PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2009) **276**, 3591–3599
doi:10.1098/rspb.2009.0994
Published online 22 July 2009

Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia

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Recent data revealed that metazoans such as mites and springtails have persisted in Antarctica throughout several glacial–interglacial cycles, which contradicts the existing paradigm that terrestrial life was wiped out by successive glacial events and that the current inhabitants are recent colonizers. We used molecular phylogenetic techniques to study Antarctic microchlorophyte strains isolated from lacustrine habitats from maritime and continental Antarctica. The 14 distinct chlorophycean and trebouxiophycean lineages observed point to a wide phylogenetic diversity of apparently endemic Antarctic lineages at different taxonomic levels. This supports the hypothesis that long-term survival took place in glacial refugia, resulting in a specific Antarctic flora. The majority of the lineages have estimated ages between 17 and 84 Ma and probably diverged from their closest relatives around the time of the opening of Drake Passage (30–45 Ma), while some lineages with longer branch lengths have estimated ages that precede the break-up of Gondwana. The variation in branch length and estimated age points to several independent but rare colonization events.

Keywords: Antarctica; biogeography; endemism; glacial refugia; green algae; molecular phylogeny

1. INTRODUCTION

Recent work on terrestrial metazoans in Antarctica has questioned the long-held 'recolonization hypothesis' that expanded ice-cover during successive Neogene and Late Pleistocene glacial maxima has resulted in almost complete extinction of biota, followed by extensive colonization after glacial retreat (e.g. Stevens *et al.* 2006; Convey & Stevens 2007; Convey *et al.* 2008; McGaughran *et al.* 2008; Pugh & Convey 2008). Instead, the data support the 'glacial refugia hypothesis', with various groups of organisms, including mites, springtails and copepods, showing a high degree of endemism and regionalization, suggesting that they were able to survive in isolated ice-free refugia (see Convey & Stevens 2007; Convey *et al.* 2008 for an overview). To date, the focus has largely been on terrestrial organisms for which coastal oases and nunataks, the Transantarctic mountains and specific regions such as the McMurdo Dry Valleys—some of which are known to have been ice-free since at least the Mid-to-Late Miocene (up to approx. 14 Ma; Boyer 1979; Prentice *et al.* 1993)—probably acted as important refuges.

Evidence for the widespread persistence of refugia in which aquatic organisms could survive glaciation (e.g. coastal low-latitude regions) is, however, still elusive (Hodgson *et al.* 2001, 2005; Gibson & Bayly 2007). Life in Antarctic aquatic ecosystems is largely microbial and confined to benthic mats consisting of cyanobacteria, and to a lesser extent diatoms and green algae (Sabbe *et al.* 2004; de los Rios *et al.* 2004). Micro-organisms form an interesting test case in the light of the refugium hypothesis in that they are expected, within the limits of their environmental tolerance, to show ubiquitous distribution patterns as a result of their virtually unlimited dispersal capacity (but see ongoing discussion on microbial biogeography in, for example, Martiny *et al.* 2006). Molecular studies of cyanobacteria, using 16S rRNA gene sequencing, suggest that most isolates have a long association with the Antarctic environment (Taton *et al.* 2003, 2006). Likewise, morphological studies on diatoms suggest that in some areas at least 40 per cent of the species are Antarctic endemics (Schmidt *et al.* 1990; Sabbe *et al.* 2004; Spaulding *et al.* in press). These high levels of endemism may point to the importance of relatively low dispersal rates and long-term survival in suitable habitats confined to isolated glacial refugia. In contrast to diatoms and cyanobacteria, the green algal component of microbial mats has remained virtually unstudied. The available data are largely restricted to morphological taxonomic inventories on the continent, such as Victoria Land (Cavacini 2001; Adams *et al.* 2006), the Antarctic Peninsula (Mataloni & Pose 2001) and maritime Antarctica (Fermani *et al.* 2007; Zidarova 2007). Broady (1996) suggested that most Antarctic

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2009.0994 or via http://rspb.royalsocietypublishing.org.

Received 9 June 2009
Accepted 3 July 2009

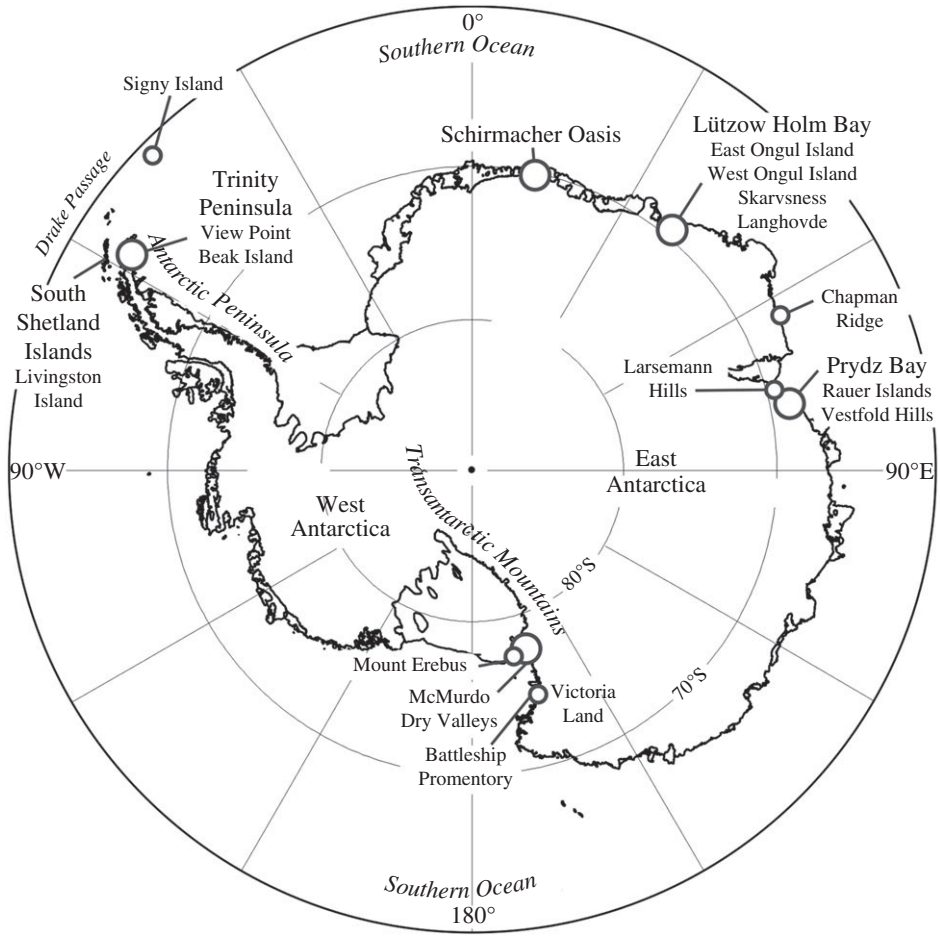


Figure 1. Map of Antarctica highlighting the five major sampling areas (large circles) and the original isolation source for the sequences from GenBank (small circles).

terrestrial green algae are cosmopolitan taxa. Morphological delimitation of green algal species and higher taxa is, however, contentious, especially in groups that are morphologically depauperate and exhibit convergent evolution towards reduced morphology (e.g. coccoid forms). Several cases of incorrect taxon delineation and cryptic species diversity are being disclosed by application of molecular phylogeny. A well-known example is that of the coccoid alga *Chlorella*, which has been shown to be polyphyletic, with the different taxa placed in separate green algal classes (Huss *et al.* 1999). DNA sequence data are therefore well placed to provide more insight into the green algal diversity on the Antarctic continent. The currently available molecular data are, however, fragmentary, consisting of a number of isolated taxonomic and ecophysiological studies on individual taxa (e.g. *Pyramimonas australis*: Moro *et al.* 2002; *Scenedesmus* sp.: Lesser *et al.* 2002; Pocock *et al.* 2004; lichen symbionts: Romeike *et al.* 2002; eukaryotic clone libraries: Christner *et al.* 2003; de la Torre *et al.* 2003; Fell *et al.* 2006).

The current study was designed to assess whether Antarctic freshwater green algae are mainly cosmopolitan species as a result of their fast colonization rates (Broady 1996), supporting the recolonization hypothesis, or are endemic to Antarctica or particular Antarctic regions, supporting the 'glacial refugia hypothesis' (cf. Convey *et al.* 2008). To this end, we employed molecular phylogenetic techniques, including molecular clock analysis of nuclear encoded 18S rRNA gene sequences of original Antarctic microchlorophyte isolates, along with an extensive set of other green algal sequences.

2. MATERIAL AND METHODS

(a) *Sampling and culturing*

Samples were obtained from 33 lakes in maritime and continental Antarctica: The Rauer Islands and Vestfold Hills (Prydz Bay, Princess Elizabeth Land), the McMurdo Dry Valleys (Victoria Land), View Point and Beak Island (Trinity Peninsula, Antarctic Peninsula), Schirmacher Oasis (Dronning Maud Land) and East Ongul, West Ongul, Langhovde and Skarvsness in the Lützow Holm Bay area (Enderby Land) (figure 1, table 1). Microbial mats or sediment samples were obtained using a custom-made scoop or a UWITEC gravity corer (Mondsee, Austria). Sample containers were topped up with water, cooled during transport and stored at 5°C.

Samples were incubated in liquid Bold modified basal freshwater medium (commercial stock Sigma-Aldrich, USA), liquid or agarized WC (Guillard & Lorenzen 1972) and desmid medium (recipe at utex.org). Cultures were grown at 12–15°C, with a 14 : 10 day : night cycle and a light intensity of 25–35 μmol photons $m^{-2} s^{-1}$.

From each sample a number of single cells or colonies with different morphology were picked out for monoclonal growth (table 1). These isolates were initially screened using light microscopy and amplified ribosomal DNA restriction analysis (ARDRA).

Table 1. Overview of the studied samples, collection and incubation data and the number of isolates obtained.

sample codes	lake name	region	region code	sampling date	number of isolates	number sequenced (unique)
R8	Rauer Island Lake 8	Rauer Islands, Prydz Bay (Princess Elisabeth Land)	PB	22 Dec 1997	2	1
ACE [1–5]	Ace Lake	Vestfold Hills, Prydz Bay	PB	18 Feb 1999	45	8 (1)
FRY [1/2]	Lake Fryxell	McMurdo Dry Valleys (Victoria Land)	McM	Feb 1999	39	10 (3)
B4/6/6L	Beak Island Lakes 4 and 6	Trinity Peninsula/Prince Gustav channel, Antarctic Peninsula (Graham Land)	TP	16 Jan 2006	14	4
VPL1/1A/2/3/4/5/6/9/9B/M	View Point Lakes 1–6, 9	Trinity Peninsula/Prince Gustav channel, Antarctic Peninsula (Graham Land)	TP	12 Jan 2006	83	14 (4)
EO2/3/4/5/7	East Ongul Lakes 2–5, 7	Lützow Holm Bay (Enderby Land)	LB	08 Jan 2007	44	11 (1)
LA1/3/4/6/8	Langhovde Lakes 1, 3–4, 6, 8	Lützow Holm Bay (Enderby Land)	LB	17–19 Jan 2007	15	1
SK2/5/6/9	Skarvsness Lakes 2, 5–6, 9	Lützow Holm Bay (Enderby Land)	LB	11–16 Jan 2007	25	3
WO1/4/8S/8L/10	West Ongul Lakes 1, 4, 8, 10	Lützow Holm Bay (Enderby Land)	LB	21–25 Jan 2007	35	6
SC1/2/6	Schirmacher Oasis Lakes 1, 2, 6	Schirmacher Oasis (Dronning Maud Land)	SC	29 Jan 2007	6	3 (1)

(b) *DNA extraction, PCR amplification, amplified ribosomal DNA restriction analysis screening and sequencing*

DNA was extracted as described in Zwart *et al.* (1998). The 18S rRNA gene was amplified using the primers P2 (5′-CTGGTTGATTCTGCCAGT-3′) and P4 (5′-TGAT CCTTCYGCAGGTTCAC-3′; Moon-van der Staay *et al.* 2000). The PCR reaction mixtures contained 2 µl of DNA, 0.5 µM of each primer, 200 µM of each deoxynucleoside triphosphate, 2.5 U of Taq DNA polymerase (Ampli Taq), 10× PCR buffer (100 mM Tris/HCl, pH: 8.3; 500 mM KCl; 15 mM $MgCl_2$; 0.01 per cent (w/v) Gelatin) and 400 ng of bovine serum albumin. The PCR reaction consisted of an initial denaturation of 2 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 55°C and 1.5 min at 72°C, and a final extension of 10 min at 72°C. ARDRA screening was performed using the restriction enzymes *Alu*I, *Hin*fI and *Taq*I following the protocol by Ventura *et al.* (2001). ARDRA images were analysed using BioNumerics 4.6 (Applied Math, Kortrijk, Belgium) with a curve-based (Pearson's correlation) clustering technique in order to define groups for further analysis. At least one strain from each ARDRA group (identical patterns) or morphological group (based on general characteristics such as size, shape and pigmentation) from each region was selected for sequencing.

Sequencing of the 18S rRNA gene was performed using the primers P2 and P4 (see above) and the internal primers P5, P12, P14, P15 and P16 (respectively, 300>, 1055<, 528>, 960> and 536<; Huss *et al.* 1999). Sequences were automatically assembled and visually checked in BioNumerics 4.6 and were deposited in GenBank under accession numbers FJ946881–FJ946908.

(c) *Sequence alignment and phylogenetic analyses*

The newly obtained Antarctic microchlorophyte sequences were combined with the closest NCBI BLAST hits, along with previously published sequences from Arctic and Antarctic regions and a broad diversity of sequences, representing the main classes and orders of Chlorophyta. Five representatives of Streptophyta were selected as outgroup. Taxa, locality details and GenBank numbers are listed in table S1 in the electronic supplementary material. While taxon sampling for the construction of the phylogenetic tree may influence our results, this is mainly constrained by the availability of sequences on GenBank. Arctic and alpine microchlorophytes sequences remain relatively under-represented in the public databases and, to our knowledge, no comparable studies have been carried out in these regions. The Antarctic sequences II22, II11 and V13 contained two putative group I introns: S516 and S943 (numbers reflect the homologous position in *Escherichia coli* 16S rRNA gene; Haugen *et al.* 2005). After introns had been removed, the sequences were aligned using Muscle 3.6 with standard parameters (Edgar 2004) and visually inspected. A FASTA-file of the alignment can be requested from the authors.

Phylogenetic analyses consisted of maximum likelihood (ML) and Bayesian inference (BI) tree searches under a general time-reversible model with a proportion of invariable sites and gamma distribution split into four categories ($GTR + I + \Gamma$), as determined by the Akaike Information Criterion in PAUP/Modeltest 3.6 (Posada & Crandall 1998; Swofford 1999). ML analysis was carried out with PhyML 2.4.4 (Guindon & Gascuel 2003). The reliability of each internal branch was evaluated based on 1000 bootstrap replicates. For BI, two independent runs, each consisting of four incrementally heated, Metropolis-coupled chains, were run for seven million generations using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Parameter values and trees were sampled every thousand generations. Convergence and stationarity of the runs was assessed using Tracer 1.4 (Rambaut & Drummond 2007), and a burn-in sample of

Table 2. Overview of the sequenced strains, with GenBank accession number (acc), detailing the regions (codes as in table 1) from which strains with identical 18S rRNA gene sequence were isolated.

strain in tree	strain name(s) identical 18S sequences	acc.	region				
			PB	McM	TP	LB	SC
VII3	Lake Fryxell (VII3, VII4)	FJ946904		x			
VI12	Lake Fryxell (VI12)	FJ946905		x			
II4	Ace Lake (II4) Lake Fryxell (VI8)	FJ946902	x	x			
VPL9-6	View Point (VPL9-6)	FJ946907			x		
VPL9-5	View Point (VPL9-5)	FJ946901			x		
I5	Ace Lake (I5, I6)	FJ946892	x				
VPL4-4	View Point (VPL4-4)	FJ946900			x		
II11	Ace Lake (II11, II12, II22, III1, V13), Rauer (R8-2), West Ongul (WO1L-3, WO1S-2, WO1S-5, WO8L-2, WO8L-6), East Ongul (EO2-2, EO2-3, EO2-6, EO2-11, EO2-14, EO3-3, EO4-4, EO4-10 EO5-7), View Point (VPL1-5, VL2-4, VL2-6, VPL6-4), Beak (B4-1, B6-1, B6-6, B6-8), Skarsness (SK2L-4, SK2L-11, SK5S-6), Langhovde (LA6L-6)	FJ946893	x		x	x	
VI11	Lake Fryxell (VI11, VI13, VI26, VI4), West Ongul (WO10-1), View Point (VPL1-1, VPL1A-2, VPL9B-2, VPL9B-5, VPL9B-6), East Ongul (EO5-4c)	FJ946884		x	x	x	
VI2	Lake Fryxell (VI2, IX4)	FJ946883		x			
VPL1-3	View Point (VPL1-3)	FJ946890			x		
EO7-4	East Ongul (EO7-4)	FJ946882				x	
SC2-2	Schirmacher (SC2-1, SC2-3)	FJ946881					x
VPL5-6	View Point (VPL5-6), East Ongul (EO2-17)	FJ946891			x	x	

1000 trees was removed before constructing the majority rule consensus tree.

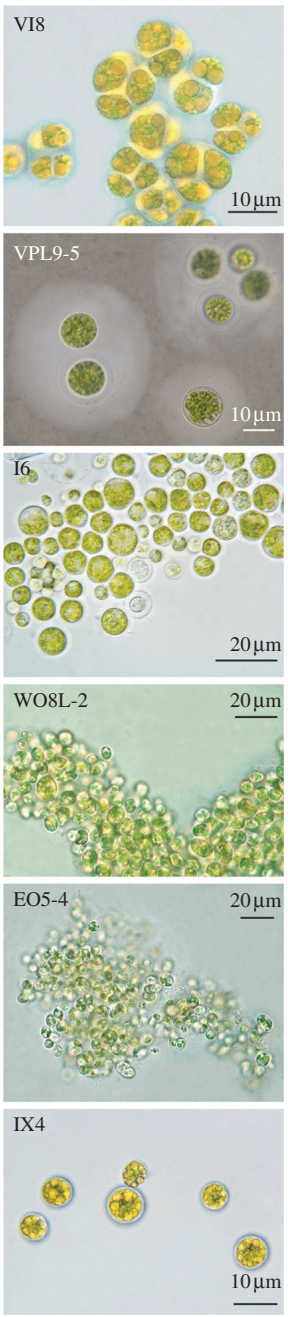
Relative node ages were estimated using r8s 1.7 (Sanderson 2002) by rate-smoothing the ML tree using penalized likelihood and a log-smoothing parameter of 5, selected as optimal by cross-validation (Sanderson 2003). Absolute ages were estimated by setting the minimum and maximum age of the Chlorophyta–Streptophyta split at 700 and 1500 Ma, based on the fossil record and molecular clock estimates (Douzery *et al.* 2004; Hedges *et al.* 2004; Yoon *et al.* 2004; Berney & Pawlowski 2006; Cavalier-Smith 2006; Roger & Hug 2006; Zimmer *et al.* 2007; Herron *et al.* 2009).

3. RESULTS

We characterized 61 isolates of green algae from 13 maritime and 30 continental Antarctic samples (table 1). All strains were isolated as non-flagellated unicells (coccal morphology). Sequencing of the 18S rRNA gene yielded 14 distinct sequences. Ten of these were detected in one Antarctic region only, two were detected in two regions and two were detected in three regions (table 2). No sequences were found in more than three of the five regions sampled. Twelve additional GenBank sequences of freshwater and terrestrial green algae from different Antarctic regions were incorporated in our dataset, along with a broad representation of green algae from other regions. The alignment of 191 sequences was 1854 sites in total, including 950 phylogenetically informative characters. ML and BI phylogenetic analyses yielded similar tree topologies, which are congruent with published 18S phylogenies of green algae (Lewis & Lewis 2005; Pröschold & Leliaert 2007). The ML phylogram, with indication of ML bootstrap values and BI posterior probabilities, is shown in fig. S1 in the electronic supplementary material. The chronogram, obtained by rate-smoothing the ML tree using penalized likelihood and calibrated by a conservative and more liberal node age of the Chlorophyta–Streptophyta split (700 and 1500 Ma, respectively), is shown in figure 2.

Phylogenetic analysis shows that the freshwater Antarctic isolates are mainly distributed among the chlorophytan classes Chlorophyceae and Trebouxiophyceae, and only a single Antarctic strain (*Trichosarcina mucosa*, a filamentous green alga) belongs to the Ulvophyceae (figure 2). Apart from the *Chlorella vulgaris* NJ-7 strain, identical to an isolate from a eutrophic pond in The Netherlands, none of the Antarctic sequences was identical to non-Antarctic sequences currently available in GenBank. Uncorrected *p*-distances with the most closely

Figure 2. (*Opposite.*) ML tree of the green algae inferred from 18S rDNA sequences with branch lengths fitted to a molecular clock using penalized likelihood, and absolute ages estimated by setting the split of Chlorophyta and Streptophyta at 700 and 1500 Ma, respectively. Numbers at nodes indicate statistical support: ML bootstrap proportions (more than 50) and BI posterior probabilities (more than 85). Phylogenetic positions of freshwater and terrestrial Antarctic sequences are indicated in red, Arctic sequences in blue. Strains obtained during this study are highlighted and indicated by strain name. In addition to species names, climatic region (NT, north temperate; ST, south temperate; Tr, tropical; Ar, Arctic; An, Antarctic), environment (FW, freshwater; Mar, marine; Ter, terrestrial) and GenBank accession numbers are given within parentheses. Regions from which identical sequences were retrieved are indicated in parentheses (McM, McMurdo Dry Valleys; VH, Vestfold Hills; VP, View Point; R, Rauer Islands; WO, West Ongul; EO, East Ongul; Beak, Beak Island; SK, Skarvsness; LA, Langhovde; SC, Schirmacher Oasis). Inset: light microscopic images of a selection of Antarctic strains.



Colour legend of taxa in tree
Antarctic freshwater and terrestrial (bold: this study)
Arctic

Chlorophyceae
Ulvophyceae
Trebouxiophyceae
Prasinophyceae
Streptophyta

Ma 700 600 500 400 300 200 100 0
Proterozoic | Paleozoic (Cambrian, Ordovic., Silurian, Devonian, Carbonif., Permian) | Mesozoic (Triassic, Jurassic, Cretaceous) | Cenozoic
60 40 20 0
Cenozoic (Paleo, Eocene, Oligo, Miocene, Plio)
Ma 1500 1000 500 0
Proterozoic | Paleozoic (Camb, Ord, Sil, Devon, Carbon, Perm) | Mesozoic (Trias, Jura, Cretac) | Cen

related non-Antarctic sequences ranged from 0.002 to 0.034 (table S2, electronic supplementary material). Of the 26 distinct Antarctic sequences, 15 are Trebouxiophyceae. Within this clade, six strains (VI11, VI2, VPL1-3, NJ-18, NJ-7 and Ant-1) belong to the Chlorellaceae (Huss *et al.* 1999) and are intermixed with temperate members of the clade. Likewise, the remaining trebouxiophycean Antarctic isolates are scattered among temperate freshwater green algae. One strain, VPL5-6, was most closely related to a *Paradoxia* isolate from the Siberian permafrost, although its exact phylogenetic position could not be determined with satisfactory statistical support. The 18S sequence of isolate SC2-2 from the Schirmacher Oasis was extremely divergent, resulting in a deep branch with uncertain phylogenetic affinity within the trebouxiophycean lineage. Fourteen unique sequences are distributed in the Chlorophyceae clade. Within the Chlorococcales clade, two isolates from Lake Fryxell (VII3 and VI12) are most closely related to strain II4 from Ace Lake, which was found to be sister to a snow alga from Spitzbergen. Strain VPL9-6 is also resolved within the Chlorococcales but its phylogenetic affinity within this clade is uncertain. Four distinct sequences fall within the *Scenedesmus*/*Desmodesmus* clade along with temperate and Arctic members. The remaining Antarctic chlorophycean strains are spread mainly among temperate freshwater representatives.

While the phylogram (fig. S1, electronic supplementary material) reflects the different levels of sequence divergence found between the Antarctic sequences and their closest non-Antarctic relatives, the time-calibrated phylogeny (chronogram; figure 2) provides absolute age estimates for evolutionary events. Estimated ages of the Antarctic lineages (determined as the node separating the Antarctic sequence from its non-Antarctic sister) range from 2.7–9.9 Ma for a number of chlamydomonad isolates (Chlorophyceae) to over 17–84 Ma for the majority of the sequences and 330–708 Ma for the trebouxiophycean isolate SC2-2.

4. DISCUSSION

From five well-distributed regions in both maritime and continental Antarctica, 14 distinct microchlorophyte sequences were recovered. Although there is no absolute threshold of 18S sequence divergence for defining green algal taxa, most Antarctic sequences are divergent enough to be considered distinct species, genera or even higher-order taxa (Lewis & Flechtner 2004; Lewis & Lewis 2005). Our results thus indicate a wide phylogenetic diversity of apparently endemic Antarctic lineages at different taxonomic levels, which has several important implications.

First, our results clearly contrast with morphological studies, which have suggested that Antarctic green algal communities are dominated by cosmopolitan species (Broady 1996), and instead support the notion that Antarctica has developed a distinct regional flora. Given that convergent evolution has led to high morphological similarity between unrelated microchlorophyte species, it is not unusual that molecular data contrast with morphological data. For example, Fawley *et al.* (2004) detected a high number of novel 18S rRNA genotypes during a molecular diversity study in freshwater systems in North America. Similarly, in desert areas of western North America, Lewis & Lewis (2005) discovered that green algae are not merely accidental visitors from aquatic environments, but long-term inhabitants of these extreme environments. Our results thus corroborate the recent studies that have revealed a high degree of endemism in other Antarctic micro-organisms such as cyanobacteria (e.g. Taton *et al.* 2006) and diatoms (Sabbe *et al.* 2003), and refute the hypothesis that for micro-organisms everything is everywhere (e.g. Finlay & Clarke 1999).

Second, our results point to several independent but rare colonization events over a long time frame and long-term survival in glacial refugia as evidenced by the different branch lengths of the Antarctic lineages. Owing to the lack of reliable green algal fossils, our phylogenetic tree is only calibrated at a single node, using a minimum and maximum age of the Chlorophyta–Streptophyta split at 700 and 1500 Ma, respectively (Douzery *et al.* 2004; Hedges *et al.* 2004; Yoon *et al.* 2004; Berney & Pawlowski 2006; Cavalier-Smith 2006; Roger & Hug 2006; Zimmer *et al.* 2007; Herron *et al.* 2009). Nonetheless, this approach allowed us to obtain rough estimates for the divergence times of the different Antarctic microchlorophyte strains. The majority of the lineages (16 out of 26) have estimated ages between 17 and 84 Ma and, based on available sequence data, probably diverged from their closest relatives around the time of the opening of Drake Passage (30–45 Ma) during the Eocene, which initiated the first transient glaciations on the continent. The lineages with longer branch lengths, including SC2-2 (330–708 Ma), have estimated ages that precede the break-up of Gondwana (65–100 Ma). These findings therefore support the hypothesis of the existence of refugia being present during successive glacial cycles (Convey & Stevens 2007; Convey *et al.* 2008) and conflict with the recolonization hypothesis, which proposes that fast colonization rates have resulted in the dominance of cosmopolitan species on Antarctica. Green algal refugia may include ice-free terrestrial habitats as well as ice habitats such as cryoconites and supraglacial ponds. Geological data do not rule out the presence of glacial refugia for aquatic micro-organisms in at least three out of the five major regions included in our study. The McMurdo Dry Valleys have probably had ice-free areas since at least the Mid-to-Late Miocene (up to approx. 14 Ma; Boyer 1979; Prentice *et al.* 1993), with large glacial lakes being present since the Last Glacial Maximum (LGM; Wagner *et al.* 2006). In Prydz Bay, parts of the Larsemann Hills, less than 80 km away from the Rauer Islands and the Vestfold Hills, were ice-free during the LGM (Hodgson *et al.* 2001). Some lakes in this region were shown to contain relict populations of diatoms and copepods (Hodgson *et al.* 2005; Cromer *et al.* 2006), while in other lakes taxa currently present in other Antarctic and sub-Antarctic regions became locally extinct during the LGM (Hodgson *et al.* 2006). In the Lützow Holm Bay region, ice-free conditions in some coastal areas during the LGM can be inferred from ^{14}C dates of *in situ* fossils in raised beach deposits (Miura *et al.* 1998). However, whether freshwater habitats escaped glaciation in this region remains unclear.

Third, our data shed some light on green algal dispersal within the Antarctic continent. Most phylotypes (10 out of 14) were only retrieved from a single ice-free region. This implies that, on the one hand, dispersal

rates within Antarctica could be low and that immigrants are competitively excluded (priority effects), and/or, on the other hand, that our sampling of the Antarctic microchlorophyte flora is currently limited by a small number of sampling sites that may differ in their limnological properties. Only two taxa (II11 and VI11), belonging to the genera *Chlorella* and *Scenedesmus*, were detected in three of the five regions, suggesting that these taxa may have more easily dispersed over the Antarctic continent. This is in agreement with Lawley *et al*. (2004), who found a similar lack of overlap between the eukaryotic biota of 'patterned ground soils' using a clone library approach from several widely separated Antarctic locations, with only a few taxonomic units showing apparently wider distributions. However, extended taxon sampling and more variable molecular markers (such as rDNA ITS) will be required to elucidate phylogeographic patterns of green algal taxa within Antarctica.

While the methods used for both cultivation and tree construction may have biased our results, they clearly highlight that (Antarctic green algae show a remarkable divergence from taxa from other regions). We are therefore confident that our main conclusion, namely that Antarctica is characterized by a distinct microchlorophyte flora, probably as a result of ancient patterns of isolation and long evolutionary history on the continent, will hold as new data become available.

This research was funded by the EU project MICROMAT and the Belgian Federal Science Policy (BelSPO) project AMBIO (Antarctic Microbial Biodiversity: the importance of geographical and ecological factors). We thank S. Cousin, K. Vanhoutte and A. De Beer for performing culture work, S. D'Hondt for obtaining sequence data, S. Boitsios for commenting on the manuscript and V. Chepurnov for providing pictures. F.L. and E.V. are post-doctoral research fellows with the Research Foundation—Flanders (FWO). The British Antarctic Survey, the Australian Antarctic Division and the Japanese Antarctic Research Expedition 48 (S. Imura, Hai Kanda and S. Kudoh) are thanked for the logistical support. Two anonymous reviewers are thanked for their valuable comments on an earlier version of the manuscript.

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