Rediscovery of the enigmatic fungus-farming ant "Mycetosoritis" asper Mayr (Hymenoptera: Formicidae): Implications for taxonomy, phylogeny, and the evolution of agriculture in ants

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Abstract

We report the rediscovery of the exceedingly rarely collected and enigmatic fungus-farming ant species *Mycetosoritis asper*. Since the description of the type specimen in 1887, only four additional specimens are known to have been added to the world's insect collections. Its biology is entirely unknown and its phylogenetic position within the fungus-farming ants has remained puzzling due to its aberrant morphology. In 2014 we excavated and collected twenty-one colonies of *M. asper* in the Floresta Nacional de Chapecó in Santa Catarina, Brazil. We describe here for the first time the male and larva of the species and complement the previous descriptions of both the queen and the worker. We describe, also for the first time, *M. asper* biology, nest architecture, and colony demographics, and identify its fungal cultivar. Molecular phylogenetic analyses indicate that both *M. asper* and *M. clorindae* are members of the genus *Cyphomyrmex*, which we show to be paraphyletic as currently defined. More precisely, *M. asper* is a member of the *C. strigatus* group, which we also show to be paraphyletic with respect to the genus *Mycetophylax*. Based on these results, and in the interest of taxonomic stability, we transfer the species *M. asper, M. clorindae*, and all members of the *C. strigatus* group to the genus *Mycetophylax*, the oldest available name for this clade. Based on ITS sequence data, *Mycetophylax asper* practices lower agriculture, cultivating a fungal species that belongs to lower-attine fungal Clade 2, subclade F.
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Introduction

Although many species are easily assignable to existing genera based on morphology, some are not. Rather than assign them the status of "incertae sedis," taxonomists often group such species into so-called "dust-bin" genera erected for species of uncertain relationship, even when the species within such genera bear little resemblance to one another. Yet numerous studies have demonstrated that such phylogenetically isolated species may be especially important for understanding deeper relationships of genera, tribes, and subfamilies [1–8]. Including those species in phylogenetic analyses has significant effects on topology, ancestral character-state reconstruction, divergence-time estimation, and inferences of evolutionary rates [9–11].

Within the fungus-farming ants (Myrmicinae, Attini, Attina; hereafter “attine” ants [12–14]), the genus *Mycetosoritis* Wheeler has historically served as a "dust-bin" genus. Since its creation, *Mycetosoritis* has been regarded as a "degenerate and simplified *Trachymyrmex* or an aberrant *Cyphomyrmex*" ([15]: 716) or as transitional between the genera *Cyphomyrmex* Mayr and *Trachymyrmex* Forel [16–18]. The genus was first established by Wheeler in 1907 [15] as a subgenus of *Atta* Fabricius to accommodate the previously described species *Cyphomyrmex asper* (Mayr) and the newly described *M. hartmanni* (Wheeler). Later, in 1913 and in 1922, Emery ([19]: 251; [20]: 343–344) transferred *Mycetosoritis* to *Cyphomyrmex*, which in his definition also contained *Trachymyrmex* (as a subgenus). In 1922, Wheeler ([21]: 669) transferred the subgenus *Mycetosoritis* to *Trachymyrmex*. In 1949, Kusnezov [22] described the species *Cyphomyrmex* (*Mycetosoritis*) *clorindae* from Argentina, pointing out its resemblance to other members of *Cyphomyrmex* except for its erect pilosity, which contrasted with the appressed and fine, scale-like pilosity of *Cyphomyrmex s. str.* In addition, Kusnezov [22] suggested that the morphological characters of *clorindae* failed to fully agree with those of the genus *Mycetosoritis*. As a result, in 1950, Creighton ([16]: 317–318) elevated *Mycetosoritis* to genus status, arguing that "to include such an obviously transitional species in either genus [*Cyphomyrmex* and *Trachymyrmex*] weakens the distinctions by which they may be separated.” As part of his revision of the *Cyphomyrmex strigatus* group in 1964, Kempf [17] acknowledged that the species *Mycetosoritis asper* [23] and *Mycetosoritis clorindae* [22] are rather different from *Mycetosoritis hartmanni* and that both species share some affinities with members of the *C. strigatus* group, including the presence of a well-developed antennal scrobe. Kempf, however, argued against including those species within *Cyphomyrmex "as a provisional and temporary measure" due to the presence of erect hairs in both species (hairs are appressed and scale-like in *Cyphomyrmex*) and the absence of males for comparison.

Since Creighton [16] elevated *Mycetosoritis* to genus status, two additional species have been described based on workers: *M. explicatus* from Brazil in 1968 [18] and *M. vinsoni* from Costa Rica in 1998 [24]. In his description of *M. explicatus*, Kempf [18] drew attention to the high variability of the genus *Mycetosoritis* as defined by Emery [20] and discussed the difficulty of placing *M. explicatus* into either *Cyphomyrmex* or *Mycetosoritis*, arguing that it shares character states with both, but he ultimately chose the latter based on pilosity. Most recently, the monophyly of *Mycetosoritis* has been questioned [25].

In this study, we focus on the species *Mycetosoritis asper*, which hitherto has remained exceedingly rare in insect collections. To date, the species is known in the literature from the type specimen, an alate queen from Santa Catarina described by Mayr in 1887 [23], and a single worker subsequently collected in Puerto Piray, Misiones, Argentina, and described by Emery in 1906 [26]. Since then, only three additional workers have been collected (one in 1957 and two in 1999) in Santa Catarina, Brazil. These last three records remain unpublished and the biology of the species remains, heretofore, entirely unknown. In an attempt to clarify the phylogenetic position of this species within the fungus-farming ants, as well as to learn more...
about its biology, we conducted field research in the Floresta Nacional de Chapecó, Santa Catarina, Brazil. Here, for the first time, we: (i) describe the male and larva of *Mycetosoritis asper*, (ii) document its nest architecture and demographics based on twenty-one colonies collected, (iii) report on the phylogenetic position of *M. asper* within the subtribe Attina by generating new DNA sequences and conducting multilocus phylogenetic analyses, and (iv) report on the identity of its fungal cultivar and the agricultural system to which it belongs.

**Materials and methods**

**Field observations and nest excavations**

Field work was conducted 18–21 October 2014 in the Floresta Nacional de Chapecó (henceforth FLONA Chapecó), located between the municipalities of Guatambú and Chapecó in the west of the state of Santa Catarina, Brazil. The FLONA Chapecó is divided into three zones or Glebas: Glebas I and III are located in the municipality of Guatambú, whereas Gleba II is located in the municipality of Chapecó [27, 28]. The FLONA Chapecó contains remnants of Atlantic Forest, including *Araucaria angustifolia*, as well as plantations of *A. angustifolia*, pines (*Pinus elliottii, P. taeda*), and *Eucalyptus* species. The FLONA Chapecó is surrounded by intensively disturbed habitat used mostly for agriculture, pasture, and silviculture [28]. The most common types of soil in the FLONA Chapecó are Cambisols and Latosols. For more information regarding the FLONA Chapecó see ICMbio [28]. The FLONA Chapecó Gleba I, where we located twenty-one nests of *Mycetosoritis asper* (at 27.10306˚ S 52.77898˚ W, elevation 595–601 m above sea level), has an estimated area of 1300 ha and is considered a remnant of Atlantic Forest (containing mixed ombrophilous and seasonal deciduous forests), with a mean annual rainfall of 2007 mm and a mean annual temperature of 22˚ C [27–29].

Foraging activity by workers of *Mycetosoritis asper* was observed during the day. Foragers were located by baiting the area with Cream of Rice® cereal spread generously on the ground. The workers carrying the bait were then followed to their nest entrances. Nest entrances were marked with flagging and dug up when a substantial number of nest entrances had been located. Nests were excavated following Schultz [30], Rabeling et al., [31], and Sosa-Calvo et al., [32]. The twenty-one excavated colonies were transferred, using flame-sterilized forceps and spoons, from their subterranean chambers into plastic nest boxes containing a layer of plaster at the bottom, which was saturated with water [32]. Eleven of the twenty-one colonies collected are still maintained alive in artificial nest boxes in the AntLab at the Smithsonian Institution in Washington, DC (Table 1).

<table>
<thead>
<tr>
<th>Nest</th>
<th>Coll. Code</th>
<th>Date</th>
<th>Depth (cm)</th>
<th>Height (cm)</th>
<th>Width (cm)</th>
<th>Depth (cm)</th>
<th>Field notes</th>
<th>Laboratory notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AJ141018–01</td>
<td>Oct 18, 2014</td>
<td>44</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>Large garden, several workers and brood present, <em>queen present</em></td>
<td>Produced mostly gynes and a single male. Production of reproductives from mid Feb to mid Mar. Colony expired Nov 2015</td>
</tr>
<tr>
<td>2</td>
<td>AJ141018–02</td>
<td>Oct 18, 2014</td>
<td>48</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td><em>Queen present</em></td>
<td>Colony still alive in laboratory. Did not produce reproductives</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Nest</th>
<th>Coll. Code</th>
<th>Date</th>
<th>Depth (cm)</th>
<th>Height</th>
<th>Width</th>
<th>Depth</th>
<th>Field notes</th>
<th>Laboratory notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>AJ141018–03</td>
<td>Oct 18, 2014</td>
<td>36</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>Large garden, several workers and brood present, queen present</td>
<td>Colony still alive in laboratory. Did not produce reproductives</td>
</tr>
<tr>
<td>4</td>
<td>AJ141018–04</td>
<td>Oct 18, 2014</td>
<td>39</td>
<td>4.5</td>
<td>7</td>
<td>9</td>
<td>Large garden, partially yellowish with some parts greenish, several workers and brood present, queen present</td>
<td>Produced 7 alate gynes and 3 males. Production of reproductives from mid Feb to beginning of Mar. Colony still alive in laboratory</td>
</tr>
<tr>
<td>5</td>
<td>AJ141018–05</td>
<td>Oct 18, 2014</td>
<td>42</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>Large garden, several workers and brood present, queen present</td>
<td>Colony still alive in laboratory. Did not produce reproductives</td>
</tr>
<tr>
<td>6</td>
<td>AJ141018–08</td>
<td>Oct 18, 2014</td>
<td>30</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>Chamber was accidentally opened from the top, garden partially ruined. Large garden, several workers and brood present, queen present</td>
<td>Did not produce reproductives. Colony expired Jul 2016</td>
</tr>
<tr>
<td>7</td>
<td>AJ141018–09</td>
<td>Oct 18, 2014</td>
<td>40</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td>Large garden, several workers and brood present, queen present</td>
<td>Did not produce reproductives. Colony expired Feb 2016</td>
</tr>
<tr>
<td>8</td>
<td>AJ141018–11</td>
<td>Oct 18, 2014</td>
<td>29</td>
<td>6</td>
<td>5</td>
<td>7</td>
<td>Large garden, partially yellowish with some parts greenish and a large pellet of wet dirt/refuse located on chamber floor, several workers and brood present, queen present</td>
<td>Colony still alive in laboratory. Did not produce reproductives</td>
</tr>
<tr>
<td>10</td>
<td>AJ141020–02</td>
<td>Oct 20, 2014</td>
<td>47</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>Large garden, large pellet of wet dirt/refuse located on chamber floor, several workers and brood present, queen present</td>
<td>Produced only alate gynes. Production of reproductives from mid Feb to mid Mar. Colony still alive in laboratory</td>
</tr>
<tr>
<td>11</td>
<td>JSC141018–01</td>
<td>Oct 18, 2014</td>
<td>60</td>
<td>7</td>
<td>5.7</td>
<td>4.5</td>
<td>Single chamber containing small compact garden, several workers present, queen present</td>
<td>Colony still alive in laboratory. Did not produce reproductives</td>
</tr>
<tr>
<td>12</td>
<td>JSC141018–04</td>
<td>Oct 18, 2014</td>
<td>65</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td>Chamber found by accident and destroyed during excavation, queen present</td>
<td>Colony still alive in laboratory. Did not produce reproductives</td>
</tr>
<tr>
<td>13</td>
<td>JSC141018–08</td>
<td>Oct 18, 2014</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>Incipient nest, small chamber, few workers present, queen present</td>
<td>Did not produce reproductives. Colony expired Mar 2015</td>
</tr>
<tr>
<td>14</td>
<td>JSC141018–10</td>
<td>Oct 18, 2014</td>
<td>34</td>
<td>2.5</td>
<td>3.5</td>
<td>1.5</td>
<td>Very small chamber. Small garden, few workers present, queen present</td>
<td>Did not produce reproductives. Colony expired May 2016</td>
</tr>
<tr>
<td>15</td>
<td>JSC141020–01</td>
<td>Oct 20, 2014</td>
<td>48</td>
<td>6</td>
<td>7</td>
<td>5.5</td>
<td>Large chamber with large fungus garden. Chamber convex on top and somewhat flattened on bottom. A large pellet of wet dirt/refuse located on chamber floor, several workers and brood present, queen present</td>
<td>Produced only alate gynes. Production of reproductives from mid Feb to mid Mar. Colony still alive in laboratory</td>
</tr>
<tr>
<td>16</td>
<td>JSC141020–03</td>
<td>Oct 20, 2014</td>
<td>40</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>Large chamber with large fungus garden. Chamber convex on top and somewhat flattened on bottom. A large pellet of wet dirt/refuse located on chamber floor, several workers and brood present, queen present</td>
<td>Produced only males. Production of reproductives from mid Mar to mid Apr. Colony still alive in laboratory</td>
</tr>
<tr>
<td>17</td>
<td>JSC141020–04</td>
<td>Oct 20, 2014</td>
<td>65</td>
<td>4</td>
<td>6</td>
<td>5</td>
<td>Large garden, several workers and brood present, queen present</td>
<td>Produced only alate gynes. Production of reproductives only in Feb. Colony still alive in laboratory</td>
</tr>
</tbody>
</table>

(Continued)
Fungus garden fragments were isolated and axenically cultured on PDA (potato dextrose agar) medium with three antibiotics (Penicillin G, Streptomycin sulfate, and Chloramphenicol) in the Smithsonian Institution AntLab. Mycelia were transferred from agarose to PDA liquid broth medium with no antibiotics and cultured at 30˚C under constant agitation in a New Brunswick Scientific Series 25 Incubator Shaker, after which the tissues were filtered, lyophilized, and placed into cryo-storage for later DNA extraction.

Nest architecture was recorded, photographed, and measured following Sosa-Calvo et al., ([32]; page 307) Fungus garden fragments and a subset of workers were preserved in 95% ethanol in the field. Fungal vouchers are deposited in the USNM and ant vouchers are deposited in the insect collections of the following institutions:

<table>
<thead>
<tr>
<th>Nest</th>
<th>Coll. Code</th>
<th>Date</th>
<th>Depth (cm)</th>
<th>Height</th>
<th>Width</th>
<th>Depth</th>
<th>Field notes</th>
<th>Laboratory notes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>JSC141021–01</td>
<td>Oct 21, 2014</td>
<td>10</td>
<td>1</td>
<td>1.5</td>
<td>0.5</td>
<td>Incipient nest, very small chamber, workers not observed, <em>queen present</em></td>
<td>Collected into alcohol at time of excavation</td>
</tr>
<tr>
<td>19</td>
<td>JSC141021–02</td>
<td>Oct 21, 2014</td>
<td>50</td>
<td>6</td>
<td>7.5</td>
<td>6.5</td>
<td>Large chamber with large fungus garden. Fungus garden hanging from ceiling of chamber, probably attached to rootlets. A large pellet of wet dirt/refuse located on chamber floor, several workers and brood present, <em>queen present</em></td>
<td>Produced 33 alate gynes and 3 males. Production of reproductives from beginning of Feb to mid of Mar. Colony expired Apr 2016</td>
</tr>
<tr>
<td>20</td>
<td>JSC141021–04</td>
<td>Oct 21, 2014</td>
<td>43.5</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>Large chamber with large fungus garden. Chamber convex on top and somewhat flattened on bottom. A large pellet of wet dirt/refuse located on chamber floor, several workers and brood present, <em>queen present</em></td>
<td>Produced 9 alate gynes and 3 males. Production of reproductives from end of Jan to mid of Feb. Colony expired Apr 2015</td>
</tr>
<tr>
<td>21</td>
<td>TRS141017–01</td>
<td>Oct 17, 2014</td>
<td>52</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>Large garden, several workers present, brood not observed, <em>queen present</em></td>
<td>Colony still <em>alive</em> in laboratory. Did not produce reproductives</td>
</tr>
</tbody>
</table>

* Observations conducted from Nov 2014 to October 2015 when feeding colonies (three times a week). Numbers in bold in the “Nest” column indicate colonies that remain alive in the Smithsonian AntLab. In addition to males and gynes, lab colonies also produced workers, but worker numbers were not recorded.

https://doi.org/10.1371/journal.pone.0176498.t001

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<table>
<thead>
<tr>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC</td>
<td>C. Rabeling Collection, Arizona State University, Tempe, AZ, U.S.A.</td>
</tr>
<tr>
<td>DZUP</td>
<td>Coleção Entomológica “Padre Jesus Santiago Moure,” Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil.</td>
</tr>
<tr>
<td>ICN</td>
<td>Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, DC, Colombia.</td>
</tr>
<tr>
<td>MPEG</td>
<td>Museu Paraense ‘Emilio Goeldi,’ Belém, Pará, Brazil.</td>
</tr>
<tr>
<td>MZSP</td>
<td>Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil.</td>
</tr>
<tr>
<td>MBC-UFU</td>
<td>Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.</td>
</tr>
<tr>
<td>USNM</td>
<td>United States National Museum of Natural History, Washington, DC, U.S.A.</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0176498.t002
Material examined

In addition to the material collected by us in the FLONA Chapecó, we examined the type specimen, a dealate queen from Santa Catarina, Brazil, described by Mayr [23], and a worker from Puerto Piray, Misiones, Argentina, described by Emery [26], but in that publication erroneously recorded as collected in Chubut (see below). Other material examined includes a single worker collected in 1957 by F. Plaumann in Chapecó, Santa Catarina, and two workers collected in 1999 by Rogerio da Silva in a Winkler sample in Seara, Santa Catarina. These specimens are deposited in the following institutions:

<table>
<thead>
<tr>
<th>Institution</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSNG</td>
<td>Museo Civico di Storia Naturale “Giacomo Doria,” Genoa, Italy.</td>
</tr>
<tr>
<td>MZSP</td>
<td>Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil.</td>
</tr>
<tr>
<td>NHMW</td>
<td>Naturhistorisches Museum, Wien, Austria.</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0176498.t003

Morphological measurements and specimen preparation

All measurements were taken to the nearest 0.001 mm and, unless otherwise noted, are in millimeters. Composite images were generated at the USNM Ant Lab using a JVC KY–F75U digital camera mounted on a Leica Z16 APO stereomicroscope attached to a Dell Optiplex GX620 computer. Composite images were assembled using Auto-Montage Pro® (Version 5.03.0061 BETA) software (Synoptics Ltd.). Wings of males and queens were removed from the left side of the specimen, placed on microscope slides with Euparal mounting medium, and covered with a circular cover glass. The slides were labeled with the name of the species, sex, country, and locality of collection, and the unique USNMENT number of the specimen to which the wings belong.

The larvae were dehydrated sequentially through a series of ethanol concentrations to 100% absolute and then critical-point dried in a Balzers CPD–030 using liquid CO₂ at the Scanning Electron Microscopy (SEM) Lab in the SI–NMNH and mounted on aluminum stubs. The prepared larvae, as well as a worker, a queen, and a male, were sputter-coated with 60:40 wt% gold: palladium alloy on a Cressington Scientific 108 auto/SE sputter coater to a thickness of 20–25 nm. Scanning Electron Micrographs (SEMs) of these specimens were generated using a Philips XL–30 ESEM with Lanthanum Hexaboride (LaB6) source and with a backscatter detector.

Measurements, indices, abbreviations, and morphological terminology follow longstanding standard protocols [7, 13, 33–38] and literature cited therein, with modifications where noted. All images were edited using Adobe Photoshop CC 2015.0.1 (Version 20150722.r.168 x64) (Adobe Inc.). The following abbreviations are used in the description: w = worker, dq = dealate queen, m = male.

Anatomical abbreviations are as follows:

Permits

Permits to conduct field work were issued to JSC, AJ, HLV, and TRS by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Processo EXC 039/07; Portarias 267, 359) and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio; Permits 14789–1, 14789–2, 14789–9). Permits to export and import live ant colonies were issued to TRS and MB by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis.
Molecular phylogenetics

Ant and fungal cultivar DNA extraction, amplification, and sequencing were conducted at the Laboratories of Analytical Biology (LAB) at the National Museum of Natural History, Smithsonian Institution, Washington, DC. Genomic DNA was extracted using the Qiagen DNEasy Blood and Tissue kit (Quiagen, Inc.) for the ants and the Plant DNeasy kit (Quiagen, Inc.) for the fungus. For the ants, five nuclear protein-coding genes (EF1α-F1, EF1α-F2, wg, LW Rh, and TOP1) were amplified and sequenced following the methodology outlined in previous studies [12, 39, 40]. For the fungal cultivar, a ribosomal gene fragment, internal transcribed...

(IBAMA; live export Permit 14BR015572/DF) and to TRS by the USDA APHIS PPQ (Permit to Move Live Plant Pests, Noxious Weeds, and Soil P526P-14-01931).

Molecular phylogenetics

Ant and fungal cultivar DNA extraction, amplification, and sequencing were conducted at the Laboratories of Analytical Biology (LAB) at the National Museum of Natural History, Smithsonian Institution, Washington, DC. Genomic DNA was extracted using the Qiagen DNEasy Blood and Tissue kit (Quiagen, Inc.) for the ants and the Plant DNeasy kit (Quiagen, Inc.) for the fungus. For the ants, five nuclear protein-coding genes (EF1α-F1, EF1α-F2, wg, LW Rh, and TOP1) were amplified and sequenced following the methodology outlined in previous studies [12, 39, 40]. For the fungal cultivar, a ribosomal gene fragment, internal transcribed...
Spacer (ITS), was amplified and sequenced following [41–43]. New sequences generated for this study are deposited in GenBank under accession numbers KY809160–KY809180 for the fungal cultivar and KY828479–KY828592 for the ants. Nexus and tree files can be found in Treebase (http://purl.org/phylo/treebase/phylows/study/TB2:S20864).

Ant DNA sequences, consisting of ~3.3 kbp, were added to the aligned data set of Schultz and Brady [39] and Sosa-Calvo et al., [7] and aligned first by eye in Mesquite [44] and subsequently by using MAFFT v7.017 [45–47] as implemented in Geneious R9 v8.1.8 [48]. Data were partitioned and modeled using the program PartitionFinder v1.1.0 [49] under the Bayesian Information Criterion (BIC) with 15 data blocks consisting of the first, second, and third codon positions of each of the five gene fragments and with a user tree resulting from an unpartitioned maximum-likelihood best-tree analysis conducted in RAxML v.8.2 [50]. The eight partitions and models identified by PartitionFinder were employed in Bayesian analyses using MrBayes 3.2.2 [51] with nucmodel = 4by4, nruns = 2, nchains = 8, samplefreq = 1000, and 20 million generations, with a burn-in of 2 million generations. To address known problems with branch-length estimation in MrBayes [11, 52–56], we set brlenspr = unconstrained: Exp (100). Burn-in, convergence, and stationarity were assessed using Tracer, version 1.5 [57], by examining potential scale reduction factor values and.stat output files in MrBayes, and by using Bayes factor comparisons of harmonic-mean marginal likelihoods of pairs of runs with standard error estimated using 1,000 bootstrap pseudoreplicates in Tracer 1.5 [57], which employs the weighted likelihood bootstrap estimator of Newton and Raftery [58] as modified by Suchard et al., [59].

Fungal ITS sequences for *M. asper* were added to a preexisting data set of agaricaceous (Basidiomycota: Agaricales: Agaricaceae: Leucocoprineae) ant-associated and free-living fungi and aligned in MAFFT, producing a matrix consisting of 506 taxa and 1281 characters, including indels. Data were partitioned and modeled using the program PartitionFinder 2.0 under the kmeans algorithm [60], which does not require initial data blocks, and the corrected Akaike information criterion (AICc). The two partitions and models identified by PartitionFinder were employed in Bayesian analyses using MrBayes 3.2.2 as described above for the ant phylogenetic analyses, except that burn-in was set to 5 million generations.

Results and discussion

Systematics

Etymology of "Mycetosoritis." Despite a tradition of treating it as feminine, the genus name "Mycetosoritis" should be regarded instead as masculine and should be combined with masculine-form adjectives such as "asper" and "explicatus." W. M. Wheeler [15] originally described *Mycetosoritis* as a subgenus of *Atta*. A rereading of Wheeler’s [15] description of *Mycetosoritis hartmanni* provides no guidance on the gender of *Mycetosoritis* because (i) "hartmanni" is genitive and (ii) the adjective "aspera," which Wheeler uses when he transfers the former *Cyphomyrmex asper* into *Atta* (subgenus *Mycetosoritis*), matches the (feminine) gender of *Atta*, the genus name, and not that of *Mycetosoritis*, the subgenus name. Because there is thus no indication of gender in the original description of *Mycetosoritis*, Section 30.1.4.2 of the International Code of Zoological Nomenclature [61] applies, requiring the use of the masculine gender for genus-group names with endings of ambiguous gender for which the author did not indicate the gender either explicitly or via a clear adjectival species-group name. The assertion by G. Wheeler [62] that the second part of "Mycetosoritis" is derived from "Soritis," an alternate name for the Greek goddess Ceres, is not, even if true, sufficient to supersede the ICZN rule. Wheeler’s assertion is, moreover, unsupported based on the opinion (pers. comm.) of E. Adler, Associate Professor of Classics, University of Maryland, College Park, who (i)
confirmed that the noun ending "-is" can be masculine or feminine and (ii) could find no reference to a god or goddess "Soritis" in The Oxford Latin Dictionary [63] or in Liddell and Scott’s Greek-English Lexicon [64].

**Mycetophylax Emery (1913).** The multilocus analyses presented here (Fig 1), as well as phylogenomic analyses of 950 UCE loci [14], indicate with strong support that the species *Mycetosoritis asper* and *Mycetosoritis clorindae* are members of the genus *Cyphomyrmex* as currently defined and, more specifically, of the *C. strigatus* group. Both data sets further indicate that, as currently defined, the genus *Cyphomyrmex* is paraphyletic with respect to *Mycetophylax, Mycetagroicus,* and the higher Attina, and that the *C. strigatus* group, as currently defined, is paraphyletic with respect to the species *Mycetophylax conformis, Mycetophylax morschi,* and *Mycetophylax simplex,* which are derived members of the clade containing the *C. strigatus* group. Based on these results, and in the interest of taxonomic stability, we here recognize the *C. strigatus* group as a separate genus. *Mycetophylax conformis* is the nominal species for the genus name *Mycetophylax* [19] and "Mycetophylax" is the oldest available species-group name for the clade containing the *C. strigatus* group. We therefore transfer all members in the *C. strigatus* group, including *Mycetosoritis asper* and *Mycetosoritis clorindae,* to the genus *Mycetophylax.*

**Taxonomic synopsis of the species in Mycetophylax Emery.** The species list presented here is modified from Kempf [17] and Klingenberg and Brandão [34]:

*Mycetophylax conformis* Mayr 1884[65]
*Mycetophylax asper* (Mayr 1887)[23] new combination
*Mycetophylax auritus* (Mayr 1887)[23] new combination
*Mycetophylax strigatus* (Mayr 1887)[23] new combination
*Mycetophylax morschi* (Emery 1888)[66]
*Mycetophylax simplex* (Emery 1888)[66]
*Mycetophylax olitor* (Forel 1893)[67] new combination
*Mycetophylax bigibbosus* (Emery 1894)[68] new combination
*Mycetophylax lectus* (Forel 1911)[69] new combination
*Mycetophylax bruchi* (Santschi 1917)[70] new combination
*Mycetophylax faumulus* (Wheeler 1925)[71] new combination
*Mycetophylax paniscus* (Wheeler 1925)[71] new combination
*Mycetophylax daguerrei* (Santschi 1933)[72] new combination
*Mycetophylax clorindae* (Kusnezov 1949)[22] new combination
*Mycetophylax lilloanus* (Kusnezov 1949)[22] new combination
*Mycetophylax vallensis* (Kusnezov 1949)[22] new combination
*Mycetophylax nemei* (Kusnezov 1957)[73] new combination
*Mycetophylax plaumanni* (Kempf 1962)[74] new combination
*Mycetophylax occultus* (Kempf 1964)[17] new combination
*Mycetophylax andersoni* (Mackay & Serna 2010)[75] new combination
**Mycetophylax snellingi** (Mackay & Serna 2010) new combination

**Mycetophylax asper** (Mayr 1887) new combination

Figs 2–8.


**Diagnosis.** Inner margin of mandibles with 6–7 teeth; preocular carina extending posterad to cephalic corner, forming a well-developed antennal scrobe (shared with members of the former *Cyphomyrmex strigatus* group, *Mycetophylax clorindae* and *Mycetophylax morschi*, but not with *Mycetophylax conformis* and *Mycetophylax simplex*, in which the scrobe is secondarily reduced); anterior margin of antennal scape with a broad carina, gradually expanding apically; body covered with short, erect, simple hairs; single median pronotal tubercle present, well-developed (shared with members of the former *Cyphomyrmex strigatus* group, but vestigial in species of the former *Mycetophylax s.s.*); node of petiole with a pair of tubercles or spines; gastral tergite I strongly rugose and with small tubercles from each of which arises a single, short, erect hair.

**Description.** The worker of *Mycetophylax asper* was first described by Emery [26] from a single specimen collected in Argentina. Here, we complement Emery’s description of the worker based on the specimen studied by him and additional workers from multiple nest series collected in the municipality of Chapecó, Santa Catarina, Brazil.

**Head:** in full-face view, and excluding mandibles, as long or slightly longer than wide (HL 0.75–0.83, HW 0.72–0.83, CI 96–105); in full-face view, cephalic margin medially emarginate (Figs 2B, 3G and 4A); in full-face view, preocular carina extending posterad to cephalic corner, meeting frontal carina to form conspicuous antennal scrobes (Figs 2B and 2D; 3A and 3G; 4A and 4D); in lateral view, antennal scrobes reticulate (Fig 4D); in full-face view, supraocular tooth triangular, carina-like (Figs 2B, 3G and 4A); in profile, supraocular tooth carina-like and separated from preocular carina by shallow groove (Fig 4D); in profile, dorsum of head covered with short, simple, erect hairs (best seen in Fig 4D); in full-face view, frontal lobes broadly expanded (FLD 0.52–0.60, RFLDI 70–75, RFLDII 71–76), covering antennal insertions (Figs 2B, 3G and 4A); upper margin of frontal carina with short simple hairs (Fig 4A); dorsoapical margin of head with short, erect, simple hairs; dorsoapical margin of head rugose (Fig 4A); in full-face view, lateral margin of body of clypeus with pair of fronoctoclypeal teeth directly underneath frontal lobes (Figs 4A and 5A); anterior margin of clypeal apron smooth and convex, interrupted medially by conspicuous notch from which a short, stout, median clypeal seta arises (Figs 4A and 5A); mandibles long (ML 0.47–0.53, MI 59–68); masticatory margin of mandibles 6–7-toothed, with teeth 3 and 4 (counting from base) slightly smaller than basal teeth 1 and 2, apical tooth largest (Fig 5A); outer margin of mandibles sinuous; dorsoapical margin of mandibles striate (Figs 4A and 5A); eyes (EL 0.12–0.13; OI 15–17) convex, with 7–8 ommatidia in longest row (34–42 ommatidia total) (Fig 4D); antennal scapes not surpassing cephalic corner when in repose (SL 0.49–0.59, SI 63–75) (Figs 2B and 2D); apical half of antennal scapes gradually broader than basal half (Figs 2B and 3G); leading margin of antennal scape with broad carina apically (Figs 3G...
and 4A); dorsum of antennal scapes covered with erect, simple hairs, leading margin of scapes with decumbent simple hairs, posterior margin of scapes with appressed simple hairs. Palpal formula 4,2 (Fig 4D).

Mesosoma: In fronto-dorsal and lateral view, pronotum with single median anterior pyramidal tubercle, connected by thin carinæ to robust lateral pronotal tubercles, somewhat separating pronotum from mesonotum (Figs 2D and 2F; 3A and 3B; 4G and 4J); dorsum of pronotum rugulose-foveolate (best seen with high magnification, see Fig 4G and 4J); pronotum lacking humeral tubercles; in lateral view, inferior corner of pronotum armed with

Fig 2. The queen (holotype) and first-described worker of Mycetophylax asper. a) Queen, full-face view; b) worker, full-face view; c) queen, lateral profile; d) worker, lateral profile; e) queen, dorsal view; f) worker, dorsal view; g) queen, specimen labels; h) worker, specimen labels.

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triangular tooth (Figs 2D, 3A and 4G); in ventral view, propleural plates whitish, almost cer-
tainly due to the presence of actinomycete bacteria ([76]; Fig 5C). In dorsal view, lateral meso-
notal tubercles carina-like, connected anteriorly by thin transverse carina (Figs 2F, 3B and 4I),

Fig 3. Worker, queen, and male of Mycetophylax asper. a) Worker, lateral profile; b) worker, dorsal view; c) queen, lateral profile; d) queen, dorsal view; e) male, lateral profile; f) male, dorsal view; g) worker, full-face view; h) queen, full-face view; i) male, full-face view.

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anterior mesonotal margin broadly convex; in dorsal view, area circumscribed by lateral and posterior mesonotal tubercles slightly concave and weakly sculptured; in lateral view, posterior mesonotal tubercles triangular (Fig 4G); in lateral view, mesopleural margin (katepisternum) with thin but conspicuous carina (Fig 3A); metanotal groove deep. In lateral view, anterior portion of propodeum with pair of small tubercles (Figs 2D, 3A and 4G); posterior propodeal tubercles larger and acute (Figs 2D, 3A and 4G); anterior and posterior propodeal tubercles connected by lateral carinae; declivity of propodeum shorter than base of propodeum and lacking lateral carinae; in dorsal view, lateral face of propodeum with conspicuous carina, arising anterior to propodeal spiracle to form small but conspicuous tubercle; second, smaller, rounded tubercle arising posterior of spiracle, near the margin of metapleural gland bulla;
propodeal lobes vestigial to absent. Hind femur and, to a lesser extent, mid femur with pair of conspicuous ventral carinae, produced in the basal one-third into strong ventro-posterior lobe (Fig 5E). Outer margin of mid and hind tibia with short, erect hairs (Fig 5E).

**Metasoma:** In lateral view, peduncle of petiole vestigial, with small antero-ventral tooth (Figs 2D and 3A); ventral margin of petiole sinuous (Figs 2D and 3A); in dorsal view, antero-
dorsal portion of petiole flattened (Figs 2F and 3B); node of petiole with anterior pair of tubercles rounded at tip and posterior margin of petiolar node with strong transverse carina, giving the impression of posterior tubercle in lateral view (Figs 2D and 3A). In dorsal view, petiole somewhat subquadrate, anterior angles tooth-like (Figs 2F and 3B); lateral portion of petiole with short, erect, simple hairs. In dorsal view, postpetiole wider than long (PPL 0.15–0.17, PPW 0.32–0.34, PPI 196–222), broadly convex anteriorly (Figs 2F and 3B). Dorso-lateral view with pair of carinae (Figs 2F and 3B); area demarcated by carinae weakly concave medi ally. Gastral tergite I strongly rugulose and with small tubercles, from each of which arises a single, short, erect hair (Figs 2D and 2F; 3A and 3B; 4J; 5D).

**Color:** Individuals mostly yellowish to ferrugineous in color. Integument matte, rugose, and foveolate (Figs 2B, 2D and 2F; 3A, 3B and 3G; 4A, 4D, 4G and 4J; 5D).

**Measurements.** WORKER (specimen from Argentina [26]). EL (0.13) 0.12–0.13, FLD (0.58) 0.52–0.60, GL (0.79) 0.71–0.81, HFL (0.76) 0.71–0.81, HL (0.80) 0.75–0.83, HTL (0.46) 0.42–0.48, HW (0.79) 0.72–0.83, ML (0.48) 0.47–0.53, MSL (0.05) 0.05, PL (0.34) 0.28–0.40, PPL (0.17) 0.15–0.17, PPW (0.32) 0.32–0.34, PW (0.56) 0.51–0.59, SL (0.58) 0.49–0.59, TL (3.58) 3.34–3.74, WL (1.01) 0.97–1.10, CI (98) 96–105, MI (59) 59–68, MSI (11) 6–11, OI (17) 15–17, PPI (196) 203–222, RFLDI (73) 70–75, RFLDII (74) 71–76, SI (73) 63–75 (n = 12).

**Queen:** Similar to the worker with modifications expected for the caste and with the following differences:

**Head:** Dorsum of head densely rugulose, dark brown in color (Figs 2A, 3H and 4B). Anterior ocellus embedded in deep pit, carinate posteriorly (Figs 2A, 3H and 4B). A pair of carinae originating at level of posterior ocelli and extending posterad to cephalic margin (Figs 2A, 3H and 4B).

**Mesosoma:** In dorsal view, anterior portion of pronotum with weakly impressed rugae (Figs 2C, 3C and 4H). Median pronotal tubercle absent (Figs 2C, 3C and 4H); lateral pronotal tubercles connected by a conspicuous carina, forming an obtuse angle at the midline, i.e., where the median pronotal tubercle occurs in the worker (Figs 2C, 3C and 4H). Dorso-lateral view with pair of carinae (Figs 2C, 3C and 4H); lateral carinae strongly rugose (Figs 2E, 3D and 4K); parapsidal lines present, discrete (Figs 2E, 3D and 4K); parascutal lobes with irregular lateral carinae (Figs 2E, 3D and 4K). Scutellum lacking lateral projections (Figs 2E, 3D and 4K); posterior margin of scutellum lacking tubercles, straight or slightly concave (Figs 2E, 3D and 4K). Base of propodeum with small but conspicuous lateral carinae (Fig 4H); declivity of propodeum lacking lateral carinae (Fig 4H); propodeal tubercles present, triangular; base of propodeum shorter than declivity of propodeum (Figs 2E, 3D and 4K).

**Color:** Head and body mostly yellowish to ferrugineous; dorsum of head, mesosoma, and gaster tend to be darker in color. Pilosity as in the worker.

Wings smoky, covered with minute pilosity. Forewing lacking pterostigma and with five closed cells present. Hindwing with reduced venation, a single closed cell present (Fig 6A).

**Measurements.** QUEEN (Holotype). EL (0.18) 0.17–0.19, FLD (0.73) 0.65–0.74, GL (1.18) 1.13–1.28, HFL (0.91) 0.84–0.94, HL (0.90) 0.82–0.93, HTL (0.56) 0.47–0.57, HW (0.91) 0.88–0.98, ML (0.60) 0.53–0.61, MSL (0.05) 0.03–0.05, PL (0.48) 0.36–0.49, PPL (0.17) 0.15–0.22, PW (0.53) 0.50–0.54, SL (0.65) 0.61–0.68, TL (4.72) 4.26–4.76, WL (1.39) 1.20–1.34, CI (101) 102–109, MI (67) 58–70, MSI (9) 4–9, OI (20) 19–20, PPI (308) 230–359, RFLDI (81) 78–82, RFLDII (79) 73–77, SI (72) 65–77 (n = 17).

**Male:** Excluding eyes, slightly longer than wide (HL 0.53–0.65, HW 0.49–0.60, CI 98–100); in full-face view, posterior cephalic margin strongly convex (Figs 31 and 4C); preocular carina present, extending posterad almost to level of anterior ocellus, failing to reach cephalic margin (Figs 31 and 4C); dorsum of head (frons) areolate with torulose interspace sculpture (Fig 4C); median carina absent (Fig 4C). Mandibles triangular, inner margin 5- to 6-toothed, increasing in size towards apex (Figs 4C and 5B); dorsum of mandibles imbricate...
and with appressed simple hairs (Fig 5B). Clypeal apron thin, shiny, and strongly notched medially (Fig 5B); dorsum of clypeal apron weakly reticulate (Fig 5B); unpaired median seta long (MSL 0.07–0.09, MSI 11–27), emerging from dorsal portion of clypeal apron well removed posterad from border (Fig 5B). In full-face view, body of clypeus subquadrate, circumscribed by lateral carinae, each of which is produced into a small frontoclypeal tooth, directly underneath each frontal lobe (Fig 4C); median portion of body of clypeus, between frontal lobes, with 1 or 2 minute tubercle(s) or blunt projection(s), best seen when looking from above from a viewpoint in which both the occipital collar and the posterior ocelli are visible; posterior margin of clypeus forming deep groove between frontal lobes (Fig 4C). In full-face view, frontal lobes narrow or vestigial, failing to cover antennal insertions (FLD 0.20–0.26, RFLDI 37–41, RFLDII 40–46) (Fig 4C and 4F); frontal carinae extending posterad to join rugae on either side of anterior ocellus (Fig 4C). Antennal scapes long (SL 0.52–0.63, SI 104–110), longer than length of funicular segments I–III combined; antennal scapes surpassing cephalic margin by ~1/2 of their length (Figs 3E and 3I; 4C); antennal scapes thin, lacking expanded carinae as in worker and queen, attaining maximum width near apex (Fig 3I); dorsal of antennal scapes covered with appressed, simple hairs, becoming decumbent at apex (Figs 3I and 4C); integument of antennal scapes as on frons; antennae 12-segmented. Eyes large (EL 0.22–0.25, OI 40–46), convex. In lateral view, gena with prominent carina that extends from base of mandibles to occipital collar (Fig 4F). In lateral view, hypostoma lacking teeth (Fig 4F). Palp formula 4,2.

**Mesosoma:** Pronotum lacking humeral tubercles; in dorsal view, lateral tubercles of pronotum reduced to carinae, at most forming obtuse angles (Figs 3E and 3F; 4I); in lateral view and, to a lesser extent, in fronto-dorsal view, a minute tooth present at position of median pronotal tubercle, very close to posterior margin of pronotum (Figs 3E and 4I); antero-inferior margin of pronotum, at most, angulate, not produced into a tubercle or tooth (Figs 3E and 4I). In dorsal view, median sulcus and median line of mesoscutum inconspicuous, visible under high magnification (Fig 4L); notaui present, deep and costulate (Fig 4L); parapsidal lines present (Fig 4L); in dorsal view, mesoscutum rugulose in addition to areolate with torulose interspace sculpture (Fig 4L). Oblique mesopleural sulcus (= anapleural sulcus; [77, 78]) deep, with some costulae, excavating the anepisternum so that its lower edge slightly overhangs the katepisternum (Fig 4L). Mesoscutellum with deep, transversely costate scutoscutellar sulcus (Fig 4L); posterior margin of mesoscutellum bidentate (Fig 4L). In lateral view, propodeum lacking teeth, at

![Fig 6. Wings of Mycetophylax asper. a) Queen, fore and hind wings; b) male, fore and hind wings. Scale bars in figures represent 1.0 mm in length.](https://doi.org/10.1371/journal.pone.0176498.g006)
most with small carinae forming obtuse angles (Figs 3E and 4I); base of propodeum larger than declivity of propodeum (Figs 3E and 4I).

Legs very long, length of hind femur longer than mesosoma (HFL 1.25–1.60, WL 1.18–1.39, HFI 106–116); ventral margin of mid and hind femur with erect simple hairs on basal half, apical half lacking hairs or with very short, appressed hairs.

**Metasoma:** In lateral view, peduncle of petiole cylindrical in appearance and with a minute antero-ventral petiolar process (Fig 3E); petiole dorsoventrally nearly flattened, weakly convex dorsally (Fig 3E and 3F), subquadrate; in dorsal view, node of petiole lacking tubercles, at most a pair of very inconspicuous tumosities (Fig 3F); in dorsal view, lateral margin of petiole with thin lateral carina; dorsum of petiole finely reticulate, lacking hairs (Fig 3F). Postpetiolar wider than long (PPL 0.20–0.26, PPW 0.36–0.46, PPI 169–203); in dorsal view, postpetiolar dome-shaped (Fig 3F); dorsum of postpetiole lacking hairs, except for a pair located near posterior margin. Dorsum of gastral segment I finely and strongly reticulate (Fig 3F).

Fore and hind wings as in the queen (Fig 6B).

**Color:** Antennal funicular segments, mandibles, and tarsomeres yellowish to light brown, rest of body dark brown (Fig 3E, 3F and 3I).

**Measurements.** MALE. EL 0.22–0.25, FLD 0.20–0.26, GL 1.16–1.46, HFL 1.25–1.60, HL 0.53–0.65, HTL 1.04–1.30, HW 0.49–0.60, ML 0.32–0.38, MSL 0.07–0.09, PL 0.35–0.44, PPL 0.20–0.26, PW 0.36–0.46, PW 0.51–0.66, SL 0.52–0.63, TL 3.76–4.54, W 1.18–1.39, CI 90–94, HI 59–61, MSI 11–27, OI 40–46, PPL 169–203, RFLDI 37–41, RFLDD 40–46, SL 104–110 (n = 5).

**Larva:** Description based on SEM study of three prepupal worker larvae from a single nest (AJ141020–02).

Body profile “attoid” sensu Wheeler [79] and Wheeler & Wheeler [80], i.e., longitudinally curved, bean-shaped, with ventral profile shorter than dorsal (Fig 7A). Thoracic-abdominal articulation absent, thoracic intersegmental constrictions superficial, deep lateral depressions associated with abdominal spiracles absent, and leg vestiges present and visible as open slits ventrally on thorax. Dorsal and lateral body surfaces without setae (Fig 7A); setae on head and venter mostly simple, a few with multifurcate tips (Fig 7B). Genal lobes apparently absent. Supra-antennal and supraclypeal setae absent, four setae on each gena and two on clypeus. A few papilliform spinules present on head, restricted to clypeus and genae. Labrum monolobate, narrow, and inflated, with two distinctly setiform anterior setae (Fig 7B–7D). Thoracic and abdominal segments each with six long, simple hairs ventrally. First and second abdominal segments each with a non-lobiform ventromedian protuberance, protuberance on first abdominal segment more pronounced than that on second (Fig 7D). Four long, simple hairs arise ventrally on each side of protuberance on abdominal segment one, and two to three hairs (varying between specimens) arise on each side of protuberance on abdominal segment two (Fig 7D). Abdominal segment three with one or two pairs of setae; ventral setae absent on abdominal segments four to nine. A single pair of setae
anterior to anal opening and another pair lateral to opening, all four setae arising on abdominal segment ten (Fig 7F and 7G). Ventral anal lip absent.
**Comments.** The species *Mycetophylax asper* was first described by Mayr (23) (as *Cyphomyrmex asper*) from a single alate queen collected in Santa Catarina, Brazil. Subsequently, Emery (26) described a worker of *M. asper* collected by F. Silvestri during his travels in southern South America, including Argentina. In his publication, Emery (26) gives the locality of the specimen as Puerto Piramides in Chubut, Argentina. However, the label on the specimen studied by Emery, which is deposited in Genoa, Italy (Fig 2B, 2D, 2F and 2H), indicates the locality “Puerto Piray, 13.vii.900” (Fig 2H). Filippo Silvestri’s “Ricordi e itinerary scientifichi” (published posthumously in 1959 [81]), which includes the diary of his travels, indicates that Silvestri visited both localities in Argentina: Puerto Piramides, Chubut Province, southwest of Buenos Aires, at the beginning of December 1899; and Pampa (or Puerto) Piray, Misiones Province, on two occasions, one from 11–13 and another from 19–23 of July, 1900. Both the diary dates and the label data indicate that Silvestri collected the *M. asper* worker specimen in Piray, Misiones, Argentina, rather than in Chubut as erroneously reported in Emery (26) (Maria Tavano, pers. comm.).

**Material examined:** ARGENTINA: [Provincia de Misiones], Puerto Piray, [26.468686˚ S 54.715880˚ W; elev. 149 m], 13.vii.1900, (F. Silvestri) [1w, MSNG, Emery Collection, USNMENTNo.00921154]. BRAZIL: Santa Catarina, (Hečko) [1aq, NHMW, Mayr Collection, USNMENTNo.00923112]; Santa Catarina, Chapecó, v.1957, (F. Plaumann) [1w, MZSP]; Seara, 27.1166667˚ S 052.300˚ W, vi–vii.1999, (R. da Silva), Winkler (soil) [2w, MZSP]; Santa Catarina, Chapecó, Floresta Nacional de Chapecó, 27.10306˚ S 52.77898˚ W, elev. 601 m, 18.x.2014, (A. Jesovnik, T. R. Schultz, & J. Sosa-Calvo), nest series, AJ141018–01 [2aq, USNM]; same locality information but AJ141018–04 [1m, USNM]; same locality information but, 27.10307˚ S 52.77904˚ W, elev, 595 m, 20.x.2014, (J. Sosa-Calvo, A. Jesovnik, & T. R. Schultz), nest series, JSC141020–03 [1w, USNM]; same locality information but, (A. Jesovnik, T. R. Schultz, & J. Sosa-Calvo), nest series, AJ141020–02 [1aq CRC; 5aq, USNM]; same locality information but, 21.x.2014, (J. Sosa-Calvo, A. Jesovnik, & T. R. Schultz), nest series, JSC141021–04 [1aq, 1m, 1w, CRC; 1w, ICN, 1w MPEG; 1aq, 1m, 1w MZSP; 1w, MBC-UFU; 1aq, 1m, 2w, USNM].

**Natural history**

**Habitat.** All but one collection of *Mycetophylax asper* are from the southern state of Santa Catarina, Brazil, including the twenty-one colonies collected by us at the Floresta Nacional de Chapecó (this study). The collections were made from lower to mid elevations (~150–600 m elevation) in remnants of the Atlantic forest in Brazil and, in a single case, in Argentina.

Our field observations indicate that workers of *Mycetophylax asper* forage individually throughout the day for substrate, mainly frass from green-plant-consuming insects. As in many other Attini and fungus-farming ants, workers of *M. asper* feign death when disturbed [13, 82].

Twenty-one nests of *Mycetophylax asper* were excavated at the Floresta Nacional de Chapecó. Information regarding nest architecture and nest demography is summarized in Table 1. These colonies were located in and at the edge of an unpaved dirt road through the forest, covered by grass and apparently rarely used (Fig 8F and 8G).

**Nest architecture.** Nest entrances of *Mycetophylax asper* consisted of a single hole in the ground (3–4.5 mm in diameter) surrounded by a mound of excavated soil (Fig 8A and 8B). In some cases, the area surrounding the nest entrance was covered by what appeared to be insect frass (Fig 8B). All excavated nests (Table 1) consisted of a single subspherical or dome-shaped (broadly convex on the top and flattened on the bottom) chamber, 1–7 cm in height and 1.5–8 cm in diameter (Fig 8C), located from 9–65 cm below the surface (Table 1). Each chamber contained a compact, small to large fungus garden suspended from the ceiling of the chamber.
by rootlets. Most of the fungus gardens extracted from these colonies were of a light yellow color and honeycomb-like in appearance, with some parts of the garden greenish in color due to the presence of newly added substrate (Fig 8D and 8E). In six of the larger colonies, a wet mud pellet was found at the bottom of the chamber, possibly consisting of dirt or refuse (Fig 8E), differing from the infrabuccal pellet piles described by Little et al., [83].

Demography. Colonies of Mycetophylax asper are relatively small, containing up to 100 workers. A single, dealate queen was found in every excavated colony, indicating that M. asper is monogynous. As of April 2017, eleven of the twenty-one colonies remain alive in artificial nest boxes at the USNM (see Table 1). Observations during a one-year period in the laboratory

Fig 8. Mycetophylax asper, field images. a) Nest entrance, nest TRS141017-01; b) nest entrance, nest AJ141018-11; c) subterranean garden chamber; d) fungus garden and ants in field nest boxes; e) fungus garden and ants in field boxes showing pellet of wet dirt or refuse; g) and f) habitat.

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suggest that *Mycetophylax asper* produces reproductive forms from January to April, corresponding to the austral summer when temperatures are warmest.

### Ant morphology

Morphologically, *Mycetophylax asper* shares with most members of the genus *Mycetophylax*, as defined here, the circumscribed antennal scrobes formed by the joining of the frontal carinae and the posterad-directed preocular carinae at the occipital corners (secondarily reduced in *Mycetophylax conformis* and *Mycetophylax simplex*) (Figs 2B and 2D; 3A and 3G; 4A and 4D), six or more mandibular teeth (Fig 5A), and the presence of a single mid-pronotal tubercle [17, 75], secondarily reduced in the species *M. conformis*, *M. morschi*, and *M. simplex*. In the males of *Mycetophylax asper* the antennal scapes are long (SL 0.52–0.63, SI 104–110), longer than length of funicular segments I–III combined, a condition that is shared with other neoattine species and that contrasts with that of the paleoattines, in which the male antennal scape is shorter than the combined length of funicular segments I–III [19, 35]. In addition, the males of *Mycetophylax asper*, which were unknown at the time of Kempf (17) revision of the *Cyphomyrmex strigatus* group, have 12-segmented antennae, a deviation from the ancestral condition of 13 found in most fungus-farming ants. Although this condition occurs in other members of the former *Cyphomyrmex strigatus*-group (*Mycetophylax faunulus*, *Mycetophylax auritus*) and in *Mycetophylax conformis* [34], it remains unclear whether this shared reduction is due to homology or homoplasy because (i) *Mycetophylax morschi* and *Mycetophylax simplex* males are reported to have 13-segmented antennae [34] and (ii) the 12-segmented condition has arisen independently multiple times in the Attina, including in *Mycetagroicus inflatus*, species of *Sericomyrmex*, *Trachymyrmex opulentus* [23, 34, 36], and some social parasites [84–86].

Based on larval characters, *Mycetophylax asper* is clearly a member of the Neoattina and is more closely related to members of the former *Cyphomyrmex strigatus*-group (= *Mycetophylax* s.l.) than to those of the *C. rimosus* (yeast-farming) group. Character states shared with all other fungus-farming ants include the absence of a thoracic-abdominal articulation, the absence of deep lateral depressions associated with the abdominal spiracles, the superficiality of the thoracic intersegmental constrictions, the leg vestiges visible as open slits ventrally on the thorax, the mandibles fleshy and subconical, and, shared with most other attines, the absence of setae on the dorsal and lateral body surfaces (Fig 7A). Character states shared with other neoattines include the fusion of the basal portion of the maxilla with the head capsule combined with the position of the maxillary palp, widely removed laterad from the galea; the labium feebly protruding; the absence of lateral sericteral protuberances; and the reduction of the labial palps to sensilla.

A close relationship of *Mycetophylax asper* to *Mycetophylax* s.l. (i.e., as here redefined) is indicated by the states of no less than six larval characters. First, the presence of non-lobiform ventromedian protuberances on the second and third abdominal segments is similar to the condition observed in the species *Mycetophylax auritus* and *M. faunulus* [87], whereas it differs from the condition in the yeast-cultivating *C. rimosus*-group species in which the first abdominal segment (and, in some species, the second and even third abdominal segments) bears a triangular, lobiform appendage [79, 87]. Second, the hypopharyngeal spinules are predominantly unidentate, a condition previously described only in *M. auritus* and *M. faunulus* [87] (Fig 7D). Third, a single pair of setae arise anterior to the anal opening, another pair arise lateral to the opening, and all four setae arise on abdominal segment ten (Fig 7F and 7G). This pattern is apparently related to the conditions observed in both the *Cyphomyrmex strigatus*- (as formerly defined, = *Mycetophylax* s.l.) and *Cyphomyrmex rimosus*-group species [87], in which there are four ventral setae, but it may also be related to the condition reported in *Mycetophylax*...
conformis, in which there are only two ventral setae [87]. Fourth, the ventral anal lip is absent, differing from the condition in most but not all other known Cyphomyrmex species, including some members of the newly defined Mycetophylax, but shared with M. auritus and M. conformis. Fifth, the presence of a distinct mandibular apical tooth is shared with all known Mycetophylax s.l. species but not with C. rimosus-group species, in which the apical tooth is reduced to a spine. Sixth, the spinules on the head are papilliform as in other known Mycetophylax s.l. species, rather than serrate as in most known C. rimosus-group species.

The larva of Mycetophylax asper differs from those of other neoattines in having the galea remarkably enlarged and covered with denticles (Fig 7C). It differs from other Mycetophylax species in having the hypopharyngeal spinules densely distributed, an apparent symplesiomorphy shared with most attine ants, including members of the C. rimosus group. The larva of
Mycetophylax asper is further distinct from all other known Cyphomyrmex and Mycetophylax species in (i) lacking ventral setae on the ninth abdominal segment and (ii) the apparent absence of genal lobes.

Phylogeny

Results of the ant molecular phylogenetic analyses, which, compared to those of Schultz and Brady [39] and Sosa-Calvo et al., [7], include an additional nuclear gene fragment and additional taxa, place Mycetophylax asper in the former strigatus group of the genus Cyphomyrmex (rendered paraphyletic with respect to Mycetophylax) with strong support (Fig 1). Moreover, Mycetophylax asper is closely related to the recently described species Cyphomyrmex andersoni [75] from Central America (Fig 1), here transferred to Mycetophylax. This curious distribution suggests (i) that either or both Mycetophylax asper (in southern Brazil) and M. andersoni (in Mesoamerica) are more widespread than is currently known or (ii) that the distribution of their unknown most recent common ancestor is or was at some time in the past more widespread than either species is today. This pattern of peripherally and disjunctly distributed relict taxa is common across multiple sister clades in the Neoattina and is certainly deserving of further inquiry [14].

Results from the fungal molecular phylogenetic analyses indicate that the fungal species cultivated by Mycetophylax asper falls within Clade 2 of the lower attine cultivars (Fig 9, left and center trees) [42, 88]. More specifically, the fungal cultivar of Mycetophylax asper belongs to Clade 2, subclade F (Fig 9, center and right trees) of Mehdiaabadi et al., [41], likely a single fungal species that is also cultivated by Cyatta abscondita [7], Kalathomyrmex emeryi, Mycetagroicus cerradensis [43], Mycetagroicus inflatus [36], Mycocepurus smithi, Myrmicocrypta buenzii, and two species in the former Cyphomyrmex strigatus group (Mycetophylax faunulus and Mycetophylax strigatus) [41]. Curiously, the ITS fungal strains most closely related to those cultivated by the forest-dwelling Mycetophylax asper are also cultivated by cerrado-dwelling species (Cyatta abscondita, Mycetagroicus inflatus, Mycetagroicus cerradensis, and Kalathomyrmex emeryi), in some cases thousands of kilometers away.

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