



Biological notes, nest architecture, and morphology of the remarkable ant *Hylomyrma primavesi* ULYSSÉA, 2021 (Hymenoptera: Formicidae: Myrmicinae)

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Abstract

Natural history discoveries have contributed to broad applications in the fields of taxonomy, ecology, evolution, and conservation of the species. Knowledge on the biology of species of the Neotropical ant genus *Hylomyrma* FOREL, 1912 remains incipient despite the substantial data reported during its last taxonomic revision. Here, based on fieldwork carried out in an area of Campo Rupestre (Rupestrian Field) in Minas Gerais, Brazil, we present the first biological data of the recently described species *Hylomyrma primavesi* ULYSSÉA, 2021, including the descriptions of the ergatoid queen, male, larva, female reproductive tract, nest architecture, and feeding behavior. *Hylomyrma primavesi* forage solitarily and prefer very small insects (Collembola and larvae, pupae, and imagos of Diptera and Coleoptera). Its nests are found in sandy soil and are not deep. One straight tunnel connects the tube entrance to a final chamber. The colonies comprised approximately 7 - 65 workers and intercaste specimens and one ergatoid queen per colony. The structure of the female reproductive tract of *H. primavesi* is similar to that in other Myrmicinae. Workers and intercaste specimens have one pair of ovarioles and lack the spermatheca, while the ergatoid queen has three pairs of ovarioles and spermatheca. Most remarkably, it is the only *Hylomyrma* species that has only ergatoid queens. Lastly, we comment on the evolution of new castes in *Hylomyrma* from the perspective of the hourglass model and the standard model. The findings reported here fill gaps in the understanding of the natural history of cryptobiotic species and shed light on the caste development and evolution in *Hylomyrma*.

Key words: Ergatoid queen, male, larva, Pogonomyrmecini, ovary, spermatheca, taxonomy.

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Introduction

The ant genus *Hylomyrma* FOREL, 1912 is a member of the ant tribe Pogonomyrmecini (WARD & al. 2015), along with its sister genus *Pogonomyrmex* MAYR, 1868 and the genus *Patagonomyrmex* JOHNSON & MOREAU, 2016. The natural history of many species of the seed-harvester ant genus *Pogonomyrmex* is well-known (JOHNSON 2015), and the knowledge about the biology of the three granivorous species of *Patagonomyrmex* is restricted to Kusnezov's observations (JOHNSON & MOREAU 2016), while our knowledge about the biology of the *Hylomyrma* species is still incipient. The rare observations of live specimens made by Wilson (i.e., *Hylomyrma versuta* KEMPF, 1973

in WHEELER & WHEELER 1960) and recently by Ulysséa (i.e., *Hylomyrma blandiens* KEMPF, 1961, *Hylomyrma immanis* KEMPF, 1973, and *Hylomyrma reitteri* (MAYR, 1887) in ULYSSÉA & BRANDÃO 2021), morphological traits (i.e., *H. reitteri* in SILVA & BRANDÃO 2010), and label data (KEMPF 1964, 1973, ULYSSÉA & BRANDÃO 2021), mention *Hylomyrma* ants as being cryptic, epigeic generalist predators, inhabitants of the leaf-litter of wet and dry environments in forested habitats or cropping areas, with small nests and colonies, queen-like and worker-like intercaste specimens, and workers that feign death (thanatosis) when disturbed.

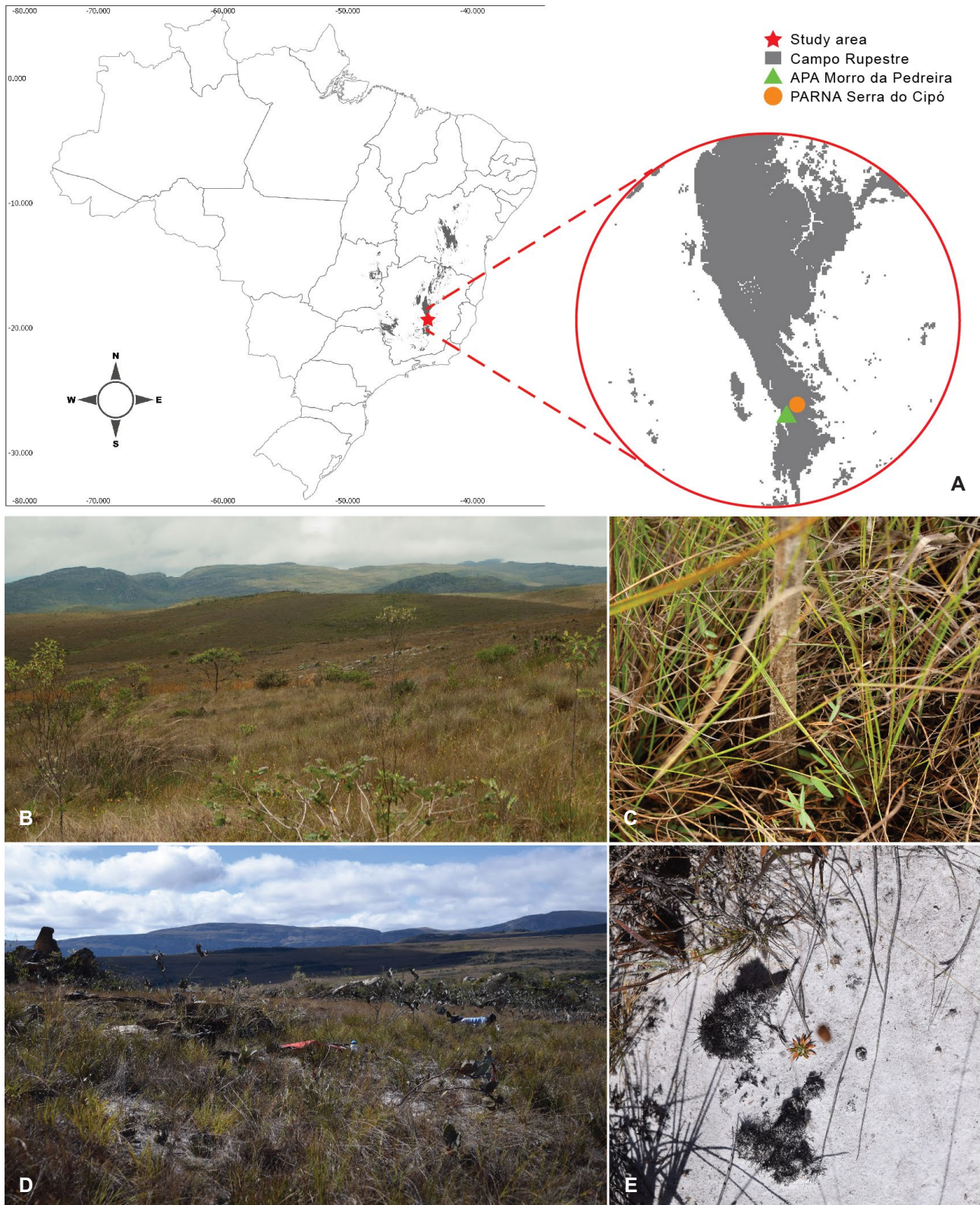


Fig. 1: Study area (red asterisk). **A)** Map showing the two sampled localities – PARNAserra do Cipó (orange circle) and APA Morro da Pedreira (green triangle), Santana do Riacho, Minas Gerais, Brazil; **B - C)** landscape and ground detail in Alto Palácio region at PARNAserra do Cipó; and **D - E)** landscape and ground detail in Q16 region at APA Morro da Pedreira.

Hylomyrma primavesi ULYSSÉA, 2021 is an endemic Brazilian ant, occurring in higher elevations in areas of Campo Rupestre (Rupestrian Field) ecoregion at San-

tana do Riacho, State of Minas Gerais (MG), and can be easily distinguished from its 29 congeners mainly due to its blackish body (ULYSSEÁ & BRANDÃO 2021). From



Fig. 2: Field observations on *Hylomyrma primavesi* behavior at the APA Morro da Pedreira, Santana do Riacho, Minas Gerais, Brazil.

pitfall trap material, the species' description was based on few workers ($n = 6$) and a higher number of specimens identified as intercaste specimens ($n = 23$), which were identifiable by an inconspicuous central ocellus or a more or less pronounced depression on the region where winged queens have the central ocellus, and the promesonotal junction and metanotal groove were distinguishable by a slight depression more or less accentuated and by altered sculpture. Due to the broad presence of specimens identified as intercaste specimens and the unknown winged queen, ULYSSÉA & BRANDÃO (2021) hypothesized that in this species, intercaste specimens may perform reproduction. Hence, we conducted a study on the natural history of the species *H. primavesi*. Here, with the aim of advancing knowledge about the natural history of cryptobiotic ant species, for the first time we (i) document *H. primavesi* nest architecture and colony size based on ten colonies collected in the Campo Rupestre, (ii) describe the previously unknown ergatoid queen, male, and immature stadia for the species, (iii) report the morphology of the female reproductive tract, (iv) record the behavior of the forage workers and their feeding preferences based on field and laboratory observations, and (v) we hypothesize that the phenotypes of *H. primavesi* and a closely-related species, *Hylomyrma reitteri*, fit in the recently proposed model of caste development and evolution by TRIBLE & KRONAUER (2017, 2021a, b).

Material and methods

Study area

The study was conducted at the PARNA Serra do Cipó (= Serra do Cipó National Park) ($19^{\circ} 15' 51''$ S, $43^{\circ} 32' 02''$ W), in an area called Alto Palácio, and at the APA Morro da

Pedreira (= Morro da Pedreira Environmental Protection Area) ($19^{\circ} 17' 50.11''$ S, $43^{\circ} 35' 39.69''$ W), also referred to as Q16, being both in the municipality of Santana do Riacho, State of Minas Gerais, Brazil (Figs. 1A - E). These two contiguous areas correspond to the southern ending of the Espinhaço Mountain Range, classified as a Biosphere Reserve by UNESCO (SILVEIRA & al. 2016). The landscape is defined as Campo Rupestre, whose climatic regime consists of markedly dry winters and wet summers (SILVEIRA & al. 2016). At both sampling sites, in highland areas (1300 - 1400 m), the core landscape is a vegetation mosaic composed of grassland and shrub species associated with rocky outcrops, surrounding patches of gallery forests, whereas Cerrado (= Savannah) and Caatinga (= Dry Forest) occur at the lower elevations (SILVEIRA & al. 2016, NUNES & al. 2020).

Ant sampling and field observations

The field work was carried out in two field expeditions, one during the rainy season – from 23 February to 6 March 2021, and one during the dry season – on 13 and 14 July 2021. Five nests of *Hylomyrma primavesi* (N6, N7, N11, N12, N17) were located and marked in the field, not being excavated. Three nests (N1, N2 + N3, N10) were excavated and collected on the first field expedition on the same day they were found. These three colonies were transferred to plastic containers, taken to the field lodging at Reserva Vellozia, and then preliminary observations were made on the female reproductive tract. Four nests (N5, N8, N15, N16) were collected on the second field expedition, a compact block of the soil containing each nest was transferred to a plastic container and then taken to the Laboratório de Ultraestrutura Celular at the Federal University of Viçosa (UFV), MG, Brazil. In the laboratory, the soil blocks were

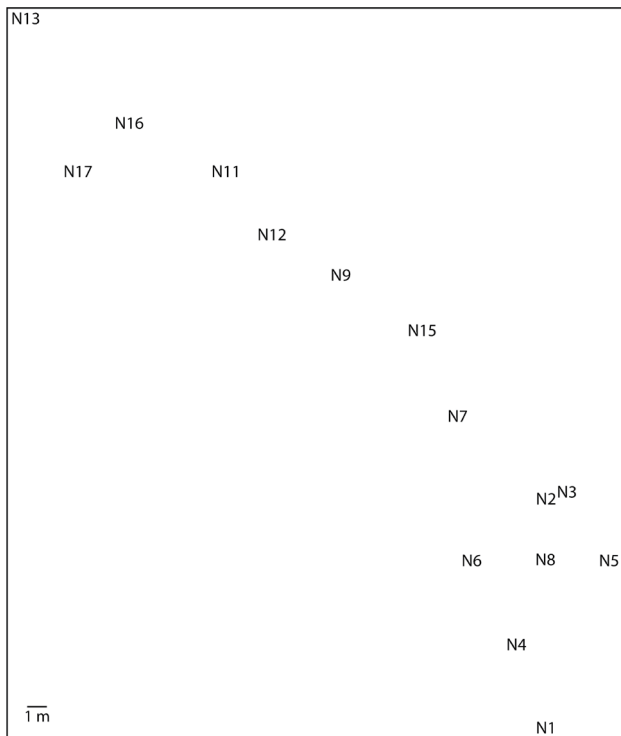


Fig. 3: Nest distribution at the APA Morro da Pedreira, Santana do Riacho, Minas Gerais, Brazil.

carefully split in half from the top opening, exposing the chamber with the ergatoid queen, workers, intercaste specimens, and immature stadia. The ergatoid queen and 2 - 10 workers / intercaste specimens of these four nests were dissected to quantify differences in the female reproductive tract. After dissection, the nests (N1, N2 + N3, N5, N8, N10, N15, N16) were taken to the Laboratório de Sistemática, Evolução e Biologia de Hymenoptera at the Museum of Zoology, University of São Paulo (MZSP), SP, Brazil, and kept alive for laboratory observations. The nest N14 was collected on 6 March 2021, and kept alive until 24 June 2023 by Fabiano F. Albertoni. Field observations were made by two researchers, M.A.U. and L.P.P., on the behavior of *H. primavesi* to determine its diet preferences. In total, 32 hours of observation on foragers from six nests (N4, N5, N6, N7, N8, N9) were carried out (Figs. 2A - B, Fig. 3). The nest N4 was excavated only at the end of the first field expedition and the colony was collected and kept alive at MZSP for laboratory observations. The nest N9 was found on the first field expedition, marked in the field, and on the second field expedition, it was excavated, but no specimens of *H. primavesi* were found. The nest N13 was excavated on the same day it was found and all the colony was fixed in EtOH 95% due to the presence of males, which have not yet been described for this species.

Nest excavation

To determine the external and internal structure of *Hylomyrma primavesi* nests, they were excavated using hand shovel and hand hoe by first digging a trench approximately 10 cm away from the nest entrance (Figs. 4A - I).

The trench was extended by carefully shaving away soil with a shovel, brushes, and spatula in the direction of the nest entrance. Since the nest structure of *H. primavesi* was initially unknown, after locating a tunnel, excavation continued very carefully downward in order to locate possible additional tunnels and chambers. Ants were collected with sterilized soft forceps and dry brush. Nest entrance, tunnel, and chamber dimensions were recorded, including: the entrance measures, length (maximum length along vertical axis), width (maximum length along horizontal axis parallel to the excavation plane), and depth (maximum length along horizontal axis perpendicular to the ground surface, excluding the entrance tunnel) (Tab. S1, as digital supplementary material to this article, at the journal's web pages).

Data processing

The specimens were identified using keys to ant genera from BACCARO & al. (2015) and the *Hylomyrma*'s species key in ULYSSÉA & BRANDÃO (2021). All adults (workers, intercaste specimens, males, and ergatoid queens), immature stadia (eggs, larvae, and pupae), and nest entrances were deposited at the Ant Collection of the MZSP. Most of the material were fixed with 95% alcohol; four larvae (MZSP77139, MZSP77140, MZSP77141, MZSP77142), two males (MZSP77143, MZSP77144), two ergatoid queens (MZSP77145, MZSP77146), and 11 workers / intercaste specimens were dry-mounted (MZSP77147, MZSP77148, MZSP77149, MZSP77150, MZSP77151, MZSP77152, MZSP77153, MZSP77154, MZSP77155, MZSP77156, MZSP77157).

The terminology of external morphology, measurements, and categorization (i.e., young or nearly adult larvae) for larvae follows WHEELER & WHEELER (1960, 1976, 1977) and for adults follows HÖLLDOBLER & WILSON (1990), BOLTON (1994, 2000), SERNA & MACKAY (2010), KELLER (2011), BOUDINOT (2015), and AIBEKOVA & al. (2022). To characterize pilosity, WILSON (1955) was used. The forewing venation terminology follows the proposal of YOSHIMURA & FISHER (2011, 2012), hindwing terminology follows YOSHIMURA & FISHER (2012), and MELO & al. (2012) was used for vein terminology. The integument sculpture follows HARRIS (1979) and ULYSSÉA & BRANDÃO (2021), and the terminology of the female reproductive tract follows FARDER-GOMES & al. (2019a, b).

The adults were examined with a Leica (Heerbrugg, Switzerland) MZ9.5 stereomicroscope at magnifications up to 60 × at MZSP in the Laboratório de Sistemática, Evolução e Biologia de Hymenoptera. All measurements were taken using this stereomicroscope with an ocular micrometer scaled in 0.1 mm and are given in millimeters (minimum - maximum). The following standard measurements were used (ULYSSÉA & BRANDÃO 2021: fig. 1):

CI – Cephalic index, $(HW \times 100) / HL$.

GL – Gaster length: maximum length of gaster in lateral view, excluding sting.

GS1L – First gastral segment length: maximum length of the first gastral segment in lateral view.



Fig. 4: *Hylomyrma primavesi* nest. **A)** nest excavation tools; **B)** nest entrance marked; **C)** nest entrance detail; **D - E)** nest excavation process; **F - H)** images of the excavated nests, final chamber marked on N15, N5, and N8, respectively; and **I)** nest N2 + N3 without the final chamber.

GW – Gaster width: maximum width of gaster in dorsal view.

HL – Head length: maximum length of head in full-face view, from the median anterior clypeal margin to the posteriormost margin of the head medially.

HW – Head width: maximum width of head in full-face view, measured posterior to compound eyes.

ML – Mandibular length: maximum distance from the outer base of mandible to its apex in dorsal view.

MOD – Maximum ocular diameter: maximum diameter of the eye measured with the head in lateral view, with the margins of the eye in the same plane of focus.

OI – Ocular index, $(MOD \times 100) / HW$.

PNW – Pronotal width: maximum width of pronotum in dorsal view.

PSL – Propodeal spine length: maximum length from the propodeal spine apex to the middle of a transversal imaginary line across the base of propodeal spine, in lateral view.

PL – Petiolar length: maximum length of petiole in lateral view, from the anteriormost visible point of the tergo-sternal petiolar suture to the insertion of the postpetiole.

PW – Petiolar width: maximum width of petiole in dorsal view.

PPL – Postpetiolar length: maximum length of postpetiole in lateral view, from the anteriormost visible point of the tergo-sternal postpetiolar suture to the insertion of the gaster.

PPW – Postpetiolar width: maximum width of postpetiole in dorsal view.

SI – Scape index, $(SL \times 100) / HW$.

SL – Scape length: maximum chord distance from the base (excluding radicle) to the apex of the antennal scape, with the head in full-face view.

TL – Total length, sum of the following measures: ML, HL, WL, PL, PPL, and GL.

WL – Mesosomal length or Weber's length: maximum diagonal length of the mesosoma from upper curvature of the pronotum to the posteroventral corner of the metapleuron, in lateral view.

Four ergatoid queens, 10 intercaste specimens and six workers, all dark-colored, were dissected under the Olympus (Tokyo, Japan) SZ40 stereomicroscope at magnifications up to $40 \times$ at the Laboratório de Ultraestrutura Celular, UFV, in saline solution (0.1 M NaCl, 0.1 M KH_2PO_4 , 0.1 M Na_2HPO_4) to obtain their reproductive tracts, which were transferred to Zamboni's fixative solution (STEFANINI & al. 1967) 24 h at $5^\circ C$. Afterwards, the reproductive tracts were dehydrated in a graded ethanol series (70 - 99%) and embedded in Historesin (Leica Biosystems Nussloch GmbH, Heidelberg, Germany). Sections ($4 \mu m$ thick) were stained with hematoxylin and eosin; they were analyzed and photographed using a light microscope Leica DM4000 B LED.

High-resolution images of the whole female reproductive tracts, larva, ergatoid queen, male, wings, and intercaste specimens were taken with a Leica M205 C stereomicroscope with coupled camera Leica MC190 HD and the software LAS V4.12 at the Laboratório de Sistemática, Evolução e Biologia de Hymenoptera, MZSP. Larval morphology was also examined with Scanning Electron Microscopy in the Laboratório de Microscopia Eletrônica, MZSP, with a Carl Zeiss (Cambridge, England) microscope LEO440. The specimens were sputter-coated with gold. Larval measurements were taken using the ImageJ software (SCHNEIDER & al. 2012). Photos of living *Hylomyrma primavesi* were taken using a Canon (Tokyo, Japan) 5D Mark II with Canon MP-E 65 MM f / 2.8 1 - 5 \times Macro lens and Canon MT-24E \times Macro Twin Lite flash. All images were cropped and edited using Adobe Photoshop software and plates were organized using Adobe Illustrator software. The map was created using Quantum GIS version 2.18.7-1 (QGIS.org 2021).

Results

Nest location and architecture

After finding foragers by chance, crumbs of cream crackers, almonds, or grape raisins were offered to them. All 17 nests (Tab. S1) were located by following the foraging workers returning to their nest with the bait (Videos S1 - 2). Fifteen out of the 17 nests located were clustered

in a small area at the Q16 region (Fig. 4). A straight line between the most distant nests, N1 and N13, was approximately 45 m long. Only two nests, N10 and N14, were found at the Alto Palácio region.

Ten nests were excavated and measured. All nests had a very inconspicuous tube entrance (Figs. 4B - C), with a single entrance hole approximately 0.2 - 0.5 cm in diameter. The tube entrances were mainly curved (invertedly U-shaped), built at the vegetation tuft's base, and composed by grains of sand and small pieces of grass. In the colony kept alive in the laboratory (N14), foragers built a straight tube entrance (Figs. 5G - H). The tube entrances of N5 and N6 were built horizontally between rotten leaves on the floor. Each nest had one tunnel that was mostly straight and perpendicular to the surface and terminated with one final chamber that contained eggs, immature stadia, and adults (Figs. 4F - I, Fig. 5K). The tunnel varied from 0.3 - 0.9 cm in width and 4.9 - 8.6 cm in length, while the oval chamber was flattened or slightly domed with the horizontal dimension greater than the vertical, and varied from 0.8 - 1.7 \times 1 - 3.3 cm in width and 0.4 - 1 cm in height. The deepest nest was 9 cm deep and the shallowest was 5.5 cm deep. N2 and N3 were 23 cm apart and belonged to the same colony. N3, understood as a temporary nest, was a small horizontal nest with a tunnel and a final chamber built at the base and between the roots of a shrub. From the nest entrance to the chamber's final edge, N3 measured 3.5 cm. Foragers were observed leaving N3, going into N2, excavating N2, and returning to N3. N2 had a tube entrance, a straight tunnel 8 cm deep, and no final chamber. The colonies comprised approximately 7 - 65 workers and intercaste specimens per colony, and all colonies had just one ergatoid queen (Figs. 5L - M, Tab. S1).

Biological notes

Standardized field observations on the behavior of *Hylomyrma primavesi* were performed at the Q16 region over three days (1 to 3 March 2021) during the rainy season under windy and cloudy weather, high humidity, and elevated temperatures between 10:00 and 15:00. Four nests (N5, N6, N8, N9) and its foragers were observed during four straight hours in the morning (08:30 to 12:30, 8:36 to 12:36), while two (N4, N7) were observed for eight consecutive hours (09:38 to 17:38), totaling 32 hours. The observations were carried out during the day, because in the first days in the field it was observed that this was their period of activity. Observational data are summarized on Table S1.

Workers and intercaste specimens foraged solitarily around the nest over short distances (9 - 98 cm), up to 4.10 m from the nest entrance. No recruitment was recorded. Only for a few minutes were 3 - 4 foragers observed exploring together (Fig. 5N, Video S3). They tended to avoid interactions with other ants, especially with *Linepithema* MAYR, 1866 ants, which are very abundant in the sampling sites and highly active during day times. *Linepithema* are recognized not only for their territorial behavior and aggressiveness, often attacking other ant species, but



Fig. 5: Images of *Hylomyrma primavesi*. **A - M)** taken in captivity by Fabiano F. Albertoni (N14); and **N)** taken at the APA Morro da Pedreira, Santana do Riacho, Minas Gerais, Brazil, by M.A.U. (N5). Central ocellus of the ergatoid queen (EQ) marked on **L**; reproductive (EQ) and non-reproductive workers / intercaste specimens (W / I) marked on **M**; and foragers inside the yellow circle on **N**.

also for their superior mobility, recruitment ability, and omnivory (TOUYAMA & al. 2003, COSTA-MILLANEZ & al. 2014). For example, one *Hylomyrma primavesi* forager was returning to the nest carrying its prey. Upon encountering a *Linepithema* worker, the *H. primavesi* forager promptly retreated, hid between the grass, and subsequently resumed its trajectory towards the nest. During aggressive interactions, the *Linepithema* workers first

quickly inspected the *H. primavesi* foragers and attacked their body appendages. It was observed that a *H. primavesi* forager lost one antenna during one of these encounters. A single worker of *Linepithema* (TL about 2 - 3 mm) can dominate a *H. primavesi* forager (TL 5.32 - 5.78 mm), which protects itself by standing and bending the gaster downwards and forwards while trying to escape by going in the opposite direction of the attacker. A *Linepithema*

worker entering inside the *H. primavesi* nest was threat enough for one *H. primavesi* worker / intercaste to immediately leave the nest with a pupa between its mandibles, returning when the *Linepithema* left.

Most of the time, the foragers leave the nest and return to it without carrying anything between their mandibles. However, when they captured something, there was a preference ($n = 19$) for very small insects, such as Diptera larvae, Collembola, pupae of Diptera and Coleoptera, and adults of Scarabaeidae and Nitidulidae. The foragers also brought minute pieces of vegetable material and grains of sand to the nest. Few collected materials remained undetermined. In captivity, foragers of *Hylomyrma primavesi* accepted live larvae of clothes moths, cocoons of parasitoid wasps (Fig. 5F), living and dead larvae of beetles (e.g., Tenebrionidae, *Tenebrio molitor* LINNAEUS, 1758), dead mosquitos, and a dead fly offered to the foragers outside their nest (Figs. 5I - J). The dead *Tenebrio* larva, cut in two pieces, was offered to them during the night and in the morning seven foragers were seen exploring the resource. The dead fly was found by one *H. primavesi* forager and later by two other foragers, then all three moved the fly to a more hidden place, but it did not seem like a coordinated activity. One forager cut off the fly's head, and each of the other two pulled off a fly's leg. Live mosquito larvae were not accepted by the foragers.

No garbage pile was observed outside the nests. In the field, the workers / intercaste specimens were observed carrying grains of sand (Fig. 5C) from the nest and discarding them 5 to 18 cm from the nest entrance. In captivity, one dead worker / intercaste specimen was taken out of the nest (Fig. 5D).

In the laboratory, foragers from N15 were put into N16, and no conspecific aggression was observed. The N16 foragers only displayed antennation behavior while the N15 foragers which were antennated showed no special behavior. In captivity, foragers outside the nest tended to run and hide under or between the leaf-litter when disturbed. When the nest was disturbed, the workers / intercaste specimens left the nest carrying eggs, larvae (Fig. 5B), and pupae (Fig. 5A).

Morphological descriptions

Eggs: Whitish, semi-transparent, smooth, and oval as typically found in other ants; length:width circa 3:1 (Fig. 6A).

Larvae: Profile aphaenogastroid (Figs. 6B - C): narrowed at abdominal segments A3 - A4, diameter increases gradually both ways toward thoracic segment T3 and A5 - A8, body arched ventrally. **Young larvae** ($n = 2$, N13; MZSP77141, MZSP77142) (Figs. 6B - C): Total length 1.5 - 1.8 mm. **Nearly adult larvae** ($n = 2$, N15; MZSP77139, MZSP77140; $n = 1$, N13; MZSP-HYM-Lote15; Fig. 6B): Total length 3.8 - 4.1 mm.

Body segmentation visible, including three thoracic and 10 abdominal segments. Body setae sparse, of two types (Figs. 6C - D, F, H): (1) 0.021 - 0.077 mm, abundant, slightly curved, filiform with few or multiple short

branches of equal size giving a serrated appearance, tapered at the apex, on every segment; (2) 0.118 - 0.220 mm, few, sinuous, long, unbranched, with a fan-shaped apex, 4 - 6 present medially on the dorsal surface from T2 to A7, 2 present medially on the ventral surface from A3 - A7. Spiracles small (Fig. 6G). Cranium subhexagonal, occipital corners broadly rounded (Fig. 6J). Antenna (ant) small, with 3 spinulose sensilla arranged in a cluster (Fig. 6J). Clypeus (cl) subtriangular, with 2 pairs of setae (st) on the latero-anterior region near labrum (Fig. 6J). Labrum (lb) bilobed, divided by a thin longitudinal sulcus; each lobe with 3 spinulose sensilla on dorso-anterior region (ssd) arranged in a row, 4 spinulose sensilla on the edges between dorsal and ventral surfaces (ssdv), about 6 - 7 spinulose sensilla on ventral surface (ssv) (Figs. 6J - K). Mandible (md) pogonomymecoid; apical tooth heavily sclerotized, slightly curved and with rounded apex, longer than the 2 subapical teeth; anterior region of masticatory margin irregular (Figs. 6D, J, L). Maxilla (mx) paraboloidal, spinulose sensilla sparse, isolated or in short rows; maxillary palps (mxp) and galeae (ga) weakly sclerotized, digitiform, subequal in size, elongated and narrow; maxillary palps with 1 spinulose sensilla (ss) at the lateral margin and about 3 encapsulated sensilla (es) at the apex; galea with 2 encapsulated sensilla at the apex (Figs. 6J - K). Hypopharynx and labium (la) with numerous spinules in short arcuate transverse rows; labial palp (lap) with 3 spinulose and 2 encapsulated sensilla; opening of sericteries (se) a short transversal slit (20 - 24 μ m) (Fig. 6I). Anus (an) ventral, anal opening straight to convex (108 - 184 μ m) (Fig. 6E).

Worker ($n = 2$; holotype MZSP67412, paratype MZSP67413): GS1L (0.866 - 0.897), GW (0.866). As described by ULYSSÉA & BRANDÃO (2021).

Intercaste ($n = 1$; MZSP77147; Figs. 7B, D, F - G, I): CI 94.736, GL 1.393, GS1L 0.990, GW 1.021, HL 1.176, HW 1.114, ML 0.681, MOD 0.309, OI 27.777, PL 0.650, PNW 0.743, PPL 0.340, PPW 0.402, PSL 0.185, PW 0.294, SI 69.444, SL 0.773, TL 5.619, WL 1.377.

The reproductive tract of the non-reproductive females (workers $n = 6$ and intercaste specimens $n = 10$, from N5, N8, N15, N16) has a pair of small and narrowed ovaries connected by a pair of lateral oviducts, opening into a common oviduct. It was observed 1 ovariole (Ovr) per ovary (Ov), varying in the degree of maturation, with few intercaste specimens presenting developed eggs (Oc) (Figs. 8A - B, 9F).

Ergatoid queen ($n = 2$; MZSP77145, MZSP77146; Figs. 7B, D, F - G, I): CI (88.000 - 90.540), GL (1.393 - 1.733), GS1L (1.052 - 1.083), GW (1.269), HL (1.145 - 1.160), HW (1.021 - 1.037), ML (0.650 - 0.681), MOD (0.278 - 0.294), OI (27.272 - 28.358), PL (0.696 - 0.727), PNW (0.758 - 0.820), PPL (0.402 - 0.417), PPW (0.479), PSL (0.185), PW (0.325 - 0.340), SI (67.164 - 72.727), SL (0.696 - 0.743), TL (5.727 - 6.052), WL (1.377 - 1.393).

Color body, pilosity, and sculpture characters shared with conspecific workers (ULYSSÉA & BRANDÃO 2021), only slightly larger body size and more robust gaster. Cephalic dorsum with 3 small ocelli, the central ocellus is more

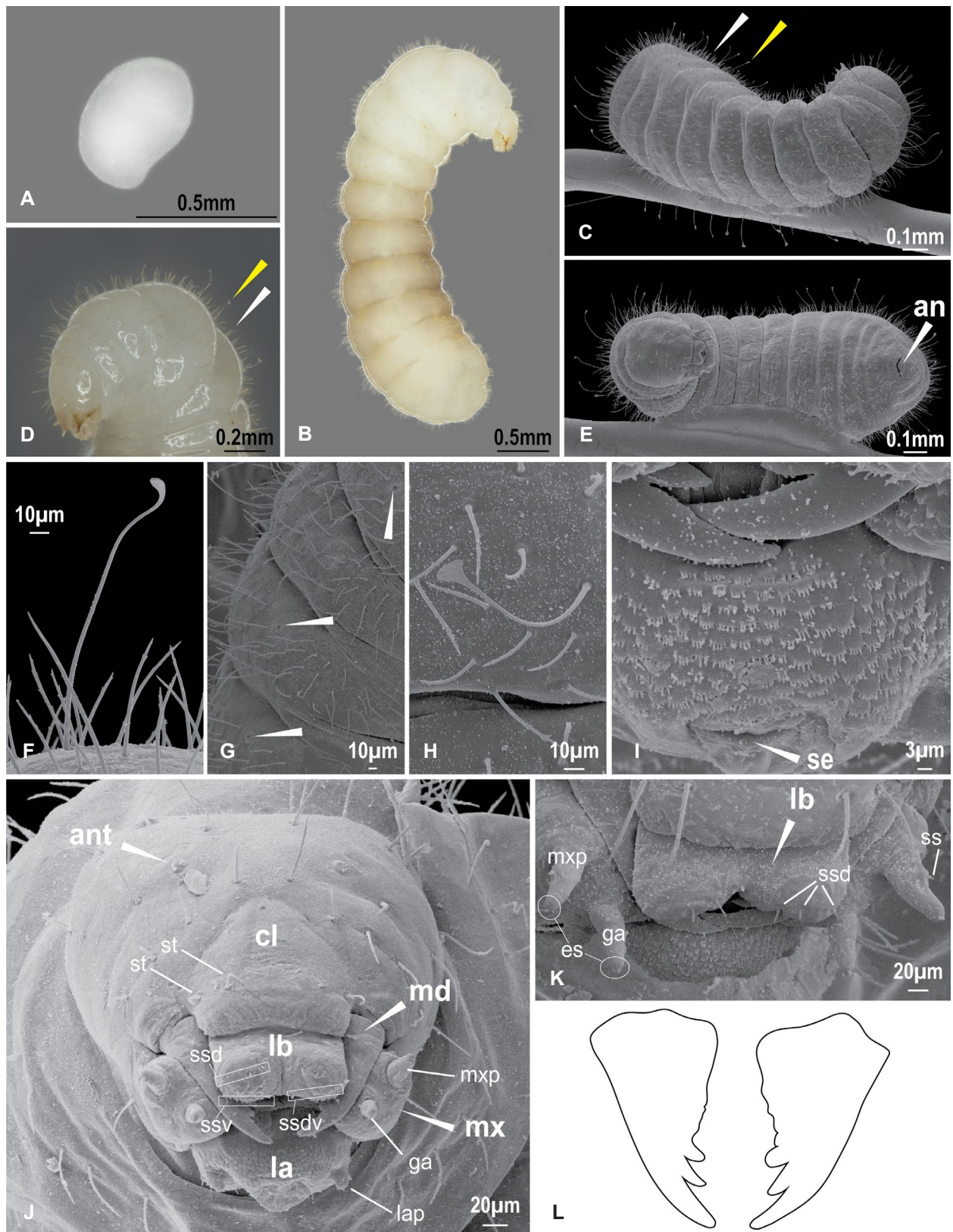


Fig. 6: Egg and larvae of *Hylomyrma primavesi*. **A)** egg; **B)** larva lateral profile; **C - D)** oblique view, larva setae type 1 (white) and type 2 (yellow) marked; **E)** ventral view, larva anus marked; **F, H)** detail of larva setae; **G)** larva spiracles marked; **I - K)** head frontal view; and **L)** drawing of the larva mandibles. Images A, B, D: N13; C, E, F: MZSP77142; G, H, I, K: MZSP77141; and J: MZSP77139. an: anus, ant: antenna, cl: clypeus, es: encapsulated sensilla, ga: galeae, la: labium, lap: labial palp, lb: labrum, md: mandible, mx: maxilla, mxp: maxillary palps, ss: spinulose sensilla, ssd: spinulose sensilla on dorso-anterior region, ssdv: spinulose sensilla on the edges between dorsal and ventral surfaces, ssv: spinulose sensilla on ventral surface, se: opening of sericteries, and st: seate.

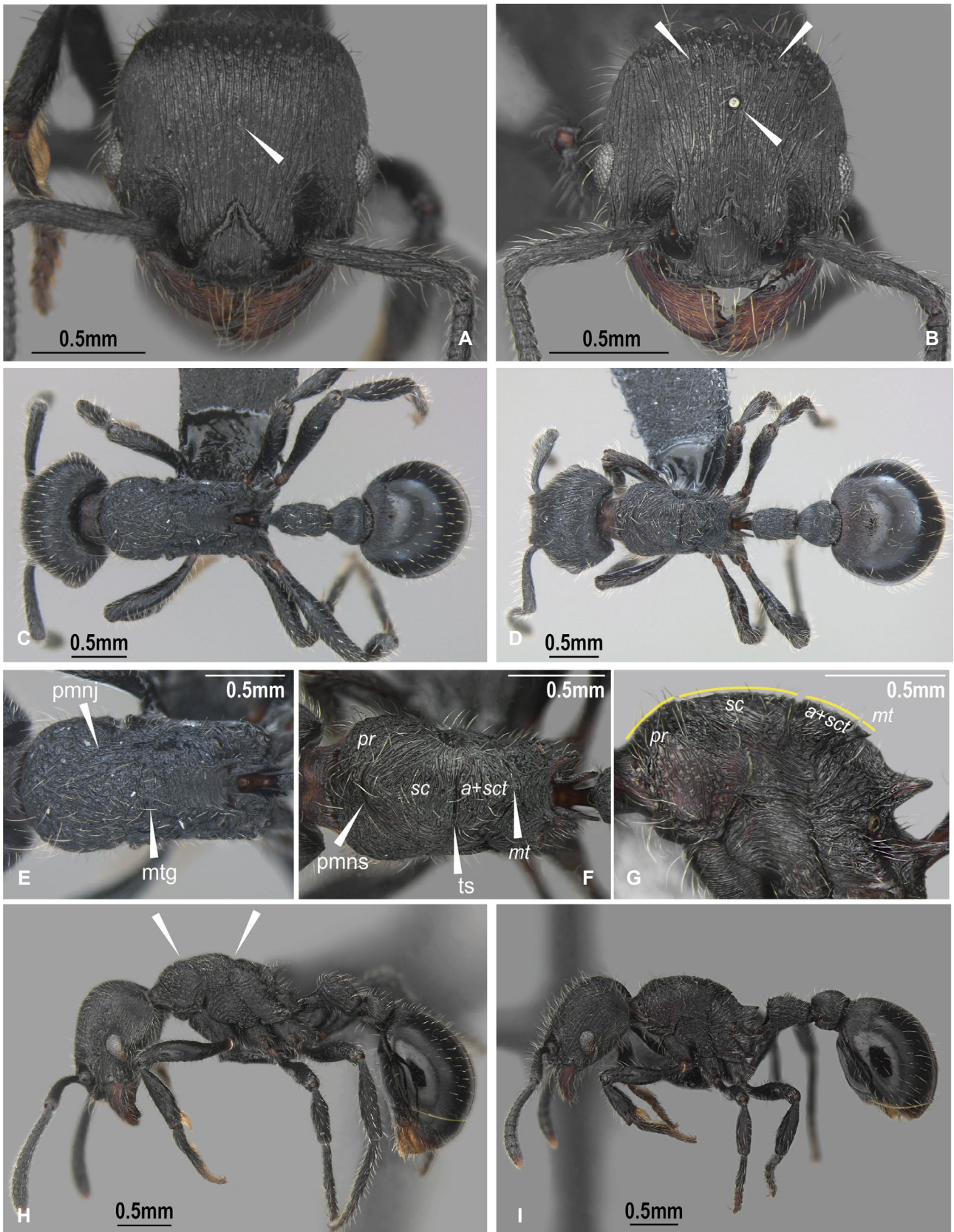


Fig. 7: Intercaste (A, C, E, H: MZSP77147) and ergatoid queen (B, D, F, G, I: MZSP77145) of *Hylomyrma primavesi*. **A - B)** frontal view, ocelli marked; **C - F)** dorsal view; and **G - I)** lateral view. a + sct: axilla and scutellum, mt: metanotum, mtg: metanotal groove, pmnj: promesonotal junction, pmns: promesonotal suture, pr: pronotum, sc: scutum, and ts: transscutal suture.

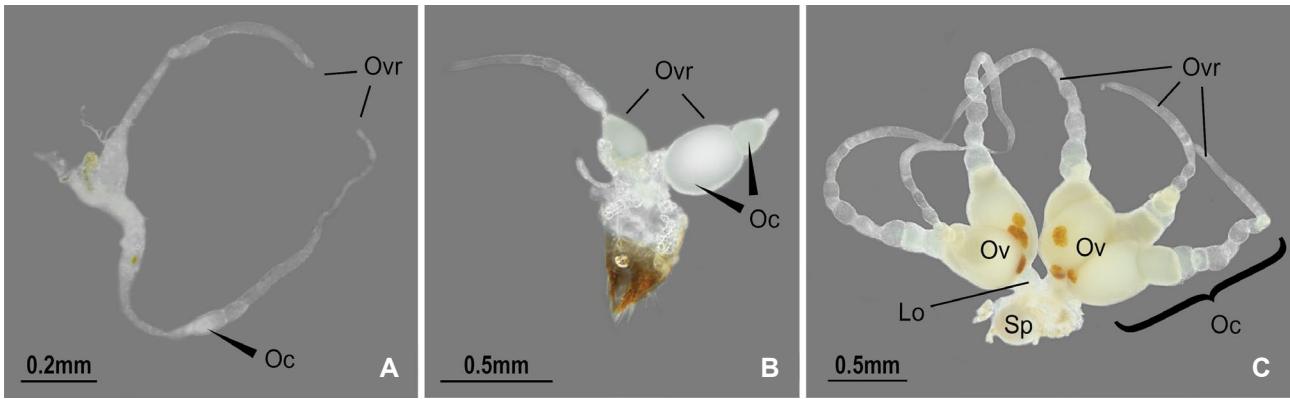


Fig. 8: The reproductive tract of *Hylomyrma primavesi*. **A)** undeveloped ovary of the worker (N5) showing the young oocyte; **B)** ovary of the intercaste with more developed oocytes (N8); and **C)** ovary of the ergatoid queen showing the oocytes in different developmental stages (N15). Lo: lateral oviduct, Oc: oocyte, Ov: ovary, Ovr: ovariole, Sp: spermatheca.

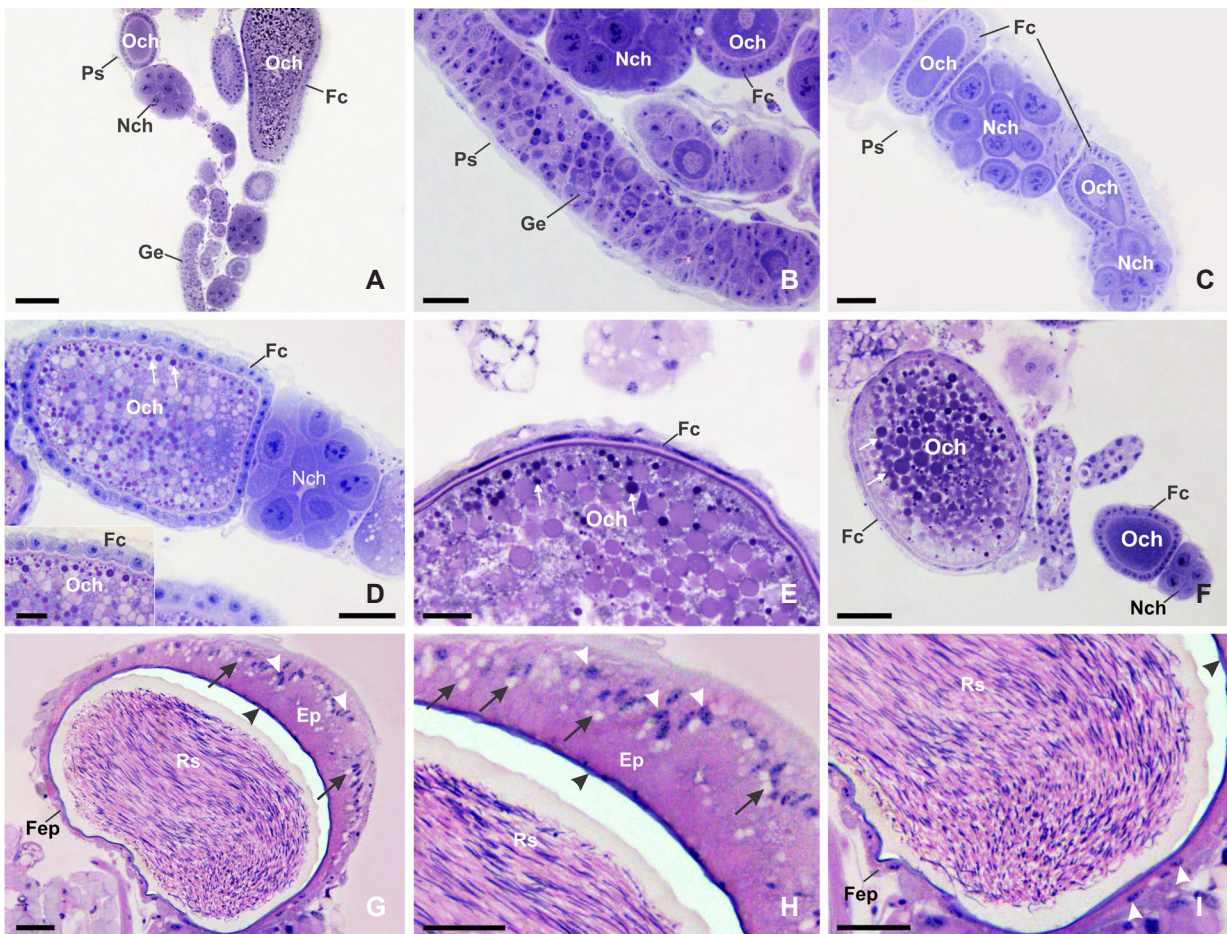


Fig. 9: Histological sections of the reproductive tract of *Hylomyrma primavesi* worker (F) and ergatoid queen (A - E, G - I). **A)** ovarioles showing the germarium, nurse cells, oocytes in different developmental stages, follicular cells, and peritoneal sheath; **B)** detail of the ovarioles; **C)** follicle at the early stage of development; **D)** oocytes in the late maturation stage surrounded by cuboidal follicular cells, yolk granules marked; **E)** detail of the oocyte surrounded by flattened follicular cells and the cytoplasm filled with yolk granules (white arrow); **F)** worker ovariole showing the nurse cells, oocytes in different developmental stages, and follicular cells, yolk granules marked; **G)** spermatheca showing the reservoir lumen lined by cuticle (black arrowhead), the columnar epithelium with many vacuoles (black arrow), the flattened epithelium, and the cell nuclei (white arrowhead); **H)** detail of the spermatheca columnar epithelium showing the nuclei rich in decondensed chromatin (white arrow) and many vacuoles (black arrow), and the reservoir cuticle (black arrowhead); and **I)** detail of spermatheca flattened epithelium showing the nuclei rich in decondensed chromatin (white arrow) and the reservoir cuticle (black arrowhead). Ep: columnar epithelium, Fc: follicular cells, Fep: flattened epithelium, Ge: germarium, Nc: nurse cells, Nch: nurse chamber, Oc: oocyte, Och: oocyte chamber, Ps: peritoneal sheath, Rs: spermathecal reservoir.

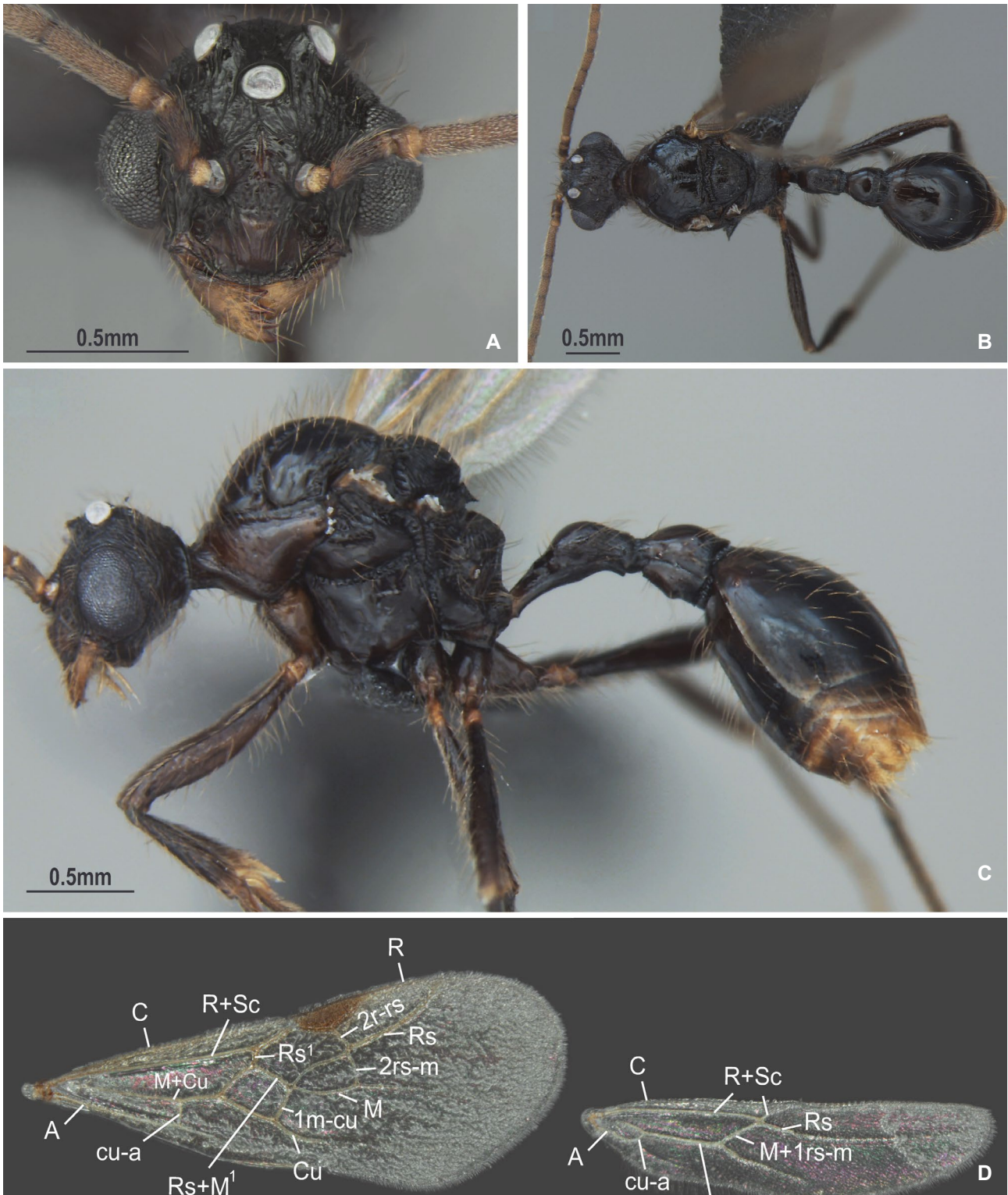


Fig. 10: Male (MZSP77144) of *Hylomyrma primavesi*. **A)** frontal view; **B)** dorsal view; **C)** lateral view; and **D)** wings. Venation – A: anal, C: costa, Cu: cubitus, cu-a: cubitus-anal crossvein, M: media, M + Cu: media-cubitus, M + 1rs-m: media-first radial sector-media, R: radial, Rs: radial sector, Rs + M¹: radial sector-media 1, R + Sc: radial-subcosta, 1 m-cu: first media-cubitus crossvein, 2rs-m: submarginal 1 + 2, and 2r-rs: second radial-radial sector crossvein.

developed; eye larger in diameter with 13 - 15 ommatidia. Dorsal margin of mesosoma convex. Promesonotal suture (pmns) well-marked. Scutum (sc) with regular-irregular to rugose striation, interspaces distinguishable; regular-

irregular striae assuming multiple directions. Parapsidal line and tegulum absent. Transscutal suture (ts) weakly marked (better observed in LV). Mesepisternal sulcus absent; mesepisternum covered with regular to irregular

and longitudinal striae, interspaces distinguishable. Axilla and scutellum (a + sct) not differentiated, scutoscutellar sulcus absent. Dorsal margin of mesoscutellar disc straight in lateral view. Metanotum (mt) small. Apterous.

The reproductive tract of the ergatoid queen (n = 4; N5, N8, N15, N16) has a pair of ovaries (Ov) connected by lateral oviducts (Lo), opening in a common oviduct associated with a rounded-shaped spermatheca (Sp) (Fig. 8C). Each ovary has 3 ovarioles (Ovr) (Fig. 8C) surrounded by a thin peritoneal sheath (Ps); each follicle of the ovaries has an oocyte (Oc) and a nurse chamber (Nch) (merostic polytrophic ovary) (Fig. 9A). The apical portion of the ovariole has a large cluster of germ cells, the germarium (Ge), near these cells the oocyte chamber (Och) is smaller than the nurse chamber, both chambers are covered by a layer of follicular cells (Fc), which are cuboidal in the oocyte chamber (Figs. 9B - C). In addition, the cluster of nurse cells has nuclei with decondensed chromatin (Figs. 9C - D).

As the oocyte moves toward the oviduct, it begins to increase in size due to the yolk accumulation (Fig. 9D). The follicular cells flatten in the mature oocyte chamber, and the cytoplasm is filled with yolk granules (Fig. 9E).

The spermathecal reservoir (Rs) has an epithelial cell layer lined by a cuticle that forms the internal coat (Figs. 9G - I); the upper region of the epithelium is formed by columnar cells (Ep) with many vacuoles, and the remainder is formed by flattened cells (Fep) (Figs. 9G - I); the cells presented rounded nuclei rich in decondensed chromatin (Figs. 9G - I).

Male (n = 1; MZSP77144; Figs. 10A - C): CI 84.000, GL 1.207, GW 0.897, HL 0.773, HW 0.650, ML 0.433, MOD 0.417, OI 64.285, PL 0.619, PNW 0.990, PPL 0.433, PPW 0.371, PW 0.278, SI 42.857, SL 0.278, TL 4.922, WL 1.455.

Black to dark brown body, lighter legs, mandibles, and antennae. Mandible mostly smooth, some longitudinal striae weakly marked, 4 teeth. Antennal scape short. Anterior margin of clypeus laterally rounded, unarmed. Eye large, oval. Head dorsum striate; regular to irregular striae assuming distinct directions, interspaces distinguishable; ventral surface partly smooth and partly covered with concentric striae. Promesonotal articulation is well-marked. Scutum mostly smooth. Notaulus well-marked, Y-shaped. Parapsidal line well-marked. Tegulum subtriangular and translucent. Mesepisternal sulcus well-marked; upper and lower mesopleural areas (previously named anepisternum and katepisternum) mostly smooth. Axilla subtriangular, laterally rounded. Scutoscutellar sulcus marked dorsally, continuous, longitudinally striate, striae regular, interspaces distinguishable. Scutellum longitudinally striate, striae regular, interspaces distinguishable. Mesoscutellar disc trapezoidal, convex in lateral view. Propodeum mostly longitudinally striate, regular striae weakly marked, interspaces distinguishable. Propodeal lobe rounded. Petiole dorsum partly smooth and partly longitudinally striate, striae regular to irregular, interspaces distinguishable. Postpetiole smooth. Gaster shiny, smooth.

On forewing, well-developed pigmented pterostigma; venation well-developed, with costa I, radial I, radial sector (Rs), media (M), cubitus (Cu), anal (A), radial-subcosta (R + Sc), media-cubitus (M + Cu), cubitus-anal crossvein (cu-a), first media-cubitus crossvein (1 m-cu), radial sector-media 1 (Rs + M¹), second radial-radial sector crossvein (2r-rs), and second radial sector-media (2rs-m); basal (B), sub-basal (BS), discal (D), sub-marginal 1 (SM¹), sub-marginal 2 (SM²), and marginal (M) cells present. Hindwing with 7 hamuli; venation underdeveloped, with costa (C), radial-subcosta (R + Sc), radial sector (Rs), media-cubitus (M + Cu), media-first radial sector-media (M + 1rs-m), anal (A), and cubitus-anal (cu-a) present; jugal lobe absent. Wings covered with unbranched and short setae with sharp apex (Fig. 10D; MZSP77143).

Discussion

This study adds important biological information to what little is currently known about the species of *Hylomyrma*. It describes for the first time the ergatoid queen, male, immatures, female reproductive tract, nest architecture, and feeding preferences of *Hylomyrma primavesi*, being also the first formal description of a nest, the female reproductive tract, and the ergatoid queen for the genus.

Feeding

Our knowledge on the feeding behavior of *Hylomyrma* was restricted to observations made in the laboratory by Wilson (WHEELER & WHEELER 1960), to the nest of *Hylomyrma blandiens* collected by M.A.U. (ULYSSEÁ & BRANDÃO 2021), and also inferred from a morphometric approach (SILVA & BRANDÃO 2010). In captivity, *Hylomyrma versuta* accepted *Drosophila* spp., Collembola, and a few other selected small invertebrates (WHEELER & WHEELER 1960). Inside the nest of *H. blandiens*, plant remains and parts of an adult Curculionidae were found (ULYSSEÁ & BRANDÃO 2021). Additionally, *Hylomyrma reitteri* was characterized as a midsized epigeaic generalist predator based on its medium size, triangular mandibles, well-developed eyes set at borders of comparatively large heads, and eyes placed relatively high on the head, away from the mandibles (SILVA & BRANDÃO 2010).

In the field, *Hylomyrma primavesi* has a preference for very small insects, but in captivity they accepted small to bigger items such as *Tenebrio molitor* larvae (Figs. 5I - J). Our observations on *H. primavesi* corroborate the definition of *Hylomyrma* ants as generalist predators of small arthropods. In short, the Pogonomyrmecini include species apparently exclusively granivorous (i.e., *Patagonomyrmex*, JOHNSON & MOREAU 2016, FERNANDEZ & SERNA 2019), carnivores (i.e., *Hylomyrma*) and harvester-scavenger ants (i.e., *Pogonomyrmex*, BELCHIOR & al. 2012, JOHNSON 2015, JOHNSON & MOREAU 2016).

The *Hylomyrma primavesi* workers / intercaste specimens are solitary foragers and non-aggressive, like species of the other genera of Pogonomyrmecini (JOHNSON 2015, JOHNSON & MOREAU 2016). Foragers explore an area close to the nest entrance, whereas foragers of *Pogonomyrmex*

excelsior JOHNSON, 2021 can travel up to 30.00 - 40.00 m from the nest (JOHNSON 2021). Only for a few minutes, 3 - 4 foragers were observed exploring the same resource together, but not in a coordinated way. This behavior was observed more often in the laboratory, and it is probably associated with the limited foraging area and food resources when in captivity. Foragers also captured minute pieces of plant material and grains of sand, apparently used for nest-tube-entrance maintenance.

Nesting

Scattered observations of live specimens and a few label data indicated that *Hylomyrma* nests are located in fallen twigs and rotten logs in the leaf-litter (ULYSSEÁ & BRANDÃO 2021). In 2013, one small colony of *Hylomyrma blandiens* was collected inside a dead twig (10 cm length × 4 cm diameter) found among the leaf-litter in Madre de Dios, Peru (ULYSSEÁ & BRANDÃO 2021). More recently, parts of two colonies of *Hylomyrma reitteri* were found in the same circumstances. One was collected in Florianópolis, Brazil (P. Hönle, unpubl.; Fig. S1) and contained only two workers with brood. The second had workers and a queen, and was found in a rotten log in Salesópolis, Brazil (L.P. Prado, unpubl.). Additionally, in Igaratá, Brazil, two queens of *H. reitteri* were found nesting inside a rotten log (32 cm length × 52 cm diameter), which also had other ant species' nests (*Solenopsis* sp.8 and *Pheidole sigillata* WILSON, 2003) (T.T. Fernandes, unpubl.). These species shared the same log, but not the same nest. Regarding *Hylomyrma balzani* (EMERY, 1894), in 2021, two nests of this species were found at the Federal University of Viçosa, Brazil. One was inside a 15 cm hollow branch at Mata da Biologia (ANTWEB1053900), and the second nest was inside a small log at Mata Paraíso (ANTWEB1053901) (G. Figueiredo, unpubl.). In 2022, also a nest of *H. balzani* was found inside a small log at Dores do Rio Preto, state of Espírito Santo, Brazil (ANTWEB1047385) (E.M. de A. Vieira, unpubl.).

The nesting preferences of *Hylomyrma primavesi* are different from those of *Hylomyrma blandiens* and *Hylomyrma reitteri*. *Hylomyrma primavesi* nests in sandy soils, and often the nest is located near the base of grasses, similarly to some soil-nesting species of the genus *Patagonomyrmex* (JOHNSON & MOREAU 2016) and the *Hylomyrma*-sister-genus *Pogonomyrmex*, as the Argentine harvester ant *Pogonomyrmex mendozanus* CUEZZO & CLAVER, 2009 (CUEZZO & CLAVER 2009) and the in Brazil widely distributed *Pogonomyrmex abdominalis* SANTSCHI, 1929 and *Pogonomyrmex naegelii* EMERY, 1878 (BELCHIOR & al. 2012, JOHNSON 2015). Most of the ten excavated nests of *H. primavesi* had similar characteristics – a curved tube entrance, one straightened tunnel perpendicular to the surface, and a final oval chamber between 5.5 and 9 cm deep (Figs. 4B - C, F - I). Comparatively, the nests entrance of *P. abdominalis* and *P. naegelii* vary, ranging from a cryptic entrance to a 15 cm tumulus (JOHNSON 2015). The last has nests with one or two inconspicuous entrances and five to seven interconnected oval chambers

located 3 - 70 cm deep (BELCHIOR & al. 2012), while nests of *Pogonomyrmex pencosensis* FOREL, 1914, a species also occurring in South America, can lack a tumulus, have 10 - 30 chambers and reach a depth of 60 - 70 cm (JOHNSON 2015). The nest of a mature colony of the Nearctic species *Pogonomyrmex occidentalis* (CRESSON, 1865) can reach 39.37 m and have five main tunnels each with additional short branches (LAVIGNE 1968). The tube entrances of two nests of *H. primavesi* (N5, N6) were built horizontally between the rotten leaves on the floor. Part of a nest of *Hylomyrma* aff. *montana* PIERCE, BRANSTETTER & LONGINO, 2017 with few workers and brood among the rotten leaves was also collected on the forest floor in Ecuador (P. Hönle, unpubl.; Figs. S2 - S3). Thus, possibly *H. primavesi* is not the only species of the genus that nests in the soil.

The higher or the lower number of nests found at the sampling sites may not be related with the frequency of nests at these localities, but with the ease of locating a forager (Figs. 1C - D). The area at the Q16 region is a mosaic composed of exposed whitish sand soil and sparse vegetation, mainly grassland and few shrub species, while the Alto Palácio region is characterized by dense grassland vegetation covering all the blackish sand soil. As *Hylomyrma primavesi* has black color, it was easier to find the foragers on the whitish soil background than at the Q16 region. In addition, as part of the Long-Term Ecological Research PELD Campos Rupestres da Serra do Cipó, a study recorded the presence of *H. primavesi* in 22 out of the 60 samples from Alto Palácio region and in eight out of the 60 samples from Q16 region (i.e., "*Hylomyrma prox*(=near) *reitteri*", LANA 2015).

Colony size

The colony size of *Hylomyrma* is relatively small when compared with *Patagonomyrmex* – around 300 - 400 workers (JOHNSON & MOREAU 2016), and *Pogonomyrmex* – ranging from hundreds to thousands of workers, like *Pogonomyrmex naegelii* with 166 - 580 workers (BELCHIOR & al. 2012) and mature colonies of *Pogonomyrmex barbatus* (SMITH, 1858) with 10,000 – 12,000 workers (GORDON 1986). The biggest colony of *Hylomyrma primavesi* had 65 workers / intercaste specimens in addition to one ergatoid queen. The smallest colonies, with only 7 and 8 workers / intercaste specimens, were those that had the shallowest nest (N8 4.8 cm deep) and the nest without the final chamber (N2 + N3), respectively. Presumably, these were nests in early stages of the colony. The majority of the colonies had more than 43 workers / intercaste specimens, twice the number registered in the known colonies of *Hylomyrma blandiens* (21 workers) (ULYSSEÁ & BRANDÃO 2021), *Hylomyrma balzani* (ANTWEB1053900 = 23 workers; ANTWEB1047385 = 17 workers), and the *Hylomyrma reitteri* from Salesópolis (20 workers / intercaste specimens). These differences among the colony size and nest architecture of these three species may be due to the limitations imposed by extrinsic factors, like the nesting site (soil or twig / log), or by intrinsic factors, like genotype and evolutionary history (O'FALLON & al. 2022).

However, knowledge about nesting, as well as variations in the structures of the species' nests promoted by environmental and ecological factors remains incipient for ants in general. Most colonies of *H. primavesi* also contained eggs and immatures, but just one colony (N13) had males. The N14, which was maintained for approximately 28 months, did not produce any male or queen.

Castes: occurrence, morphology, and development

One important result of this study is the recognition of the first *Hylomyrma* species which has only ergatoid queens and no winged queens. The known nest of *Hylomyrma blandiens* had one dealated queen and two callow queens with wings (ULYSSEÁ & BRANDÃO 2021), the *Hylomyrma reitteri* nest from Salesópolis and two nests of *Hylomyrma balzani* (ANTWEB1053900, ANTWEB1047385) had one dealated queen, but all excavated nests of *Hylomyrma primavesi* had just one ergatoid queen. This fact can explain why the nests have a clustered distribution (Fig. 3), since the ergatoid queen disperses on foot to found a new colony, usually near its natal nest (PEETERS & MOLET 2010, PEETERS & ADAMS 2016).

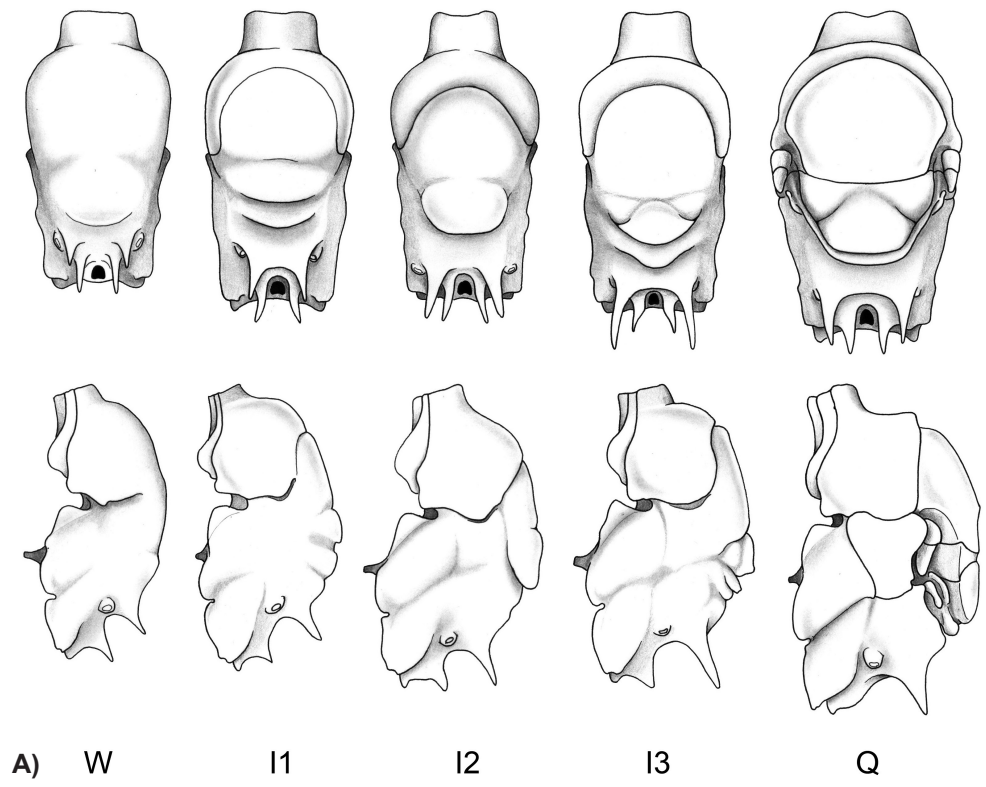
In the literature, the presence of ergatoid queens is recorded for *Hylomyrma immanis* by PEETERS (2012) and JOHNSON (2015). The last author also inferred that *Hylomyrma blandiens*, *Hylomyrma dentiloba* (SANTSCHI, 1931), *Hylomyrma dolichops* KEMPF, 1973, *Hylomyrma sagax* KEMPF, 1973, and *Hylomyrma transversa* KEMPF, 1973 have ergatoid queens. PEETERS (2012) and JOHNSON (2015) based their deductions from the external morphological description of the *Hylomyrma* queens made by KEMPF (1973) (C. Peeters and R.A. Johnson, both pers. comm.). Both relied on two of KEMPF's quotes: "Ocelli present but inconspicuous", and "Wings unknown". M.A.U., after studying almost all material studied by KEMPF plus recent material of *Hylomyrma*, observed that all queen specimens of these species exhibit evidence of the presence of wings, and therefore the record of ergatoid queens in the literature is based on misinterpreted evidence. Beside *Hylomyrma primavesi*, intercaste specimens having queen-like morphology, which may be understood as ergatoid queens in the future, are present in four species of *Hylomyrma* – *Hylomyrma balzani*, *Hylomyrma lopesi* ULYSSÉA, 2021, *Hylomyrma montana*, and *Hylomyrma reitteri* (Figs. 11A - C; ULYSSÉA & BRANDÃO 2021: figs. 2A - C). These four species also have winged queens, and there is no further information if the queen-like intercaste specimens occur together with winged queens in the same nest and which role it performs in the colony. Additional research should examine the nest, reproductive biology, and colony foundation behavior of these species.

Regarding the internal morphology, the structure of the female reproductive tract of *Hylomyrma primavesi* is similar to that described in other Myrmicinae ants, such as *Acromyrmex* MAYR, 1865 (ANTUNES & al. 2002, FARDER-GOMES & al. 2019a), *Eutetramorium* EMERY, 1899

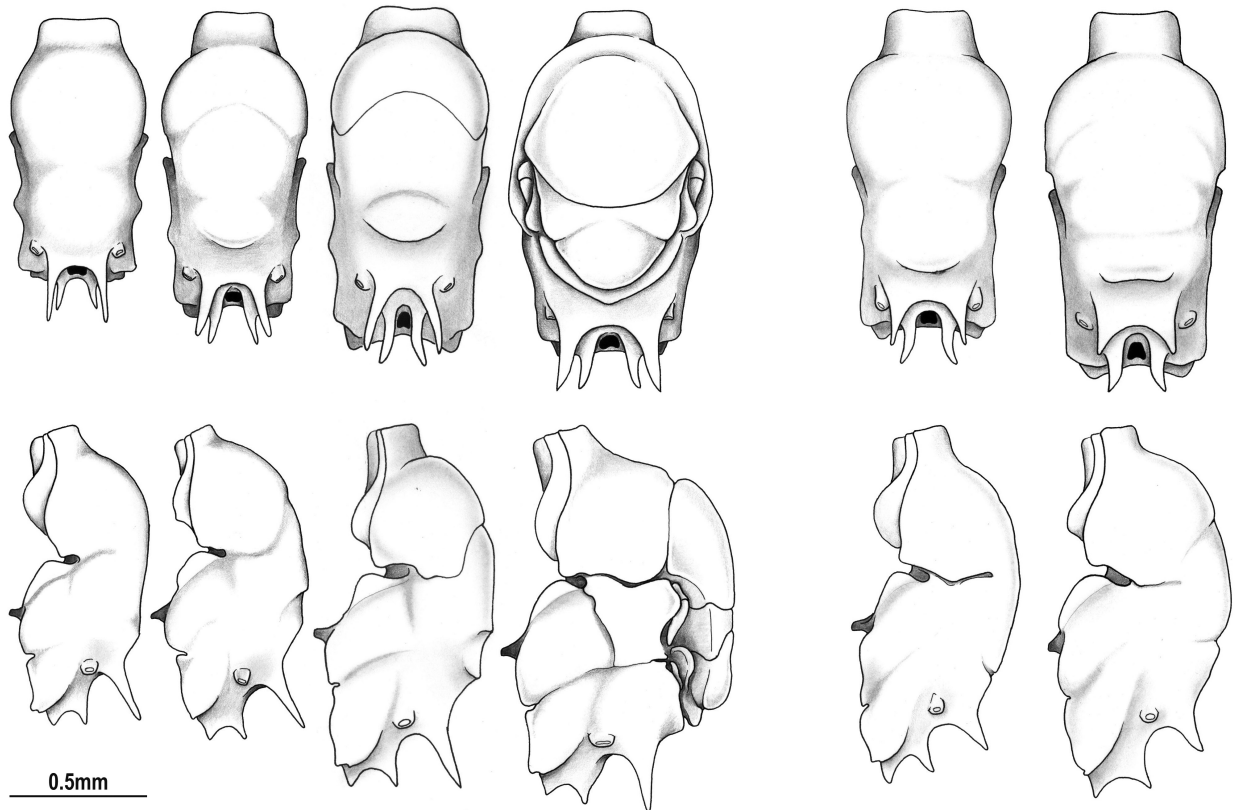
(HEINZE & al. 1999), and *Pogonomyrmex* (see JOHNSON 2010). However, the number of ovarioles of the ergatoid queen of *H. primavesi* (3 ovarioles) is much lower than that observed in other ergatoid queens of the same tribe, such as *Pogonomyrmex naegelii* with 8 ovarioles (JOHNSON 2015), *Pogonomyrmex pencosensis* with 8 - 13 ovarioles (JOHNSON 2010, 2015) and *Pogonomyrmex laticeps* SANTSCHI, 1922 with 12 - 15 ovarioles (PEETERS & al. 2012). The workers and intercaste specimens of *H. primavesi* had a lower number of ovarioles than the ergatoid queens and lacked the spermatheca, similar to that reported for *P. pencosensis*, whose workers and ergatoid queens also display a high degree of morphological similarity (JOHNSON 2010). Thus, it is possible to suggest that workers and intercaste specimens lay unfertilized eggs used to feed the brood, the ergatoid queen, and other workers and intercaste specimens. The spermatheca is of paramount importance for the adaptative success of ants, as it guarantees sperm storage and viability until oocyte fertilization (MALTA & al. 2014, GOTOH & al. 2017). Although *H. primavesi* workers and intercaste specimens lack a spermathecal gland, the reservoir wall composed of columnar cells with many vacuoles suggests a secretory epithelium, which synthesizes substances to maintain sperm viability (ORTIZ & CAMARGO-MATHIAS 2007, PASCINI & MARTINS 2017, FARDER-GOMES & al. 2019a). Moreover, epithelial cells of the reservoir secrete the cuticle that protects and keeps the sperm isolated from the hemolymph (PASCINI & MARTINS 2017, FARDER-GOMES & al. 2019b).

Hylomyrma primavesi was described based on workers and intercaste specimens (Figs. 7A, C, E, H, Fig. 11C), both having similar sizes and external morphology, but intercaste specimens are recognized by the presence of a minute central ocellus or a more or less pronounced depression on the region of the central ocellus, and the promesonotal junction and metanotal groove distinguishable by a slight depression more or less accentuated and by altered sculpture (ULYSSEÁ & BRANDÃO 2021). According to the authors, the majority of all the known specimens of *H. primavesi* were classified as intercaste specimens, leading them to the hypothesis that those intercaste specimens could perform reproduction. On the contrary, this study demonstrates that the reproduction in this species is performed by an ergatoid queen, which presents a higher degree of mesosoma's differentiation when compared with the intercaste specimens or workers – that is, pronotum, promesonotal suture, scutum, transscutal suture, axilla + scutellum, metanotal groove, and metanotum (Figs. 7F - G).

In the field and in the laboratory, it was observed that the ergatoid queen moves slowly, sometimes dragging the gaster along the ground. The maximum width of the gaster (GW) as well as the length of the first gastral segment (GS1L) of the ergatoid queen is slightly bigger (GW 1.269, GS1L 1.052 - 1.083) than in intercaste specimens (GS1L 0.990, GW 1.021) or workers of *Hylomyrma primavesi* (GS1L 0.866 - 0.897, GW 0.866). However, for almost all measurements taken, workers, intercaste specimens and ergatoid queens of *H. primavesi* overlap to some extent



A) W I1 I2 I3 Q



B) W I1 I2 Q C) W I1

Fig. 11: Female external morphology of mesosoma, showing worker (W), intercaste specimens (I1 - I3) and queen (Q). **A)** *Hylomyrma balzani*; **B)** *Hylomyrma montana*; and **C)** *Hylomyrma primavesi*.

(worker: TL 5.320 - 5.780; intercaste: TL 5.619; ergatoid queen: TL 5.727 - 6.052), being difficult to categorize them when they are alive. The same pattern, ergatoid queens being very similar in size to conspecific workers, is observed in other genera especially of Myrmicinae (*Eutetramorium*, *Ocymyrmex* EMERY, 1886, *Pogonomyrmex*, and *Terataner* EMERY, 1912), but also in Amblyoponinae (*Mystrium* ROGER, 1862) and Proceratiinae (*Proceratium* ROGER, 1863) (PEETERS 2012). Under the microscope, identifying the ergatoid queen is effortless due to its higher degree of mesosoma's differentiation. On the other hand, separating workers from intercaste specimens is extremely arduous and impractical because of the gradual morphological variation displayed by the intercaste specimens regarding the central ocellus, the promesonotal junction, the metanotal groove, and the size of the gaster. The last depends on the degree of development of the reproductive tract, which varies within workers and intercaste specimens, and between both.

TRIBLE & KRONAUER (2017, 2021a, b) proposed that caste morphology of female adults varies based on size or other factors that are closely related to size. Their hypothesis on the evolution and development of castes proposes that castes emerge or disappear due to changes in the distribution of body sizes within a species that alter the occupied regions of phenotypic space between workers and winged queens. In opposition, the standard model (WHEELER 1986), also called bifurcate or tree model, states that the development of queen, worker, and soldier castes are determined through a series of developmental thresholds mediated by juvenile hormone associated with nutritional switches, environmental factors, such as temperature, and genotype. Depending on the taxon, within threshold 1, at egg stage, the developmental path will bifurcate into queen (reproductive caste) and minor worker-soldier (non-reproductive caste) in response to temperature and photoperiod; within threshold 2, at larval stage, the developmental path of non-reproductive caste will bifurcate into minor worker and soldier in response to nutrition (ABOUHEIF 2021). The subsequent developmental trajectory of each caste, queen, worker, and soldier, can be evolutionarily modified independently of others (ABOUHEIF 2021).

Intercaste individuals in the genus *Hylomyrma* are not rare; 11 out of the 30 species present intercaste specimens (ULYSSEÁ & BRANDÃO 2021). Four species that belong to the same clade – *Hylomyrma balzani*, *Hylomyrma lopesi*, *Hylomyrma primavesi*, and *Hylomyrma reitteri* (ULYSSEÁ 2017: figs. 6 - 8, where *Hylomyrma* sp.J = *H. primavesi* and *Hylomyrma* sp.V = *H. lopesi*) – display the widest-documented range of adult caste phenotypes (Figs. 11A - C; ULYSSÉA & BRANDÃO 2021: figs. 2A - B). Within this clade, *H. primavesi* is unique because it replaced the winged queen by the ergatoid queen. *Hylomyrma reitteri* retains winged queens and displays intercaste specimens, the largest of which are morphologically comparable with the reproductive ergatoid queens in *H. primavesi* colonies. While studying specimens of *H. reitteri*, our first inter-

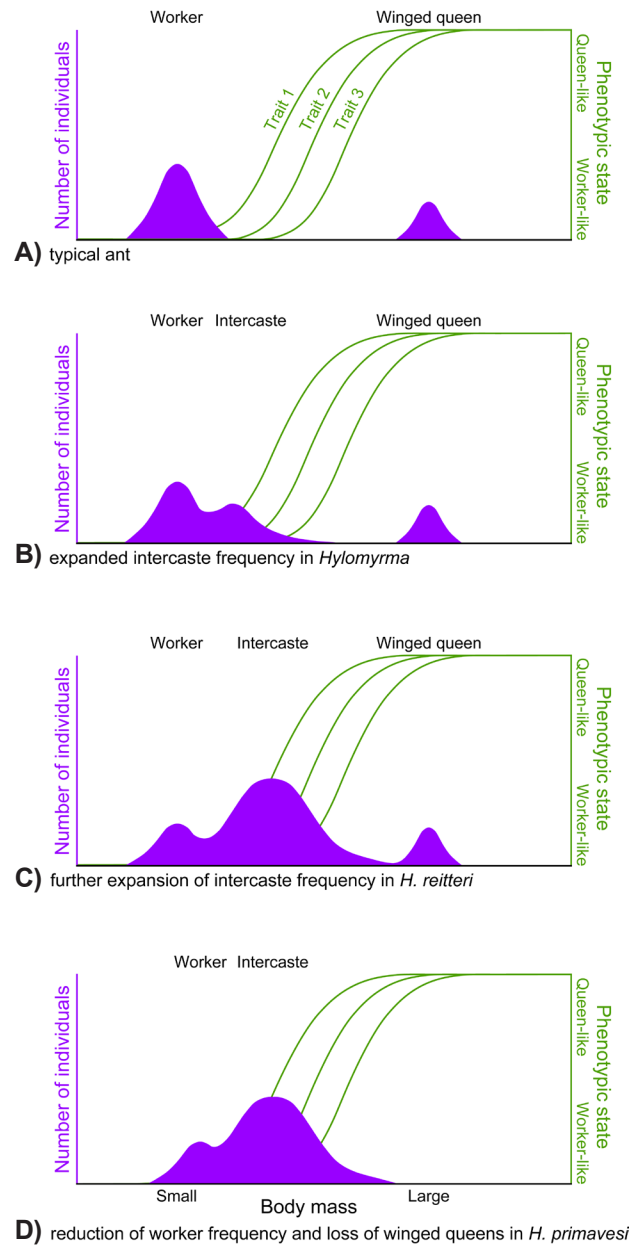


Fig. 12: Caste reaction norm and cartoons of adult phenotypes according to the hourglass model of caste development. **A)** The ancestral worker / winged queen system (adapted from TRIBLE & KRONAUER 2021); **B)** *Hylomyrma balzani* and *Hylomyrma lopesi*; **C)** *Hylomyrma reitteri*; and **D)** *Hylomyrma primavesi*. Traits 1, 2, and 3 refer to the reproductive, visual, and flight system, respectively.

pretation was to consider that this species had minor and major workers (M.A. Ulysséa, unpubl.), but further observations on external morphology allowed the recognition of those phenotypes as workers (n = 211) and intercaste specimens (n = 730) (ULYSSEÁ & BRANDÃO 2021: fig. 2A: W, I1, and I2).

Under the perspective of TRIBLE & KRONAUER (2017, 2021a, b), we may infer that changes in the occupancy of phenotypic space between workers and winged queens can

describe the evolution of the novel ergatoid queen caste in *Hylomyrma primavesi* (Fig. 12D). Relative to other ants, *Hylomyrma* are more likely to possess intercaste specimens that are slightly larger than workers (Figs. 12A - B). Relative to these *Hylomyrma*, *Hylomyrma reitteri* occupies an expanded region of the phenotype space that includes large intercaste specimens with flight sclerites on the mesosoma and more well-developed ocelli while retaining the winged queen (ULYSSEÁ & BRANDÃO 2021: fig. 2A; Fig. 12C). These large intercaste specimens might function as ergatoid queens, but this has not been investigated in this species. The caste system of *H. primavesi* may arise via a further modification that replaces the winged queen with large intercaste specimens that function as ergatoid queens (Fig. 12D). Interestingly, the replacement of winged queens with ergatoid queens in *H. primavesi* was accompanied by a large frequency of intercaste specimens, raising the possibility that the gain of ergatoid queens may have been achieved by increasing the body size of the worker population (TRIBLE & KRONAUER 2017, 2021a, b). Thus, the caste differences between species such as *Hylomyrma balzani*, *Hylomyrma lopesi*, *H. primavesi*, and *H. reitteri* could be explained using a series of changes in the occupancy of the phenotypic space between workers and winged queens. On the other hand, from the perspective of the standard model, the presence of the ergatoid queen in *H. primavesi* is a consequence of reprogramming events that occurred during the developmental pathway of the winged queen caste reducing its body size (queen degeneration). Whereas worker-like intercaste specimens emerge in response to changes that occurred during worker-caste differentiation. Doubtless, more studies focusing on the development and the distribution of female populations are required for better understanding the evolution of novel castes and the phenotypic plasticity encountered today in many species of the remarkable genus *Hylomyrma*.

As far as we know, *Hylomyrma primavesi* has only ergatoid queens, and several *Hylomyrma* species have intercaste specimens. Similarly, seven *Pogonomyrmex* species, all occurring in South America, have only ergatoid queens (JOHNSON 2015, 2021). However, the genus *Pogonomyrmex* exhibits the most diverse range of queen phenotypes found in ants (i.e., winged queens, brachypterous queens, and ergatoid queens), and the reproductive female can be exclusively monomorphic in some species, while dimorphic in others (JOHNSON 2015). For example, the queen phenotypes of *Pogonomyrmex laticeps* have a non-random geographic distribution: Within the northern portions of the species' range, colonies exclusively have brachypterous queens, while in the southern portions, colonies only have ergatoid queens (PEETERS & al. 2012, JOHNSON 2015). In *Hylomyrma balzani*, *Hylomyrma lopesi* and *Hylomyrma reitteri*, large intercaste specimens occur randomly within the species' range, not being restricted to a specific biome or habitat. Although *Pogonomyrmex* species have been more intensively studied than *Hylomyrma*, we have not yet unraveled what mechanisms might be driving this female phenotype diversity. Commonly, the explanation

for the emergence and maintenance of non-flying queen phenotypes is based on the lower cost per capita (i.e., the reduction or absence of flight muscles reduces production costs), and the lower mortality rate (i.e., short-distance dispersal decreases the risk of predation, is thought to increase the probability of survival) (MOLET & al. 2009, PEETERS & al. 2012). All this female phenotype diversity in both genera may reflect a phylogenetic signal of sister groups, and we may expect to recognize few *Hylomyrma* species as dimorphic in the future.

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Author contributions

M.A.U.: conceptualization; funding acquisition; data collecting and processing; writing – original draft of the manuscript, editing, and review. L.P.P.: data collecting; writing – maps, editing, and review. C.F.F.G.: data processing – analysis of the reproductive tracts; writing – the reproductive tracts, editing, and review.

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