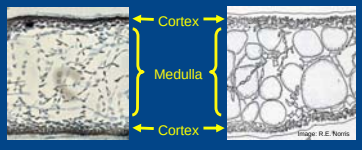
Delimiting species of the red algal family Kallymeniaceae (Rhodophyta) in British Columbia, Canada using molecular & morphological characters

Bridgette Clarkston & Gary W. Saunders
Dept. of Biology, University of New Brunswick, Fredericton, N.B.

Introduction:

Along the coast of B.C. the majority of species in the family Kallymeniaceae are taxonomically challenging. Morphological and anatomical traits traditionally used for identification are often subject to variability, making species discrimination difficult. As such, there are potentially many cryptic species in the flora that have been overlooked. We utilize a taxonomic approach that involves both molecular and morphological characters to delimit species. Here, we present a combination of molecular, morphological and anatomical results for members of the genera *Pugetia* and *Euthora*. Currently, two species of *Pugetia* (*P.firma* & *P.fragilissima*) and one species of *Euthora* (*E.cristata*) are recognized in B.C.

a) Cell type/arrangement in medulla



b) Number of carpogonial branches / supporting cell

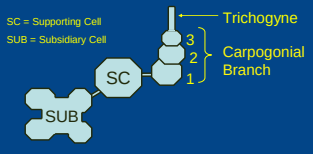


Fig.1. Some characters traditionally used to delimit members of the Kallymeniaceae include a) medullary cell characters (eg. loose filaments vs. filaments and densely packed isodiametric cells), b) carpogonial branches / supporting cell (one to many), & vegetative traits (branching pattern, branching order, etc.)[1].

Molecular Tools:

- Assign individual collections to genetic 'species' groups based on DNA sequences[2]
- Have been shown to delimit effectively species of macroalgae[3]
- Many tools available. We use the DNA barcode (Standardized portion of the *Cox*I gene).

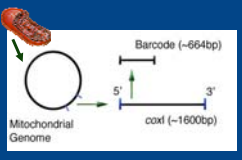


Fig. 2. Diagram of DNA barcode location in the mitochondrial genome.

Objectives:

1. Resolve species diversity of *Pugetia* and *Euthora* in B.C. using the DNA barcode
2. Establish consistent traits for species identification
   - morphological - compare traits by eye
   - anatomical - examine vegetative & reproductive sections

Methods: Acquiring DNA Sequences

- Voucher specimen pressed on day of collection
- Sample for DNA dried in silica
- PCR and sequence target gene region
- Edit & align sequence by eye

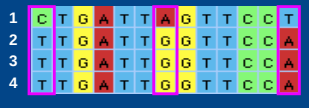


Fig. 3. Portion of DNA alignment. Boxes highlight nucleotide sites where sequence **1** differs from **2**, **3** & **4**.

Fig. 4. a) Satellite map of sampling sites in British Columbia, Canada b) Collecting sites from Vancouver Is.

Methods: Identifying genetic 'species' groups

Phylogram generated based on distances between sequences[4]

- Use visual displays of DNA barcode variation within and between 'species'[5]

Low nucleotide variation within species:
0-3 differences (~0 - 0.5%)

High nucleotide variation between species:
> ~30 differences (> ~ 4.5 - 5%)

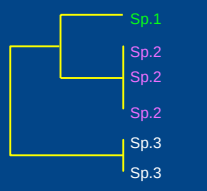


Fig. 5. Sample UPGMA tree demonstrating how similar sequences cluster together into 'species' groups.

Results: Molecular

DNA Barcode indicates there are more spp. within *Pugetia* & *Euthora* in B.C. than currently recognized:

- *Pugetia*: 5 spp. (2 recognized)
- *Euthora*: 2 spp. (1 recognized)

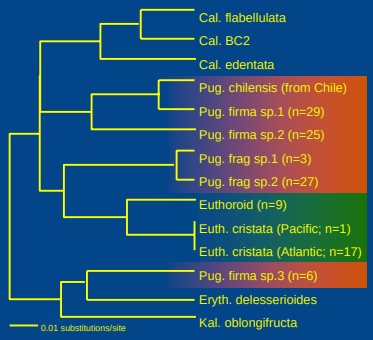


Fig. 6. UPGMA phylogram of DNA Barcode results. Each taxon represents a separate 'species' group (n = # of samples within a group).

Results: Morphological / Anatomical

	E.cristata	'Euthoroid '
Habitat	- Subtidal / low intertidal - Epilithic & epiphytic - Atlantic & Pacific	- Subtidal - On worm tubes - Pacific; all one site
Thallus Height	47.6mm	52.8mm
Axes Width	1mm or less, even at base	1mm or greater
Cortex	1 cell layer; cells 'taller ' than 'wide '	1-2 cell layers; cells 'wider ' than 'tall '
Misc.	Very few encrusting animals	Majority of thallus covered in animals

Table 1. Preliminary results of differences between *Euthora cristata* and Euthoroid 'species' groups.

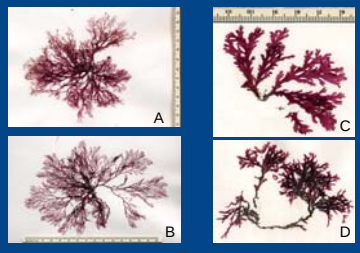


Fig. 7. Voucher specimens of *Euthora cristata* (A & B), and 'Euthoroid' (C & D).

	P.firma sp. 1	*P.firma* sp. 2
Habitat	- Low intertidal / subtidal - Epilithic	- All subtidal -Epilithic & epizoic
Thallus Height Cortex	47.7 mm 1-2 cell layers	80.0mm 3-4 cell layers
Tetrasporangia	In cortex, cruciate , scattered	In cortex, cruciate , scattered

Table 2. Preliminary results of differences between *Pugetia firma* sp.1 and *P.firma* sp.2 'species' groups.

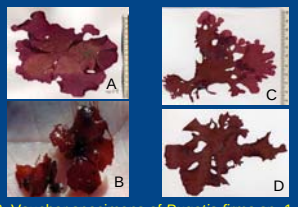


Fig. 8. Voucher specimens of *Pugetia firma* sp. 1 (A & B), and *P. firma* sp. 2 (C & D).

	P.frag . sp.1	*P.frag* . sp.2
Habitat	-Subtidal -Epilithic	-Subtidal -Epilithic, -phytic , -zoic
Thallus: Height Width Thickness Colour Cortex	40.4mm (127mm) 55.0mm (189mm) 108 µm Brick-red (dried) 1 layer, rarely 2	44.4mm (138mm) 53.2mm 202 µm Deep fuchsia (dried) 1-2 layers
Tetrasporangia	Cruciate , embedded in cortex	Cruciate , embedded in cortex

Table 3. Preliminary results of differences between *Pugetia fragilissima* sp.1 and *P.frag.* sp.2 'species' groups.

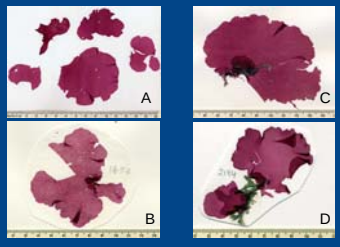


Fig. 9. Voucher specimens of *Pugetia fragilissima* sp. 1 (A & B), and *P. frag.* sp. 2 (C & D).

Conclusions & Future Work:

- Cryptic diversity exists within the Kallymeniaceae of Canada that until now has been overlooked: 1 sp. *Euthora* to 2 spp. and 2 spp. of *Pugetia* to 5 spp.
- An integrative taxonomic method using both molecular and morphological data is an effective way to delimit species of red algae
- Other genera in the Kallymeniaceae need to be examined in detail

Acknowledgements & References:

J. Lawrence, A. Nedelcu S. Clayden, S. Hamsher, H. Kucera, A. Johnson, L. Le Gall, D. McDevit, B. McDonald, T. Moore, H. Randhawa

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