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RESEARCH ARTICLE - TERMITES

The use of Tympanic Arena as an Alternative for Behavioral Vibroacoustic Essays in Termites (Blattodea: Isoptera)

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Abstract

In termites, substrate-borne vibrations play an important role in communication among nestmates. The adaptive significance of such an ability has led to an ever-increasing number of studies aimed at improving knowledge on vibroacoustic communication in these insects. Such studies are commonly carried out in laboratory arenas consisting of Petri dishes made of plastic or glass. However, the rigidness of such materials may limit the transmission of vibrational waves impairing accurate records of the feeble vibrations produced by termites. This is one of the reasons why such experiments must be carried out under strictly controlled conditions, using extremely sensitive equipment, usually connected to amplifiers. If, instead, arenas bear a flexible floor (hence simulating a tympanum), vibrations might not be dampened or even easily amplified, thereby overcoming the need for such a specialized setup. Here we test such a hypothesis, using an accelerometer to measure and record vibrations whose intensity was tailored to mimic the feeble vibrations of a small termite species, *Constrictotermes cyphergaster*. Results support the notion that tympanic arenas portray such vibrations far more accurately than arenas made of plastic or glass. We hence recommend this type of arena as a cheap, albeit accurate, alternative in studies of vibroacoustic behaviors of termites and other insects of comparable size, especially in situations where noise is minimally controlled. These arenas, then, can be useful in conducting such studies just after termite collection in remote regions where well-equipped labs are not available. In doing so, we minimize the stress involved in transporting termites over long distances.

Introduction

Communication is an important life trait of organisms, allowing the exchange of information among intraspecific, and in some cases the interception of valuable information from interspecific (Evans et al., 2009; Cristaldo et al., 2016; Mark & Rufus, 2013; Šobotník et al., 2010). In termites, communication occurs basically by mechanical and chemical channels (Cristaldo et al., 2015; Šobotník et al., 2010; Costa-Leonardo & Haifig, 2014; Bagnères & Hanus, 2015). The mechanical channel is transmitted by substrate-borne vibration. When producing vibroacoustic signals, termites perform vertical and longitudinal oscillatory movements (respectively called “drumming” and “shaking”), which transmit vibrations to the substrate when the individual hits the ground and/or ceiling with its head or abdomen (Howse, 1964; Stuart, 1963; Hager & Kirchner, 2013; Cristaldo et al., 2015). Because substrate vibrations disseminate information quickly (Hunt & Richard, 2013) this pathway of communication has been reported to be important alarm signals inside and outside termite colonies (Howse, 1965; Cristaldo et al., 2015). It has been also demonstrated that vibroacoustic cues can be used by termites to assess food items (Evans et al., 2005) and to eavesdrop their competitors and predators (Evans et al., 2009; Oberst et al., 2017). The undeniable adaptiveness of such an ability has boosted the amount of studies on termite vibroacoustic behavior in recent years (Costa-Leonardo & Haifig, 2014; Bagnères & Hanus, 2015).

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Vibroacoustic bioassays involving termites are commonly performed using arenas consisting of glass or plastic Petri dishes (Howse, 1965; Hager & Kirchner, 2014; Cristaldo et al., 2015; Oberst et al., 2017). Rigid materials such as plastic and glass, however, are known to limit the transmission of vibrational waves (Joyce et al., 2008). It follows that vibroacoustic bioassays using such arenas need to be carried out on anechoic conditions, using high sensitivity accelerometers connected to amplifiers in order to reveal oscillatory sequences in full detail. This could be particularly true for bioassays involving some termites which, being small, produce feeble signals.

Very often, however, termite collections occur in remote regions, away from well-equipped laboratories. Taking termites thousands of kilometers away to where sophisticated setup is available is not always feasible, due to both biological and bureaucratic constraints. Additionally, legal permits must be obtained for the transportation of live specimens (sometimes across country borders) and, on top of that, termites may get too stressed and die before the bioassay is actually run.

In the absence of a sophisticated setup, an arena which mimics a tympanum seems a suitable alternative. Structures known as "tympanums" consist of a flexible membrane anchored to a solid frame and whose main function is to pick up waves and transmit them as vibratory stimuli (Sosa et al., 2002; Errobidart et al., 2014). As opposed to rigid materials with their low transmission of waves (Joyce et al., 2008), tympanums are sensitive to the intensity and frequency of the waves coming from a stimulus being, hence, a suitable flooring for arenas used in vibroacoustic bioassays.

Here we test the hypothesis that the high sensitivity of tympanums would pick feeble vibratory signals – equivalent to those produced by small termites – even in environments where noise is only minimally avoided. Ultimately, we aim to establish an alternative protocol for vibroacoustic bioassays, using an arena flooring which portrays authentic vibratory signals even under rough conditions, e.g., out of an anechoic room.

Materials and Methods

Overall rationale

To test our hypothesis, we connected an accelerometer sensor to distinct arenas and subjected them to a known vibratory stimulus whose intensity was equivalent to that of termites performing typical vibratory behavior. This testing stimulus was inflicted on the inner surface of the arenas' floor, right on the spot corresponding to the external place of attachment of the accelerometer's sensor. Readings thereby obtained were compared to those from this same stimulus inflicted directly on the accelerometer's sensor. The arena whose readings better approximated the direct readings was taken as the most viable arena for the analysis of such behavior in this group of insects, in that condition. Tested arenas consisted of plastic or glass Petri dishes and a homemade tympanic arena, and all assays have been carried out in a normal lab room with only minimal noise control (details are given below).

The experiment was conducted in two steps: (i) we first identified the best model object to simulate termite vibrations (Fig 1) and then (ii) we used the stimulus produced by this model object to compare the arenas (Fig 2). The use of such a model object guaranteed that every tested arena would receive precisely the same stimulus, at the same spot, and with the same intensity. This would not be possible if we had used actual termites as they move around the arena and perform vibrations at random spots with varying intensity.

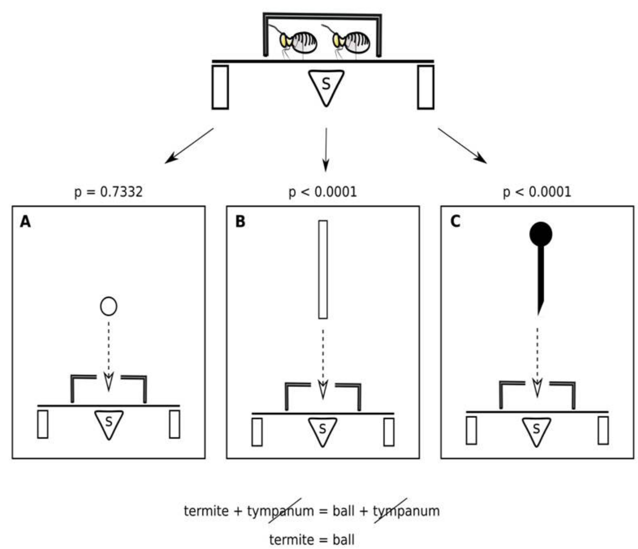

Fig 1. The pilot test: comparing the vibrations produced by termites on a tympanic arena with those produced by (A) a styrofoam ball, (B) a wooden stick, (C) an entomological pin. Vibrations were recorded by the an accelerometer's sensor (S) attached to the under surface of the tympanum. Of those objects, only styrofoam ball produced vibrations similar to those produced by termites. It was hence concluded that termites can be modeled by styrofoam balls in this type of bioassay. Fig 5 presents these results more formally.

After choosing the best arena, we performed an additional test subjecting it to the object which was found most dissimilar to termites in the step "(i)" above. In doing this we checked whether the other arenas could still be useful in assays involving stronger stimuli such as those produce by bigger termites (Fig 3).

Pilot test: validating the model object

In the search of a model object that best simulated termite vibrations we tested (i) a styrofoam ball (Ø = 0.5 mm), (ii) an entomological pin (number 1, Ø = 0.4 mm), and (iii) a wooden stick (25 cm long; Ø = 4 mm). Such test is detailed at Fig 1.

The test consisted in comparing the intensity of the readings produced by such objects with those produced by *Constrictotermes cyphergaster* (Termitidae: Nasutitermitinae) termites on the floor of an arena specially built to combine rigid and tympanic elements. Such an arena consisted of the lid of plastic Petri dish (Ø = 53 mm) covering a piece of tracing paper kept taut by a frame. The accelerometer's sensor was attached to the lower (external) surface of the arena's

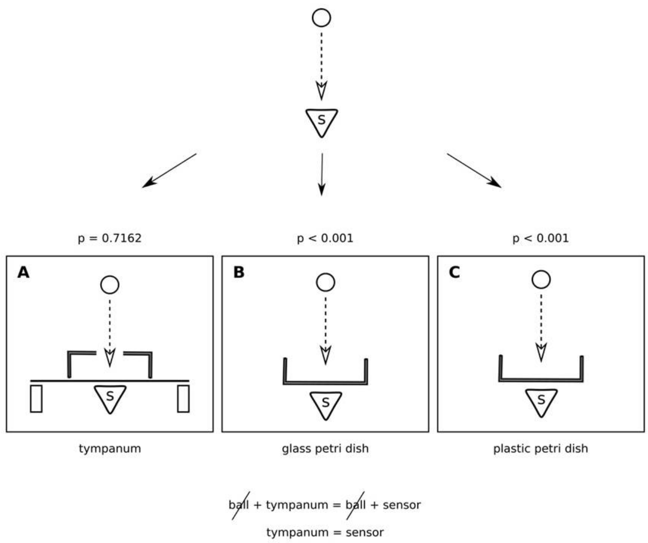

Fig 2. The main test using styrofoam ball: comparing the vibrations produced when such a ball was dropped directly onto the accelerometer's sensor (S) with the vibrations produced by this same ball on (A) a tympanic arena, (B) an arena consisting of a glass Petri dish, (C) an arena consisting of a plastic Petri dish, all these having an accelerometer's sensor attached to their under surface. Vibrations produced on the tympanic arena did not differ from those produced directly on the sensor. It was hence concluded that losses of stimulus' intensity due to the substrate are insignificant for tympanic arenas, confirming their suitability for this type of bioassays. Fig 6 presents these results more formally.

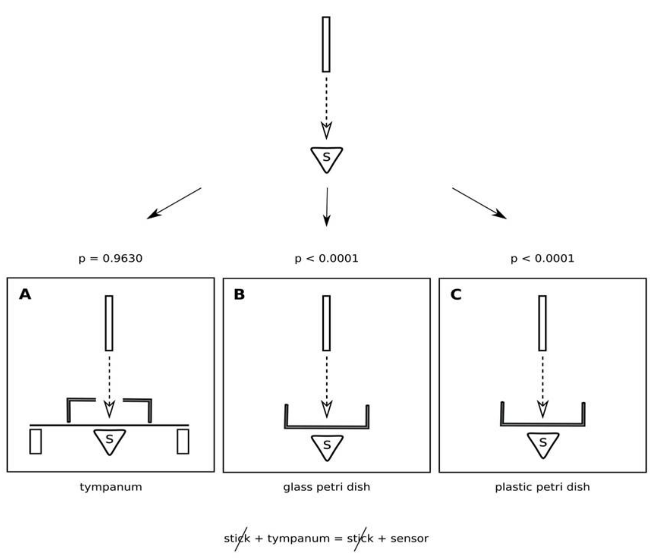

Fig 3. The main test using a styrofoam ball: comparing the vibrations produced when such a stick was dropped directly onto the accelerometer's sensor (S) with the vibrations produced by this same stick on (A) a tympanic arena, (B) an arena consisting of a glass Petri dish, (C) an arena consisting of a plastic Petri dish, all these having an accelerometer's sensor attached to their under surface. Vibrations produced on the tympanic arena did not differ from those produced directly on the sensor. It was hence concluded that losses of stimulus' intensity due to the substrate are insignificant for tympanic arenas, confirming their suitability for this type of bioassays. Fig 7 presents these results more formally.

floor. Vibroacoustic stimuli were produced on the arena's floor, allowing termites to bang their heads or by dropping the testing objects from a height of nine centimeters onto this floor. A small hole was drilled on the center of the Petri dish serving as a lid, to allow objects to be dropped onto the arena's floor.

We have chosen as the model object the one whose readings recorded by the accelerometer resembled closer those readings originated from termites. A total of 40 independent trials have been conducted in the pilot test, producing 20,480 readings. Each of these trials produced one vibrational profile with 512 readings, similar to the one depicted at Fig 4. These correspond to 10 independent trials for each of the three objects under test plus 10 trials for termites. Each of these trials corresponded to a given profile with 512 readings, similar to the one depicted at Fig 4. See "Statistical analyses" below for details on such measurements.

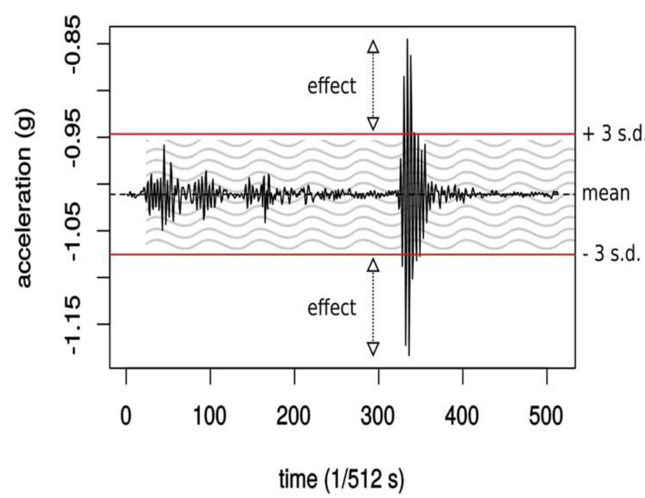

Fig 4. A typical vibratory profile, as recorded by an accelerometer's sensor fixed underneath an experimental arena. In order to calculate the effect of the respective arena on the accelerometer's readings, we first extracted from this profile all values within the range mean ± 3 standard deviations, as these are too affected by residual oscillations besides those due to the treatment alone. Then we summed the amplitudes corresponding to the extreme upper and lower values ("effect") remaining in the series, to be taken as the treatment effects in the analyses.

The main test: comparing arenas

After selecting the model object, we proceeded to the main test which actually compared arenas (Fig 2). To do so, we used two types of rigid arenas and one type of tympanic arena, each of them having the accelerometer's sensor attached to the lower (external) surface of their floor. Rigid arenas consisted of the lower part of either a plastic or a glass Petri dish. Tympanic arenas consisted of an embroidery hoop lined with tracing paper. Embroidery hoops consist of a pair of concentric circular wooden rings which hold taut a piece of fabric, thereby helping artisans in their activities of cutting and sewing. This closely resembles a tympanum or a shallow drum, being a readily available and cheap apparatus.

The tympanic arenas here described had, as their floor, a piece of tracing paper held by the concentric rings of the embroidery hoop. Diameters of arenas varied as follows: plastic arenas from 49.4 to 144.4 mm, glass arenas from 42.0 to 140.0 mm, and tympanic arenas of 150.0 and 200.0 mm.

The test consisted of dropping the model object onto the floor of each arena (precisely on the spot below which the sensor was attached to) and comparing the amplitude of such readings with that of the readings produced by dropping this same object directly onto the accelerometer's sensor. The model object was dropped at the height of nine centimeters from the arenas floor or from the sensor.

The arena whose readings resembled closer the readings produced by the model object directly on the sensor was defined as the best (among those tested) for vibroacoustic studies using these insects in that condition.

A total of 51 independent trials have been conducted in the main tests, producing 26,111 readings. Each of these trials produced one vibrational profile with 512 readings, similar to the one depicted at Fig 4. These correspond to 27 trials for the main test involving styrofoam balls, conducted with three repetitions for direct stimulus on the sensor, six repetitions for tympanic arena, eight repetitions for plastic arenas and ten repetitions for glass arenas. The remaining 24 trials have been conducted with a wooden stick, including two repetitions for direct stimulus on the sensor, four repetitions for tympanic arena, eight repetitions for plastic arenas and ten repetitions for glass arenas.

Technical specifications

In order to minimize noise and vibrations from human trafficking and other activities in nearby laboratories, all testing setups were mounted into a wooden box lined with a five centimeters layer of glass wool. Arenas were placed inside this box over a pair of egg crate foam strips laying on a styrofoam hollowed cubic structure.

Vibratory stimuli have been measured and recorded using an USB accelerometer (Gulf Coast Data Concepts, LLC ™ model X2-2 logger) equipped with a Kionix KXRB5-2050 ™ sensor at 2.5 volts, which results in a sensitivity factor of 500 mv/g. The sensor registers the readings in three axes (X, Y and Z) separately. We have used only the values recorded for vertical axis Z. To facilitate the essays, the sensor was removed from the accelerometer's case while keeping it connected to the recording unit by electric wires. In doing so, we could attach this sensor directly to the external bottom surface of the arenas.

Focal species

We used soldier and workers of *Constrictotermes cyphergaster*, a neotropical termite species common in Brazil, Paraguay, Bolivia and Northern Argentina (Mathews, 1977). Vibroacoustic behavior is one of the alarm responses known to this species' defense arsenal (Cristaldo et al., 2015). It consists of vertical and longitudinal oscillatory movements performed by a termite individual which result in alternate banging of its head and abdomen on the substrate, thereby transmitting vibrations which are interpreted as alarm by the nestmates. Both soldiers and workers exhibit this mechanical alarm behavior.

Statistical analyses

Statistical analyses proceeded in R using Generalized Linear Modeling (GLM) under normal errors. Model simplification was performed by stepwise deletion with F tests, lumping together treatment levels as long as these did not provoke significant changes ($P<0.05$) in the model (Crawley, 2005). Residual analyses confirmed the choice of the error distribution.

For both, pilot test and main test, the amplitude of the accelerometer's readings in a given treatment was used as y-var. This value was obtained using the extreme values from a given reading after residual oscillations have been extracted from the corresponding oscillatory profile, as detailed in Fig 4.

For the pilot test, a categorical x-var representing the stimulus held four levels, each one corresponding to a given object under test ("styrofoam ball", "pin", "wooden stick") or to termites themselves. We aimed here to determine which of these objects, when dropped on the arena's floor, would produce amplitude values most similar to those produced by termites exhibiting vibroacoustic behavior. The object thereby selected was used in the main experiment.

The full model for the pilot test was hence:

amplitude ~ stimulus

For the main experiment, a categorical x-var representing the substrate held four levels, each one corresponding to a given arena under test ("tympanum", "plastic", "glass") or to the sensor of the accelerometer onto which the object was directly dropped. The amplitude of vibration is known to depend on the extension of the substrate's free span: the larger the substrate span, the lower the amplitude. This happens because of the loss of energy during the propagation of vibratory waves along the surface, favoring small Petri dishes (Ø = 44 mm) over large embroidery hoops (Ø = 200 mm). In order to account for such an effect, the diameter of the arenas was included as a co-variate in the model. We aimed here to determine which of these arenas would produce amplitude values most similar to those produced by the model object dropped directly onto the sensor. The arena thereby selected was defined as the best one for this type of study in that condition.

The full model for the main experiment was hence:

*amplitude ~ substrate + diameter + substrate * diameter*

Results

Pilot test: validating the model object

In tests to validate the model object, termites produced the lowest average amplitude of vibrations (3.4 ± 0.93 units; mean ± s.e.), being followed by the styrofoam ball (430.6 ± 61.07 units), the pin (11645.9 ± 641.7 units) and the wooden stick (15056.1 ± 598.2 units). A summary of these is given at Figs 1 and 5.

In such trials, the averaged amplitude of the vibrations produced by termites did not differ from the averaged amplitude produced by the styrofoam ball ($F(1,37) = 0.118$, $p = 0.7332$), but these differed from those produced by the pin ($F(1,38) = 115.4$, $p < 0.0001$) which in turn also differed from the amplitudes due to the wooden stick ($F(1,38) = 7.7022$, $p = 0.0086$).

These results support the notion that styrofoam balls, but not the other objects, can be used as an object to model termites in the tests here conducted.

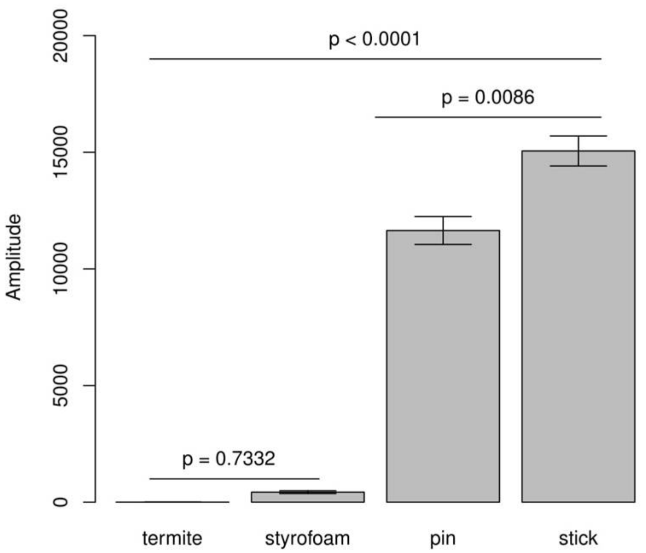

Fig 5. Defining the model object to be used in the main test. Vibrational amplitudes (as defined in Fig 4) produced by styrofoam ball do not differ from those produced by termites, but differ from the amplitudes produced by the pin and the wooden stick.

The main test

In trials using styrofoam balls as model object, the average amplitude of the vibrations produced directly on the sensor was affected by the type of arena ($F(3,23) = 25.289$, $p = 5.20e\text{-}07$) but not by their diameter ($F(1,22) = 1.206$, $p = 0.285$) nor by the interaction between arena type and diameter ($F(2,20) = 1.077$, $p = 0.360$). As summarized in Table 1, the averaged amplitude of vibrations produced directly on the sensor did not differ from those produced on the tympanum ($F(1,24) = 0.136$, $p = 0.7162$), but these differed from those produced on the plastic arena ($F(1,24) = 15.696$, $p < 0.001$) which in turn also differed from the amplitudes recorded in the glass arena ($F(1,24) = 13.428$, $p = 0.0013$). Whereas the tympanums transmitted 94% of the stimulus, plastic arenas transmitted only 43% and glass arenas transmitted 1%. In other words, plastic and glass arenas severely dampened stimuli, hence underestimating the readings. These results are summarized at Table 1 and Fig 6, supporting the notion that the loss of a feeble stimulus when using tympanic arena was negligible but that was not so for plastic or glass arenas.

In the additional trials involving wooden sticks, the average amplitude of the vibrations produced directly on the sensor was affected by the type of arena ($F(3,20) = 70.395$, $p < 8.59e\text{-}10$) but not by their diameter ($F(1,19) = 3.375$, $p = 0.084$) nor by the interaction between arena type and diameter ($F(2,17) = 0.263$, $p = 0.772$). As summarized in Table 1, the averaged amplitude of vibrations produced directly on the sensor did not differ from those produced on the tympanum ($F(1,21) = 0.002$, $p = 0.963$), but these differed from those produced on the plastic arena ($F(1,21) = 58.503$, $p < 0.001$) which in turn also differed from the amplitudes recorded in the glass arena ($F(1,21) = 4.416$, $p = 0.049$). In these trials, while the tympanums transmitted 100% of the stimulus, plastic arenas transmitted 38% and glass arenas transmitted 28%. These results are summarized at Table 1 and Fig 7, supporting the notion that the loss of a strong stimulus while negligible in tympanic arenas, was still significant when using plastic or glass arenas.

Table 1. The percentage of stimulus transmitted or absorbed by the arenas' flooring to the accelerometer's sensor as compared to this same stimulus provoked directly onto the sensor. Stimuli were produced dropping either a styrofoam ball or a wooden stick onto the sensor or onto the arenas. More statistical details are given at Figs 6 and 7.

Substrate	Reading	% Transmitted	% Absorbed	Statistical significance
		Styrofoam ball:		
Sensor	229.70 ± 44.2	100	0	
Tympanum	215.39 ± 41.5	94	6	n.s.
Plastic	97.72 ± 18.8	43	57	***
Glass	2.14 ± 0.4	1	99	***
		Wooden stick:		
Sensor	22551.5 ± 4603.3	100	0	
Tympanum	22645.0 ± 4622.4	100	0	n.s.
Plastic	8628.6 ± 1761.3	38	62	***
Glass	6333.6 ± 1292.8	28	72	***

In summary, considering losses of stimulus to be transmitted to the accelerometer's sensor, tympanum is better than plastic which, in turn, is better than glass. This is true for both, weak or strong stimuli.

Discussion

As predicted by our hypothesis, tympanic arenas have revealed themselves as an excellent alternative to either glass or plastic arenas for lab bioassays of vibroacoustic signals emitted by small termites in a condition where noise is minimally controlled. Losses of such stimuli when using tympanic arenas were insignificant (Table 1) to the point of not being distinguishable from stimuli inflicted directly on the accelerometer's sensor (Fig 6, Table 1). The other arenas did absorb much of the stimulus, failing to transmit it accurately to the accelerometer's sensor.

It must be warned that this is not to imply that previous vibroacoustic studies on termites, using Petri dishes and alike,

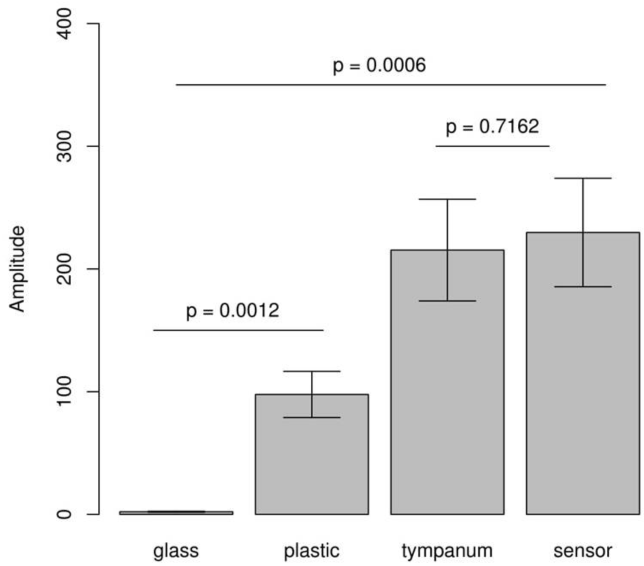

Fig 6. The main test using styrofoam ball: vibrations produced when such a ball was dropped directly onto the accelerometer's sensor did not differ from those produced onto a tympanic arena, but they did differ from those produced dropping the ball onto the floor of an arena consisting of a glass Petri dish or of a plastic Petri dish.

would be invalid. In general, these have been conducted using a strictly controlled setup and highly sensitive equipment (*e.g.* Cristaldo et al., 2015; Oberst et al., 2017) which would certainly compensate for the rigidity of the experimental arena. What we want to show here is an alternative which, while cheap, is still highly suitable and accurate. The high sensitivity of the tympanic arena here reported seems to compensate for the absence of, *e.g.*, an anechoic chamber or a high sensitive accelerometer.

These results find support on theoretical expectations (Cocroft et al., 2006; Michelsen et al., 1982; Miklas et al., 2001) according to which the substrate material interferes on the transmission of the vibrational stimuli, mainly when these materials are rigid. In fact, Joyce et al. (2008) found that the amplitudes of waves coming from vibrational stimuli are influenced by the substrate: in rigid materials as plastic and glass, the transmission of stimuli is lower than in flexible materials, as maize and bean leafs.

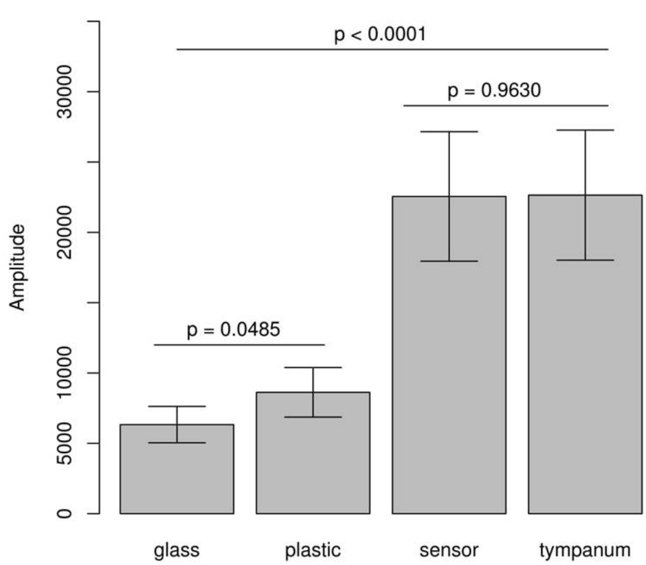

Fig 7. The main test using wooden stick: vibrations produced by a wooden stick dropped directly onto the accelerometer's sensor did not differ from those produced onto a tympanic arena, but they did differ from those produced dropping the stick onto the floor of an arena consisting of a glass Petri dish or of a plastic Petri dish.

Because plastic and glass are denser and more rigid than tracing paper, the floor of the tympanic arena is more elastic than that of Petri dish arenas. It is then expectable this latter to convey stimulus to the accelerometer's sensor less accurately. This qualifies these tympanic arenas as a very suitable apparatus to the study of vibroacoustic signals in termites in an environment where noise is only minimally controlled.

The fact, however, that these arenas were also more accurate in transmitting even stronger stimuli makes these arenas even more recommendable. As depicted in the lower panel of Table 1, as well as in Fig 3, vibrations produced by the wooden stick were also severely dampened by plastic (62% lost) and glass arenas (72%), but not by the tympanum (0%). This is highly surprising specially considering that the stimulus produced by this stick is about 100 times stronger than the stimulus produced by the styrofoam ball (from "Sensor" lines in Table 1: 22551.5/229.7=98.17). Since the stimulus produced by the styrofoam ball was indistinguishable from that produced by termites (Fig 1 and 5), it follows that tympanic arenas could be recommended for vibroacoustic studies even for termites much larger than *C. cyphergaster*.

The suitability of the tympanic arenas here studied goes beyond their accuracy in transmitting stimuli to accelerometer's sensor. Being made out of embroidery hoops lined with tracing paper, these are readily available and relatively inexpensive. A single 150 mm glass Petri dish would cost not less than US$ 8 while a set of five hoops, from 130 to 230 mm, can cost as little as US$ 10 (http://www.amazon.com, retrieved: 12 Aug 2017).

Concluding, the tympanic arenas here describe may be a suitable alternative for vibrational studies on termites, especially in situations where the noise is only minimally controlled. This could be useful, for instance, to run such bioassays directly in field stations just after the termites have been collected, avoiding the stresses resulted from transporting termites over long distances to better equipped laboratories.

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