Taxonomic Update of Historic Nori Specimens Using Molecular Methods

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Introduction

Nori is the common name for two genera of red algae: *Porphyra* and *Pyropia.* It is the seaweed that is used to make sushi, which is a 1.5 billion dollar industry in the US alone, making these very economically important organisms (Reddy et al. 2010). Recently, *Porphyra* underwent a taxonomic update, and some species were reclassified as *Pyropia* (Sutherland 2011). *Porphyra* and *Pyropia* species can sometimes have identical morphology, so misidentification is common (Reddy et al. 2010). Also, it is possible that individuals of the same species can look vastly different, again resulting in misidentification (Reddy et al. 2010). Molecular methods can assist in updating Herbaria collections as well as global species distribution

In 2015, an undergraduate student used molecular data to identify four species of *Porphyra* and *Pyropia* (Table 1) that had never before been observed in the Long Island Sound (Nencetty 2015).

Table 1. The historic and new distribution of four non-native *Pyropia* species, as observed by Nencetty in 2015.

Species	Native Distribution	New Records
Pyropia parva	Europe	Connecticut, R.I.
Pyropia tenipedalis	Asia	Connecticut, Mass.
Pyropia pulchella	Asia	Connecticut, R.I.
Pyropia yezonesis	Europe and Asia	Connecticut, Mass.

The objectives of this study were to:

- Identify historic distributions of non-native nori to determine when it arrived in the Long Island Sound.
- Revise species designation of Herbaria specimens as needed using molecular methods.

Materials and Methods

Sixty specimens of *Porphyra* and *Pyropia* species were sampled from the New York Botanical Garden Herbarium (Figure 1). Genomic DNA was then extracted from the samples using a Qiagen DNeasy Plant Mini Kit, modified so the samples were left to lyse overnight. Primers specific to these species of algae were designed, for both the nuclear encoded SSU rRNA and chloroplast encoded *rbcL* (Table 2). These primers were designed to yield short fragments to ensure they would amplify older, potentially degraded samples. Using these primers, Polymerase Chain Reaction (PCR) was then used to amplify the sequences and gel electrophoresis was used to confirm size. PCR products were sent to Yale to be sequenced, and the sequences were compared to known sequences to determine species identification.

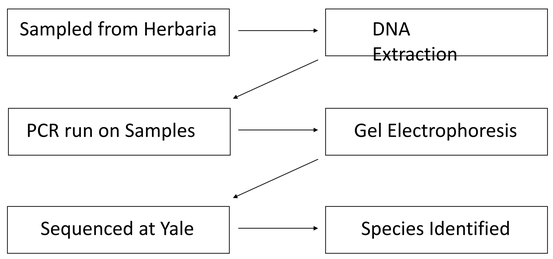

Figure 2. A flow chart of the process performed on each sample.

Figure 1. Examples of *Porphyra* herbarium presses of two different species, highlighting their similar morphology (Macroalgal Herbarium Portal).

Results and Discussion

Of the 60 samples, 40 have been sequenced and 27 were successful in yielding an identification. Out of the 8 primer pairs that were used, three were successful in yielding a positive identification, while only one almost always sequenced fungus or bacteria (Table 2). Three out of the four species new to the Long Island Sound area observed by Nencetty (2015) were identified among these old specimens, and have been here for as long as 135 years (Table 3). Almost none of the identifications on the Herbaria labels were correct, revealing the need for molecular work to be done on older specimens. Many of the species identified were first reported in the New England area in the early 2000's; this study shows that these species have actually been here over a century. The oldest specimen sequenced was one of the species never previously recorded in North America, further justifying the need for molecular identification on cryptic species and the utility of historic herbarium specimens in examining species distributions..

Table 2. The primers used in this study and their rate of success sequencing historic algae. Primer PCW321 was taken from Carlile et al (2010).

Gene	Primer Pair		Length of Segment	Success Rate
	Forward	Reverse		
SSU	192 FOR	350 REV	158	17%
SSU	330 FOR	517 REV	187	84%
SSU	496 FOR	668 REV	172	100%
rbcL	92 FOR	240 REV	148	93%
rbcL	221 FOR	417 REV	196	100%
rbcL	398 FOR	546 REV	148	80%
rbcL	795 FOR	951 REV	156	100%
rbcL	PCW321	417 REV	96	75%

Table 3. Select specimens identified through molecular methods and their distribution. A * indicates the species was observed in the study by Nencetty in 2015. The distribution lists only the oldest identified specimen in each location. Bold font indicates the oldest specimens recorded in species newly observed in North America.

Species	Previously Recorded Distribution		My Study Distribution
	North America	Global	
Porphyra purpurea	Alaska and Western Canada Maine (2001)	Europe, Asia	MA (1884)
Pyropia elongata	South Carolina (1991) Connecticut (2007)	Europe	CT (1886) NY (1902)
*Pyropia parva**	**Never in NA**	Spain	**NY (1882)** RI (~early 1900s)
*Pyropia yezoensis**	Alaska (1977) New England (2008)	Asia, Europe	CT (1999)
*Porphyra tenipudalis**	**Never in NA**	Japan	**NJ (1891)** RI (1905) MA (1908) NY (1929)
*Pyropia koreana**	New England (2007)	Korea, Europe	NY (1901)
Porphyra umbicalis	Maine (2001)	Europe, Asia, Africa, S.A	ME (1900)
Porphyra leucosticta	Maine (1957)	Europe, Asia, Africa, S.A	ME (1904)

Conclusion and Future Work

Conclusions

- Herbaria are very important in updating species distribution patterns.
- Numerous *Porphyra* and *Pyropia* species have been in this area since 1882, but since they are all morphologically similar they have been misidentified for years.
- Seven of the eight primers designed were very successful in identifying historic specimens.

Future Work

- Continuation of this project should include sequencing the remaining 20 samples collected from the NYBG.
- All *Porphyra* and *Pyropia* specimens at the New York Botanical Gardens should be taxonomically updated using molecular methods.
- Further species and Herbaria should be updated using molecular methods to ensure correct species identification and species distribution.

References

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