Range expansion of *Marinomyxa marina*, a phytomyxid parasite of the invasive seagrass *Halophila stipulacea*, to the Caribbean[☆]

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ABSTRACT

Halophila stipulacea, a small seagrass species native to the Indo-Pacific, is a Lessepsian migrant and a high-profile invader that has successfully colonized two exotic regions, the Mediterranean (first observed in 1894) and the Caribbean (2002). In 1961, an intracellular phytomyxid parasite, *Marinomyxa marina* (SAR: Rhizaria: Endomyxa: Phytomyxea) was discovered in the petioles of *H. stipulacea* in the Red Sea, and three decades later, it was reported off the coast of Sicily (Mediterranean), suggesting parallel migration of the two organisms. In 2018, infected petioles of *H. stipulacea* were also observed in St. Eustatius (Caribbean), but the identity of the causative agent remained unresolved. Here, we provide information on four new localities of phytomyxid-infested populations of *H. stipulacea* in Greece (Mediterranean), and Bonaire and Martinique (Caribbean), including notes on infection prevalence and seasonal dynamics. Using the 18S rRNA barcoding gene, we bring molecular evidence that the disease is caused by a genetically uniform variant of *M. marina* at all the examined sites. We conclude that the parasite is now widespread throughout both invaded regions and has been present in the Caribbean since 2013 at the latest. For the first time, the production of fruits in infected plants is observed, indicating a non-lethal nature of the symbiosis. While the arrival of *M. marina* to the Caribbean is unlikely to alleviate the current invasiveness of *H. stipulacea*, we emphasize the need for its further monitoring since the host-specificity and general biology of seagrass-associated phytomyxids are still poorly understood.

1. Introduction

Biological invasions and introductions of non-native species into new habitats are considered a great threat to the already declining biodiversity and functioning of ecosystems worldwide (Sala et al., 2000). They are often exacerbated by human activities like increased global traffic (Hulme, 2009; Galil et al., 2015), which both directly and indirectly damage the health and balance of long-established biocenoses (Blackburn et al., 2004). In affected areas, inflicted changes may result in deterioration of existing ecosystem services, and subsequently a loss of income to local residents, despite economic resources being spent in

(often inefficient) attempts to eradicate the invaders, slow down their spread or reduce their impact (Bradshaw et al., 2016; Walsh et al., 2016). In marine environments with indistinct geographical barriers and limited human awareness and management, artificial species introductions are particularly difficult to detect in time, restrict or all the more prevent (Giakoumi et al., 2019). As a result, the number of recognized marine invaders tends to be underestimated and rising, leading to previously distinct aquatic communities becoming altered and homogenized (Layman et al., 2014; Morri et al., 2019).

Halophila stipulacea (Alismatales: Hydrocharitaceae; Fig. 1a), a small (sub)tropical seagrass native to the Indian Ocean, Persian Gulf and the

Red Sea (den Hartog, 1970), is a textbook example of a marine organism that has colonized vast areas beyond its original habitat due to human 'help.' After the opening of the Suez Canal in 1869, *H. stipulacea* was one of the first documented Lessepsian migrants in the Mediterranean Sea (Lipkin, 1975). As the earliest observed meadows were established mainly in or near Eastern Mediterranean ports (den Hartog, 1972; Lipkin, 1975), it is likely that the seagrass was dragged through the Canal via shipping. In the same way, it was eventually introduced into the Caribbean, where it has spread rapidly since the first observation in Grenada in 2002 (Ruiz et al., 2004; Willette et al., 2014). Currently, *H. stipulacea* commonly occurs in the Mediterranean; throughout the Eastern Mediterranean Basin, along parts of southwest Italy (Winters et al., 2020; di Genio et al., 2021) and in the south of France (Thibaut et al., 2022). In the Caribbean, its range is continuously expanding and includes the coasts of Puerto Rico, the Lesser Antilles and continental Venezuela (Vera et al., 2014; Ruiz et al., 2017; Winters et al., 2020).

The invasive nature of *H. stipulacea* appears to be promoted by its phenotypic plasticity and physiological adaptability to a broad spectrum of environmental conditions (Winters et al., 2020). The seagrass shoots adapt to varied irradiance levels by altering their leaf surface area (Schwarz and Hellblom, 2002), increasing the synthesis of photosynthetic pigments and fatty acids to promote chloroplast membrane fluidity (Beca-Carretero et al., 2019), and by chloroplast clumping (a mechanism to protect against photodamage) (Drew, 1979). *Halophila stipulacea* can also shift its thermal optimum in response to the prevailing ambient water temperature (Wesselmann et al., 2020) and thrives in eutrophic conditions where it forms much denser mats than other deeper rooting seagrass species (van Tussenbroek et al., 2016). All these attributes enable *H. stipulacea* to fill free niches when introduced to exotic areas and in some cases displace indigenous seagrass populations, as reported in the Caribbean (Willette and Ambrose, 2012). While in the Mediterranean its invasive potential appears to be limited by the low winter water temperatures (Wesselmann et al., 2020), in the Caribbean, the similarity in climate to its native habitat has allowed *H. stipulacea* to become a serious threat to local coastal ecosystems (Hayes, 2008; Willette and Ambrose, 2012).

Recently, we brought to attention the long-overlooked fact that *H. stipulacea* serves as a host to the phytomyxid parasite *Marinomyxa marina* (Kolátková et al., 2021) (Fig. 1b). Phytomyxids (SAR: Rhizaria: Endomyxa: Phytomyxea) are microscopic obligately biotrophic protists who complete their complex life cycles within cells of various photosynthesizing organisms or their parasites such as oomycetes (Bulman and Neuhauser, 2017). Although greatly understudied, they appear to be ubiquitous in the marine environment (Neuhauser et al., 2011) and

closely associated with narrow ranges of hosts (Kolátková et al., 2021). No experimental studies have been carried out to understand their pathogenic potential in the oceans or the possibility of transmission to other host taxa, although several cross-kingdom host shifts have already occurred during their evolution (Neuhauser et al., 2014). Once introduced to a particular locality, infection often becomes well-established and can be repeatedly found at affected sites, sometimes even after decades (Schnepf et al., 2000; Kolátková et al., 2020). This phenomenon has been well described in terrestrial phytomyxid representatives, which infest economically significant crops, whereby resting spores dispersed in the soil stay viable and capable of infecting suitable hosts after several years of dormancy (Balendres et al., 2017; Ernst et al., 2019).

Since its discovery in the Red Sea in 1961 (Lipkin and Avidor, 1974), the presence of *M. marina* in the leaf petioles of *H. stipulacea* has been confirmed at two distant localities in the Mediterranean (Marziano et al., 1995; Vohník et al., 2017; Kolátková et al., 2020). Furthermore in 2018, Maitz et al. (2021) noted an apparent phytomyxid infection in *H. stipulacea* specimens from St. Eustatius, Caribbean, suggesting that the invasive seagrass may host a similar disease in this region also, although the identity of the phytomyxid was not determined. While a trans-oceanic dispersal has been previously observed in some marine phytomyxids (Mabey et al., 2021), it remains unclear whether this is the case for *M. marina* or whether a different organism causes the infection observed in the Caribbean specimens. As parasite co-introductions continue to be a highly discussed topic given the complexity of potential ecological impacts, the former scenario raises earnest concerns, all the more so when the impact of phytomyxids on the seagrass hosts remains not understood.

To shed more light on the ecological amplitude and geographical range of *M. marina*, we examined several populations of *H. stipulacea* outside the Indo-Pacific, evincing signs of phytomyxid infection. We analyzed the specimens on a molecular level and compared them with available sequences of *M. marina* from the Red Sea (i.e., the native region of its host). Where possible, we also recorded data on the infection rates and monitored the seagrass populations throughout the season to better understand the dynamics of the symbiosis and determine whether the parasite is established at the site.

2. Materials and methods

2.1. Sampling and data collection in the Mediterranean

In late October 2018, during routine fieldwork off the coast of Samos, Greece, near the Pythagoreio port (N 37.69745, E 26.96998), shoots of
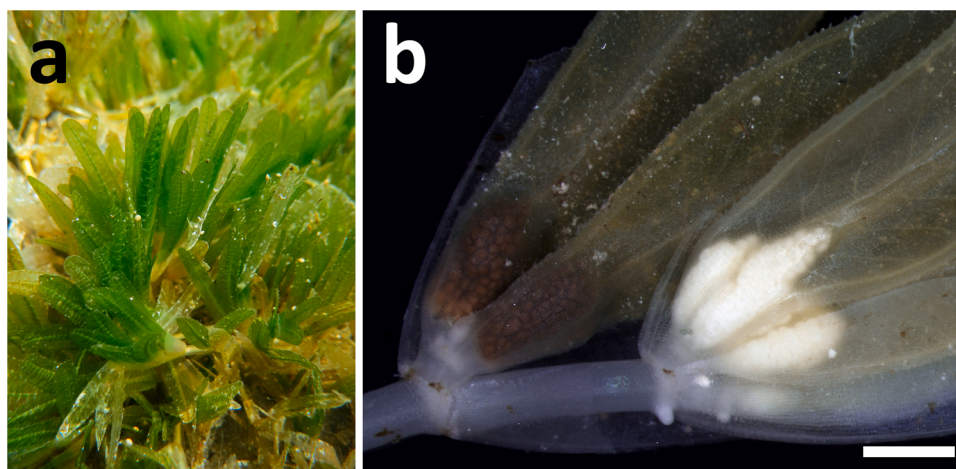
Fig. 1. Morphology of the invasive seagrass *Halophila stipulacea* and its phytomyxid infection. (a) Population of *H. stipulacea* in a small municipal harbour on the west coast of Crete (Greece, Mediterranean Sea). (b) Detail of *H. stipulacea*'s petioles infected with the protist parasite *Marinomyxa marina* (Phytomyxea). Coloration of formed galls varies from white to dark brown based on the parasite's maturation stage (see Vohník et al., 2017). Scale bar = 2 mm.

Halophila stipulacea were observed in ~6 m depth with visibly swelled petioles resembling phytomyxid infection. The water temperature at the site was recorded using a HOBO logger (Onset Computer Corporation, USA) placed 20 cm above the seafloor. The infection prevalence was evaluated by collecting biomass from 10 benthic chambers (surface area 0.071 m^2 per chamber) installed in the surrounding seagrass meadow for other research purposes (Ward, 2020). All the leaves from the chambers were dried, counted and screened for the presence of phytomyxids. The percentage of infected leaves was determined. In November 2018, the site was revisited, and samples of the infected shoots were collected via free diving and stored in 70% EtOH for further analyses. The locality was further monitored, and screening dives were performed in February/March and September 2019. The infection prevalence was calculated again during the latter, although this time, using the protocol described in Kolátková et al. (2020) to unify the methods. Briefly, leaves were collected from three microsites with confirmed presence of the infection, all collected leaves were screened, and the percentage of infected ones was determined (Supplementary Table 1). This calculation method was also used in all the onward mentioned surveys. The main difference between the two applied approaches lies in examining random *H. stipulacea* microsites (October 2018) vs. only examining microsites where the parasite was actually found (September 2019). To allow further comparison, microsites not containing any infected leaves were excluded from the former when calculating average infection prevalence.

In September 2019, another locality with *H. stipulacea* phytomyxid infection was discovered off the Greece mainland coast, near the town Kineta (N 37.97467, E 23.24077) at 6–7 m depth. Water temperature was recorded with a Freedom diving computer (Divesoft Ltd., Czech Republic), and infection prevalence was calculated. Samples were collected and fixed by mixing local seawater with 96% ethanol (final solution ~30–50% EtOH) and transported to the laboratory, where they were kept in a refrigerator (4–7 °C) before further examination. The material collected at both sites was later transferred into 70% EtOH in deionized water for long-term storage.

2.2. Sampling and data collection in the Caribbean

Hypertrophied petioles in *H. stipulacea* were first observed in the Caribbean in May/June 2013 in Lac Bay, Bonaire (N 12.10556, W 68.22250) (B. van Tussenbroek, pers. comm.) and later in November 2015 on the opposite side of the same bay (N 12.10065, W 68.24009) but the infection was not further investigated at those times. Infected leaves were spotted again during fieldwork at the latter locality in September 2018 in ~2 m depth. The prevalence was calculated, water temperature measured with a HOBO logger, and specimens collected via scuba diving and transported to the laboratory. There, fragments were fixed in 30% EtOH (and later transferred into 70% EtOH for long-term herbarium storage), 3% formalin in seawater, and pressed and mounted on paper sheets (also for herbarium storage). A number of screening dives were subsequently carried out during January, February and April 2019 and January 2020.

In July 2018, several infected leaves were also found in *H. stipulacea* meadow near the Fort de France's harbour, Martinique (N 14.59994, W 61.06805). The infection at this site was too scarce to calculate its prevalence. The few discovered specimens were fixed and treated as described above.

After completing all analyses, samples from all four newly discovered localities were deposited in the Herbarium of the Institute of Botany, Czech Academy of Sciences, Průhonice (PRA; Table 1), and are available for loan. Photographs of the examined material were taken in situ using a Sealife DC1400 underwater camera and a Canon G10 camera in a WP-DC28 underwater case and in the laboratory using an Olympus SZX12 stereomicroscope equipped with an Olympus DP70 camera and Quick-PHOTO MICRO 3.2 software (Promicra Ltd., Czech Republic).

2.3. DNA extraction, amplification and sequencing

Three to five infected *H. stipulacea* petioles per locality were selected for molecular identification. The DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN Inc., the Netherlands) according to the manufacturer's instructions except for the final elution step when 10 mM Tris was used instead of the provided buffer. The barcoding 18S rRNA gene was amplified following a nested PCR protocol described in Kolátková et al. (2020). The amplicons were purified using a QIAquick PCR Purification Kit (QIAGEN), and Sanger sequenced by SEQme Ltd. (Czech Republic) using the following internal primers: 577F (GCCAGCAGCCGCGGT), 577R (ACCGCGGCTGCTGGC), 1055F (GGTGGTGCATGGCCG) and 1055R (CGGCCATGCACCACC) (Elwood et al., 1985). Newly obtained sequences were visually inspected for quality and trimmed using Finch TV 1.4.0 (Geospiza Inc., www.geospiza.com). They were then manually assembled in BioEdit 7.0.4.1 (www.mbio.ncsu.edu/BioEdit/bioedit.html) and deposited in GenBank (www.ncbi.nlm.nih.gov/genbank/) under the accession numbers MW558606–MW558609.

3. Results

3.1. The Mediterranean

In agreement with the data presented in Kolátková et al. (2020), the phytomyxid infection in *Halophila stipulacea* petioles was only observed during the late summer/autumn months in the Mediterranean localities (Table 1). In February/March 2019, no infected *H. stipulacea* leaves were found in Samos, although >5000 were examined (Supplementary Table 1). In fact, many rhizomes bore no leaves at all, and pale leaves with dark veining were highly abundant (Fig. 2a). The ambient water temperature was lower during this time period and fluctuated between 16 and 17 °C compared to the 21–23.8 °C measured in the late summer months. In Samos, the infection prevalence ranged from 0.8% to 9.9% per microsite in October 2018 and from 0.8% to 33.1 % in September 2019. In Kineta, 3.8–4.3 % of the examined leaves were infected in September 2019. Notably, galls were also developed in plants producing fruits (Fig. 2b), indicating that the parasite does not necessarily interfere with the seagrass' life cycle or viability.

3.2. The Caribbean

Although the measured water temperature remained constant (28–29 °C) all year round in the Caribbean, most of the infected petioles were interestingly also discovered during the second half of the year (i. e., Atlantic hurricane season). In September 2018, the infection prevalence in Lac Bay, Bonaire, ranged from 1.5% to 3.1%. In January/February 2019 as well as in January 2020, no phytomyxid galls were observed at the site, and in April 2019, only two infected *H. stipulacea* fragments were found during a two-week-long survey. Dwarfism of the plants, previously reported by Maitz et al. (2021), was partially observed in the form of shortened internodes (Fig. 2c), but this mainly applied to the youngest infected shoots and not to the entire length of the rhizome. No co-occurrence of galls and fruits was recorded at either of these sites, corresponding with the reality that *H. stipulacea* is not known to reproduce sexually in the Caribbean so far (Smulders et al., 2020).

3.3. DNA extraction, amplification and sequencing

All four newly obtained phytomyxid sequences were identical in all 1503 bp to the 18S rRNA sequences of *Marinomyxa marina* previously published in Kolátková et al. (2020, 2021) originating from the Strait of Messina (Mediterranean) and the Gulf of Aqaba (Red Sea), respectively. The only noticeable genetic variability was represented by subtle chromatogram double peaks in two adjacent base pairs (position 99 and 100), common for both sequences from the Greek localities. Given the
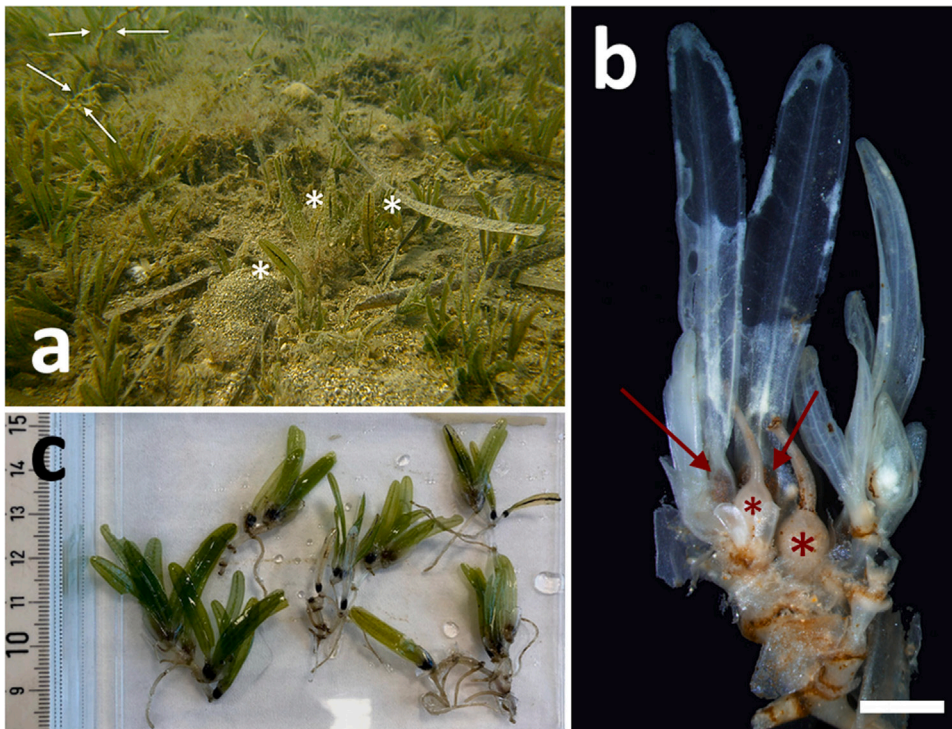
Table 1
List of all *Marinomyxa marina* reports and relevant data arranged in chronological order. First observations in each region (Red, Mediterranean or Caribbean Sea) are in bold. Observations reported in this study are highlighted in grey.

date of observation	region	location	GPS	depth (m)	water temperature (°C)	average infection prevalence[a]	substrate	herbarium[b]	reference sequence #	source
1961 Jun	**Red Sea**	**Israel, Eilat**	**N/A**	**0.5–7**	**N/A**	**<1%**	**coralligenous sand**	**N/A**	**N/A**	Lipkin and Avidor (1974)
1963 Jul	Red Sea	Israel, Eilat	N/A	0.5–7	N/A	<1%	coralligenous sand	N/A	N/A	Lipkin and Avidor (1974)
1969 Mar	Red Sea	Egypt, Taba	N/A	0.5–7	N/A	<1%	coralligenous sand	N/A	N/A	Lipkin and Avidor (1974)
1969 Dec	Red Sea	Egypt, Shorat El Manqata	N/A	0.5–7	N/A	<1%	coralligenous sand	PHYT 2261	N/A	Lipkin and Avidor (1974)
1973 Jul	Red Sea	Egypt, Shorat El Manqata	N/A	0.5–7	N/A	<1%	coralligenous sand	N/A	N/A	Lipkin and Avidor (1974)
1973 Aug	Red Sea	Egypt, Ras Burqa	N/A	0.5–7	N/A	<1%	coralligenous sand	N/A	N/A	Lipkin and Avidor (1974)
1990s	**Mediterranean**	**Italy, Giardini Naxos /Riposto**	**N/A**	**N/A**	**N/A**	**<1%**	**N/A**	**lost**	**N/A**	Marziano et al. (1995)
2015 Aug	Mediterranean	Turkey, Marmaris	N36.79082 E28.30403	7–8	22	high (similar to Messina, see below)	coarse sand	PRA-12834 (dried); PRA-12835 (EtOH)	N/A	Vohník et al. (2017)
2013 May/ June	**Caribbean**	**Bonaire, Lac Bay**	**N 12.10556 W 68.22250**	**0.5–2.5**	**N/A**	**N/A**	**sand, coarse sand**	**N/A (photos available)**	**N/A**	**B. van Tussenbroek (pers. comm., 2021)**
2015 Nov	Caribbean	Bonaire, Lac Bay	N12.10065 W68.24009	~ 2	29	N/A	sand, coarse sand	N/A (photos available)	N/A	this study
2017 Sep	Mediterranean	Italy, Messina	N38.22915 E15.57089	11–14	26	~40%	sand, coarse sand	PRA-13417, PRA-13418 (dried); PRA-13419 (EtOH)	MN128644	Kolátková et al. (2020)
2018 Jan	Red Sea	Israel, Eilat, Taba border crossing	N29.49764 E34.91328	8–24	23	~4%	coralligenous sand	PRA-13846 (dried); PRA-13847 (EtOH)	MT482407	Kolátková et al. (2021)
2018 Mar	Red Sea	Israel, Eilat, North-western beach	N 29.53986 E 34.94780	9	22	~4%	sand	TAU-3098 (dried)	N/A	Kolátková et al. (2021)
2018 May	Red Sea	Israel, Eilat, North beach	N 29.53466 E 34.97285	0.5–1	26	~4%	sand	TAU-3321, TAU-3330, TAU-3350 (dried)	N/A	Kolátková et al. (2021)
2018 Jul	Caribbean	Martinique, Fort de France	N14.59994 W61.06805	~ 2	28	<1%	muddy sand	PRA-18052 (EtOH)	MW558606	this study
2018 Sep	Mediterranean	Italy, Messina	N38.22915 E15.57089	11–14	23	~30%	sand, coarse sand	N/A	N/A	Kolátková et al. (2020)
2018 Sep	Caribbean	Bonaire, Lac Bay	N12.10065 W68.24009	~ 2	29	~2%	sand, coarse sand	PRA-15024 (dried); PRA-15117 (EtOH)	MW558607	this study
2018 Oct	Mediterranean	Greece, Samos	N37.69745 E26.96998	~ 6	22	~4%	sand, coarse sand	PRA-15920 (dried); PRA-15921 (EtOH)	MW558608	this study
2018 Oct	Caribbean	St. Eustatius	N17.47987 W62.99402	18	N/A	N/A	N/A	N/A	N/A	Maitz et al. (2021)
2019 Feb	Red Sea	Egypt, Nuweiba, Maagana Camp	N29.10598 E34.67545	~ 6	21	<1%	coralligenous sand	PRA-18051 (EtOH)	MT482410	Kolátková et al. (2021)
2019 Sep	Mediterranean	Greece, Samos	N37.69745 E26.96998	~ 5	22	~13%	sand, coarse sand	PRA-16126 (EtOH)	N/A	this study
2019 Sep	Mediterranean	Greece, Kineta	N37.97467 E23.24077	6–7	25	~4%	sand	PRA-16127 (EtOH)	MW558609	this study
2020 Feb/ Mar	Caribbean	St. Eustatius	N17.47987 W62.99402	18	N/A	N/A	N/A	N/A	N/A	Maitz et al. (2021)

[a] Percentage of infected leaves at examined microsites.

Fig. 2. Remarkable features observed in phytomyxid-infested populations of *Halophila stipulacea*. (a) Meadow showing signs of deterioration such as dark veining (asterisks) and naked shootless rhizomes (arrows). Observed in Samos (Greece) in February 2019. (b) Fragment of *H. stipulacea* infected with *Marinomyxa marina* (arrows) bearing developed fruits (asterisks). Collected in Kineta (Greece) in September 2019, decolorized due to preservation in ethanol. Scale bar = 3 mm. (c) Specimens of *H. stipulacea* with visible dark swellings in leaf petioles and unusually shortened internodes. Collected in Bonaire (Caribbean) in September 2018.

biogeographical range of the collected samples (Fig. 3a,b), the genetic uniformity of *M. marina* contrasts with the previously described distinct 18S rRNA gene variants of *Marinomyxa halophilae* (99.28 % sequence identity) found less than 200 km apart (Kolátková et al., 2021).

4. Discussion

Marinomyxa marina, the phytomyxid parasite of the invasive seagrass *Halophila stipulacea* first discovered in the Red Sea in 1961 (Lipkin and Avidor, 1974) and in the Mediterranean Sea no later than 1993 (Marziano et al., 1995), is now present at several distant localities in the Lesser Antilles (Caribbean) (Fig. 3b), and well-established at least at one of them (Lac Bay, Bonaire, repeated observations from 2013 to 2020). The first known observation of *M. marina* in the Caribbean dates to May/June 2013 (Lac Bay, Bonaire; B. van Tussenbroek, pers. comm.). The initial identification of the parasite based on the characteristic morphology of the galls was confirmed at the same locality in 2018 using molecular analyses of the 18 S rRNA gene. The parasite has also
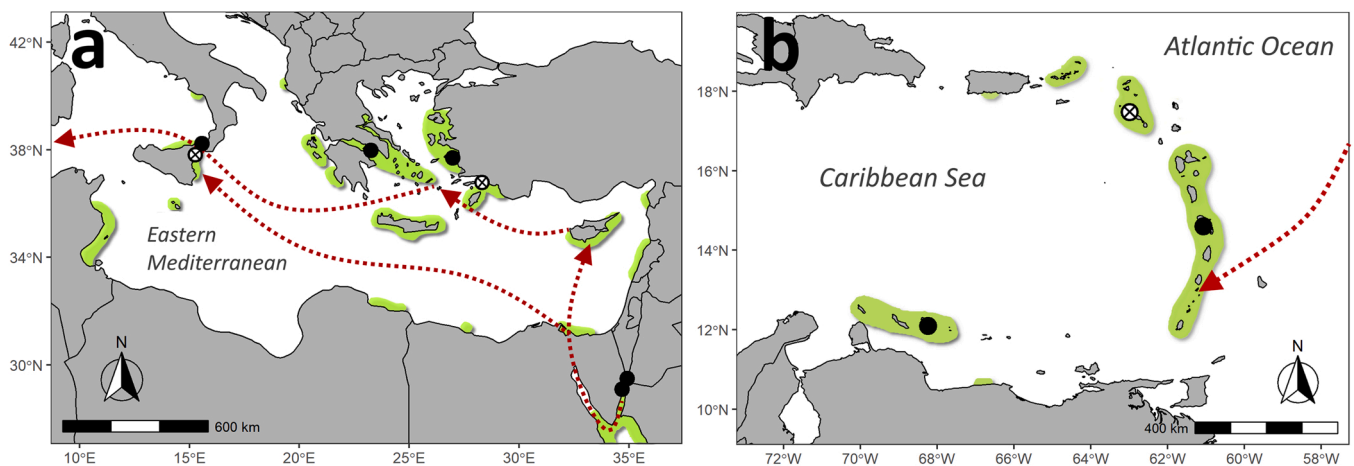
Fig. 3. Geographical distribution of *Marinomyxa marina*. (a) Map of the Eastern Mediterranean Basin showing the current distribution of *Halophila stipulacea* meadows (green) and the reported occurrences of its phytomyxid parasite *M. marina* based on genetic/morphological (black dot) or solely morphological (crossed circle) evidence. Red discontinuous arrows indicate hypothetical introduction routes of *H. stipulacea* based on the data gathered by Chiquillo (2021). (b) Map of eastern Caribbean Sea showing the current distribution of *H. stipulacea* meadows (green) and the reported occurrences of *M. marina* based on genetic/morphological (black dot) or solely morphological (crossed circle) evidence. Red discontinuous arrow indicates the arrival of *H. stipulacea* from the Mediterranean Sea via the trans-Atlantic route (see Chiquillo, 2021).

been recorded (chronologically, see Table 1) in a harbour of Fort de France, Martinique (sequence-based identification; this study), and on St. Estuatius (morphology-based identification; Maitz et al., 2021). In addition, while putting together data for this study, we learned that a morphologically similar phytomyxid infection in *H. stipulacea* had also been observed in Spanish Water, Curaçao in November 2020 (N. Slikboer, pers. comm.) and Barcadera bay, Aruba repeatedly since May 2018 (S. T. Becker, pers. comm.). Unfortunately, the infected specimens were discarded due to the lack of knowledge of such parasite's existence.

Although the presence of *M. marina* in the Caribbean before the arrival of *H. stipulacea* cannot be ruled out, the genetic uniformity of the available 18 S rRNA gene sequences of the parasite from the Red, Mediterranean and Caribbean Sea, together with the increasing evidence that *H. stipulacea* arrived in the Caribbean from the Mediterranean (Chiquillo, 2021), indicate that *M. marina* was more likely co-introduced to the region together with its host. In the Mediterranean, the parasite is now known to occur and prosper along the northern coast of the Eastern Basin (Vohník et al., 2017; this study), as well as the heavily trafficked Strait of Messina, one of the two gates to the Western Basin (Kolátková et al., 2020, 2021) (Fig. 3a). Since the Mediterranean is responding strongly to ongoing climate change (Bianchi and Morri, 2003; Borghini et al., 2014), with the range of *H. stipulacea* slowly expanding northwards and beyond the geographical borders of the Eastern Basin (Procaccini et al., 1999; Gambi et al., 2009; Thibaut et al., 2022), a simultaneous spread of *M. marina* into those waters may be expected as well.

The observed seasonal pattern in the infection incidence is presumably linked to *H. stipulacea*'s population dynamics. Biological rhythms have been hypothesized to represent key elements of host-parasites interactions (Martinez-Bakker and Helm, 2015). Annual and daily fluctuations in environmental conditions and available resources imprint in the hosts' immunity variation, which leads to cycles in infection (Martinez-Bakker and Helm, 2015). In the Mediterranean, *H. stipulacea* undergoes its growth peak (i.e., the highest cover percentage, shoot density, leaf surface area and total above-ground biomass) during autumn months (Nguyen et al., 2020; di Genio et al., 2021), which correlates with the reported infection occurrence. Furthermore, the seagrass flowering in this region appears to reach its maximum during July-September (Nguyen et al., 2018; Dural et al., 2020), preceding the phytomyxid infection outbreak. In *H. stipulacea*'s native habitat in lower latitudes, sea surface temperatures remain above 20 °C throughout the year, and seasonal changes in phenology are much subtler within its meadows (Nguyen et al., 2018; Beca-Carretero et al., 2020). We speculate this is why leaves with phytomyxid infection can be spotted year-round in the Gulf of Aqaba (Lipkin and Avidor, 1974; Kolátková et al., 2021) (Table 1). In the Caribbean, where climate and physical conditions are somewhat similar to those in the Red Sea, we expected the infection also to be present year-round, yet seasonal patterns still seem to play a role regarding *M. marina*'s activity in this newly invaded region. Since seasonal variation in immunity is often based on genetic components (Versteegh et al., 2014), the infection seasonality in the Caribbean might be a physiological relict inherited from seagrass populations responding to the variable Mediterranean habitat.

At present, *M. marina* does not appear to cause harm significant enough to threaten the survival or reproductive ability of *H. stipulacea*. The fruits observed on the infected plants from Samos, Greece (Fig. 2b) represent the first piece of evidence for low pathogenicity of phytomyxids in their natural seagrass hosts. However, co-introduction events account for radical interventions in symbiotic relationships, and the established equilibrium can be broken in such circumstances. In addition, a question arises whether *M. marina* represents a threat to any of the seagrass species native to the two invaded regions. So far, marine phytomyxid representatives are considered relatively host-specific (den Hartog, 1965, 1989; Kolátková et al., 2021), but recent studies on *Maullinia*, the only genus of phytomyxids known to parasitize brown algae (Phaeophyceae), provide a signal that closely related parasite

strains can be found in phylogenetically distant algal hosts (Maier et al., 2000; Blake et al., 2017). Particularly *Maullinia ectocarpii* has now been confirmed to form lesions in the tissue of several southern bull kelp species (*Durvillaea* spp., Fucales) (Blake et al., 2017) as well as to infect *Ectocarpus* spp. (Ectocarpales) and other filamentous brown algae growing on rocks or epiphytically on other algae belonging to Fucales and Laminariales (Maier et al., 2000). It is, therefore, possible that direct proximity may also play a partial role in phytomyxid transfers.

Considering the environmental value of seagrass meadows, which rank among some of the most productive and complex marine ecosystems (Duffy, 2006; Duarte et al., 2010), but which are undergoing worldwide decline (Orth et al., 2006; Short et al., 2006, 2014), we propose that the occurrence of their microbial parasites should be further monitored. Since *H. stipulacea* is already a point of scientific interest due to its invasive nature, it represents a convenient model organism for research of such complex trophic interactions. We suggest a close eye is kept on the presence of *M. marina* in the Caribbean, as there are four other *Halophila* species found in the region (i.e., *H. baillonis*, *H. decipiens*, *H. engelmannii* and *H. ovalis* – also known as *H. johnsonii*) (Creed et al., 2003; Waycott et al., 2021) which may be susceptible to this or similar phytomyxid parasites (Kolátková et al., 2021). Understanding the relationships between seagrasses and their parasites may be a crucial component in our endeavour to conserve these valuable ecosystems and is not to be taken lightly.

CRediT Authorship Contribution Statement

VK: Investigation (Molecular Analysis), Data curation, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition. **FOHS:** Investigation (Field work & Data collection), Resources, Writing – review & editing. **EAW:** Investigation (Field work & Data collection), Resources, Writing – review & editing. **MV:** Investigation (Field work & Data collection), Resources, Writing – review & editing, Funding acquisition, Supervision. **All authors** gave final approval for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Balendres, M.A., Tegg, R.S., Wilson, C.R., 2017. Resting spore dormancy and infectivity characteristics of the potato powdery scab pathogen *Spongospora subterranea*. J. Phytopathol. 50, 323–330. https://doi.org/10.1111/jph.12565.

Beca-Carretero, P., Guihéneuf, F., Winters, G., Stengel, D.B., 2019. Depth-induced adjustment of fatty acid and pigment composition suggests high biochemical plasticity in the tropical seagrass *Halophila stipulacea*. Mar. Ecol. Prog. Ser. 608, 105–117. https://doi.org/10.3354/meps12816.

Beca-Carretero, P., Rotini, A., Mejia, A., Migliore, L., Vizzini, S., Winters, G., 2020. *Halophila stipulacea* descriptors in the native area (Red Sea): a baseline for future comparisons with native and non-native populations. Mar. Environ. Res. 153, 104828 https://doi.org/10.1016/j.marenvres.2019.104828.

Bianchi, C.N., Morri, C., 2003. Global sea warming and "tropicalization" of the Mediterranean sea: biogeographic and ecological aspects. Biogeographia 24, 319–329. https://doi.org/10.21426/B6110129.

Blackburn, T.M., Cassey, P., Duncan, R.P., Evans, K.L., Gaston, K.J., 2004. Avian extinction and mammalian introductions on oceanic islands. Science 305 (5692), 1955–1958. https://doi.org/10.1126/science.1101617.

Blake, C., Thiel, M., López, B., Fraser, C., 2017. Gall-forming protistan parasites infect southern bull kelp across the Southern Ocean, with prevalence increasing to the south. Mar. Ecol. Prog. Ser. 583, 95–106. https://doi.org/10.3354/meps12346.

Borghini, M., Bryden, H., Schroeder, K., Sparnocchia, S., Vetrano, A., 2014. The Mediterranean is becoming saltier. Ocean Sci. 10, 693–700. https://doi.org/10.5194/os-10-693-2014.

Bradshaw, C.J.A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J.-M., Simard, F., Courchamp, F., 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7, 12986. https://doi.org/10.1038/ncomms12986.

Bulman, S., Neuhauser, S., 2017. Phytomyxea. In: Archibald, J.M., Simpson, A.G.B., Slamovits, C.H., Margulis, L., Melkonian, M., Chapman, D.J. (Eds.), Handbook of the Protists. Springer International Publishing, Switzerland, pp. 783–803.

Chiquillo, K.L., 2021. Understanding the ecological and evolutionary dynamics of tropical seagrasses and the factors driving habitat distribution (Dissertation). University of California.

Creed, J.C., Phillips, R.C., van Tussebroek, B.I., 2003. The seagrasses of the Caribbean. In: Green, E.P., Short, F.T., Frederick, T. (Eds.), World Atlas of Seagrasses. University of California Press, Berkeley, pp. 234–242.

Drew, E.A., 1979. Physiological aspects of primary production in seagrasses. Aquat. Bot. 7, 139–150. https://doi.org/10.1016/0304-3770(79)90018-4.

Duarte, C.M., Marba, N., Gacia, E., Fourqurean, J.W., Beggins, J., Barrón, C., Apostolaki, E.T., 2010. Seagrass community metabolism: assessing the carbon sink capacity of seagrass meadows. Glob. Biogeochem. Cycles 24, GB4032. https://doi.org/10.1029/2010GB003793.

Duffy, J.E., 2006. Biodiversity and the functioning of seagrass ecosystems. Mar. Ecol. Prog. Ser. 311, 233–250. https://doi.org/10.3354/meps311233.

Dural, B., Okudan, E.Ş., Demir, N., Şenkardeşler, A., Erduğan, H., Aysel, V., 2020. Observations on the flowering and fruit developments in *Halophila stipulacea* (Hydrocharitaceae) in the Aegean Sea (Turkey). J. Black Sea Mediterr. Environ. 26 (1).

Elwood, H.J., Olsen, G.J., Sogin, M.L., 1985. The small-subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. Mol. Biol. Evol. 2, 399–410. https://doi.org/10.1093/oxfordjournals.molbev.a040362.

Ernst, T.W., Kher, S., Stanton, D., Rennie, D.C., Hwang, S.F., Strelkov, S.E., 2019. *Plasmodiophora brassicae* resting spore dynamics in clubroot resistant canola (*Brassica napus*) cropping systems. Plant Pathol. 68, 399–408. https://doi.org/10.1111/ppa.12949.

Galil, B., Boero, F., Fraschetti, S., Piraino, S., Campbell, M., Hewitt, C., Carlton, J., Cook, E., Jelmert, A., Macpherson, E., Marchini, A., Occhipinti-Ambrogi, A., Mckenzie, C., Minchin, D., Ojaveer, H., Olenin, S., Ruiz, G., 2015. The enlargement of the Suez Canal and introduction of non-indigenous species to the Mediterranean Sea. Limnol. Oceano Bull. 24, 41–43. https://doi.org/10.1002/lob.10036.

Gambi, M.C., Barbieri, F., Bianchi, C.N., 2009. New record of the alien seagrass *Halophila stipulacea* (Hydrocharitaceae) in the western Mediterranean: a further clue to changing Mediterranean Sea biogeography. Mar. Biodivers. Rec. 2, 1–7. https://doi.org/10.1017/S175526720900058X.

di Genio, S., Gaglioti, M., Meneghesso, C., Barbieri, F., Cerrano, C., Gambi, M.C., 2021. Phenology and ecology of the alien seagrass *Halophila stipulacea* in its northern range limit in the Mediterranean Sea. Aquat. Bot. 168, 103304 https://doi.org/10.1016/j.aquabot.2020.103304.

Giakoumi, S., Katsanevakis, S., Albano, P.G., Azzurro, E., Cardoso, A.C., Cebrian, E., Deidun, A., Edelist, D., Francour, P., Jimenez, C., Mačić, V., 2019. Management priorities for marine invasive species. Sci. Total Environ. 688, 976–982. https://doi.org/10.1016/j.scitotenv.2019.06.282.

den Hartog, C., 1965. Some notes on the distribution of *Plasmodiophora diplantherae*, a parasitic fungus on species of *Halodule*. Persoonia 4, 15–18.

den Hartog, C., 1970. The seagrasses of the world. Verh. K. Ned. Akad. Wet. Afd. Nat. 2 (59), 1–275.

den Hartog, C., 1972. Range extension of *Halophila stipulacea* (Hydrocharitaceae) in the Mediterranean. Blume Biodivers. Evol. Biogeogr. Plants 20 (1), 154–156.

den Hartog, C., 1989. Distribution of *Plasmodiophora bicaudata*, a parasitic fungus on small *Zostera* species. Dis. Aquat. Organ 6, 227–229.

Hayes, K.R., Barry, S.C., 2008. Are there any consistent predictors of invasion success? Biol. Invasions 10 (4), 483–506. https://doi.org/10.1007/s10530-007-9146-5.

Hulme, P.E., 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46 (1), 10–18. https://doi.org/10.1111/j.1365-2664.2008.01600.x.

Kolátková, V., Čepička, I., Gargiulo, G.M., Vohník, M., 2020. Enigmatic phytomyxid parasite of the alien seagrass *Halophila stipulacea*: new insights into its ecology, phylogeny, and distribution in the Mediterranean Sea. Micro Ecol. 79 (3), 631–643. https://doi.org/10.1007/s00248-019-01450-3.

Kolátková, V., Čepička, I., Hoffman, R., Vohník, M., 2021. *Marinomyxa* gen. nov. accommodates gall-forming parasites of the tropical to subtropical seagrass genus *Halophila* and constitutes a novel deep-branching lineage within Phytomyxea (Rhizaria: Endomyxa). Micro Ecol. 81, 673–686. https://doi.org/10.1007/s00248-020-01615-5.

Layman, C.A., Jud, Z.R., Nichols, P., 2014. Lionfish alter benthic invertebrate assemblages in patch habitats of a subtropical estuary. Mar. Biol. 161 (9), 2179–2182. https://doi.org/10.1007/s00227-014-2491-x.

Lipkin, Y., 1975. *Halophila stipulacea*, a review of a successful immigration. Aquat. Bot. 1, 203–215. https://doi.org/10.1016/0304-3770(75)90023-6.

Lipkin, Y., Avidor, A., 1974. *Tetramyxa marina* a new plasmodiophoraceae marine parasite. Nova Hedwig. 72, 799–810.

Mabey, A.L., Parvizi, E., Fraser, C.I., 2021. Pathogen inferred to have dispersed thousands of kilometres at sea, infecting multiple keystone kelp species. Mar. Biol. 168 (4), 1–7. https://doi.org/10.1007/s00227-021-03853-8.

Maier, I., Parodi, E., Westermeier, R., Müller, D.G., 2000. *Maullinia ectocarpii* gen. et sp. nov. (Plasmodiophorea), an intracellular parasite in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) and other filamentous brown algae. Protist 151 (3), 225–238. https://doi.org/10.1078/1434-4610-00021.

Maitz, A., Kitson-Walters, K., Boman, E.M., 2021. Phytomyxid infection in the non-native seagrass *Halophila stipulacea* in St Eustatius, Caribbean Netherlands. Aquat. Bot. 168, 103321 https://doi.org/10.1016/j.aquabot.2020.103321.

Martinez-Bakker, M., Helm, B., 2015. The influence of biological rhythms on host–parasite interactions. Trends Ecol. Evol. 30 (6), 314–326. https://doi.org/10.1016/j.tree.2015.03.012.

Marziano, F., Villari, R., Tripodi, G., 1995. A plasmodiophorid fungal parasite of the seagrass *Halophila stipulacea*. Mycotaxon 55, 165–170.

Morri, C., Montefalcone, M., Gatti, G., Vassallo, P., Paoli, C., Bianchi, C.N., 2019. An alien invader is the cause of homogenization in the recipient ecosystem: a simulation-like approach. Diversity 11 (9), 146. https://doi.org/10.3390/d11090146.

Neuhauser, S., Kirchmair, M., Gleason, F.H., 2011. Ecological roles of the parasitic phytomyxids (plasmodiophorids) in marine ecosystems–a review. Mar. Freshwat Res. 62 (4), 365–371. https://doi.org/10.1071/MF10282.

Neuhauser, S., Kirchmair, M., Bulman, S., Bass, D., 2014. Cross-kingdom host shifts of phytomyxid parasites. BMC Evol. Biol. 14 (1), 1–3. https://doi.org/10.1186/1471-2148-14-33.

Nguyen, H.M., Kleitou, P., Kletou, D., Sapir, Y., Winters, G., 2018. Differences in flowering sex ratios between native and invasive populations of the seagrass *Halophila stipulacea*. Bot. Mar. 61 (4), 337–342. https://doi.org/10.1515/bot-2018-0015.

Nguyen, H.M., Yadav, N.S., Barak, S., Lima, F.P., Sapir, Y., Winters, G., 2020. Responses of invasive and native populations of the seagrass *Halophila stipulacea* to simulated climate change. Front Mar. Sci. 6, 812. https://doi.org/10.3389/fmars.2019.00812.

Orth, R.J., Carruthers, T.J., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., 2006. A global crisis for seagrass ecosystems. Bioscience 12, 987–996. https://doi.org/10.1641/0006-3568(2006)56[987:AGCFSE]2.0.CO;2.

Procaccini, G., Acunto, S., Famà, P., Maltagliati, F., 1999. Structural, morphological and genetic variability in *Halophila stipulacea* (Hydrocharitaceae) populations in the western Mediterranean. Mar. Biol. 135 (1), 181–189. https://doi.org/10.1007/s002270050615.

Ruiz, H., Ballantine, D.L., 2004. Occurrence of the seagrass *Halophila stipulacea* in the tropical west Atlantic. Bull. Mar. Sci. 75 (1), 131–135. https://doi.org/10.1017/S1755267213000961.

Ruiz, H., Ballantine, D.L., Sabater, J., 2017. Continued spread of the seagrass *Halophila stipulacea* in the Caribbean: documentation in Puerto Rico and the British Virgin Islands. Gulf Caribb. Res. 28 (1), SC5–SC7. https://doi.org/10.18785/gcr.2801.05.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M., Wall, D.H., 2000. Global biodiversity scenarios for the year 2100. Science 287, 1770–1774. https://doi.org/10.1126/science.287.5459.1770.

Schnepf, E., Kühn, S.F., Bulman, S., 2000. *Phagomyxa bellerocheae* sp. nov. and *Phagomyxa odontellae* sp. nov., Plasmodiophoromycetes feeding on marine diatoms. Helgol. Mar. Res. 54 (4), 237–241. https://doi.org/10.1007/s101520000056.

Schwarz, A.M., Hellblom, F., 2002. The photosynthetic light response of *Halophila stipulacea* growing along a depth gradient in the Gulf of Aqaba, the Red Sea. Aquat. Bot. 74 (3), 263–272. https://doi.org/10.1016/S0304-3770(02)00080-3.

Short, F.T., Koch, E.W., Creed, J.C., Magalhaes, K.M., Fernandez, E., Gaeckle, J.L., 2006. SeagrassNet monitoring across the Americas: case studies of seagrass decline. Mar. Ecol. 27 (4), 277–289. https://doi.org/10.1111/j.1439-0485.2006.00095.x.

Short, F.T., Coles, R., Fortes, M.D., Victor, S., Salik, M., Isnain, I., Andrew, J., Seno, A., 2014. Monitoring in the Western Pacific region shows evidence of seagrass decline in line with global trends. Mar. Pollut. Bull. 83 (2), 408–416. https://doi.org/10.1016/j.marpolbul.2014.03.036.

Smulders, F.O., Chiquillo, K.L., Willette, D.A., Barber, P.H., Christianen, M.J., 2020. Inconclusive evidence of sexual reproduction of invasive *Halophila stipulacea*: a new field guide to encourage investigation of flower and fruit production throughout its invasive range (ahead-of-print). Bot. Mar. 1. https://doi.org/10.1515/bot-2020-0046.

Thibaut, T., Blanfuné, A., Boudouresque, C.F., Holon, F., Agel, N., Descamps, P., Deter, J., Pavy, T., Delaruelle, G., Verlaque, M., 2022. Distribution of the seagrass

Halophila stipulacea: a big jump to the northwestern Mediterranean Sea. Aquat. Bot. 176, 103465 https://doi.org/10.1016/j.aquabot.2021.103465.

van Tussenbroek, B.I., van Katwijk, M.M., Bouma, T.J., van der Heide, T., Govers, L.L., Leuven, R.S.E.W., 2016. Non-native seagrass *Halophila stipulacea* forms dense mats under eutrophic conditions in the Caribbean. J. Sea Res. 115, 1–5. https://doi.org/10.1016/j.seares.2016.05.005.

Vera, B., Collado-Vides, L., Moreno, C., van Tussenbroek, B.I., 2014. *Halophila stipulacea* (Hydrocharitaceae): a recent introduction to the continental waters of Venezuela. Caribb. J. Sci. 48 (1), 66–70. https://doi.org/10.18475/cjos.v48i1.a11.

Versteegh, M.A., Helm, B., Kleynhans, E.J., Gwinner, E., Tieleman, B.I., 2014. Genetic and phenotypically flexible components of seasonal variation in immune function. J. Exp. Biol. 217 (9), 1510–1518. https://doi.org/10.1242/jeb.097105.

Vohník, M., Borovec, O., Özgür Özbek, E., Okudan Alsan, E., 2017. Rare phytomyxid infection on the alien seagrass *Halophila stipulacea* in the southeast Aegean Sea. Mediterr. Mar. Sci. 18, 433–442. https://doi.org/10.12681/mms.14053.

Walsh, J.R., Carpenter, S.R., Vander Zanden, M.J., 2016. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc. Natl. Acad. Sci. 113, 4081–4085. https://doi.org/10.1073/pnas.1600366113.

Ward, E.A., 2020. Productivity and Carbon Sequestration Potential of Seagrass Ecosystems in the Eastern Aegean Sea (Dissertation). University of Essex.

Waycott, M., van Dijk, K., Calladine, A., Bricker, E., Biffin, E., 2021. Genomics-based phylogenetic and population genetic analysis of global samples confirms *Halophila johnsonii* Eiseman as *Halophila ovalis* (R.Br.) Hook.f. Front Mar. Sci. 8, 740958 https://doi.org/10.3389/fmars.2021.740958.

Wesselmann, M., Anton, A., Duarte, C.M., Hendriks, I.E., Agustí, S., Savva, I., Apostolaki, E.T., Marbà, N., 2020. Tropical seagrass *Halophila stipulacea* shifts thermal tolerance during Mediterranean invasion. Proc. R. Soc. B 287 (1922), 20193001. https://doi.org/10.1098/rspb.2019.3001.

Willette, D.A., Ambrose, R.F., 2012. Effects of the invasive seagrass *Halophila stipulacea* on the native seagrass, *Syringodium filiforme*, and associated fish and epibiota communities in the Eastern Caribbean. Aquat. Bot. 103, 74–82. https://doi.org/10.1016/j.aquabot.2012.06.007.

Willette, D.A., Chalifour, J., Debrot, A.D., Engel, M.S., Miller, J., Oxenford, H.A., Short, F.T., Steiner, S.C., Védie, F., 2014. Continued expansion of the trans-Atlantic invasive marine angiosperm *Halophila stipulacea* in the Eastern Caribbean. Aquat. Bot. 112, 98–102. https://doi.org/10.1016/j.aquabot.2013.10.001.

Winters, G., Beer, S., Willette, D.A., Viana, I.G., Chiquillo, K.L., Beca-Carretero, P., Villamayor, B., Azcárate-García, T., Shem-Tov, R., Mwabvu, B., Migliore, L., 2020. The tropical seagrass *Halophila stipulacea*: reviewing what we know from its native and invasive habitats, alongside identifying knowledge gaps. Front. Mar. Sci. 7, 300. https://doi.org/10.3389/fmars.2020.00300.