

Life history and morphological studies of *Punctaria tenuissima* (Chordariaceae, Phaeophyceae), a new record for the Azores

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Abstract

Punctaria tenuissima (Chordariaceae, Phaeophyceae) is reported for the first time from the Azores. Erect thalli were collected on the Island of São Miguel; they were up to 3 cm long, flattened and often twisted. The plurilocular sporangia were formed from surface cells that were quadrate or rectangular in surface view. Unilocular sporangia were not observed in the field. In culture, the plurispores of *P. tenuissima* developed into *Hecatonema*-like, tufted, prostrate thalli that formed plurilocular sporangia. The plurispores of the prostrate thalli cultured at 15°C with a long day (LD) photoperiod developed into new prostrate thalli, which in turn formed plurilocular sporangia. This cycle was repeated 5 times, resulting in several generations of reproductive prostrate thalli. When these cultures were cooled to 10°C with a short day (SD) photoperiod, new erect thalli developed from the prostrate thalli resembling the thalli collected in the field. These erect blades produced unilocular and plurilocular sporangia on the same or on different thalli. The plurispores and unispores produced by the erect thalli at 10°C under SD conditions once again developed into new prostrate thalli. This

is the first report of unilocular sporangia formed in cultures of *P. tenuissima*. Sexual reproduction was not observed. The culture conditions, particularly temperature and daylength, appeared to influence the formation of erect blades. The *Punctaria*-like thalli were produced in 10°C/SD conditions, whilst the *Hecatonema*-like thalli were produced under 10°–15°C/LD and SD conditions. These results are similar to those reported for *P. tenuissima* from other locations and suggest the occurrence of both direct and heteromorphic life histories in the Azorean algae.

Keywords: *Hecatonema terminale*; life history; morphology; Phaeophyceae; *Punctaria tenuissima*.

Introduction

Punctaria tenuissima (C. Agardh) Greville was originally described as *Zonaria tenuissima* C. Agardh (Agardh 1824) based on specimens from the Kattegat, an area of sea between Sweden and Denmark. *Zonaria tenuissima* was subsequently transferred by Greville (1830) to the genus *Punctaria*. *Punctaria tenuissima* is characterized by the presence of dorso-ventrally flattened, solitary or gregarious, simple, thin and narrow erect blades arising from a small discoid holdfast (Fletcher 1987). This species has been reported from several locations in the temperate-to-cold north Atlantic, the Mediterranean, and the Black Sea, as well as from Burma (Myanmar) in the Indian Ocean (see Guiry and Guiry 2009), but this latter record must be considered doubtful. *Hecatonema terminale* (Kützinger) Kylin was reported from the Azores by Parente et al. (2000) (as *H. terminalis* Kylin) and is the only other member of the ‘Punctariaceae’ that has been described from this region. *Hecatonema* species are solitary or, more commonly, confluent microscopic tufts consisting of a basal layer that is mostly monostromatic, but in places distromatic, from which arise unbranched or scarcely branched erect filaments (Fletcher 1987). *H. maculans* (F.S. Collins) Sauvageau, a species very similar to *H. terminale*, has frequently been recognized as an alternate stage in the life history of blade forming members of the Punctariaceae (Clayton and Ducker 1970, Clayton 1974, Fletcher 1984, Pedersen 1984), a family currently placed in the Chordariaceae (Peters and Ramírez 2001).

Life history studies of *Punctaria tenuissima* have generally revealed the occurrence of an asexual, direct, monophasic life history with spores liberated from erect thalli germinating into a filamentous prostrate system from which new erect blades are produced (Kylin 1933, Rhodes 1970, Rietema and van den Hoek 1981, Lockhart 1982, Pedersen

1984). For many isolates, environmental conditions appeared to play an important role in determining the production of the erect blades and the fertility of the microthalli (Pedersen 1984). According to Fletcher (1987), *P. tenuissima* may occur in different genotypes since isolates from different geographical regions vary in their responses to various environmental conditions, and he suggested that *P. tenuissima* could be subsumed under *Punctaria latifolia* Greville. The present study reports the occurrence of *P. tenuissima* in the Azores and describes the morphology of the algae collected as well as aspects of their development and life history in culture. Similarities between *P. tenuissima* and *P. latifolia* are discussed.

Materials and methods

Thalli of *Punctaria tenuissima* were collected in the intertidal in March 1999 at Feteiras, in March 2002 at Santo António Além Capelas and in March 2005 at Maia on São Miguel (coordinates 37°–40° N and 25°–31° W), the largest island of the Azorean Archipelago.

For morphological studies, thalli were examined, numbered, stored in 5% buffered formaldehyde-sea water solution and deposited in the Department of Biology, University of the Azores. The code numbers of the more representative specimens are given in the text. The entire thallus, portions or sections of specimens were observed under stereo and compound microscopes. Measurements of cells and other structures were made using a micrometer eye piece. Sections were made manually using a razor blade. In some circumstances, preparations were also made by squashing portions of thalli between slides and cover slips.

Culture studies were carried out from one thallus collected in March 2005 at Maia. The entire alga was washed in clean seawater and the surface wiped clean with portions of damp cotton wool (bating). Segments of thallus bearing plurilocular sporangia were cut out and spores were isolated following Wynne's (1969) 'Hanging Drop' technique and using Grund's (von Stosch 1963) culture medium. Cultures were incubated in culture cabinets (Gallenkamp, Loughborough, UK: cooler incubator with timed cycling, regime 1 and ARALAB, Oeiras, Portugal: Fitoclima 4600, regime 2). A total of ten Petri dishes was set up, five for each regime, all containing a large number of spores. When a subsequent generation was required, fertile material was subcultured, as described previously.

Initially, the cultures were submitted to regime 1, i.e., 15°C, 16–8 h light–dark; after two months, six Petri dishes were deposited in another culture cabinet, representing regime 2, 10°C, 8–16 h light–dark. Regime 1 was selected to reflect the climate conditions in the Azores at the time the cultured specimen was collected, while regime 2 was selected to reflect colder regions where the species is common. In both regimes, light was provided by white fluorescence tubes at a photon irradiance of $\sim 45\text{--}50 \mu\text{mol cm}^{-2} \text{day}^{-1}$.

The different phases of development of the life cycle and the reproductive organs were photographed using an OLYM-

PUS-PM (Tokyo, Japan) 10–35 AD-1 microphotography system.

Results

Punctaria tenuissima is reported from the Azores for the first time, based on collections from São Miguel Island. It represents the only species of the genus described for the Azores.

Morphology and phenology

Specimens studied: SMG-99-497, SMG-02-105; SMG-05-49. Thallus epiphytic and/or epilithic, consisting of erect blades arising from a small discoid holdfast. Blades up to 3 cm long, light to greenish brown in colour, dorso-ventrally flattened, solitary or gregarious, simple, thin and narrow, frequently spirally twisted with crisp margins. In surface view, cells are quadrate or rectangular, measuring 15–35×13–23 μm , each containing large numbers of discoid chloroplasts with associated pyrenoids (Figure 1A). In transverse section, blades are 1–4 cells thick, and the quadrate to rectangular cells measure 17–30×10–15 μm . Phaeophycean endogenous hairs with a basal sheath are frequent, measuring 13–15 μm in diameter. Plurilocular sporangia are quadrate or rectangular in surface view (25–30×18–28 μm) and formed from surface cells (Figure 1A). Unilocular sporangia were not observed. Epiphytic thalli grew on *Pterocladia capillacea* (S.G. Gmelin) Santelices et Hommersand and *Cladostephus spongiosus* (Hudson) C. Agardh. Fertile thalli bearing plurilocular sporangia were only found in March 2005 at Maia.

Cultures established from field-collected *Punctaria tenuissima*, and growth patterns in regime 1 [15°C, 16–8 h light–dark/long days (LD)]

The spontaneously released spores from plurilocular sporangia were pyriform, with two dissimilar flagella and contained several discoid chloroplasts and a prominent red eyespot. After 1–4 h, they began to settle, and rounded up (Figure 1B). After spore settlement, a number of cultures was set up. Within 1–3 days, each spore developed a small germ tube (Figure 1C). This culture regime resulted in the development of microthalli. Transverse divisions along the germ tube produced a short, uniseriate, branched system, often with a hair (Figure 1D). After 1–2 weeks, all sporelings produced numerous ectocarpoid plurilocular sporangia that originated from the basal cells and from cells of the erect filaments (Figure 1E, F). They were sessile or had short stalks, were 1–6 cells in length and were lateral or terminal on erect filaments. At about the same time, most of the sporelings produced long uniseriate filaments (Figure 2A) that terminated in a true hair (Figure 2B) resembling the early development of an erect blade. However, these structures never became parenchymatous. After 1 week, these filaments developed lateral uniseriate filaments and resembled an extension of the prostrate system, but after 1–2 weeks these

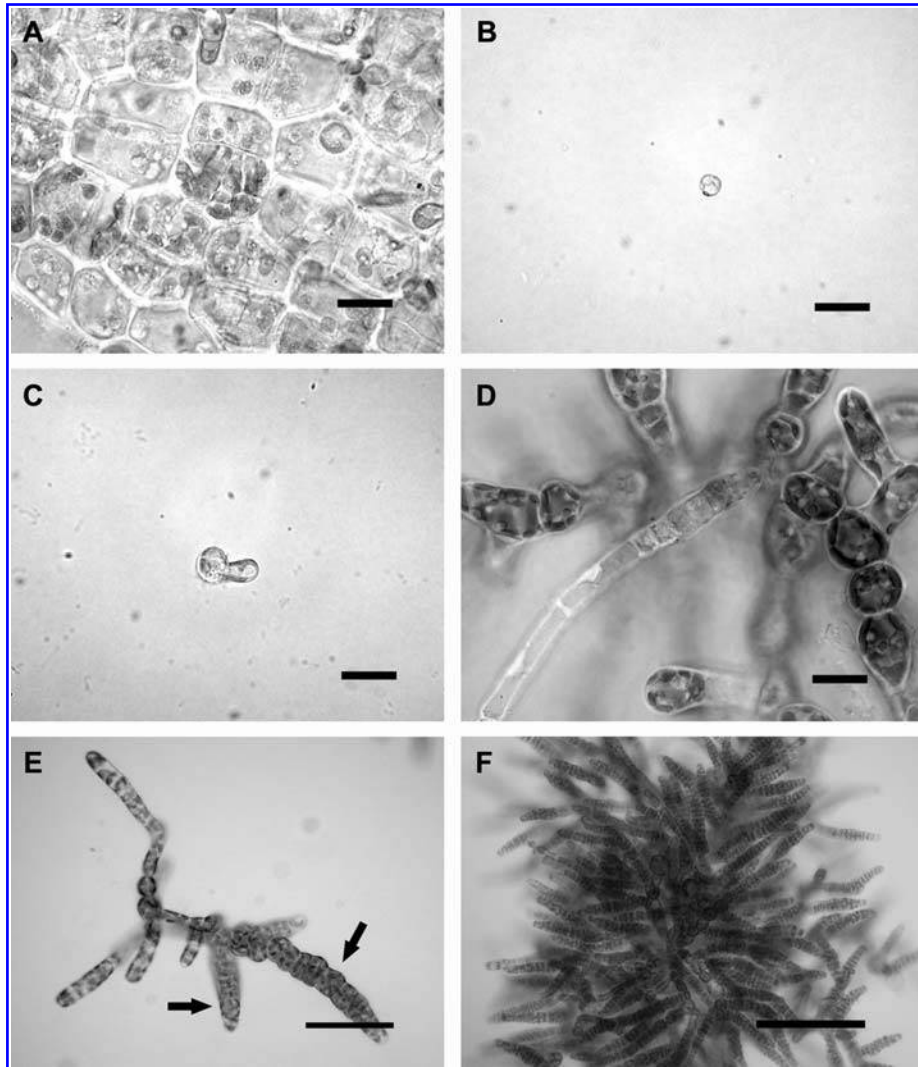


Figure 1 *Punctaria tenuissima*: surface view of mature thallus (collected from the sea) bearing plurilocular sporangia. (A) Development in culture under regime 1, 15°C/LD (progeny derived from plurispores); bar=10 μ m. (B–F): (B) Settled spore from plurilocular sporangia (plurispore); bar=10 μ m. (C) Spore with small germ tube; bar=10 μ m. (D) Microthalli with a hair; bar=10 μ m. (E, F) Microthalli with ectocarpoid plurilocular sporangia (arrows); (E) bar=40 μ m, (F) bar=100 μ m.

filaments became colourless and died. The swarmer from the plurilocular sporangia produced in culture underwent a type of development similar to that described above for plurispores of erect thalli. All liberated spores developed directly into new microthalli. This type of life history was repeated through five generations.

Cultures established from 2-month-old microthalli with plurilocular sporangia from regime 1 (15°C, 16–8 h/LD), and growth patterns in regime 2 [10°C, 8–16 h light-dark/short days (SD)]

After a few weeks in regime 2, the microthalli stopped producing plurilocular sporangia and erect thalli were produced directly from the microthallus, represented by uniseriate, erect filaments with endogenous hairs with a basal sheath, as described previously under regime 1. Later, after longi-

tudinal divisions, mainly in one plane, these erect filaments became parenchymatous (Figure 2C) forming flattened and twisted blades (Figure 2D). Some blades increased in width up to eight cells, and gradually grew to resemble the erect vegetative thalli characteristic of field-collected specimens (Figure 2D); others never reached this size and remained 1–3 cells in width. The cells of the erect blades contained several discoid chloroplasts with associated pyrenoids. After two months under this regime, the erect blades developed several unilocular and plurilocular sporangia from the surface cells on the same thalli (Figure 3A). The unilocular sporangia were ovate in surface view (Figure 3B). The plurilocular sporangia were quadrate or polygonal in surface view, conical (and sometimes bifurcate) (Figure 3C, D) and protruding above the surface in vertical section.

Thallus portions with only unilocular sporangia were isolated, and the liberated unispores developed into new pros-

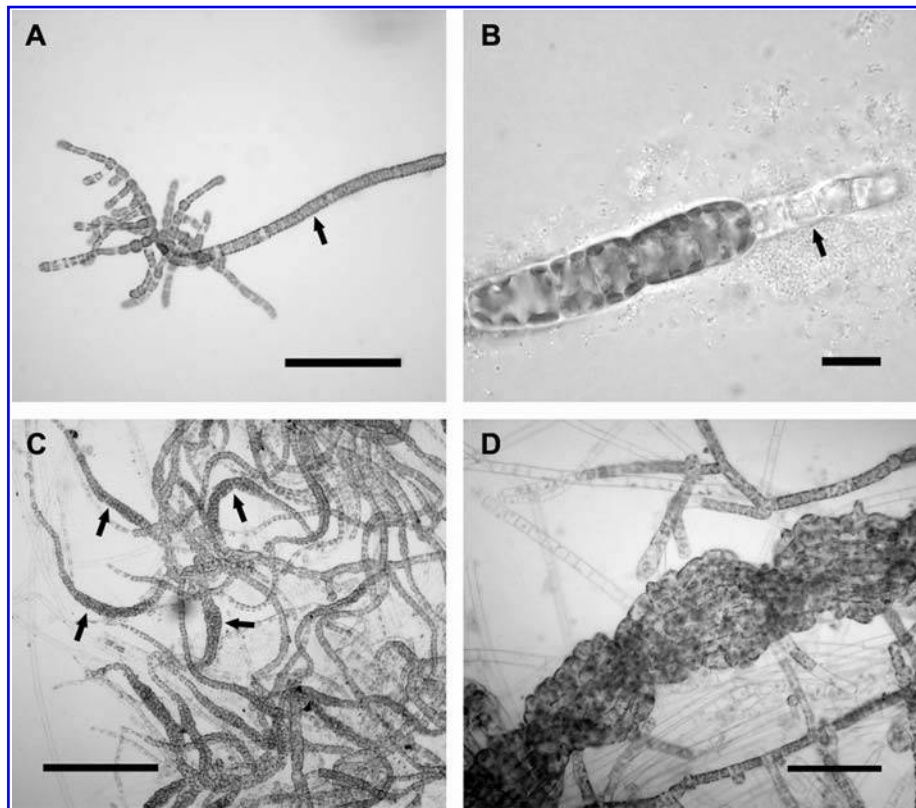


Figure 2 *Punctaria tenuissima*: development in culture under regime 1, 15°C/LD (progeny derived from plurispores). (A, B): (A) Sporelings with long uniseriate filament (arrow); bar=100 µm. (B) Long uniseriate filament terminated by a true hair (arrow). Development in culture under regime 2, 10°C/SD; bar=10 µm. (C) Numerous erect blades produced from microthalli (arrows); bar=200 µm. (D) Flattened and twisted erect blades characteristic of field-collected specimens; bar=40 µm.

trate thalli, as previously described. The same developmental pattern occurred for portions of thalli with plurilocular sporangia, in which the plurispores produced prostrate thalli.

Sexual fusion was not observed in any culture experiment, but no cross experiments were possible with field thalli due to the small number of specimens collected.

Discussion and conclusion

Punctaria tenuissima is newly recorded for the Azores, which now represents its southern limit of distribution in the Atlantic. The Azorean specimens are in agreement with the descriptions of *P. tenuissima* given by Fletcher (1987) and others. No major differences were observed in measurements of cells, hairs and plurilocular sporangia between Azorean and British specimens of *P. tenuissima*.

The present life history study of Azorean *Punctaria tenuissima* (Figure 4) revealed the involvement of a prostrate phase resembling a *Hecatonema*-type of thallus. This result finds support in the investigations of other authors (Kylin 1933, Loiseaux 1969, Clayton and Ducker 1970, Rhodes 1970, Clayton 1974, Fletcher 1984, Pedersen 1984) who have reported the involvement of *Hecatonema* sp. and *Hecatonema*-like microthalli in the life histories of species of the macroscopic genera *Asperococcus* and *Punctaria*. Earlier

studies by Kylin (1933) on *P. tenuissima* [as *Desmotrichum undulatum* (J. Agardh) Reinke] revealed the occurrence of fertile microthalli in the life history similar to both *H. maculans* and *Ectocarpus terminalis* Kützinger (Guiry and Guiry 2009). Later, Pedersen (1984) identified the microthalli produced in his cultures of *Asperococcus fistulosus* (Hudson) Hooker as either *H. reptans* Sauvageau and *Ectocarpus reptans* Reinke or *H. maculans* and *H. terminalis*, depending on the light level under which they were grown. Loiseaux (1967, 1969, 1970) undertook culture studies with a number of different species of the genus *Hecatonema* [*H. maculans*, *H. foecundum* (Strömf.) Loiseaux, *H. hispanicum* (Sauvageau) Loiseaux and *H. primarium* (Setchell et N.L. Gardner) Loiseaux and *H. streblonematoides* (Setchell et N.L. Gardner) Loiseaux] collected from California and France (Roscoff, Brittany). She reported that *H. maculans* had a life history link with a macroscopic phase of the genus *Myriotrichia*. According to Pedersen (1984) and Fletcher (1987), the *Myriotrichia* species (probably *Myriotrichia clavaeformis* Harvey) identified by Loiseaux (1969) was misidentified, the correct determination being *Asperococcus*, probably *A. fistulosus*. Cultures of *H. maculans* were also established by Clayton (1974) from specimens collected in South Australia and by Fletcher (1984) from specimens collected in the British Isles. These gave rise to macrothalli resembling *Punctaria latifolia*/*P. tenuissima* and *P. tenuissima*, respectively.

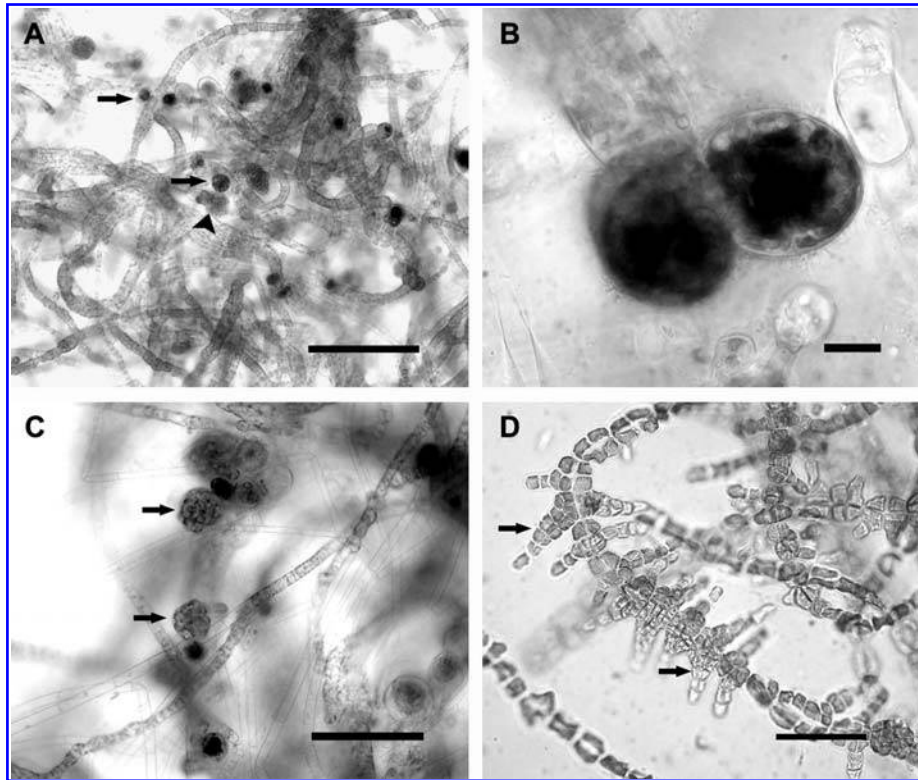


Figure 3 Development of *Punctaria tenuissima* in culture under regime 1, 15°C/SD (progeny derived from plurisporos). (A, B): (A) Erect blades with uni- (arrows) and plurilocular (arrowhead) sporangia on the same thallus; bar=200 μ m. (B) Erect blades with rounded unilocular sporangia; bar=10 μ m. (C) Erect blades with quadrate to polygonal plurilocular sporangia (arrows); bar=100 μ m. (D) Erect blades with bifurcate, protruding plurilocular sporangia (arrows); bar=40 μ m.

Pedersen (1984) also reported the presence of microthalli identified as *Hecatonema* and *Streblonema effusum* Kylin in cultures of *P. latifolia* and *P. tenuissima* (as *Desmotrichum undulatum*), respectively, from Denmark. According to Clayton (1974), *H. maculans* is a heterogeneous assemblage of genotypes having, at an early stage in the development, a more or less uniform phenotype.

Interestingly, the only two ‘‘Punctariaceae’’ species reported for the Azores are *Hecatonema terminale* (Parente et al. 2000) and *Punctaria tenuissima* which are now found to be connected by life history. A comparison between the *Hecatonema*-like thalli developed in the present cultures and *H. terminale* found in nature in the Azores and other specimens from different localities (Kuckuck 1897, Børgesen 1926, Levring 1937, 1940, Fletcher 1987) revealed them to be sim-

ilar (Table 1). Nevertheless, Loiseaux (1970) showed that some species of *Hecatonema* from California (excluding *H. streblonematoides*) developed directly into thalli identical to their parents. Similar results were obtained by Edelstein et al. (1971) working with *H. maculans* from eastern Canada and Pedersen (1984) working with specimens from Denmark. It seems, therefore, that *Hecatonema* species are phases in the life histories of many erect ‘‘Punctariaceae’’, although *Hecatonema* thalli also occur independently in nature.

The *Hecatonema*-like thallus produced in the present culture study agrees in almost all respects with the description of *H. terminale* (as *Ectocarpus terminalis*) given by Kuckuck (1897), Børgesen (1926) and Hamel (1931–1939). According to Kuckuck (1897) and Børgesen (1926), plurilocular sporangia can be lateral and terminal on the erect filaments. In the Azorean cultures, the plurilocular sporangia occurred in a terminal and lateral position on erect filaments and were also formed directly on the prostrate filaments which were typically monostromatic, and partly distromatic. This latter feature was not mentioned by the above authors. A partly distromatic base was, however, evident in drawings by Kuckuck (1897) of *H. maculans* from Germany and was also reported for *H. maculans* (Collins) Sauvageau by Fletcher (1987) from the British Isles and Hamel (1931–1939) from France. According to Levring (1937, 1940), *H. maculans* is a synonym of *H. terminale* and the presence of a distromatic

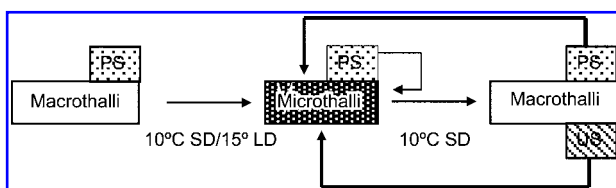


Figure 4 *Punctaria tenuissima*: schematic life history of Azorean specimens. LD, long days; PS, plurilocular sporangia; SD, short days; US, unilocular sporangia.

Table 1 Non-exhaustive historical review of the life history studies in culture of *Hecatonema*, *Punctaria latifolia* (sometimes referred to as a synonym of *Punctaria tenuissima*) and *Desmoirichium undulatum* (a synonym of *P. tenuissima*).

Source	Studied plant (inocula)	Type of life history	Geographic origin of the inocula	Range of temperature in culture (°C)	Development of macrothalli		Development of microthalli		Identification of the alternate thallus produced
					Temperature (°C)	Reproductive structures produced	Temperature (°C)	Reproductive structures produced	
Kylin (1933)	Unispores and plurispores of <i>D. undulatum</i>	Het., mon.	Sweden (Kristineberg)	6	6	PS (at 6)	6	PS (at 6)	
Loiseaux (1969)	Plurispores of <i>H. maculans</i>	Het., mon.	France (Roscoff)	12 and 18–20	18–20	PS	12	US and PS	<i>M. claviformis</i> ^a
Rhodes (1970)	Plurispores of <i>D. undulatum</i>	Het., mon.	USA (Virginia)	6 and 21	6 and 21	PS (at 6 and 21)	21	PS	
Clayton and Ducker (1970)	Unispores and plurispores of <i>P. latifolia</i>	Het., mon.	Australia (Victoria)	15–16	15–16	PS	15–16	PS and US	
Edelstein et al. (1971)	Plurispores of <i>H. maculans</i>	Iso., mon.	Canada (Halifax)	13	13	PS			
Clayton (1974)	Plurispores of <i>H. maculans</i>	Het., mon.	Australia (Victoria)	10, 16 and 21	16	PS (at 10, 16 and 21)	10, 16 and 21	PS (at 10, 16 and 21)	<i>P. latifolia</i> / <i>D. undulatum</i>
Rietema and van den Hoek (1981)	Plurispores of <i>D. undulatum</i> or <i>P. latifolia</i>	Het., mon.	Netherlands	4, 8, 12, 16, 20, 25, 30 and 35	4, 8, 12, 16, 20, 25 and 30	PS (at 16, 20, 25 and 30)	4, 8, 12, 16, 20, 25 and 35	PS (at 4, 8, 12, 16, 20, 25 and 30)	
Lockhart (1982)	Plurispores of <i>D. undulatum</i>	Het., mon.	USA (Massachusetts)	5, 10, 15 and 20	5, 10, 15 and 20	PS (at 5 ^b , 10, 15 and 20)	5, 10, 15, and 20 ^c	PS (at 5 ^b , 10, 15 and 20)	
Fletcher (1984)	Plurispores of <i>H. maculans</i>	Het., mon.	British Isles	10 and 15–20	10	PS	15–20		<i>D. undulatum</i> or <i>A. fistulosus</i>
Pedersen (1984)	Unispores of <i>P. latifolia</i> (US)	Het., mon.	English Channel (Lihou Island)	4 and 15	4 and 15	PS	4 and 15	US (at 15) ^d , PS (at 4 and 15)	
Pedersen (1984)	Plurispores of <i>D. undulatum</i>	Het., mon.	Denmark (Kalø Vig)	4, 5 (±2.5) and 15	4, 5 (±2.5) and 15	PS [at 4, 5 (±2.5) and 15]	4, 5 (±2.5) and 15	PS (at 4, 5 (±2.5) and 15)	<i>Hecatonema</i> sp.
Pedersen (1984)	Plurispores of <i>H. maculans</i>	Iso., mon.	Denmark (North Zealand)	4, 5 (±2.5) and 15	4, 5 (±2.5) and 15	PS [at 4, 5 (±2.5) and 15]			
Pedersen (1984)	Plurispores of putative <i>H. maculans</i>	Het., mon.	Denmark (The Sound)	4, 5 (±2.5) and 15	4, 5 (±2.5) and 15	PS [at 4, 5 (±2.5) and 15]	15	PS (at 15)	<i>D. batricum</i> / <i>D. undulatum</i>

(Table 1 continued)

Source	Studied plant (inocula)	Type of life history	Geographic origin of the inocula	Range of temperature in culture (°C)	Development of macrothalli		Development of microthalli		Identification of the alternate thallus produced
					Temperature (°C)	Reproductive structures produced	Temperature (°C)	Reproductive structures produced	
Pedersen (1984)	Unispores of <i>A. fistulosus</i>	Het., mon.	Denmark (Island of Hirsholm)	4, 5 (±2.5) and 15	4, 5 (±2.5) and 15	PS [at 4, 5 (±2.5) and 15]	4, 5 (±2.5) and 15	US (at 4, 5 (±2.5) and 15)	<i>H. reptans</i> , <i>E. repens</i> , <i>H. maculans</i> , and <i>H. terminalis</i>

^a According to Pedersen (1984) and Fletcher (1987) *M. claviformis* is probably *A. fistulosus* and not *M. claviformis* as suggested by Loiseaux (1969).

^b Only a few scattered PS were produced at 5°C under long day conditions.

^c Erect blades produced at 20°C only under short day conditions.

^d The author only mentions the production of US in culture under *D. undulatum* discussion (pp. 23).

A., *Asperococcus*; het., heteromorphic; iso., isomorphic; M., *Myrionema*; mon., monophasic; PS, plurilocular sporangia; US, unilocular sporangia.

base is influenced by environmental conditions. Wynne (1998) suggests that *H. terminale* would have priority with the basionym *E. terminalis* Kützting. This classification has been used by Parente et al. (2000) and Guiry and Guiry (2009), but was not used by John et al. (2004) who still considered *H. terminale* and *E. terminalis* Kützting *sensu* Kuckuck as synonyms of *H. maculans*. According to John et al. (2004), *E. terminalis* Kützting is a young filamentous stage of *Spongonema tomentosum* (Hudson) Kützting, while *H. terminale* is based on *E. terminalis* Kützting *sensu* Kuckuck.

A particularly noteworthy point emerging from the present study was the occurrence of unilocular sporangia in the cultures of Azorean *Punctaria tenuissima*, which has not been reported previously. Unilocular sporangia were produced on the macrothalli at 10°C with SD conditions, and the liberated unispores developed into new microthalli in the same culture regime. *P. tenuissima* from the field has been described with both unilocular and plurilocular sporangia (e.g., Rietema and van den Hoek 1981, Lockhart 1982, Fletcher 1987), but the role of the unilocular sporangia and unispores has never been understood in this species. The results of the present study have revealed that the unilocular sporangia are capable of perpetuating the microthallus. It seems that both uni- and pluri-spores can play a similar role in the life history of *P. tenuissima*, although differences in ploidy were not investigated. Currently, there is no evidence of the life history being ploidy-driven in this species. It seems, however, that the production of macrothalli is facultative and influenced by environmental conditions (particularly temperature and day-length), rather than by differences in ploidy, as suggested previously by Fletcher (1987). In fact, in the present study, the *Punctaria*-like thallus development was observed only in 10°C/SD conditions, whilst the *Hecatonema*-like thallus was produced in both 10°C/SD and 15°C/LD conditions. These results are in agreement with those obtained previously by Rhodes (1970, Virginia, USA). The lowest mean seawater surface temperatures in the Azores are reached in February/March, being around 16.5°C since 1981 (NASA 2009). In fact, the macrothallus was initiated under regime 1 (15°C/LD) in the present cultures, but never became polystromatic. However, the lowest seawater surface temperatures may exceptionally reach 13°C (Instituto Hidrográfico 1981), and in the upper shallow pools the temperature could slightly decrease below this value, as minimum local air temperatures in winter are about 8–10°C (Instituto Hidrográfico 1981). The sporadic occurrence of the macrothallus of *P. tenuissima* in the Azores could be due to temperature responses. In fact, *P. tenuissima* has its Atlantic southernmost limit of distribution in the Azores. *P. tenuissima* was collected only in March, whereas *Hecatonema* has been reported to be present in the Azores all year round (Parente et al. 2000). These are different from the results obtained by Rietema and van den Hoek (1981, Netherlands) and Pedersen (1984, Denmark) working with (continental) European material. These authors showed that macrothallus formation was not temperature controlled. In the present study, however, isolates of *P. tenuissima* from the Azores responded differently to envi-

ronmental conditions in comparison with isolates from different geographical regions, which is in agreement with observations made by Pedersen (1984) and Fletcher (1987).

Pertinent to this discussion is the supposition of Rietema and van den Hoek (1981) and Fletcher (1987) that *Punctaria tenuissima* could be a synonym of *P. latifolia*. This was not accepted by Pedersen (1984) who compared the two species in culture and found some differences between them, for example the influence of temperature on the number of plurilocular sporangia formed in culture, the absence of fertile juvenile stages in cultures of *P. latifolia*, and the absence of unilocular sporangia produced in cultures of *P. tenuissima*. This latter difference is questioned in view of the results of the present study, where unilocular sporangia were produced on erect macrothalli under regime 1 conditions (10°C 8:16 h light-dark/SD). These results suggest that *P. tenuissima* and *P. latifolia* are conspecific and the former should be regarded as synonym of *P. latifolia*. The influence of temperature on the number of plurilocular sporangia formed and the absence of fertile juvenile stages in cultures of *P. latifolia* seem insufficient to distinguish the two species. The limited DNA sequence data that are available also supports conspecificity of *P. tenuissima*, *P. latifolia*, and *Hecatonema maculans*. A Danish isolate of *P. tenuissima* (GenBank accession number AF207816) was found to differ by only two base substitutions from a Korean isolate of *P. latifolia* (AY095322) in a stretch of 520 base pairs (bp) of the *rbcL* gene, and was found to be identical to a Danish isolate of *Hecatonema* sp. [AF055401, tentatively named *H. maculans* by Siemer (1998)]. In the complete *rbcL* (1467 bp), Danish *Hecatonema* sp. still differs by only two bp from Korean *P. latifolia*. The two bp difference (<0.14%) can be due to isolation by distance and falls well within the intraspecific variation for this gene in brown algae (Kogame et al. 1999, Cho et al. 2003, 2004, Cho and Boo 2006). *P. plantaginea* (Roth) Greville (AF055410, Greenland) differs by 28 bp (1.91%) from both *Hecatonema* sp. and *P. latifolia*.

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