#### SHORT NOTE



# New confirmed depth limit of Antarctic macroalgae: *Palmaria decipiens* found at 100 m depth in the Southern Ocean

Ben Jamie Owen Robinson<sup>1</sup> · Simon A. Morley<sup>1</sup> · Anastasia Rizouli<sup>2</sup> · Joanne Sarantopoulou<sup>2</sup> · George A. Gkafas<sup>2</sup> · Athanasios Exadactylos<sup>2</sup> · Frithjof C. Küpper<sup>3,4,5</sup>

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#### Abstract

Living specimens of the macroalga *Palmaria decipiens* were collected from 100 m depth, representing a new confirmed depth record, considerably exceeding the previous record of 42 m depth. Previous deeper collections (below conventional SCUBA depths) have relied on dredge/grab samples or drop camera surveys. Remote techniques cannot conclusively prove that macroalgae are living at these depths, as algae detach from shallower substrata, e.g., through ice scouring, and drift to depths below their growth limit. This, combined with a low rate of decay of macroalgae around Antarctica, requires validation that algal samples from depth have grown in situ. Estimates of macroalgal biomass, energy fluxes, and the potential energy fixation may need adjusting to consider the deeper growing depths particularly with glacial retreat along the Antarctic Peninsula revealing areas of rocky substrata for macroalgal colonisation. The confirmed extension of depth where macroalgae can grow will have implications for assessments of benthic productivity and food webs in Antarctica.

Keywords Macrophytobenthos · Molecular phylogeny · Rhodophyta · ROV · Depth limit · Algae · Benthos

# Introduction

Antarctic macroalgae have their highest diversity and biomass along the Western Antarctic Peninsula (Wiencke & Amsler 2012), with multiple authors reporting macroalgal communities in the region (Moe & De Laca 1976; Mystikou et al. 2014; Wiencke et al., 2014). Reports relying on direct collection and observation, using SCUBA diving, are limited in depth range to ~ 30–40 m deep (Mystikou et al. 2014). The

Ben Jamie Owen Robinson benrobinson@live.co.uk

- <sup>1</sup> British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, UK
- <sup>2</sup> Department of Ichthyology & Aquatic Science, University of Thessaly, Fytokou street, 38 446 Volos, Greece
- <sup>3</sup> School of Biological Sciences, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK
- <sup>4</sup> Department of Chemistry, Marine Biodiscovery Centre, University of Aberdeen, Aberdeen AB24 3UE, Scotland, UK
- <sup>5</sup> Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182-1030, USA

deepest direct macroalgal collection to date has been from 42 m (Delépine et al. 1966). Below these depths macroalgal research has relied on dredge collection and indirect observation such as by Zielinski (1990), who reported the collection of *Desmarestia anceps* and *Himantothallus grandifolius* at depths between 90 and 100 m.

One of the few video observations of algae growing in deeper waters comes from an ROV recording at 70 m depth (Wiencke et al. 2014). Other observations, using dredge and grab samples (Cormaci etal. 2000), found the encrusting red alga Phymatolithon foecundum (species complex, Amsler et al. 1995) at 70 m depth. This agrees with previous work on minimum light requirements for Antarctic algae which postulates a physiological limit, which is deeper than 200 m (Wiencke, 1990). Antarctic macroalgae are known to grow at depths of 40 m, but with the potential for deeper growth. This expectation is due to their highly shade-adapted nature (Gómez et al. 2009; Wiencke & Amsler 2012), which allows growth and survival with only half the annual cumulative light exposure of equivalent temperate species (Runcie & Riddle 2006). At these high latitudes sunlight is strongly seasonal, however even during summer (Bischof et al. 2006), when there is 24 h of daylight, sea-ice and the phytoplankton

bloom can restrict light from reaching the benthos (Clark et al. 2013; Venables & Meredith 2014; Vernet et al. 2008).

Zaneveld (1966, 1968) reported the depth distribution of multiple species, including *Desmarestia menziesii*, from dredge samples from deeper than 650 m, however he argued that such records were undoubtedly algae that had been torn loose by ice scour and drifted into deeper water. As growth at these depths was not supported by physiological data (Gómez et al. 2009; Wiencke & Amsler 2012), and the rate of decay of macroalgal fronds is so low in the cold of the Antarctic (Amsler et al. 1995; Brouwer 1996), the lack of decay does not indicate growth. This lack of decay presents a challenge when trying to establish the depth range of species, as traditional methods and observations cannot definitively determine whether algal specimens are living at deeper depths.

Palmaria decipiens is a common and endemic species in the sublittoral zone of Antarctica (Lamb & Zimmermann 1977; Lüderet al. 2002; Ricker 1988). Typically P. decipiens is a "seasonal anticipator" (Lüder et al. 2002), it develops new blades in August following circannual rhythms (Weykam & Wiencke 1996) preparing to grow and reproduce in late winter/spring (Weykam & Wiencke 1996; Wiencke, 1990; Wiencke et al. 1993). Previously collected specimens of P. decipiens were dredged from 311 m (J. Zaneveld 1968; J. S. Zaneveld 1966) but, as previously discussed, were considered to contradict theoretical depth limits and it was, therefore, concluded that they had sunk from shallow water (Wiencke, 1990). This study aims to further understand the depth range of *P. decipiens* through photographic surveys and sample collections via ROV, at 100 m depth at Adelaide Island, WAP (Western Antarctic Peninsula).

## **Materials and methods**

Samples were collected from Rothera Point, Adelaide Island, WAP (67° 34′ 50″ S, 68° 07′ 00″ W) on steep rocky slopes, adjacent to the Rothera Research Station (British Antarctic Survey), using a Deep Trekker Generation 2 Worker ROV. The ROV allowed individual specimens to be inspected closely and from multiple angles, unlike methods such as video sledges or drop cameras. Each collection dive began with an active search of 1000 m<sup>2</sup> area for any algae followed by a close-up inspection of any potential specimens. When potential specimens were found, they were manipulated using both the claw and the thrusters of the ROV, with the aim to manipulate the specimen and test whether there are attached. If it required less thrust of the ROV to remove or there was any ambiguity over the outcome, the process was repeated until it could be confirmed that the specimen was attached, or another specimen was chosen.

Initial morphological examination using the key provided by Wiencke and Clayton (2002) identified the samples as P. decipiens. Total genomic DNA was extracted using the Pure-Link<sup>TM</sup> Genomic DNA Mini Kit (Invitrogen, Waltham, MA, USA), following the manufacturers protocol. PCR fragments were amplified using primer pairs targeting the cytochrome oxidase subunit I (Cox1) gene (Saunders 2005). PCR reactions were performed in 20 µl reaction mixtures containing 10 ng template DNA, using the GoTaq® Green Master Mix (Promega, WI, USA). PCR amplification was applied under the following cycling conditions: an initial denaturation at 95 °C for 10 min followed by 35 cycles. Each cycle included the steps below: a denaturation at 95 °C for 45 s, an annealing at 50 °C for 45 s, and an extension at 72 °C for 1 min. A final extension at 72 °C for 10 min was applied. The PCR amplification Cox1 products were separated in 1.5% (wt/ vol) agarose gels using 1X Tris Borate EDTA (TBE) and photographed on a UV transilluminator.

PCR amplification products of both regions were purified using the NucleoSpin Extract Kit (Macherey Nagel, Düren, Germany) in order to remove secondary metabolites prior to sequencing. All sequences were determined on an ABI PRISM ® 3700 DNA Analyzer (Applied Biosystems). Each fragment used was sequenced in both directions in order to maximize the accuracy of the sequence.

## Results

Several potential specimens were manipulated during the three survey dives (Fig. 1) and they required little force to remove. Two specimens, however, were confirmed as attached macroalgae and could not be easily moved by use of thruster or claw manipulation at 100 m depth. With the claw gripping a section of an algal thallus, it required nearly full thrust to remove a section, indicating that the holdfast attachment to the benthos was secure. These two specimens were collected. Each dive could only collect one specimen and return to the surface at a time. Due to this constraint, multiple specimens that were potentially attached were not collected (Table 1).

Of the two specimens collected from 100 m depth both were identified as *P. decipiens* through taxonomic keys with one being further sampled for sequencing. Phylogenetic data resulted in the aligned cytochrome oxidase subunit I sequence revealed a length of 654 bp (Genbank accession number: No. OL944595). Blast search (Morgulis et al. 2008) revealed that our specimen is 100% identical with *P. decipiens*.

Fig. 1 Video stills from ROV footage during an algal collection at 100 m depth, 31st January 2018 (**A**, **B**). High detailed ROV imagery of macroalgae attached on hard substratum at 90 m. February 2016 (**C**, **D**). Herbarium specimen (310118-10 FCK) collected via ROV on Jan 2018, scale bar is 5 cm (**E**)



 Table 1
 Table of rhodophyta specimen collection and testing across

 3 ROV dives

Dive #	Depth (m)	Speci- mens tested	Unattached specimens	Potentially attached	Con- firmed attached
1	101	4	2	2	0
2	89	5	1	3	1
3	102	2	1	0	1

Rhodophyta specimens were collect via ROV manipulators, with specimens firmly attached to the seafloor collected (confirmed attached, total: 2). Specimens were collected from Rothera Point, Adelaide Island, WAP ( $67^{\circ}$  34' 50" S,  $68^{\circ}$  07' 00" W)

# Discussion

The collection of *P. decipiens* at 100 m depth represents a new depth record for living Antarctic macroalgae. Previous attempts to describe the lower depth limit of *P. decipiens* have been inconclusive and disagreed with their theoretical limit (Gómez et al. 2009; Wiencke & Amsler 2012). *P. decipiens* can propagate at these depths due to being a "seasonal anticipator", developing new blades in August (Weykam & Wiencke 1996), a time of no or little light at 100 m depth. This life cycle allows it to exploit the short period in late winter/early spring, between the breaking up of the seasonal sea ice and start of light depletion by the phytoplankton bloom, as light requirements for photosynthesis are low and not temperature dependent for this species (Wiencke & Tom Dieck, 1989). ROV dives during this period did observe down welling blue light on low-quality cameras at depths down to 100 m, however the seasonal availability of this down welling light is variable [pers. obs.; (Wiencke, 1990)].

Due to the multiple patches of *P. decipiens* (Fig. 1) being reliably found on each dive (max.  $1000 \text{ m}^2$  area) it is rather unlikely that 100 m depth is the extreme lower limit of this species. Rhodophytes have generally patchy distributions, a characteristic of the Antarctic benthos (Smale 2008; Thrush et al. 2006). Patches of rhodophytes were often found on areas with a gentler slope but this is also a characteristic of fragmented sections of algae collecting in seabed hollows (Braeckman et al. 2019). These

collections exceed previous estimates of  $49 \pm 22$  m depth limit, however it was noted at the time that its depth distribution of macroalgae likely exceeded this (Wiencke 1990). Many specimens that looked attached were often found to be only partially buried fragments (Table 1), which means that previous observations, particularly at depth, may not be able to identify attached and growing algae. *P. decipiens* has variable morphology and although *P. decipiens* was been identified there could be other rhodophytes growing within this depth range.

This source of macroalgal carbon production is an important source of food for the benthic Antarctic community (Huang et al. 2006; Iken et al. 1998). Within Antarctica the shallow (above 40 m depth) hard substrate can be dominated by macroalgae and suspension-feeder assemblages (Quartino et al. 2005; Robinson et al. 2021; Wahl 2009). Through macroalgal decomposition and fragmentation this biomass in the form of macroalgal detritus (or fragments), plays a key role in carbon flux to greater depths or in benthic soft-sediment communities (Cordone et al. 2020; Dunton 2001; Gillies et al. 2012; Norkko et al. 2007, 2004). The establishment of the lower depth limit of algae along the Western Antarctica Peninsula is of particular interest as new suitable rocky substrate is opening up to further macroalgal colonisation (Braeckman et al. 2019), as glacial retreat is occurring at unprecedented rates (A. Cook et al. 2016; A. J. Cook et al. 2015).

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Author contributions BJOR completed the sampling, FCK identified *Palmaria decipiens*, AR, JS, GAG, and AE all contributed to molecular identification and analysis. With BJOR, SMOR, FCK, and GAG contributing to drafting and finalizing the written text.

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**Data availability** All data can be made available upon request to BJOR (benson@bas.ac.uk).

#### **Declarations**

Conflict of interest The authors declare no competing interest.

**Ethical approval** The authors declare no potential conflict of interests, this research involved no human participants or animals and fully complied with *Polar Biology* ethical standards.

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