

Brood development and nest parasitism of *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier (Hymenoptera: Apidae), a promising crop pollinator in Argentina

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Short title: Brood development and parasitism in *Xylocopa*

ACKNOWLEDGMENTS

We thank Amy Comfort and two anonymous reviewers for their insightful comments that improved the manuscript, and Daniel Aquino and Jens-Hermann Stuke for their help identifying the parasitic wasp and conopid fly. The Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET) provided financial support for this work. V.H. Gonzalez's participation was supported through The National Science Foundation's REU program (DBI 1560389).

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/JEN.12773](https://doi.org/10.1111/JEN.12773)

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Article type : Original Contribution

Abstract – Pollination of passion fruit and other crops by species of carpenter bees of the genus *Xylocopa* Latreille significantly increases both the quality and quantity of fruits. To enhance pollination services, bee nests are either introduced into the crop area or females are encouraged to nest using trap-nests. Thus, knowledge of trap-nest preference, brood development, and nest parasitism is essential for effective and sustainable nest management practices. *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier is a promising pollinator in some areas of Argentina because of its high abundance, ability to buzz pollinate, and easy acceptance of trap-nests. However, limited information is available on the biology of this pollinator. Herein we provide detailed information on its nesting cycle, brood development, and parasitism from trap-nests at an urban area in Buenos Aires province, Argentina. Our study indicates that *X. augusti* is a solitary and likely univoltine species that shares some nesting and developmental features with other species in the subgenus *Neoxylocopa*, which might facilitate the adoption of existing management techniques developed for other species and regions. Biological information on *Physocephala wulpi* Camras (Diptera: Conopidae), a parasitoid of *X. augusti*, is also given for the first time.

Pollination services / Physocephala / Parasitoid / Hyperparasitoid / Xylocopinae

1. INTRODUCTION

The economic value of animal pollination in agriculture is undeniable, as about 75% of the leading global food crops depend partially or fully on pollinators (Klein et al., 2007). For

31 example, while bees are essential for the pollination of some crops (e.g., almonds: Traynor,
32 2017), for others less pollinator-dependent crops, bees might help to increase up to 50% the
33 quality and quantity of their seeds and fruits (e.g., coffee: Roubik, 2002). Pollinators are also
34 responsible for 40% of the world's supply of nutrients (Eilers, Kremen, Greenleaf, Garber, &
35 Klein, 2011), which means that pollinator loss could significantly affect global health due to
36 the increase of malnutrition-related diseases (e.g., Chaplin-Kramer et al., 2014; Ellis, Myers,
37 & Ricketts, 2015; Smith, Singh, Mozaffarian, & Myers, 2015).

38 The management of pollinators is a sustainable alternative to the maintenance of
39 agricultural production, particularly in view of recent declines of native pollinators
40 worldwide and honey bee colony losses (e.g., Bommarco, Kleijn, & Potts, 2013; Junqueira &
41 Augusto, 2017). Carpenter bees of the genus *Xylocopa* Latreille (Apidae: Xylocopini) are
42 among the most promising crop pollinators due to their cosmopolitan distribution, long
43 activity season, polylectic habits, easy acceptance of artificial substrates for nesting, their
44 ability to buzz-pollinate, and being active at higher temperatures than honey bees
45 (Buchmann, 2004; Hogendoorn, 2004; Keasar, 2010).

46 *Xylocopa* includes more than 470 species in 31 subgenera worldwide, most of them
47 occurring in tropical and subtropical areas. The majority of species nest in solid wood, such
48 as tree branches and trunks, but some nest in hollow plant stalks and soft wood, or even in the
49 ground. Species are often robust and range from solitary to semisocial or primitively eusocial,
50 where the oldest female (mother or sister) feeds both young females and males via
51 trophallaxis (Michener, 2007; Lucia, Gonzalez, & Abrahamovich, 2015). A few species of
52 *Xylocopa* have already been used as efficient crop pollinators of passion fruit (*Passiflora*
53 *ssp.*), sunflowers (*Helianthus annuus* L.), squash (*Cucurbita* *ssp.*), tomato (*Solanum*
54 *lycopersicum* L.), and eggplant (*Solanum melongena* L.) (Gerling, Velthuis, & Hefetz, 1989;
55 Sihag, 1993a; Mardan, 1995; Hogendoorn, Steen, & Schwarz, 2000; Aguiar-Menezes,
56 Menezes, Cassino, & Soares, 2002; Sadeh, Shmida, & Keasar, 2007). Pollination by
57 carpenter bees increases the quantity and quality of the crop, as in the case of passion fruits
58 that result in bigger and sweeter fruits when pollinated by these bees (e.g., Junqueira &
59 Augusto, 2017).

60 To increase pollination services, natural carpenter bee nests are introduced into the
61 crop area or females are encouraged to nest using trap-nests, a method that consists of using
62 bamboo canes or wooden blocks with tunnels of varying diameter to promote their nesting
63 (e.g., Krombein, 1967; Freitas & Oliveira-Filho, 2003). Thus, knowledge on the natural

64 history as well as trap-nest preference, brood development, and nest parasitism is essential for
65 effective and sustainable nest management practices.

66 *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier is a widely distributed species in southern
67 South America, occurring in Bolivia, Uruguay, Paraguay, Argentina, and southern Brazil
68 (Moure, 2012; Lucia, Alvarez, & Abrahamovich, 2014a). In Argentina, it is more widespread
69 in the east than in the west, occurring from Buenos Aires to Formosa province (Lucia,
70 Alvarez, & Abrahamovich, 2014a). This species is a promising crop pollinator in Argentina
71 because of its high abundance, docile behavior, ability to buzz-pollinate, and easy acceptance
72 of trap-nests. In addition, the species uses a wide range of plant species to obtain pollen and
73 nectar, including several crops of economic importance, such as *H. annuus*, *S. melongena*,
74 *Medicago sativa* L. (Lucerne), *Phaseolus vulgaris* L. (beans), and *Cucurbita moschata*
75 *Duchesne ex Poir.* (Tesón, Dagoberto, Lizarralde, & Loíacono, 1976; Tellería, 1999, Alvarez,
76 Lucia, Ramello, Del Pino, & Abrahamovich, 2014; Lucia, Tellería, Ramello, &
77 Abrahamovich, 2017).

78 Despite the potential of *X. augusti* as a crop pollinator, limited information is
79 available on its biology to facilitate the development of effective and sustainable management
80 practices. Thus, the aim of this work is to provide detailed information on its nesting cycle,
81 brood development, and parasitism from trap-nests established at an urban area in Buenos
82 Aires province, Argentina.

83

84 2. MATERIAL AND METHODS

85 2.1. Study area and trap-nests

86 We conducted observations at the Unidad de Vivero Forestal of the Universidad
87 Nacional de La Plata (34°54'39"S, 57°55'37"W, 18 m.a.s.l.) and Vivero Forestal, Facultad de
88 Ciencias Naturales y Museo, an urban area of the city of La Plata located in the northeastern
89 Buenos Aires province, Argentina. This area, about 4 ha in extension, possess a variety of
90 cultivated trees accompanied by weeds and adventitious species. The climate of the region is
91 humid temperate, with the cold season extending from May to October, and the mean
92 monthly temperature not reaching 18 °C. There is no a dry season and the average annual
93 precipitation ranges from 800 to 1000 mm. Lucia et al. (2017) provide detailed information
94 on the foraging behavior as well as local floral resources of *X. augusti* at the study area.

95 Each September, between 2015 and 2018, we deployed about 1500 trap-nests at the
96 study site, each consisting of an internode of wild cane (*Arundo donax* L., Poaceae) closed at
97 one end by the node and open at the other. We cut and collected canes from plants growing

98 along roads and streams near the study area, and chose those with a best fit for *X. augusti* as
99 approximated by its body size. Due to availability in the field, we obtained a variable number
100 of internodes of different lengths and diameters. Thus, the canes we used as trap-nests ranged
101 from 13.6 to 27.0 cm in length and from 12.50 to 17.00 mm in inner diameter. We placed
102 trap-nests horizontally, in groups of 15–25, inside hollow bricks and empty plastic containers
103 on top of open shelves, located between 1 and 2 m above ground. Each group of trap-nests
104 contained a variable, random number of trap-nests of different dimensions (Figure 1). We
105 recorded the presence or absence of bees daily by inspecting the entrance of each trap-nest.
106 During these inspections, we also noted their guarding behavior at random times through the
107 day, as well as sometimes early in the night. Bees occupied trap-nests as early as 5 to 10 days
108 after we deployed them in the field. Because we intended to maintain a permanent population
109 of *X. augusti* at the study area for other studies, we replaced the trap-nests we used for this
110 study and left unoccupied nests for the following nesting season.

111 To observe brood development, we opened completed nests lengthwise and secured
112 both halves with tape. We reopened nests daily until adult emergence and kept them in the
113 laboratory at room conditions (18–25 °C). We recorded the following nest data: number,
114 width, and length of brood cells, cell partition thickness (center and periphery), inner
115 diameter of nest entrance, and internode length. We took measurements of internal nest
116 features using a caliber. We estimated sex ratio as the number of emerged females over the
117 number of emerged males. To determine the average wet mass of larval provisions, we
118 carefully removed them from the brood cells, weighed them with an electronic scale, and
119 return them to the same cell. We documented details of the nest architecture, emergence
120 phenology, and sex ratio from 52 trap-nests. We took brood cell dimensions from 79 trap-
121 nests and used a Panasonic® FZ18 digital camera (Panasonic, Japan) to record external nest
122 features.

123 We obtained adults of *Physocephala* (Diptera: Conopidae) from larvae developing
124 inside the metasoma of dead female bees that we found inside the nest or near the nest
125 entrance. We placed the metasoma of each bee individually in acrylic containers and kept
126 them in the laboratory until emergence of adult conopid flies. We euthanized conopids 24 h
127 after emergence, sexed, labeled, and numbered them with the corresponding bee specimen.
128 We estimated bee body size by measuring the minimum intertegular distance (Cane, 1987)
129 with an ocular micrometer on a Nikon SMZ745 stereomicroscope. To estimate relative age of
130 carpenter bees that were parasitized by conopid flies, we record the degree of wing wear in a
131 scale of one to five, with one being unworn and five heavily worn, as in Camillo and

132 Garófalo (1989). Associated organisms were identified using taxonomic keys and by
133 comparison with specimens deposited in the División Entomología of Museo de La Plata,
134 Argentina (MLP), as in the case of Coleoptera (i.e., Pinto & Bologna, 1999), or by specialists
135 (Hymenoptera and Diptera). Voucher specimens of bees and associated organisms are in
136 MLP.

137

138 2.2. Data analysis

139 We used an independence test (Chi-square analysis) to evaluate the relationship
140 between the sex of the emerged bees and the position of the brood cell inside the nest from
141 which they emerged. For comparison purposes, we used data of bees that emerged from the
142 first four cells because nests ranged from one to six in their number of cells. We estimated
143 total mortality of immature stages as the sum of the number of cells attacked by either
144 parasites or pathogens, plus the number of cells containing dead larvae or pupae of unknown
145 cause. To test for association between bee body size and sex of the emerged conopid fly, we
146 used a Goodness of fit test. In addition, we tested for differences in the body size between
147 sexes of the conopid flies using a non-parametric Wilcoxon test. We tested assumptions of
148 each statistical test and conducted analyses with R statistical software, version 3.3.1 (R Core
149 Team 2016).

150

151 3. RESULTS

152 3.1. Nest cycle and brood development

153 Overwinter females started to build their nests in early October and began to close the
154 first cells in mid-October. Adult emerge occurred from early/mid-December throughout mid-
155 January but it peaked in late December. All nests consisted of a single adult, which defended
156 the nest by blocking the nest entrance with either the dorsum of the metasoma or with the
157 head. Blocking the nest with the head occurred throughout the day, primarily in response to
158 other female of *X. agusti* trying to enter the nest. Bees blocked their nest entrance with their
159 metasoma at night (Figure 1b), sometimes expelling a light brown or yellowish fluid from
160 their anal opening, when we disturbed the nests.

161 Bees used trap-nests of all diameters. However, about half (58.4%) of trap-nest used
162 by bees had diameters ranging between 14 and 16 mm. Each nest contained a linear series of
163 one to six ($\bar{x} = 3.0 \pm 1.35$, $n = 52$ trap-nests) barrel-shaped brood cells. Brood cells ranged
164 from 15.35 to 24.23 mm ($\bar{x} = 19.27 \pm 1.49$, $n = 178$ cells) in length and their position inside
165 the nest did not have an effect on its length (Table 1, Figure S1 in supporting information).

166 Cell partitions are smooth and concave on their outer surface, rough and flat on their inner
 167 surface. Cell partitions were thicker at the periphery ($\bar{x} = 5.14 \pm 0.90$ mm, 3.14–7.5, $n = 113$)
 168 than at the center ($\bar{x} = 1.65 \pm 0.33$ mm, 0.9–2.4, $n = 102$).

169 The egg-to-adult development time ranged from 44 to 66 days and was similar
 170 between sexes ($W = 25$, $p = 0.11$, $n = 18$). However, the larva-pupa period was shorter in
 171 males than in females (Table 2). The larvae feeding period lasted 8–18 days in females ($\bar{x} =$
 172 15.4 ± 3 , $n = 19$) and 12 to 15 days in males ($\bar{x} = 12.8 \pm 1.6$, $n = 5$) after hatching. In females,
 173 pupae pigmentation began between 10 to 18 days after pupation ($\bar{x} = 14.8 \pm 2.3$, $n = 23$).
 174 Mass provision varied from 1.12 to 2.00 gr among cells ($\bar{x} = 1.57 \pm 0.29$, $n = 16$).

175

176 3.2. Sex ratio

177 The overall sex ratio was female-biased ($3\text{♀}:1\text{♂}$) based on the 52 nests we kept in the
 178 laboratory (83 ♀, 31 ♂), which was significantly different from a theoretical sex ratio 1:1 ($\chi^2 =$
 179 24.85 , $df = 1$, $p < 0.001$). Both sexes of bees emerged from cells at different positions
 180 inside the nest. However, females were more likely to emerge from the two innermost cells (1
 181 and 2) than males (Cell 1: $\chi^2 = 32.4$, $df = 1$, $p < 0.0001$, $n = 38\text{♀}$, 2♂ ; Cell 2: $\chi^2 = 5.76$, $df = 1$,
 182 $p < 0.05$, $n = 24\text{♀}$, 10♂). In contrast, the outermost cells were not sex-biased (Cell 3: $\chi^2 =$
 183 0.18 , $df = 1$, $p = 0.67$, $n = 10\text{♀}$, 12♂ ; Cell 4: $\chi^2 = 2.57$, $df = 1$, $p = 0.11$, $n = 10\text{♀}$, 4♂) (Table
 184 3, Figure 2, Figure S2 supporting information).

185

186 3.3. Brood mortality

187 Overall brood mortality was approximately 30%. Mortality was highest in the larval
 188 stage (25.5%, 41 out of 161 brood cells of 52 trap-nests) likely due to viruses, fungi, and
 189 unknown causes. The beetle *Cissites maculata* (Swederus) (Coleoptera: Meloidae) (Figure 3)
 190 and the sapid wasp (Hymenoptera: Sapiidae, likely *Huarpea*) were the only brood parasites
 191 we observed. The former species parasitized six cells of four nests while the latter two cells
 192 of two nests.

193

194 3.4. *Xylocopa* adult mortality

195 The conopid fly *Physochepala wulpi* Camras (Diptera: Conopidae) significantly
 196 attacked young bees ($\chi^2 = 29.45$; $df = 4$, $p < 0.0001$), as inferred by the bees' wing condition
 197 (1–3 stage of wing wear according Camillo and Garófalo (1989). Bee relative age and
 198 number of parasitized females was inversely correlated ($cor = -0.77$). The emergence period

199 of the conopid fly varied depending on the season in which we collected them. Larvae
200 collected between late-spring (mid-November) and mid-summer (January) showed a
201 significantly shorter developing period (σ : 29–43 days, \bar{x} = 35.38, median = 32, n = 19; ρ :
202 29–46 days, \bar{x} = 34.07, median = 32, n = 15) than those collected during the late summer
203 (mid-March) and beginning of autumn (early April) (σ : 186–296 days, \bar{x} = 235.6, median =
204 237, n = 17; ρ : 191–280 days, \bar{x} = 235.6, median = 235, n = 17). The sex of the conopid fly
205 was independent of the size of the host bee (OR = 2.12; IC = 0.31, 14.38; Wald statistic, p =
206 0.44; n = 41).

207

208 3.5 Conopid adult mortality

209 The eulophid wasp *Pediobius williamsoni* (Girault) (Hymenoptera: Eulophidae)
210 parasitized 11 pupae of *Physocephala wulpi*. The development of *P. williamsoni* in the
211 laboratory lasted from 25 to 79 days (\bar{x} = 46.62 \pm 19.1, n = 8). However, in two cases, it
212 showed a longer development period (130 and 218 days) (Table S1 supporting information).

213

214 4. DISCUSSION

215 Cavity-nester bees, such as carpenter bees, are suitable pollinators for sustainable
216 pollination in agroecosystems because they accept trap-nests, which then can be moved in
217 order to increase pollination services. A few types of trap-nests have been employed to
218 promote nesting of carpenter bees, namely bamboo canes, wood trunks, and wooden boards
219 inside of Langstroth honey bee hives (Camillo & Garofalo, 1982; Freitas & Oliveira-Filho,
220 2003; Pinilla-Gallego & Nates-Parra, 2015). However, as used in this work, canes appear to
221 be preferable because they are affordable and easy to deploy and replace, as well as readily
222 accepted by bees (Chaves-Alves, Junqueira, Rabelo, Oliveira, & Augusto, 2011). Despite the
223 importance of carpenter bees for agriculture, their diversity, and worldwide distribution, only
224 a few species are currently managed. This is a perhaps a reflection of our limited knowledge
225 of many aspects of their biology. Although basic information on the nesting biology and nest
226 architecture of carpenter bees is available for several species worldwide (e.g., Anzenberg,
227 1977; Gerling, Hurd Jr., & Hefetz, 1983; Sihag, 1993a, 1993b; Vicidomini, 1996; Raju &
228 Rao, 2006; Hongjamrassilp & Warrit, 2014; Ali, Shebl, Alqarni, Owayss, & Ansari, 2016),
229 most studies are restricted to a few common species and from particular regions. For
230 example, most information in South America is from Brazil (e.g., Sakagami & Laroca, 1971;
231 Camillo & Garofalo, 1982; Camillo, Garófalo, & Muccillo, 1986; Viana, Kleinert, & Silva,

232 2002; Oliveira-Filho & Freitas, 2003; Bernardino & Gaglianone, 2008; Marchi & Melo,
233 2010; Pereira & Garófalo, 2010), with few studies available from other regions (e.g.,
234 Gonzalez, Gonzalez, & Cuellar, 2009; Lucia et al., 2014a, 2015, 2017). Such a limitation
235 impedes the effective development and implementation of sustainable practices for a wide
236 range of species. For example, efforts to establish carpenter bees in bamboo canes in
237 Colombia have had little to no success due to the incipient knowledge of the bees' biology
238 (Pinilla-Gallego and Nates-Parra 2015). Thus, the biological data present herein for *X.*
239 *augusti* will add to this dearth of knowledge.

240 In general, our findings on the life cycle and nest structure are similar to those
241 recorded by Pereira and Garofalo (2010), Marchi and Melo (2010), and Camillo and Garofalo
242 (1982) for other species of the subgenus *Neoxylocopa* Michener. The number of cells per nest
243 in *X. augusti* and the egg-to-adult developing time are within the ranges of those reported for
244 *X. frontalis* (Olivier) (1–6 cells; 53 days) and *X. grisescens* Lapeletier (2–5 cells; 55 days)
245 (Pereira & Garofalo 2010). The female-biased sex ratio we observed in *X. augusti* has also
246 been documented in *X. frontalis* (Marchi & Melo, 2010) and *X. suspecta* Moure & Camargo
247 (Camilo et al., 1986). Similarly, the emergence of females from the innermost cells and males
248 from the outermost cells in *X. augusti*, follows the pattern observed in other carpenter bees
249 (e.g., Sakagami & Laroca, 1971; Pereira & Garofalo, 2010), as well as other solitary species
250 (e.g., Jesus & Garófalo, 2000; Pitts-Singer & Cane, 2011). Lastly, the dimensions of the
251 brood cells and partitions documented from trap-nests are similar to those reported in natural
252 nests for *Xylocopa augusti* (Lucia et al., 2014a). Thus, the features of the nesting biology of
253 *X. augusti* studied here seem highly conserved among the species of the subgenus
254 *Neoxylocopa*.

255 Several pathogens and natural enemies are associated with carpenter bees during their
256 life cycle, including brood parasites, parasitoids, virus, and entomopathogenic fungus (Hurd
257 Jr., 1978; Lucia, Aquino, Hansson, & Abrahamovich, 2010, 2014b, 2015; Avalos-Hernández,
258 Lucia, Álvarez, & Abrahamovich, 2011; Stuke, Lucia, & Abrahamovich, 2011; Reynaldi,
259 Lucia, & Garcia, 2015; Lucia, 2016). However, infestation by fungus is among the most
260 important causes of immature mortality (Gerling et al., 1989; Pereira & Garofalo, 2010). The
261 entomopathogenic fungus *Ascosphaera apis* (Maasen ex Claussen) L.S. Olive and Spiltoir
262 (Onygenales: Ascosphaeraceae) causes chalkbrood disease and was recently reported for *X.*
263 *augusti* in Argentina (Reynaldi et al., 2015).

264 The beetle *Cissites maculata* (Coleoptera: Meloideae) and sapygid wasps
265 (Hymenoptera: Sapydidae) have also been reported as brood parasites in other species of

266 carpenter bees. For example, the sapygid wasp *Huarpea fallax* (Gerstaecker) was recorded
267 from *X. (Schonherria) splendidula* Lepeletier, *X. (Neoxylocopa) augusti*, and *X.*
268 (*Neoxylocopa*) *atamisquensis* (Friese, 1923; Hurd Jr. & Moure, 1961) while *H. wagneriella*
269 (duBuy) was recorded from nests of *Xylocopa (Nanoxylocopa) ciliata* Burmeister (Lucia,
270 2016).

271 Conopid flies are solitary and internal parasites of insects, usually in aculeate wasps,
272 and especially in bees (Camras & Hurd, 1957). Species of the genus *Physocephala* Schiner
273 have been recorded from several genera of neotropical bees, including carpenter bees. These
274 flies attack adult female bees at flowers while they are foraging for nectar or pollen (Lucia et
275 al., 2010; Stuke et al., 2011), a behavior we also observed in two occasions at our study site.
276 However, little is known about the biology of these parasites. To date, most biological studies
277 on conopid flies are from temperate areas and relating to the effect on species of bumble bees
278 (e.g., Schmid-Hempel, Müller, Schmid-Hempel, & Shykoff, 1990; Schmid-Hempel &
279 Schmid-Hempel, 1988). The interval between the collection date of the dead host bee and the
280 emergence of the conopid fly in *Xylocopa augusti* varied according to the season, from 29–46
281 days if collected in the late-spring and mid-summer to 186–296 days if collected in the late
282 summer and beginning of the autumn. This is likely related to changes in the temperature as
283 the winter season approaches, as it has been observed by Santos et al. (2008) in other species
284 of *Physocephala* attacking the solitary bee *Centris (Heterocentris) analis* (Fabricius).

285 Enhancing pollination services by using high numbers of trap-nests of *Xylocopa*
286 *augusti* might increase the incidence of chalkbrood disease, conopids, and other parasites.
287 Chalkbrood is commonly associated with larvae of both solitary and social species and could
288 be devastating for the production of managed bees (Pitts-Singer & Cane, 2011). Likewise,
289 conopid parasitism is common (30–70%) in natural populations of bumble bees in Europe
290 and Canada, sometimes affecting colony size by increasing worker mortality and altering
291 their foraging behavior (Schmid-Hempel et al., 1990; Otterstatter, Whidden, & Owen, 2002).
292 Thus, further studies should address the impact of these diseases and parasites on managed
293 populations of *X. augusti*. The eulophid wasp *Pediobius williansoni*, a parasite of the conopid
294 fly *Physocephala wulpi* (Lucia et al., 2010), might also prove useful in the biological control
295 of this species, if this ever becomes a problem. In summary, our study indicates that *X.*
296 *augusti* is a solitary and likely univoltine species with some nesting and developmental
297 features similar to other species in the subgenus *Neoxylocopa*. Knowing that the nesting
298 biology of these species is conserved could be useful from a practical point of view, as it
299 might facilitate the development and implementation of similar management techniques

300 across multiple species and regions. Future studies on *X. augusti* should focus on
301 productivity aspects, such as the effect of trap-nest dimensions on both rates of occupancy
302 and the number of brood cells. Although we did not set up our experiment to assess
303 occupancy rate, this value ranged from about 5 to 30 % each year and bees tended to occupy
304 trap-nests with diameters between 14 and 16 mm. However, resource availability at the study
305 site might have influenced the rate of nest establishment each season, and trap-nest
306 preference might have resulted from having an abundance of those diameters. Trap-nest
307 diameters that are closer to the bee's body size are expected to have greater number of brood
308 cells when compared to those that are much wider than the bee, as the latter requires an
309 additional energetic cost to build and provisione. Similarly, longer trap-nests might allow
310 females to build more brood cells than short trap-nests. However, at least for some species of
311 cavity-nesters, tunnel diameter appears not to have a significant effect on brood productivity
312 (Vitale, Gonzalez, & Vázquez, 2017). Likewise, at least in natural nests of some carpenter
313 bees, the number of brood cells is independent of the tunnel length (Lucia et al. 2015). These
314 observations suggest that for some bee species, and at least under natural conditions, tunnel
315 dimensions might not be the main reproductive constrain. Doubtless, these aspects ought to
316 be investigate for *X. augusti*.

317

318 **Conflict of interest Statement**

319 The authors declare that they have no potential conflict of interest in relation to the study in
320 this paper.

321 **Authors Contribution**

322 ML and PJR conceived this research and designed experiment; VHG participated in the
323 interpretation of the data. ML and VHG wrote the manuscript and participated in the
324 revisions of it. All authors read and approved the final manuscript.

325

326 **Data Availability Statement**

327 Raw data supporting the findings of this study (e. g. nest cycle and brood development, sex
328 ration and adult mortality) are available from the corresponding author on request.

329

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537 **Figure 1.** Open shelves showing hollow bricks and plastic containers with trap-nests of
538 *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier in Buenos Aires province, Argentina. a, each
539 shelf was protected from direct sunlight and precipitation by a roof; b, photograph taken at
540 night (20:30 h) showing bees guarding the nest entrances with the dorsum of the metasoma
541 (black spots inside traps).

542

543 **Figure 2.** Number of bees of each sex emerged from trap-nests of *Xylocopa* (*Neoxylocopa*)
544 *augusti* Lepeletier in relation to the position inside the trap-nest in Buenos Aires province,
545 Argentina

546

547 **Figure 3.** Detail of the trap-nest and associated organisms of *Xylocopa* (*Neoxylocopa*)
548 *augusti* Lepeletier in Buenos Aires province, Argentina. a, sagittal section of a trap-nest
549 showing differential development stages; b, larva of conopid fly *Physocephala wulpi* Camras
550 (Diptera: Conopidae) inside the metasoma of *X. augusti*; c–d, larva and pupa of *P. wulpi*; e,
551 larvae of the host bee and the brood parasite *Cissites maculata* (Swederus) (Coleoptera:
552 Meloideae) indicated by the arrow in the middle cell.

553

554 **Table 1.** Brood cell length (mm) in relation to the position inside the trap-nest of *Xylocopa*
555 (*Neoxylocopa*) *augusti* Lepeletier in Buenos Aires province, Argentina. Cells are numbered
556 from 1 to 6 according to their relative position inside the nest, with one being the innermost
557 cell, far from the nest entrance. n = number of cells measured.

558

559 **Table 2.** Duration (days) of each developmental stage of *Xylocopa* (*Neoxylocopa*) *augusti*
560 Lepeletier using trap-nests in Buenos Aires province, Argentina.

561

562 **Table 3.** Brood cell content, mortality, and sex emerged in relation to the position inside the
563 trap-nest of *Xylocopa* (*Neoxylocopa*) *augusti* Lepeletier in Buenos Aires province, Argentina.
564 n = number of cells examined.

1 **Table 1.**

2

Cell number	\bar{x}	Max	Min	Desv.	n
1	19.61	24.23	17.00	1.51	60
2	19.32	21.50	17.00	1.29	51
3	19.27	22.56	15.35	1.79	36
4	18.57	21	15.90	1.39	21
5	18.67	18.90	18.10	0.29	7
6	17.88	18.32	17	0.76	3

3

4

5 **Table 2.**

	Female			Male		
	Min	Max	$\bar{x} \pm Dsv$	Min	Max	$\bar{x} \pm Dsv$
Egg-Larva	4	6	4.6 \pm 0.65 (n=13)	4	6	4.8 \pm 0.8 (n=5)
Larva-Pupa	16	37	24.6 \pm 6.2 (n=14)	18	21	19.2 \pm 2.57 (n=7)
Pupa-Adult	18	28	21.7 \pm 2.5 (n=42)	18	30	22 \pm 3.2 (n=17)
Complete cycle	46	66	53.6 \pm 6.5 (n=13)	44	50	47.2 \pm 2.1 (n=5)

6

7

8 **Table 3.**

Cell number	Cells content					n	%	%
	Female	Male	Dead larvae	Dead pupae	% mortality			
1	38	2	11	1	23	52	95.0	5.0

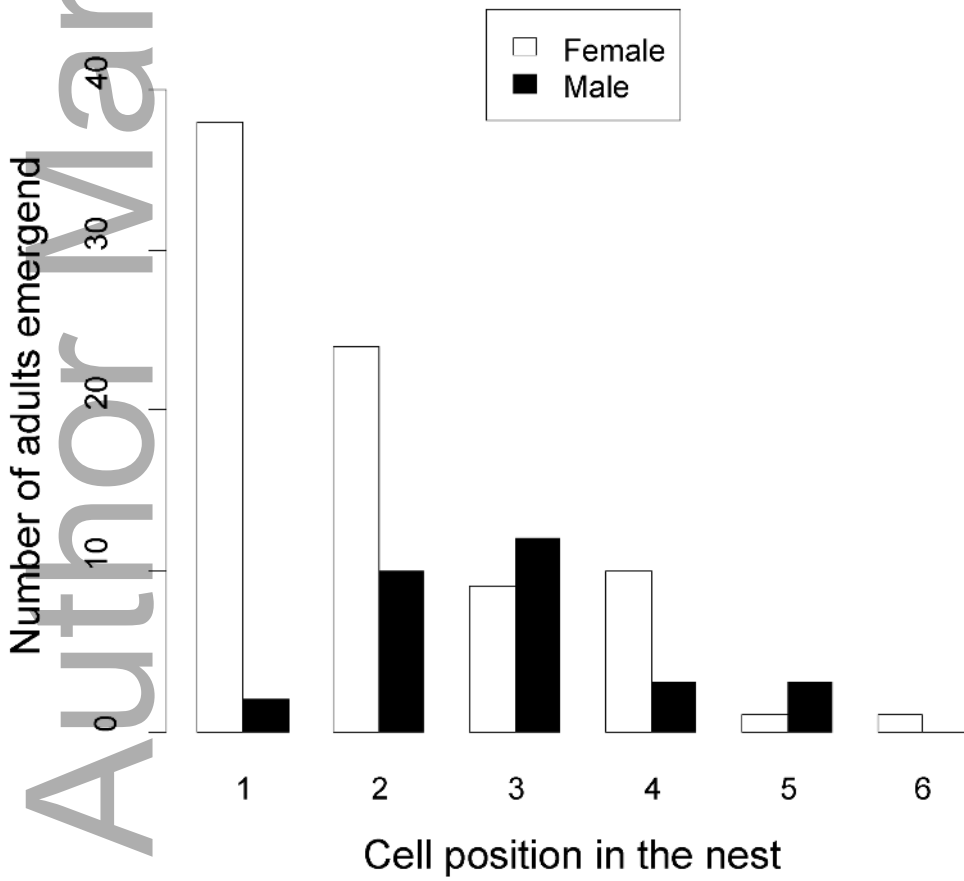
2	24	10	9	2	24	45	70.6	29.4
3	10	12	12	1	38	35	45.5	55.5
4	9	4	7	–	33	21	71.0	29.0
5	1	3	1	1	33	6	30.0	70.0
6	1	–	1	–	50	2	100	–
Total	83	31	41	5		161		

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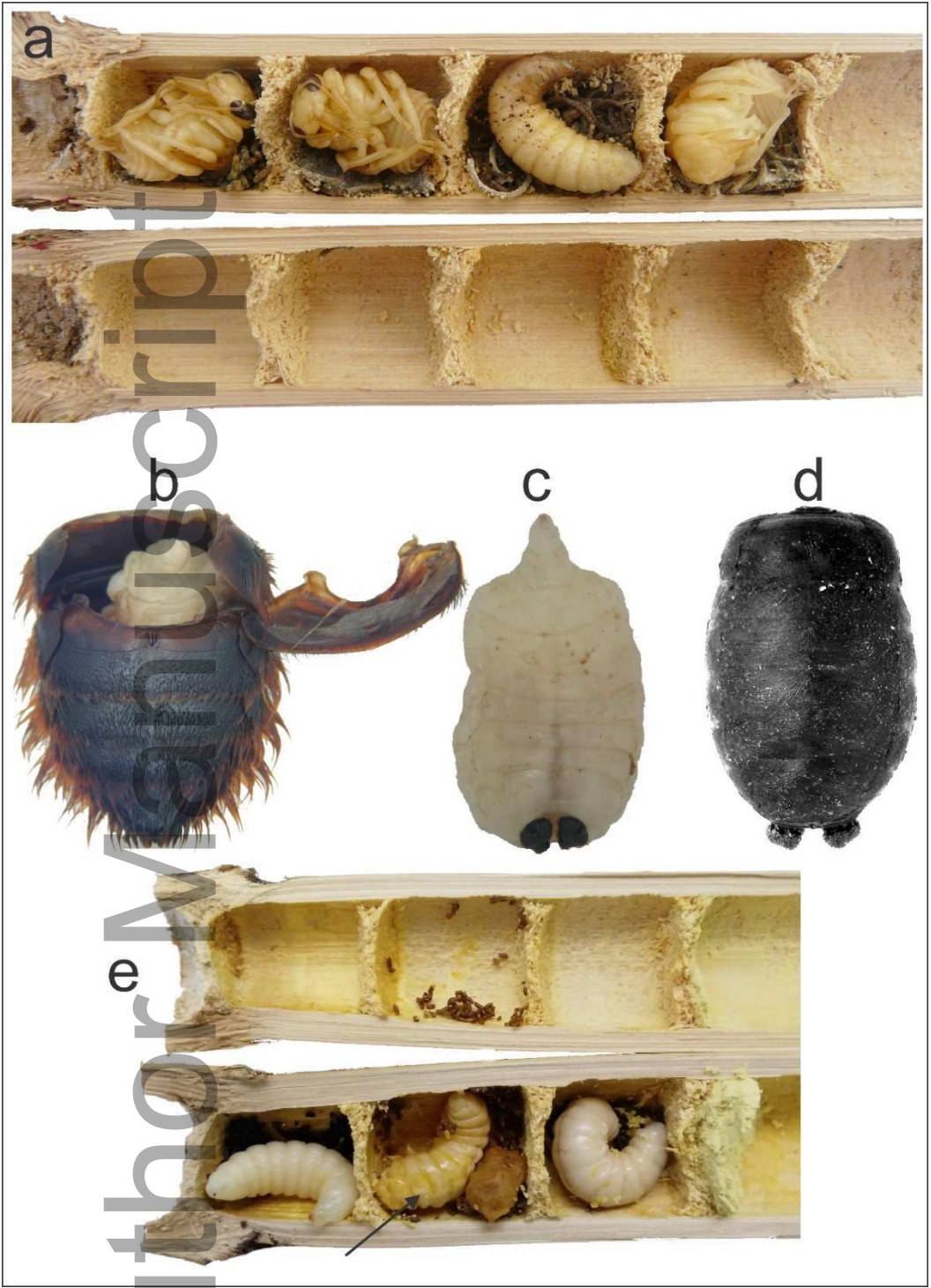


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