# The Occurrence of the Thermophilic Blue-green Alga, Mastigocladus laminosus, on Surtsey in 1970

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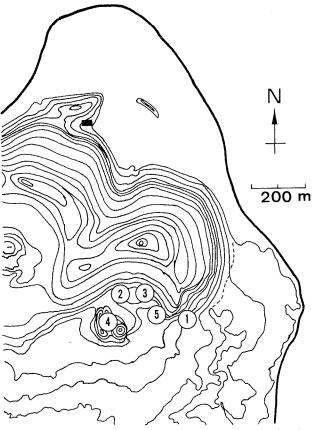
#### INTRODUCTION

Although several species of blue-green algae and other algae have been reported from moist areas surrounding steam vents on Surtsey, none of these appear to be true thermophiles exhibiting growth optima above 45°C (Schwabe, 1970a, 1970b; Behre & Schwabe, 1969). The volcanic island of Surtsey was first formed in late 1963 off the south coast of Iceland.

Mastigocladus laminosus (Ag.) Cohn is a cosmopolitan thermophilic blue-green alga (Schwabe, 1960) and its various forms are very common in almost all of the hot springs and steam vents of Iceland which have a neutral to alkaline pH and are below a temperature of about 63-64°C (Castenholz, 1969a). Two very distinct forms are common in Icelandic hot water. One is a seldombranching trichome which grows at temperatures constantly as high as 63-64°C, thus representing the upper limit photosynthetic blue-green alga of Iceland. The other is the typical branching form which does not grow above about 57°C (Castenholz, 1969a).

On June 26, 1970 I examined several of the steam vents of Surtsey, particularly those near the old (or easternmost) crater. From these I collected presumptive algal specimens in the moist ash which recorded temperatures as high as 65°C. The greatest abundance of steam vents occurred on the inner (southern) slopes of the old crater, on the lava flats on the southeastern side of the crater, and within the north-south fissure created during the 1966-67 eruptions. The major collection stations are numbered on Fig. 1. Almost all collections were examined in the field with Fig. 1. Map of the northeastern portion of Surtsey indicating a Cooke-McArthur field microscope fitted with collection stations in the eastern crater region.

a phase contrast system with 400x total magnification. Blue-green algal trichomes were quite abundant in some of the collections, particularly Station 2, and most of the material from the thermal collections looked like the high-temperature form of Mastigocladus. The purpose of this short study was to prove that this material was indeed Mastigocladus and that it was capable of growth at various temperatures above 45°C



and even at 60°C. For this, culture isolations and growth experiments in the laboratory were carried out.

Some of the hot moist areas around steam vents were a faint green color due to the density of blue-green algae. Mosses and other algae were also restricted to moist areas near steam vents but at lower temperatures and in more protected areas such as the fissure of the 1966–67 eruptions (Station 4, Fig.1).

## MATERIALS AND METHODS

The collections were made and treated as follows. Sterile vials were used throughout and forceps were resterilized between collections in the high temperature steam of the vents (> 90°C). The samples containing algal material and moist ash were stored in darkness at between 15° and 25°C for approximately 12 days before culture enrichment and isolation techniques were applied. These conditions of storage have proved quite satisfactory for thermophilic blue-green algae in past work (see Castenholz, 1969b, 1970). Duplicates of some of the samples were preserved with formalin on the collection day.

The culture techniques used for the samples in my laboratory at the University of Oregon were those standardly used for the isolation of *Mastigocladus* (Castenholz, 1969a, 1969b, 1970) except that medium free of combined nitrogen was not required in these cases for the initial enrichment of the organism. The high-temperature forms were sought for, in particular, by the methods used.

- (1). Tubes and fasks containing medium D were inoculated with samples from 7 of the collections and incubated at  $60^{\circ}$ C under approximately 2,000 lux (coolwhite fluorescent lamps) continuous light. Similar inoculations were made of all the collections in liquid medium D with incubations at  $45^{\circ}$ C. Some were treated similarly at  $30^{\circ}$ C.
- (2). Inocula from almost all collections and enrichment cultures were also placed or streaked on medium D solidified with 1.5% (w/v) agar in 20x150 mm plastic petri plates. Incubations were primarily at  $45^{\circ}$ C, but some plates were also held at  $60^{\circ}$ C or  $30^{\circ}$ C. Individual trichomes which subsequently radiated from inoculum as motile hormogonia or by growth processes were then easily isolated with watch-maker's forceps on small pieces of agar. These were transferred to liquid medium D to establish clone cultures.

The decisions as to what forms of *Mastigo-cladus* were present at the various sites on Surtsey

are based primarily on the culture isolations rather than the fresh or preserved collections of ash. The taxonomy of blue-green algae (including Mastigocladus) is currently in a confused state, principally because it has traditionally been based solely on morphology, a highly variable criterion in some groups. Thus, clonal culture should be used whenever possible together with collections.

# COLLECTIONS (FIG. 1)

- S-1: steam vent on flat ground; ash temperature 58–60°C at lip of orifice; slightly green cover unbranched *Mastigocladus*-like trichomes in clusters on ash particles.
- S-2: crescent-shape ridge on crater slopes with vents on and under crest; a = east vent,  $40 50^{\circ}\text{C}$  (collection site); b = center vents,  $35 50^{\circ}\text{C}$ , pH 7.8 (determined by W. Doemel), slightly green cover of unbranched *Mastigocladus*-like trichomes; c = west vents,  $45 55^{\circ}\text{C}$  and  $15 20^{\circ}\text{C}$ .
- S-3: on crater slopes, several vents, 45–65°C; no algae found in collection.
- S-4: large north-south oriented fissure and crater from 1966–67 eruptions; many steam vents at base and on walls of fissure; a = steamy area on top of north end,  $< 20^{\circ}\text{C}$ , visible moss cover; b = near top of north end,  $20 25^{\circ}\text{C}$ , crevices in rock with moss and algal cover; c = wet wall below steam vents near base of fissure,  $ca. 55^{\circ}\text{C}$ , no visible algal cover, but unbranched Mastigocladus-like trichomes in clusters on same ash particles.
- S-5: several steam vents on flat lava flow below inner wall of crater;  $a=45-55^{\circ}\text{C}$ , unbranched Mastigocladus-like trichomes and more typical branching Mastigocladus also on ash particles;  $b=55-65^{\circ}\text{C}$ , no algae found on particles.

#### **CULTURES**

The results of the original culture enrichments in liquid medium D at  $30^{\circ}$ ,  $45^{\circ}$ , and  $60^{\circ}$  are summarized in Table 1. Most collections incubated at  $45^{\circ}$ C gave rise to Mastigocladus but only the unbranched form. Two of the collections of material made below  $25^{\circ}$ C did not. Almost all of the collections which gave rise to Mastigocladus at  $45^{\circ}$ C also did so when incubated at  $60^{\circ}$ C. This again was the unbranched "high-temperature" form. None of the collections gave rise to this form of Mastigocladus when incubated at  $30^{\circ}$ C instead of either of the higher temperatures. Other filamentous and coccoid blue-green algae came up at  $30^{\circ}$ C in addition to unicellular and

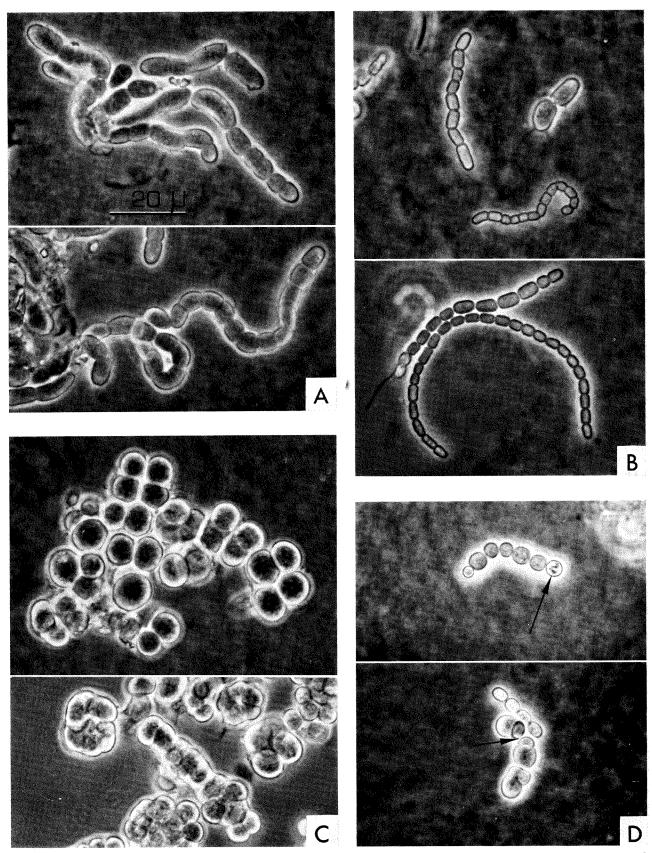


Fig. 2. Culture I-S<sub>2</sub>-m Clone 3— the non-branching form of Mastigocladus, originally isolated from a 60°C enrichment (collected from station S-2b, 35°—50°C). (A). grown in complete medium at 60°C. (B). grown in complete medium at 45°C. (C). grown in complete medium at 45°C — slow growth conditions. (D). grown in medium lacking combined nitrogen at 45°C; developing heterocysts are indicated.

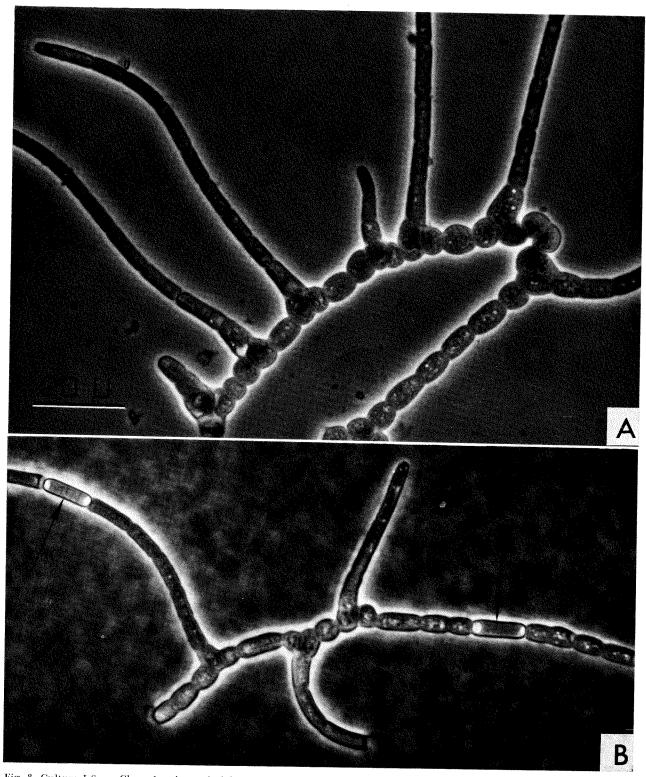


Fig. 3. Culture I-S<sub>5</sub>-m Clone 1— the typical branching form of Mastigocladus, originally isolated at 30°C (collected from station S-5a, 45—55°C). (A), grown in complete medium at 45°C. (B), grown in medium lacking combined nitrogen at 45°C; heterocysts are indicated.

TABLE 1
Enrichments of Mastigocladus (M = unbranched form; Mt = typical branched form) from samples collected at stations on Surtsey. A = other blue-green and green algae.

Station	30°C	45°C	60°C
S-1 (58-60°C)	_	M	M
S-2a (40-50°C)		$\mathbf{M}$	$\mathbf{M}$
S-2b (35-50°C)	A	M	$\mathbf{M}$
S-2c (15-20°C)	A	0	
S-2c (45-55°C)	$\mathbf{A}$	$\mathbf{M}$	$\mathbf{M}$
S-3 (45-65°C)	0	0	0
S-4a (20°C)	A	M	******
S-4b (20-25°C)	A	0	_
S-4c (55°C)	_	M	$\mathbf{M}$
S-5a (45-55°C)	Mt, A	$\mathbf{M}$	
S-5b (55-65°C)	_	M	0

colonial green algae. In one case, however (S-5a), a typical branching form of *Mastigocladus* arose at 30°C. This was later isolated and cloned. The same inoculum gave rise to the unbranched form of *Mastigocladus* at 45°C (Table 1). Many of the enrichments of *Mastigocladus* at 45° and 60°C were subsequently cloned from agar plates by manually removing single trichomes (under a dissecting microscope), then inoculating each into liquid medium in culture tubes. Although several clones of most cultures were successful only those listed in Table 2 have been retained in the culture collection.

The two principal forms of Mastigocladus laminosus occurring on Surtsey are illustrated in Fig 2 and 3. The species as interpreted by Fremy (1936) and Schwabe (1960) is highly polymorphic. However, it may be seen that even clonal cell lines are highly plastic in morphology. The growth form depends to a large degree on the environmental conditions in culture and on the age of the culture (Castenholz, 1970, and unpublished data) The complications of morphological variability in Mastigocladus laminosus have been most recently discussed by Schwabe (1960) and are also being investigated at present in my laboratory. Any further discussion now, however, would be out of place for the purposes of this report.

The unbranched form (Fig.2) grows well in culture at both 60°C and 45°C; growth is slow, however, at 30°C. The branched form (Fig 3) from Surtsey did not grow at 60°C; even at 55°C no growth was apparent. The upper growth

TABLE 2
Cultures of Mastigocladus from Surtsey presently maintained at the University of Oregon (November, 1970).

Culture	Clone	Culture Temp. °C	Collection Station	Collection Temp. °C	Notes	
I-S <sub>2</sub> -m	1	45,60	S-2c	45-55	unbranched "high-temp." form	
	2	45,60	S-2a	40-50	**	,,
	3	45,60	S-2b	35-50	,,	,,
$I-S_4-m$	1	45,60	S-4c	55	,,	**
I-S <sub>5</sub> -m	1	45	S-5a	45-55	f. typica, branching; does not grow above 53°C.	

limit in this case appears to be about 53°C. Growth also occurred at 45° and 30°C.

#### DISCUSSION

The principal result reported here is simply the occurrence of at least two genetic types of *Mastigocladus* on Surtsey, the first report of a truly thermophilic blue-green alga on this new island. The "high-temperature" form, at least, was fairly well distributed around most of the steam vents examined (Table 1). This suggests that an initial successful inoculation may have occurred a year or two earlier and that the organism has since spread to other steam vents. This appears more likely than the simultaneous inoculation of several steam vents from a fairly distant source.

At this point it would be mere speculation to suggest the manner in which Mastigocladus inoculum was first transported to Surtsey. The manner in which most micro-algae are dispersed is largely speculation, but at least there has been some work (see Maynard, 1968; Proctor et al., 1967) which indicates that both air and birds are important long distance agents. In the case of Mastigocladus laminosus and other true thermophiles on Surtsey the inoculum must have originated from mainland Iceland hot springs or steam vents and have been carried more or less directly to the new site. This "rare source" condition makes such a colonization a less frequent possibility than that involving more ubiquitously distributed mesophilic algae, many of which now occur on Surtsey (Schwabe, 1970, 1972).

There are no thermal waters or steam vents on the Westman Islands of which Surtsey is a part. The hot springs closest to Surtsey are approximately 75—90 km distant near the mouth of Ölfusá, near mid-portions of Thjórsá, and in the Torfajökull region. There is a single warm well near the southern slope of Eyjafjallajökull which is only about 50 km from Surtsey (Barth, 1950), but it is unknown whether *Mastigocladus* occurs there.

Mastigocladus laminosus is a very hardy species and can tolerate desiccation and freezing (Castenholz, 1969a, 1969b, 1970). This should allow fairly easy dissemination if a dispersing agent is present. The world-wide distribution of Mastigocladus in hot springs of alkaline to neutral pH supports the hypothesis that Mastigcaldus is one of the most easily dispersed thermophiles. Thermo-biologists and other scientists working in thermal areas can become the principal agents for dispersal of thermophilic micro-organisms if precautions are not taken, such as thorough cleaning of boots and collecting equipment. The original introduction of Mastigocladus to Surtsey by this means cannot be excluded.

#### ACKNOWLEDGEMENTS

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