

## PORTERINEMA FLUVIATILE (PORTER) WAERN (PHAEOPHYCEAE) IN THE NETHERLANDS

A. J. DOP

Vakgroep Plantensystematiek, Biologisch Laboratorium, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam

### SUMMARY

Details are given of *Porterinema fluviatile* from freshwater habitats in The Netherlands; experiments with cultured material are described and the Dutch algae are compared with previously published records. It is suggested that a freshwater record of *Apistonema pyrenigerum* from England (BELCHER 1959) and a species of *Apistonema* described from the Neusiedler See, *A. expansum* Geitler 1970, actually represent material of *Porterinema fluviatile*.

### 1. INTRODUCTION

Vegetative material of *Porterinema fluviatile* (Porter) Waern (Phaeophyceae, Ralfsiales) was first found in The Netherlands in April 1974 in the Botshol, a peat pond S. of Amsterdam (chlorinity 0.4‰, see DOP & VROMAN 1976), growing on or in several macro-algae. During the last three years it has also been collected from glass slides submerged in the pond of the Hortus Botanicus of the Free University of Amsterdam, in the Alkmaarder Meer, and in ditches in the Amsterdamse Bos; these waters are eutrophic and have a chlorinity of about 0.1‰. It was also found in samples from a ditch on the Frisian island Schiermonnikoog (chlorinity 5‰).

*P. fluviatile* has so far been recorded from habitats with salinities varying from near zero to 35.5‰ (JAASUND 1965; PORTER 1894; RAUTENBERG 1960; RHODES 1972; RIETH 1969; WAERN 1952; WEBBER 1967; WILCE et al. 1970).

### 2. MATERIALS AND METHODS

A clone of *Porterinema fluviatile* was isolated in April 1974 from a floating mass of *Rhizoclonium hieroglyphicum* in the Botshol, south of Amsterdam. Culturing media were an Erd-Schreiber solution based on Botshol water, and Wood's Hole artificial freshwater medium (STEIN 1973).

To induce reproduction, culture vessels were placed at 8 and 16°C, under light/dark cycles of 8 hours light/16 hours darkness and 16 hrs light/8 hrs darkness, light intensity being approximately 1500 Lux (fluorescent tubes).

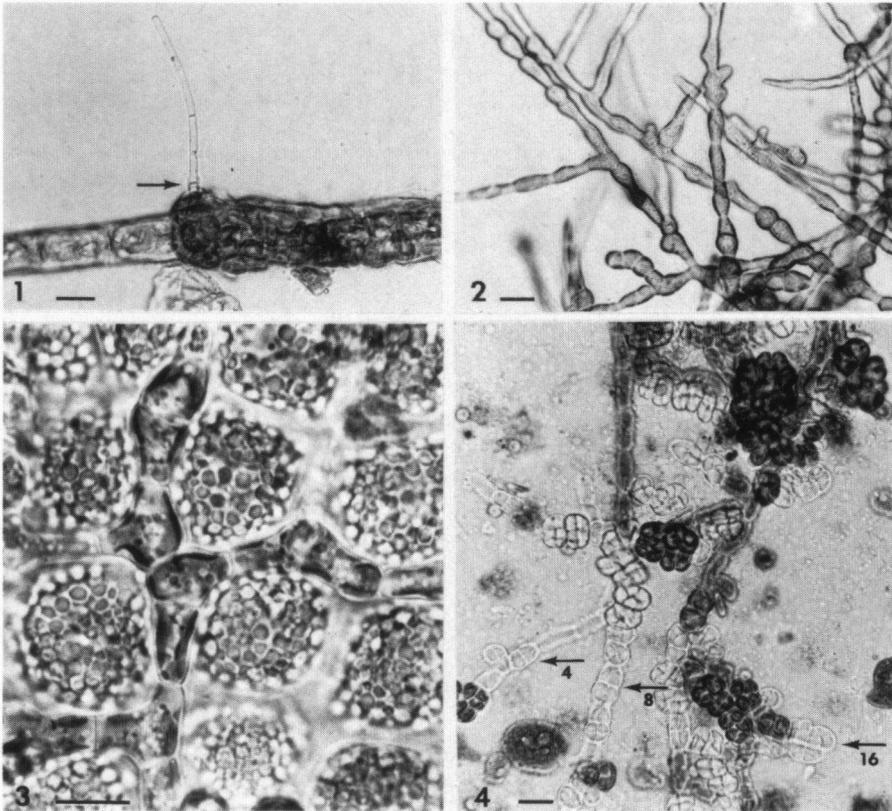
To determine the chlorinity range of the alga, culture solutions with chloride concentrations varying from 0 to 55‰ were made up by adding NaCl to Wood's Hole medium, and placed at 12°C under an L/D cycle of 12/12.

### 3. OBSERVATIONS

#### 3.1. Habit of field material

In *fig. 1* the isolated alga is shown as found in the field. It consists of creeping filaments comparable in habit to other epiphytic algae. Cell dimensions are variable: from  $14 \times 8 \mu\text{m}$  in thallus centre to  $19 \times 6,5 \mu\text{m}$  in rhizoid-like tops of filaments (see *fig. 2*). Chloroplasts are parietal, lobed, usually two in number and variably shaped.

In *fig. 3* portion of a thallus from the same location, epiphytic or endophytic on *Enteromorpha* sp., is illustrated. It shows a striking net-like growth pattern by following the cell borders of the host thallus.



Scale bar equals  $20 \mu\text{m}$ .

**Fig. 1.** The alga used in the experiments, as found in the field, epiphytic on *Rhizoclonium hieroglyphicum*. Note basal sheath of hair (arrow).

**Fig. 2.** Cultured material (16 C, L/D 18/6); free-floating thalli showing variability in cell shape and size.

**Fig. 3.** Epiphytical filaments of *Porterinema* creeping along cell borders of *Enteromorpha* sp. (field material).

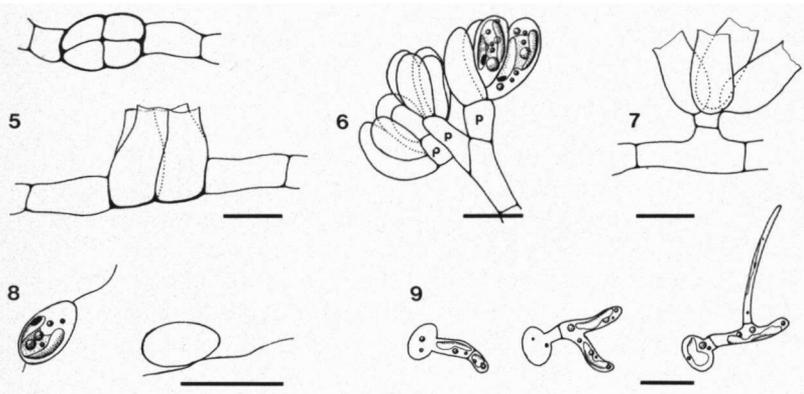
**Fig. 4.** Thallus growing on slide from ditch in the Amsterdamse Bos, consisting mainly of intercalary plurilocular sporangia. Four-, eight- and sixteen-celled sporangia are seen (arrows).

Fig. 4 shows a young thallus growing on a microscope slide submerged for two weeks in a ditch in the Amsterdamse Bos. Habit varies with age: irregularly branched creeping filaments of young thalli later form roughly circular patches. In fig. 4 filaments consist mainly of intercalary plurilocular sporangia. The basic type appears to consist of four cells, while eight- or sixteen-celled ones can also be observed, next to compact crown-shaped terminal sporangia.

Like *Pleurocladia lacustris* (found in the same localities), the present alga accumulates calcium carbonate crystals in its thallus. The two species are distinguished by sporangium shape, growth habit of mature thalli (mostly monostromatic, roughly circular in *Porterinema*; hemispherical through numerous short vertical branches in *Pleurocladia*) and cell size (larger in *Pleurocladia*: 19–29 × 10–13 µm).

3.2. Influence of photoperiod on reproduction and thallus form  
After two weeks, 5–20 young thalli were found in all cultures, presumably due to release of zoids upon environmental shock when being transferred. Large-scale sporangium formation started after four weeks in the L/D 16/8 cultures at 16 °C and after two more weeks at 16 °C, L/D 8/16. At 8 °C, cultures followed suit in the same order.

Sporangia were mostly of the intercalary, four-celled type (rarely eight-celled). In fig. 5 top and side view of a four-celled plurilocular sporangium are shown. In the centre of older thalli, globular masses of terminal sporangia were found (see



Scale bar equals 10 µm.

Fig. 5. Top and side view of an empty intercalary sporangium. Note (as in fig. 4) that zoidocysts are closely connected.

Fig. 6. Part of a stand of crown-shaped terminal sporangia (cultured material, 16 °C, L/D 18/6) showing three clusters of four zoidocysts on pedicel (p). Cell contents (single chloroplast with stigma; lipid globules) are shown in two zoidocysts.

Fig. 7. Intercalary crown-shaped sporangium, empty. Note, as in fig. 6, that zoidocysts are connected in the lower half only.

Fig. 8. Top and side view of zoid, showing oval shape, lateral flagellum insertion, chloroplast with stigma and lipid globules.

Fig. 9. Young germlings (cultured material, L/D 18/6); note lateral germination. Zoid mostly loses its cell content; wall remains visible.

fig. 6). Only once a pedicellate, loosely built four-celled sporangium was found (see fig. 7).

Issuing zoids are pear-shaped with flagella inserted laterally towards the truncate anterior end. The long flagellum is directed anteriorly; the short one backwards, close to the cell body, and is seen to emerge from under the zoid when viewed dorsally (fig. 8). Zoids swim alternately in circles and in a straight line. After settling on the substratum with a vibrating movement, the flagella are retracted.

Zoids germinate directly to form *Porterinema* thalli again (fig. 9); young thalli in zoid-producing cultures consisted largely of plurilocular sporangia.

Mature thalli under long day conditions are more lax and feather-shaped than under short photoperiod; hair formation is stimulated by long photoperiod.

### 3.3. Influence of chlorinity on growth and reproduction

A qualitative assessment is given in table 1. Hair formation is negatively influenced by high  $\text{Cl}^-$  concentrations; thallus form varies from lax and feather-shaped at low concentrations to compact and disc-like with clumped palmelloid

Table 1. Qualitative assessment of influence of chlorinity on hair formation, growth and zoid production.

$\text{‰ Cl}^-$	0	0.3	0.6	2	4	8	12	16	22	30	55
hair formation	+	+	+	+	+	-	-	-	-	-	-
growth	+++	+++	+++	++	++	+	+	+	+	+	+
zoid production	+	++	+++	+++	++	+	+	+	+	+	+

explanation of symbols:

+++ = abundant; ++ = moderate; + = scarce; - = none.

centre from  $16\text{‰ Cl}^-$  upwards. At highest concentration tested ( $55\text{‰}$ ) growth was extremely slow and stunted, thalli consisting of a shapeless clump of cells, no hairs being formed. Only a few young thalli were observed apart from the inoculated thallus; after two months these still proved viable upon transferring to lower chloride concentrations. Zoids were produced at all concentrations, the optimum being at 0.6 and  $2\text{‰ Cl}^-$ .

## 4. DISCUSSION

### 4.1. Morphology and distribution

The structure of the plurilocular sporangia is comparable to that reported by earlier authors. No correlation was found between environmental factors and formation of a given sporangium type. The two types of sporangia are rather different. The intercalary type is formed by partition, the original cell wall surrounding the whole sporangium; the crown-shaped type seems to be the result of cleavage, resulting in four loosely connected zoidocysts on a common stalk (see figs. 6, 7).

Unilocular sporangia, whose presence in the field has been reported by WAERN (1952) and WILCE et al. (1970), were neither present in our field collections, nor were they produced in culture.

Judging by the photoperiod experiments, sporangium formation is induced after a sufficient amount of light energy is absorbed. Photoperiod does influence thallus form, as does chloride concentration of the culturing medium.

The chlorinity-range experiments show our clone of *Porterinema fluviatile* to have a broadly euryhaline character; this is consistent with the field observations published (see table 2).

Until now, *P. fluviatile* has been found in Germany, on the Baltic and North Norwegian coast and on the North American Atlantic coast. A distribution map is presented in WILCE et al. (1970), to which the records of RAUTENBERG (1960), RIETH (1969), RHODES (1972) and those presented in this paper should be added.

#### 4.2. Other records, attributable to *Porterinema fluviatile*

BELCHER (1959) mentions a find of *Apistonema pyrenigerum* Pascher, epiphytic on *Cladophora* sp. in a gravel pit at Chestnut in the Lee valley, England, and compares it with PASCHER's (1939) original description and that given by WAERN (1952).

The alga described by Belcher differs from these descriptions in possessing long cellular hairs. His illustration (see fig. 10) shows remarkable similarity to drawings of *Porterinema fluviatile* as presented by WAERN (1952, p. 137, fig. B), WILCE et al. (1970, p. 131, fig. 4e), RHODES (1972, p. 118, figs. 4, 10) and RIETH (1969, fig. 2a-c). These authors show endogenous cellular hairs, however (as does RAUTENBERG 1960), which are not apparent in Belcher's illustration. The typically short cells of the hair base and the remaining protoplasmic contents of one long cell are clearly visible in his drawing however. The hair sheaths are not always easily distinguished; in our cultures they are visible in only  $\pm$  sixty percent of all hair bases examined. In all other cases they are either absent or so closely appressed as to go unnoticed. The failure to observe this distinctly Phaeophycean character may account for Belcher's opinion of dealing with a "Chrysotrichalean" alga. In the absence of sporangia and endogenous cellular hairs there is no easy way of distinguishing a filamentous Chrysophyte and a primitive Phaeophyte at the light microscopical level. For comparison of characters of Belcher's alga with reported records of *Porterinema fluviatile*, see table 2. The large pyrenoids mentioned by Belcher but not observed by others may have been the one or more strongly refringent vesicles present in most cells and illustrated in figures and photographs of most authors.

Another case where *P. fluviatile* may be concerned is Geitler's (1970) description of an alga from the Neusiedler See (salinity 1-3‰) in Austria. It was a branched epiphytic filamentous alga (growing on *Cladophora* sp.), and described as a new species of *Apistonema*: *A. expansum*.

Its creeping filaments are free or pseudoparenchymatically appressed; cells are extremely variable in dimensions, being broadly barrel-shaped to long and cylindrical. When cultured on agar, it grew for weeks and produced sporangia.

Table 2. Summary of data given by previous authors and present communication.

Author	Thallus shape	Cell dimensions	Chloroplasts	Cellular hairs	Salinity	Zoidangia	Zoids
Belcher 1959	Creeping filaments	7 × 15 µm	Mostly two, golden yellow	Multicellular, up to 200 µm long	Freshwater	-	-
Geitler 1970	Creeping filaments	6-19 µm diam	Two, parietal disc-shaped, incised in the middle	Multicellular, up to 66 µm long, 3-3.5 µm diam	1-3‰	In groups of four; cell diam. 4-4.5 µm	-
Jaasund 1965	Creeping endophytic filaments	4-6 µm diam; up to 4 times as long	-	Multicellular, with long sheath; 4.5-5.5 µm diam	Marine	Crown-like or intercalary, the latter 8-8.5 µm broad. Cells 4-5 µm diam	-
Porter 1894	Creeping filaments, free to almost pseudoparenchymatous	5-8 µm diam	One or several plates	-	0.3-8‰	Four-celled, 10-18 µm broad	-
Rautenberg 1960	Discoid	-	One to three	Present in large numbers	8.3‰	Intercalary, sessile or pedicellate; cells 5-7 × 7-12 µm; four-celled	-
Rhodes 1972	-	8-10 × 8-30 µm	Two or three lobed parietal discs	Multicellular, sheathed, 6 µm diam	29.8‰	Terminally clustered on few-celled lateral branches with a tendency to cluster in tetrads	8 × 10 µm, with two unequal laterally inserted flagella and a single parietal chloroplast with eyespot
Rieth 1969	Disc-shaped in culture	6-8 × 55 µm at periphery; 6-9 × 21 µm in thallus centre	One, mostly two	Multicellular with basal sheath, 3-5 µm diam. and up to 1600 µm long	Less than 0.5‰	Intercalary, four-celled or terminal, crown-shaped and multicellular	Ellipsoid with two unequal apically inserted flagella, one parietal plate-shaped chloroplast with stigma; 5-7 × 8-13 µm

Waern 1952	Irregularly branched or disc-shaped	3.75–10 µm diam	One or two, parietal, disc-shaped	Multicellular, sheathed, 3.75–8.75 µm diam	0.16–1.7‰	Mostly four-celled; intercalary, sessile or pedicellate in branched, crownlike system; cells 6–7.5 × 10.4 µm	6.25 × 8 µm broad, with one chloroplast
Webber 1969	Thin brown crust, filamentous	6.2–9.9 µm diam	One, platelike	Sheathed, sparingly present	0–0.5‰	Four-parted, intercalary, sessile or pedicellate.	—
Wilce et al. 1970	Crust of prostrate, loosely anastomosing and highly branched filaments with few-celled upright branches	6–10 µm diam.	Single, platelike	Sheathed, sparingly present	Less than 5‰	Four-parted, intercalary, sessile or pedicellate; cells 6–8 µm diam.	Laterally biflagellate
Present comm.	Field material: irregularly branched creeping filaments Cultured material: irregularly branched to disc-shaped plates	8 × 14–6.5 × 19 µm	Two, parietal, lobed and variously shaped	Multicellular, sheathed. Presence dependent upon environmental factors	0.1–5‰	Mostly four-celled (8 and 16 observed), intercalary, sessile or crown-shaped in branched stands; cells 4–7 × 10–14 µm	Pear-shaped with laterally inserted flagella and one parietal, plate-like chloroplast with stigma. 6.5 × 8 µm

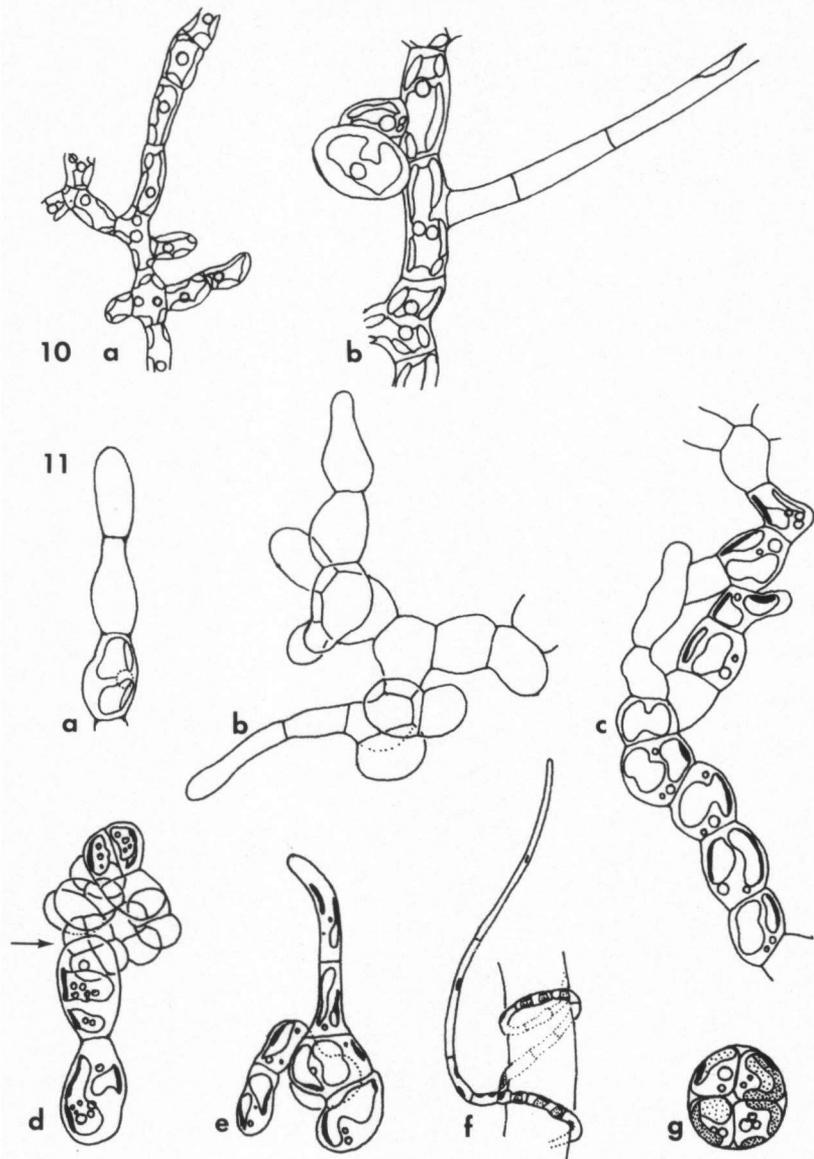


Fig. 10. Illustrations of Belcher's record of *Apistonema pyrenigerum* (after BELCHER 1959). a-filaments of *Apistonema* creeping on the cell wall of *Cladophora*. b-cells of *Apistonema* with hair on *Cladophora* with *Cocconeis*.

Fig. 11. *Apistonema expansum*, after GEITLER 1970.

a-top of filament, normally developed. b-thallus fragment. c-intertwining filaments of two thalli. d-thallus fragment with groups of cells developing zoids. e-young thallus. f-low-power view of hair-like outgrowth of filament creeping on *Cladophora*. g-enlarged view of group of four cells with zoid in each cell. After live material.

Note similarity with our figs. 2, 3 and 4, and shape of sporangia in fig. d (arrow).

Zoids were not observed; no pyrenoids were present. Long cellular hairs are formed.

In his excellent illustrations, Geitler shows variability in cell shape, the hairs, chloroplast shape and sporangium formation (see *fig. 11*). The striking resemblance to earlier published reports of *Porterinema fluviatile* is apparent. The illustrated sporangium is a top view of an intercalary plurilocular structure.

Sheathed hair bases were not observed by Geitler.

For comparison of characters, see *table 2*.

Considering the likeness in habit, dimensions, chloroplast shape and cellular hairs (except for the unobserved basal sheaths) of the aforementioned material to *Porterinema fluviatile*, and taking into account the broadly euryhaline character of the latter, it is highly probable that both Belcher and Geitler collected specimens of *Porterinema fluviatile*.

It is suggested that BELCHER's (1959) record of *Apistonema pyrenigerum* applies to *Porterinema fluviatile*. Its range would thus be extended to freshwater habitat in England; quite probably it can be found in coastal areas in the British Isles as well.

As for *Apistonema expansum* Geitler, prof. Geitler kindly informed the author that his *fig. 2* (GEITLER 1970, p. 22) is to be considered the iconotype. On the evidence presented here, it is proposed that *Apistonema expansum* Geitler be treated as a synonym of *Porterinema fluviatile* (Porter) Waern.

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