**Supplementary Material File S1.** Barcoding sequencing.

Exploratory analysis of genetic divergence between Puerto Rican populations of *C. sandstedei* was carried out by generating single-locus data for three samples from Maricao and three samples from Vega Baja. This work entailed obtaining sequences for the Translation Elongation Factor 1-Alfa (TEF-1α) and the RNA polymerase I subunit II (RPB2). Internal Transcribed Spacer (ITS) sequences for these and two additional *C. sandstedei* subsp. *cubana* specimens were also generated but were not part of the exploratory analysis (Table S3).

Primers and PCR conditions used in this study are described in Table S1. PCR amplification and sequencing followed protocols described in Mercado-Díaz et al., (2020). Reference sequences were downloaded from GenBank or obtained from Rebecca Yahr (Table S2).

Table S1. Primers and PCR conditions used for single-locus sequencing.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Locus** | **Primer** | **Primer sequence 5’-3’** | **PCR protocol** | **Reference** |
| Translation elongation factor 1-alpha(~ 1,000 bp)Program: EF1TD | EF1-526fEF1-1567R | GTC GTY GTY ATY GGH CAY GTACH GTR CCR ATA CCA CCR ATC TT | 94°C for 4 mins;10 cycles: 94 °C for 30 s, 66 °C for 30 s (decreasing 1 °C per cycle), 72 °C for 90 s; 30 cycles: 94 °C for 30 s, 56 °C for 30 s, 72 °C for 90 s; 72 °C for 7 mins | Rehner, 2001 |
| RNA polymerase II subunit 2 (RPB2) (~ 800 bp)Program: IGS52\_2 | RPB2-5fRPB2-7cR | GAY GAY MGW GAT CAY TTY GGCCC ATR GCT TGY TTR CCC AT | 94˚C for 3 min; 34 cycles: 94˚C for 45 s, 50˚C for 60 s, 72˚C for 90 s; 72˚C for 7 min | Liu et al., 1999 |
| Internal Transcribed Spacer (ITS) (~600 bp). Program: ITS48 | ITS1FITS4 | CTTGGTCATTTAGAGGAAGTAATCCTCCGCTTATTGATATGC | 94˚C for 5 min; 39 cycles: 94˚C for 30 s, 48˚C for 30 s, 72˚C for 90 s; 72˚C for 5 min | Zoller et al. (1999) |

Table S2. Samples and GenBank accession numbers used for exploratory phylogenetic analysis. New GenBank accession numbers in bold. Exclamation marks show sequences obtained from R. Yahr that are not available in GenBank.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ID** | **Species** | **Area** | **EF1** | **RPB2** |
| LK46 | *Cladonia confusa* | Brazil |  | KP941559 |
| Burgaz 96193 | *Cladonia rangiformis* | Spain | JN811444 | JF288838 |
| DNA15497 | *Cladonia sandstedei* subsp*. landroniana* | Maricao, PR | **PQ217219** | **PQ217213** |
| DNA15498 | *Cladonia sandstedei* subsp*. landroniana* | Maricao, PR | **PQ217220** | **PQ217214** |
| DNA15499 | *Cladonia sandstedei* subsp*. landroniana* | Maricao, PR | **PQ217221** | **PQ217215** |
| DNA15500 | *Cladonia sandstedei* subsp*. landroniana* | Vega Baja, PR | **PQ217222** | **PQ217216** |
| DNA15501 | *Cladonia sandstedei* subsp*. landroniana* | Vega Baja, PR | **PQ217223** | **PQ217217** |
| DNA15502 | *Cladonia sandstedei* subsp*. landroniana* | Vega Baja, PR | **PQ217224** | **PQ217218** |
| RY1004 | *Cladonia subtenuis* | Florida, USA | DQ490098 | DQ522287 |
| RY1123 | *Cladonia subtenuis* | North Carolina, USA | DQ490096 |  |
| RY1128 | *Cladonia subtenuis* | North Carolina, USA | DQ490101 |  |
| RY1129 | *Cladonia subtenuis* | North Carolina, USA | DQ490093 |  |
| RY1151 | *Cladonia subtenuis* | North Carolina, USA | DQ490095 |  |
| RY1189 | *Cladonia subtenuis* | North Carolina, USA | DQ490105 |  |
| RY1190 | *Cladonia subtenuis* | North Carolina, USA | DQ490104 | DQ522286 |
| RY1208 | *Cladonia subtenuis* | Georgia, USA | ! | DQ522282 |
| RY1210 | *Cladonia subtenuis* | Georgia, USA | ! | DQ522283 |
| RY1213 | *Cladonia subtenuis* | Georgia, USA | ! | DQ522284 |
| RY1215 | *Cladonia subtenuis* | Georgia, USA | DQ490102 |  |
| RY1216 | *Cladonia subtenuis* | Georgia, USA | DQ490100 |  |
| RY1224 | *Cladonia subtenuis* | Pennsylvania, USA | ! | DQ522289 |
| RY909 | *Cladonia subtenuis* | Florida, USA | DQ490103 |  |
| RY910 | *Cladonia subtenuis* | Florida, USA | DQ490091 |  |
| RY911 | *Cladonia subtenuis* | Florida, USA | DQ490097 |  |
| RY913 | *Cladonia subtenuis* | Florida, USA | DQ490092 |  |
| RY941 | *Cladonia subtenuis* | Florida, USA | DQ490094 |  |
| RY942 | *Cladonia subtenuis* | Florida, USA | ! | DQ522285 |
| RY943 | *Cladonia subtenuis* | Florida, USA | DQ490099 |  |
| RY999 | *Cladonia subtenuis* | Florida, USA | ! | DQ522288 |

Table S3. Samples and GenBank accession numbers for Internal Trancribed Spacer sequences generated during sequencing efforts for the present study.

|  |  |  |  |
| --- | --- | --- | --- |
| **ID** | **Species** | **Locality** | **ITS** |
| DNA15498 | *Cladonia sandstedei* subsp*. landroniana* | Maricao, PR | PQ219004 |
| DNA15499 | *Cladonia sandstedei* subsp*. landroniana* | Maricao, PR | PQ219003 |
| DNA 17998 | *Cladonia sandstedei* subsp*. landroniana* | Maricao, PR | PQ219005 |
| DNA15500 | *Cladonia sandstedei* subsp*. landroniana* | Vega Baja, PR | PQ219006 |
| DNA15501 | *Cladonia sandstedei* subsp*. landroniana* | Vega Baja, PR | PQ219008 |
| DNA15502 | *Cladonia sandstedei* subsp*. landroniana* | Vega Baja, PR | PQ219007 |
| DNA18400 | *Cladonia sandstedei* subsp*. cubana* | Cuba | PQ219002 |
| DNA18401 | *Cladonia sandstedei* subsp*. landroniana* | Cuba | PQ219001 |

**References**

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Rehner, S.A., 2001. Primers for Elongation Factor 1-alpha (EF1-alpha).