Revista Brasileira de Entomologia xxx (2017) xxx–xxx

SOCIEDADE BRASILEIRA DE ENTOMOLOGIA
FUNDADA EM 1937

REVISTA BRASILEIRA DE
Entomologia
A Journal on Insect Diversity and Evolution
www.rbentomologia.com

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

ARTICLE INFO

Article history:
Received 17 October 2017
Accepted 13 November 2017
Available online xxx
Associate Editor: Rodrigo Gonçalves

Keywords:
Apidae
Apoidea
Brood cells
Methodology
Solitary bees

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), 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 performed during four hours on two different dates: on February 6th,

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

https://doi.org/10.1016/j.rbe.2017.11.003

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 (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

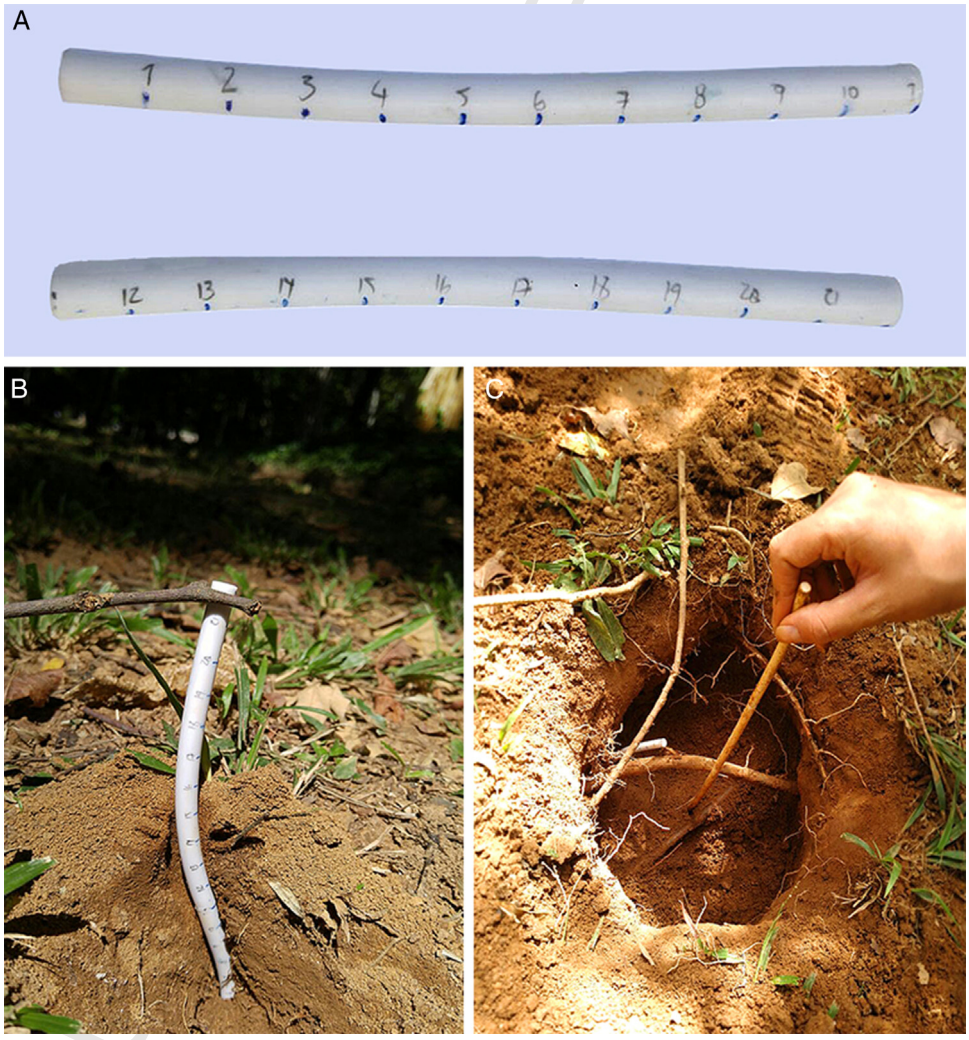

Fig. 1. Rubber refills used as tracer for excavation: (A) with graded marks, and (B) glued to each other by their extremities tracing an *Epicharis fasciata* nest gallery. (C) Posterior gallery excavation in of *Epicharis fasciata* nests in order to obtain randomly placed brood cells around the rubber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

Please cite this article in press as: Marinho, D., et al. A new technique in the excavation of ground-nest bee burrows (Hymenoptera: Apoidea). Rev. Brasil. Entomol. (2017). https://doi.org/10.1016/j.rbe.2017.11.003