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Short Communication

A new technique in the excavation of ground-nest bee burrows (Hymenoptera: Apoidea)

Diego Marinho[a,*], Juliana Andrade[a], Rodrigo O. Araujo[b], Felipe Vivallo[a]

[a] *Universidad Federal do Rio de Janeiro, Museu Nacional, Departamento de Entomologia, Quinta da Boa Vista, Rio de Janeiro, RJ, Brazil*
[b] *Universidad Católica del Maule, Facultad de Ciencias Básicas, Departamento de Ciências Biológicas y Químicas, Talca, Chile*

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ABSTRACT

Bees have a diversified natural history, thus the methods applied to study such diversity are varied. When it comes to studies of nesting biology, bees which nest in pre-existing cavities have been reasonably well studied since researchers started using trap-nests. However, bees whose nests are built underground are poorly studied due to the difficulty of finding natural nesting areas and the absence of a method that facilitates bee nest excavation. The latter is evidenced by the lack of accurate descriptions in literature of how nests are excavated. In this study we tested cylindrical rubber refills of eraser pen as a new material to be used as a tracer of underground nest galleries in a natural nesting area of two species of *Epicharis* Klug, 1807 (Apidae). We compared this technique directly with plaster in powder form mixed with water and our results with other methodological studies describing alternative methods and materials. The rubber refill technique overcame the main issues presented by materials such as plaster, molten metal alloys and bioplastic, namely: death of the organisms by high temperatures and/or formation of plugs and materials unduly following the roots inside the galleries.

Bees are insects with a very diversified natural history. Most species are solitary (about 85%) and each female takes care of its own brood cells (Batra, 1984; Michener, 2007). Their nests are built with several materials, like resin, leaves, Dufour's gland secretions, etc. and on several substrates, such as rocks, wood, plant branches, inside pre-existing cavities or directly in the ground (Batra, 1984). Considering this bionomical diversity, the methods used to study these organisms are also diversified (Linsley et al., 1952; Michener et al., 1955).

Apids and Megachilids which nest in pre-existing cavities in the wood, for instance, have their biology better understood due to the advent of the trap-nest method (Krombein, 1967). However, the majority of bees build their brood cells underground (Batra, 1984), and subsequently, little is known about the process of nest construction and associated aspects of the biology of these species, mostly due to the difficulty of finding natural nesting areas and to obtain data collection from their underground nests.

Several methodological studies were developed regarding Hymenoptera ground-nesting habits (Michener et al., 1955; Williams and Lofgren, 1988), but the majority of them were focused on ants (Tschinkel, 2010, 2013). For studies that propose specific methods for collecting underground bee nests, the materials used are plaster (Linsley et al., 1952; Michener et al., 1955; Norden et al., 1994), molten metal alloys, paraffin wax (Linsley et al., 1952) and bioplastic (Howell, 1960).

From 1960 onwards, to the best of our knowledge, no methodological studies have been published focusing on methods of collecting underground bee nests, evidencing the lack of literature concerning alternative ways to study such nests. Furthermore, several studies on the biology of ground-nesting bees do not describe in detail how excavation is performed (see Gaglianone, 2005; Gaglianone et al., 2015; Rocha-Filho et al., 2008; Roubik, 1980; Rozen, 2016; Thiele and Inouye, 2007), hindering the possibility of comparison of the methods used. In this context, we aim to propose a new practical method for tracing the nest tunnels and collecting brood cells from underground nests. Additionally, we compare it with other methodological studies present in literature.

Nests excavation

The study was carried out in a nest aggregation area of *Epicharis analis* Lepeletier, 1841 and *Epicharis fasciata* Lepeletier & Serville, 1828 at the "Jardim Botânico do Rio de Janeiro" (22 58′14″ S, 43 13′18″ W), State of Rio de Janeiro, Brazil. Nest excavations were

* Corresponding author.
E-mail: diego.mp89@gmail.com (D. Marinho).

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performed during four hours on two different dates: on February 6th, 2017 when *E. analis* was at its peak activity and on April 6th, when *E. fasciata* was nesting. Several females of both species shared the single aggregation area, but nesting activities were independent.

In the first expedition, we used powder of plaster and plaster mixed with water, in order to track the gallery of the nests until the brood cell chambers built by the bees according with the methodology originally proposed by Michener et al. (1955) and subsequently widely used by others researchers (Danforth, 1989; Eickwort and Eickwort, 1969; Osgood, 1989; Wcislo et al., 1993).

During the second visit to the nesting area we tested rubber refills from the Gom Pen Maped® brand (Fig. 1A), used in eraser pens. Each rubber refill has a cylindrical shape, 0.7 cm diameter and 11.0 cm height, high flexibility, an average cost of US$ 2.50 and can be easily found at stationers. Across its height, we made graded marks in centimeters using a ballpoint pen. For deeper brood cells where a single rubber refill cannot reach, we glued several of them to each other by their extremities using cyanoacrylate of the Super Bonder® brand (Fig. 1B). We tested the tracer with up to four rubber refills due to the depth of the brood cells in the ground.

Excavations of *E. analis* cells were performed during the first visit, when we tested plaster, but with little progress. The track of the nest gallery was easily lost using that material in powder form; the debris and soil around the tunnel kept falling during the excavation using the shovel. When the plaster was applied mixed with water, the cast hardened in about one hour, but acquired a fragile consistency and broke easily during excavation. We obtained six brood cells of *E. analis* using this method.

The excavation using rubber refill (henceforth "rubber") was employed during the second visit to the same area, when *E. fasciata* was nesting. The procedure was performed as follows: the entrance of the nest tunnel was spotted visually; the rubber was inserted and conducted inside the gallery. Using the rubber as a tracer, we dug a parallel transect using a shovel (Fig. 1B); this way, the nest architecture was preserved in order to be drawn and measured, since the rubber retains the tunnel structure, serving as a tracer for the deeper parts of the nest gallery.

After the scheme of the nest architecture was done, an area of approximately 35 cm of diameter was dug around the gallery traced by the rubber (Fig. 1C). This posterior excavation was performed in order to find the brood cells placed randomly around the main gallery by the founder bees. Then, brood cells were removed manually and we were able to collect 14 of them, four of *E. analis* and ten of *E. fasciata*. No harm was done to the bee cells when excavating through this technique. Vouchers of the brood cells and emerged

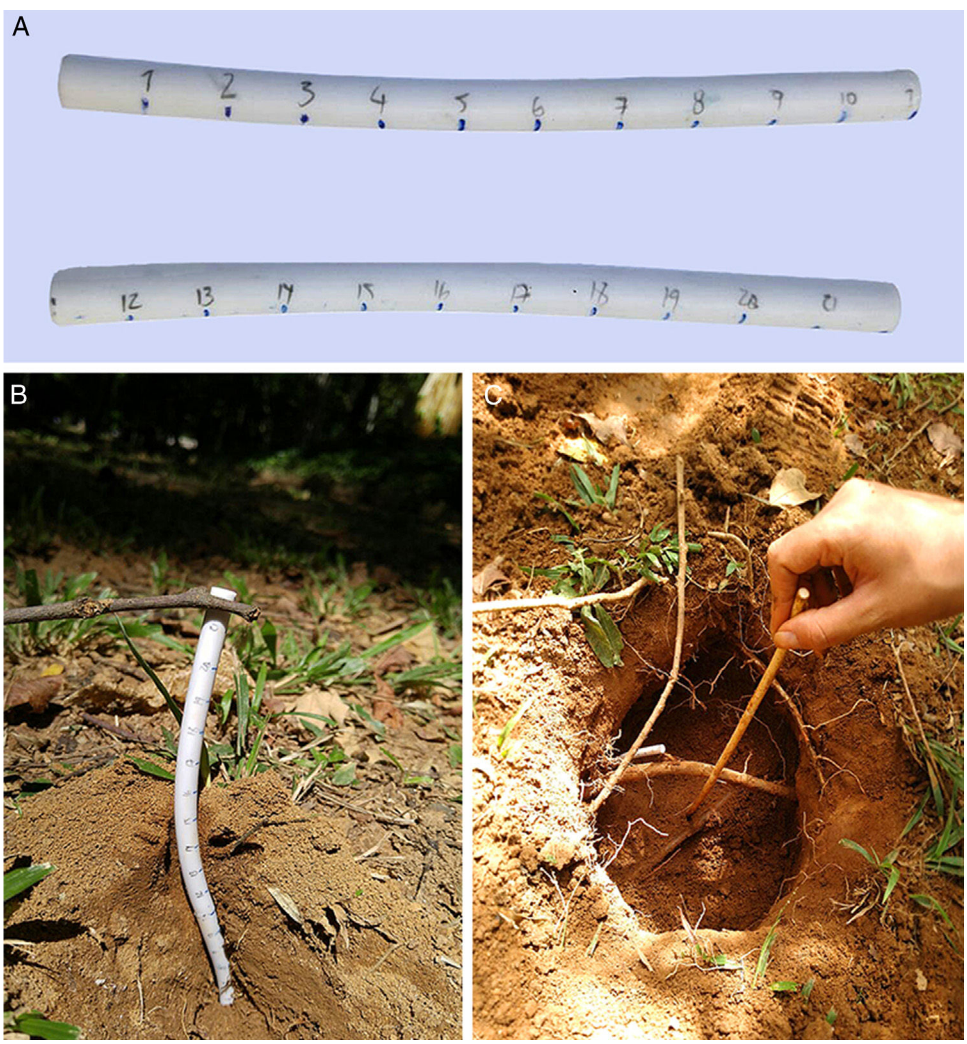

Fig. 1. Rubber refills used as tracer for excavation: (A) with graded marks, and (B) glued to each other by their extremities tracing an *Epicharis fasciata* nest gallery. (C) Posterior gallery excavation in of *Epicharis fasciata* nests in order to obtain randomly placed brood cells around the rubber refill tracer.

specimens were deposited in the Entomological Collection of the Museu Nacional do Rio de Janeiro (MNRJ).

Tracer methods for nest excavation

The method chosen for excavating nests may vary greatly depending on the scope and the available infrastructure to perform the study. The most used material is plaster, mainly because is easily obtained and handled (Linsley et al., 1952). However, we noticed the same problems reported by Howell (1960): we continually lost the track of the tunnels due to loose debris that kept falling from the gallery; the cast formed when we applied it with water was easily broken; and much time was spent waiting for it to harden. The procedure employed using rubber share the same advantages and overcomes all the disadvantages of plaster: there is no need for water, no waiting time and the tunnel is neither lost nor the rubber is broken during the excavation.

Other methods described in literature are metal alloys, dental plaster, paraffin wax and bioplastic which cast the structure of the underground nests; however, the eggs or immature larvae may be killed due to the high temperature of these materials when they are employed (Michener et al., 1955; Tschinkel, 2010), with the exception of bioplastic (Howell, 1960). In this study, the cell architecture of both species observed would favor those materials to flood into brood cells, since we observed some apertures, probably for larval-breathing. In studies aiming to study the biology of bees or even other insects that nest in the ground, this side effect is not desirable, since the collected brood cells are taken to the laboratory for further observations such as monitoring and studying immature development. Casts of the tunnel structures cannot be made using the rubber technique, but we consider it a lesser disadvantage. Additionally, the use of metal alloys is difficult because it requires much safety equipment when handling it in the field (Tschinkel, 2010).

In spite of bioplastic being a better alternative for the cast methods discussed above, Howell (1960) observed some issues: difficulty to excavate the cast formed, bio-plastic readily following roots in the path of the gallery, and the formation of plugs because of its uneven solidification. Our method also overcame these disadvantages, since the rubber was not impeded to go through the gallery by roots, and as long as it is already solid, no plug-formation issues occur.

The new method presented can be used to study underground nesting bees with several sizes, as we can find rubber with diameters varying from 0.2 to 0.7 cm. We also point out that this technique can be used to other ground-nesting Hymenoptera besides bees. This is also a low cost method, due to unit values ranging from US$ 2.2 to US$ 3.8, depending on the desired diameter. In comparison with other methods such as molten metal alloys, the cost is reasonably small, since one would not need tools such as S-hooks, stir bars and hot gloves, which may turn field work costly (Tschinkel, 2010).

Studies on other ground-nesting Hymenoptera, such as ants, provide several comparable techniques regarding specific objectives on ant biology studies and such comparisons are stated as beneficial for the field (Tschinkel, 2010). Such comparable methods are lacking in underground bee nest investigations and even in other field research regarding pre-existing cavities bee nesters, as pointed out by MacIvor (2017) in his review on cavity-nest boxes for solitary bees. Over time, several tools may be usable for the study of ground-nesting bees and we point out the need for methodology descriptions employed in nesting biology studies. The detailed description on how brood cells are excavated from underground nests may contribute for the establishment of better methods to study ground-nesting bees.

Contributors

All authors conceived the research and went to field to perform the experiments. The first author wrote the paper and all authors contributed equally reviewing critically the manuscript for important intellectual content. All authors approved the final version of this manuscript.

Conflicts of interest

None declared.

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