Distribution and Pathogenicity of the Protist *Labyrinthula* sp. in western Mediterranean Seagrass Meadows

Neus Garcias-Bonet · Timothy D. Sherman · Carlos M. Duarte · Núria Marbà

Abstract The presence of the pathogenic protist *Labyrinthula* sp., the causative agent of seagrass wasting disease and mass mortality events, was assessed in 18 seagrass meadows in the Balearic region (western Mediterranean). This protist was found in 70% of seagrass meadows investigated and in all seagrass species present in the region (i.e., *Posidonia oceanica*, *Cymodocea nodosa*, and *Zostera noltii*). *Labyrinthula* spp. cultures isolated from seven *P. oceanica* and one *Thalassia testudinum* meadows were used as inocula to perform cross-infection experiments in order to test seagrass vulnerability to *Labyrinthula* spp. infection. These isolates produced lesions on *P. oceanica* and other seagrass species (*Zostera marina*, *Z. noltii*, and *C. nodosa*). *P. oceanica* and *Z. noltii*, both species autochthonous to the Mediterranean Sea, were the seagrasses most vulnerable to infection by the tested isolates. One of the *P. oceanica* isolates of *Labyrinthula* sp. also infected the Atlantic seagrass *Z. marina*, and all of the Mediterranean seagrasses were infected by *Labyrinthula* sp. isolated from the *T. testudinum*, native to the Caribbean and Gulf of Mexico. This work confirms that *Labyrinthula* sp. is commonly found on seagrasses of the Mediterranean Sea and demonstrates that *Labyrinthula* sp. can infect seagrasses in different genera, in contrast to previous studies where *Labyrinthula* sp. was considered to be genus-specific. This finding points out the broadly pathogenic nature of some *Labyrinthula* sp. isolates. Finally, this work identifies *Labyrinthula* sp. as a possible detrimental agent for *P. oceanica*.

N. Garcias-Bonet (✉) · C. M. Duarte · N. Marbà
Department of Global Change Research. IMEDEA (CSIC-UIB),
Institut Mediterrani d'Estudis Avançats,
Miquel Marquès 21,
07190 Esporles, Spain
e-mail: neus@imedea.uib-csic.es

C. M. Duarte
e-mail: carlos.duarte@uwa.edu.aus

T. D. Sherman
Department of Biology, University of South Alabama,
307 N University Blvd.,
Mobile, AL 36688, USA

C. M. Duarte
The UWA Oceans Institute, The University of Western Australia,
MBD: M470, 35 Stirling Highway,
Crawley 6009, Australia

Introduction

Seagrasses rank among the most productive ecosystems on earth (Duarte and Chiscano 1999) and play important functions in the marine environment, such as increasing biodiversity, protecting coastline, and sequestering carbon and other nutrients (Orth et al. 2006). *Posidonia oceanica* is the dominant seagrass in the Mediterranean Sea and, despite its very slow clonal growth (rhizome extension rates ranging from 1 to 6 cm year^{-1} apex^{-1}; Marbà and Duarte 1998), develops meadows that exist for millennia (Mateo et al. 1997). *P. oceanica* meadows are experiencing a general decline across the Mediterranean Sea (Marbà et al. 2005), as reported for seagrass meadows elsewhere (Waycott et al. 2009). This decline has been associated with disturbances such as altered sediment dynamics, climate change, and nutrient and organic matter inputs to coastal waters (Duarte 2002). However, damage to *P. oceanica*

shoots and subsequent meadow decline could also be caused by pathogenic diseases, a possibility that is relatively unexplored.

Historically, there has been evidence of mass mortality of seagrass due to pathogenic disorders. The widespread decline of *Zostera marina* meadows along the Atlantic coasts during the 1930s was associated with infection by the marine protist *Labyrinthula zosterae* which caused the so-called wasting disease (Renn 1936; Young 1943; Muehlstein et al. 1991). Similarly, *Labyrinthula* sp. was apparently associated with mass mortality of *Zostera capricorni* in New Zealand in the early 1960s (Armiger 1964) and *Thalassia testudinum* in Florida Bay during the late 1980s (Robblee et al. 1991). Interestingly, *Labyrinthula* spp. have been found ubiquitously in seagrasses (Muehlstein et al. 1988; Short et al. 1993; Vergeer and den Hartog 1994; den Hartog et al. 1996), yet outbreaks of disease are uncommon. Some authors have suggested that *Labyrinthula* spp. infections only contribute to seagrass mass mortality when plant survival is already compromised by other detrimental factors, such as hypoxia or light limitation (e.g., Robblee et al. 1991; Vergeer et al. 1995). While this may be true, factors that affect the potential pathogenicity of *Labyrinthula* spp. (e.g., salinity and temperature) are also likely to play a role (e.g., Tutin 1938; McKone and Tanner 2009). Moreover, it has been suggested that there are inherent differences in pathogenicity among *Labyrinthula* sp. isolates (Muehlstein et al. 1988; Boettcher and Martin, personal communication).

Labyrinthula spp. produce lesions on seagrass leaves through enzymatic degradation of the cell wall, condensation and destruction of chloroplasts and cytoplasm, and vesiculation of cell membranes, thereby spreading inside the leaf and even reaching the vascular tissues (Muehlstein 1992). Infections by *Labyrinthula* spp. strongly decrease photosynthesis in the lesions and in adjacent, apparently healthy leaf areas (Ralph and Short 2002).

Labyrinthula spp. have been reported to be associated with most marine seagrasses, including: *Z. marina*, *Zostera mucronata*, *Zostera noltii*, *Zostera japonica*, *Heterozostera tasmanica*, *P. oceanica*, *Halodule universis*, *Halodule wrightii*, *Cymodocea nodosa*, *Phyllospadix scouleri*, *Syringodium isoetifolium*, *Thalassodendron ciliatum*, *Ruppia cirrhosa*, *Ruppia maritima*, *T. testudinum*, and *Halophila ovalis* (Vergeer and den Hartog 1991; Vergeer and den Hartog 1994; Martin and Boettcher personal communication). In the Mediterranean Sea, *Labyrinthula* spp. have been isolated from *P. oceanica*, *C. nodosa*, and *Z. marina* meadows along the southern and eastern Italian coasts (Vergeer and den Hartog 1994; den Hartog et al. 1996), but *Labyrinthula* sp. remains unreported for the western Mediterranean.

Although *Labyrinthula* spp. appear to be widespread, previous infection experiments with these protists sug-

gested that the occurrence of infection is genus-specific with regard to *Labyrinthula* spp. isolates (Muehlstein et al. 1988; Vergeer and den Hartog 1991; Short et al. 1993; Vergeer and den Hartog 1994). However, more recent work showed that an isolate from *T. testudinum* cross-infected *Z. marina* (Caldwell 2006). Given the possibility of cross-infection among seagrass genera, determination of the vulnerability of Mediterranean seagrasses to *Labyrinthula* spp. is particularly important because of the increased potential hazard associated with globalization and global transport (Ruiz et al. 2000) of invasive species introduction and spread of pathogens in aquatic ecosystems.

Here, we examine the presence of *Labyrinthula* spp. in seagrass meadows along the Balearic Island coasts (western Mediterranean) to evaluate its occurrence on seagrass species in this region. In addition, we use cross-infection experiments to assess the vulnerability of seagrasses from the Mediterranean (*P. oceanica*, *C. nodosa*, and *Z. noltii*), Gulf of Mexico (*T. testudinum*), and Atlantic (*Z. marina*) to infection by *Labyrinthula* sp. isolated from *P. oceanica*.

Methods

Study Sites

The Balearic Islands comprise four main islands (Mallorca, Menorca, Ibiza, and Formentera) and the Cabrera Archipelago, with small islands and inlets. *P. oceanica* meadows are the dominant subtidal ecosystems along the Balearic
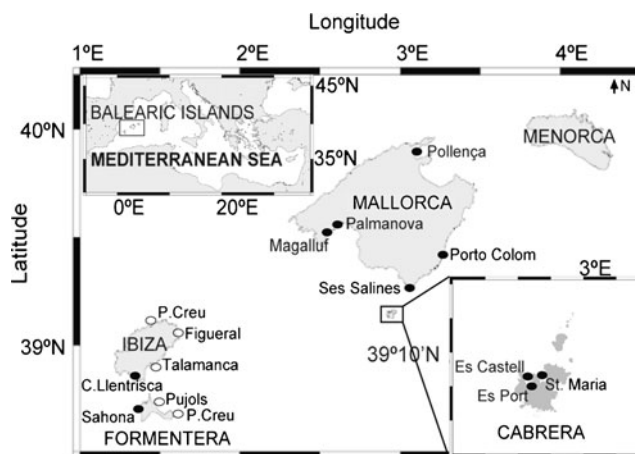

Fig. 1 Location of 18 seagrass meadows of *P. oceanica* (14), *Z. noltii* (one), and *C. nodosa* (three) sampled for presence of *Labyrinthula* spp. on the Balearic Islands (Mallorca, Ibiza, Formentera, and Cabrera). *Filled circles* represent meadows where *Labyrinthula* sp. was isolated. *Open circles* represent absence of *Labyrinthula* sp. in seagrass tissues sampled

Island coasts, covering ~1,200 km². These seagrass stands are monospecific and occur at depths of 0–45 m. *C. nodosa* meadows are restricted to shallow bays and below the depth limit of *P. oceanica*, and *Z. noltii* is relatively rare and occurs in shallow bays.

In August–September 2006, when seawater temperature was 26.3±0.02°C (Marbà, personal communication) and salinity was 37.4±0.1 (Lasternas et al. 2010), we sampled 18 seagrass meadows distributed along the 950 km of the Balearic Islands coast (Fig. 1). These included five meadows of *P. oceanica* and two meadows of *C. nodosa* along the coast of Mallorca Island, seven meadows of *P. oceanica* along the coast of Formentera and Ibiza Islands, and two *P. oceanica* meadows, one of *C. nodosa* and one of *Z. noltii* in Cabrera Island (Table 1). The *P. oceanica* meadows were growing at 3–13 m, and those of *C. nodosa* and *Z. noltii* at 2–3 m water depth. The study sites were distributed from relatively pristine areas (e.g., Es Castell and Santa Maria in the National Park of Cabrera Archipelago; Cap Salines) with low coastal human pressure to areas with intense touristic development (e.g., Magalluf, Palmanova, Pollença, Porto Colom, Talamanca, and Es Figueral). The Balearic Islands receive around ten million tourists annually (data from the Spanish Institute of Statistics), which are mainly concentrated in coastal zones, reaching 6,082 vessels around Mallorca Island on the busiest day in high summer (Balaguer et al. 2010).

At each sampling site, SCUBA divers collected three shoots of each seagrass present. The plant materials were placed in plastic bags underwater to avoid cross-contamination of the samples during transport to the laboratory.

Isolation of *Labyrinthula* sp.

Labyrinthula spp. were isolated from seagrass leaves as previously described (e.g., Porter 1990). Briefly, pieces of seagrass leaves were cut into 1–2 cm length fragments immediately after return to the laboratory. The fragments were dipped in 0.5% sodium hypochlorite solution for 2 min and rinsed in sterile distilled water for 2 min and then in sterile seawater for 2 min. These surface-sterilized leaf pieces were placed on serum-seawater agar (SSA) medium (1.2% agar in filtered (0.4 μm) and autoclaved seawater, 3 mg L^{-1} germanium dioxide (GeO_2), 25 ml L^{-1} penicillin/streptomycin [10,000 units penicillin G/mL and 10 mg mL^{-1} streptomycin], 1% (*v/v*) horse serum) in plastic Petri dishes and maintained at 25°C. When the growth of *Labyrinthula* sp. was visible, a portion of the agar containing the culture was transferred to a new plate in order to isolate the protists from marine fungi, which sometimes grow on the plates. The cultures were maintained by sub-culturing on to new plates every 2 weeks.

Identification of *Labyrinthula* sp.

The *Labyrinthula* spp. were identified by light microscopy of the cultures using phase contrast optics (Zeiss). Representative isolates of *Labyrinthula* sp. were recognized based on gross cell morphology and growth patterns on SSA medium (Muehlstein et al. 1988), cell ultrastructure using transmission electron microscopy (Porter 1969; Muehlstein et al. 1991), and the presence of an ectoplasmic

Table 1 List of 18 sampled seagrass meadows at 15 sites in the Balearic Islands

Source (seagrass)	Island	Location	*Labyrinthula* sp. presence	Water depth (m)
Z. noltii	Cabrera	Es Port	+	3
C. nodosa	Cabrera	Es Port	+	2
C. nodosa	Mallorca	Palmanova	+	2
C. nodosa	Mallorca	Pollença	+	2
P. oceanica	Cabrera	Es Castell	+	12
P. oceanica	Cabrera	Sta. Maria	+	13
P. oceanica	Mallorca	Magaluf	+	6
P. oceanica	Mallorca	Palmanova	+	5
P. oceanica	Mallorca	Porto Colom	+	6.5
P. oceanica	Mallorca	Pollença	+	4
P. oceanica	Mallorca	Cap Salines	+	5
P. oceanica	Ibiza	Cap Llentisca	+	6
P. oceanica	Ibiza	Es Figueral	-	6.5
P. oceanica	Ibiza	Talamanca	-	3
P. oceanica	Ibiza	Pta Sa Creu	-	4.5
P. oceanica	Formentera	Cala sahona	+	7
P. oceanica	Formentera	Pta Sa Creu	-	5
P. oceanica	Formentera	Els Pujols	-	5.5

Source indicates seagrass species from which *Labyrinthula* spp. were isolated, indicating location of the meadow (Island and Location). Water depth of sampled meadows is indicated

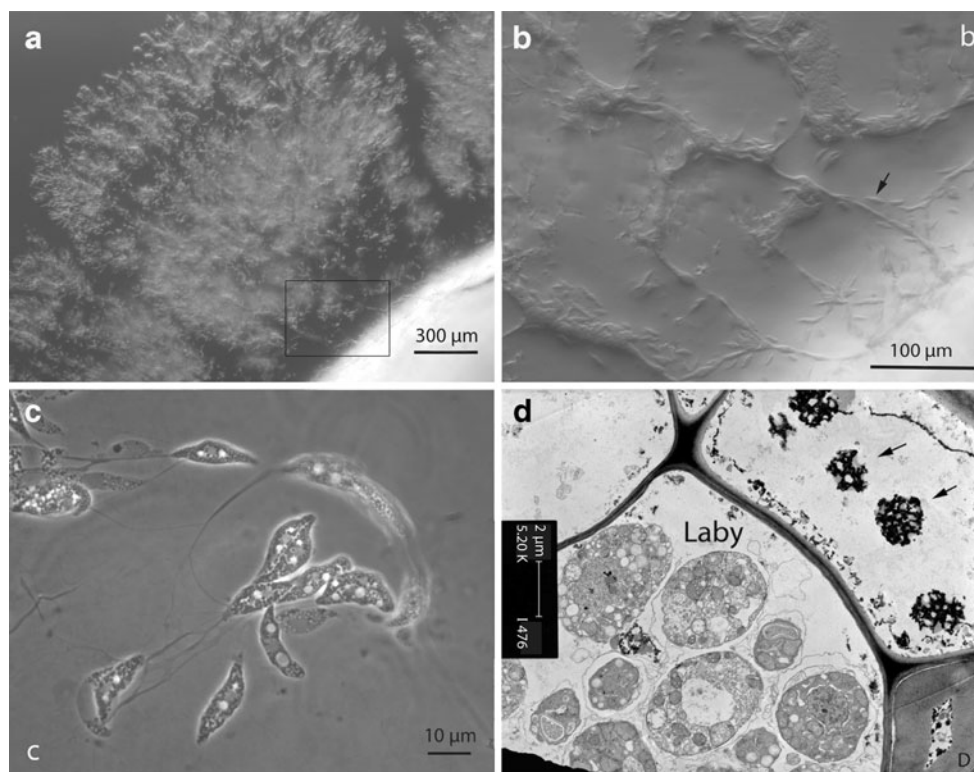*Plus sign* indicates presence, *minus sign* indicates absence of *Labyrinthula* spp. on seagrass leaves

Fig. 2 Representative images of *Labyrinthula* sp. used in this work. All images are of *T. testudinum* isolate. **a**–**c** Represent light microscope images of cultures growing on serum seawater agar (SSA) medium. **a** Cells display typical growth morphology of *Labyrinthula* sp. in culture, with cells on the surface and growing into the agar medium. **b** An enlargement of boxed area of **a**. Plane of focus images cells on surface of agar, where cells can be seen following the ectoplasmic network (*arrow*). **c** Higher magnification network (Fig. 2). Additionally, in 2009, sequence analysis shows typical features of *Labyrinthula* sp. cell structure and ectoplasmic network. **d** TEM of *Z. marina* tissue in area of leaf associated with a lesion caused by *Labyrinthula* sp. isolate from *T. testudinum*. Note numerous *Labyrinthula* sp. cells within the plant cell at bottom of figure and extensively degraded cytoplasmic structures in the surrounding plant cells (*arrows* denote remnants of degraded chloroplasts)

network (Fig. 2). Additionally, in 2009, sequence analysis of a 240-bp diagnostic region of the 18S rDNA (Martin et al. 2006) was used to identify the *P. oceanica* isolate from Pollença, Mallorca, Spain (Martin unpublished data). The sequences in the18S rRNA gene were defined by the primers 960bF (a eukaryote-specific primer without the GC clamp) and 1200R (a universal 18S primer) described by Gast et al. (2004). Blastn analysis yielded best matches with *Labyrinthula* spp. accession numbers AB290459.1 and AB290458.1 (score=304, E=2e-79, Identity=89%, gaps= 9). Of the top 20 hits, all were for *Labyrinthula* sp. or *Aplanochytrium* sp. Although both groups are closely related, colony growth morphologies of the two are quite different and easily discernible. Unfortunately, at the time of this sequencing, the *P. oceanica* isolate from Pollença, (Mallorca, Spain) was the only isolate available because all other Mediterranean isolates ceased to grow in culture. Based on our experience with numerous isolates taken globally over the past 5 years, cessation of growth in culture is common with this protist (Sherman and Martin, unpublished data).

Cross-Infection Experiments

To evaluate the pathogenicity (i.e., capacity to produce lesions on healthy seagrass shoots) of *Labyrinthula* spp. isolated from seven *P. oceanica* meadows in the Balearic Islands, we conducted five cross-infection experiments using the following target seagrass species: *T. testudinum* collected from the coast of Florida, USA (30° 18.186′ N, 87° 24.264′ W), *Z. marina* collected from the temperate Western Atlantic (37° 15.50′ N, 76° 01.30′ W), and *P. oceanica*, *Z. noltii* and *C. nodosa* from three meadows around Mallorca and Cabrera Islands (*P. oceanica* from Es Cargol 39° 16.62′ N, 3° 2.449′E; *Z. noltii* from Es Port-Cabrera 39° 9.18′ N , 2° 55.86′ E; and *C. nodosa* from Cala Estancia 39° 32.13′ N, 2° 42.65′ E). From each meadow containing the target seagrass species, we collected ~100 healthy shoots. These were maintained in aerated seawater to allow them to acclimate to aquarium conditions for 3–4 days before the onset of the experiments.

We conducted the infection experiments according to the procedures of Muehlstein et al. (1988). The infection

vectors used were autoclaved pieces of seagrass leaves that were placed on *Labyrinthula* sp. cultures in Petri dishes for 7 days at 25°C. The leaf sections carrying the *Labyrinthula* sp. were clamped onto healthy leaves of seagrass shoots with a piece of clear, flexible PVC tubing. Negative controls were sterile leaf pieces that had no contact with *Labyrinthula* sp. cultures. These were fixed to healthy leaves with PVC tubing. In addition, we used a *Labyrinthula* sp. isolated from *T. testudinum* (Steele et al. 2005) as a positive control as it was previously reported to infect not only *T. testudinum* (≈100% efficiency), but also *Z. marina* (≈70% efficiency) (Caldwell 2006). Only one leaf per seagrass plant was inoculated with the *Labyrinthula* sp. vector clamp. We distributed the six to ten replicated seagrass shoots per treatment (Table 2) between two aquaria (2 L) filled with filtered (0.4 µm), aerated seawater (salinity 38). There was no water flow among aquaria to avoid contamination. We placed the aquaria in an incubator at 25°C for 2 weeks with a 12 h light/12 h dark photoperiod, receiving light intensity of 150 µmol m^{-2} s^{-1}.

We monitored the plants daily for lesions and, at the end of the experiment, we assessed the virulence of the *Labyrinthula* spp. by calculating the percentage of shoots with lesions out of the total number of shoots inoculated per treatment. All lesions observed in the experiments were restricted to an area in or around the *Labyrinthula* spp. inoculation point. *Labyrinthula* spp. were re-isolated from randomly selected lesions that appeared on the seagrass leaves at the end of the infection experiments, following the methodology described above, in order to satisfy Koch's postulates. These isolates were examined for colony growth morphology as well as protist size and shape to ascertain the presence of *Labyrinthula* sp. in the lesions.

Electron Microscopy

During infection experiments on *Z. marina* (conducted at the University of South Alabama), *Labyrinthula* sp. isolated from *T. testudinum* readily produced lesions, and these lesions were processed for transmission electron microscopy to confirm the presence of the pathogen in the tissue. Samples of both uninfected and infected tissue at the margins of lesions were cut into thin sections while immersed in 3% (v/v) glutaraldehyde in seawater and allowed to stand on ice for 2 h. Samples then were washed twice in 100 mM cacodylate (pH 7.2) for 15 min each, post-fixed in 2% osmium in cacodylate (pH 7.2) for 2 h, and then given two water rinses before incubation in 2% uranyl acetate overnight at 4°C. Samples were washed in water, dehydrated in a graded acetone series, and transferred to propylene oxide. A 1:1 (v/v) mixture of Spurr's low-viscosity resin and Polybed 812 (Polysciences, Warrington, Pennsylvania) was added in increasing increments to 75% plastic, with the remaining 25% of propylene oxide allowed to evaporate through small holes in aluminum foil covers over the sample vials. The samples then were oriented in Teflon flat molds (Ted Pella Inc., Redding, California) and Epon/Spurr's was used to completely fill the cavity. A coverslip was applied and the

Table 2 Results of cross-infection experiments using *Labyrinthula* spp. isolated from *P. oceanica* with different targeted seagrass species (*T. testudinum, Z. marina, P. oceanica, Z. noltii,* and *C. nodosa*)

Treatment	Seagrass source	Seagrass collection site	Percentage of shoots infected					Target spp. infected
			T. testudinum	*Z. marina*	*P. oceanica*	*Z. noltii*	*C. nodosa*	
Positive Control	*T. testudinum*	Perdido Bay (Florida)	100 (7)	100 (7)	90 (10)	100 (8)	100 (6)	5
Isolate 1	*P. oceanica*	St. Maria (Cabrera)	0 (7)	0 (7)	0 (7)	0 (8)	0 (7)	0
Isolate 2	*P. oceanica*	Es Castell (Cabrera)	0 (7)	0 (7)	100 (7)	83 (6)	0 (7)	2
Isolate 3	*P. oceanica*	C. Sahona (Formentera)	0 (7)	0 (7)	100 (7)	0 (7)	nd	1
Isolate 4	*P. oceanica*	Palmanova (Mallorca)	0 (7)	100 (7)	67 (6)	100 (7)	0 (9)	3
Isolate 5	*P. oceanica*	Magaluf (Mallorca)	0 (7)	0 (7)	0 (7)	100 (8)	0 (6)	1
Isolate 6	*P. oceanica*	Pollença (Mallorca)	0 (7)	0 (7)	50 (6)	100 (7)	100 (6)	3
Isolate 7	*P. oceanica*	Cap Salines (Mallorca)	nd	nd	63 (8)	50 (6)	100 (6)	3
Negative Control			0 (7)	0 (7)	0 (7)	0 (7)	0 (7)	0
P. oceanica isolates producing lesions			0	1	5	5	2	

Occurrence of infection (percentage of shoots presenting lesions out of total shoots inoculated) on each treatment and target seagrass species are indicated. Number of replicates is shown in parenthesis under "Percent of shoots infected." Additionally, the total number of target seagrass species infected by each *Labyrinthula* sp. isolate and total number of isolates that infected a particular target seagrass species are indicated. Frequencies of infection differed significantly among target species and sources of *Labyrinthula* sp. (three-way log-linear analysis, $G=70.35$, df= 9, $p<0.0001$)

nd no data

samples polymerized at 60°C for 16 h. Thin sections (gray-silver reflectance) were cut with a Delaware Diamond Knife on a LKB 8800 Ultratome III, post-stained with uranyl acetate and lead citrate and observed with a Phillips CM 100 electron microscope.

Statistical Analysis

Data from the cross-infection experiments were evaluated via three-way log-linear analysis (G-test of independence; Sokal and Rohlf 1995; Quinn and Keough 2002) to look for differences in rates of infection between seagrass species and differences in infection due to different *Labyrinthula* spp. isolates.

Results

Geographic Distribution of *Labyrinthula* spp.

Labyrinthula spp. organisms were found in 13 of the 18 seagrass meadows examined. Specifically, *Labyrinthula* spp. were isolated from seagrass leaves from all four meadows at Cabrera Island and in all seven of those around Mallorca Island. Conversely, *Labyrinthula* spp. were isolated in only two of the seven meadows examined at Ibiza and Formentera Islands (Fig. 1). *Labyrinthula* spp. were cultured from nine of the 14 *P. oceanica* meadows, from all three *C. nodosa* meadows, and from the single *Z. noltii* meadow investigated (Table 1). As determined by light microscopy, cell shape of the various isolates from the Mediterranean was in keeping with the morphology ascribed to *Labyrinthula* sp., with spindle-shaped cells within a filamentous network (as shown in Fig. 2).

Cross-Infection Experiments

No lesions were observed on the negative control treatments for any of the infections conducted (Table 2). Conversely, the *Labyrinthula* sp. used as a positive control (isolate from *T. testudinum*) produced lesions on the leaves of all replicated shoots of all seagrass species tested. This isolate was the most pathogenic of all isolates tested. *Labyrinthula* sp. was observed within the infected tissue of *Z. marina* with evidence of extensive cellular damage in the surrounding seagrass cells (Fig. 2).

The most virulent *Labyrinthula* spp. isolates from *P. oceanica* were all from meadows around Mallorca Island (Palmanova, Pollença, and Cap Salines). *Labyrinthula* spp. from Pollença and Cap Salines were capable of producing lesions on all three Mediterranean seagrass species tested. *Labyrinthula* sp. isolated from the Palmanova location produced lesions on *Z. marina* from the temperate Western

Atlantic, as well as the Mediterranean seagrasses *P. oceanica* and *Z. noltii*. The least virulent *Labyrinthula* sp. isolate was that from Santa Maria Bay (Cabrera Island), which did not produce lesions in any of the examined seagrass species.

The seagrass species most vulnerable to infection by *Posidonia*-derived *Labyrinthula* spp. isolates were *P. oceanica* and *Z. noltii*, with five out of the seven *Labyrinthula* spp. isolates producing lesions in the cross-infection experiments. Only two of the seven isolates from *P. oceanica* produced lesions on *C. nodosa*, and only one of them produced lesions on *Z. marina*. It is also of note that *T. testudinum* was the seagrass most resistant to infection, since none of the seven *Labyrinthula* spp. isolated from *P. oceanica* produced lesions on its leaves (Table 2).

Analysis of these data confirms that the various seagrass species tested had significantly different susceptibilities to the pathogens and that there were differences in pathogenicity of the various *Labyrinthula* spp. isolates (three-way log linear analysis, $G=70.35$, df=9, $p<0.00010$).

Discussion

This is the first report of *Labyrinthula* spp. in the western Mediterranean Sea, and it was found on leaves of all three seagrass species in the Balearic Islands: *P. oceanica*, *C. nodosa*, and *Z. noltii*. The presence of *Labyrinthula* spp. on *P. oceanica* was previously reported in the Ionian Sea (Gallipoli, Italy), on *C. nodosa* in Taranto, Italy (Vergeer and den Hartog 1994) and in the Lagoon of Venice, Adriatic Sea (den Hartog et al. 1996), and in association with *Z. noltii* in The Netherlands and France (Vergeer and den Hartog 1991). In this study, we found *Labyrinthula* spp. in most (70%) of the sampled meadows, indicating that *Labyrinthula* spp. are widespread in the seagrass meadows of the Balearic Islands and very common in the Mediterranean Sea, with some of them producing lesions like those described previously during wasting disease events (Muehlstein 1992).

Our cross-infection experiments revealed that *Labyrinthula* sp. infectivity was not specific to the seagrass species from which it was isolated. *Labyrinthula* sp. isolates from *T. testudinum* produced lesions on 90–100% of all leaves in experiments with *T. testudinum, P. oceanica, Z. marina, Z. noltii*, and *C. nodosa*. Those from *P. oceanica* produced lesions on 50–100% of all treated leaves of *P. oceanica, Z. marina, Z. noltii*, and *C. nodosa*. These results contrast with those in previous studies (Muehlstein et al. 1988; Short et al. 1993; Vergeer and den Hartog 1994), where the capacity for infection by *Labyrinthula* sp. was restricted to the seagrass species from which it was isolated, or at most, to a congeneric species. However, our results parallel those of

Caldwell (2006), where *Labyrinthula* sp. isolates from *T. testudinum* cross-infected *Z. marina*, but not vice versa.

The target host range for the pathogen could be much broader than was thought previously. The *Labyrinthula* sp. isolated from *T. testudinum* in Perdido Bay, Florida, infected the shoots of all seagrass species tested here, including representatives of the western Mediterranean Sea, the temperate western Atlantic, and Florida coast. The *Labyrinthula* spp. isolates from *P. oceanica* were, to varying degrees, somewhat less virulent, but still produced infections on species from other genera. Additionally, *Labyrinthula* spp. used in the present study had different capacities to produce lesions, indicating a range of virulence among isolates from a given seagrass species, consistent with the findings of Muehlstein et al. (1988). Our results also reveal that vulnerability to *Labyrinthula* spp. infections depends on the seagrass species as evidenced by the fact that the two seagrass species not autochthonous to the Mediterranean Sea (*T. testudinum* and *Z. marina*) were the most resistant to infection by *Labyrinthula* sp. isolated from *P. oceanica*. Collectively, these results highlight the fact that virulence of some *Labyrinthula* spp. isolates may not be defined by phylogenetic or geographical boundaries.

In summary, this work demonstrates that *Labyrinthula* spp. are prevalent in seagrass meadows of the western Mediterranean Sea and can damage seagrass leaves. *P. oceanica* may be particularly vulnerable to *Labyrinthula* spp. infections, as it is very slow-growing and supports a large belowground biomass, so that any loss of photosynthetic capacity may compromise its carbon budget and, therefore, plant survival. As some *Labyrinthula* spp. isolates can infect seagrasses from outside their geographical region, Mediterranean seagrass meadows may be threatened by *Labyrinthula* spp. infections. Increasing worldwide maritime traffic may contribute to enhanced vulnerability to *Labyrinthula* spp. infections of these valuable seagrass meadows. Implications for avoiding propagation of this disease are then apparent, and further examination of the conditions favoring disease transmission and development are needed.

References

Armiger, L.C. 1964. An occurrence of *Labyrinthula* in New Zealand *Zostera*. *New Zealand Journal of Botany* 2: 3–9.

Balaguer, P., A. Diedrich, R. Sardá, M. Fuster, B. Cañellas, and J. Tintoré. 2010. Spatial analysis of recreational boating as a first key step for marine spatial planning in Mallorca (Balearic Islands, Spain). *Ocean and Coastal Management* 54: 241–249.

Caldwell, M.M. 2006. Characterization of the pathogen *Labyrinthula* sp. isolated from the host seagrass *Thalassia testudinum*. M.S. Thesis. University of South Alabama.

Den Hartog, C., L.H.T. Vergeer, and A.F. Rismondo. 1996. Occurrence of *Labyrinthula zosterae* in *Zostera marina* from Venice Lagoon. *Botanica Marina* 39: 23–26.

Duarte, C.M. 2002. The future of seagrass meadows. *Environmental Conservation* 29: 192–206.

Duarte, C.M., and C.L. Chiscano. 1999. Seagrass biomass and production: a reassessment. *Aquatic Botany* 65: 159–174.

Gast, R.J., M.R. Dennett, and D.A. Caron. 2004. Characterization of protistan assemblages in the Ross Sea, Antarctica by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology* 70: 2028–2037.

Lasternas, S., S. Agustí, and C.M. Duarte. 2010. Phyto- and bacterioplankton abundance and viability and their relationship with phosphorus across the Mediterranean Sea. *Aquatic Microbial Ecology* 60: 175–191.

Marbà, N., and C.M. Duarte. 1998. Rhizome elongation and seagrass clonal growth. *Marine Ecology Progress Series* 174: 269–280.

Marbà, N., C.M. Duarte, E. Diaz-Almela, J. Terrados, E. Alvarez, R. Martinez, R. Santiago, E. Gacia, and A.M. Grau. 2005. Direct evidence of imbalanced seagrass (*Posidonia oceanica*) shoot population dynamics in the Spanish Mediterranean. *Estuaries and Coasts* 28: 53–62.

Martin, D., R. Ross, L.B. Quetin, and A. Murray. 2006. Molecular approach (PCR-DGGE) to diet analysis in young Antarctic krill *Euphausia superba*. *Marine Ecology Progress Series* 319: 155–165.

Mateo, M.A., J. Romero, M. Perez, M.M. Littler, and D.S. Littler. 1997. Dynamics of millenary organic deposits resulting from the growth of the Mediterranean seagrass *Posidonia oceanica*. *Estuarine, Coastal and Shelf Science* 44: 103–110.

McKone, K.L., and C.E. Tanner. 2009. Role of salinity in the susceptibility of eelgrass *Zostera marina* to the wasting disease pathogen *Labyrinthula zosterae*. *Marine Ecology Progress Series* 377: 123–130.

Muehlstein, L.K. 1992. The host-pathogen interaction in the wasting disease of eelgrass, *Zostera marina*. *Canadian Journal of Botany* 70: 2081–2088.

Muehlstein, L.K., D. Porter, and F.T. Short. 1988. *Labyrinthula* sp, a marine slime mold producing the symptoms of wasting disease in eelgrass, *Zostera marina*. *Marine Biology* 99: 465–472.

Muehlstein, L.K., D. Porter, and F.T. Short. 1991. *Labyrinthula zosterae* sp. nov, the causative agent of wasting disease of eelgrass, *Zostera marina*. *Mycologia* 83: 180–191.

Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott, and S.L. Williams. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56: 987–996.

Porter, D. 1969. Ultrastructure of *Labyrinthula*. *Protoplasma* 67: 1–19.

Porter, D. 1990. Phylum Labyrinthulomycota. In Handbook of Protoctista. Margulis, L, Corliss, J.O., Melkonian, M., Chapman, D.J. Boston, Jones and Bartlett, 388–398.

Quinn, G.P., and M.J. Keough. 2002. *Experimental Design and Data Analysis for Biologists*, 1st ed. Cambridge: Cambridge University Press.

Ralph, P.J., and F.T. Short. 2002. Impact of the wasting disease pathogen, *Labyrinthula zosterae*, on the photobiology of eelgrass *Zostera marina*. *Marine Ecology Progress Series* 226: 265–271.

Renn, C.E. 1936. The wasting disease of *Zostera marina.* I, A phytological investigation of the diseased plant. *The Biological Bulletin* 70: 148–158.

Robblee, M.B., T.R. Barber, P.R. Carlson, M.J. Durako, J.W. Fourqurean, L.K. Muehlstein, D. Porter, L.A. Yarbro, R.T. Zieman, and J.C. Zieman. 1991. Mass mortality of the tropical seagrass *Thalassia testudinum* in Florida Bay (USA). *Marine Ecology Progress Series* 71: 297–299.

Ruiz, G.M., T.K. Rawlings, F.C. Dobbs, L.A. Drake, T. Mullady, A. Huq, and R.R. Colwell. 2000. Global spread of microorganisms by ships - Ballast water discharged from vessels harbours a cocktail of potential pathogens. *Nature* 408: 49–50.

Short, F.T., D. Porter, H. Iizumi, and K. Aioi. 1993. Occurrence of the eelgrass pathogen *Labyrinthula zosterae* in Japan. *Diseases of Aquatic Organisms* 16: 73–77.

Sokal, R.R., and F.J. Rohlf. 1995. *Biometry: The principles and practice of statistics in biological research*, 3rd ed. New York: W. H. Freeman.

Steele, L., M. Caldwell, A.E. Boettcher, and T.M. Arnold. 2005. Seagrass-pathogen interactions: pseudo-induction of phenolics near wasting disease lesions. *Marine Ecology Progress Series* 303: 123–131.

Tutin, T.G. 1938. The autecology of *Zostera marina* in relation to its wasting disease. *The New Phytologist* 37: 50–71.

Vergeer, L.H.T., and C. den Hartog. 1991. Occurrence of wasting disease in *Zostera noltii*. *Aquatic Botany* 40: 155–163.

Vergeer, L.H.T., and C. den Hartog. 1994. Omnipresence of *Labyrinthulaceae* in Seagrasses. *Aquatic Botany* 48: 1–20.

Vergeer, L.H.T., T.L. Aarts, and J.D. deGroot. 1995. The wasting disease and the effect of abiotic factors (light-intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. *Aquatic Botany* 52: 35–44.

Waycott, M., C.M. Duarte, T.J.B. Carruthers, R.J. Orth, W.C. Dennison, S. Olyarnik, A. Calladine, J.W. Fourqurean, K.L. Heck Jr., A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, F.T. Short, and S.L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 106: 12377–12381.

Young, E.L. 1943. Studies on *Labyrinthula*, the etiologic agent of the wasting disease of eel-grass. *American Journal of Botany* 30: 586–593.