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COMPARISON OF THREE MOONMILK CAVE HABITATS ASSOCIATED WITH TROGLOBITIC BEETLES


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This study focuses on three different hydrated secondary calcium carbonate moonmilk deposits associated with troglobitic beetles, Cansitiella spp. (Leptodrininae). Three caves systems were evaluated (Grotta della Foos, Vecchia Diga, and Bus della Genziana, North Eastern Italy). In Grotta della Foos, up to 8-12 specimens per m² Cansitiella spp. are found associated with moonmilk. Grotta della Foos moonmilk had 0.01-0.06% (w/w) organic nitrogen and 0.1-0.52% (w/w) organic carbon, and little to no chlorophyll within the moonmilk or percolating waters associated with the moonmilk, indicating limited surface-derived organic carbon in the beetle habitat. Although it is well known that Cansitiella spp. have modified mouth parts (hoe-shaped mandibles and spoon-shaped galeas), there has not been thorough evaluations of its food source. We hypothesized that the mouth parts represent adaptations to browsing the moonmilk, and specifically feeding on microbes incorporated within the moonmilk structure. Observations of beetles from Grotta della Foos confirm that moonmilk fragments are ingested. The possibility of moonmilk-based foodwebs may offer some insight into mechanisms that have dictated novel troglobitic adaptations in nutrient-limited conditions.

1. Introduction

Cave and karst habitats are generally characterized as having limited amounts of food for troglobitic invertebrates, due in part to hydrological isolation that restricts the influx of surface-derived organic matter. An interesting troglobitic beetle, Cansitiella spp., is found in three caves in northeastern Italy (Grotta della Foos, Vecchia Diga, and Bus della Genziana) (Gasparo, 1971). These beetles have been previously described to have peculiar, semi-aquatic feeding behavior and body characteristics that differ greatly from the majority of other troglobitic Leptodrininae, including a feeding apparatus with distinct mandibles, galeas, and distinct short apical labial article shapes (Paoletti, 1973, 1980; Piva, 2000) (Fig. 1). Based on the habitat locations, and our observations of beetles browsing the moonmilk, we hypothesize that the beetles consume microbes within the moonmilk and at the moonmilk-water interface, otherwise known as the cave hygropetric habitat (Skei, 2004).

Vecchia Diga Cave and Genziana Cave contain Cansitiella tonieloi, but most of our research to date has been in Grotta della Foos where we found Cansitiella servadeci (Paoletti et al., 2009). Microbial biomass from the Grotta della Foos moonmilk was estimated to be ~10⁷ microbial cells/ml and ~10⁴ micro- and meiofaunal individuals per m², suggesting significant standing stock (Engel et al., 2009). The bacterial diversity of the moonmilk was evaluated by screening 16S rRNA gene sequence clone libraries. The majority of clones were affiliated with the Proteobacteria phylum (57%), among which the Betaproteobacteria class (26%) dominated, followed by the Bacteroidetes/Chlorobi (34%), and rarer sequences (<10% total of all clones, and in order of decreasing relative abundance) represented by the candidate division TM7, Planctomycetacia, Ferruginicobia, Acidobacteria, Actinomycetacia, Firmicutes, Nitrospirae, and

Figure 1: Cansitiella servadeci on hydrated moonmilk in Grotta della Foos cave, Italy. The beetle is approximately 2.8 mm long.
the candidate division WS3. Some of these microbial
groups have not been identified from cave or karst habitats
and consequently their roles in the moonmilk system are
not known. The purpose of the current investigation was
to compare the moonmilk and habitat geochemistry of
the three beetle cave habitats to begin to evaluate possible
foodweb interactions.

2. Materials and Methods
The beetles were filmed using digital and video cameras to
assess movements and feeding modalities and to document
foraging and feeding behavior. Moonmilk samples and
associated water were collected aseptically from each of the
caves and maintained at 4°C. Water samples were filtered
to 0.2 μm and analyzed for major ions, pH, temperature,
and other parameters were measured immediately in the
cave (Table 1). Unprepared and hydrated moonmilk
samples were examined using a Philips XL 30 ESEM-TEM
environmental (E-) scanning electron microscope (SEM)
under water vapor conditions were normally at 5°C at
4.5 to 6.5 Torr in the instrumental chamber. The ESEM
was capable of X-ray fluorescence (XRF), induced by the
electron primary beam and detected in Energy Dispersive
Spectroscopy (EDS) mode. *Cansiliella* spp. individuals
were analyzed with SEM. Samples were either air-dried
or washed in alcohol and covered with a thin gold layer.
ESEM observations of the invertebrates were also done by
mounting them on a thermoregulated holder.

3. Results and Discussion
*Cansiliella* spp. were associated with moonmilk deposits in
all caves, and individuals were observed under 2-8 mm of
percolating water flowing over the moonmilk. *Cansiliella*
spp. were not seen on bare limestone in any system, although
the moonmilk in Genziana Cave was markedly thinner and
patchier than the moonmilk from the other caves.
Moonmilk had a marzipan-like structure from Grotta
della Foos and Vecchia Diga, and the associated waters had
comparable geochemistry (Table 1). The water entering into
the moonmilk area of Genziana cave, which is located at
a higher elevation than the other two caves, had lower pH
and temperature and higher dissolved chloride and sodium
concentrations (Table 1). In fact, the Genziana moonmilk
appeared to be in a state of erosion rather than deposition,
which may be due to the higher Na-Cl levels. Despite the
slight differences in water composition, the moonmilk in the
three caves had essentially the same elemental composition
from XRF analyses, although there was a slightly elevated
silica concentration in the Vecchia Diga moonmilk (Table
2).

<table>
<thead>
<tr>
<th>Date</th>
<th>Grotta della Foos</th>
<th>Vecchia Diga</th>
<th>Genziana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air temp</td>
<td>8.6°C</td>
<td>7.3°C</td>
<td>6.2°C</td>
</tr>
<tr>
<td>Water temp</td>
<td>8.8°C</td>
<td>8.8°C</td>
<td>7°C</td>
</tr>
<tr>
<td>pH</td>
<td>8.25</td>
<td>8.21</td>
<td>7.89</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>10.8 mg/L</td>
<td>8.8</td>
<td>7.87</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Conductivity</td>
<td>225 μS/cm</td>
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<td>390</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.2 mg/L</td>
<td>0.6</td>
<td>25.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>&gt;0.05 mg/L</td>
<td>&lt;0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.3 mg/L</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>45.2 mg/L</td>
<td>37.8</td>
<td>46</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>&gt;0.05 mg/L</td>
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<td>1</td>
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<tr>
<td>Cl⁻</td>
<td>1.2 mg/L</td>
<td>0.8</td>
<td>58.6</td>
</tr>
<tr>
<td>HCO₃⁻</td>
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<td>131.8</td>
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<tr>
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<td>&lt;0.05</td>
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<td>4</td>
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<tr>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>3.5 mg/L</td>
<td>1.8</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Table 1: Physicochemistry of percolating waters from the three study caves.

When *Cansiliella* spp. were found associated
with the moonmilk, or within the percolating
waters, their mouth parts
were engaged against
the moonmilk, so much
so, in some instances,
that physical disruption
of the moonmilk surface
could be seen. From
Grotta della Foos,
moonmilk particles were
observed in the beetle
mouth (Fig. 2). Microbial
cells, and predominately
filamentous cells, were
observed to be an
integral component of the
moonmilk (Fig. 3). The
role of filaments has been
previously considered
essential to moonmilk
formation (e.g., Borsato et
al., 2000).
to elevate presence of extraneous ions, do not present the better conditions to support the fragile presence of *Cansiliella*.

References


### Table 2: Elemental composition (in weight percent) of moonmilk by XRF. BDL = below detection limit.

<table>
<thead>
<tr>
<th></th>
<th>Grotta della Foos</th>
<th>Vecchia Diga</th>
<th>Genziana</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt %</td>
<td>w t %</td>
<td>w t %</td>
<td>w t %</td>
</tr>
<tr>
<td>C</td>
<td>14.9</td>
<td>15.38</td>
<td>14.13</td>
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<tr>
<td>O</td>
<td>38.57</td>
<td>43.28</td>
<td>41.5</td>
</tr>
<tr>
<td>Mg</td>
<td>0.38</td>
<td>0.82</td>
<td>0.43</td>
</tr>
<tr>
<td>Al</td>
<td>1.94</td>
<td>3.97</td>
<td>1.47</td>
</tr>
<tr>
<td>Si</td>
<td>2.57</td>
<td>5.33</td>
<td>2.72</td>
</tr>
<tr>
<td>P</td>
<td>0.92</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>S</td>
<td>0.95</td>
<td>BDL</td>
<td>BDL</td>
</tr>
<tr>
<td>K</td>
<td>0.5</td>
<td>0.76</td>
<td>0.47</td>
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<tr>
<td>Ca</td>
<td>39.16</td>
<td>28.66</td>
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</tr>
<tr>
<td>Fe</td>
<td>1.99</td>
<td>1.8</td>
<td>1.62</td>
</tr>
</tbody>
</table>

![Figure 2: Arrows point to carbonate particles in the mouth parts of C. servadaii from Grotta della Foos cave, Italy.](image1)

![Figure 3: SEM photomicrographs of microbial cells intermixed with carbonate minerals from moonmilk in Grotta della Foos (1B), microbial cells from the moonmilk-water interface in Grotta della Vecchia Diga (2B).](image2)