

On the identity and geographical distribution of *Ammatoidea normanii* W. et G.S. West (Oscillatoriales, Cyanoprokaryota)

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Résumé – Identité et distribution géographique d'*Ammatoidea normanii* W. et G.S. West (Oscillatoriales, Cyanoprokaryota). La cyanoprocaryote filamenteuse *Ammatoidea normanii* W. et G.S. West est une espèce inhabituelle, rarement identifiée dans quelques lieux très éloignés les uns des autres dans les régions froides et tempérées du monde entier. L'espèce n'a été signalée qu'une seule fois pour l'Antarctique. Le présent travail élargit la distribution géographique dans l'Antarctique, contribue à de nouvelles observations sur la morphologie d'*A. normanii* et complète la description de l'environnement où elle habite. Sur ces bases, l'identité des mentions pour l'Antarctique et la distribution géographique dans le monde est discutée.

Cyanoprokaryota / *Ammatoidea* / distribution géographique / écologie

Abstract – The filamentous cyanoprokaryote *Ammatoidea normanii* W. et G.S. West is a rather uncommon species, recorded from a small number of distant locations around the temperate and cold areas of the world, and only once from Antarctica. This paper contributes to our knowledge of this species through a new Antarctic record, accompanied with morphological observations and a complete description of the environment in which it was found. On this basis, the identity of former probable records from Antarctica and throughout the world is discussed.

Cyanoprokaryota / *Ammatoidea* / geographical distribution / ecology

INTRODUCTION

Ammatoidea normanii W. et G.S. West is an uncommon oscillatorialean species. First described by W. & G.S. West (1897) from southern England, it has been found only in a small number of widely disjunct locations around the world. One single sample from an ecologically isolated nunatak – a mountain peak emerging from an icy plain – near the South Pole constitutes the only confirmed record of *A. normanii* from Antarctica to date (Broady & Ingerfeld, 1999).

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Anagnostidis & Komárek (1988) recognized eight species of the genus *Ammatoidea*, including *A. normanii* as the type. These are: *A. breviarticulata* Hirose et Chung, *A. multispora* Yoneda, *A. murmanica* Petrov, *A. olivacea* Yoneda, *A. simplex* Voronich., *A. singularis* Hirose et Chung and *A. yellowstonensis* Copeland. Later, Komárek & Anagnostidis (2005) added to this list *A. xizangensis* Zhu et Li, *A. sinensis* Ley and *A. aegea* Anagnostidis et Pantazidou, although they recommend a comparison of the latter two species with the genus *Homeothrix*.

Filaments of *Ammatoidea* spp. usually form tufts or mats visible to the naked eye (Komárek *et al.*, 2003) and their general aspect resembles that of *Scytonematopsis* spp. (Scytonemataceae). A key difference between these two genera is the ability of the latter to produce nitrogen-fixing heterocysts in nitrogen-poor environments. Nevertheless, Broady & Ingerfeld (1999) obtained normal specimens of *A. normanii* in nitrogen-poor cultured media. They also demonstrated that the lack of heterocysts in this population was not simply a consequence of the high concentration of bioavailable nitrogen in the environment, but a consistent, taxonomically valuable feature justifying the position of this species in the order Oscillatoriales. On the other hand, these authors questioned whether "...should an aquatic alga epiphytic on *Batrachospermum* in a pool in southern England really be assigned to the same species as specimens growing in a fissure on an exposed rock close to the South Pole". Through new records that enlarge the geographic distribution, and the discussion of the ecological conditions of this and former findings, this paper aims to contribute an answer to this question.

Study site

Cierva Point, Danco Coast, Antarctic Peninsula (Fig. 1), encompasses SSSI (Site of Special Scientific Interest) No. 15, nominated by the 'Scientific Committee on Antarctic Research' on account of its high biodiversity. As part of the management plan for this special area, the algal diversity of Cierva Point has been studied for more than a decade. Regarding freshwater environments, Tesolín *et al.* (1997) made a preliminary characterization of a number of small ponds, while Mataloni *et al.* (1998) studied the phytoplankton diversity of the main water body of this area. Izaguirre & Pizarro (2000) and Mataloni *et al.* (2005) studied the epilithic algal communities from Torrente and Pingüinera streams, respectively. Mataloni & Tesolín (1997) surveyed the cryobiontic flora. The algal flora of recently exposed mineral soils was analyzed by Mataloni *et al.* (2000), while Mataloni & Tell (2002) studied the algal communities of ornithogenic soils. A floristic survey of the islands around Cierva Cove was also performed by Mataloni & Pose (2001). To date, a total of 210 freshwater and terrestrial algal species has been reported from this area (Mataloni, unpub.)

In spite of this extensive survey, the large microenvironmental diversity characterising Cierva Point (Agraz *et al.*, 1994) suggests that additional species might be found in the area. This is the case for the population of *Ammatoidea normanii* studied in this paper.

MATERIALS AND METHODS

Samples were collected inside the area of Argentinean Primavera Station on three occasions: February 1998, April 2002 and March 2003. Visible mats of *A. normanii* were growing along cracks on the large granodiorite stones that

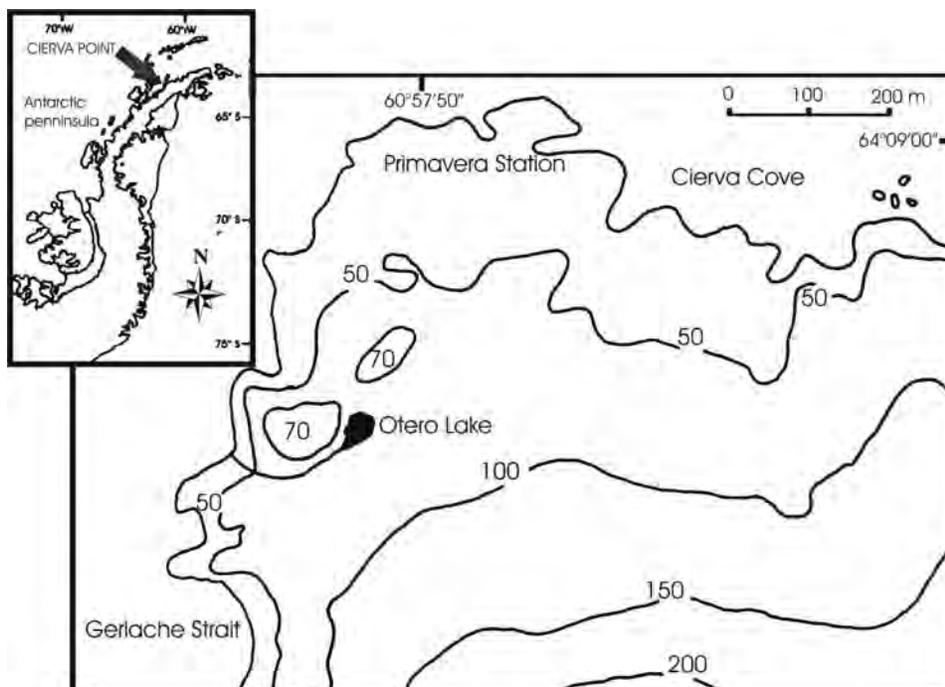


Fig. 1. Location map of Cierva Point, showing the collection site of *A. normanii*.

constitute the pathway between the helicopter landing area and the main building of the station. These rocks were constantly irrigated by the thawing of a snow field located higher above sea level. On the first occasion, an algal sample was taken by carefully brushing the rock surface. In 2002 and 2003, algae were collected together with small amounts of the running water by brushing the algae out of the rock surface and then sucking them in a sterile rubber tube connected to a 50 ml sterile syringe. This procedure also allowed us to take water samples in acid-washed PVC bottles for chemical analyses. Temperature was measured *in situ* and samples immediately carried to the Station Laboratory. After allowing them to reach room temperature, conductivity and pH were measured with electronic combined sensors. Then, samples were filtered through Whatman GF/F filters, and $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations measured using a Hach DR/890 colorimeter (Hach, USA), with the appropriate Hach reactives for each analysis. Algal specimens were observed *in vivo* with an Olympus CX-31 microscope transmitted light microscope and drawn with a *camera lucida*. Portions of samples were fixed in 1% formaldehyde and the rest maintained at 4 °C in both full strength and 1/20 dilution BG-11 liquid medium (Rippka *et al.*, 1979). Back in Buenos Aires, cultures were established under controlled conditions (10 °C, 50 $\mu\text{mol photons. m}^{-2}\text{.sec}^{-1}$, 16:8 light:dark cycle) in 1% agarised medium at both concentrations. Microphotographs of the preserved material were taken using an Olympus BX-40 with an attached Olympus Camedia digital camera.

RESULTS

Natural populations of *A. normanii* from Cierva Point consisted of clusters or tufts of filaments frequently growing prostrate with erect branches, thus forming a mat on the rock surface. The filaments were 5-11 μm wide, each containing a single isopolar trichome tapering toward both ends and encased in a mucilaginous sheath that was firm, lamellated, golden-brown to colourless, and open at both ends. Cells in the old parts of the filaments were shorter than wide, slightly constricted at the cross walls, and sometimes forming simple (Fig. 2) or geminated (Fig. 5) false branches. Younger cells at the apices were either longer than wide and slightly constricted at the cross walls (Fig. 6), or more-or-less quadrate and strongly constricted at the cross walls (Fig. 3). Some filaments showed cells with a few, large granules (Figs 2, 8). Reproduction occurred through homogonia (Figs 3, 4) formed by fragmentation of the filament due to the presence of necridia (Fig.7).

Apart from the described features, which generally coincide with the detailed observations by Broady & Ingerfeld (1999), we also observed a few filaments with another type of cells (Fig. 8), these biconvex in shape, without visible contents and with thick walls, yet being neither heterocysts nor akinetes. These cells were very rare and identical in aspect to those observed by Corte (1962) from Hope Bay, in a specimen identified as *Calothrix* sp. (Figs 9-11).

Table 1 shows values of important environmental parameters at a single location in Cierva Point where *A. normanii* was found. Very low temperatures and slightly acid pH values are typical from meltwaters, and conductivity values are within the range given for enriched Pingüinera Stream (Mataloni *et al.*, 2005). Although $\text{NH}_4\text{-N}$ concentrations are very low, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ concentrations would certainly characterize this environment as nutrient-rich.

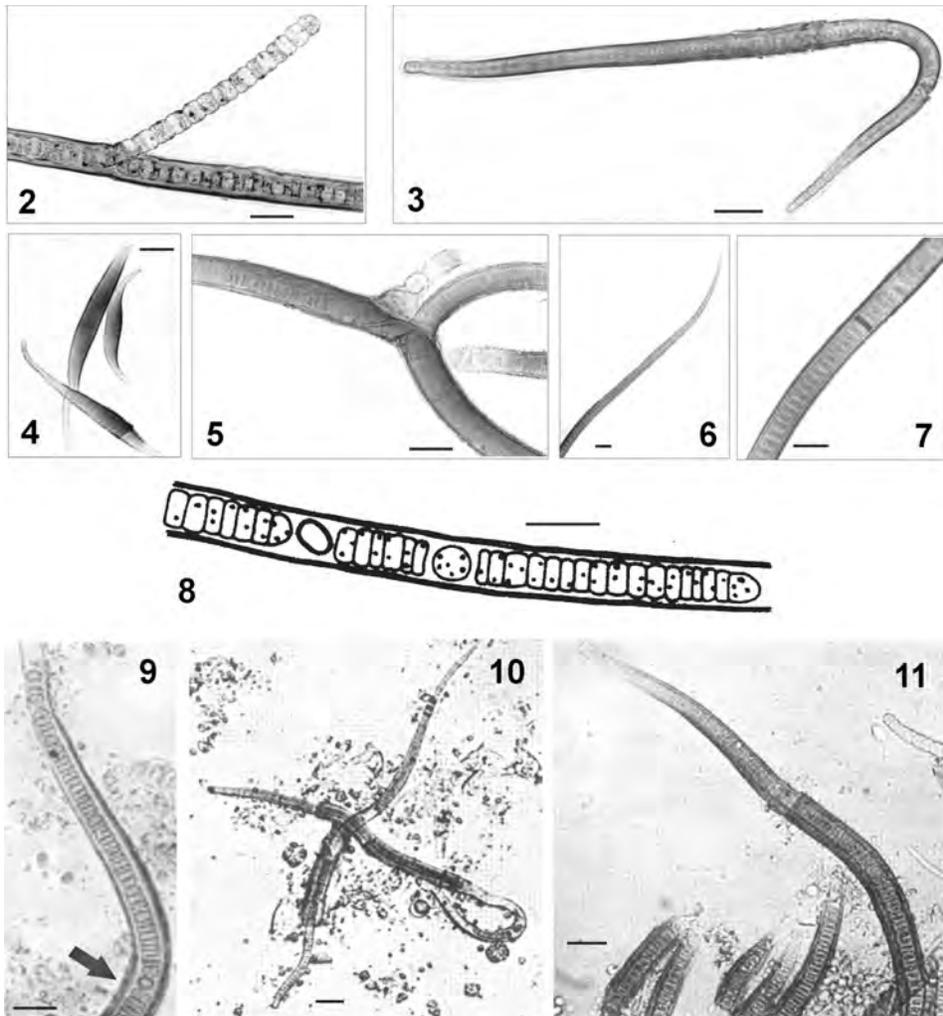
Attempts to grow cultures of *A. normanii* were unsuccessful, both in normal BG-11 medium as well as in the low concentration recommended by Broady (pers. comm.). No growth was observed in inocula consisting of single filaments, and small, fast-growing accompanying individuals of *Gloeocapsopsis aurea* Mataloni *et* Komárek and *Chamaesiphon subglobosus* (Rostaf.) Lemm. (Chroococcales) invariably took over in larger inocula.

DISCUSSION

Like at Punta Cierva, mat-forming specimens of *A. normanii* recorded by Broady & Weinstein (1998) were accompanied by a chroococcalean cyanobacterium, in this case *Gloeocapsa* sp., and the chlorophyte *Stichococcus bacillaris*

Table 1. Values of key environmental features at the collection site of *A. normanii*, Cierva Point, in two consecutive field seasons.

Season	2002	2003
Temperature ($^{\circ}\text{C}$)	0.2	0
pH	5.27	6.08
Conductivity ($\mu\text{S/cm}$)	157	156
$\text{PO}_4\text{-P}$ (mg/l)	> 2.5	12.8
$\text{NO}_3\text{-N}$ (mg/l)	1.42	5.8
$\text{NH}_4\text{-N}$ (mg/l)	0.04	< 0.01



Figs 2-8. Filaments of *A. normanii* from Cierva Point. **2.** Single false branch. **3-4.** Hormogonia. **5.** Geminated false branch. **6.** Terminal portion of a filament. **7.** Central portion of a filament showing a necridic cell. **8.** Filament with a thick-walled cell.

Figs 9-11. Filaments of *A. normanii* from Hope Bay (after Corte, 1962). The arrow in Fig. 9 shows a thick-walled cell identical to that of Fig. 8. All scales = 10 μm .

Nägeli. The only difference between the specimens of *A. normanii* studied by these authors and our material is the rare presence of the thick-walled cells depicted in Fig. 8. While their function is yet to be investigated, their position in the filament and the subsequent separation of terminal portions of the trichome as hormogonia (Fig. 8) suggest that they could act as necridia.

Specimens described by Corte (1962) under *Calothrix* sp. from Hope Bay are identical with ours. Corte (1962) interpreted the only thick-walled cell he found in the entire sample (Fig. 9) as a heterocyst. He therefore misidentified his specimens as the *Calothrix* sp. recorded by West & West (1911) from Cape Royds,

Ross Is., Continental Antarctica. Nevertheless, drawings of the latter taxon clearly show heteropolar filaments with basal heterocysts, while both young and adult stages of Corte's specimens taper toward both ends. We therefore identify specimens from Hope Bay as *A. normanii*, taking into account that the habit and environmental features of his records (among thalli and filaments of *Prasiola* sp. in the littoral of Boeckella and Esperanza lakes, temperature = 0.5 - 1 °C, pH = 6.6 - 6.8) agree with those recorded by us at Cierva Point. Also, Fermani & Mataloni (2004) found filaments of *A. normanii* on one wet soil sample from Deception Is., South Shetland Islands. These were interspersed with *Leptolyngbya fragilis* (Gom.) Anagn. et Kom., *L. frigida* (Fritsch) Anagn. et Kom., *Nodularia harveyana* Thur., *Oscillatoria amphibia* Ag. and *Phormidium murrayi* (W. et G.S. West) Anagn. et Kom., forming a thick cyanobacterial mat.

The co-occurrence of *Ammatoidea* and *Homeothrix* species in some Antarctic environments, together with the similar aspect of their thalli, led to some confusion in identification of specimens (Broady & Ingerfeld, 1999). Still, both genera can be distinguished on the basis of the isopolar growth of *Ammatoidea* as opposed to the heteropolar filaments of *Homeothrix*.

The question posed by Broady & Ingerfeld (1999) regarding the taxonomic identity of specimens growing in such distant locations and different habitats as a shallow pool in England and a fissure on a rock surface near the South Pole deserves close attention in the context of the modern definition of cyanobacterial species proposed by Komárek *et al.* (2003), which would include

Table 2. Comparison of morphological features of *A. normanii* populations from Europe (Komárek & Anagnostidis, 2005), La Gorce Mts. (86°30'S) (Broady & Ingerfeld, 1999) and Antarctic Peninsula (this study).

Features	Komárek & Anagnostidis, 2005	Broady & Ingerfeld, 1999	this study
Thallus	brush-like	?	brush-like
Filament shape	coiled and bent in the middle	bent into a hook shape	coiled or hooked at both ends
Filament width (µm)	5.4 to 13.7	up to 11	5 to 11
Sheath	colourless to yellow-brown, thin or thick, lamellated in the central part	colourless to dark golden-brown, lamellated, firm, up to 5 µm thick	colourless to golden-brown, firm, lamellated, open at both ends
False branching	common	rare	not very common
Trichome width	3.5 to 7.5 (10)	up to 6	up to 6.5
Constrictions at cross-walls	central part: slightly constricted apices: not constricted	slightly constricted throughout	central parts: slightly constricted apices: slightly to strongly constricted
Cell shape	central part: isodiametric or shorter than wide apices: up to 6 × longer than wide	central part: quadrate to discoid apices: generally longer than wide	central part: quadrate to shorter than wide apices: longer than wide
Granules	?	few, large, in some filaments	few, large, in some filaments

Table 3. Previous records of *A. normanii* from cold and temperate areas of the world, probable Antarctic records according to Broady & Ingerfeld (1999), and new Antarctic records confirmed in this study. The location and habit of the species are specified in every case.

<i>Previous worldwide records Reference</i>	<i>Location</i>	<i>Habit</i>
W & GS West, 1897	Dartmoor, Devonshire, England	small tufts epiphytic on <i>Batrachospermum</i> sp. in a bog
Lemmermann, 1907	Chatham Is., New Zealand	epilithic or epiphytic, in running or stagnant waters (in Geitler, 1932)
Bachmann, 1921	West Greenland	epiphytic on <i>Rhizoclonium</i> sp. in a stream
Starmach, 1927	Tatra Mts., Poland	fixed on littoral rocks from mountain lakes, together with other algae and mosses
Copeland, 1936	Yellowstone, USA	epiphytic on other algae, not in thermal springs
Smith 1950	Yellowstone, USA	epiphytic on plants or mucilaginous algae
Guerrero, 1949	Sierra do Gerés, Portugal	epiphytic on <i>Batrachospermum</i> sp. in a mountain river
Golubic, 1966	Titisee, Schwarzwald, Germany	well-developed tufts fixed on littoral rocks
Cocke, 1967	North Carolina, USA	in a shallow pool at the base of a drinking fountain
Bourrelly, 1970	Prims, Saarland, Germany	fixed on littoral rocks, epiphytic, on the sheath of <i>Batrachospermum</i> sp.
Starmach, 1973	Tatra Mts., Poland	littoral of ponds with water level variations
Kawecka, 1980	Tatra Mts., Poland	epilithic among other algae in a cold mountain stream
Broady & Ingerfeld, 1999	La Gorce Mountains, Antarctica	thin black mat in a rock fissure receiving percolating water
<i>Probable Antarctic records according to Broady & Ingerfeld, 1999 - Reference</i>	<i>Location</i>	<i>Habit</i>
Broady, 1981 (as <i>Homeothrix</i> sp.)	Vestfold Hills	black epilithic incrustation wetted by trickling water
Broady, 1989 (as <i>Homeothrix</i> cf. <i>rivularis</i>)	Edward VII Peninsula	black epilithic incrustation wetted by trickling water, dark brown wefts amongst mineral material in a shallow lake
Ling & Seppelt, 1998 (as <i>Hammatoidea</i> sp.)	Windmill Is.	Aquatic in a small shallow tarn
<i>New confirmed Antarctic records - Reference</i>	<i>Location</i>	<i>Habit</i>
Corte, 1962	Hope Bay, Antarctic Peninsula	filaments among <i>Prasiola</i> sp. in the littoral of lakes
Fermani & Mataloni, 2004 this study	Deception Is., South Shetlands Cierva Point, Antarctic Peninsula	cyanobacterial mat on wet soil black epilithic mat irrigated by meltwater

populations belonging to the same genotype (genus), with stable and recognizable phenotypic features, and identical ecological demands. Comparisons of the main morphological features of European populations with those of well-studied Antarctic ones (Table 2) show similarities in the shape of thalli, filaments and cells. On the other hand, filaments of *A. normanii* from Antarctic natural populations have fewer false branches, are rather thinner, and some cells possess large granules. Nevertheless, as Broady & Ingerfeld (1999) point out, branching can change according to culturing conditions.

Table 3 lists a number of locations encompassed in a wide distribution area along temperate and cold areas of the world. Nevertheless, Komárek (2000) states that the ubiquitous character has never been demonstrated in any cyanobacterial species, calling the attention to the fact that even many of the so-called “cosmopolitan taxa” are in fact widely distributed but always in given, ecologically similar locations.

This ecological restriction holds true for *A. normanii* as well as for most algal groups. As Mataloni (1997) demonstrated, the algal flora of peat bogs from Tierra del Fuego is more closely related to that of Canadian and European peat bogs than to the flora from surrounding lakes. The problem is: are the environments listed in Table 2 similar enough to guarantee that all these similar morphotypes from different populations belong to the same species? A closer look shows that recorded specimens are always mat forming/epilithic/epiphytic (but never free-living), and live in wet soils, irrigated stones or the littoral of water bodies from temperate to cold areas of the world. There is therefore a common trait between these environments. It is also worth noting that the environment sampled by Broady & Weinstein (1998) received water from melting ice while in full sun, even when air temperature was as low as -16°C . This stresses once more the importance of the high microenvironmental variability in Antarctica, which allows for unexpectedly favorable conditions in small areas. This results in an enhancement of the local microbial diversity (Mataloni & Pose, 2001) and the subsequent widening of biogeographical distribution areas.

In the case of *A. normanii*, as well as in other cyanobacteria, conclusive evidence on the identity of all different strains can only be attained through a multidisciplinary approach which, as Komárek *et al.* (2003) rightly state, will require the integration of traditional taxonomy, ecology and molecular biology, and it is toward this end that we wish to contribute with this study.

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