

Supplement 1**Table S1:** Additional guidelines for conducting reliable and reproducible coralline algal species identifications

Method	Notes	Key References
Molecular Identification		
Collecting for DNA sequencing	<p>Collection: Be sure that the sample represents a single species as it is common for margins between corallines to blur and species overgrowth to occur. Only a small subsample (~1x1cm) is needed for DNA extraction.</p> <p>Cleaning: Carefully remove epiphytes and check for endophytes to reduce chances of contamination for DNA extraction. Although markers used are often designed for coralline algae, they can often amplify other species.</p> <p>Preservation: The best way to preserve specimens for DNA extraction is through rapid desiccation. This is usually achieved through the use of silica gel. Wrap the coralline in tissue or thin cloth before placing in silica gel to avoid losing crumbs as the sample becomes brittle on drying. Store specimen in container or sealable bag with silica gel in a dry, cool place out of the light until ready for DNA extraction.</p>	Collection and storage protocols (see Harvey et al. 2005 pg 24-29; Farr et al. 2009 pg 20-22)
Marker selection	<p>The three commonly used markers in coralline algae research are:</p> <p><i>psbA</i> (photosystem II D1 protein)- 852bp length.</p> <p><i>rbcL</i> (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) - 1467bp length. Typically amplified in two parts. Note that often smaller segments of <i>rbcL</i> ~200bp in length are usually used for old specimens with degraded DNA</p> <p>COI-5P (cytochrome oxidase subunit 1)- 664bp length</p>	<p>Commonly used <i>psbA</i> primers - <i>psbA</i>-F1 in combination with <i>psbA</i>-R2 or <i>psbA</i>-R1 (Yoon et al. 2002).</p> <p>Commonly used <i>rbcL</i> primers - Many different primer combinations (see Freshwater & Rueness 1994; Gabrielson et al. 2011; Hughey & Gabrielson 2012; Hernandez-Kantun et al. 2016; Twist et al. 2019)</p> <p>Commonly used COI-5P primers - Many different primer combinations (see Le Gall & Saunders 2010; Clarkston & Saunders 2012; Saunders & Moore, 2013)</p>

DNA extractions	Many different commercially available DNA extraction kits have been used successfully for coralline algae. These include but are not limited to; Qiagen DNeasy kits, GenElute DNA kits, QuickExtract and NucleoSpin tissue kit	Extractions protocols for coralline algae (see e.g. Hughey et al. 2001; Broom et al. 2008; Gabrielson et al. 2011; Rösler et al. 2016; Anglès d’Auriac et al. 2019; Pezzolesi et al. 2019; Twist et al. 2019)
PCR amplifications	PCR reagents can be sourced from a number of commercial vendors. Protocols for PCR amplifications are dependent on the marker being used, often with different annealing temperatures and times.	<i>psbA</i> - (e.g. Broom et al. 2008; Richards et al 2014; Adey et al. 2015a; Twist et al. 2019) <i>rbcL</i> - (e.g. Adey et al. 2015a; Hernandez-Kantun et al. 2016; Twist et al. 2019) COI-5P - (e.g. Richards et al 2014; Peña et al. 2015)
Sequencing	Various commercial agencies exist for Sanger sequencing of amplified PCR products.	A search of Sanger sequencing will reveal several agencies where PCR products can be sent to.
Species Identification		
Sequence trimming and cleaning	Often sequences need to be assessed for quality and the ends trimmed before these are compared to other sequences in an online database. There are various programs designed for this (e.g. Geneious, MEGA, BioEdit, ClustalW2).	Additional information on sequence editing can be found in MacManes 2014
GenBank (sequence databases)	GenBank and other sequence databases (e.g. BOLD) are good places to compare sequence data. However, the names applied to sequences in these databases are sometimes out of date and/or unreliable.	GenBank entries can be searched using a BLAST query of a nucleotide sequence (https://blast.ncbi.nlm.nih.gov/)
Taxonomy and Taxonomic guidelines	Algaebase is a great starting point for up to date taxonomy on coralline algae research.	https://www.algaebase.org/
Morpho-anatomical identification	Due to coralline algae taxonomy rapidly changing, published	Primary literature and Algaebase

<p><i>(Not recommended)</i></p>	<p>identification guides often include outdated information on taxonomic names therefore should be used with extreme caution. Recent taxonomic primary literature can be consulted for updated species descriptions and defining characteristics (although often there are very few defining characters for newly described coralline species). Additionally, Algaebase can be a great resource for the status of currently accepted names and also provides links to key references.</p>	
<p>Voucher specimen storage</p>	<p>Depositing voucher specimens in a recognized herbarium can be done at relatively low cost and provides a long-term record long after an article has been published. These vouchers can therefore be compared and re-examined many years later.</p>	<p>A list of globally registered herbarium can be found at http://sweetgum.nybg.org/science/ih/ A detailed outline of best practices for herbarium care of coralline algae can be found in Appendix 1 of Nelson et al. 2019</p>

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