A marine invasive benthic diatom species [Licmophora normaniana (Greville) Wahrer, 1985] in an inland oasis mineral spring in Egypt

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Abstract
Sampling campaigns associated with the ongoing PhyBiO project revealed the new presence of the benthic pinnate heterovalvar araphid diatom Licmophora normaniana. This species is considered cosmopolitan and is common on marine coasts. Based on literature searches, the following identification represents the first known occurrence in an inland mineral spring in the Siwa Oasis, the Western Desert of Egypt, and of this diatom species in mineral desert springs generally, or from any inland waterbody. The presence of L. normaniana was confirmed using light and scanning electron microscopy. Environmental variables (including major ions and metals) of this Saharan biotope were measured to characterize the habitat of this population. We hypothesize that the introduction of this marine species in the Siwa Oasis was related to fish farming activities carried out in the springhead by local inhabitants. We further hypothesize that the primary negative consequence of this invasion is likely to be the alteration of diatom community composition, with possible replacement of characteristic native taxa. Biological invasions place further pressure on spring habitats in oases, where they are affected by numerous growing human impacts, including global climatic changes. Therefore, legislation to protect these springs and enable sustainable and adaptive management, coupled with raised public awareness, is urgently needed to safeguard and conserve these unique biodiversity hotspots.

Key words: diatoms, invasive species, Saharan springs, conservation, the Siwa Oasis

Introduction
Over the last decades, the ecological integrity of inland aquatic ecosystems, particularly desert springs, has been intensely impaired by local human activities through nutrient enrichment due to land-use activities and fisheries management practices (e.g., Jeppesen et al. 2007). One of the main consequences on springs in arid climatic settings is the alteration of the community structure of autochthonous microorganisms such as diatoms (Cantonati et al. 2020). Generally, non-indigenous species (NIS) are continuously increasing today as a result of global climate changes and
direct human pressures (Simberloff et al. 2013). Of the many species that get introduced into new environments, only a small portion can acclimate physiologically to the new habitat and become established abundantly and permanently, generally with negative consequences for the colonized ecosystem: these are termed “invasive species” (Sakai et al. 2001; Colautti and MacIsaac 2004). Identification of invasive species and the study of their biological characteristics afford greater understanding of their ecophysiological adaptive traits, ecological niches, and potential distributions (Stachowicz et al. 2002; Facon et al. 2006).

The main goal of this study was to report the establishment of a cosmopolitan benthic marine diatom [the araphid *Licmophora normaniana* (Greville) Wahrer] in a groundwater-fed habitat (an inland mineral desert spring in the Siwa Oasis, the Western Desert of Egypt). We characterized the invasive diatom by studying morphology and ultrastructure, and the colonized habitat by measuring hydrochemical and environmental characteristics.

**Materials and methods**

**Study site**

The Siwa Oasis (~ 800 km² total area), with a hot hyper-arid desert climate, represents one of the smallest oases in the Western Desert of Egypt. It is located ca. 10–17 m below the sea level (El-Sabbagh et al. 2017). Groundwater (GW), the only available water source in this Saharan ecosystem, is mainly discharging from the world’s largest non-renewable GW resource, the Nubian Sandstone Aquifer System (NSAS), and the Fissured Complex Carbonate Aquifer (Powell and Fensham 2016). The samples including the invasive diatom species were collected on 6 May 2018 from the thermal limno-rheocrenic mineral spring “Ain Pirizi” (29°12′43″N; 25°30′44″E) at an elevation of ~16 m b.s.l. The springhead is surrounded by a concrete wall (Figure 1). Besides fish farming in the springhead, the spring is mainly used to carry out agricultural activities in this harsh desert habitat.

**Diatom sampling, processing, and identification**

Benthic diatoms, including *Licmophora normaniana*, were sampled following the European standard methods for sampling phytobenthos in shallow running waters (EN 15708 2009). The brown covers on the submerged rocky surfaces of the springhead were scraped using a special brushing syringe (Spitale et al. 2011). Five circular areas in different points were brushed, and all diatom materials were eventually combined in a composite sample representative for the spring. Diatoms were cleaned using hot 37% H₂O₂ and hydrochloric acid (HCl) for about one hour. The reaction was completed by the addition of K₂Cr₂O₇ (Cantonati et al. 2007). The resulting clean material was diluted with distilled water to avoid excessive diatom concentrations. Cleaned diatom valves were mounted in Naphrax®
Figure 1. Landscape view of the springhead of Ain Pirizi in the Siwa Oasis, the Western Desert of Egypt. Photograph by Abdullah A. Saber.

(refractive index = 1.74). Light (LM) and scanning electron microscopy (SEM) observations were conducted at the MUSE – Museo delle Scienze, Limnology & Phycology Section, Trento, Italy, using an Axioskop 2 microscope (Zeiss, Jena, Germany) equipped with an Axiocam digital camera, and a LEO XVP electron microscope (Carl Zeiss SMT Ltd., Cambridge, UK) at 10 kV on gold coated prepared material, respectively. To assess the relative abundances of the dominant and subdominant species, 400 valves were counted. Measurements of the morphological and ultrastructural features were conducted on 24 different specimens representative of the size-diminution series. As concerns *L. normaniana*, identification followed Wahrer et al. (1985), and terminology for valve morphology Round et al. (1990). For the identification of the dominant and subdominant species we used (Krammer and Lange-Bertalot 1991; Moser et al. 1998; Cantonati et al. 2017). The slides and prepared materials are deposited at the Phycology Unit (No. 341), the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt, and the MUSE.

*Hydrochemistry*

*In situ* water temperature, pH, ion conductivity, and total dissolved solids (TDS) were measured with a calibrated HANNA HI 991301 meter. The hydrochemical variables were analysed following standard methods adopted by Chapman and Pratt (1978), and Clesceri et al. (2000). Anions (Cl⁻ and SO₄²⁻), major cations (Na⁺, K⁺, Ca²⁺, and Mg²⁺), and metals were estimated using ionic chromatography (ICS 1500 Dionex Corp.). Nutrients (NO₃⁻, NO₂⁻, NH₄⁺, and SRP) were determined by molecular absorption spectrometry. Silicates (SiO₂) were assessed by the molybdosilicate method.
Results

Morphological and ultrastructural characteristics of the invasive benthic diatom

The invasive benthic diatom was identified as *Licmophora normaniana* (Greville) Wahrer (Class Bacillariophyceae, Order Licmophorales, Family Licmophoraceae, Genus *Licmophora*).

With LM, non-beaked and beaked valves were observed (Figure 2A, B). Valves arcuate, heteropolar, gradually tapering from the apex to the base, with rounded basal pole, and an asymmetrical rounding of the apical pole; 81–123 µm apical axis, 8.3–10.7 µm transapical axis near the apical pole, and 3.0–4.2 µm transapical axis near the basal pole. Striae parallel, typically opposite to slightly alternate, becoming radiate at the apical pole; 17–20(21) in 10 µm.

Under the SEM, externally striae uniseriate, composed of rounded to slightly transversely elongated areolae (Figure 2C–E). Areolae 30–32 in 10 µm. Sternum narrow and running along the entire valve surface (Figure 2F). Marked apical elongate slits present at the basal pole (Figure 2E). Internally, rimoportulae sessile, only present at the apical pole, obliquely (Figure 2G–H) or apically oriented (Figure 2I). External labiate process opening at the head pole not observed.

Teratological structure and arrangement of striae and areolae were also occasionally observed (Figure 2I–K).

Occurrence and autecology in the present study

The invasive benthic marine diatom was subdominant (with a relative abundance of 5%) among the other epilithic diatoms in the mineral spring Ain Pirizi (the Siwa Oasis, the Western Desert of Egypt). The dominant diatom species was *Gomphosphenia oahuensis* (Hustedt) Lange-Bertalot (81%), and other subdominants were *Achnanthes brevipes* C.Agardh (9%), and *Melosira moniliformis* var. *subglobosa* (Grunow) Hustedt (1%). The remaining diatom species accounted for only 4% of the assemblage composition. The tubular green macroalga *Ulva flexuosa* subsp. *paradoxa* (C.Agardh) M.J.Wynne was the main occurring macroalga. Based on Glazier’s (2009) classification system, who identified thermal springs as those with water temperatures above the mean annual air temperature (MAA, which is ca. 22 °C in the Siwa Oasis), Ain Pirizi is categorized as a “thermal” spring with relatively stable water temperature around 29 °C. The water had circumneutral pH value of 7.18 and high conductivity (41.4 mS.cm⁻¹). Sodium, chlorides, and sulphates constituted the major dissolved ions (7900, 14750, and 1250 mg.l⁻¹ for Na⁺, Cl⁻, and SO₄²⁻, respectively), reflecting the nature of the groundwater and bedrocks of the aquifer in this region. Noteworthy levels of other cations were also detected, i.e. K⁺, Ca²⁺, and Mg²⁺ had 400, 250, 575 mg.l⁻¹, respectively. Nutrients
Table 1. Physical and chemical variables of the inland thermal mineral spring Ain Pirizi (Siwa Oasis, the Western Desert of Egypt).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ain Pirizi</th>
</tr>
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<tbody>
<tr>
<td>Temperature (°C)</td>
<td>29</td>
</tr>
<tr>
<td>pH</td>
<td>7.18</td>
</tr>
<tr>
<td>Conductivity (mS.cm⁻¹)</td>
<td>41.4</td>
</tr>
<tr>
<td>T.D.S. (mg.l⁻¹)</td>
<td>20800</td>
</tr>
<tr>
<td>Na⁺ (mg.l⁻¹)</td>
<td>7900</td>
</tr>
<tr>
<td>K⁺ (mg.l⁻¹)</td>
<td>400</td>
</tr>
<tr>
<td>Ca²⁺ (mg.l⁻¹)</td>
<td>250</td>
</tr>
<tr>
<td>Mg²⁺ (mg.l⁻¹)</td>
<td>575</td>
</tr>
<tr>
<td>Cl⁻ (mg.l⁻¹)</td>
<td>14750</td>
</tr>
<tr>
<td>SO₄²⁻ (mg.l⁻¹)</td>
<td>1250</td>
</tr>
<tr>
<td>NH₄⁺ (µg.l⁻¹)</td>
<td>440</td>
</tr>
<tr>
<td>Fe (µg.l⁻¹)</td>
<td>132</td>
</tr>
<tr>
<td>Zn (µg.l⁻¹)</td>
<td>20</td>
</tr>
<tr>
<td>Mn (µg.l⁻¹)</td>
<td>52</td>
</tr>
<tr>
<td>Cu (µg.l⁻¹)</td>
<td>10</td>
</tr>
</tbody>
</table>

were in general very low in concentration, except NH₄⁺ (440 µg.l⁻¹), confirming the oligo–mesotrophic condition of this spring. The heavy metals Fe, Mn, Zn, and Cu showed notable concentrations, i.e. 132, 52, 20, and 10 µg.l⁻¹, respectively (Table 1).

Discussion

In arid, strongly water-limited regions, natural springs sustain important landscape functions (Parker et al. 2021), being biodiversity hotspots, keystone ecosystems, and evolutionary refugia (Cartwright et al. 2020). In spite of this, desert springs, like Ain Pirizi spring in the Siwa Oasis, are severely threatened by multiple stressors including water overexploitation (i.e., recreation and fish farming practices in the main springhead, and water abstraction for agricultural purposes), groundwater deterioration, and climatic changes (Cantonati et al. 2020). In the present study, the documentation of the benthic diatom *Licmophora normaniana*, typically cosmopolitan on marine and brackish coasts (e.g., Hernández-Almeida et al. 2013; López-Fuerte and Siqueiros-Beltrones 2016), for the first time in an inland mineral desert spring can most likely be attributed to rapidly-growing fish farming activities and other human impacts on this isolated, azonal biotope. Generally, in the oases, springs and their artificial counterparts, i.e. drilled wells, are the only available sources of water necessary for settlement and development. Being widely distributed on marine coasts, the diatom *L. normaniana* investigated in this study is considered an invasive species in this unique inland-desert spring ecosystem because: 1) previous studies on the diatom flora of the Siwa Oasis did not record this species (Shaaban 1985, 1994; Hamed 2008); 2) *L. normaniana* is fairly abundant in the diatom assemblage (being a large-celled species, its quantitative importance is under-expressed by the standard valve counts). From the taxonomic point of view, the Siwa Oasis *L. normaniana*
specimens resemble the holotype population described by Wahrer et al. (1985). In a similar way and in the frame of our in-depth multifaceted studies on the algal communities inhabiting the Siwa Oasis within the PhyBio project, a characteristic *Ulva* (Chlorophyta) population, *U. flexuosa* subsp. *paradoxa*, has recently been reported as an invasive species in Ain Abu Sherouf (Saber et al. 2018).

The *Asterionella formosa* Hassall incursion into New Zealand is an informative example of a diatom species invasion coupled with rapid expansion (Harper 1994), and of the significant negative effects that such a diatom incursion can cause. *Asterionella formosa* is a widespread planktonic diatom in eutrophic lakes (Krammer and Lange-Bertalot 1991). The analysis of lake sediment cores from New Zealand showed a complete absence of *A. formosa* in pre-European sediments, although it is now widespread (present in 45% of lakes for which phytoplankton records are available). Harper (1994) explained this diatom-invasion process via the introduction of salmon eggs into New Zealand lakes in the second half of the 19th century. Other convincing evidences for human-mediated introductions of invasive diatoms include the reporting of *Thalassiosira baltica* (Grunow) Ostenfeld in the Laurentian Great Lakes (Edlund et al. 2000), of the North-American species *Gomphoneis minuta* (Stone) Kociolek & Stoermer (*Gomphonema kociolekii* R.Jahn & N.Abarca according to current taxonomy) and *Encyonema triangulum* (Ehrenberg) Kützing in France (Coste and Ector 2000), and of *Coscinodiscus wailesii* Gran & Angst in the English coastal waters, where it is supposed to have arrived via ship’s ballast or by aquacultural practices (Edwards et al. 2001). Generally, the potential vectors responsible for passive dispersal of algae, including diatoms, fall into four main categories: water currents, animals, wind, and humans (Kristiansen 1996). From the biodiversity and ecological standpoints, Vanormelingen et al. (2008) and Cantonati et al. (2020) also stressed the significance of the protection and conservation of relatively isolated areas against human-mediated introductions of non-indigenous and invasive species.

Cantonati and Lowe (2014) noted that the mid-depth zone of lakes has several environmental characteristics typical of permanent springs, such as stable and favorable conditions, and the deep zone is as well stable, but light limited. Schütz et al. (2021) recently showed that zebra (and quagga) mussel invasion devastated the peculiar macroalgal association colonizing the littoriprofundal of Lake Constance: in particular, the world-wide rare brown macroalga *Bodanella lauterbornii* was displaced from its type habitat (submersed rockwalls). The algal communities and other biota of peculiar habitats, such as springs and the littoriprofundal, should thus be particularly protected from invasive species.

The major negative implications and consequences of this diatom-invasion process are the alteration of the native diatom and algal community
structure, with replacement of native and characteristic species with widely distributed halophilic taxa. Unfortunately, oasis desert springs, besides biological invasions, are also affected by other increasingly growing and diverse human impacts and by the detrimental effects of global climatic changes. Therefore, spring-protection legislation that foresees sustainable and adaptive management, and the raise of public awareness for these peculiar biodiversity hotspots are urgently needed to safeguard and conserve the role of these unique desert springs, and their capability to offer shelter to a rich and characteristic biodiversity.

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Authors’ contribution

Conceptualization, A.A.S., M.C.; methodology, A.A.S., M.C.; validation, A.A.S., M.C.; formal analysis, A.A.S., M.C.; investigation, A.A.S; resources, A.A.S.; data curation, A.A.S., M.C.; writing – original draft preparation, A.A.S., A.B., H.S., M.C.; writing – review and editing, M.C., M.E., A.A.G.; visualization, A.A.S., M.C.; supervision, M.C., M.E., A.A.G.; project administration, M.C.; funding acquisition, A.A.S., M.C. All authors have read and agreed to the published version of the manuscript.

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