Acoustic analysis of vocalization and the behavioral response associated to sound production of the nine banded armadillo *Dasypus novemcinctus* (Mammalia, Cingulata, Dasypodidae) in an agonistic context

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Abstract. Although communication capabilities are displayed by many vertebrate groups, some repertoires are poorly known, such as the case of xenarthrans, particularly armadillos, for which vocalization as a source of communicating to others remains poorly understood and relies on punctual reports of sounds. Here we provide the first description of a behavioral response associated with sound emission of two subjects of *Dasypus novemcinctus*. Both audio and visual registration was performed to subsequent analyses of expressed behaviors and emitted calls, which accounted for 76 vocalizations from a total of eight video recordings randomly collected from 2017 to 2019. Sound is acoustically characterized by both inhale and exhale phases composed of two vocal units, and no harmonic structure was observed. Once the subjects have always produced these vocalizations while cornered and exhibiting defensive behavior against another subject/human disturbance, these vocalizations were termed as distress. Subjects produced a hiss-purr-like sound while trying to avoid contact with another by bowing or lowering their bodies, humping, or even moving elsewhere when sound production ceased. This shows that the sound repertoire of armadillos is still to be unveiled and seems to be much more complex than previously thought.

Keywords. Bioacoustics; Ethology; Behavioral repertoire; Xenarthra; Cingulates.

INTRODUCTION

Acoustics within the biological field regards the science known as Bioacoustics, which aims at studying the production, dispersion, and reception of sound by/to animals (Lubis *et al.*, 2016) whose cognitive and social skills are much revealed by the development level of their acoustic communication behavior (Ladich & Winkler, 2017). These capabilities are displayed by most vertebrate groups (Ladich & Winkler, 2017) and continuously constitute the focus of many studies that describe and classify emitted calls into vocal repertoires (Garcia & Favaro, 2017).

Communication can be defined as the way one mind affects another (Shannon & Weaver, 1949), which is achieved by vocalization as the sound produced by means of the respiratory system of a vertebrate animal, typically by the action of vocal cords (Köhler *et al.*, 2017) either triggered spontaneously and/or by a response when the animal is handled (Cooper & Vierck-Jr., 1986). These acoustic repertoires are thus produced under certain behavioral circumstances, from distressful
to competitive (feeding/space/mate) situations (Ladich & Myrberg, 2006), and their acoustic properties differ depending on the caller’s emotional state (Szpij et al., 2017). Notwithstanding, explaining them can be even a more difficult task when dealing with animals that do not commonly emit sounds in the field since, besides the lack of sound records, it is difficult to match communication with behavior. Extensive research has attempted to link sound production by a species with the context in which the species produced this sound (acoustic signals with their function) and the associated behavioral response triggered by this sound, as well as to understand the mechanisms involved in the vocal communication (Garcia & Favaro, 2017).

Xenarthrans (armadillos, sloths, and anteaters) are a conspicuous group of mammals, but despite mammalian vocalization structures having been widely studied for many groups, information regarding this group is scarce and much relies on punctual reports of sounds produced by its members (e.g., Montgomery & Sunquist, 1974; Greene, 1988, Rocha & Mourão, 2006; Bezerra et al., 2007; Schmidt, 2012). Sloths produce sounds by diverting air through their snouts, which makes a whistle (Goffart, 1971; Montgomery & Sunquist, 1974). They are known to have quite intense mother-young communication (e.g., Choloepus hoffmani and Bradypus variegatus) during infant dependence, and a hissing sound has already been recorded to call the mother’s attention (Soares & Carneiro, 2002). Conspecifics of B. variegatus also communicate during the breeding season (Bezerra et al., 2007; Gilmore et al., 2001; Soares & Carneiro, 2002). Sloths are also known to vocalize during agonistic contact (Greene, 1988). Some of these sounds have already been recorded in the field (Emmons et al., 1998), but a description of this acoustic repertoire is yet lacking.

Anteaters exhibit a behavioral repertoire that comprises an unusual, short “low-frequency whistle” reported by Schmidt (2012) for the giant anteater Myrmecophaga tridactyla, although it could not be described, nor audio recorded. Smith (2007a) has referred to these sounds as “quiet grunts” produced under a disturbance situation, “long, drawn-out harrrr sound” in agonistic encounters (Rocha & Mourão, 2006), or even “sharp whistles” of offspring to their mothers (Emmons, 1999). There are also records of some sounds (a repertoire of whistles, snorts, grunts, clicks, sniffs, and hisses) for the silky anteater Cyclopes didactylus under similar circumstances (mother/offspring communication, aggressive interaction, distressful situation with potential risk) (Shabel, 2011; Wainwright, 2002), but few is known about the communication skills of this species.

Despite anteaters and sloths vocalize to communicate with others, armadillos are not considered to communicate primarily by sound, but by chemical signaling (olfaction) (Loughry & McDonough, 2013). Although sound communication does not seem to essentially constitute the main source of transmitting to others an armadillo emotional state, it indeed consists in a (poorly known) way to communicate, and long reported since the ‘50s. Members of the Dasypodidae (the only living family of cingulates) are reported to vocalize in different forms and contexts: while foraging (grunting sound), pairing (chuck sound), communicating with mother/young (buzz sound), or being disturbed (scream or growl sound) (Talmage & Buchanan, 1954; Anderson & Jones-Jr., 1984; Nowak, 1999; Feldhamer et al., 2003). However, despite much of these sounds having been reported in previous studies (e.g., Superina, 2008; Smith, 2011; Abba & Superina, 2015; Sousa et al., 2016), very few studies have actually described them. Except for the classical paper of Christensen & Waring (1980) who provided a sound spectrogram to detail the “chuck” sound of Dasypus novemcinctus, and the more recent study of Amaya et al. (2019) who presented a detailed description of the weeping vocalization of Chaetophractus vellerosus, studies of this kind are lacking. Herein, we describe a specific vocalization of D. novemcinctus and also associate it to a behavioral response during the interaction of two subjects.

**MATERIAL AND METHODS**

**Data collection**

Subjects were two male D. novemcinctus (hereinafter referred to as M1 and M2) kept in the vivarium of Lauro de Souza Lima Institute (ILSL) at Bauru County in São Paulo State, Brazil. The first one (M1) was a wild-trapped adult from the savannah reserve of ILSL on April 22nd, 2017, and since then been kept at the vivarium for study purposes on the species’ behavior. At that year other seven nine-banded armadillos were being kept at the vivarium undergoing with M1 experiments on social interaction when a non-reported sound production and its response was recorded between M1 and a long-term captive adult male subject (trapped in March 23rd, 2015) on October 20th, 2017. Two video recordings were then made in sequence (02:06 and 05:03 min, starting at 17:56 and 18:05 h, respectively) by a camera (SONY® Cyber-shot DSC-H50 model, 9.1 megapixels) for both audio and visual registration. At first, M1 was recorded during the social interaction experiment, and then the other subject was removed from the bay and replaced by someone’s hand that pretended to simulate the contact of M1 with another subject. Afterward, a digital recorder (Marantz PMD-620) was provided to refine sound acquisition and posterior analyses, and on April 5th, 2018, during when these armadillos were undergoing cognitive tests, another video plus a sound recording of M1 performing the same sound behavior was accomplished (01:39 min, starting at 16:30 h).

Similar sound production and its response were then reported on February 28th, 2019, by a different subject from the seven others and during when armadillos were still being tested on their cognitive skills. The second subject (M2) was a captive-born young male on October 10th, 2017 (about a year and four months old at that time) that has just started to exhibit the same sound/behavior during a test that did not account for any contact with others. Besides this video recording, seven others
were produced by camera (lasting between 15 sec and 06:00 min, depending on the duration of the performed behavior, along the day – from 09:30 to 17:00 h) together with sound registering by the digital recorder (same equipment). It is worth mentioning that other armadillos did not vocalize nor exhibit the associated behavior, and that M2 behavior and sound production were recorded with the subject not interacting with others. All experiments were conducted in accordance with the Research Ethics Committee of ILSL under the procedure number ILSL004/16.

### Sound Analysis and Associated Behavioral Response

All recorded behaviors (three video recordings from M1 and eight from M2) were analyzed with QuickTime Player (version 10.3, Apple Inc.) and described. Sound recordings were housed at Sound Collection (HCLP-S) of the Herpetology Laboratory (LHERP) of São Paulo State University (UNESP), Institute of Biosciences, São Vicente. Four vocalization recordings (vouchered HCLP-S 1028, 1029, 1030, and 1035) from M1 (HCLP-S 1028) and M2 (HCLP-S 1029, 1030, and 1035) were taken with a digital recorder (Marantz PMD-620). Other available recordings (vouchered HCLP-S 1024-27 from M1; HCLP-S 1031-34 and 1036 from M2) were discarded and not analyzed due to excessive background noise (e.g., bird calls, dog barks, and other animal sounds) that overlapped with the frequency of the armadillo’s vocalizations. Sound analysis was performed with Raven Pro 1.5 software (Bioacoustics Research Program, 2014), with FFT of 512 points, overlap 93%, and Hann’s sampling window for spectrograms. The following temporal and spectral acoustic variables were measured: dominant frequency (Hz), duration 90% (s), energy (dB), number of pulses, and pulse interval duration (s). Temporal variables were measured from the oscillogram while spectral variables were measured from the spectrogram.

### RESULTS

### Acoustic characterization

A total of 76 vocalizations were recorded and analyzed for the recognition of a sound pattern. Generally, *Dasypus novemcinctus* vocalizations are characterized by the presence of two phases, each composed of two vocal units or notes:

#### A: inhale phase
This phase can be subdivided into two units or notes: Unit I, where the animal initiates inhaling, and unit II, where a strong inhaling is accomplished. This phase produces a spectrally structured sound emitted over a wide frequency band that resembles a “hiss.”

#### B: exhale phase
This phase also can be subdivided into two units or notes. Unit I, where the animal initiates exhaling, is characterized also as a spectrally structured sound emitted over a wide frequency band and resembles a low “hiss.” Unit II, a continuation of exhaling, is a pulse repetition sound where a series of low-frequency pulses is emitted, resembling a “purr” like sound.

No harmonic structure was observed. The pattern of how each individual vocalization is composed can vary, with two general patterns being observed regarding vocal unit emission: A-I, A-II, B-I, B-II (Fig. 1A) or A-I, A-II, B-I, B-II, B-I, B-II (Fig. 1B). As such, a single vocalization would comprise one repetition of either the former or latter pattern. Table 1 shows the parameters measured for the vocalizations. These vocalizations have always been produced when the subject was cornered and exhibiting defensive behavior.

### Behavioral response description

During the first M1 interaction with another male the subject placed itself at one of the corners of the bay (1.50 × 1.20 m) where both subjects were kept, with the head slightly downturned to the ground and hidden against the wall (with only the ears exposed). At this position, the back is turned against both the center of the bay and the other male (that initially approaches from the back), with its whole body slightly arched as the subject hides its head by ducking it down to be protected by the lateral projections of the anterior part of the carapace (Fig. 2A). By assuming this stance, M1 barely exposes its flesh (uncovered) parts (i.e., limbs and belly), but the ears, which seems to be the point of preventing these parts to be accessed. From this point on the other male started...
the tactile contacts, meaning that it touches M1 with one or both forelegs while sniffs it, which has already been described for armadillos when interacting (Contato tátil of Costa et al., 2011). This contact can be performed in three different ways: approaching from the back, flank, or front regarding from which position the other male comes to start sniffing M1. When the approach comes from the back of M1 (Figs. 2B, C), it can advance to covering (Cobertura of Costa et al., 2011), which means that the other male bipedally projects its belly against M1’s back and holds on to this position for a while (Fig. 2D). Depending on how intense the subsequent contact with forelegs is (from sniffing to sniff/touch and sniff/touch/cover), the response/reaction of M1 can be the latter bowing its body right or left depending on which side it is being touched by the other subject’s nose while sniffing (i.e., if the contact comes from the right side, the whole body responds by bowing to the same direction as to keep the animal’s back always against the other subject). When the other male approaches from the right/left flanks or back by covering, holding on by standing upwards on two legs to touch the back of the M1’s carapace, M1 humps as to get rid of its contact. This behavior (hump) has been described before (Movimentação em Corcoveio of Costa et al., 2011) and means M1 suddenly moving the back upwards, which causes the other to leave its position and cease the contact. This movement was reported as a response to the physical contact that could be followed by a sniff (the male sniffs osteoderms or between moveable bands, which can be succeeded (or not) by the subject trying to scratch the osteoderms or the uncovered bands). Some attempts at physically contacting M1 were made from below, with the other male lowering down and pedaling both forelegs against M1’s belly as to turn it over and expose it. This movement usually provoked a reaction/response of M1 turning its back toward the right/left side depending on which side the other male performed it. Whenever cornered and physically contacted by the other, the abovementioned responses of M1 are simultaneously followed by its vo-

Figure 1. Oscillogram (top) and spectrogram (bottom) of the agonistic vocalizations of Dasypus novemcinctus, HCLP-S 1028, recorded from individual M1. (A) A single vocalization composed of the pattern A-I, A-I, B-I, B-II; (B) A single vocalization composed of the pattern A-I, A-II, B-I, A-II, B-I, B-II.
calization (a hiss-purr-like sound). Vocalization seems to increase as the other male’s approach intensifies (i.e., a more abrupt physical contact). When the approach comes from the back to the tail (particularly its basis) (Figs. 2B, C), with the other male eventually scratching it (and also seeming to try to turn M1 over) (Fig. 2C), the reaction/response, as already mentioned, could be lowering down the back or humping as to get rid of the other male, together with the same vocalization pattern. If these attempts are continuous and intense, M1 could move from its place to another (corner) to avoid and run away from the other (Fig. 2D). During M1 displacement contacts could still be avoided, but it is worth mentioning that no sound is produced until M1 gets to another corner and assumes the former position of hiding itself against the wall and turning its back against the center of the bay. Indeed, under no different circumstance from M1 placing itself against the corner, a sound was produced.

The same set of behaviors was also recorded for M2, with the difference of this subject having vocalized while being disturbed by human contact. The subject had been released at a contiguous bay just after being submitted to a cognitive test (that did not involve interacting with another subject) and was about to be conducted to its bay when assumed the position of leaning against one of the corners and started to vocalize. From this moment on M2 was purposely disturbed to induce its vocalization, and the subject equally behaved as M1 by (1) bowing its body right/left depending on where it is touched by someone’s hand, (2) humping to get rid of hand contact when touched at the flanks or back, (3) lowering the body when touched from below. At all contacts, as in M1, vocalization was notably increasing at every contact, when the subject produced the same hiss-purr-like sound.

**DISCUSSION**

Clark (1951) called “pig-like grunts” the noise produced by armadillos while foraging, which seems to be the “low wheezy grunts” of Christensen & Waring (1980) recorded while armadillos were rooting and digging. Different sounds are also produced when armadillos are alarmed. Clark (1951) has further noticed a buzzing noise that could be heard when individuals were “completely terrorized”, and in a similar situation a “pig-like squeal” was reported by Lowery-Jr. (1974). The “chuck” sound was studied by Christensen & Waring (1980) and described as being a low-frequency (1.85 kHz average peak frequency), low-frequency (below 1.0 kHz), and short-duration (range 0.02- to 0.14-sec) sound (bouts of 5-8 notes) given by males and females while pairing (contact call), which was posteriorly recovered (although not to all observed pairs of armadillos) by McDonough (1997). The authors have also reported a “weak purring” from a young to “nurse an unrelated female”, but they raised doubts about it being indeed a vocalization (Smith, 2007b). A further mentioned noise reported by the authors is related to a distress call (a “wheezy grunt”) from struggling individuals when captured.

Distress calls are a specific vocalization that signs a great deal of physical stress by an individual when been handled/disturbed, thus related to an antipredator behavior (Manteuffel et al., 2004; Caro, 2005). Being recorded in many phylogenetically unrelated vertebrate groups, these calls are supposed to trigger different behavioral responses that can provide an opportunity to escape from predators (Amaya et al., 2019). Their first acoustic characterization provided to an armadillo has been recently published by Amaya and colleagues (2019). The authors have tested if the “weeping sound”
of Chaetophractus vellerosus when purposely disturbed while handled constituted such a call. Indeed, by its acoustic structure, similar to that expected to constitute sounds produced when an individual is under physical stress, as well as the behavioral context that has motivated its production, this call could be functionally identified as a distress signal. It is worth mentioning that the authors called attention to this sound not being produced in the field, but in the only circumstance of the individual being hand-held. This seems to be the case of the sound produced under the described circumstances in which both M1 and M2 vocalized, which is thus here termed as distress calls.

Both M1 and M2 had been handled to be put under specific experiments (interaction with a conspecific and cognitive test for M1 and M2, respectively), with the difference of M1 having started to vocalize after being put together with another individual and just after assuming the already described head-against-wall position, and M2 just after being removed from the bay test to another. As the latter was alone at the moment it leaned against the corner and started to vocalize, we assume that the subject was probably manifesting high-stress levels. This has already been reported for individuals of Euphractus sexcinctus (six-banded armadillo) submitted to electroejaculation (EEJ) to obtain semen, after which they showed intense vocalization (Sousa et al., 2016). This vocalization is not explained nor described, but these armadillos are known to produce a low, continuous, purr-like sound while vibrating when handled (Costa, pers. comm.). A purring sound has also been reported for Dasypus species, such as D. novemcinctus (as here reported), D. hybridus (Abba & Superina, 2015), and even for other armadillos such as Zaedyus pichy (Superina, 2008), with the latter presenting a grunt or scream as a progression of this purrr sound similar to that described for C. vellerosus (Superina, 2008). It is worth noticing that this type of sound is related to both these species (D. novemcinctus and E. sexcinctus) being hand-held disturbed. Male individuals of Cabassous unicinctus (southern naked-tailed armadillo) emit a “pig-like grunting noise” when handled, although females are reported to be silent (Smith, 2011). Thus, a stressful/dangerous context that could represent a threat is plausible to motivate these sounds in armadillos, although a distress call has just been described by Amaya et al. (2019) for C. vellerosus up to now.

This study identified two vocal units/notes for the two analyzed subjects of D. novemcinctus: an inhale phase, which is emitted over a wide frequency band resembling a “hiss” sound, and an exhale phase, which is also emitted over the same frequency band and resembles a low “hiss” sound ending in low-frequency pulses of “purr” like sound. Inhale and exhale notes have also been described for the distress call of C. vellerosus, as well as crying and grunt notes, with the former (crying notes) concentrating the main communicative function (Amaya et al., 2019). The sound produced by the subjects here analyzed of D. novemcinctus did not present a crying nor a grunt note, but the continuous purr sound succeeded by the inhalation phase of a hissing sound simultaneously being produced while the subject expresses specific behaviors shows that it could have a communicative function. As that concluded for C. vellerosus, it also seems to be a kind of distress call.

Different from the study of Amaya and colleagues (2019), in which the analyzed subjects were purposely induced to vocalize, subjects of D. novemcinctus here studied have spontaneously started to vocalize, which was perceived by chance and then recorded. This allowed us to report and describe the set of behaviors involved in/during sound emission. As the approaching of other to the position where the subject is set begins and is followed by physical/tactile contact, the subject behaves as to avoid it. Behavioral responses are mainly bowing the body to the side from where the contact comes or humping to get rid of the other bipedally touching with the front paws its dorsal carapace. These responses simultaneously given with the subject vocalizing are presumably an aspect of defensive behavior in this context. While pairing, some of the reported behaviors are also expressed, especially by the male to access the female. Males have been observed to touch the female’s carapace dorsally with front paws (McDonough, 1997), which has also been performed by the other armadillo relative to M1, although in a different context from mating. Thus, we could say that this behavior also applies to situations other than breeding and could be associated with approaching and contacting to know the other (male/female).

Moreover, the behavior named “tail wag” of McDonough (1997) (Movimentação sinoidal da cauda of Costa et al., 2011) reported for females reacting to males’ contact by a side-to-side movement of the tail has also been performed by M1. Details of this movement were not provided by McDonough (1997), but it seems to be the same movement observed for M1 while being contacted by the other armadillo. If so, this is a slow sinusoidal movement (Costa, pers. obs.) different from the quick, whip movement of the tail (Movimentação em chicote da cauda of Costa et al., 2011) that seems to be associated with a distress response to an interaction (with both subjects contacting each other). Thus, despite the meaning of this wavy-wagging movement of the tail is not known, it could be related to a broader range of situations, which thus dissociate it from a merely mating behavior.

Studies on animal behavior and communication consider as categorically discrete vocalizations produced in different contexts (Lingles et al., 2012). As above mentioned, armadillos produce a range of calls in many behavioral contexts, and although further studies are needed to recognize and understand the diversity and acoustic structure of these calls, some of them are already been identified and starting to be decoded, particularly those produced in stressful behavioral contexts. The here described distress call of D. novemcinctus, despite not being the first of these calls produced by armadillos to be acoustically characterized, is the first associated with a set of different behaviors expressed during the sound.
production. It is also worth mentioning that the same vocalization and behavioral responses were expressed both for M1 and M2, with the former being a recently wild-caught specimen at the time it was recorded, and the latter a captivity-born specimen that has never experimented an agonistic encounter before.

CONCLUSION

Although it is the first time a distress call plus the associated set of behaviors have been more detailed for *D. novemcinctus*, many further studies are required to indeed provide the behavioral repertoire of this species during agonistic encounters. However, the fact that the two subjects here studied having performed many similar calls and related behaviors when submitted to the same conditions at different times (and without having interacted with each other before) might be indicative of some pattern to be confirmed for the species. Moreover, as for *C. vellerosus*, which has been reported to consistently call when trapped by dogs (Amaya et al., 2019), *D. novemcinctus* could also vocalize in similar situations apart from hand-held disturbance, and thus express the here described behaviors if cornered somewhere without the possibility of escaping. Thus, as armadillos seem to be able to perform/display plenty of behaviors that are still being unveiled, as well as their poorly known ability to produce sounds is yet to be better understood, we conclude that their repertoire is likely to be much more complex than previously thought.

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