

DNA Barcoding: a tool to distinguish species in three taxonomically difficult genera of marine macroalgae.

DNA barcoding for species identification

A consortium of researchers is undertaking a large-scale project to discover and identify all eukaryotes in Canada using the DNA barcode (www.bolnet.ca). The DNA barcode (~650 bp of the 5' end of the mitochondrial cytochrome c oxidase 1 gene; *cox1-5'*) has been used to distinguish species and discover new species (1). DNA barcoding will become a standardized, rapid species identification tool, with a relatively low cost. As part of this project our lab is using the DNA barcode to identify all algae in Canada. Marine macroalgae are ubiquitous and critically important components of coastal ecosystems.

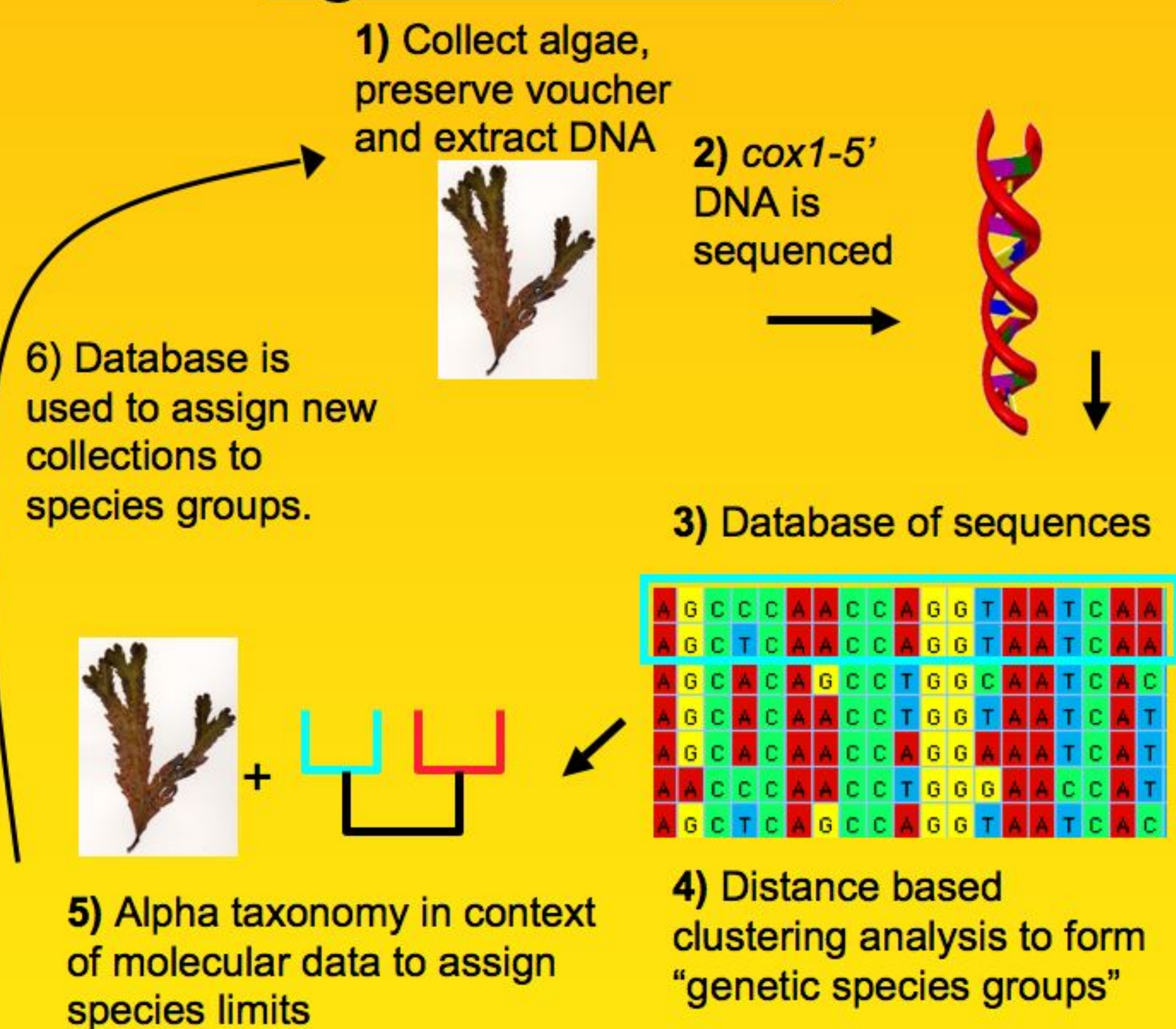


Fig. 1 The diverse Canadian intertidal.

Why barcode algae?

The Canadian coastline, the longest of any country in the world, represents habitat for an estimated 700 species of intertidal and subtidal marine algae (Fig. 1). Unlike many plants and animals, algae have a paucity of morphological characteristics on which to base taxonomy, exhibit morphological plasticity and convergent evolution leading to the presence of cryptic species and making identification extremely difficult or often impossible in the field—even for the expert. *Porphyra*, *Ulva* and *Fucus* are particularly prone to these difficulties and the DNA barcode provides an objective tool for species identification.

Developing the DNA barcode for algal identification



Collections

Collections from the Atlantic and Pacific and Arctic coasts of Canada, are made throughout the year and represent the full range of habitats and seasons for each genus. Atlantic collection localities include: the Bay of Fundy, Newfoundland, Nova Scotia south shore and Cumberland straight. Pacific sites include: Vancouver Island, Burrard Inlet and Prince Rupert.

Fucus

Fucus spp. (rockweed; Fig. 4) are some of the most common intertidal brown seaweeds. Traditionally, ten taxa were recognized in Canada, however, a recently published mitochondrial phylogeny (3) recognized only four.

Our DNA barcoding results (Fig. 5) were able to assign collections to species in all cases except for the closely related *F. spiralis* and *F. vesiculosus*.



Fucus results

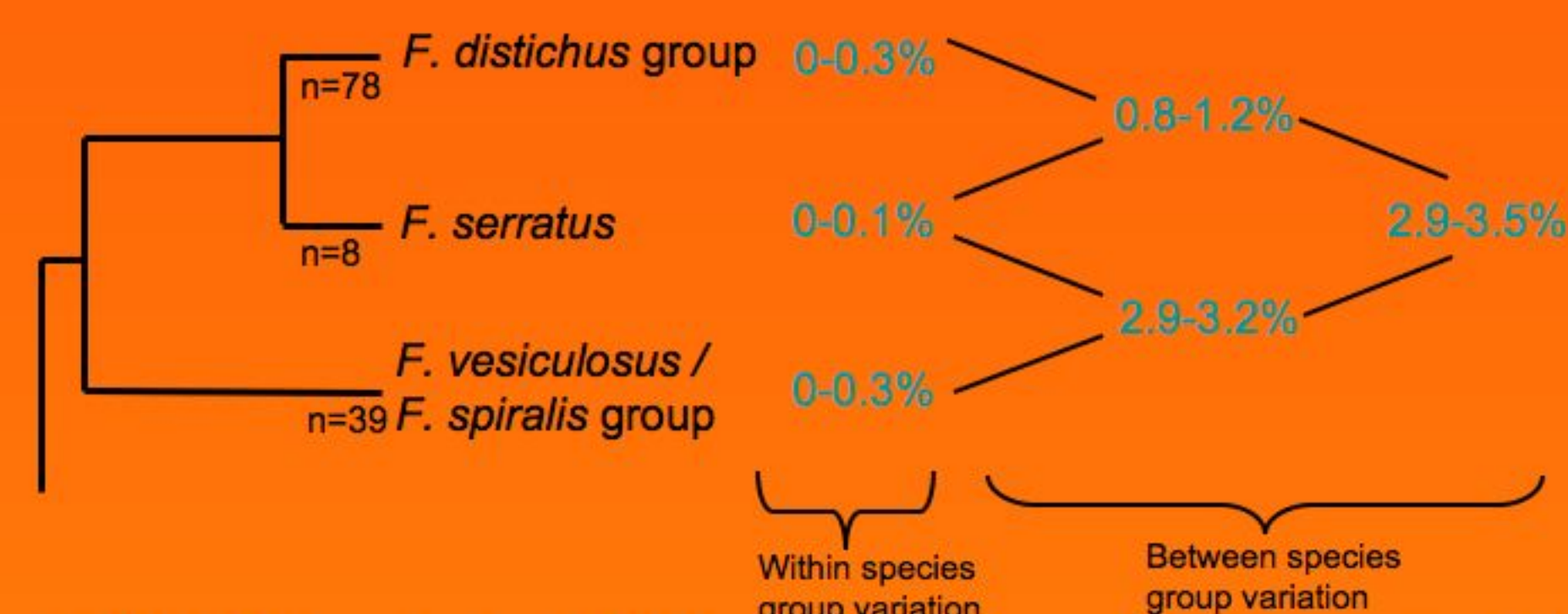


Fig. 5. UPGMA analysis of DNA barcode sequence data. Percent variation within and between species groups shown on right.

Phenotypic plasticity in Pacific *F. distichus*

- very large strap-like morphs on exposed rocky coasts
- indistinguishable from the relatively rare *F. spiralis* in several habitats
- tiny moss-like fronds embedded in estuarine mud

Niche exclusion in Pacific *F. distichus*

- in absence of competition, fills estuarine mud niches that are filled by *Fucus spiralis* and *F. vesiculosus* in the Atlantic



Fig. 6. A few examples of morphological variation in Pacific *F. distichus*. a) morph indistinguishable from *F. spiralis* b) mud embedded form c,d) typical long, strap-like morph



Porphyra

- Known world wide as nori, the sushi seaweed
- In Canada: 8 species recognized in the Northwest Atlantic; 20 in the Northeast Pacific
- Species all have the same general blade-like morphology.

Difficulties in identifying specimens

Characteristics used to distinguish species:	Problems with these characters can include:
Number of cell layers in blade	limited utility (only 2 states)
Blade colour and shape	Subjective, variable
Adherence to herbarium paper	Difficulty in field assessment
Aspects of reproduction: a) monoecy v.s. dioecy b) arrangement of reproductive cells in thallus	Absence of reproductive characteristics in vegetative collections

Porphyra results

The DNA barcode works well to delimit species of *Porphyra*, and has also revealed cryptic species diversity (Fig. 3 and (2)). In general, we observe ~ 10-18% sequence difference between species and ~0-1% within species.

Porphyra results continued

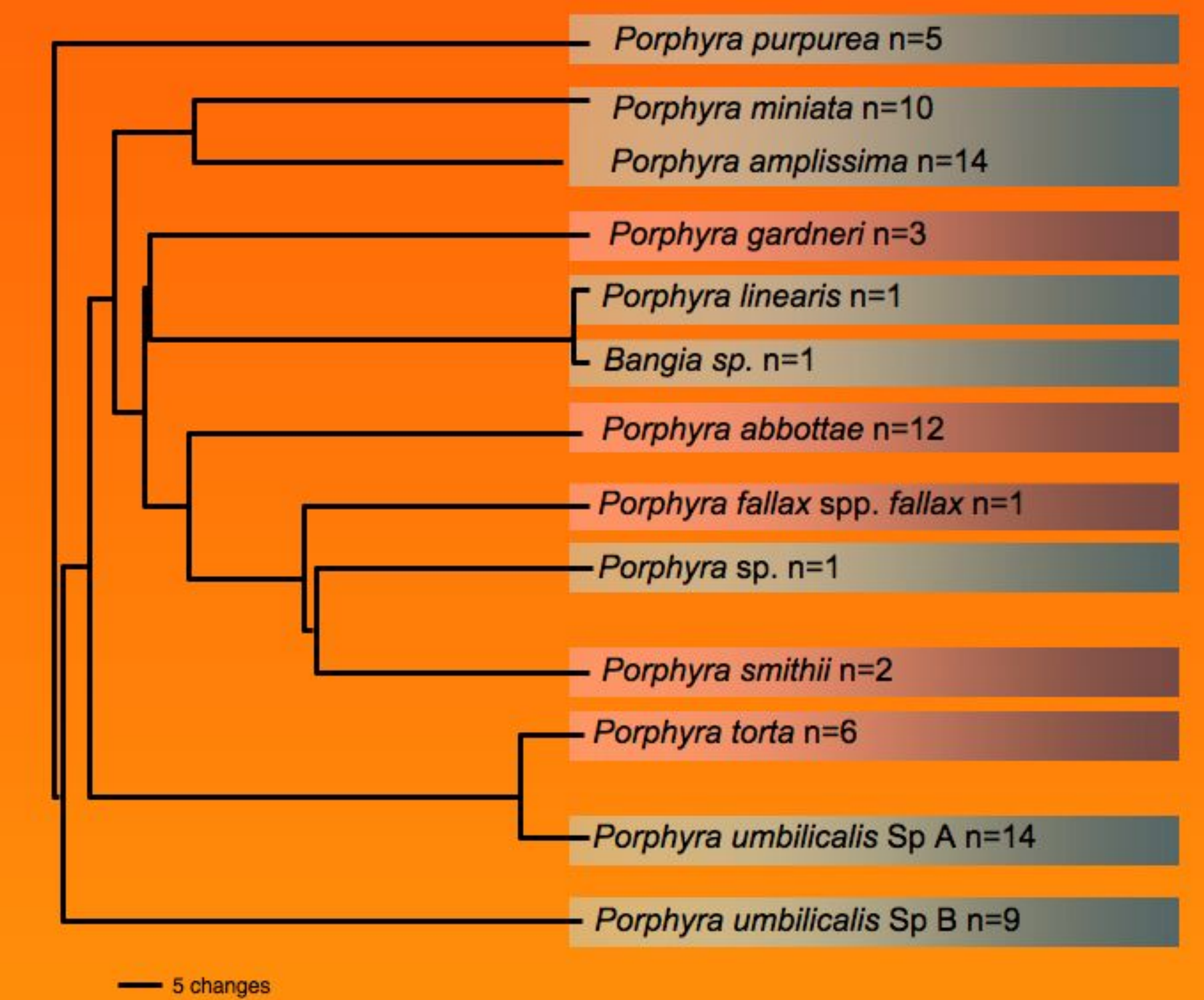


Fig. 3. UPGMA analysis of DNA barcode data for *Porphyra*. Atlantic collections shaded grey, Pacific shaded pink. *P. umbilicalis* forms two genetic species groups suggesting that cryptic species exist.

Ulva

There are ten species recognized in the Northeast Pacific, and nine in the Northwest Atlantic. *Ulva* species are distinguished based on morphological characters such as: whether thalli are tubular or blade-like (Fig. 7), number of cell layers (Fig. 8a) in and along the length of the thallus, presence of teeth-like margins (Fig. 8b) on the blades, number of pyrenoids, cell size and dimensions, and branching.



Fig. 7 Blade-like morphology of *Ulva*

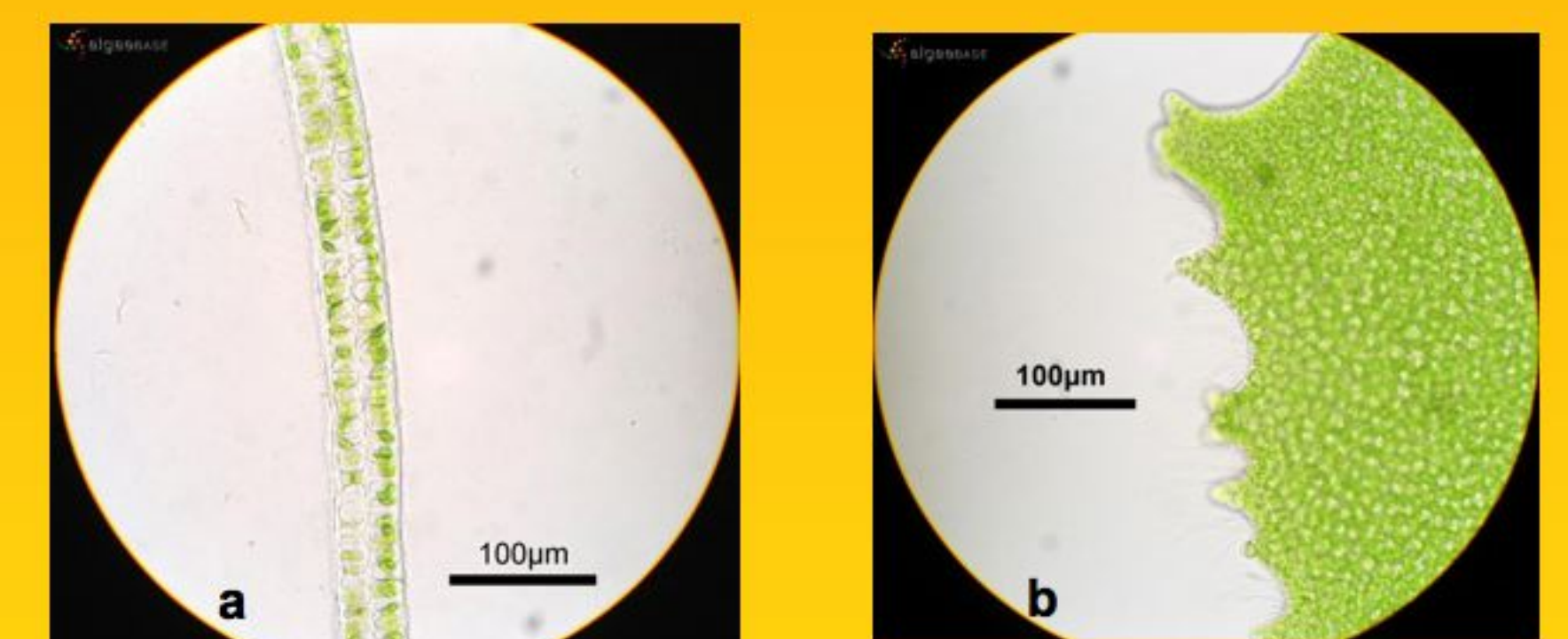


Fig. 8. Examples of characters used to distinguish *Ulva* species. a) cross-section of blade-like species b) tooth-like margins

However, recent molecular studies have questioned the taxonomic utility of these characteristics (4). The need for a consistent, rapid, and objective species diagnostic tool is paramount in this genus.

Green algal DNA barcoding future work:

We have designed a number of *cox1-5'* primer combinations based on published ulvophyte mitochondrial genomes. However, the presence of introns has led us to abandon *cox1-5'* as a suitable marker for green algal DNA barcoding.

For various reasons, the *cox1* is not suitable for DNA barcoding in land plants. Alternate genes such as *rbcl*, *matK*, and *rpoC1* as well as a few others are under consideration. We will investigate the feasibility and utility of these genes for use in DNA barcoding of green algae.

References

1. Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 313-321.
2. Robba L, Russell SJ, Barker GL, Brodie J (2006) Assessing the use of the mitochondrial *cox1* marker for use in DNA barcoding of red algae (Rhodophyta). *American Journal of Botany* **93**, 1101-1108.
3. Coyer JA, Hoarau G, Oudot-Le Secq MP, Stam WT, Olsen JL (2006) A mtDNA-based phylogeny of the brown algal genus *Fucus* (Heterokontophyta; Phaeophyta). *Molecular Phylogenetics and Evolution* **39**, 209-222.
4. Hayden HS, Blomster J, Maggs CA, et al. (2003) Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycology* **38**, 277-294.

Acknowledgements

Funding was provided by the Natural Science and Engineering Research Council of Canada, the Canada Research Chair Program, and Genome Canada through the Canadian Barcode of Life Network. Several figures are courtesy of www.algaebase.org. We thank past and present members of the Saunders lab (Bridgette Clarkston, Susan Clayden, Line LeGall, Sarah Hamsher, Dan McDevit, Brian McDonald, Marina Morabito, Haseeb Randhawa) as well as Graham Cox, Colin Curry, Chris Kolacz, Vanessa Paesani, and Dennis Wong for helpful discussions and advice. Particular thanks go to Andrew Blakney for generating sequence data. Field and technical assistance provided by: Ali Johnson, Hana Kucerova, Tanya Moore, Dave Riddell, Jose Utge Buil, Adrian Utge LeGall.