

PHYLOGENETIC RELATIONSHIPS OF THE BANGIALES FROM KING GEORGE ISLAND, ANTARCTICA

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ABSTRACT

Members of the **Bangiales** (Rhodophyta) are distributed worldwide from tropic to Antarctic and Arctic waters. Three species of the **Bangiales**; *Bangia* sp. (as *B. atropurpurea*), *Porphyra plocamiestris* and *Pyropia endiviifolia* (as *Porphyra endiviifolium*), have been reported in the Antarctic. Morphological and molecular data were investigated for the **Bangiales** from the Antarctic and its adjacent waters. Each six sequences of SSU rDNA, plastid *rbcl* and mitochondrial *cox1* gene were newly determined in this study. Molecular data from over 72 taxa of the **Bangiales** worldwide including previously published sequences, indicated that the genera *Bangia*, *Dione*, *Pyropia*, *Porphyra*, *Wildemanina* and other related genera be recognized in the **Bangiales** as in the previous molecular study. *Bangia fuscopurpurea* from the Antarctic was different from *B. fuscopurpurea* from north Pacific (Korea and Japan) by 12 bp in *cox1* gene sequence. *Porphyra plocamiestris* growing on other macroalgae in sub-tidal zone grouped into the genus *Wildemanina* with the species having one or two cell layers in molecular data. *Pyropia endiviifolia* is olive green in color and it allied to a clade with *P. aedois* from South Africa, *P. cinnamomea* and *P. virididentata* from New Zealand. The taxonomic issues and phylogenetic relationships of the Antarctic members of the **Bangiales** were discussed.

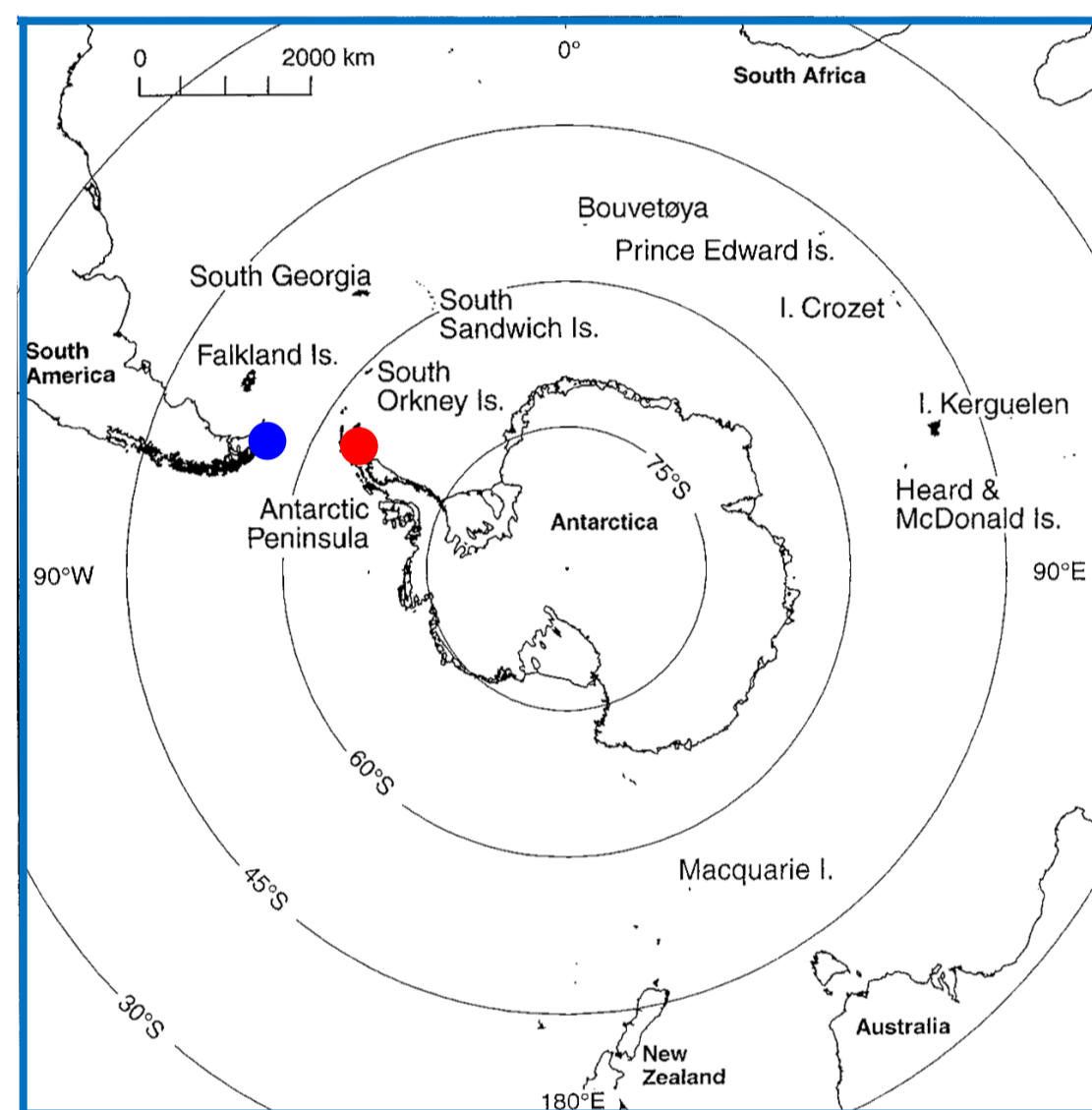


Fig. 1. Sampling sites of the **Bangiales**: ●: King George Island, Antarctica for *Bangia* sp., *Pyropia endiviifolia* and *Porphyra plocamiestris*; ●: Punta Arenas, Chile for *Porphyra woolhousiae* and *Pyropia* sp.

INTRODUCTION

Bangia and *Porphyra* belonging the order **Bangiales** are distributed world wide from the Arctic or Antarctic to tropical waters. Three species of the **Bangiales** have been reported from the Antarctica: *Bangia* sp. (as *B. fuscopurpurea*), *Porphyra endiviifolium* and *P. plocamiestris*, and several species have been added from sub-Antarctic waters (Clayton *et al.* 1997, Kim *et al.* 2001). Recently, the studies of materials from New Zealand, South Africa and sub-Antarctic islands have revealed unexpectedly high generic diversity in members of the **Bangiales** from the southern hemisphere regions (Nelson *et al.* 2006, Sutherland *et al.* 2011). In this study, nuclear SSU rDNA, plastid *rbcl* and mitochondrial *cox1* gene sequences were examined for six entities of *Bangia* and *Porphyra* collected from the Antarctica and Chile in order to get some implications for the phylogenetic relationships with other members.

GENERIC REVISION OF THE BANGIALES BY THE BANGIALES CONSORTIUM

- Bangia-type 7 genera**
- Minerva* W.A. Nelson in Nelson *et al.* 2005
- Dione* W.A. Nelson in Nelson *et al.* 2005
- Pseudobangia* K.M. Müll. et Sheath in Müller *et al.* 2005
- Bangia* Lyngb. 1819
- '*Bangia*' 1
- '*Bangia*' 2
- '*Bangia*' 3
- Porphyra-type 9 genera**
- Miuraea* N. Kikuchi, S. Arai, G. Yoshida, J.A. Shin et M. Miyata gen. nov.
- Clymene* W.A. Nelson gen. nov.
- Porphyra* C. Agardh 1824
- Sorora* W.A. Nelson & J. Brodie gen. nov.
- Lysithea* W.A. Nelson gen. nov.
- Fuscifolium* S.C. Lindstrom gen. nov.
- Boreophyllum* S.C. Lindstrom, N. Kikuchi, M. Miyata et Neefus gen. nov.
- Wildemanina* De Toni 1890
- Pyropia* J. Agardh 1899

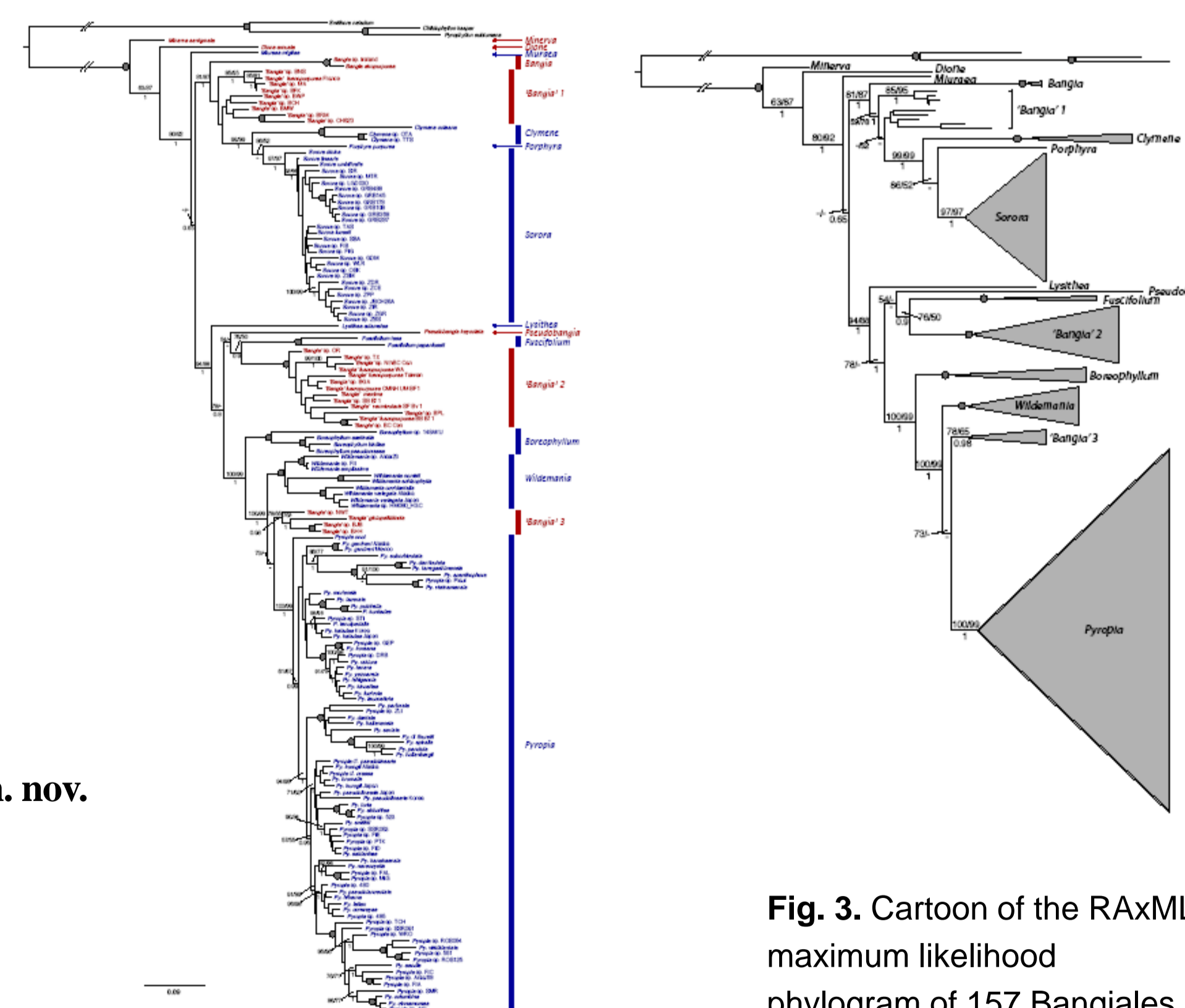


Fig. 2. Maximum likelihood phylogram of 157 **Bangiales** taxa calculated from the concatenated dataset under RAxML. Bootstrap values for RAxML and GARLI are shown above and Bayesian PP values below the nodes. Some internal support values are omitted for clarity. Gray circles indicate nodes supported at 100/100/1 (Sutherland *et al.* 2011).

Fig. 3. Cartoon of the RAxML maximum likelihood phylogram of 157 **Bangiales** taxa calculated from the concatenated dataset showing existing and proposed new genera with support for nodes along the backbone of the tree. Gray circles indicate nodes supported at 100/100/1 (Sutherland *et al.* 2011).

RESULTS AND DISCUSSION

1. Phylogenetic relationships of Antarctic **Bangiales**

Pyropia endiviifolia from the Antarctica was different from the material of *Pyropia* sp. from Chile by 2 bp in SSU, 18-19 bp in *rbcl* and 28 bp in *cox1* gene sequences, showing that two materials would be the different species. This species grouped into a clade with *Py. aedois* from South Africa, *Py. virididentata* and *Py. cinnamomea* from New Zealand and *Pyropia* sp. from Chile and Falkland Island based on combined SSU rDNA and *rbcl* data.

Wildemanina plocamiestris from the Antarctica and Chile which has one cell layer of blade grouped into a clade with *P. miniata* and *P. amplissima* from north Atlantic having two cell layer in SSU rDNA, and with five species from north Pacific and north Atlantic having two cell layer based on combined data.

Porphyra woolhousiae from Chile grouped into a clade with *P. dioica*, *P. lucasii*, *P. purpurea* and *P. umbilicalis* based on combined data.

Bangia sp. from the Antarctica showed the same sequence with *B. fuscopurpurea* from north Pacific and north Atlantic in SSU rDNA, whereas it was different from *B. fuscopurpurea* by 1-2 bp in *rbcl* and from *B. fuscopurpurea* from north Pacific (Korea and Japan) by 11-12 bp in *cox1* gene sequence. These results imply that this species would be a different one from *B. fuscopurpurea*.

2. Cox1 barcoding

Cox1 barcoding would be a powerful method in the identification of the members of the **Bangiales** such as in cases of other red algal groups (Saunders 2005). In all cases of this study intraspecific divergence values ranged from 0 to 5 bp, whereas interspecific divergences were more than 12 bp.

3. Taxonomic issues

Important taxonomic characters such as cell layer, sexuality (monoecious or dioecious), arrangement of reproductive cells (mixed or sectored vertically) do not reflect the molecular phylogeny.

Morphology

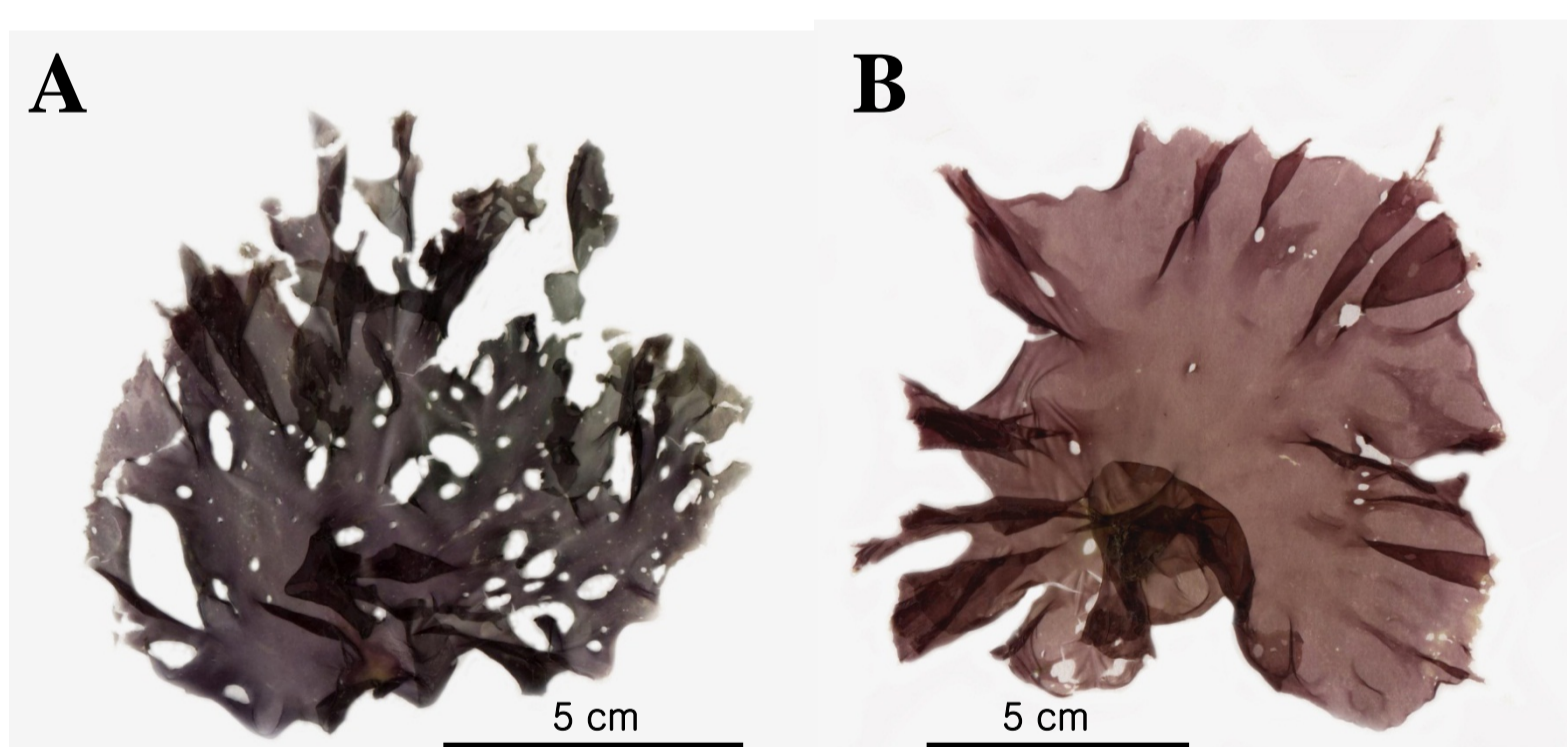


Fig. 4. *Pyropia endiviifolia* and *P. sp.*: A: A plant from King George Island, Antarctica. B: A plant from Punta Arenas, Chile.

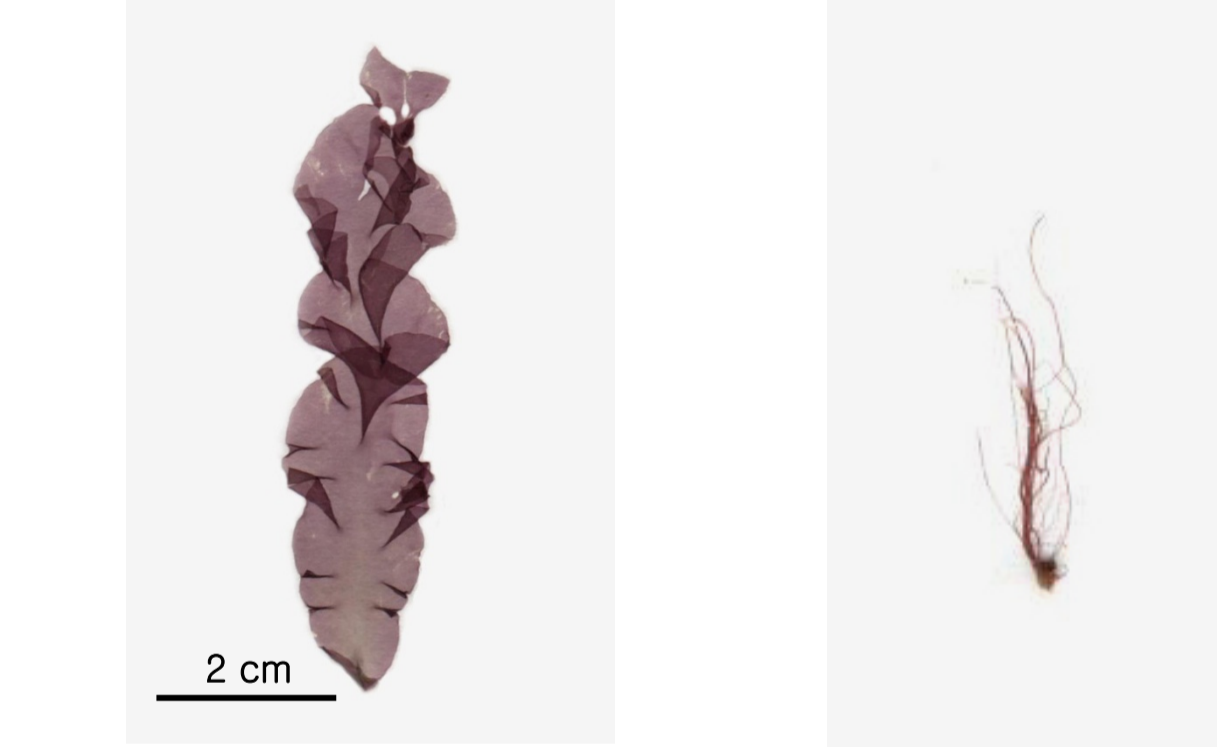


Fig. 5. *Porphyra woolhousiae* from Punta Arenas, Chile.

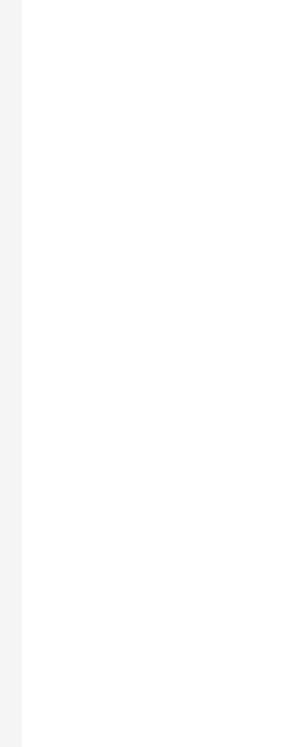


Fig. 6. *Bangia* sp. from King George Island, Antarctica.

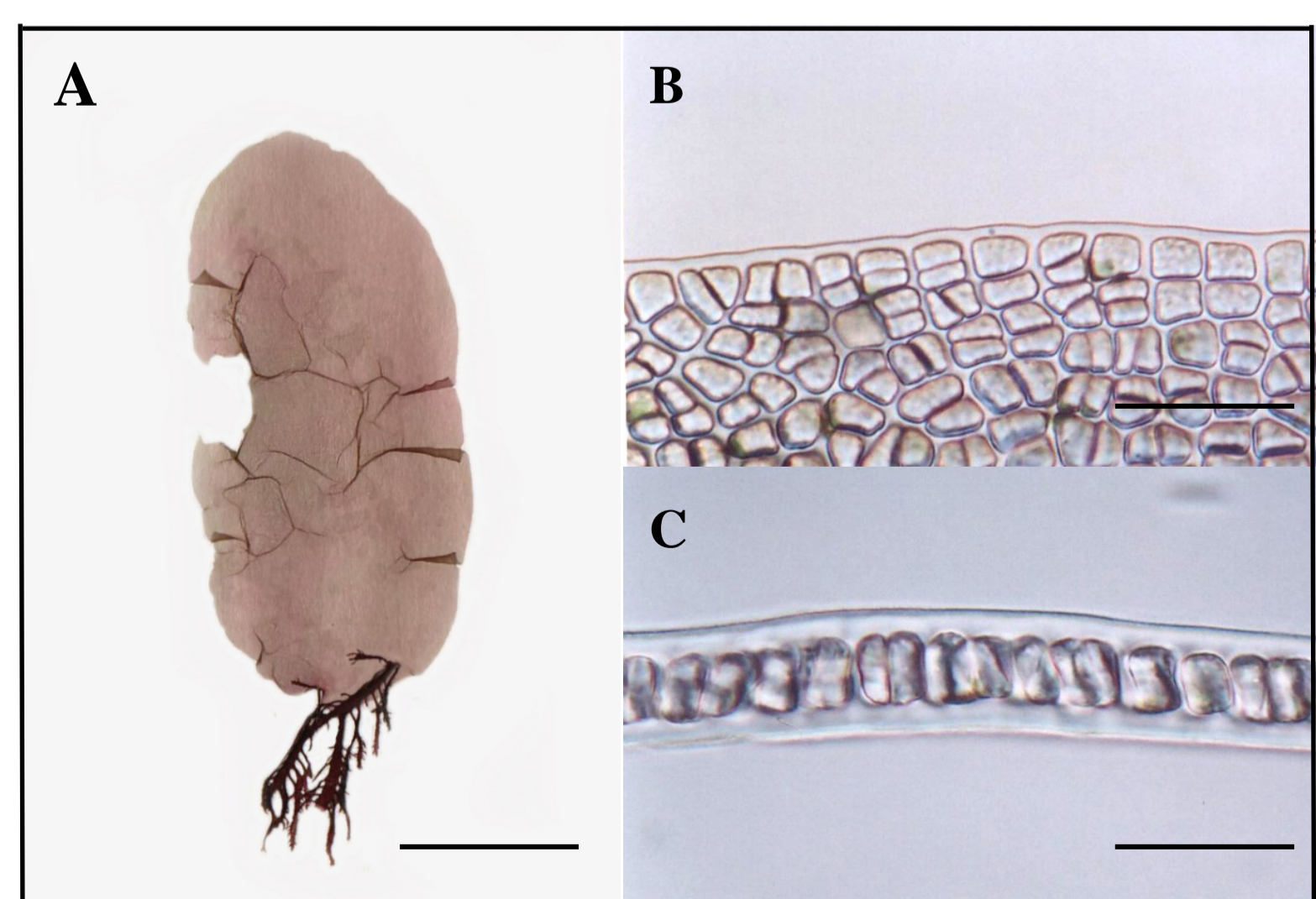


Fig. 7. *Wildemanina plocamiestris* from King George Island, Antarctica. A: Habit, B: Surface view of vegetative cells and margin of blade, C: Cross-sectional view of vegetative cells. Scale bars: 2 cm (A), 40 μ m (B-C).

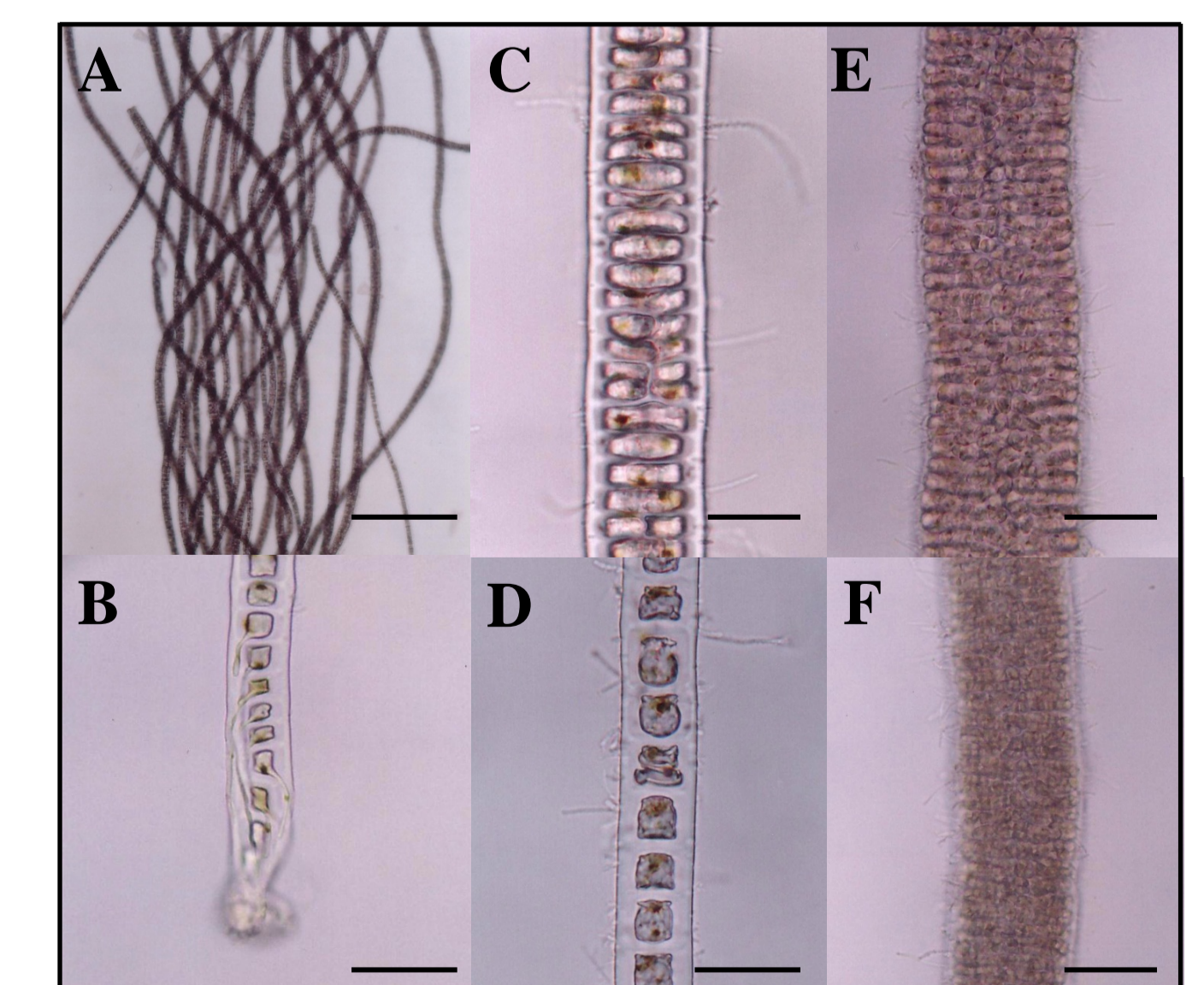


Fig. 8. *Bangia* sp. from King George Island, Antarctica. A: Habit, B: Rhizoidal cells, C: Cells of middle parts, D: Cells of lower part, E: Zygotosporangia, F: Spermatangia. Scale bars = 200 μ m (A), 50 μ m (B-D), 100 μ m (E-F).

Molecular Phylogeny

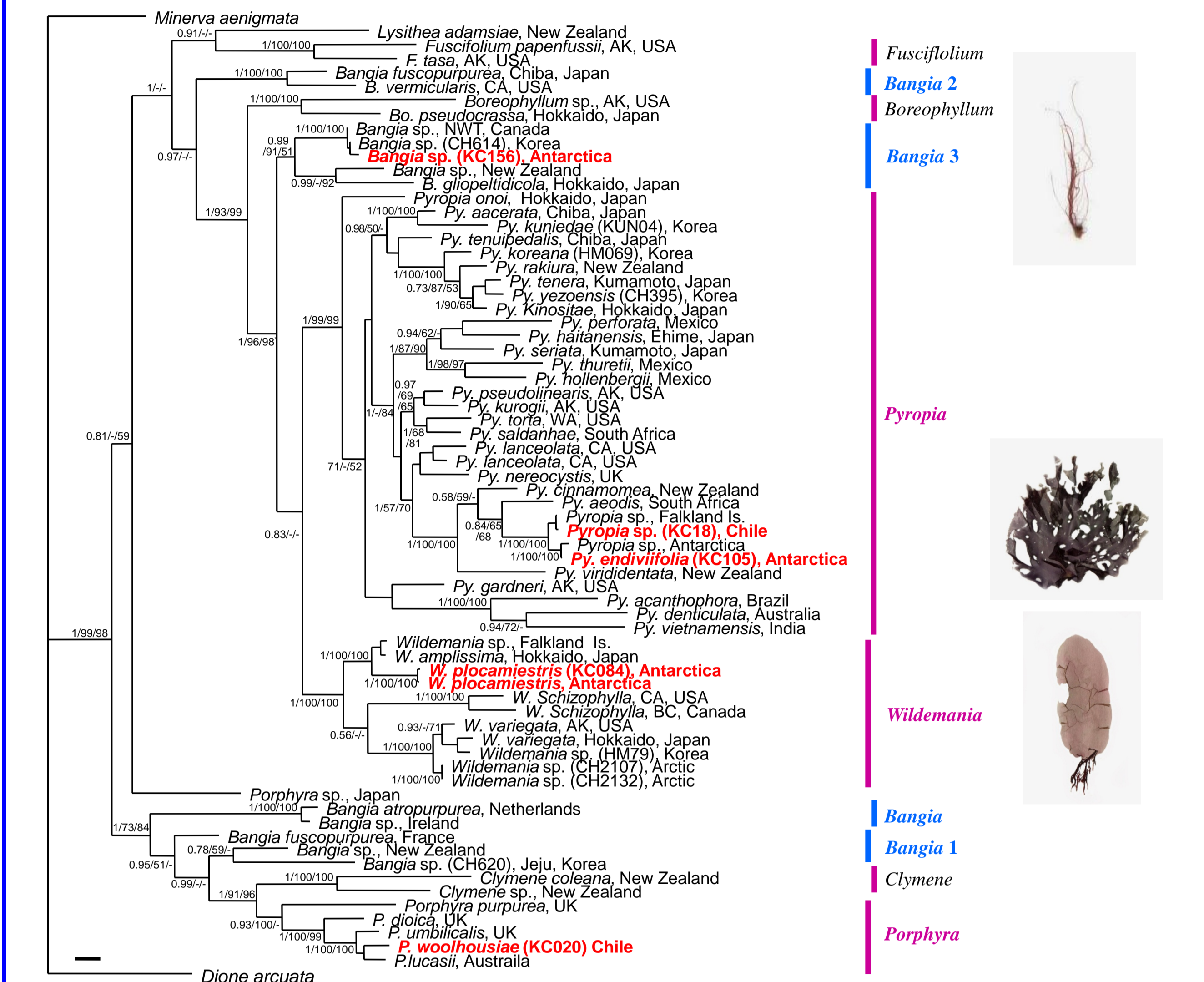


Fig. 9. Tree constructed with Bayesian inference for the concatenated nuclear SSU rDNA and plastid *rbcl* data set (GTR+I+G model). Values at branches represent Bayesian posterior probabilities (left value), 2000 and 1000 bootstrap replicates each for distance and maximum parsimony (center and right values, respectively) analyses. Branches lacking values received less than 50% support. Scale bar = 0.01 substitutions/site.

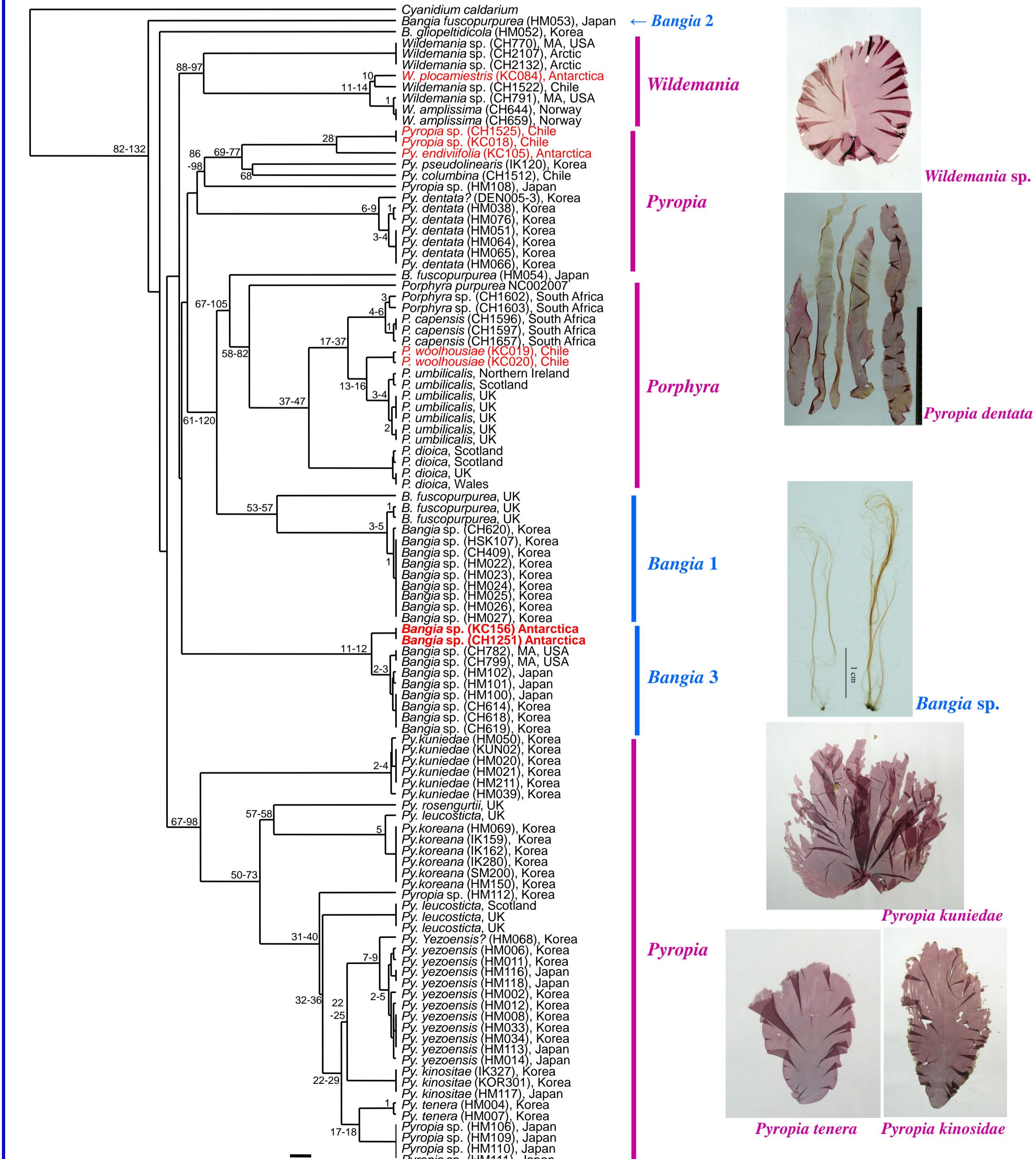


Fig. 10. Phylogram (UPGMA) displaying clustering of the included species for DNA barcoding by *cox1* data in this study. Numbers in the right side of each node indicate numbers of nucleotide changes between related taxa. Scale bar = 5 changes.