

1 **Original Article**

2  
3 **Title**

4 The first fossil replete ant worker establishes living food storage in the Eocene

5  
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## 32 **Abstract**

33 Worker specialization extends the behavioral and ecological repertoire of ant colonies.  
34 Specialization may relate to colony defense, brood care, foraging, and, in some taxa, storage.  
35 Replete workers swell the crop and gaster to store liquid food, which can be accessed by other  
36 colony members through trophallaxis. This storage ability, known as repletism, has  
37 independently evolved across several ant lineages, but the temporal history of this trait has not  
38 yet been investigated. Here, we describe the first fossil replete in the extinct species  
39 *Leptomyrmex neotropicus* Baroni Urbani, 1980 preserved in Miocene-age Dominican amber.  
40 Together with new evidence of repletism in *L. neotropicus'* extant sister species, *L. relictus*  
41 Boudinot et al., 2016, we reconstruct the pattern of acquisition and descent in this storage-  
42 linked trait. Our ancestral state reconstruction suggests that *Leptomyrmex* acquired replete  
43 workers in the Eocene and may therefore represent the earliest instance of so-called  
44 “honeypot” ants among all known ants, both living and extinct.

## 47 **Introduction**

48 Eusociality is a profound phenotypic phenomenon that shapes morphology as well as  
49 behavior. Division of labor is central to advanced sociality in insects and workers may exhibit  
50 a range of behavioral or morphological specializations related to task performance (WILSON &  
51 HÖLLDOBLER 2005). A striking example of caste specialization is repletism. Replete workers  
52 serve as living food storage within the colony by retaining liquid food within their gaster  
53 (terminal abdominal segments). Food storage takes place in the crop, a region of the  
54 alimentary canal in the foregut between the esophagus and the proventriculus. The crop swells  
55 to accommodate large amounts of food, distending the gaster to large proportions via elastic  
56 intersegmental membranes located between each tergite (CARNEY 1969, WILSON 1974). This  
57 elasticity enables repletes to distend their gaster dramatically in some species, which renders  
58 replete individuals visibly distinct from other workers (CONWAY 1994) and may limit mobility  
59 (CHARBONNEAU & al. 2017). During times of scarcity, the stored contents of the replete crop  
60 are redistributed to colony members. Food is regurgitated from one ant to another, a process  
61 known as trophallaxis. Prior to trophallaxis, ants concentrate the stored material by reducing  
62 its water content and they add components of their internal fluids in the crop (MEURVILLE &  
63 LEBOEUF 2021). This creates a network of fluid and nutrient exchange in the colony. Because  
64 trophallaxis is a common feature across ant lineages, many taxa have the capacity to distend  
65 the crop and gaster as part of a colony-wide “social stomach” (MEURVILLE & LEBOEUF 2021).  
66 Taxa with a replete caste are ostensibly less vulnerable to fluctuating resource availability,  
67 particularly during seasons when food sources are limited (VAN ELST & al. 2021). Trophallaxis  
68 may regulate the flow of nutrients among the colony with repletes, in particular, providing a  
69 reliable source of a “higher quality” of food (BORGESSEN 2000).

70  
71 Repletism is a convergently evolved trait that has been observed in several ant lineages. While  
72 a precise definition of repletism is lacking, well-documented replete castes are reported from  
73 20 genera (ANDERSEN 2002, BORGESSEN 2000, CASADEI-FERREIRA & al. 2020, COSENS &  
74 TOUSSAINT 1985, GLANCEY & al. 1973, EYER & al. 2012, CONWAY 1992, KHALIFE & PEETERS  
75 2020, LORINCZI 2016, MOFFETT 1986, 1988, RUANO & TINAUT 1999). Repletes are typically  
76 classified as either crop repletes, storing liquid carbohydrates in the social stomach (i.e., crop),  
77 or fat body repletes, containing lipids in hypertrophied fat bodies (CHARBONNEAU & al. 2017,  
78 TSCHINKEL 1987). Both types of repletes play a role in regulating nutrient storage in the colony  
79 and can provide sustenance to the colony during times of scarcity (BORGESSEN 2000,  
80 CHARBONNEAU & al. 2017, KHALIFE & PEETERS 2020). Crop repletes are often referred to as  
81 honeypot ants or honey ants, while fat body repletes are referred to as corpulents or “false  
82 honeypot ants” (BORGESSEN 2000, LORINCZI 2016). Nearly all origins of repletism occur in the  
83 subfamilies Formicinae and Myrmicinae, while a single dolichoderine genus – *Leptomyrmex*  
84 MAYR, 1862 – exhibits honeypot workers (Fig. 1).

85  
86 While most of the 29 described *Leptomyrmex* species are endemic to Australia, New Guinea,  
87 and New Caledonia, two species are known from the neotropics: a single fossil species from

88 the Dominican Republic and a recently discovered extant species in Brazil (BARDEN & al. 2017,  
89 BOUDINOT & al. 2016, LUCKY & WARD 2010). Numerous Australasian *Leptomyrmex* species  
90 exhibit replete workers, which are frequently found outside of the nest and apparently use their  
91 distended crops for liquid food transport as well as storage (WHEELER 1915). Until now, the  
92 replete status of neotropical *Leptomyrmex* species has remained unknown, obscuring the  
93 temporal and biogeographic origin of this trait. Following the recent discovery of *L. relictus* in  
94 the Brazilian cerrado (BOUDINOT & al. 2016), *Leptomyrmex* is hypothesized to have originated  
95 in the Neotropics during the Eocene before dispersing to Australasia prior to the glaciation of  
96 Antarctica (BARDEN & al. 2017), a route that has been documented in other lineages (DLUSSKY  
97 & RADCHENKO 2013, SANMARTIN & RONQUIST 2004). Were replete workers gained recently in  
98 Australasia or did repletes evolve in the Neotropics prior to their long-distance migration and  
99 diversification? Are there multiple origins of repletism in *Leptomyrmex*?

100  
101 Here, we report new fossil and extant evidence of repletism in the Neotropics. Through  
102 microCT imaging we confirm the replete status of the now extinct Caribbean species *L.*  
103 *neotropicus* Baroni Urbani, 1980 and report replete workers in the extant sister species *L.*  
104 *relictus* Boudinot et al., 2016 for the first time. With these natural history data, we estimate the  
105 approximate age and retention of repletism in the genus *Leptomyrmex* through ancestral state  
106 reconstruction. Our approach illuminates the evolutionary history of extreme morphology-  
107 assisted food storage in ants.

## 108 109 110 **Materials and methods**

### 111 **Fossil imaging:**

112 Photomicrographs were taken using a Nikon SMZ25 stereomicroscope equipped with a DS-  
113 Ri2 digital camera. Individual images were digitally stacked using Nikon NIS Elements to  
114 generate a high-resolution extended focus montage image. X-ray computed tomography data  
115 were generated at the New Jersey Institute of Technology Otto H. York Center for  
116 Environmental Engineering and Science using a Bruker SkyScan 1275 micro-CT scanner. The  
117 specimen was scanned at a voltage of 38kV and current of 190 $\mu$ A for 65ms exposure times  
118 averaged over four frames per rotation with a voxel size of 8.00 $\mu$ m. Z-stacks were generated  
119 using NRecon (Micro Photonics, Allentown, PA), segmented using 3D Slicer v4.9 (FEDOROV &  
120 al. 2012), and rendered in Blender v.3.2.1.

### 121 122 **Ancestral state reconstruction:**

123 We reconstructed ancestral states of repletism across *Leptomyrmex* workers using the  
124 phylogeny of BARDEN & al. (2017) and a survey of natural history observations. Replete codings  
125 were derived from a literature survey as well as published iNaturalist accounts of reliably  
126 identified *Leptomyrmex* species (Supplemental Table 1). Given the uncertainty associated with  
127 some species regarding the presence of repletes, species were coded in a probability matrix:  
128 terminals were assigned a 1/0 replete/non-replete status if known to have repletes, a 0/1  
129 replete/non-replete status if known to not have repletes, and assigned 0.5/0.5 if the presence  
130 of repletes was uncertain. We assumed a flat uninformative prior probability distribution for  
131 uncertain states rather than attempting to assess the probability of repletes vs non-repletes in  
132 uncertain species, given the lack of natural history information for many species. We conducted  
133 ancestral state reconstruction using stochastic character state mapping implemented with the  
134 prior probability matrix for character states. We inferred the reconstruction under the equal  
135 rates (ER) model, based on prior comparisons of the Akaike information criterion (AIC) using  
136 different character state evolution models (symmetrical (SYM) and all rates different (ARD),  
137 and ran the simulation for 200 trees (nsim=200)). State changes were summarized across all  
138 200 trees. Ancestral state construction was conducted in R version 4.2.0 using the package  
139 phytools (REVELL 2012).

## 140 141 **Results**

### 142 **A fossil replete:**

143 Specimen BALDR-0155 is a *Leptomyrmex neotropicus* worker preserved as an inclusion within  
144 amber dated to the Upper Miocene (~16 Ma; ITURRALDE-VINCENT & MACPHEE 1996) from the  
145 Northern mines of near La Cumbre, Dominican Republic. The gastral elastic intersegmental  
146 membrane is significantly distended (Fig. 2) while there are no signs of taphonomic distortion  
147 across the cuticle. X-ray computed tomography recovers a sharp difference in inclusion density  
148 in the region of the gaster and head (Fig. 3), consistent with air. This heterogeneous density is  
149 the result of void space within the cuticle, a common feature recovered through X-ray imaging  
150 as internal features degrade after an insect is entombed in resin (DIERICK & al. 2007).

### 151 152 **Extant repletes in the Neotropics:**

153 We (LC, HMM) observed replete workers of *Leptomyrmex relictus* entering and exiting a  
154 disturbed nest entrance, with some repletes carrying brood or unidentified objects in their  
155 mandibles (Supplemental Video 1 and 2). Replete gasters are conspicuously enlarged and  
156 distended relative to nearby non-repletes. This documentation in *L. relictus* confirms mobility  
157 and multiple task performance of repletes, as described in other *Leptomyrmex* species  
158 (PLOWMAN 1981). Observations took place across the months of July, August, and September  
159 2020 in Parque Cesamar (-10.209838, -48.322934) city of Palmas, state of Tocantins, Brazil.  
160 Two videos were recorded from the same nest within the park (Supplemental Video 1, 2).

### 161 162 **The evolution of repletism in *Leptomyrmex*:**

163 We found strong support for repletism as the ancestral condition of *Leptomyrmex* (Fig. 4;  
164 posterior probability 0.89 repletism). State changes were relatively infrequent; across all trees,  
165 we estimated the average number of gains and losses as 2.5. Our reconstruction suggests  
166 that once repletism evolved in *Leptomyrmex*, it infrequently or perhaps never reverted. The  
167 preponderance of ancestral nodes estimated as replete suggests that many *Leptomyrmex*  
168 species are likely to have a replete caste upon further study, though because our probability  
169 matrix used a flat uninformative prior due to lack of ecological data, this may have biased some  
170 more recent ancestral nodes towards repletism.

171  
172 In extreme cases of repletism (e.g., *Myrmecocystus* Wesmael, 1838), replete workers tend to  
173 be immobile and confined to the nest, solely serving as subterranean food storage (CONWAY  
174 1977). In fat repletes, once workers have depleted the resources in their fat bodies, usually  
175 during the season after storage, they also become foragers (WILLIAMS AND LUCKY 2020). In  
176 other taxa, repletes are mobile, performing other tasks, such as carrying brood or foraging  
177 (COSENS & TOUSSAINT 1985, CONWAY 1992, PLOWMAN 1981, SKINNER 1980). Several species  
178 of *Leptomyrmex* are documented as mobile repletes, foraging on plants and transporting liquid  
179 food to the nest (PLOWMAN 1981, DAVIDSON & al. 2004). Our report of mobile repletes in *L.*  
180 *relictus*, and the presence of a *L. neotropicus* replete worker in fossil amber suggests that  
181 mobility and replete foraging was ancestral in this lineage.

### 182 183 **Discussion**

184 We recover a single origin of replete workers in the last common ancestor of all extant and  
185 extinct *Leptomyrmex* species in the Eocene ~45 Ma (Fig. 4). Our results suggest that living  
186 food storage was present in a Neotropical ancestor and that this trait was retained as the genus  
187 expanded into Australasia. The expansion of grasslands and increases in global temperatures  
188 during the Eocene-Miocene transition may have contributed to the retention of repletism even  
189 across continents and tens of millions of years (AZEVEDO & al. 2020, DLUSSKY & RADCHENKO  
190 2013). The retention of this trait in *Leptomyrmex* is unexpected because repletism is frequently  
191 ascribed to species that inhabit dry climates or are winter active (HÖLLDOBLER & WILSON 1990,  
192 KRONAUER & al. 2004), while some replete species in *Leptomyrmex* are found in wet forests.  
193 Several other genera, including *Pheidole* WESTWOOD, 1839 (TSUJI 1990), exhibit repletes  
194 within species that are endemic to wet habitats; even as climate is strongly linked to living food  
195 storage in some lineages, it does not appear to be a requirement for repletism.

196 It is notable that in our ancestral state reconstruction (Figure 4), and throughout ant lineages  
197 that contain repletism (Table 1), there remain many species with an unknown status.

198 Repletism is often difficult to demonstrate if the replete workers are immobile and confined to



199 the nest. These ants can be difficult to extract from underground, and nestmates often move  
200 repletes to deeper chambers to avoid exposure. There is therefore a bias toward  
201 underreporting replete castes, where they may exist, and it is more likely that fossilized  
202 lineages will exhibit evidence of mobile repletes since taxa with immobile repletes are  
203 unlikely to be aboveground and therefore caught in resin- or sediment-based preservation  
204 modes.

205  
206 This study marks the first ancestral state reconstruction of repletes in any genus of ants, and  
207 the estimated ages of lineages that contain replete species provide an opportunity to assess  
208 the temporal distribution of living food storage (Tab. 1). Molecular-based divergence estimates  
209 suggest that crown-group *Carebara* WESTWOOD, 1840, *Leptothorax* MAYR, 1855, and  
210 *Monomorium* MAYR, 1855 each originated in the early Eocene, prior to *Leptomyrmex*.  
211 Repletism is present but not pervasive in these older taxa, which prevents a clear  
212 reconstruction of replete origins – it is not yet known whether repletes evolved once early in  
213 the history of these lineages and were subsequently lost in several descendants, or if repletism  
214 was recently acquired across multiple distantly related species, for example. Although  
215 *Leptomyrmex* is not the oldest lineage to contain repletes, the definitive reconstruction of  
216 ancestral repletism here establishes the first clear indication that ants with “honeypot” repletes  
217 were present in the Eocene. Future ancestral reconstructions of replete workers across ant  
218 lineages will further reveal the tempo of replete evolution as a striking case of morphology-  
219 enabled division of labor.

220

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226

## 227 **References**

- 228 ANDERSEN, A.N. 2002: Common names for Australian ants (Hymenoptera: Formicidae). –  
229 Australian Journal of Entomology 41: 285-293.
- 230 AZEVEDO, J.A., COLLEVATTI, R.G., JARAMILLO, C.A., STROMBERG, C.A., GUEDES, T.B., MATOS-  
231 MARAVI, P., BACON, C.D., CARILLO, J.D., FAURBY, S. & ANTONELLI, A. 2020: On the young  
232 savannas in the land of ancient forests. In RULL, V. & CARNAVAL A.C (Eds): Neotropical  
233 Diversification. – Patterns and Processes, pp. 271-298.
- 234 AZORSA, F. & FISHER B.L. 2018: Taxonomy of the ant genus *Carebara* Westwood  
235 (Formicidae, Myrmicinae) in the Malagasy region. – ZooKeys 767: 1.
- 236 BARDEN, P., BOUDINOT, B. & LUCKY, A. 2017: Where fossils dare and males matter:  
237 Combined morphological and molecular analysis untangles the evolutionary history of the  
238 spider ant genus *Leptomyrmex* Mayr (Hymenoptera: Dolichoderinae). – Invertebrate  
239 Systematics 31: 765-780.
- 240 BLAIMER, B.B., LAPOLLA, J.S., BRANSTETTER, M.G., LLYOD, M.W. & BRADY, S.G. 2016:  
241 Phylogenomics, biogeography and diversification of obligate mealybug-tending ants in the  
242 genus *Acropyga*. – Molecular Phylogenetics and Evolution 102: 20-29.
- 243 BORGESSEN, L.W. 2000: Nutritional function of replete workers in the pharaoh’s ant,  
244 *Monomorium pharaonis*. – Insectes Sociaux 47: 141-146
- 245 BOUDINOT, B.E., BOROWIEC, M.L. & PREBUS, M.M. 2022: Phylogeny, evolution and  
246 classification of the ant genus *Lasius*, the tribe Lasiini and the subfamily Formicinae  
247 (Hymenoptera: Formicidae). – Systematic Entomology 47: 113-151.
- 248 BOUDINOT, B., PROBST, R.S., BRANDAO, C.R., FEITOSA, R.M. & WARD, P.S. 2016: Out of the  
249 Neotropics: Newly discovered relicutal species sheds light on the biogeographical history  
250 of spider ants (*Leptomyrmex*, Dolichoderinae, Formicidae). – Systematic Entomology 41  
251 658-671.
- 252 CAMMAERTS, R. 1996: Factors affecting regurgitation behavior of the ant *Lasius flavus*  
253 (Formicidae) to the guest beetle *Calivger testaceus* (Pselaphidae). – Behavioural  
254 Processes 38: 297-312.

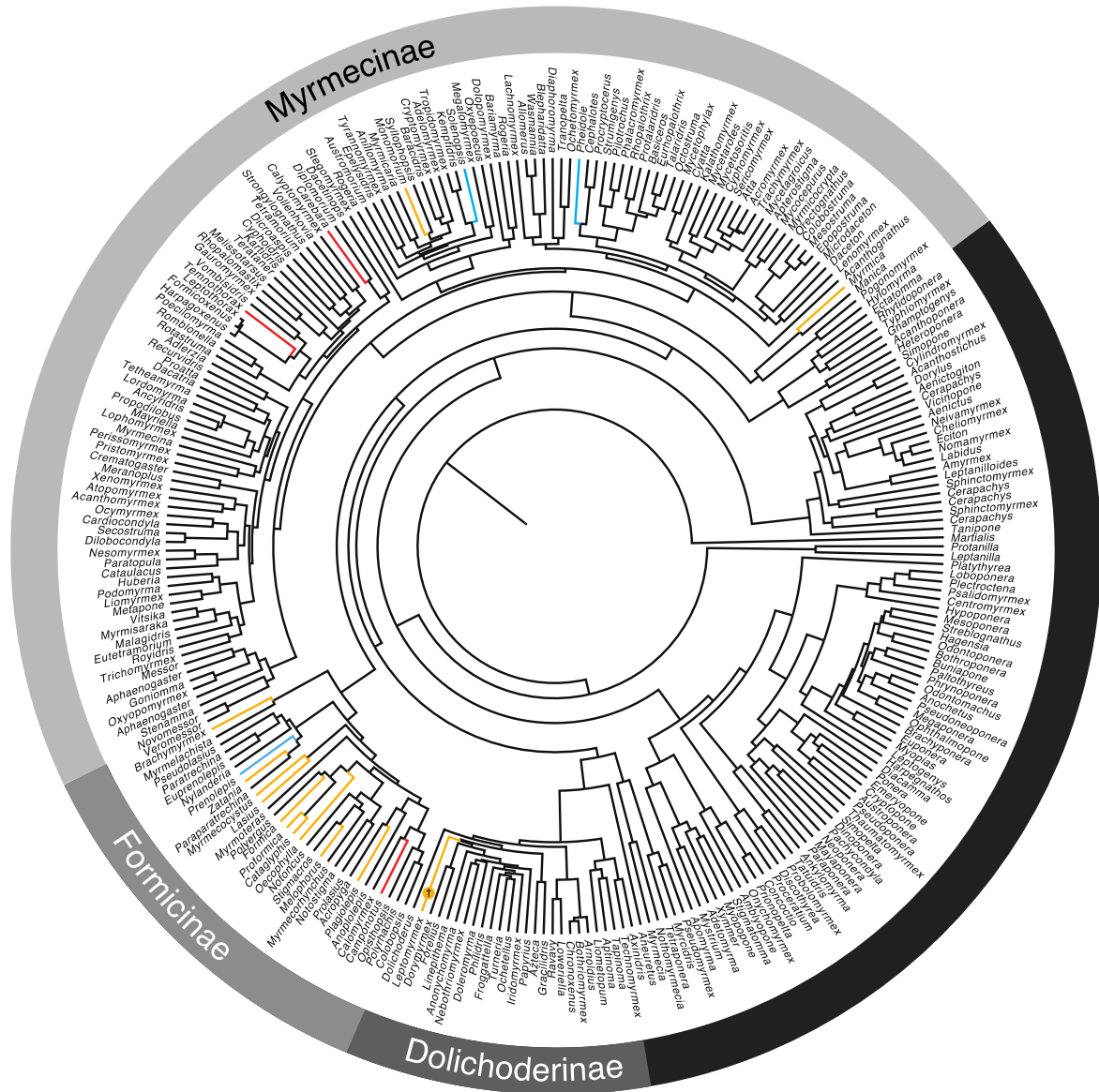
- 255 CARNEY, W.P. 1969: Behavioral and morphological changes in carpenter ants harboring  
256 *Dicrocoeliid metacercariae*. – The American Midland Naturalist 82: 605-611.\
- 257 CASADEI-FERREIRA, A., FISCHER, G. & ECONOMO, E.P. 2020: Evidence for a thoracic crop in  
258 the workers of some Neotropical *Pheidole* species (Formicidae: Myrmicinae). – Arthropod  
259 Structure and Development 59.
- 260 CHARBONNEAU, D., POFF, C., NGUYEN, H., SHIN, M.C., KIERSTEAD, K., & DORNHAUS, A. 2017:  
261 Who are the lazy ants? The function of inactivity in social insects and a possible role of  
262 constraint: inactive ants are corpulent and may be young and/or selfish. – Integrative and  
263 Comparative Biology 57: 649-667.
- 264 CONWAY, J.R. 1977: Analysis of clear and dark amber repletes of the honey ant,  
265 *Myrmecocystus mexicanus* hortideorum. – Annals of the Entomological Society of  
266 America 70: 367-369.
- 267 CONWAY, J.R. 1994: Honey ants. – American Entomologists 40: 229-234.
- 268 CONWAY, J.R. 1992: Notes on a nest of the honey ant, *Plagiolepis squamulosa* Wheeler, in  
269 the Northern Territory, Australia. – Australian Entomologist 19: 61-63.
- 270 CONWAY, J.R. 1992: Notes on the excavation of a nest of *Melophorus bagoti* Lubbock in the  
271 Northern Territory, Australia (Hymenoptera: Formicidae). – Australian Journal of  
272 Entomology 31: 247-248.
- 273 COSENS, D. & TOUSSAINT, N. 1985: An experimental study of the foraging strategy of the  
274 wood ant *Formica aquilonia*. – Animal Behavior 33: 541-552
- 275 COEVELO DE ZOLESSI, L., PETRONE DE ABENANTE, Y. & GONZALEZ, L.A. 1976: Bioethological  
276 description and observation of a new species of *Brachymyrmex* (Hymenoptera:  
277 Formicidae). – Revista de Biologica del Uruguay 4: 21-44.
- 278 DAVIDSON, D.W., COOK, S.C. & SNELLING, R.R. 2004: Liquid-feeding performances of ants  
279 (Formicidae): Ecological and evolutionary implications. – Oecologia 139: 255-266.
- 280 DIERICK, M., CNUUDE, V., MASSCHAELE, B., VLASSEN BROECK, J., VAN HOOREBEKE, L. & JACOBS,  
281 P. 2007: Micro-CT of fossils preserved in amber. – Nuclear Instruments and Methods in  
282 Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated  
283 Equipment 580: 641-643.
- 284 DLUSSKY, G. & RADCHENKO, A. 2013: A new enigmatic ant genus from the late Eocene  
285 Danish amber and its evolutionary and zoogeographic significance. – Acta  
286 Palaeontologica Polonia 59: 931-939.
- 287 EYER, P.A., FREYER, J. & ARON, S. 2012: Genetic polyethism in the polyandrous desert ant  
288 *Cataglyphis cursor*. – Behavioral Ecology 24: 144-151.
- 289 FEDOROV, A., BEICHEL, R., KALPATHY-CRAMER, J., FINET, J., FILLION-ROBIN, J.C., PUJOL, S.,  
290 BAUER, C., JENNINGS, D., FENNESSY, F., SONKA, M., BUATTI, J., AYLWARD, S., MILLER, J.V.,  
291 PIEPER, S. & KIKINIS, R. 2012: 3D Slicer as an image computing platform for the  
292 quantitative imaging network. – Magnetic Resonance Imaging 30: 1323-1341.
- 293 FROGGATT, W.W. 1896: Honey ants. – Spencer B (Ed).
- 294 GALLKOWSKI, C., LEBAS, C., WEGNEZ, P., LENOIR, A. & BLATRIX, R. 2017: Redescription of  
295 *Proformica nausta* (Nylander, 1856) (Hymenoptera, Formicidae) using an integrative  
296 approach. – European Journal of Taxonomy 290.
- 297 GLANCEY, M.B., STRINGER, C.E., CRAIG, C.H., BISHOP, P.M. & MARTIN, B.B. 1973: Evidence of  
298 a replete caste in the fire ant *Solenopsis invicta*. – Annals of the Entomological Society of  
299 America 66: 233-234.
- 300 HASEGAWA, E. 1993: Caste specialization in food storage in the dimorphic ant *Colobopsis*  
301 *nipponicus* (Wheeler). – Insectes Sociaux 40: 261-271
- 302 HETERICK, B.E. 2022: A guide to the ants of Western Australia. Part 2: Distribution and  
303 biology. - Records of the Western Australian Museum, supplement 86: 241-510.
- 304 HETERICK, B.E., CASTANELLI, M. & SHATTUCK, S.O. 2017: Revision of the ant genus  
305 *Melophorus* (Hymenoptera: Formicidae). – ZooKeys 700:1.
- 306 HÖLLDOBLER, B. & WILSON, E.O. 1990: The ants. – The Harvard University Press.
- 307 ITURRALDE-VINCENT, M.A. & MACPHEE, R.D.E. 1996: Age and paleogeographic origin of  
308 Dominican amber. – Science 273: 1850-1852.

- 309 KHALIFE, A. & PEETERS, C. 2020: Food storage and morphological divergence between  
310 worker and soldier castes in a subterranean myrmicine ant, *Carebara perpusilla*. – Journal  
311 of Natural History 54: 3131-3148.
- 312 KRONAUER, D.J.C., HOLLDOBLER, B. & GADAU, J. 2004: Phylogenetics of the new world honey  
313 ants (genus *Myrmecocystus*) estimated from mitochondrial DNA sequences. – Molecular  
314 Phylogenetics and Evolution 32: 416-421.
- 315 LORINCZI, G. 2016: Winter activity of the European false honeypot ant, *Prenolepis nitens*  
316 (Mayr, 1853). – Insectes Sociaux 63: 193-197
- 317 LUCKY, A. & WARD, P.S. 2010: Taxonomic revision of the genus *Leptomymex* Mayr  
318 (Hymenoptera: Formicidae). – Zootaxa 2688.
- 319 LUBBOCK, J. 1880: Observation of ants, bees, and wasps: with a description of a new species  
320 of honey-ant. – Journal of the Linnean Society of London, Zoology 15: 167-187.
- 321 MEURVILLE, M.P. & LEBOEUF, A.C. 2021: Trophallaxis: The functions and evolution of social  
322 fluid exchange in ant colonies (Hymenoptera: Formicidae). – Myrmecological News 31.
- 323 MOFFETT, M.W. 1988: Foraging behavior in the Malayan swarm-raiding ant *Pheidologeton*  
324 *Silenus* (Hymenoptera: Formicidae: Myrmicinae). – Annals of the Entomological Society of  
325 America 81: 356-361.
- 326 MOFFETT, M.W. 1986: Notes on the behavior of the dimorphic ant, *Oligomyrmex overbecki*  
327 (Hymenoptera: Formicidae). – Psyche 93: 107-116.
- 328 PLOWMAN, K.P. 1981: Resource utilization by two New Guinea rainforest ants. – Journal of  
329 Animal Ecology 50: 903-916 Notes on the behavior of the dimorphic ant, *Oligomyrmex*  
330 *overbecki* (Hymenoptera: Formicidae) – Psyche 93: 107-116.
- 331 PRINS, A.J. 1983: A new ant genus from South Africa (Hymenoptera, Formicidae). – South  
332 African Museum.
- 333 REVELL, L.J. 2012: Phytools: An R package for phylogenetic comparative biology (and other  
334 things). – Methods in Ecology and Evolution, pp. 217-223
- 335 RUANO, F. & TINAUT, A. 1999: Rapid process, activity pattern and influence of abiotic  
336 conditions in the slave-making ant *Rossomyrmex minuchae* (Hymenoptera: Formicidae). –  
337 Insectes Sociaux 46: 341-347.
- 338 SANMARTIN, I. & RONQUIST, F. 2004: Southern hemisphere biogeography inferred by event-  
339 based models: Plant versus animal patterns. – Systematic Biology 53: 216-243.
- 340 SKINNER, G.J. 1980: The feeding habits of the wood-ant, *Formica rufa* (Hymenoptera:  
341 Formicidae), in Limestone Woodland in North-west England. – Journal of Animal Ecology  
342 49: 417-433.
- 343 SNELLING, R.R. 1976: A revision of the honey ants, genus *Myrmecocystus* (Hymenoptera:  
344 Formicidae).
- 345 TSCHINKEL, W.R. 1987: Seasonal life history and nest architecture of a winter-active ant,  
346 *Prenolepis imparis*. – Insectes Sociaux 34: 143-164
- 347 TSUJI, K. 1990: Nutrient storage in the major workers of *Pheidole ryukyuensis* (Hymenoptera:  
348 Formicidae). – Applied Entomology and Zoology 25: 283-287.
- 349 VAN ELST, T., ERIKSSON, T.H., GADAU, J., JOHNSON, R.A., RABELING, C., TAYLOR, J.E. &  
350 BOROWIEC, M.L. 2021: Comprehensive phylogeny of *Myrmecocystus* honey ants  
351 highlights cryptic diversity and infers evolution during aridification of the American  
352 Southwest. – Molecular Phylogenetics and Evolution 155.
- 353 WARD, P.S., BRADY, S.G., FISHER, B.L. & SCHULTZ, T.R. 2015: The evolution of myrmecinae  
354 ants: Phylogeny and biogeography of a hyperdiverse ant clade (Hymenoptera:  
355 Formicidae). – Systematic Entomology 40: 61-81.
- 356 WHEELER, W.M. 1936: Ants from Hispaniola and Mona Island. – Museum of Comparative  
357 Zoology.
- 358 WHEELER, W.M. 1915: The Australian honey-ants of the genus *Leptomymex* Mayr. –  
359 Proceedings of the American Academy of Arts and Sciences 51: 255-286.
- 360 WILLIAMS, J.L. & LUCKY, A. 2020: Winter ant, false honey ant *Prenolepis imparis* (Say)  
361 (Insecta: Hymenoptera: Formicidae: Formicinae). - <  
362 <https://edis.ifas.ufl.edu/pdf/IN/IN116800.pdf>>, retrieved on 8 October 2022.
- 363 WILSON, E.O. 1974: The soldier of the ant, *Camponotus (Colobopsis) fraxinicola*, as a trophic  
364 caste. – Psyche 81: 182-188.

365 WILSON, E.O. & HOLLOBLER, B. 2005: Eusociality: Origin and consequences. – Proceedings  
366 of the National Academy of Sciences 102: 13367-13371.  
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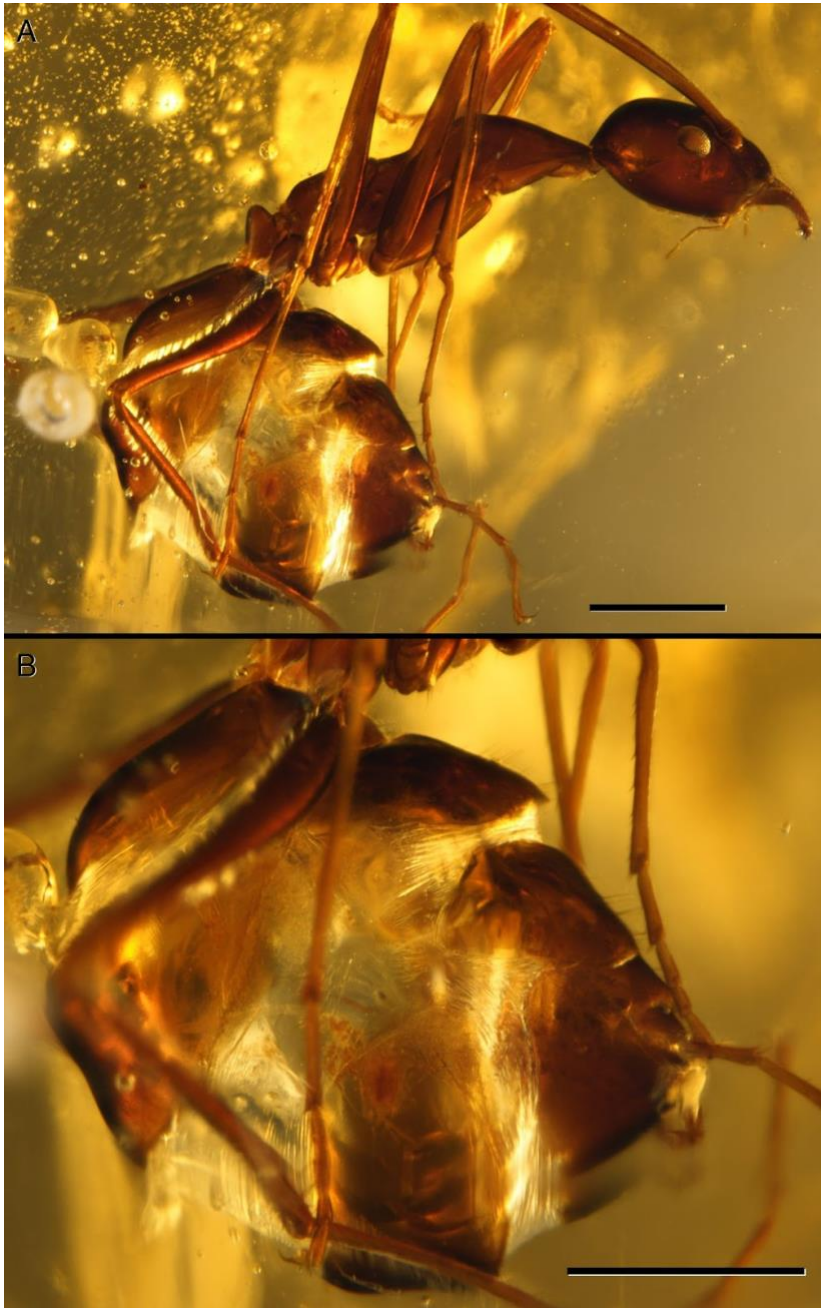


370 **Table and figure captions**  
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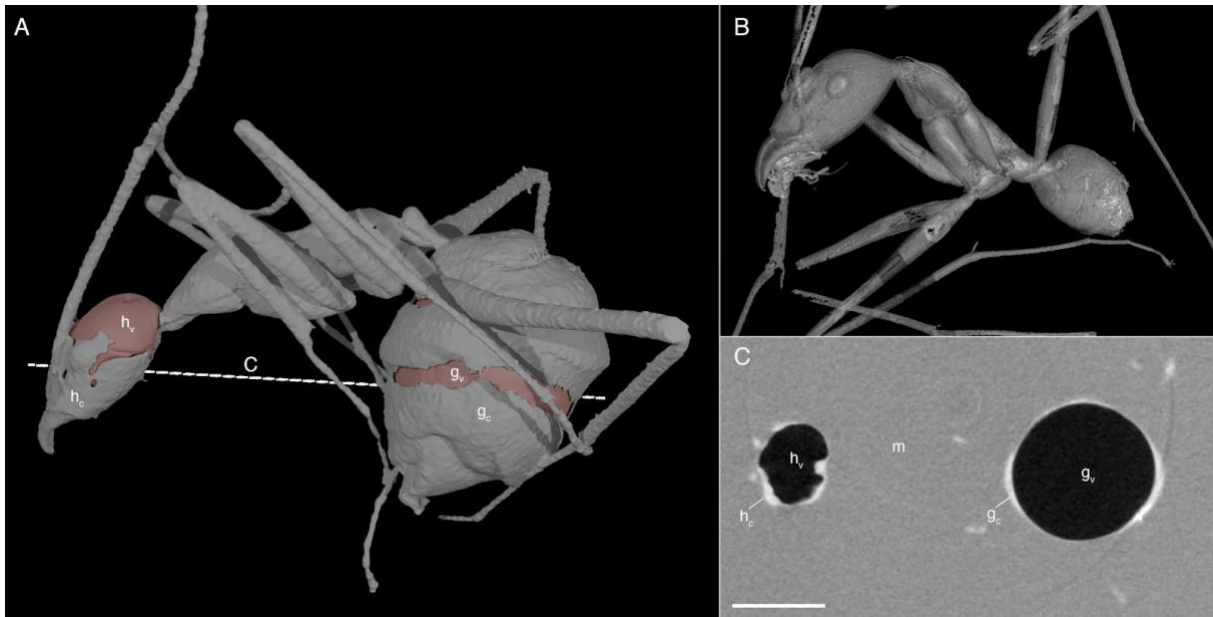
372 **Figure 1. Phylogenetic distribution of known repletes.** Topology adapted from Moreau  
373 and Bell, 2017. Lineage color reflects presence of repletes. Yellow: crop repletes; blue: fat  
374 body repletes; red: crop/fat body repletes. The fossil species *Leptomyrmex neotropicus* is  
375 denoted by the dagger † symbol.  
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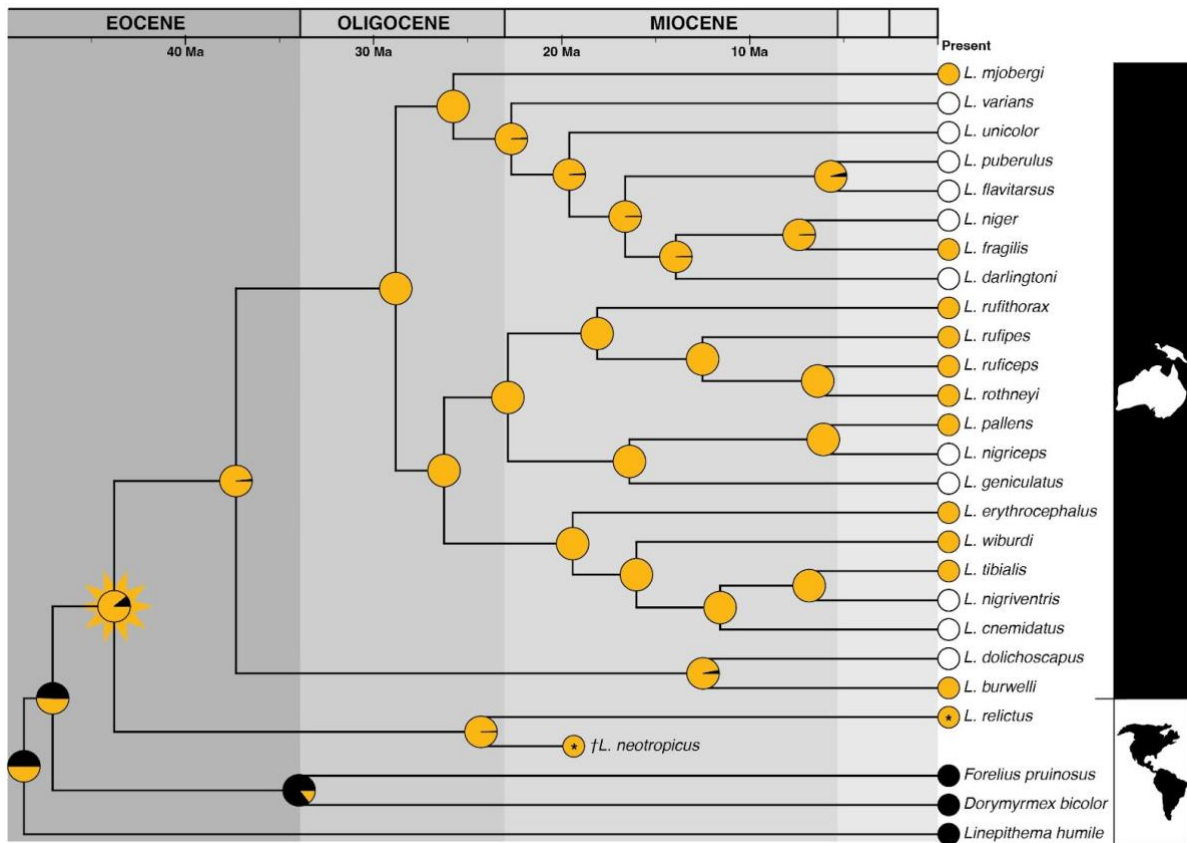
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**Figure 2. Photomicrograph of *L. neotropicus* replete specimen BALDR-0155 preserved in Miocene-age Dominican amber. (A) Lateral view. (B) Enlarged, lateral view of distended gaster. Scale = 1mm.**



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**Figure 3. X-ray computed tomography images of *L. neotropicus*** (A) Lateral view of replete *L. neotropicus* worker specimen BALDR 0155. (B) Lateral view of a non-replete *L. neotropicus* worker (AMNHDR-13-85 modified from Barden et al., 2017). (C) Z-stack cross section of specimen BALDR 0155 head and gaster denoted by dotted line in sub-panel A. hc = head cuticle; hv = voidspace of head; m = amber matrix; gc = gaster cuticle; gv = voidspace of gaster. Scale = 1mm



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**Figure 4. Ancestral state reconstruction of replete workers across *Leptomyrmex*.** Summary of 200 simulated stochastic character histories under an equal rates (ER) model. Node pie charts represent posterior probabilities of states at each node. Yellow = replete; black = no repletes; white = unknown, these were coded as ambiguous in ancestral state reconstruction. Most recent common ancestor of all *Leptomyrmex* is indicated with star icon. Topology and mean node ages from Barden et al. (2017).

**Table 1. Summary of major replete lineages and their estimated crown ages.**



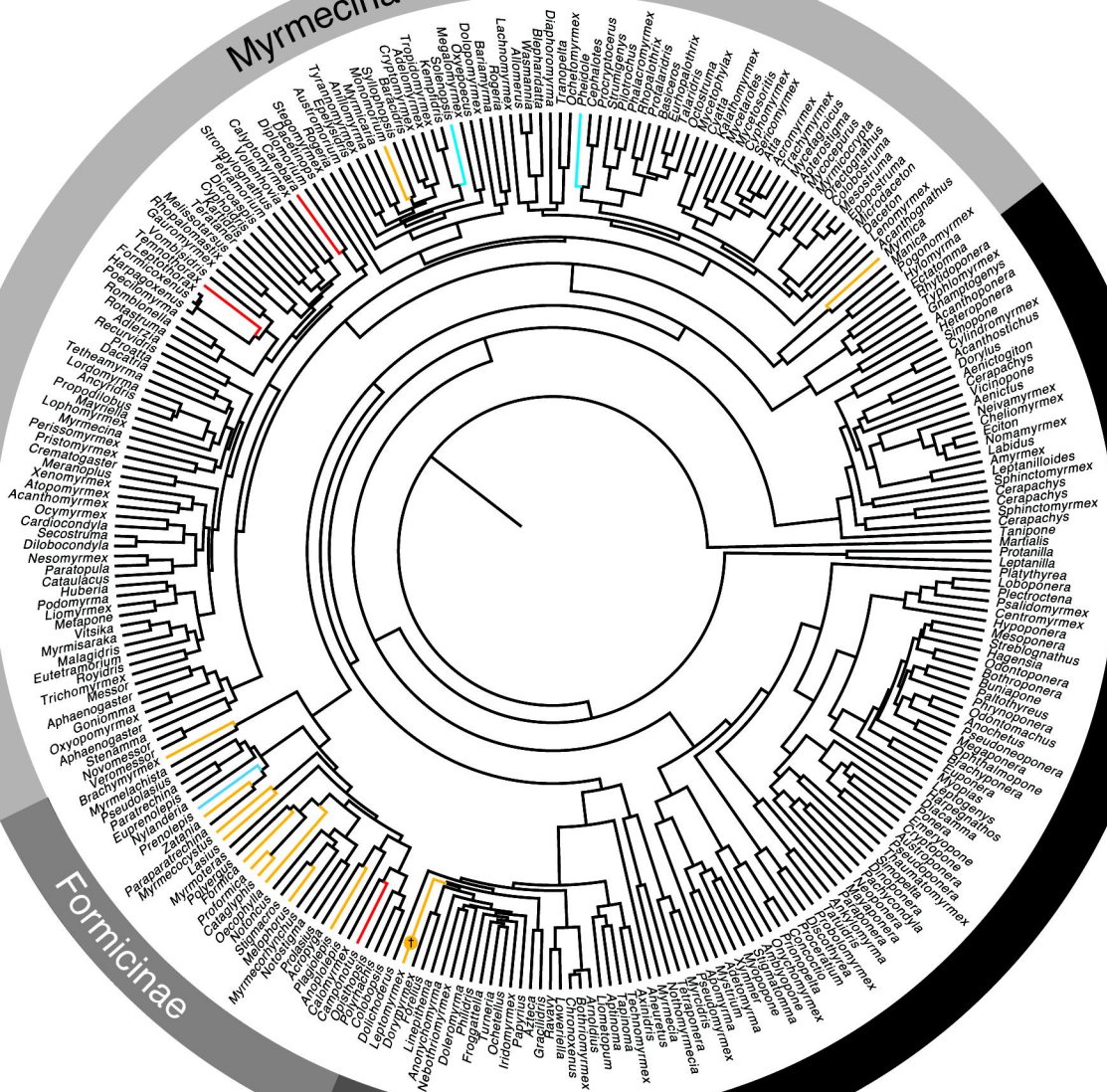
Replete genera	Type of repletism	Mean lineage age (Ma)	Age reference	Replete reference
<i>Agraulomyrmex</i>	Fat body	~23	Blaimer et al. (2016)	Prins (1983)
<i>Brachymyrmex</i>	Crop	~17 [27.5 – 7.5]	Boudinot et al. (2022)	Zolessi et al. (1978)
<i>Camponotus</i>	Crop	~24 [30 – 13]	Boudinot et al. (2022)	Lubbock (1880); Froggatt (1896) Heterick (2022)
<i>Cataglyphis</i>	Crop	~15 (divergence)	Tinaut and Ruano (2021)	Eyer et al. (2013)
<i>Colobopsis</i>	Crop	~24.5 [35 – 15]	Boudinot et al. (2022)	Wilson (1974); Hasegawa (1993)
<i>Lasius</i>	Crop	~21.9 [28.6 – 15.3]	Boudinot et al. (2022)	Cammaerts (1996)
<i>Melophorus</i>	Crop	~44.2 [51 – 20] (divergence)	Blaimer et al. (2015)	Conway (1992); Heterick (2017)
<i>Myrmecocystus</i>	Crop	~14.1 [19.9 – 10.2]	Van Elst (2021)	Froggatt (1896) Snelling (1976)
<i>Plagiolepis</i>	Crop	~11.2 [24 – 3] (low sample size)	Blaimer et al. (2015)	Heterick (2022)
<i>Prenolepis</i>	Fat body	~15 [19 – 9]	Boudinot et al. (2022)	Tschinkel (1983)
<i>Proformica</i>	Crop	~20 (divergence)	Tinaut and Ruano (2021)	Galkowski (2017)
<i>Zatania</i>	Crop	~15 [18.5 – 9]	Boudinot et al. (2022)	Wheeler (1936)
<i>Formica</i>	Crop	~17 [21 – 4]	Boudinot et al. (2022)	Cosens & Toussaint (1985)
<i>Leptomyrmex</i>	Crop	~43.8 [54 – 35.2]	Barden et al. (2017)	
<i>Solenopsis</i>	Fat body	~39.1 [31.4 – 47.1]	Ward et al. (2015)	Glancey (1973)
<i>Leptothorax</i>	Crop	[~48 – 25]	Ward et al. (2015)	Børgesen (2000)
<i>Myrmica</i>	Crop	~34	Jansen et al. (2010)	Børgesen (2000)
<i>Monomorium</i>	Crop	[~50 – 30]	Ward et al. (2015)	Børgesen (2000)
<i>Carebara</i>	Crop	~43 [50 – 30]	Ward et al. (2015)	Azorsa & Fisher (2018)
<i>Pheidole</i>	Fat body	35.2 [46.6 – 24.9]	Ward et al. (2015)	Tsuji (1990)

476 Lineage dates are derived from published molecular-based divergence estimates and indicate the age of the last common  
477 ancestor for each genus, except where otherwise noted. Lineage dates denoted with (divergence) correspond with the last  
478 common ancestor of the focal genus and its closest living relative sampled in the corresponding phylogeny – such instances  
479 reflect inadequate sampling to confidently estimate crown-ages and are therefore overestimates. Instances of single species  
480 repletes are excluded; it is not possible to estimate the age of these lineages with current available data. Color legend:  
481 Formicinae (orange), Dolichoderinae (green), Myrmicinae (blue).  
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483 **Supplemental Video 1. Replete workers of *Leptomyrmex relictus*.** Video  
484 evidencing mobile replete workers emerging from disturbed nest. Filming took place  
485 on the morning of August 20, 2020 (~9:00), at an external temperature of 29.3°C and  
486 27.8°C inside the nest (nest humidity ranging from 30% to 60%).  
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488 **Supplemental Video 2. Replete workers of *Leptomyrmex relictus*.** Video  
489 evidencing mobile replete workers emerging from disturbed nest. Filming took place  
490 on the afternoon of August 20, 2020 (~14:00), external temperature of 34.5°C and  
491 internal temperature of 29.4° (nest humidity ranging from 30% to 60%).

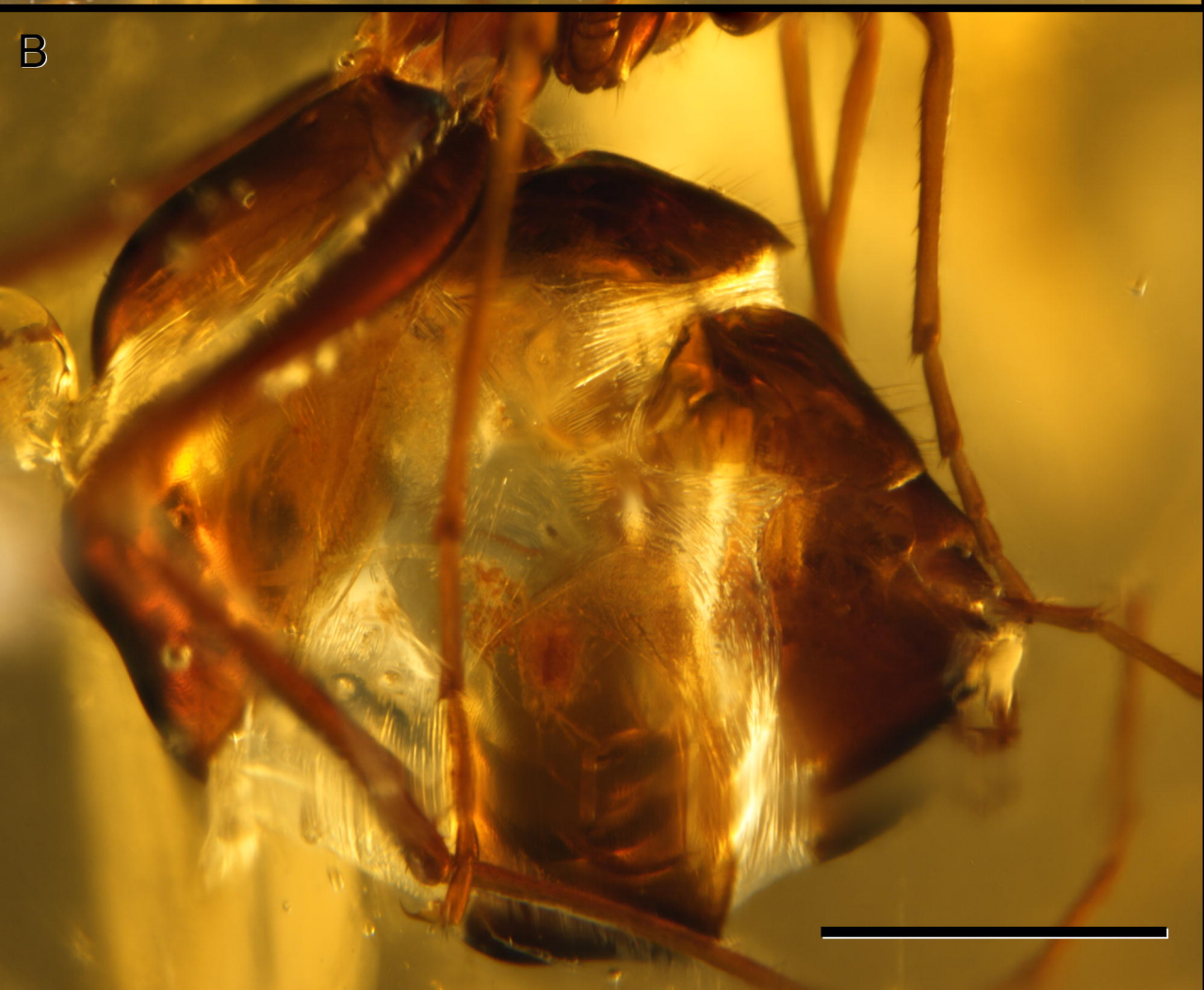
# Myrmecinae



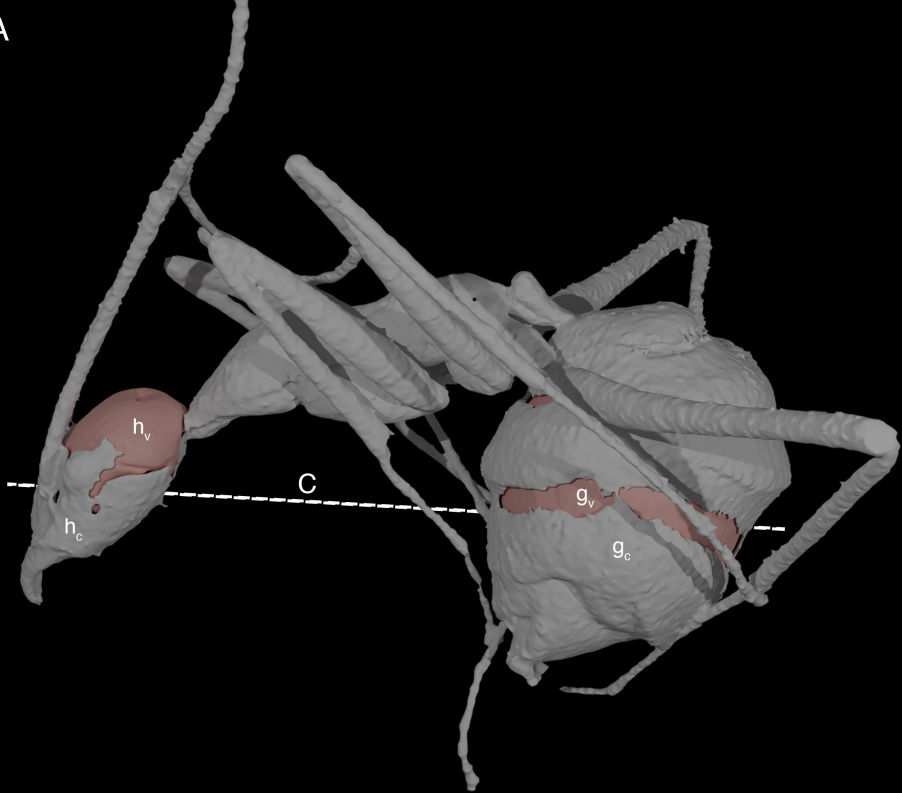
# Formicinae

# Dolichoderinae





A



B



C

