RESEARCH ARTICLE

Discovery of *Mourecotelles* (Hymenoptera, Apidae, Colletinae) in Brazil: nesting biology and pollen preferences of a remarkable new species of the genus

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Abstract

Mourecotelles Toro & Cabezas (Hymenoptera, Apidae, Colletinae) currently includes only nine valid species of cellophane bees found mostly in relatively-dry regions of western South America (Chile, Argentina, Bolivia, and Ecuador). In this paper, we describe and illustrate a new species of the genus – *M. braziliensis* Ferrari & Melo, **sp. nov.** – based on individuals of both sexes captured through trap-nesting in an environmental protection area (Araucárias Municipal Natural Park) and in flowers in different localities in southern Brazil. In total, we obtained 16 nests of *M. braziliensis*, each consisting of two to eleven brood cells arranged horizontally and lined with a cellophane-like substance. Of the 57 adult bees that emerged, 41 were male (mean weight 46.5 mg) and 16 were female (mean weight 58.9 mg), resulting in biased sex and investment ratios of 2.56:1 and 2.02:1, respectively. Both the numbers of provisioned cells and mortality rate were higher for trap nests with the narrowest bore diameter, although the differences in relation to other trap nests were not statistically significant. Pollen of nine different

plant families were found in brood cells of *M. braziliensis*, but the species showed a clear preference for Fabaceae and Polygalaceae. Indeed, some of the specimens were collected while foraging in flowers of an unidentified species of *Monnina* Ruiz & Pav. (Polygalaceae) growing in swampy areas. The evolutionary and biogeographical implications of our discovery are briefly discussed.

Keywords

Bee, Colletini, floral host, Neotropical region, sex ratio, trap nest

Introduction

Nesting biology has historically been one the most widely studied aspects of the natural world of bees (e.g. Michener 1964; Houston 1975; Rozen 1984; Roubik 2006; Martins et al. 2019; Vivallo et al. 2021), presumably due to its remarkable diversity across taxa. For instance, despite the fact that most species of bees are ground nesters (Cane 1991; Cane and Neff 2011), several other substrates are also exploited, such as twigs, dead soft stems, termite nests and previously-established cavities in concrete walls (Roubik 1989; Camargo and Pedro 2003; Fortel et al. 2016). The Colletinae are unique among bees in producing a cellophane-like waterproof substance, which is composed mainly of a mixture of macrocyclic lactones (produced by Dufour's gland) and salivary gland secretions (Albans et al. 1980; Duffield et al. 1980), to line their brood cells (Batra 1980; Torchio et al. 1988). This process is facilitated by their specialized (bilobed or bifid) glossa, a character not found in any other group of bees (McGinley 1980; Michener and Brooks 1984; Michener 2007; Ferrari and Packer 2021).

The tribe Colletini (Hymenoptera: Apidae: Colletinae; sensu Melo and Gonçalves 2005) includes approximately 540 valid species of cellophane bees (Ascher and Pickering 2021) that are divided into four genera: *Colletes* Latreille, *Hemicotelles* Toro & Cabezas, *Mourecotelles* Toro & Cabezas and *Xanthocotelles* Toro & Cabezas (Toro and Cabezas 1977, 1978; Ferrari et al. 2000; Ferrari and Packer 2021). In alternative classifications, however, only *Colletes* and *Mourecotelles* are recognized at the generic level, the latter including *Hemicotelles* and *Xanthocotelles* as subgenera (Michener 1989, 2007; Ascher and Pickering 2021). All species of Colletini are ground nesters, except *M. mixtus* Toro & Cabezas and *M. rubicola* (Benoist), which nest in dead stems (Claude-Joseph 1926 (as *C. biciliatus* Cockerell); Benoist 1942 (as *C. rubicola*)), as well as *C. rufipes* Smith and *M. triciliatus* Toro & Cabezas, which were observed nesting in trap nests (Garófalo et al. 2004; Gazola and Garófalo 2009; Dorado and Vázquez 2016). On the other hand, most species of the closest allies of the Colletini – i.e. the Euryglossini, Hylaeini, Scrapterini, and Xeromelissini (Almeida and Danforth 2009) – nest in the ground, soft wood or cavities previously excavated by other animals (see Almeida 2008 and references therein). Unlike most ground-nesting bees, females of Colletini do not possess basitibial and pygidial plates (Michener 1989), which is intriguing given that these structures are typically used in the construction of brood cells in the soil (Michener 2007). This may explain why many *Colletes* species tend to

nest in sandy soils (Batra 1980) that can be excavated with less effort and thus with less energy expenditure.

Mourecotelles currently comprises nine valid species found mostly in temperate, often xeric regions of western South America (Toro and Cabezas 1977). While the nesting biology of many *Colletes* species has already been studied and described in detail – e.g. *C. cunicularius* (Linnaeus) (Malyshev 1927), *C. michenerianus* Moure (Michener and Lange 1957), *C. ciliatoides* Stephen (Torchio 1965), *C. compactus* Cresson (Rozen and Fraveau 1968), *C. daviesanus* Smith (Scheloske 1974), *C. xerophilus* Timberlake (Batra and Schuster 1977), *C. kincaidii* Cockerell (Torchio et al. 1988) – very little is known about the other genera of Colletini (Almeida 2008).

The main goals of this paper are to describe a new species of *Mourecotelles* from southern Brazil and to document relevant aspects of its nesting biology and pollen preferences.

Methods

Study area

This study was conducted in Araucárias Municipal Natural Park (AMNP), an environmental protection area of approximately 1 km^2 located in the municipality of Guarapuava, Paraná state, Brazil (25°21'06"S, 51°28'08"W; Fig. 1A, B). The location of AMNP falls within the humid subtropical climate zone (Köppen 1900, 1918), which is characterized by warm and humid summers (mean temperature ∼25 °C) and mild winters (mean temperature ∼12 °C); precipitation is relatively evenly distributed over the year, with an annual mean of about 1900 mm (IDR-Paraná 2019).

AMNP's vegetation consists predominantly of *Araucaria* forest (43%), but also includes gallery forest (10%), grassland (7%), swamp (7%) and anthropized areas (33%). The *Araucaria* forest remnants found at AMNP, although represented mostly by *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae), are particularly diverse, comprising approximately 100 species of woody plants belonging to 73 genera in 41 families, most notably: Myrtaceae, Lauraceae, Bignoniaceae, Salicaceae, Sapindaceae, and Solanaceae (Cordeiro 2005). The grassland areas are surrounded by the *Araucaria* forest remnants and are characterized by the predominance of short species of Compositae, Cyperaceae, Leguminosae, Umbelliferae, and Verbenaceae. The swampy area is located at the lowest parts of AMNP and is primarily covered with grasses and asters (Buschini and Fajardo 2010).

Bee sampling

The studied bees were captured within AMNP through wooden trap nests between December 2001 and December 2007, following the collection methodology outlined in detail in Buschini (2006). Data from December 2001 and December 2003 were originally obtained by that author, while data from the remaining collection period
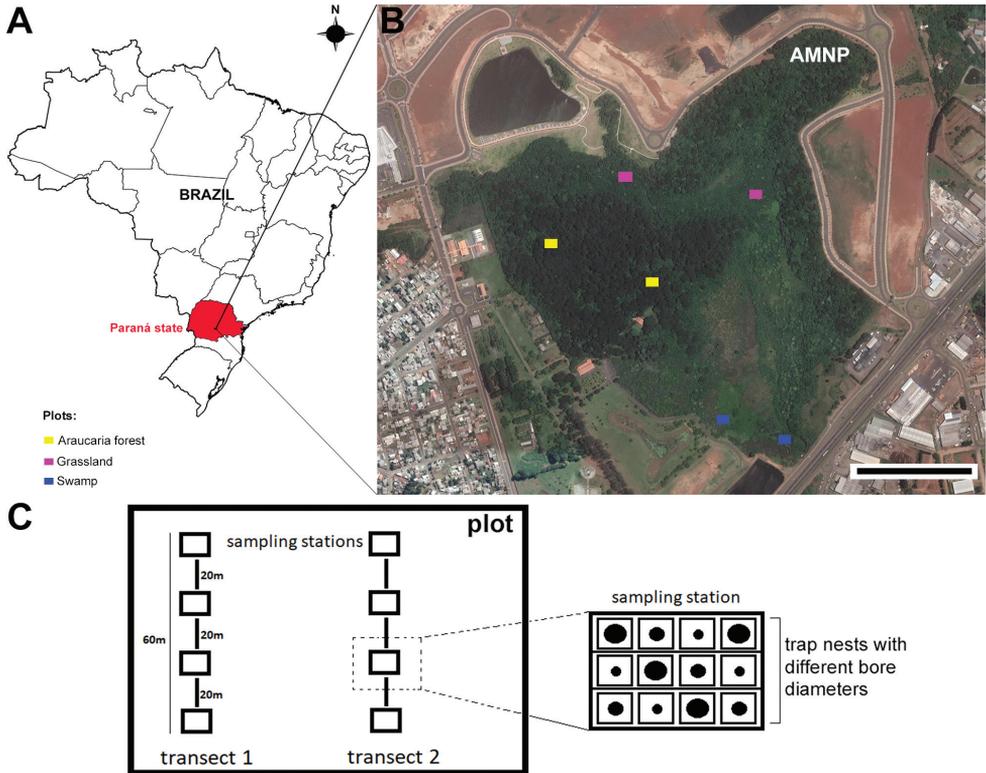
Figure 1. Study site and schematic representation of our trap-nest experiment **A** Map showing the exact location of AMNP within Paraná state, Brazil **B** Satellite image of AMNP with color-coded rectangles pointing to where the collecting plots were set up within each environment **C** Line art depicting that plots consisted of two 60-meter transects, each containing four sampling stations with 12 trap nests of different bore diameters. Scale bar: 250 m.

are new. Sampling was carried out in two plots within each of the major natural environments found at AMNP: *Araucaria* forest, grassland and swampy area. In each plot, we set eight sampling stations with 12 trap nests each along two 60-meter transects (i.e. four sampling stations per transect), totaling 96 trap nests (Fig. 1B, C). Therefore, a total of 192 trap nests were placed within each environment, 576 overall.

The trap nests were built from 120 × 25 × 20 mm (length × width × height) wooden blocks as follows. First, each block was drilled to create a one-opening cavity of 90 mm in depth. Drills of 7 mm, 10 mm and 13 mm in diameter were used to produce trap nests with different bore diameters (henceforth TN07, TN10 and TN13, respectively). Next, blocks were sawed mid-longitudinally and the resulting halves held together with adhesive tape. This allowed for ease of inspection of the interior of the trap nests both in the field and laboratory later.

The ready-to-use trap nests were placed 1.5 m above the ground mounted on natural vegetation, mostly tree branches. They were then inspected every two weeks; all colonized trap nests were removed for subsequent examination in the laboratory and replaced with new ones in order to keep the number of trap nests per sampling area constant. In the laboratory, the trap nests were re-inspected and those housing nests with immature bees were placed in rearing containers (two-liter PET bottles sealed with cotton balls) to allow for ease of capture of recently-emerged adults. The terminology used in the descriptions of nests and brood cells followed Almeida (2008).

Bee species description

All adult bees that emerged in the laboratory were killed with ethyl acetate, weighed with a precision digital scale and then pinned. Next, they were examined under a Nikon SMZ1000 stereomicroscope (maximum magnification of 112×) equipped with fluorescent light. To dissect the terminalia (i.e. genital capsule, seventh and eight metasomal sterna) of males, we first kept them inside a sealed plastic container with cotton balls soaked in water for 12 hours to relax their soft tissues. We then severed the conjunctival membrane of the metasomal apex and removed the loose terminalia from the mostly-hollow cavity with fine-tipped forceps. We subsequently cleared the terminalia of each male within separate wells of a ceramic plate containing a ~10% solution of potassium hydroxide for six hours. Using an insect pin, we separated the three structures from one another and stored them in glycerin in glass genital vials to facilitate comparative study and imaging.

We identified the bees to genus (*Mourecotelles*) using the keys of Ferrari and Packer (2021). We then attempted to identify them to species with the keys of Toro and Cabezas (1977), with special reference to the terminalia of males. Because the bees clearly did not match any of the previously known *Mourecotelles*, they are herein described as belonging to a new species. The terminology employed in this paper follows Michener (2007) for general bee morphology and Aguiar and Gibson (2010) for spatial orientation of legs. Puncture spacing is given in terms of the relative sizes of the interspaces (i) and puncture diameters (d), for example, i=2×d. Antennal flagellomeres, metasomal terga and sterna are abbreviated as F1, F2, etc., T1, T2, etc. and S1, S2, etc., respectively.

The collection data in the labels of the holotype are reproduced exactly as they are given there, as follows: data in a single label are provided between quotation marks, and the end of each line in a label is indicated by an inverted bar (\). For paratypes, data are given in the following format: country, state, municipality, collection date as dd/mm/yyyy, collector(s), number of individuals per sex [repository]. Acronyms of repositories mentioned herein are: DZUP, Coleção Entomológica Padre Jesus Santiago Moure, Universidade Federal do Paraná (Curitiba, Brazil); UNICENTRO, Universidade Estadual do Centro Oeste (Guarapuava, Brazil); PCYU, Packer Collection at York University (Toronto, Canada).

The habitus images presented in this paper were taken with a Leica DFC295 camera attached to a Leica M125 stereomicroscope. Stacking of multiple images was made using Zerene Stacker 1.4 (Zerene Systems, LLC) software. Terminalia of males were imaged with the addition of a Canon Extender EF 2× lens for a higher magnification. In all cases, we used a P-51Cam-Lift high precision Linear Actuator, which is operated by the program P51 Camlift Controller v.2.6, to take pictures from different planes of focus. First, individual pictures were imported with Adobe Lightroom v4.4 and then exported to Helicon Focus v.5.3.3, where they were stacked to produce multifocus composite images. We added scale bars and mounted the final images into plates in Adobe Photoshop CS6 v.13.0 (Adobe Inc.).

Statistical tests

Mann-Whitney nonparametric tests were performed to determine whether the bore diameters of trap nests influenced the number of brood cells constructed. The same test was used to compare whether there were statistical differences in (i) cell length, (ii) development time or (iii) body mass between males and females. The development time of bees was calculated as the time interval between the collection of trap nests from the field and the emergence of adults. Chi-square tests were performed to see whether bore diameter affected (i) the sex ratio and (ii) mortality rate. All statistical analyses were carried out in BioEstat 3.0 (Ayres et al. 2003).

Pollen analysis

The pollen samples examined for the purpose of the present study were collected from three of the nests studied by Buschini (2006). In that study, the samples were obtained from the cells containing dead immatures wherein the food provision had not yet been fully consumed. Five pollen grain slides were prepared from each nest (totaling 15 slides), following the acetolysis protocol outlined in Erdtman (1960). We also harvested pollen from known flowering plants (Cordeiro 2005) within a 500-meter radius from transects and then prepared one pollen slide for each sampled plant, following the same protocol. Next, the nest pollen provisions were identified to the lowest Linnean category possible by a palynologist (see acknowledgements) based on comparisons with pollen obtained directly from flowers under a light microscope. The Missouri Botanical Garden's online database (available at http://www.tropicos.org/) was also largely consulted. The higher-level botanical classification adopted herein follows the World Flora Online (available at http://www.worldfloraonline.org/).

To quantify the pollen from brood cell provisions, 400 grains were randomly identified per slide, totaling 1200 grains per nest. Some pollen grains were photographed with an Olympus BX 50 photomicroscope equipped with a video camera using CellSens. All pollen slides are deposited in the palynotheca of UNICENTRO.

Results

Taxonomy

Mourecotelles braziliensis Ferrari & Melo, sp. nov.
http://zoobank.org/3807CBF0-7470-4AE8-9A5A-3B3CE1F8502B
Figures 2A–D, 3A–C

Diagnosis. Both sexes of *M. braziliensis* can be readily diagnosed by having the mesosoma covered almost entirely with dark-orange pubescence (Fig. 2A–F), whereas in all other species of the genus the mesosomal pubescence is off-white to pale-yellow, which may or may not include sparse black hairs. Females of *M. braziliensis* are also unique in having the marginal zone of T6 forming a raised lip (Ferrari and Packer 2021: fig. S9E), while in females of all other *Mourecotelles* the T6 is entirely subvertical (Ferrari and Packer 2021: fig. S9E). Males of *M. braziliensis* can be further differentiated from their congeners by having the volsella with a convex digitus (Ferrari and Packer 2021: fig. S4A), whereas the digitus is always concave in the other *Mourecotelles* (Ferrari and Packer 2021: fig. S4B). *Mourecotelles braziliensis* is most similar to the sister species *M. moldenkei* Toro & Cabezas and *M. spinolae* (Crawford & Titus), but females of the former species have the gena devoid of tomentum (Fig. 2B), while the gena of females of the latter two species is covered with dense pale tomentum. Males of *M. braziliensis* have the mandible with a narrowly rounded apical tooth (Ferrari and Packer 2021: fig. S15A), thus making them very distinct from males of both *M. moldenkei* and *M. spinolae*, in which the apical tooth is broadly truncate (Ferrari and Packer 2021: fig. S15B).

Description. Female (Holotype, Fig. 2A, B, D). ***Dimensions (mm).*** Approximate body length 11.0; head width 3.9; head length 2.9; intertegular distance 3.2; forewing length 7.1.

Colouration. Black, except dark-brown on wing venation (except veins C and R of forewing black), distitarsi, tarsal claws distally; pale reddish-brown on tegula, tibial spurs, tarsal claws proximally, marginal zones of S1–S5; reddish-brown on tarsal claws distally.

Structure. Labrum with longitudinal subellipsoidal concavities. Malar area 1.4× longer than broad. Inner margins of compound eyes subparallel. F1 2.1× as long as its apical width. Facial fovea narrowly rounded below, more broadly rounded above. Dorsolateral angle of pronotum obtusely angled. Tibial spurs ciliate. Hind basitarsus 3.6× longer than broad. Marginal zone of T6 forming a raised lip.

Pubescence. Head with long, erect, off-white and black hairs intermixed; equivalent hairs longer on vertex; mandible with a fringe of long, pale-yellow setae oriented mesad; clypeus with short, suberect, pale-yellow setae concentrated below. Mesosoma with long, erect, dark-orange plumose hairs; equivalent hairs longer and paler on mesepisternum, metepisternum and lateral surface of propodeum; mesoscutum with dark-orange and black hairs intermixed anteriorly. Legs mostly with moderately long,
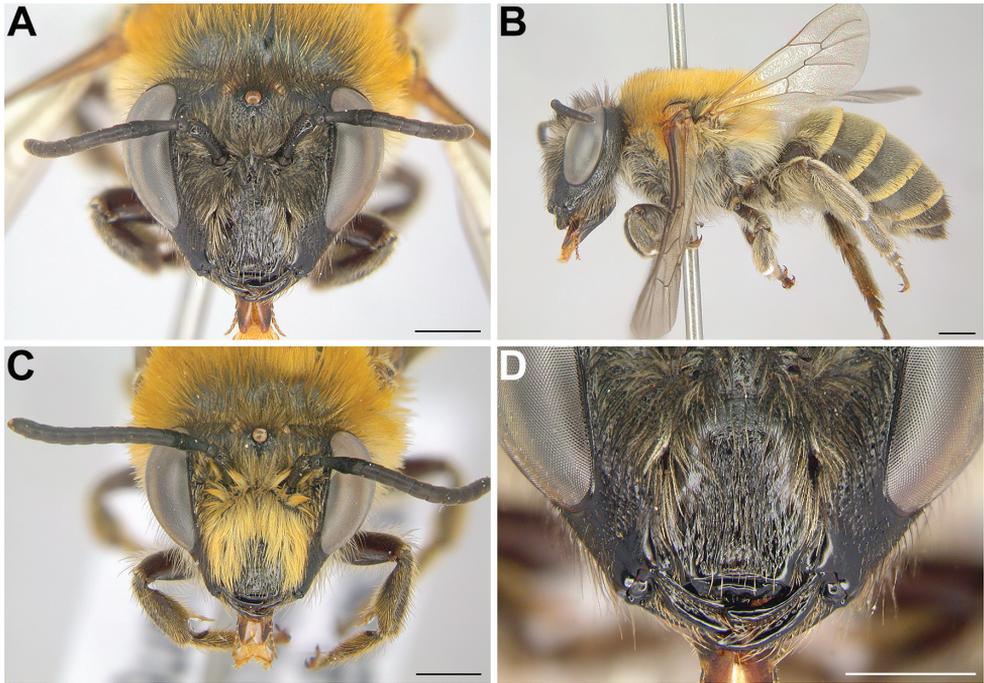
Figure 2. *Mourecotelles braziliensis* **A** head of female holotype, frontal view **B** habitus of female holotype, lateral view **C** head of male paratype, frontal view **D** lower half of head of female holotype, frontal view. Scale bars: 1 mm.

suberect, pale-yellow branched hairs; front trochanter and femur with long, erect, off-white and black hairs intermixed posteriorly; mid femur and mid and hind coxae and trochanters with very long, erect off-white hairs ventrally; mid tibia with short, erect, bright-orange thick setae forming a longitudinal line along proximal third ventrally; mid and hind tibiae and basitarsi with short, suberect, pale-yellow setae dorsally; femoral and tibial scopae with very long, pale-yellow apically-branched hairs. Metasomal terga with short, erect, pale-yellow setae on discs; T1 with very long, erect, pale-orange plumose hairs; T1–T5 apical bands with pale-orange tomentum; T6 with short, suberect, black thick setae. Metasomal sterna with minute, suberect, pale-yellow setae.

Sculpture. Clypeus with subparallel longitudinal coarse striae. Malar area unevenly punctate (i=0.5–2.0d); several punctures elongate and poorly delimited; interspaces finely imbricate. Paraocular area finely and very densely punctate (i<0.5d). Supraclypeal area largely impunctate; integument finely imbricate. Frons moderately coarsely and densely punctate (i=0.5–1.0d). Vertex finely punctate; punctures sparser (i=1.0–3.0d) medially, denser (i=0.5–1.0d) towards upper summit of eye. Mesosomal dorsum coarsely punctate; punctures sparsest (i>3d) on mesoscutum medially, densest (i=0.5–1.0d) on scutellum posteriorly, finer on metanotum; interspaces smooth, except finely imbricate on anterior third of mesoscutum. Mesepisternum coarsely and unevenly punctate, punctures densest (i=0.5–1.0d) near scrobe, sparsest (i=1.0–2.0d) towards ventral surface; interspaces finely imbricate throughout. Lateral surface of propodeum with minute
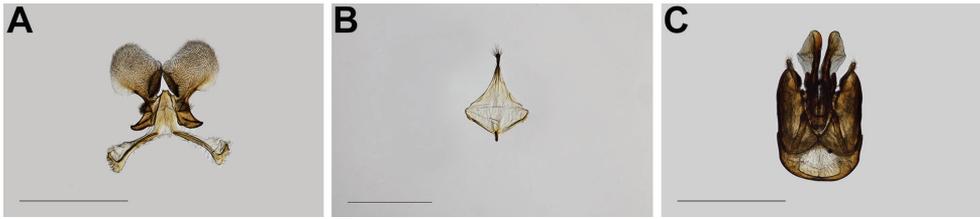
Figure 3. Terminalia of *Mourecotelles braziliensis* **A** S7, ventral view **B** S8, ventral view **C** genital capsule, dorsal view. Scale bars: 1 mm.

punctures; interspaces coarsely imbricate. Metapostnotum mostly smooth, with many short carinae along anterior margin. Metasoma minutely and sparsely (i=1.0–2.0d) punctate; interspaces corrugated on terga, finely imbricate on sterna.

Male (Fig. 2C): As in female, except for secondary sexual features and as follows. **Dimensions (mm).** Approximate body length 9.4; head width 3.6; head length 2.6; intertegular distance 3.2; forewing length 6.9.

Colouration. Tegula dark brown; metasomal sterna with black marginal zones.

Structure. Malar area 1.5× as long as basal width of mandible. F1 1.8× as long as its apical width. Hind basitarsus 3.8× longer than broad. S7, S8 and genital capsule as in Fig. 3A–C, respectively.

Pubescence. Face with mostly pale-yellow hairs, black hairs restricted to paraocular area and vertex. Supraclypeal area, gena near proboscidial fossa and lateral surface of propodeum with very long hairs. Mesoscutum with only dark-orange hairs, black hairs absent.

Sculpture. Malar area with convex interspaces. Clypeal striae somewhat finer and more irregularly oriented. Supraclypeal area more densely punctate (i=1.0–1.5d).

Type material. *Holotype* ♀: "DZUP\ 028459". "Buschini, M.L.T\ Guarapuava – PR\ Brasil – 22/11/02". "N. 283 (1)\ 22/11/02\ 30/10/03\ (0,7)". "HOLOTYPE \ *Mourecotelles\ braziliensis* Ferrari & Melo, 2021". [DZUP 028459].

Paratypes. Brazil, Paraná, Guarapuava, 24/10/2002, M.L.T. Buschini leg., 1♀ [UNICENTRO]; same data as for preceding, except 22/11/2002, 1♂ [UNICEN-TRO], 1♀ and 1♂ [DZUP 028460, 028462]; same data as for preceding, except 06/12/2002, 1♂ [PCYU]; same data as for preceding, except 19/12/2002, 1♂ [UNI-CENTRO]; same data as for preceding, except 11/10/2003, 1♀ [UNICENTRO]; same data as for preceding, except 27/10/2003, 1♀ [DZUP 028465]; same data as for preceding, except 18/12/2003, 1♀ [PCYU], 1♀ [DZUP 028461]; same data as for preceding, except 19/10/2005, 2♂ [DZUP 028463, 028464]; Brazil, Paraná, Palmas, 18/11/2009, G. Melo, K. Ramos & V. Kanamura leg., 1♀ and 1♂ [DZUP 028395, 028468]; Brazil, Rio Grande do Sul, Rio Grande, 11/2004, FURG leg., 1♀ and 1♂ [DZUP 028466, 028467].

Etymology. The only species of *Mourecotelles* currently known to occur in Brazil.

Comments. The species described herein has been referred to in several previous publications, including Buschini (2006) (as *Colletes* sp.), Diniz and Buschini (2009) (as *Rhynchocolletes* sp.), Almeida et al. (2019) (as *Mourecotelles* sp.) and Ferrari and Packer (2021) (as *Mourecotelles* sp.1).

Nesting activity

In total, females of *M. braziliensis* nested in 16 of the 576 trap nests (2.8%) placed in the field. The number of bees that emerged from each nest varied from one to ten, totaling 57 individuals (3.6 bees/nest on average).

Mourecotelles braziliensis nidified in 13 TN07 and three TN10; no nest was built in TN13. All 16 nests were founded in either the austral spring or summer, in the years of 2002 (n=10, 62.5%), 2003 (n=2, 12.5%), 2004 (n=1, 6.25%) and 2006 (n=3, 18.75%). No nest was founded in 2001, 2005 and 2007, although the year of 2001 was surveyed only in December. Of the 16 nests, 13 were founded in the sampling stations placed in the grassland area (81.25%) and three in the swampy area (18.75%).

Nest and brood cell structures

All nests founded by *M. braziliensis* are very alike in terms of general architecture. Specifically, each consisted of a series of cells arranged horizontally and separated from one another by walls built with the same cellophane-like substance used in the lining of the nest (Fig. 4A–C). Overall, the nests founded in TN10 were slightly longer (82.1 ± 0.5mm in length) than the ones founded in TN07 (81.5 ± 0.5mm in length). Regardless of the trap nest used, cells were fairly similar in shape: tubular chambers with truncate ends on both sides, except the innermost cell, the inner end of which was rounded due to the rounded end of trap-nest's bores. The mean lengths of the brood cells from which males and females emerged (irrespective of the nest's bore diameter) were 10.84 ± 0.04 mm and 10.71 ± 0.06 mm, respectively; however, the difference between them were not statistically significant (p=0.4274). After the outermost cell was constructed and provisioned, the nest entrance was plugged with a layer of the cellophane-like substance of approximately 3 mm in thickness (Fig. 4D). Larval food provisioned by *M. braziliensis* was soupy as is typical in Colletinae, although the Neopasiphaeini are known for producing semi-solid provisions (see Michener 1960). Brood cells were initially bright orange due to the color of the fresh provisions (Fig. 4A); then they gradually turned dark brown (~26 days after hatch; Fig. 4B) and subsequently black (~31 days after hatch; Fig. 4C) as the result of the accumulation of larval feces.

The number of cells constructed in each nest of *M. braziliensis* varied from two to 11, totaling 78 cells in 16 nests (4.9 cells/nest on average). Of these, 55 cells were from the 13 TN07 (4.2 cells/nest on average) and 23 from the three TN10 (7.5 cells/nest on average). This difference, however, was not statistically significant (p=0.2482). Of the 16 nests, 14 (87.5%) had at least one vestibular cell. The number of vestibular cells constructed in TN07 and TN10 was 22 and four, respectively (1.8 and 2.0 vestibular cells/nest on average, respectively).

Development time and sex/investment ratios

Overall, males (333 ± 16.4 days) developed faster than females (345 ± 16.7 days), although the difference between their mean development times was not statistically
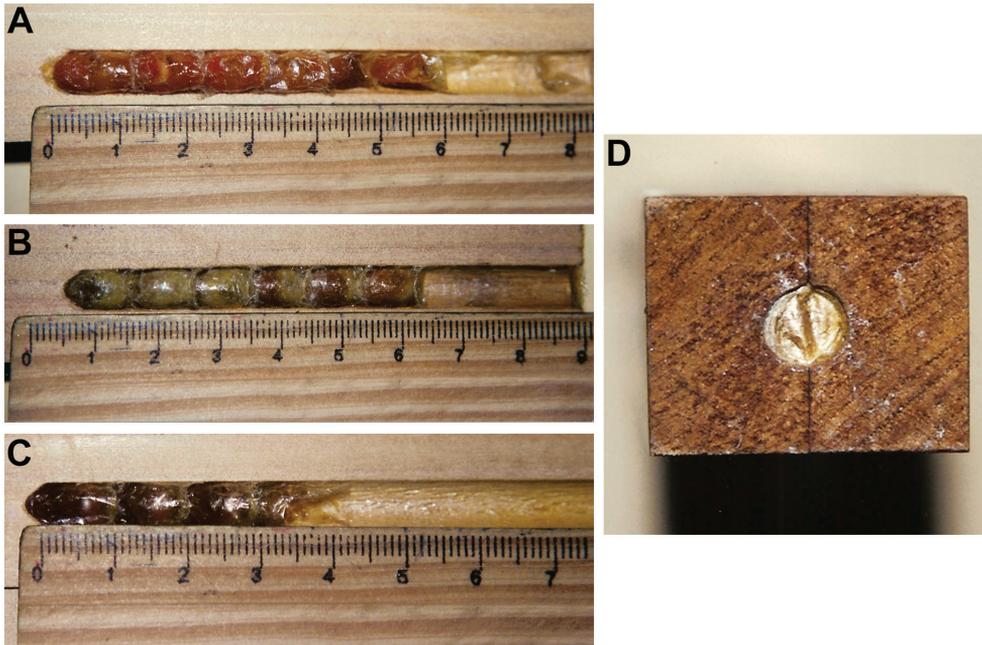
Figure 4. Nests of *Mourecotelles braziliensis* built in wooden trap nests **A** food provision looks bright orange when relatively fresh **B** brood cells become darker as larvae start to defecate (~26 days after hatch) **C** brood cells turn nearly black due to accumulation of larval feces (~31 days after hatch) **D** entrance of a TN07 plugged with cellophane-like material.

different (p=0.0341). On the other hand, females (58.9 ± 5.8 mg) were significantly heavier than males (46.5 ± 7.8 mg) (p<0.001).

Of the 57 bees of *M. braziliensis* that emerged in the laboratory, 41 were male (72%) and 16 were female (28%), resulting in a biased sex ratio towards males of 2.56:1. This is significantly different from an unbiased (i.e. 1:1) sex ratio (p<0.001). When the mean weight of individuals of each sex was accounted for, the calculated investment ratio was lower (2.02:1), albeit still statistically significant (p<0.05). The sex ratio was particularly more male biased when only TN10 was considered: 16 of the 20 bees that emerged were male (80%) and only four were female (20%), thus resulting in a sex ratio of precisely 4:1, which is also statistically different from a 1:1 sex ratio (p<0.05). The investment ratio related to only TN10 (3.15:1) was also statistically significant (p<0.05). Finally, 25 of the 37 bees that were reared from TN07 were male (67.5%) and 12 were female (31.5%), yielding sex and investment ratios of 2.08:1 and 1.64:1, respectively, both significantly different from a 1:1 ratio (p<0.05).

Mortality rate and natural enemies

No bee emerged from 20 of the 78 constructed cells, resulting in an overall mortality rate of about 26%. Of these, 13 cells contained dead larvae (65%), three were completely empty (15%), three contained dead adults (15%) and one was found with an

unidentified meloid beetle inside (5%). Although the mortality rate was, on average, higher for TN07 (17 of 55, 31%) than TN10 (three of 23, 13%), the difference between the two types of trap nests was not statistically significant (χ^2=2.715; p=0.0994).

Pollen preferences

Microscopic examination of the brood cell content obtained from the nests of *M. braziliensis* revealed 11 pollen morphotypes belonging to nine different plant families: Campanulaceae (*Lobelia* sp.), Commelinaceae (Commelinaceae sp.), Compositae (Compositae sp.1 and Compositae sp.2), Fabaceae (*Crotalaria* sp. and Fabaceae sp.), Lauraceae (*Cinnamomum* sp.), Myrtaceae (Myrtaceae sp.), Poaceae (Poaceae sp.), Polygalaceae (Polygalaceae sp.) and Styracaceae (Styracaceae sp.).

Two morphotypes, Fabaceae sp. (Fig. 5A) and Polygalaceae sp. (Fig. 5B), were largely dominant and together corresponded to roughly 98.5% of the pollen content examined (51.08% and 47.41%, respectively). However, when their relative amounts are adjusted by pollen grain size, it is revealed that the pollen mass of Polygalaceae sp. (64 µm in diameter) seemingly consumed by the larvae of *M. braziliensis* was nearly 2.5× as much as that of Fabaceae sp. (24 µm in diameter). The relative amounts of the other nine pollen morphotypes – *Crotalaria* sp. (0.55%), *Lobelia* sp. (0.25%), Myrtaceae sp. (0.16%), Styracaceae sp. (0.16%), Commelinaceae sp. (0.13%), *Cinnamomum* sp. (0.08%), Compositae sp.1 (0.08%), Compositae sp.2 (0.08%), and Poaceae sp. (0.02%) – were all negligible and most likely not actively collected by *M. braziliensis*.

Discussion

Almost all of what was previously known about the nesting biology of the Colletini was related to the widespread genus *Colletes* (*e.g.* Malyshev 1927; Stephen 1954; Michener and Lange 1957; Torchio 1965; Rozen and Fraveau 1968; Scheloske 1974; Batra and Schuster 1977; Torchio et al. 1988; Zhao et al. 2010). Thus, information on the other genera, including *Mourecotelles*, has remained comparatively much scarcer in the literature (Almeida 2008). In an early paper, females of *M. mixtus* (as *C. ciliatus*; see Toro and Cabezas 1977 for further details) were found nesting inside abandoned galleries seemingly built by xylophagous insects in dead wood in Santiago, Chile (Claude-Joseph 1926). Later, Benoist (1942) observed *M. rubicola* (as *C. rubicola*) building nests in dry twigs of brambles (Rosaceae: *Rubus*) in Quito, Ecuador. More recently, the species described herein as new was captured with wooden trap nests during two different field experiments at AMNP (Buschini 2006 [as *Colletes* sp.]; Diniz and Buschini 2009 [as *Rhynchocolletes* sp.]). In the latest publication on the nesting biology of *Mourecotelles*, Dorado and Vázquez (2016) obtained nests of *M. triciliatus* founded in trap nests near Mendoza, Argentina. There seems to be an undescribed species of *Mourecotelles* found in northern Chile that also nests in twigs (L. Packer, *pers. comm.*). Therefore, the evidence accumulated so far indicates that the *Mourecotelles* are fundamentally (if not strictly) cavity-nesting bees, although this finding may change with further investigation.
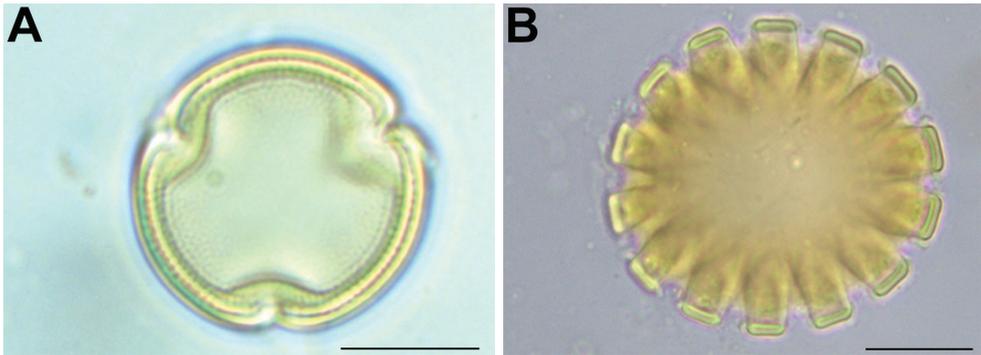
Figure 5. The two most abundant pollen types found in the food provisions of *Mourecotelles braziliensis* **A** Fabaceae sp.1 **B** Polygalaceae sp.1. Scale bars: 10 μm (**A**); 20 μm (**B**).

Assuming that the genus-level relationships recovered by Ferrari and Packer (2021) are correct, *i.e. Mourecotelles* plus *Xanthocotelles* and *Colletes* plus *Hemicotelles* as sister taxa, then the most parsimonious interpretation for the evolution of nesting behavior within Colletini is as follows. The ancestor of all Colletini might have been either a cavity- or stem-nesting species, a behavior that would have later been inherited by the ancestral *Mourecotelles* but evolved to a ground-nesting condition in the ancestral *Colletes*. This scenario, however, can only be confirmed through a rigorous ancestral trait phylogenetic reconstruction in light of additional nesting behavior data of both *Hemicotelles* and *Xanthocotelles*, which unfortunately remain unavailable. It was previously suggested that the ancestral *Colletes* may have arisen as a stem-nesting species (Almeida 2008), although this possibility was raised prior to the more recent discoveries (Dorado and Vázquez 2016).

Mourecotelles braziliensis stands out for being the sister species to the remaining species of the genus and the only one found in eastern South America (Ferrari and Packer 2021), which raises questions regarding the origin of *Mourecotelles*. Based on the available phylogenetic evidence, the ancestral *Mourecotelles* may have inhabited southern South America about 30 million years ago (Mya; see Ferrari et al. 2020), where it would have likely been relatively widespread. At that time, South America was experiencing a remarkable vegetation transformation, in which beech forests were taking over a tropical forest that had remained as the dominant biome throughout most of the Palaeocene, while drier open biomes were appearing in central portions of the continent (Iglesias et al. 2011; Meseguer et al. 2015). In the biogeographical literature, it is well established that emerging biomes may act as either corridors or dispersal barriers for some taxa (see Luebert 2021). Thus, it is possible that a dramatic change in vegetation may have resulted in a vicariant cladogenetic event that would have given rise to the lineage leading to *M. braziliensis* plus the ancestor of the remaining extant species. We argue that shedding further light on the phylogeny of *Mourecotelles* would be crucial for a better understanding of the evolution and historical biogeography of the Colletini as a whole.

Over the six-year experiment conducted by us, *M. braziliensis* nidified in trap nests that were placed only in the open environments (grassland and swampy areas), while

no nest was founded within the *Araucaria* forest remnants. Given that the forested area covers 43% of AMNP, it seems obvious that *M. braziliensis* prefers nesting in sun-exposed environments. This actually corresponds to a nesting behavior that has long known to be predominant among solitary bees (*e.g.* Sakagami and Hayashida 1960; Potts and Willmer 1997). Some bees nonetheless have a clear preference for nesting in rather shaded situations, such as *Bicolletes iheringi* (Schrottky) and *Neocorynura laevistriata* Gonçalves (Michener et al. 1958; as *N. polybioides* (Ducke)), as well as various species of *Centris* Fabricius (Frankie et al. 1988). It has been demonstrated that the development rate of immature bees is positively correlated with temperature, provided an upper limit is not exceeded (Bosch and Kemp 2000), above which high temperatures typically decrease development rate (Frankie et al. 1988) or may even be lethal to immatures (Undurraga and Stephen 1980).

The average number of cells constructed by *M. braziliensis* in our experiment (4.9 cells/nest) is very close to that reported for the Argentina-endemic *M. triciliatus* (5.7 cells/nest on average; see Dorado and Vázquez 2016). This information is relevant, among other reasons, from a conservation standpoint (see Paini and Roberts 2005; Huang et al. 2021). Note that the available report on *M. triciliatus* was based on merely three nests containing only 17 cells in total, therefore this comparison needs to be interpreted with caution. Even though the nests founded in TN07 contained less brood cells on average (4.9 cells/nest) than those founded in TN10 (7.5 cells/nest), the difference was not statistically significant. A preference for narrower cavities is further supported by the fact that *M. braziliensis* built no nest in TN13 over the experiment conducted by us. This is actually not surprising given that the head width (3.8–4.0 mm) of females, which in turn is a good proxy for body size, is more compatible with the bore diameter TN07 (i.e. 7 mm). Previous studies on the nesting biology of other bee and wasp species showed that body size is probably the most important factor influencing the selection of bore diameters of potential nesting cavities by females (Fricke 1991; Pereira et al. 1999; Aguiar and Garófalo 2004; Buschini and Farjardo 2010). It is not possible to conclude, however, that 7 mm is in fact the optimum cavity diameter for *M. braziliensis* because our experiments did not include trap nests with narrower bores.

Even though we have identified 11 different pollen morphotypes in brood cells of *M. braziliensis*, the nine least abundant of them constituted merely 1.57% of the total examined. These likely were involuntarily accumulated by females while drinking nectar or picked up as pollen secondarily deposited by other flower visitors, rather than being actively collected to nourish the larvae (see Diniz et al. 2021). Since roughly 98.5% of the observed grains belong to only two pollen morphotypes (Polygalaceae sp. and Fabaceae sp.), it appears that *M. braziliensis* may be a specialist in the order Fabales (Bello et al. 2012). However, it has been shown that even slight disparities in size among different pollen types can lead to critical differences in their respective volumes in samples (Buchmann and O'Rourke 1991). Thus, when pollen size is taken into consideration it is possible to conclude that pollen of Polygalaceae sp. corresponded to over two thirds (71%) of the total pollen mass that larvae of *M. braziliensis* apparently

consumed, which suggests a potential oligolecy. To our knowledge, no datum on floral hosts of *Mourecotelles* is currently available in the literature, although the primary author (RRF, *pers. obs.*) has observed several species of the genus visiting *Adesmia* (Fabaceae) in central Chile. It is also worth mentioning that the female and male paratypes from Palmas (Paraná state) were collected while visiting flowers of an unidentified species of *Monnina* (Polygalaceae) growing in a swampy area situated in a region of native grasslands. The female had its scopae loaded with pollen and presumably was not harvesting only nectar (GARM, *pers. obs.*).

An overall, male-biased investment ratio of roughly 2:1 observed for *M. braziliensis* in our study is intriguing and its causes are unclear. Investment ratio is an important concept in behavioral ecology, which can be defined as the relative amount of energy allocated in the production of males *vs.* females (Danforth 1990), typically calculated from the weight ratio of the emerging offspring (Paini and Bailey 2002). It has been reasoned that an investment ratio of 2:1 in favor of males would imply that the production of a female would demand twice the effort expended for the production of a male (Fisher 1930; see also Torchio and Tepedino 1980). If Fisher's rational is correct, then the investment ratio of species whose females are heavier than males (as in most hymenopterans) should always be biased towards the latter–in fact, it is well known that the amount of food provisioned for female larvae is higher than for male larvae across Hymenoptera (e.g. Frohlich and Tepedino 1986; Johnson 1990; Arvidson et al. 2018; Farder-Gomes et al. 2018; Fawcett et al. 2019). As resources become scarcer, solitary bees tend to produce even more males because decrease in body size in female offspring has a proportionally higher negative impact on maternal fitness (Peterson and Roitberg 2006). Possibly, resource availability was relatively low either during the time period when this study was conducted, or, in AMNP in comparison with surrounding areas, which may have affected the sex ratio of *M. braziliensis*. This possibility is based on the well-established understanding that resource availability plays a major role in the determination of investment ratios in solitary bees (see Kim 1999). Testing this conjecture, however, was beyond the scope of our study.

Conclusions

The remarkable new species of *Mourecotelles* described in this paper represents not only the first record of the genus in Brazil but also the first outside western South America (Chile, Argentina, Bolivia, and Ecuador). *Mourecotelles braziliensis* is morphologically very distinct from its congeners and can be easily recognized, among other features, due to its unique dark-orange mesosomal pubescence. We showed that trap-nesting is a useful method for studying relevant aspects about the nesting biology of *Mourecotelles* as the genus seems to comprise fundamentally cavity-nesting bees. Although we have found 11 different pollen morphotypes in brood cells of *M. braziliensis*, the species seems to be a specialist in the family Polygalaceae.

Acknowledgements

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