

## Research Article

## The genus *Amphistegina* (benthic foraminifera): distribution along the southern Tunisian coast

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### Abstract

The benthic foraminiferal genus *Amphistegina* is currently expanding its range in the Mediterranean Sea after being introduced through the Suez Canal from the Red Sea. Over the previous decade, *Amphistegina* spp. has colonized the eastern part of the Mediterranean Sea including the Egyptian and Libyan coasts, but the present southern limit in the Mediterranean Sea is along the southern Tunisian coast. However, this limit has never clearly been defined, and existing models predict an expansion along the entire Tunisian coastline. The Gulf of Gabes, in the southern part of Tunisia, is heavily polluted by local phosphate industries that discharge untreated waste directly into the sea. *Amphistegina* spp. is sensitive to water quality and marine pollution. Based on our surveys and recent investigations, the present distribution area of *Amphistegina* spp. along the south Tunisian coast includes the coastline east of Djerba Island, located in the Gulf of Gabes. Possible limiting factors for *Amphistegina* spp. expansion in the Gulf of Gabes may be the unfavourable environmental conditions generated by the phosphate industry.

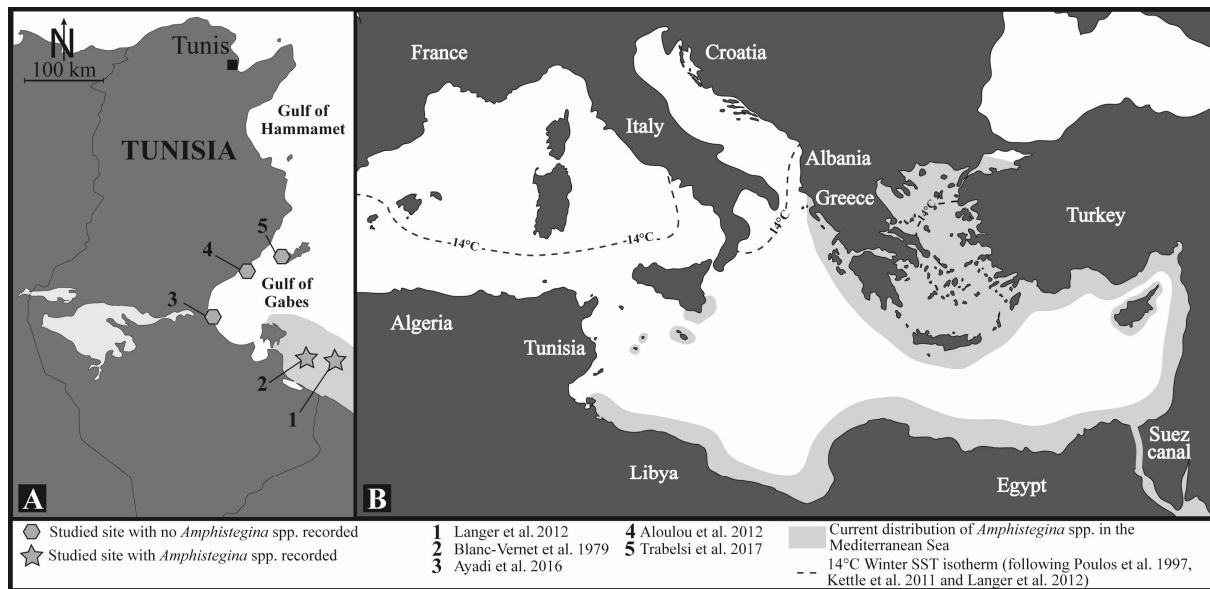
**Key words:** Gulf of Gabes, Djerba, *Posidonia oceanica*, pollution

### Introduction

The genus *Amphistegina*, which includes large symbiont-bearing foraminifera, is an excellent bio-indicator of water quality and light penetration into seawater and has been used globally to develop biotic indices to assess environmental conditions (Hallock et al. 2006; Ramirez 2008; Spezzaferri et al. 2018). *Amphistegina* spp., unicellular protists hosting diatom symbionts, are sensitive to seawater characteristics (e.g. turbidity) and can be used as a first-order indicator of water quality degradation (Koukousioura et al. 2011; Hallock 2012; de Freitas Prazeres et al. 2012). This is because *Amphistegina*'s symbionts are light dependant (Lee and Anderson 1991; Hallock 1981; Hallock 1999), and water turbidity/transparency affects their photosynthesis. Furthermore, photo-inhibitory stress related to high seawater temperatures may cause mortality of the symbiotic diatoms and bleaching in

*Amphistegina* spp. (Spezzaferri et al. 2018 and references therein). Large symbiont-bearing foraminifera are indicative of warm tropical and subtropical waters, and their latitudinal distribution is strongly related to water temperature (Hollaus and Hottinger 1997; Langer and Hottinger 2000). However, *Amphistegina* can tolerate lower temperatures, and its occurrence is delimited by winter sea surface temperature (SST) isotherms between 14 °C and 16 °C (14 °C – Langer (2008a); 15 °C – Murray (2006); 16 °C – Hollaus and Hottinger (1997)). *Amphistegina* spp. live in water depths not exceeding 130 m, depending on the species (Hallock 1999; James et al. 1999).

Five *Amphistegina* species (*A. bicirculata*, *A. lessonii*, *A. lobifera*, *A. papillosa* and *A. radiata*) are currently present in the Red Sea (Hottinger et al. 1993). In 1869, the construction of the Suez Canal provided a direct connection between the Red and Mediterranean



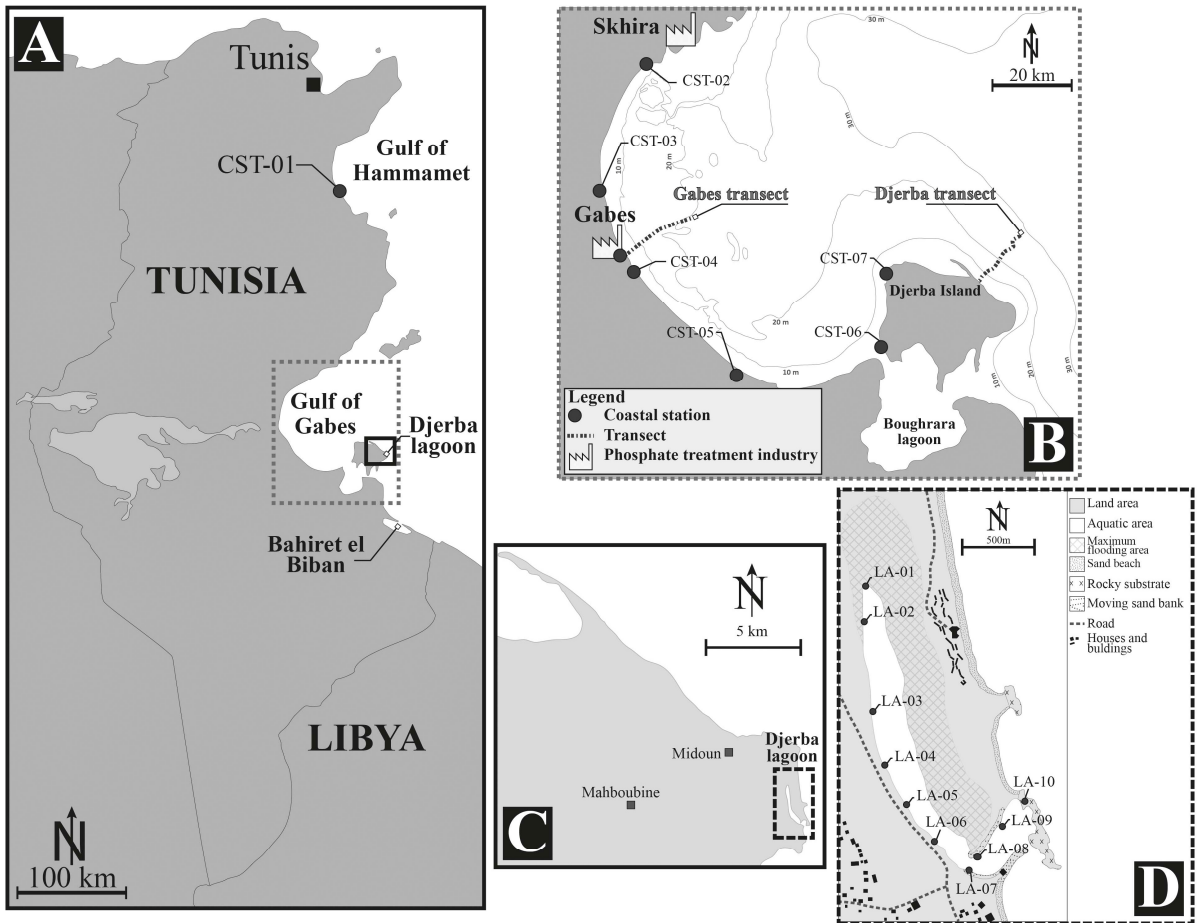
**Figure 1.** Current *Amphistegina* distribution A) along the Tunisian coast and B) in the Mediterranean Sea (including previous studies). The dashed line denotes the 14 °C winter sea surface temperature (SST) isotherm.

Seas allowing for the massive migration of taxa. This event, termed Lessepsian migration, continues today and includes fishes, molluscs, crustaceans, algae and foraminifera (Weinmann et al. 2013). It occurs predominantly in one direction, from the Red Sea to the Mediterranean Sea, and also allows the movement of *Amphistegina* spp. (Bianchi and Morri 2000; Langer 2008b; Langer et al. 2012). However, of the five *Amphistegina* species currently present in the Red Sea (Hottinger et al. 1993), only *A. lessonii* and *A. lobifera* have colonized the Mediterranean Sea (Langer 2008b; Langer et al. 2012; Weinmann et al. 2013; Caruso and Cosentino 2014 and references therein).

The reason why only two *Amphistegina* species have migrated from the Red Sea to the Mediterranean Sea has never been investigated and/or explained in the literature. However, *Amphistegina* species live at different water depths in the Gulf of Aqaba (at the northern tip of the Red Sea); *A. bicirculata*, *A. papillosa* and *A. radiata* are found in relatively deeper depths (50–100 m), while *A. lessonii* and *A. lobifera* prefer shallower depths (0–50 m) (Hohenegger 1995, 2000, 2004; Murray 2006). The shallow water depth of the Suez Canal (maximum 24 m) may have prevented the migration of the deeper species. *Amphistegina* spp. have also been found in Miocene and Pliocene sediments from the Tethys Ocean (an ocean existing during the Mesozoic Era between Gondwana and Laurasia) and the Medi-

terranean Sea (Di Bella et al. 2005; Langer 2008b). The possibility that *Amphistegina* spp. survived the late Miocene “Messinian Salinity Crisis”, in which the Mediterranean Sea underwent a desiccation process, cannot be excluded (Langer et al. 2012), and thus, current distributions may be relics from the past. In addition, *Amphistegina* spp. are currently present along the Atlantic coastline off Gibraltar (e.g., Levy et al. 1995), implying that a recolonization of the Mediterranean Sea from the Atlantic Ocean could also be possible. Additional molecular genetic methods are needed to determine whether certain *Amphistegina* spp. survived the “Messinian Salinity Crisis” or recolonized the Mediterranean Sea from the Atlantic (Langer 2008b).

The *Amphistegina* genus has been described in great detail along the eastern and southern parts of the Mediterranean, along the coast of Libya, Egypt, Israel, Lebanon, Syria, Turkey and Greece (Langer et al. 2012; Weinmann et al. 2013 and reference therein) (Supplementary material Table S1, Figure 1). However, the westernmost extent of *Amphistegina* spp. is still unknown, with sources reporting that the current distribution of *Amphistegina* spp. reaches the south of Sicily (Caruso and Cosentino 2014), the Maltese Islands (Yokes et al. 2007) and Crete (Hollaus and Hottinger 1997) (Table S1, Figure 1). *Amphistegina* spp. have been reported along the southern coast of Tunisia in a few studies without specific details on their distribution (e.g., the south-



**Figure 2.** Study area showing the sampling sites where sediment was collected in 2014. A) Tunisian coastline denoting the Gulf of Gabes and coastal station CST-01. B) Gulf of Gabes with the sampled transects (Gabes transect and Djerba transect) and coastal stations (CST-02 to CST-07). C) Location of the Djerba lagoon. D) Locations of sampled stations in the Djerba lagoon (LA-01 to LA-10).

eastern part of Djerba Island, offshore of the Bahiret el Biban lagoon during the seventies – Blanc-Vernet et al. 1979 and near the Tunisian-Libyan border – Langer et al. 2012) (Table S1, Figure 1).

The current distribution of *Amphistegina* spp. in the Mediterranean Sea is generally linked to the winter sea surface temperature (SST) isotherm (Hollaus and Hottinger 1997; Murray 2006; Langer 2008a). However, due to increases in seawater temperature as a part of climate change and global warming, it is predicted that *Amphistegina* spp. will colonize the northern Tunisian coast by the end of this century (Langer et al. 2012; Weinmann et al. 2013). Hence, the aim of this study was to document current *Amphistegina* spp. distributions along the southeastern Tunisia coastline and examine correlations between *Amphistegina* spp. densities and abiotic parameters in this region.

## Material and methods

Forty-eight stations along the southeastern Tunisian coastline were investigated. They were subdivided into the following four sites: Coastal stations (N = 7), Gabes transect (N = 16), Djerba transect (N = 15), and Djerba lagoon (N = 10) (Table S2, Figure 2). Coastal stations were sampled in January 2014 whereas the Gabes transect, Djerba transect and Djerba lagoon samples were sampled in July 2014. Coastal stations (CST-01–CST-07) were sampled parallel to the coast at shallow depths (< 1 m) (Table S2) and covered 200 km of the eastern Tunisian coast from the Gulf of Gabes to the northern edge of Djerba Island (El Kateb et al. 2018b). The Djerba and Gabes transects were sampled perpendicularly to the coastline. The Gabes transect (GBS-01 to GBC-16), located between the industrial and fishing harbours of Gabes, sampled

**Table 1.** Abundance and density (specimen/cm<sup>3</sup>) of living (stained) *Amphistegina lessonii* and *Amphistegina lobifera* in surface sediments collected from the Djerba transect (top) and the Djerba lagoon (bottom).

Djerba transect	DJB-01	DJB-02	DJB-03	DJB-04	DJB-05	DJB-06	DJB-07	DJB-08	DJB-09	DJB-10	DJB-11	DJB-12	DJB-13	DJB-14
<i>A. lessonii</i>	79	5	13	2	6	22	39	34	29	28	27	63	6	3
<i>A. lobifera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Density	3.591	0.185	0.65	0.077	0.222	0.786	1.56	2.125	2.071	1.867	1.421	2.864	0.3	0.15
Djerba lagoon	LA-10a	LA-10b												
<i>A. lessonii</i>	2	351												
<i>A. lobifera</i>	3	66												
Density	0.125	17.38												

stations approximately every 1 km along the 17.3 km transect (Figure 2). The first station (GBS-01) was located 400 m from the coast, and water depth ranged from 4.5 to 19.5 m (Table S2). The Djerba transect (DJB-01 to DJB-15), located on the eastern coast of Djerba Island, covered 13.8 km, and stations were at approximately 1 km intervals. The first station (DJB-01) was 500 m from the shoreline (El Kateb et al. 2018b), and water depth ranged from 5.1 to 26.8 m (Table S2). Stations in the Djerba lagoon were situated on the eastern edge of Djerba Island (El Kateb et al. 2018a), and samples (LA-01 to LA-10) were collected in a 2 km area at shallow depths (< 1 m) (Table S2).

All transect samples were collected using an Ekman-Birge box core (15 × 15 × 30 cm) deployed from a small fishing boat. Samples from the coastal stations and Djerba lagoon were collected by hand using plastic containers. Station LA-10, located on the western edge, was distinct from the rest of the Djerba lagoon stations as it delimited the lagoon from the open sea and was composed of an irregular rocky substrate made of beachrock with numerous cavities filled with sediment. Thus, sediments were collected from both the bottom of the cavities (LA-10a) and from the algal carpets on top of the sediment (LA-10b). At all stations, about 50 cm<sup>3</sup> of surface sediments were sampled and treated following the FOBIMO protocol (Schönfeld et al. 2012). The first centimeter of sediment was collected in plastic bottles and treated with a rose Bengal solution (2 g/L in 90% alcohol). The living (stained) *Amphistegina* spp. were picked, placed in plunger cells and counted using a Nikon SMZ18 microscope. Specimens were determined to species level based on Hottinger et al. (1993). The number of living specimens was divided by the volume of sediment to calculate the density of living specimens for each sample.

## Results and discussion

### *Distribution and density of Amphistegina spp. along the Tunisian south coast*

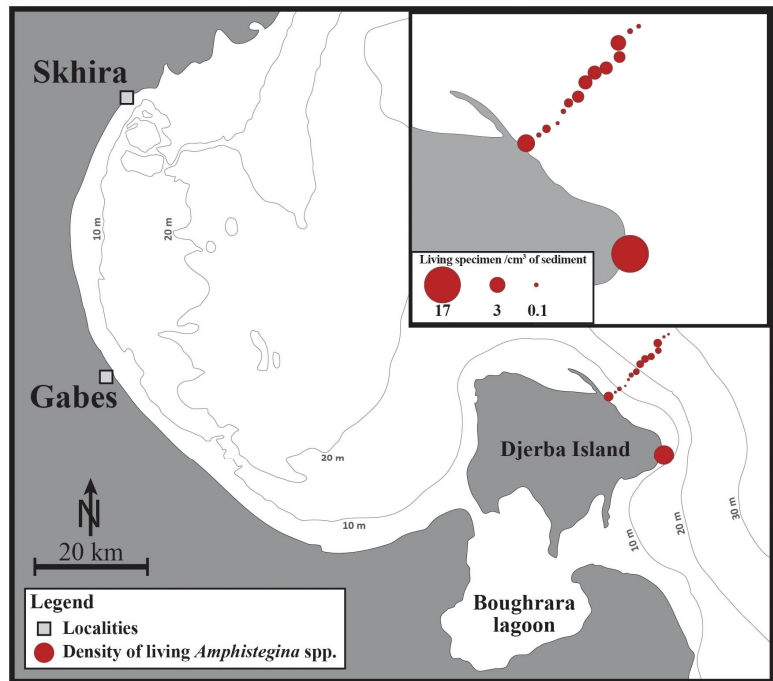
All samples from the Djerba transect and in station LA-10 from the Djerba lagoon contained *Amphistegina*.

*Amphistegina lobifera* was present only at station LA-10. Beyond the northeastern coast of Djerba Island, living *Amphistegina* were absent (Table 1, Table S2, Figure 3). The density of living *A. lessonii* varied significantly along the Djerba transect (0.1 to 3.6 specimens/cm<sup>3</sup> of sediment) (Table 1, Figure 3). Stations DJB-01 and DJB-07 to DJB-12 had relatively high densities of living *A. lessonii*. The highest living foraminifera density was in sample LA-10b, which included representatives of both *A. lessonii* (76%) and *A. lobifera* (24%), with 17.4 living specimens/cm<sup>3</sup> (Table 1, Figure 3).

Previously only Blanc-Vernet et al. (1979) reported *Amphistegina* spp. off the Tunisian coastline and only offshore of the Bahiret el Biban lagoon (Figure 1). Previous sampling of the northern part of the Gulf of Gabes, the south coast of Kerkennah Island, and the central part of the Gulf did not find *Amphistegina* spp. (Aloulou et al. 2012; Ayadi et al. 2016; Trabelsi et al. 2017; Figure 1).

### *The role of the substratum*

As an epifaunal species, hard substratum is ideal for *Amphistegina*, as confirmed by high densities of *A. lobifera* at station LA-10. *Amphistegina lobifera* is the shallowest dwelling species of the genus as it requires higher light intensity for reproduction and generally lives on hard and phytal substrates (Hallock 1981; Triantaphyllou et al. 2012 and references therein). It is also well adapted to high-energy environments due to its thick-walled shell (Triantaphyllou et al. 2012, and references therein). The shallow environment created by the beachrock barrier at Station LA-10 within the Djerba lagoon (El Kateb et al. 2018a) has maximal light penetration even during high tides, and thus is ideal for *A. lobifera*. Although both species of *Amphistegina* are very abundant on the rocky barrier, it is absent inside the lagoon. Tidal cycles regulate the hydrology in the lagoon, with seawater periodically flowing out and emptying the lagoon. *Amphistegina* spp. cannot survive under these extreme environmental conditions (El Kateb et al.



**Figure 3.** Current distribution and densities of living (stained) *Amphistegina* spp. in surface sediments collected along the southern Tunisian coast in 2014. Size plot obtained with the software Ocean Data View (Schlitzer 2016).

2018a). However, the numerous depressions, cavities and holes on the rocky barrier remain filled by seawater during low tides, protecting *Amphistegina* spp. from desiccation during low tide events.

The relatively high density of *A. lessonii* at station DJB-01 may be linked to the presence of the seagrass *Posidonia oceanica* (El Kateb et al. 2018b). The vertical growth of *P. oceanica* traps sediments among rhizomes and forms small mounds or barriers with hard substratum (e.g. Lenzi et al. 2013). Additionally, *P. oceanica* may host suitable food resources for *A. lessonii* as fungi, diatoms and bacteria (Langer 1993) colonize the seagrass leaves. Although Murray (2006) described *Amphistegina* as phytal foraminifera, the relationship between seagrass (e.g., *P. oceanica*) and *Amphistegina* is not so straightforward. High abundances of *Amphistegina* were observed amongst seaweed in Hawaii (Hallock et al. 1986) and the Pitcairn Islands (Whittaker and Hodgkinson 1995), whilst Blanc-Vernet (1969) documented *Amphistegina* on *Halophila* and *P. oceanica*. The co-occurrence of *P. oceanica* and *A. lessonii* (Table 2, Figure 3) along the Tunisian coast (e.g., along the first 8 km of the Djerba transect) suggests that they colonize environments characterized by similar ecological parameters (e.g. salinity, water depth, light intensity) (El Kateb et al. 2018b). Thus, *P. oceanica* meadows could serve as an ideal environment for the proliferation and expansion of *Amphistegina* along the Tunisian coast.

At the beginning of the 20<sup>th</sup> century, the widespread *P. oceanica* meadows were thought to be the most important ecosystems within the entire Mediterranean Sea and key nurseries for many marine species (Le Danois 1925; Guillaumont et al. 1995; Ben Mustapha et al. 1999; Aloulou et al. 2012; Hattour and Ben Mustapha 2013; El Zrelli et al. 2017). While the Gulf of Gabes historically had abundant meadows of *P. oceanica* (Aloulou et al. 2012), the phosphate industries in the Gulf of Gabes have negatively affected the marine environment and induced a significant loss of *P. oceanica* (Ben Brahim et al. 2010) especially around the industrial waste discharge (e.g. Darmoul et al. 1980; El Zrelli et al. 2017). Therefore, the decline of *P. oceanica* in the Gulf of Gabes could severely affect the colonization of *Amphistegina* spp. in this area and its expansion along the northern Tunisian coast.

Fine sediments (< 63 µm) are not the ideal substratum for *Amphistegina* spp. as they prefer seagrass and hard substrata (e.g., coral rubbles) (Murray 2006). Debenay (1988) observed that a mud content > 20% may cause a drastic decrease in the abundance of *A. lessonii*. Siltation in the Gulf of Gabes has increased over the course of the twentieth century (Zaouali 1993), and sedimentary conditions have since further degraded in the Gulf of Gabes leading to a predominance of fine particle sedimentation with grain size < 63 µm exceeding 60% (El Kateb et al. 2016).



**Table 2.** Comparisons of tolerances to abiotic conditions for *Amphistegina lessonii*, *Amphistegina lobifera* and *Posidonia oceanica*. References: (1) Murray 2006; (2) Langer 2008b; (3) Hallock and Glenn 1986; (4) Reiss and Hottinger 1984; (5) Hallock 1981; (6) Blanc-Vernet 1969; (7) Triantaphyllou et al. 2012; (8) Triantaphyllou et al. 2009; (9) Pirc 1986; (10) Pergent et al. 2012; (11) Balestri et al. 2003; (12) Sánchez-Lizaso et al. 2008; (13) Dalla Via et al. 1998; (14) Marbà and Duarte 2010; (15) Mauro et al. 2013.

	<i>A. lessonii</i>	<i>A. lobifera</i>	<i>P. oceanica</i>
Water temperature	Minimum 14 °C (2) or 15 °C, generally > 20 °C (1)	<ul style="list-style-type: none"> <li>▪ Minimum 14 °C (2) or 15 °C, generally &gt; 20 °C (1)</li> <li>▪ 13.7 °C (7)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Optimum temperature for photosynthesis: 25–30 °C (9)</li> <li>▪ Range temperatures correspond to Mediterranean Sea SSTs: 13–31 °C (10)</li> </ul>
Water depth range	0–90 m (1)	0–30m in high-energy setting (1)	0–40 m (11)
Salinity	32–35‰ (3) and 40–41‰ (4) for <i>Amphistegina</i> spp.	<ul style="list-style-type: none"> <li>▪ 35.5–39.3‰ (8)</li> <li>▪ 32–35‰ (3) and 40–41‰ (4) for <i>Amphistegina</i> spp.</li> </ul>	> 38.5 psu (12)
Light dependant	Linked to water depth (5)	Tolerant to high light levels (1) and intensity (5)	Linked to water depth (13)
Substrate	<ul style="list-style-type: none"> <li>▪ Sediment and hard substrate (1)</li> <li>▪ <i>Halophila</i> and <i>Posidonia</i> seagrass (6)</li> <li>▪ Rhodophyta alga (6)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Hard and phytal substrate (7)</li> <li>▪ Epifaunal on substrate in high energy (1)</li> </ul>	<ul style="list-style-type: none"> <li>▪ Sandy (14)</li> <li>▪ Forming barrier reef of <i>P. oceanica</i> (e.g. 15).</li> </ul>

### *Amphistegina* and water parameters

The main limiting factor for the *Amphistegina* spp. expansion in the Mediterranean Sea put forth in previous literature is the winter sea surface temperature (SST) isotherm (Hollaus and Hottinger 1997; Murray 2006; Langer 2008a). The exact temperature of this isotherm has not been clearly defined and exists somewhere between 14 °C and 16 °C (Hollaus and Hottinger 1997; Murray 2006; Langer 2008a). Within the Aegean Sea, it appears that *Amphistegina* spp. has colonized areas below the predicted 14 °C winter SST isotherm (Table S1, Figure 1). Previous laboratory experiments have demonstrated that *A. lobifera* mobility ceased below 12 °C (Zmiri et al. 1974). However, the SST should not be a limiting factor for *Amphistegina* spp. distribution, at least in the inner part of the Gulf of Gabes which does not reach winter SSTs below 14 °C (e.g. ESA/Medspiration 2012; Shaltout and Omstedt 2014). The current 14 °C winter SST isotherm limit is located south of Sardinia, which is approximately 530 km north of the Gulf of Gabes (Langer et al. 2012; Figure 1).

Increases in water turbidity around the phosphate industry waste discharge area in the Gulf of Gabes have been described since the 1970s and linked directly to the particles released by the phosphate industry (Darmoul et al. 1980). In 2014, disturbingly high turbidity was recorded 18 km from the coast (El Kateb et al. 2018b), possibly linked to phosphogypsum discharge. Phosphorus released by phosphogypsum increases nutrient concentration in the seawater, hence triggering primary production and consequently increasing water turbidity and reducing light availability, all of which negatively impact the growth rate and reproduction of *Amphistegina* spp. (Hallock 1981).

### Conclusion

Two species of *Amphistegina* are currently present along the southern Tunisian coast: *A. lessonii* and *A. lobifera*. The latter species is present only on the rocky barrier in shallow water within the Djerba lagoon. The current distribution limit of these symbiont-bearing foraminifera is the north-eastern edge of Djerba Island. Unfavourable ecological conditions (substrata and water parameters) may explain the absence of the genus *Amphistegina* within the Gulf of Gabes.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Data from previous surveys documenting *Amphistegina* spp. in the Mediterranean Sea, including location names and country with coordinates and references.

**Table S2.** Details of 2014 surveys conducted in this study including stations and coordinates, presence of *Amphistegina* spp. and sediment and water parameters.

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