The rise of the diptera-microbial mat interactions during the Cenozoic: consequences for the sedimentary record of saline lakes

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ABSTRACT

Shoreline gypsiferous sediments of an inland lake in central Spain furnish valuable insight into reconstructions of early sedimentary changes related to shore fly–microbial mat interactions in fossil gypsum precipitating saline lake systems. The association of adult and larval forms of Ephydra (Diptera) with microbial matgrounds overlying the lake margin results in the formation of gypsiferous meniscate back-filled burrows that provide an analogue for recurring, extensively developed trace fossils that occur in Cenozoic, but not older, lacustrine gypsum rocks. In this setting, sediment burrowing by ephydridae hinders significant preservation of microbialites. The overwhelming rise of Diptera at the onset of the Cenozoic resulted in extensive feeding and dwelling activity and contributed to reshape the saline aquatic habitat where microbial mats thrived, thus leading to the formation of specific trace fossils that are illustrative of the existence of microbes in the paleoenvironment.


Introduction

Although the concept of Phanerozoic stromatolite decline by grazing and burrowing animals (Garrett, 1970; Awramik, 1971) was considered too simplistic (Pratt, 1982), it is generally accepted that the appearance of three-dimensional trace fossils in Early Cambrian strata testifies to a behavioural revolution (Seilacher, 1999). The rapid evolution of efficient grazing animals constrained development of benthic mats while bioturbation disrupted buried mats and closed a taphonomic window of preservation for soft-bodied organisms (Gehling, 1999). The earliest mobile animals are interpreted to have burrowed microbially bound sediments for food resources (Gehling, 1999; Gingras et al., 2011). This interaction was conducive to a remarkable increase in the degree of bioturbation of the sediment. Buatois and Mángano (2011) have noticed that the presence of shallow feeding burrows associated with matgrounds has recurred at certain times in Earth’s history.

Some invertebrates feed and grow well on cyanobacterial substrates (Monakov, 2011). However, most examples of successful exploitation of cyanobacteria under natural conditions come from dipteran larvae (Foote, 1977). Laboratory experiments conducted by Krivosheina (2008) confirmed the ability of higher Diptera, dominantly Ephydridae, to feed on and digest cyanobacteria. Ephydridae, also called shore flies, alkaline or brine flies (Foote, 1995), are common in aquatic habitats throughout the world and they often are the sole insect species of the benthic and shoreline portions of inland saline lakes and ponds (Herbst, 1988). Ephydridae are an important food source for wildlife in saltwater pools, such as it is observed in Mono Lake, California, where millions of birds are supported almost entirely by ephydrids (Jehl, 1986; Rubega and Inouye, 1994). Other insects, such as dragonflies and chironomids are also common inhabitants of modern and ancient saline lake environments (Brennan and McLachlan, 1979; Gingras et al., 2007; Scott et al., 2009). Ephydridae are well adapted to living in arid zone shallow ephemeral waters with drastic seasonal salinity fluctuations (Warren, 2006, p. 637), but require certain concentrations for survival and normal development (Herbst, 1999).

The appearance of higher Diptera took place in the Late Cretaceous (Blagoderov et al., 2002). Throughout the Palaeogene, the dominant structure of Diptera gradually shifted to the modern one. By the beginning of the Neogene, dipteran ecological and taxonomic diversity expanded rapidly, which resulted in the appearance of the polydominant fauna of modern type. The ancestors of modern higher Diptera were adapted for life as saprophages in rotting vegetation (Ferrar, 1987). The adaptive radiation of higher Diptera, for instance Ephydridae, to different spatial and trophic resources like microbial mats, illustrates some reasons for the ample widespread success of this type of insects in wetlands (Keiper et al., 2002).

Preservation of organic remains and/or ichnofabrics in sedimentary
deposits that accumulated in saline environments is usually poor (Warren, 2006), which makes paleoecological reconstructions more challenging (Truc, 1980; Palacios-Fest et al., 1994). In contrast, strongly burrowed gypsum deposits showing a tangle-pattern structure occur in several fossil evaporite lake basins in the Cenozoic record, especially from the Mediterranean region (Truc, 1980; Ortí and Salvany, 1990; Arribas et al., 1991; Rodríguez-Aranda and Calvo, 1998; Huerta et al., 2010; Dr. Ibrahim Gündogan, 2012, pers. commun.). In the basins, up to 150-m-thick sedimentary sequences containing burrowed gypsum beds were deposited in shallow, but extensive lakes that covered areas of hundreds to thousands of km². Burrows occur throughout gypsum beds that locally reach up to 1 m in thickness and show a diffuse, mottled structure (Fig. 1A). Selective silicification enhancing morphological traces of the burrows is locally recognized (Fig. 1B) (Arribas et al., 1991; Sanz-Montero et al., 2008; Sanz-Montero and Rodríguez-Aranda, 2009). Usually, the burrow traces form a very dense network within the sediment and locally exceed 60% of the total volume of the gypsum strata (Figs 1A and B). The individual burrows are 0.5–5 mm in diameter, and up to 5 cm in length (Fig. 1B) within the tangle-pattern fabric. The burrows display typical meniscate infill composed of lenticular gypsum, faecal pellets and variable amount of micrite and/or clay (Figs 1C and D). The gypsum meniscate infill appears to have further aided in their recognition.

This article focuses on microbial mats thriving in the inland saline Lake El Longar, central Spain, that offers a chance to compare extant littoral biomats buried by Ephydridae with Cenozoic bioturbated gypsum deposits. Thus, this study provides for the first time a modern analogue for interpreting gypsiferous meniscate back-filled burrows. Moreover, observation from this modern environment furnishes valuable insight into reconstructions of early sedimentary changes related to shore fly–microbial mat interactions in fossil saline lake systems. As discussed below, these processes were especially significant after the end of the Cretaceous.

Methods

Fieldwork in Lake El Longar (altitude 683 m amsl) (Fig. 2) was conducted in five campaigns during different seasons between 2009 and 2012. Sedimentological work and sampling were mainly performed along the eastern marshy shoreline (Fig. 2). The exposed shoreline varies over the seasons and it may reach some tens of metres in the dry periods.

Water temperature, salinity and pH immediate measurements were taken with a PC300XS Sampler. Water samples were collected in duplicate directly from the lake. The water anions were determined by electrophoresis, the cations by atomic absorption and carbonates and bicarbonates by titration in the Geochemistry Laboratory of the Museo Nacional de Ciencias Naturales-CSIC (Madrid).

Diptera specimens were studied and images taken using a stereoscopic binocular microscope. Forty organosedimentary samples comprising mineral and microbial mats were collected by hand and by using a PVC dredging device dug up to 10 cm deep into the shoreline matground. The mineralogy was determined by XRD of powdered specimens on a Philips X-ray diffraction system. Preparation of thin-sections, sediment samples were dehydrated by lyophilization, embedded and impregnated with Epofix resin. Optical and fluorescence microscopies were used for measurements of bioturbated structures (Fig. 1).

Fig. 1 Examples of bioturbated gypsum from the Miocene of the Madrid Basin. Rocks shown in A and D crop out in the vicinity of Lake El Longar, their location is arrowed in Fig. 2. B and C bioturbated rocks crop out at Brea de Tajo (40°14′ 15″ N, 3°4′32″ W; altitude 725 m amsl) (Rodríguez-Aranda, 1995). (A) Close-up view of a burrowed gypsum bed characterized by a diffuse, mottled appearance, pen cap for scale = 40 mm long. (B) Hand sample showing silicified gypsum burrows with a tangle-pattern fabric, coin for scale, diameter = 23 mm. Photomicrographs showing meniscate back-filled burrows formed of gypsum (C) and silica pseudomorphs after lenticular gypsum (D).

Fig. 2 Location map and satellite view of Lake El Longar. The main sampling area is encircled. The location of Miocene gypsiferous rocks is arrowed.
examinations of thin sections were performed using an Olympus BX51 microscope. For high-resolution textural analysis, carbon-coated thin sections and fresh broken surfaces of previously air-dried and gold-coated samples were studied with scanning electron microscopy provided with X-ray energy-dispersive spectroscopy. In addition, two wet, uncoated specimens were observed by an environmental scanning electron microscope.

Setting
Lake El Longar is an inland, closed lake occupying a Quaternary flat-bottomed depression in Miocene gypsiferous mudstone located in La Mancha (Central Spain). The lake covers an area of 1 km² and has 5.36 km of perimeter. Lake water level can reach a maximum of 30 cm but usually water depth is below 20 cm. The main wildlife value of the area, mainly during the breeding season, is with waterfowl species such as terns and cranes, and also the presence of threatened steppe birds and terrestrial halophilic invertebrates. As with other lakes, dipteran larvae are a major source of food for waterfowl, shore birds and other animals in Lake El Longar. The marginal vegetation consists of annual and perennial halophytic plants (Cirujano, 1980). The local climate is Mediterranean characterized by aridity and continentality, with average temperature of 14.3 °C and average annual rainfall of 360 mm, though with significant seasonal fluctuations. The summer months are very dry, which favours desiccation of the shallower lake areas. The last complete desiccation of the lake happened in summer 2008.

Data on the hydrochemistry of the lake water is shown in Table 1. The lake brine is Mg-Na-(Ca)-SO₄·Cl type III B (Eugster and Hardie, 1978). The salinity of the water ranges from 34 to 304 °C.

The shoreline area of the lake is covered by a cohesive and tiered microbial mat. The sediment surface displays varied mat-related morphologies including desiccation polygons (Figs 3A and B), punctual, convolute, wrinkle marks, mat chips, curling up, gas domes, etc. The occurrence and distribution of the mat surface structures are related to a variety of physical, chemical and biological processes (Sanz-Montero et al., 2012). From the surface downwards, the mat is composed of a green superficial layer of cyanobacteria showing two morphologically distinct types of Oscillatoria, minor occurrences of Pseudoanabaena, LPP-forms (Guerrero and De Wit, 1992), and local presence of diatoms (Navicula and Nitzschia), a thin red layer formed by photosynthetic purple sulphur bacteria, and a lowermost cm thick black layer with evidence for sulphate reduction (Fig. 4).

Gypsum is the main precipitate from the lake brine. Lenticular gypsum crystals reaching up to 500 μm in diameter (Fig. 5) encrust the mat surface and also occur in the pigmented phototroph-dominated layers of both green and purple cyanobacteria (Fig. 5A). The gypsum crystals are embedded in a biofilm complex composed of bacterial cells with surrounding extracellular polymers and syngenetic mineral phases comprising mainly celestite (Fig. 5B), calcite, halite, hexahydrite and elemental sulphur.

Table 1 Chemical features of El Longar water.

<table>
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<tr>
<th>Date</th>
<th>T (°C)</th>
<th>pH</th>
<th>Cl⁻</th>
<th>SO₄²⁻</th>
<th>CO₃²⁻</th>
<th>HCO₃⁻</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>TDS (ppm)</th>
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<td>10778</td>
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<td>426.83</td>
<td>756</td>
<td>4000</td>
<td>3100</td>
<td>705</td>
<td>340</td>
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<tr>
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<td>7.79</td>
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<td>142520</td>
<td>1351.4</td>
<td>701.22</td>
<td>444</td>
<td>33440</td>
<td>38860</td>
<td>5700</td>
<td>304.4</td>
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<tr>
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<td>7.76</td>
<td>14307</td>
<td>21812</td>
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<td>0.00</td>
<td>674</td>
<td>6800</td>
<td>8300</td>
<td>940</td>
<td>52.9</td>
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</table>

Fig. 3 Littoral area of Lake El Longar (June 2010). (A) General view of the benthic surface which is colonized by microbial mats with polygonal desiccation cracks. (B) A high proliferation of Ephydridae flies (black blobs) is just restricted to the water’s edge. (C) Close-up view of the flies densely populating the shoreline biomats. (D) Close-up view of microbial mat degradation by grazing Ephydridae. Bar for scale is 15 cm.

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semi-aquatic flies (Diptera: Ephydridae) (Figs 3C and D) and their aquatic larvae scavenging and feeding on the microbial mats. The Diptera move following the progressive retreat of the strandline (Fig. 3C) as the brine concentration increases so that the upper layer of sediment is burrowed in an area extending at least 5 m from the water line to the back of the shore and around 500 m in width along the shoreline. Other ground-burrowing organisms inhabiting the upper part of the shoreline include salt-tolerant beetles, but they are quite subordinate in number in relation to the extremely abundant Ephydridae populations. The vast majority of flies are *Ephydra macellaria* (Diptera). The flies develop in a few weeks from egg to larva with three larval instars that display the same morphology before pupating and metamorphosing. Larvae are translucent-white maggots with eight pairs of distinct ventral prolegs, dorsal spine patterns and distensible bifurcated syphon (Figs 6A and B), which are typical characters of Ephydridae. The larvae are from 1-to-25-mm long, including the respiratory tube. The larvae and pupae remain submerged throughout development. As an adaptation to the microbial mat habitat of the Ephydridae immatures, the prolegs bear crochets that assist in grasping the substrate (Mathis and Simpson, 1981). The larval head is small and withdrawn into the thorax. The larval respiratory syphon and spiracles are fully extended (Fig. 6A), which allow to obtain atmospheric oxygen, even though they may be at least partially submerged most of the time. The mature larvae pupate, by grasping the benthic mats, with prolegs of the VI and VIII abdominal segments that act as claws. Pupae skin hardens to form a case that holds the insect in a non-feeding stage (Fig. 6C).

In Lake El Longar both larvae and adults of Ephydridae are confined to cyanobacterial mats and feed on cyanobacteria (Figs 3D and 7A). Burrows produced by feeding of the larvae on microbial mats are arranged in all directions, resulting in an extensive burrowing of the uppermost layer of the biomat that displays a characteristic tangle pattern (Fig. 7B). The burrow traces are cylindrical and internally meniscate, up to 2 cm in length and 3.5 mm in diameter (Figs 7B and 8). Single burrows are backfilled by lenticular gypsum and pellets. The wall of the burrow trace is typically delimited by gypsum (‘burrow-lining’) (Fig. 8) probably due to the reinforcement of the burrow walls by impregnation with mucus (D’Alessandro and Bromley, 1987). As a result, most of the shore sediment consists of a mixture of meniscate tubules, cyanobacterial peat, disrupted gypsum and syngenetic mineral phases, pellets, pupae and insect remains including empty cases (Figs 6C and 7).

**Discussion and conclusion**

Gypsiferous meniscate back-filled traces recurrently found in Cenozoic lacustrine deposits have distinctive infilling pattern, sizes, morphologies and architectures (Fig. 1) consistent with their construction by dipteran larvae. The first finding of similar burrows produced today by Ephydridae in Lake El Longar is noteworthy by two reasons: (i) it allows positive

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**Fig. 4** Top view showing the laminated microbial community. The mat is composed of a 1 mm-thick green superficial layer (g), a red layer formed by purple sulphur bacteria (r), and a lowermost black layer (b) several cm in thickness. On the underside of the red layer, oxygen bubbles can be seen.

**Fig. 5** Thin-section (A) and SEM-BSE (B) photomicrographs of gypsum precipitates. (A) Gypsum crystals precipitated within the microbial mat. (B) Micrometer-sized celestite crystals (white minerals) interspersed within lenticular gypsum crystals of different sizes.

**Fig. 6** Photomicrographs showing features of Ephydridae larvae. (A) and (B) lateral and ventral, respectively, views showing the typical morphology and adaptations of a third larval instar to the microbial mat ecosystem: a respiratory bifurcated siphon (r), opposite the head (h); eight pairs of prolegs-bearing crochets (p), the sixth and eighth pairs that act as claws are arrowed. (C) Ephydridae immaturity (I) and cases (arrowed) embedded in a microbial mat.
identification of the animals that produced burrowed gypsum deposits during the Cenozoic; and (ii) recognition of this type of bioturbated gypsum lithofacies can be used as evidence of former microbial mats in the environment.

In a prior overview on trace fossils in Miocene gypsum deposits, Rodríguez-Aranda and Calvo (1998) interpreted the gypsumiferous meniscate back-filled traces as feeding and dwelling structures produced by subaquatic insect larvae that were tentatively attributed to Chironomidae. The interpretation was based on the similarities in both geometry and size of the traces compared with burrow traces made by chironomids in modern and ancient, fresh to moderately saline lacustrine environments (Table 2), whilst recognizing the lack of modern analogous from gypsum-forming lakes. The present study on recent gypsumiferous lake sediments enables us to re-interpret the role played by chironomids and to recognize the importance of Ephydridae as producers of the bioturbated gypsum facies. One physiological feature that is considered meaningful is that the Ephydridae larvae possess breathing tubes that allow them to connect directly to the atmosphere. This respiratory strategy forces the larvae to stay close to the shore and likely prevents the formation of complex individual tubes such as those exhibited by chironomidae larvae that in addition need their tubes to remain open for ventilation purposes (Rasmussen, 1996).

Although pupae and cases have on occasion been found in Miocene lake deposits (Palmer and Carvalho, 1957; Pierce, 1996; Park and Downing, 2001), fossilization of Diptera remains is rare because soft tissues are destroyed by predators, scavengers and microbes. In contrast and according to the documented occurrences of mm-sized, tangle-pattern burrow structures in gypsum deposits from Palaeogene and, more commonly, from Neogene sedimentary sequences in the Mediterranean region (see references above); the potential for preservation in the geological record of gypsum backfilled, meniscate bioturbation fabrics is relatively high.

The abundance of burrowers in the environment, which is the case of Ephydridae larvae in many saline lakes, can contribute to increase the preservation potential of their traces. The development of large populations of these insects and the preservation of their body remains in the shoreline deposits of Lake El Longar is envisaged as a good modern analogue for bioturbated gypsum facies in fossil evaporite lake systems. As with other saline lakes, mainly from North America (Herbst, 1988; Schultze-Lam et al., 1996), the grazing activity of ephydrids has been found to have a significant influence on mat growth in the El Longar shoreline (Figs 3D and 7). Thereby, the activity of burrowers prevents the development of microbially induced sedimentary structures, thus reducing the preservation of microbialite in which mineralization is taking place.

This may explain the scarcity of microbial buildups in Cenozoic evaporite rocks that were deposited in lacustrine environments where the microbial mats would be expected to have thrived. Stromatolites and thrombolite, however, develop in hypersaline settings (Des Marais, 2003; Petrush et al., 2012) that are characterized by higher rates of organic production relative to grazing (Stal, 2000).

The evolution of higher Diptera from their appearance in the Late Cretaceous and further expansion through the Palaeogene and, namely, during the Miocene matches well with the presence and stratigraphic distribution of mm-sized gypsum back-filled burrows in evaporitic
Table 2 Comparison of the burrow features made by larvae of Ephydra macellaria in Lake El Longar with those of chironomid larvae in fresh to saline lake environments (data compiled from Paterson and Walker (1974), Hammer (1986), Williams et al. (1990), Gingras et al. (2007), White and Miller (2008) and Brand et al. (2012); note that chironomid larval tubes forming laminar tufa (e.g. Brasier et al., 2011) are described as a particular case of burrowing made by this group of insects.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Burrows Size (mm)</th>
<th>Shape</th>
<th>Pattern</th>
<th>Infill</th>
<th>Walls</th>
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</thead>
<tbody>
<tr>
<td>Ephydra macellaria</td>
<td>1–3.5</td>
<td>Blindly ending cylindrical tubes</td>
<td>Tangle-pattern</td>
<td>Gypsum and pellets</td>
<td>Gypsum-lined</td>
</tr>
<tr>
<td>Chironomids</td>
<td>1–3</td>
<td>Non-blindly ending, U, J, L, Y-shaped tubes</td>
<td>Thalassinoid-like</td>
<td>Meniscate backfill</td>
<td>Gypsum-lined</td>
</tr>
<tr>
<td>Chironomid larval tubes in laminar tufa</td>
<td>0.5–5</td>
<td>Lenticular to hemispherical tubes</td>
<td>Convolute networks with hemispherical cross-sections</td>
<td>Empty hollows-spar cemented</td>
<td>Calcite micrite-lined</td>
</tr>
</tbody>
</table>

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