses of forced air burst can be uncomfortable. https://drive.google.com/drive/folders/1OGYGaB3LnFRcu32DodXQ_aBFiDfdVGhW?usp=sharing

Description of the sound. The acoustic pattern usually consists of a series of 1 to 4 (maximum recorded) "compound sounds" of medium duration (0.531 seconds) followed by several short pulses (0.052 seconds) of forced air sounds. This pattern may then be repeated and interspersed in a non-uniform manner, again followed by several short pulses of forced air. Based on the classification of Alexander (1957) these sounds could be considered "distress calls" or "startle cries".

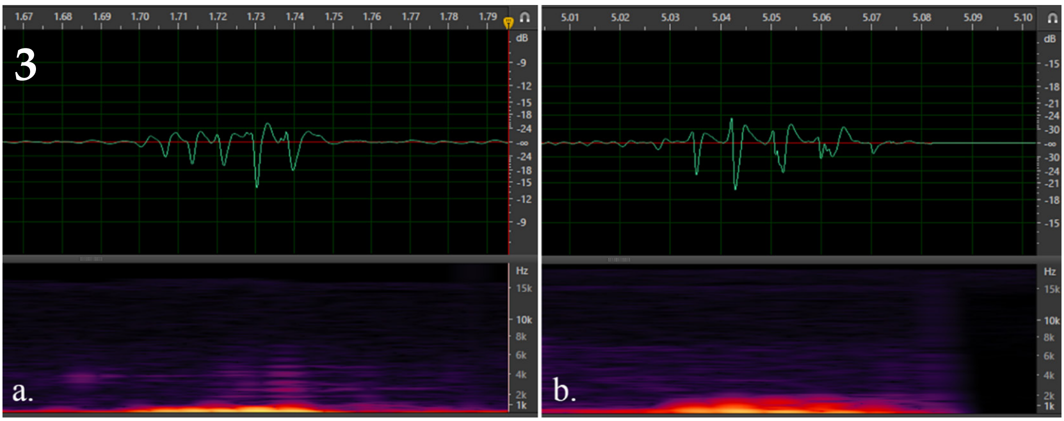
***Phileurus valgus* larvae stridulation.** The stridulation is maxillo-mandibular, and this sound is similar to a "chirp". The stridulation is produced when the larvae move the maxilla against the mandibles, causing the stridulatories teeth that are in the maxilla to rub against the stridulatory area located in the ventral region of the mandibles. Each friction movement produce a pulse that make up the stridulatory sound. The acoustic pattern of the stridulation can be composed of 4 to 12 pulses (Fig. 2). The average frequency obtained was 769 Hz (Medium frequency), with a frequency range between 600 and 800 Hz. The pulses and interpulses are constant and in the spectrogram, the stridulation is above the basal region near the mid frequencies. These help to confirm that it is a stridulation. The average duration of the stridulation (set of pulses) is 0.5 seconds. The average duration of each pulse is 0.052 seconds. The average duration of the interpulses is 0.021 seconds.



Figures 2a-2c. Comparison of numbers of pulses in stridulation, oscillogram (above). Spectrogram (down). a) 4 pulses. b) 10 pulses. c) 12 pulses. / Comparación del número de pulsos en la estridulación, oscilograma (arriba). Espectrograma (abajo). a) 4 pulsos. b) 10 pulsos. c) 12 pulsos.

Forced air sound. Pulses and interpulses are not constant, their duration may vary. As with *P. didymus* larvae, for a human to perceive the sound produced by *P. valgus* larvae, it is necessary to be silent and have it in the ear or record it with a microphone near the abdominal area, between the first and second abdominal spiracles. In the same way, the vibration they produce when making the movement is palpable when holding the larva in this region. The oscillogram of the forced air sound emitted by *P. valgus* larvae (Fig. 3a.)

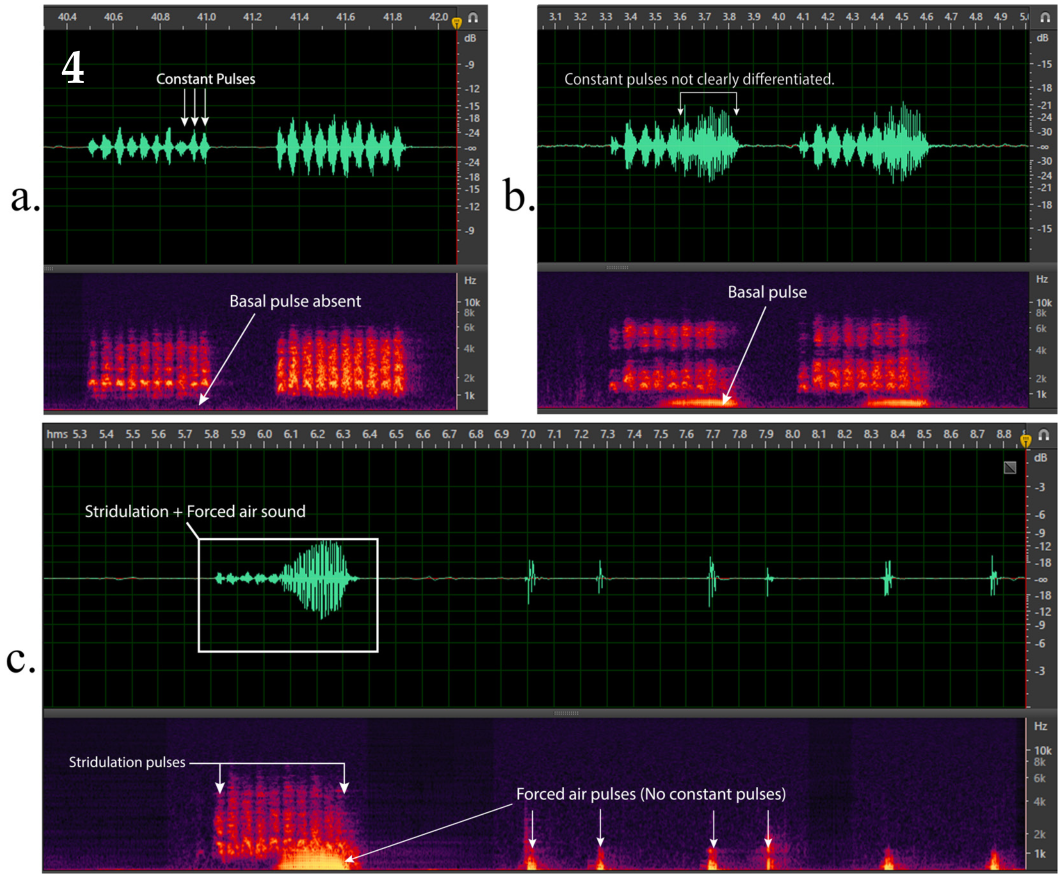
shows a clear similarity to that emitted by *P. didymus* (Fig. 3b). The larva emits an average of 4 short pulses per second at low levels of disturbance, and a maximum of 8 pulses per second at high levels of disturbance (when it is hurt or tightened). Comparing the audio data obtained from the *P. didymus* larvae bioacoustic study (Barria *et al*. 2020) with that of *P. valgus* (this work), the following was obtained: The average duration of the medium pulses (from compound sound) was 0.246 seconds. The average duration obtained from the short pulses emitted by *P. valgus* larvae was 0.057 seconds; 0.052 seconds for *P. didymus*. The average frequency produced in *P. valgus* larvae was 136.5 Hz, with frequency ranges from 111 to 178 Hz; in *P. didymus* the average is 105. 65 Hz, with frequency ranges from 84 to 120 Hz, both sounds being considered as low frequency (bass). The duration of the forced air interpulses of average duration that make up the compound sound is 0.342 seconds.



Figures 3a-3b. Comparison of oscillogram and spectrogram of forced air sound pulse. a. *Phileurus valgus*. b. *Phileurus didymus*. / Comparación del oscilograma y el espectrograma del pulso sonoro de aire forzado. a. *Phileurus valgus*. b. *Phileurus didymus*.

Compound sound: Stridulation + Air Forced (Figs. 4b-4c). The larva is able to emit both sounds almost simultaneously, producing an audible and visually distinct sound. This in a way could help the larva to create a "louder" or "aggressive" sound for defense or warning. The resulting sound being something similar to a vibrating chirp. However, the production of one is independent of the other, being able to produce them independently or together (as is the case here). The average duration of the sound is 0.531 seconds. The spectrogram and oscillogram of this combined sound are different from the other two sounds individually (Fig. 4). In the oscillogram of the compound sound (Figs. 4b, 4c) it is observed that, from the middle of the sound to the end of this one, the dynamics of the wave changes, that is to say, there is a variation in the intensity of sound, this because it is added to the stridulation, the sound by forced air and consequently it makes the amplitude of the wave to be greater. In this section, the oscillogram shows how the stridulation pulses are no longer visually constant and distinguished since they are mixed with the forced air pulse (Fig. 4b). The duration of the forced air interpulses of average duration that make up the compound sound is 0.342 seconds. On the other hand, in the oscillogram of the isolated stridulation (Fig. 4a), the pulses can be clearly differentiated along with the sound.

In the spectrogram of the compound sound (Figs. 4b, 4c) a pulse of medium duration with intensity corresponding to the forced-air sound is observed in the basal region (low frequencies). This basal pulse is absent in the isolated stridulation production (Fig. 4a). The spectrogram clearly separates the stridulation pulses in the mid-frequency region and the forced-air pulse in the lower frequencies. After the two compound sound pulses (or one in a few cases), several short forced-air pulses with variable and non-constant pulses and interpulses are observed (Fig. 4c).



Figures 4a-4c. Oscillograms (above) and spectrograms (down) of sounds emitted by *Phileurus valgus* larvae. a) Bioacoustic stridulation (isolated) patterns. b) Bioacoustic sound patterns of compound sound (stridulatory + forced air). c) Bioacoustic pattern of compound sound pulse and some forced air sound pulses (6 pulses after the compound sound). / Oscilogramas (arriba) y espectrogramas (abajo) de los sonidos emitidos por las larvas de *Phileurus valgus*. a) Patrones bioacústicos de estridulación (aislada). b) Patrones bioacústicos de sonido compuesto (estridulación + aire forzado). c) Patrón bioacústico de pulso de sonido compuesto y algunos pulsos de sonido de aire forzado (6 pulsos después del sonido compuesto).

We observe that in the initial days of the prepupa stage, when the larva is still able to move even if the epidermis is a little rough, it is able to emit the sound, although the number of pulses that compose the stridulation is less (4 to 6 generally) while the duration of the pulse by forced air is the same. This sound seems to be useful for maintaining the distance between larvae on the substrate, which benefits the larvae because resources may be scarce. Although to confirm that the sound also has anti-predatory functions it would be necessary to place the larvae with an adult or larva with predatory habits, the sound proved to be effective with larger larvae such as those of *S. aloeus*, which, although they do not have predatory habits, they do prey on smaller instar larvae when resources are scarce. The fact that larvae of their own species do not "startled" when they perceive the sounds (as is the case with *S. aloeus*), leads us to think that this may be due to the fact that they are mostly communicative so that they keep their distance and do not invade the larval chamber. While for those of other species, since it does not belong to their habits, the sound may be annoying. We do not know yet how audible or perceptible is for other larvae or adults, however, we hope that further studies will yield more answers and expand the knowledge about this type of sounds and behavior, which for the moment, are few but give us several answers of the communicative functions they have or could have.

Table 2. Comparative sounds, frequency, and sounds type of *P. didymus* and *P. valgus*. / Comparación de sonidos, frecuencia y tipo de sonidos de *P. didymus* y *P. valgus*.

Species	Sound	Average frequency, ± SD	Sound frequency type
P. didymus	Forced air	105. 65 Hz ± 10.2	Bass sound
P. valgus	Forced air	136.5 Hz ± 13.7	Bass sound
	Stridulation	769 Hz ± 68.3	Mid-sounding sound

The stridulation speed produced by *P. valgus* larvae is 20 bpm (pulses per second). The stridulation sound produced by *P. valgus* larvae is very similar (audibly) to that produced by *Passalus* (*P.*) *punctiger* LePeletier & Serville, 1825 (Coleoptera: Passalidae) larvae (personal observation), in the latter the sound is produced when the larvae move the third pair (reduced) of legs against the dorsal area of the second pair of legs. The speed of stridulation in *P.* (*P.*) *punctiger* larvae is 10 bpm (slower than *P. valgus*). In some Dynastinae and Rutelinae larvae (personal observation) we have observed that the sounds produced by maxillary-mandibular stridulation have a lower frequency (300-400 Hz) than that observed in *P. valgus* and that the sound is not as "squeaky", in these, the sound is more similar to several consecutive strokes. These variations are due to the fact that different noise frequencies can be generated according to the speed and pattern of the movement (Reyes-Castillo & Jarman 1980). It is not excluded that these sounds have some interspecific function.

Conclusion

Phileurus valgus larvae are capable of emitting a compound sound, consisting of forced air and stridulatory (maxillary-mandibular) sounds when disturbed or injured. The sounds can be emitted simultaneously (compound sound) or individually. Based on the classification of Alexander (1957) these sounds could be considered "distress calls" or "alarm cries". The production of each sound is independent of the other. The forced air sound they emit is a low-frequency, bass sound, while the stridulatory sound is a medium-frequency, mid-sounding sound. We consider that this sound is useful to keep the distance between larvae within the substrate with them and with larvae of other non-predatory species, being a communicative and warning means, something similar to what was observed by Kojima *et al*. (2012), where they observed that there is intraspecific communication since the sound signals emitted by the pupae dissuaded the larvae, preventing them from approaching or invading the pupal chambers. The experiments conducted by Buchler *et al*. (1981), where they exposed larvae and adults of *Odontotaenius disjunctus* (Illiger) (Coleoptera: Passalidae) to ravens (*Corvus brachyrhynchos* Brehm), demonstrated that the time to mortality was longer in specimens (larvae and adults) that emit sounds, dissuading predators from wanting to feed on individuals that produce sounds. It is possible that something similar may happen in the larvae of *P. valgus*, *P. didymus*, and all those that are capable of emitting sounds, causing it to decrease the percentage of mortality, because when they are wounded or sustained, the number of pulses per second increases considerably, being quite annoying for those who want to prey on them, as it is audibly. It is possible that other Phileurini larvae are capable of emitting sounds by forced air, however, it is necessary that when larvae of these are found, they are carefully examined since these sounds and movements can go unnoticed by the researcher.

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