Supplementary Materials

Unification of the type method with modern systematics using genomics

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Supplementary Methods

High-Throughput Genomics Center TruSeq Library Construction Protocol 06/28/11 mw

1- Initial fractionation step is skipped for degraded herbarium DNA

2- End-repair

Materials:

- Fractionated input DNA
- T4 DNA ligase buffer with 10mM ATP (NEB #B0202S)
- dNTP mix, 10mM each (NEB #N0447S)
- T4 DNA polymerase, 3U/ul (NEB #M0203L)
- Klenow DNA polymerase, 5U/ul (NEB #M0210L)
- T4 PNK, 10U/ul (NEB #M0201L)
- Cooling block or PCR machine
- QIAGEN MinElute kit

1. Prepare the following reaction mix:

Fractionated DNA	18.6ul
T4 DNA ligase buffer with 10mM ATP	2.5ul
dNTP mix	1.0ul
T4 DNA polymerase	1.3ul
Klenow DNA polymerase	0.3ul
T4 PNK	1.3ul
Total Volume	25ul

2. Incubate for 30min at 20^{o}C
3. Purify using MinElute column with Buffer PB. Elute in 10ul of Buffer EB.

3- Addition of an ‘A’ Base to the 3’ End of the DNA Fragments

Materials:

- DNA from last step
- 10X Klenow buffer (use NEB Buffer 2 that comes with Klenow exo^{-})
- dATP, 1mM (Fermentas #R0141)
- Klenow exo^{-} (3’ to 5’ exo minus), 5U/ul (NEB #M0212L)
- Heat block or PCR machine

1. Prepare the following reaction mix:

DNA from last step	10.0ul
Klenow buffer	1.5ul
dATP	3.0ul
Klenow exo^{-} (3’ to 5’ exo minus)	0.9ul
Total Volume	15.4ul

2. Incubate for 30min at 37^{o}C
3. Proceed directly to next step (no clean-up).

4- Ligation of TruSeq Adaptors to the Ends of the DNA Fragments

Materials:

- DNA from last step
- 2X DNA quick ligase buffer (buffer that comes with NEB Quick Ligation kit)
- Appropriate DNA Adapter index (from Illumina TruSeq kit)
- DNA quick ligase, 1U/ul (NEB #M2200L)
- Cooling block or PCR machine
- Stop Ligation Mix (from Illumina TruSeq kit)
- Agencourt AMPure XP kit (Beckman Coulter #A63881)
- Low-bind tubes (Fisher #13-6987-91)

1. Prepare the following reaction mix:

DNA from last step	15.0ul
2x DNA quick ligase buffer	18.9ul
DNA Adapter index (1:10 dilution)	1.9ul
DNA quick ligase	1.9ul
Total Volume	37.7ul

2. Incubate for 15min at 20°C.
3. Add 1ul Stop Ligation mix from Illumina TruSeq kit. Mix well.
4. Purify twice using AMPure XP beads. Elute in 20ul Buffer EB into low-bind tube.

5- AMPure Bead Clean-up

1. Let beads warm to room temp for at least 30 minutes. Mix well before using.
2. Add 1.8x volume (approx. 70ul) AMPure beads to post-ligation product and mix thoroughly by pipetting.
3. Let sit at room temp for 5 minutes.
4. Place on magnetic stand for 2-3 minutes or until clear.
5. Remove supernatant and discard. Do not disturb beads.
6. Add 180ul fresh 70% EtOH and let sit at room temp for 1 minute.
7. Remove supernatant and discard. Do not disturb beads.
8. Repeat EtOH wash two more times.
9. Remove supernatant and discard. Do not disturb beads. Let dry for 15 minutes.
10. Remove from stand. Add 22ul Buffer EB and mix thoroughly by pipetting. Let sit at room temp for 5 minutes.
11. Place on magnetic stand for 2-3 minutes or until clear.
12. Remove 20ul of supernatant and place in a low-bind tube. Do not disturb beads.

6- Enrichment of the PE Adaptor-modified DNA Fragments by PCR

Materials:

- DNA from last step
- PCR grade water
- Phusion High-Fidelity PCR Master Mix with GC Buffer (NEB #F532L)
- PCR primer cocktail, 1:2 dilution (from Illumina TruSeq kit)
- PCR machine

1. Prepare the following PCR reaction mix:

DNA	up to 20ul
Phusion DNA polymerase	25ul
PCR primer cocktail (1:2 dilution)	1ul
Water	4 - 19ul
Total Volume	50ul

2. Use following rule of thumb for library input into PCR – if amount of starting material was 75-100ng, use 5ul of post-ligation product in PCR; 50-70ng, use 10ul; 30-45ng, use 15ul; less than 25ng, use 20ul.
3. Amplify using the following PCR protocol:
    - 30” at 98°C
    - [10”at 98°C, 30” at 60°C, 30” at 72°C] for 18 cycles
    - 5min at 72°C
    - Hold at 4°C

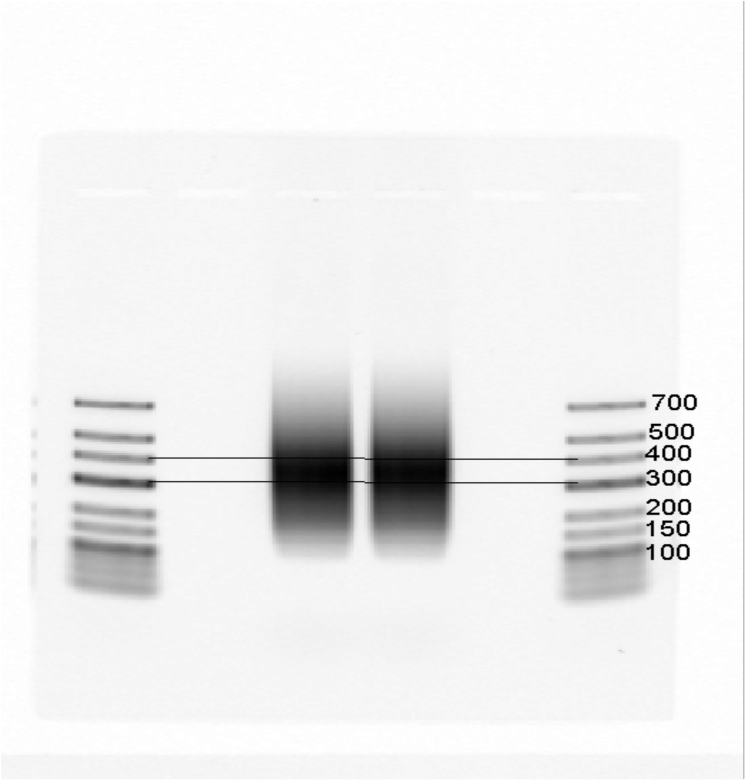
4. Store excess post-ligation product at -20°C.

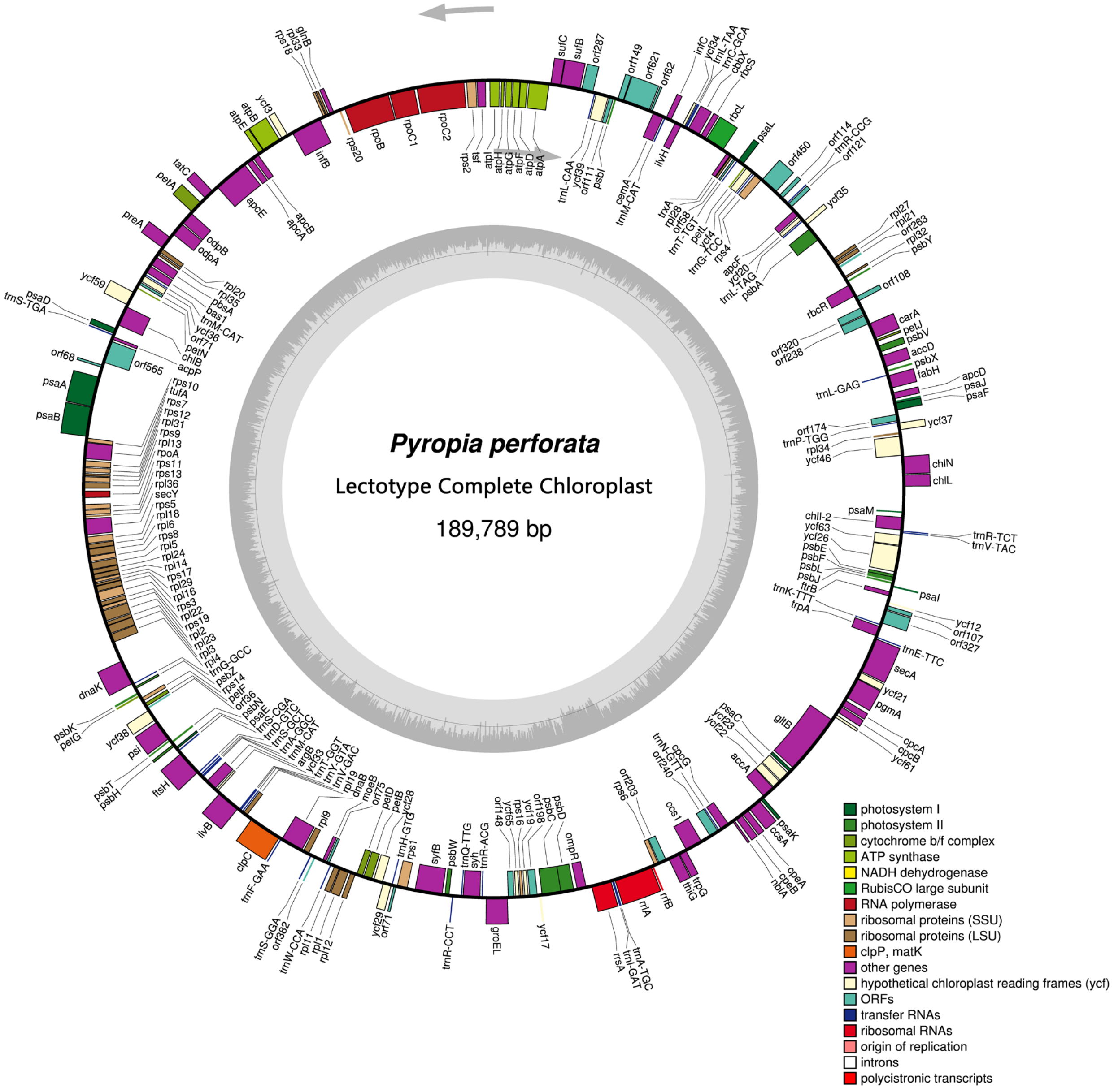
7- Final Fractionation and Clean-up

Materials:

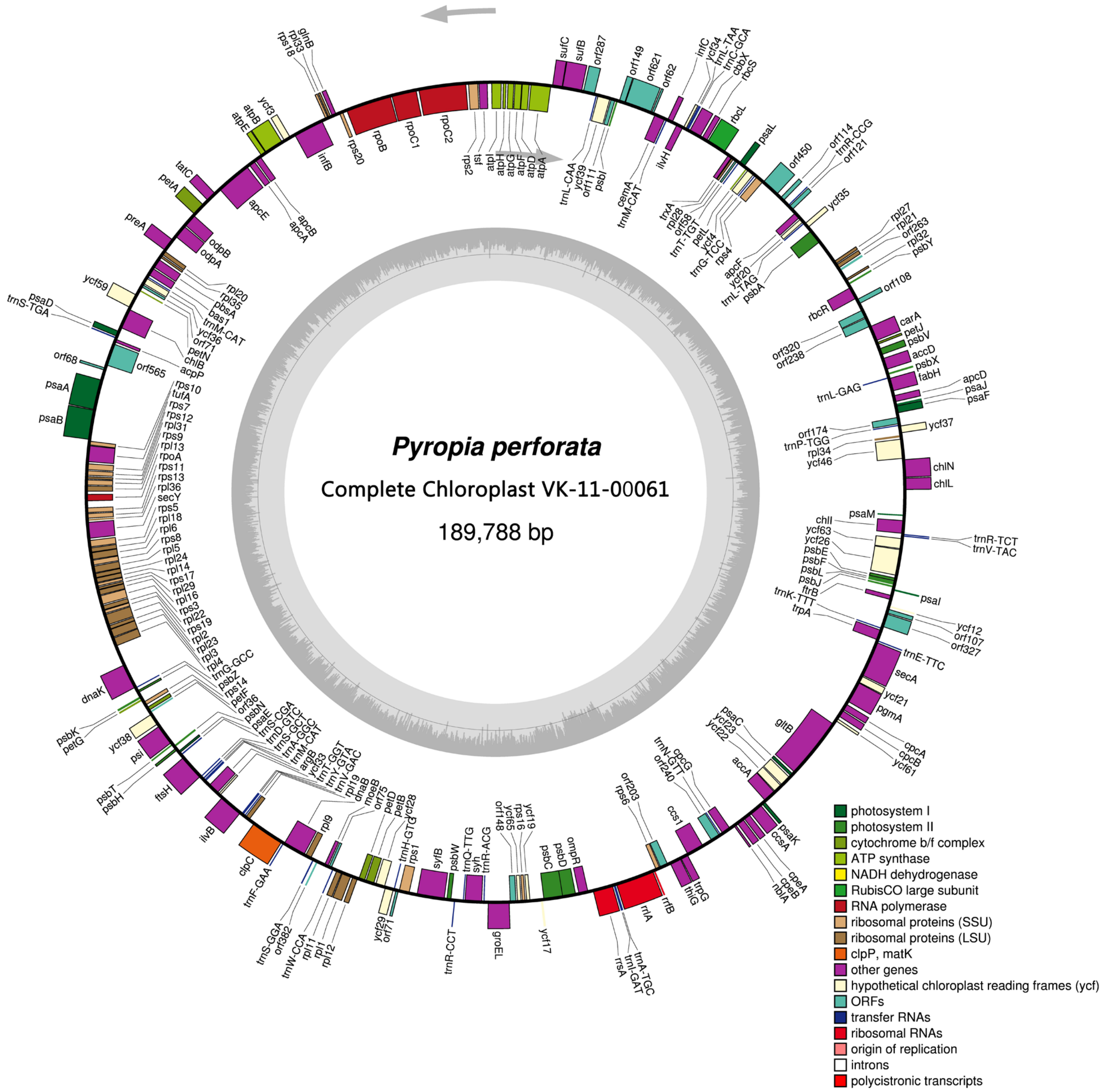
- QIAGEN MinElute gel purification kit
- Isopropanol
- Low-bind tubes (Fisher #13-6987-91)

1. Load entire enrichment product on a 2% agarose gel. Split sample into two lanes.
2. Purify by cutting out darkest band from gel (size will vary depending on input to library). See gel image below for example. TruSeq adapters add approximately 120bp to length of fragments.
3. Pool fragments from both lanes and weigh.
4. Use the MinElute gel purification kit protocol with the following modifications to purify library.
5. Add 6x volume of Buffer QG and allow gel slice to melt at room temp for 30-45min with occasional vortexing.
6. Add 2X volume of Isopropanol and mix.
7. Load sample into a QIAGEN MinElute column with syringe adaptor on vacuum manifold and let sit for 15min to allow small fragments to precipitate.
8. Load sample on to column.
9. Wash with 500ul Buffer QG.
10. Add 750ul Buffer PE and let sit for five minutes.
11. Do two more washes with Buffer PE. Spin to dry column.
12. Add 15ul Buffer EB to column and let sit five minutes. Elute in low-bind tube.
13. Repeat previous step with fresh Buffer EB (final volume is 30ul).
14. Quantify using KAPA library quantification kit with PhiX standards.
15. If making index pools, dilute each sample to 10nM with Buffer EB. Pool equal volumes of each sample for final pool of 10nM.

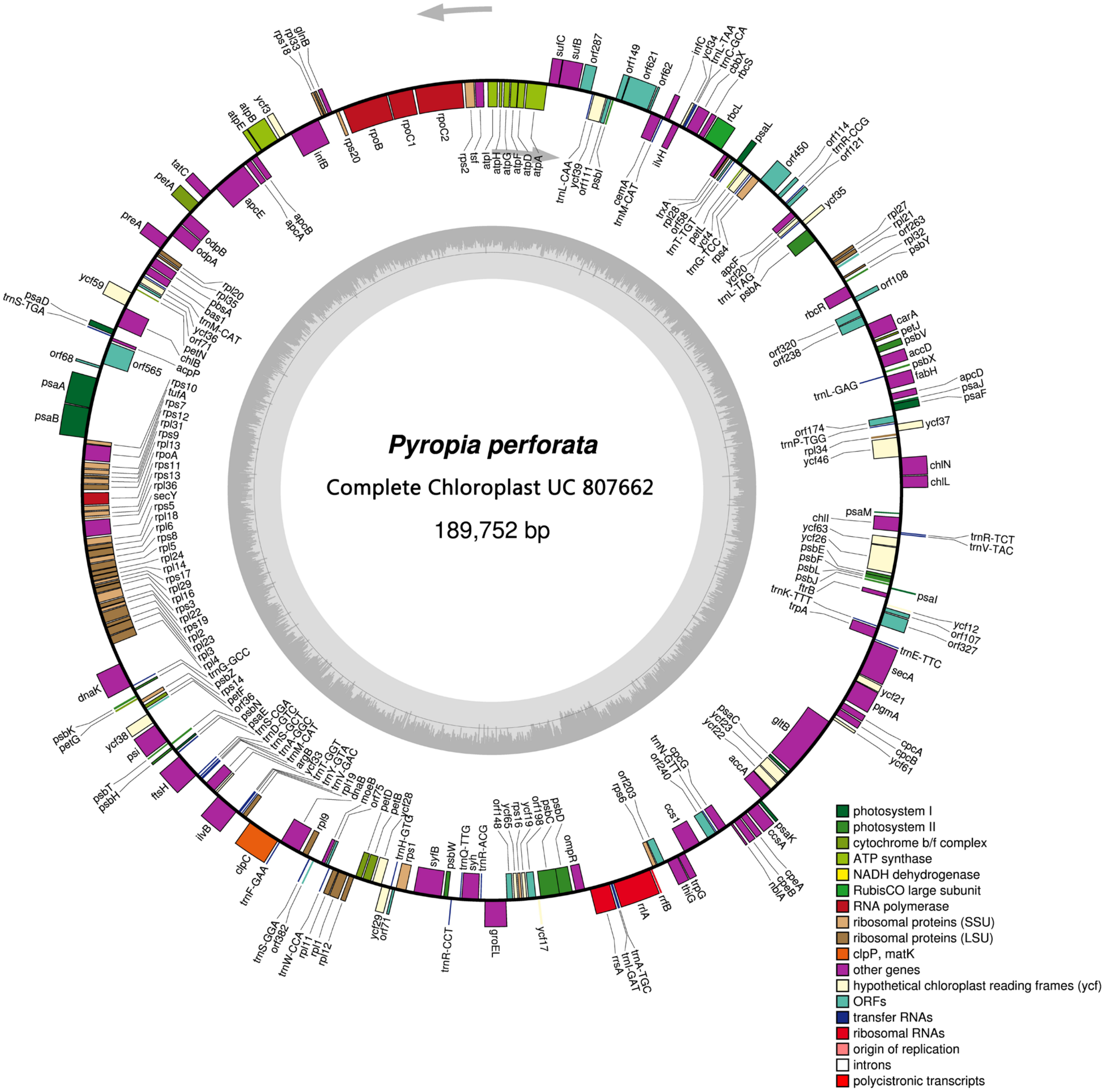




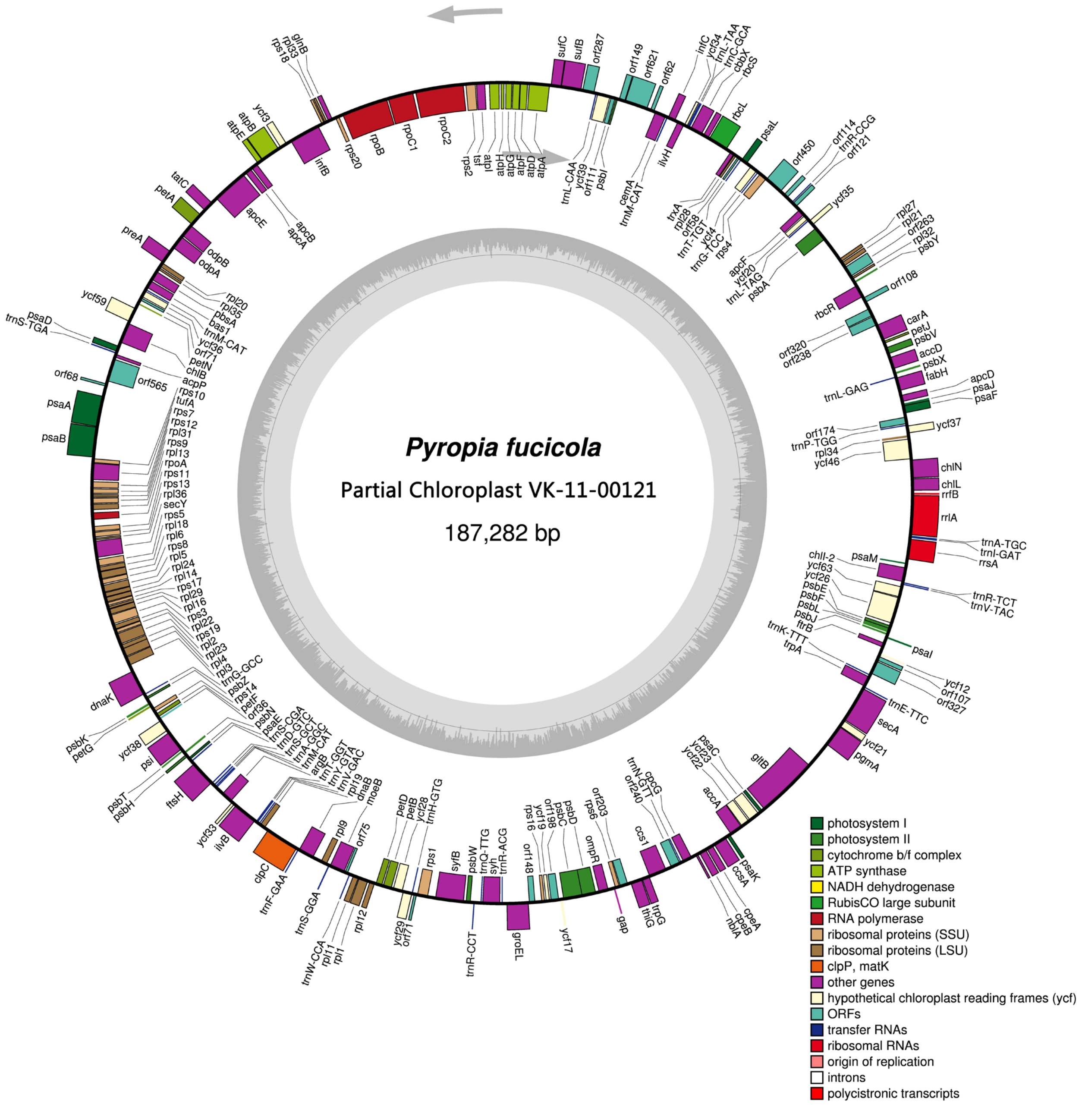
Supplementary Figure 1. The circular-mapping chloroplast genome of the lectotype specimen of *Pyropia perforata* (LD-Ag 13037, Near Golden Gate, San Francisco, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



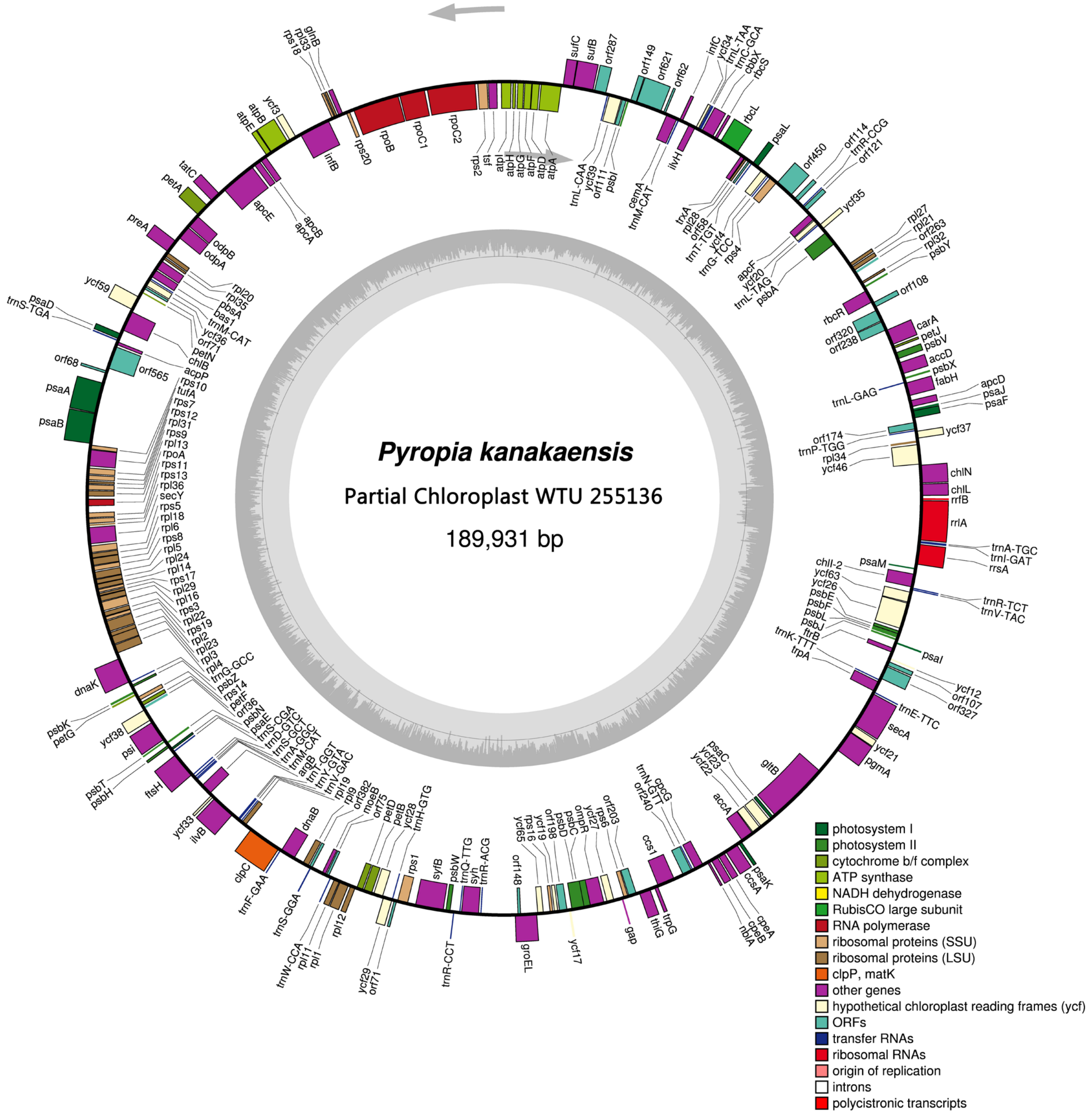
Supplementary Figure 2. The circular-mapping chloroplast genome of the holotype specimen of *Porphyra sanjuanensis* (VK-11-00061, Minnesota Reef, San Juan Island, Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



Supplementary Figure 3. The circular-mapping chloroplast genome of the syntype specimen of *Porphyra perforata* f. *segregata* (UC 807662, La Jolla, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].

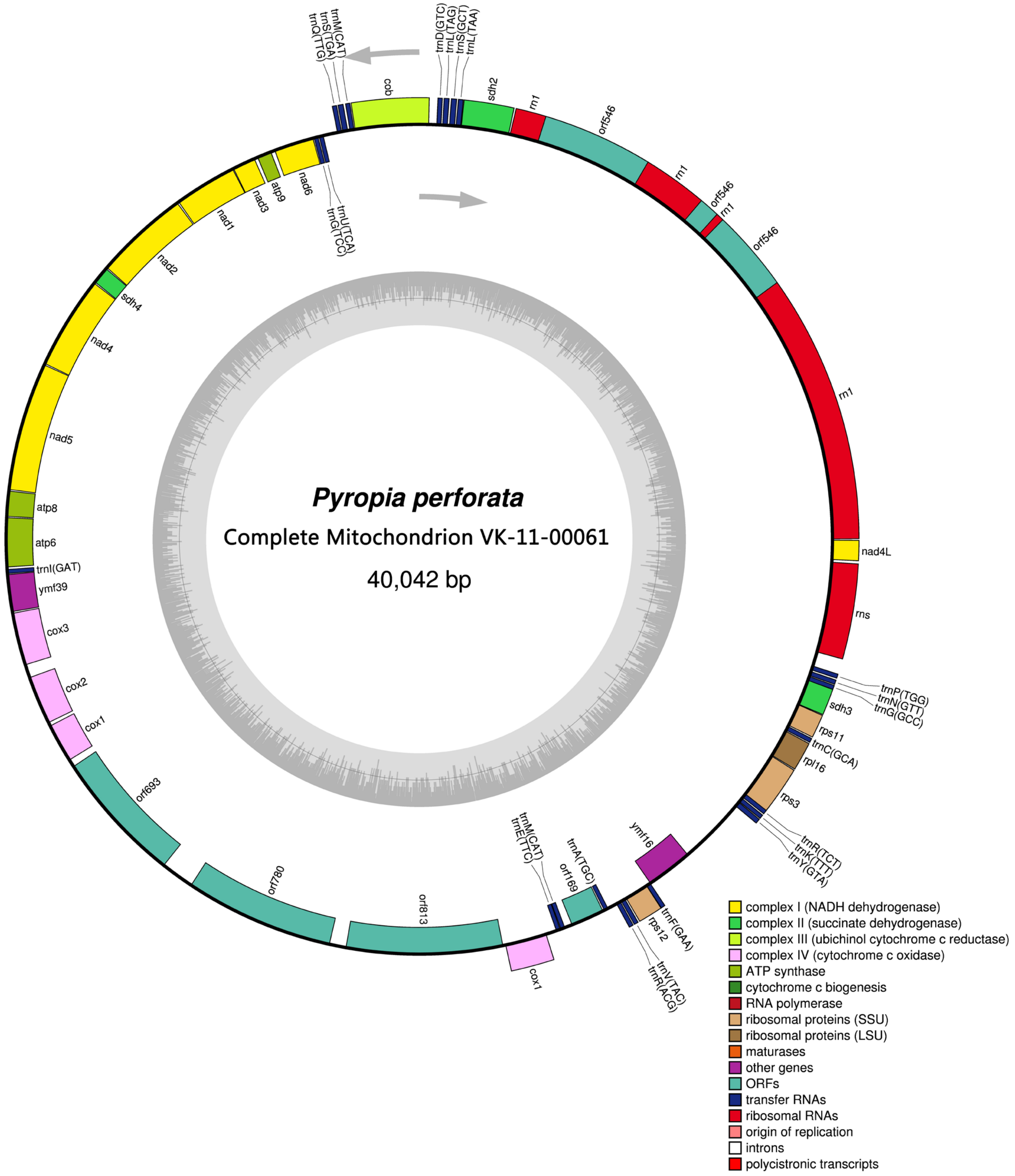


Supplementary Figure 4. The circular-mapping chloroplast genome of the holotype specimen of *Pyropia fucicola* (VK-11-00121, Makah Bay, Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The region identified as 'Gap' is predicted to represent an approximately 4.8 kb non-identical ribosomal 16S, 23S, and 5S repeat. The map was drawn using OGDRAW[1].

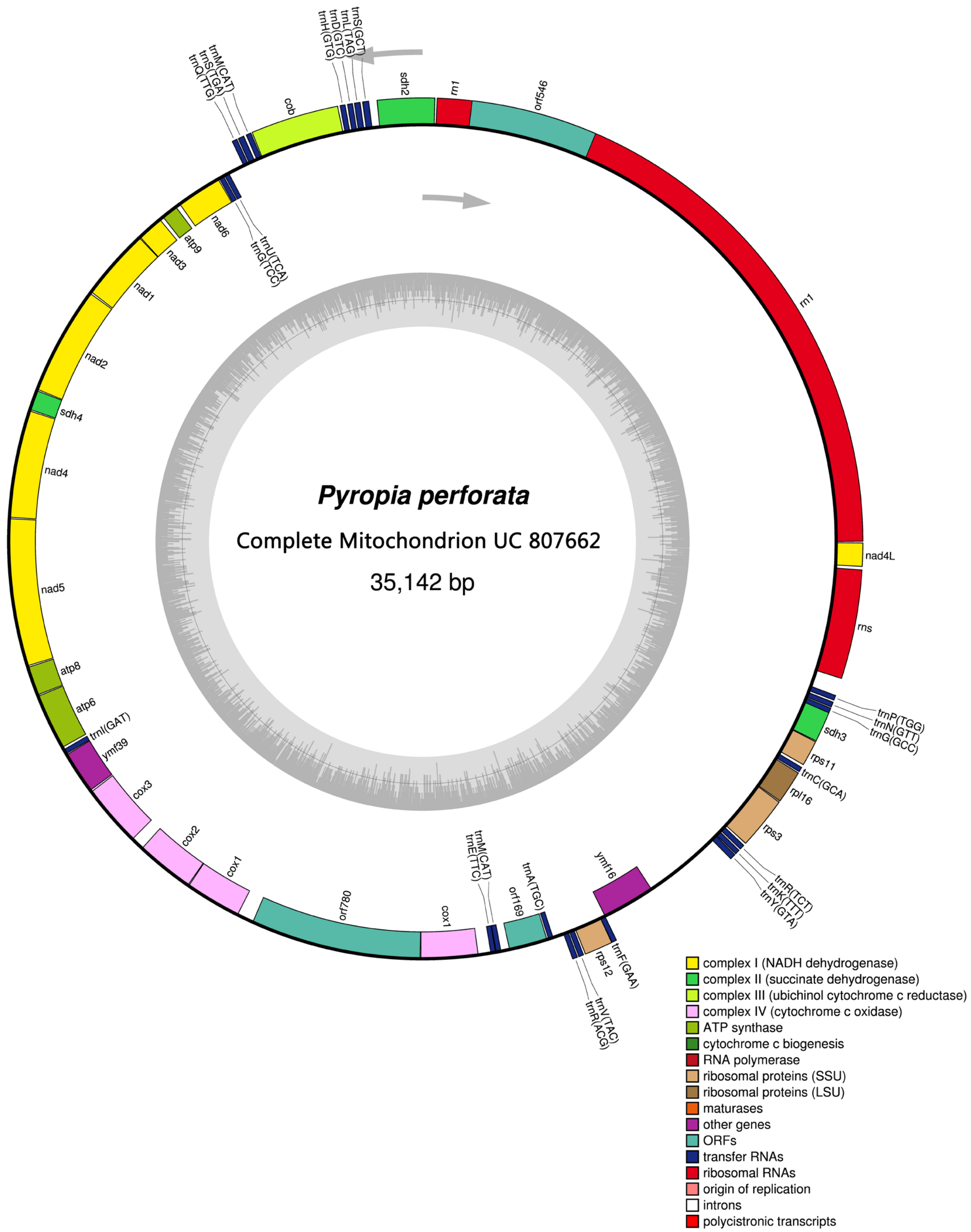


Supplementary Figure 5. The circular-mapping chloroplast genome of the holotype specimen of *Pyropia kanakaensis* (WTU 255136, Kanaka Bay, San Juan Is., Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The region identified as ‘Gap’ is predicted to represent an approximately 4.8 kb non-identical ribosomal 16S, 23S, and 5S repeat. The map was drawn using OGDRAW[1].

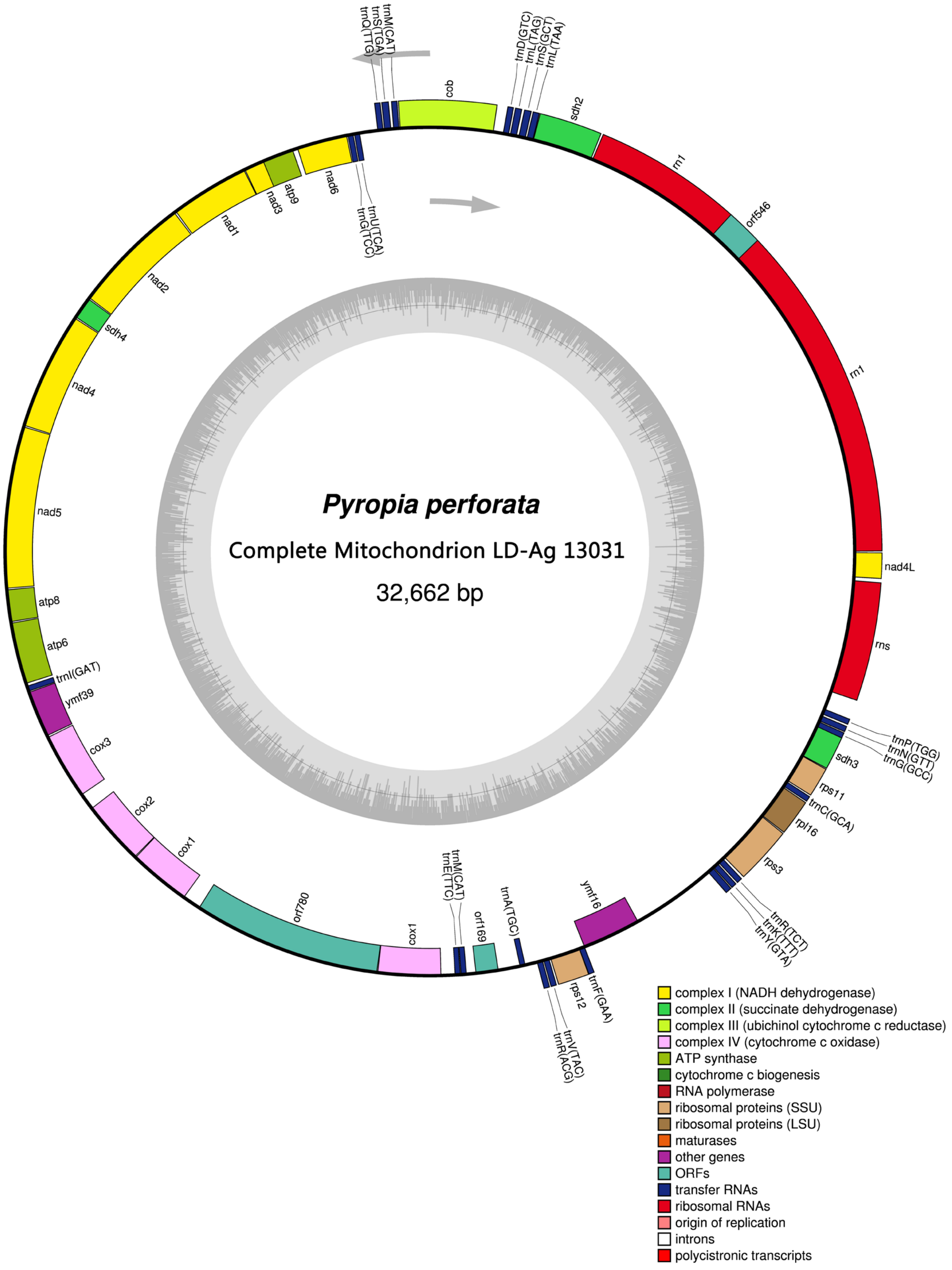
Supplementary Figure 6. The circular-mapping mitochondrial genome of the lectotype specimen of *Pyropia perforata* (LD-Ag 13037, Near Golden Gate, San Francisco, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



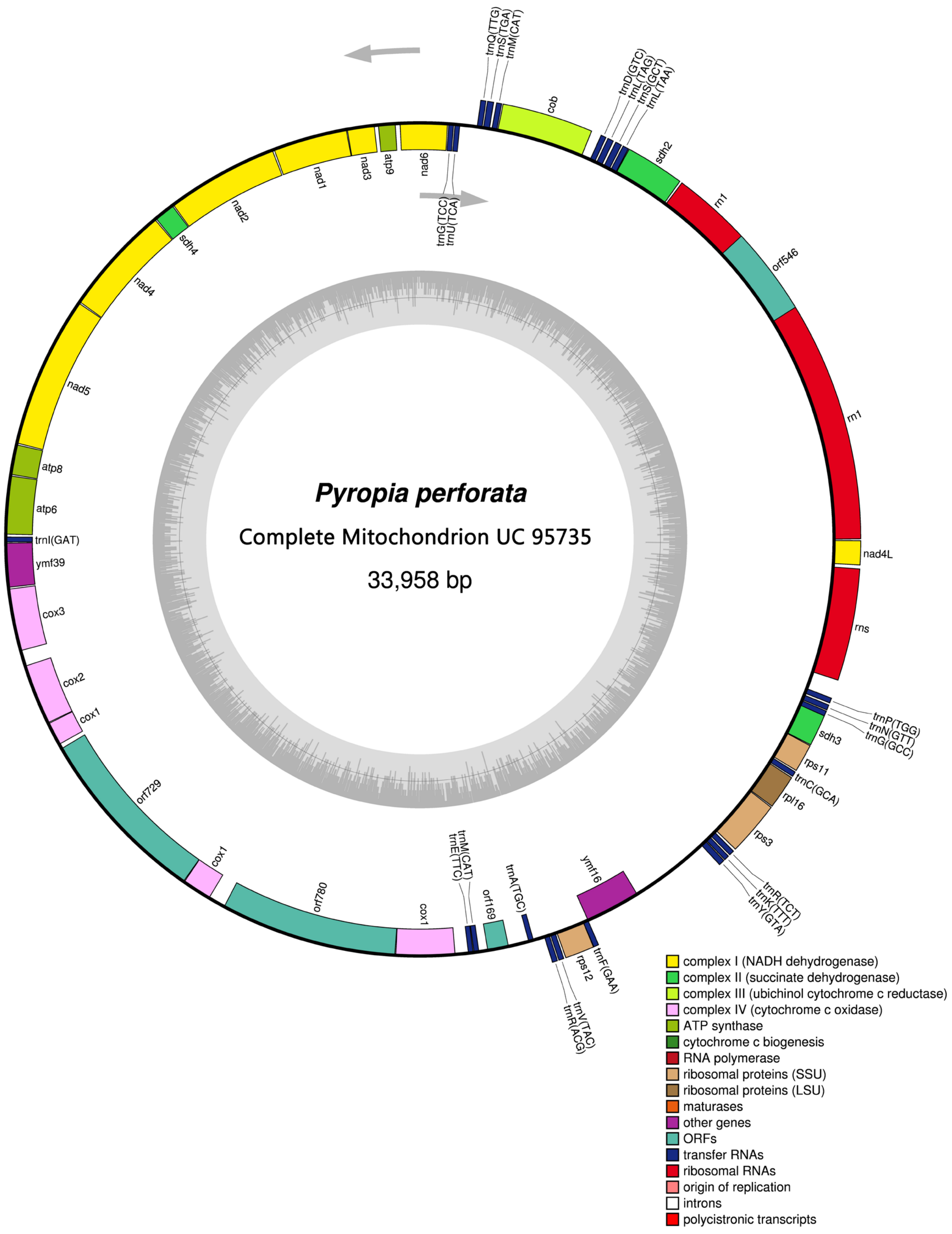
Supplementary Figure 7. The circular-mapping mitochondrial genome of the holotype specimen of *Porphyra sanjuanensis* (VK-11-00061, Minnesota Reef, San Juan Island, Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



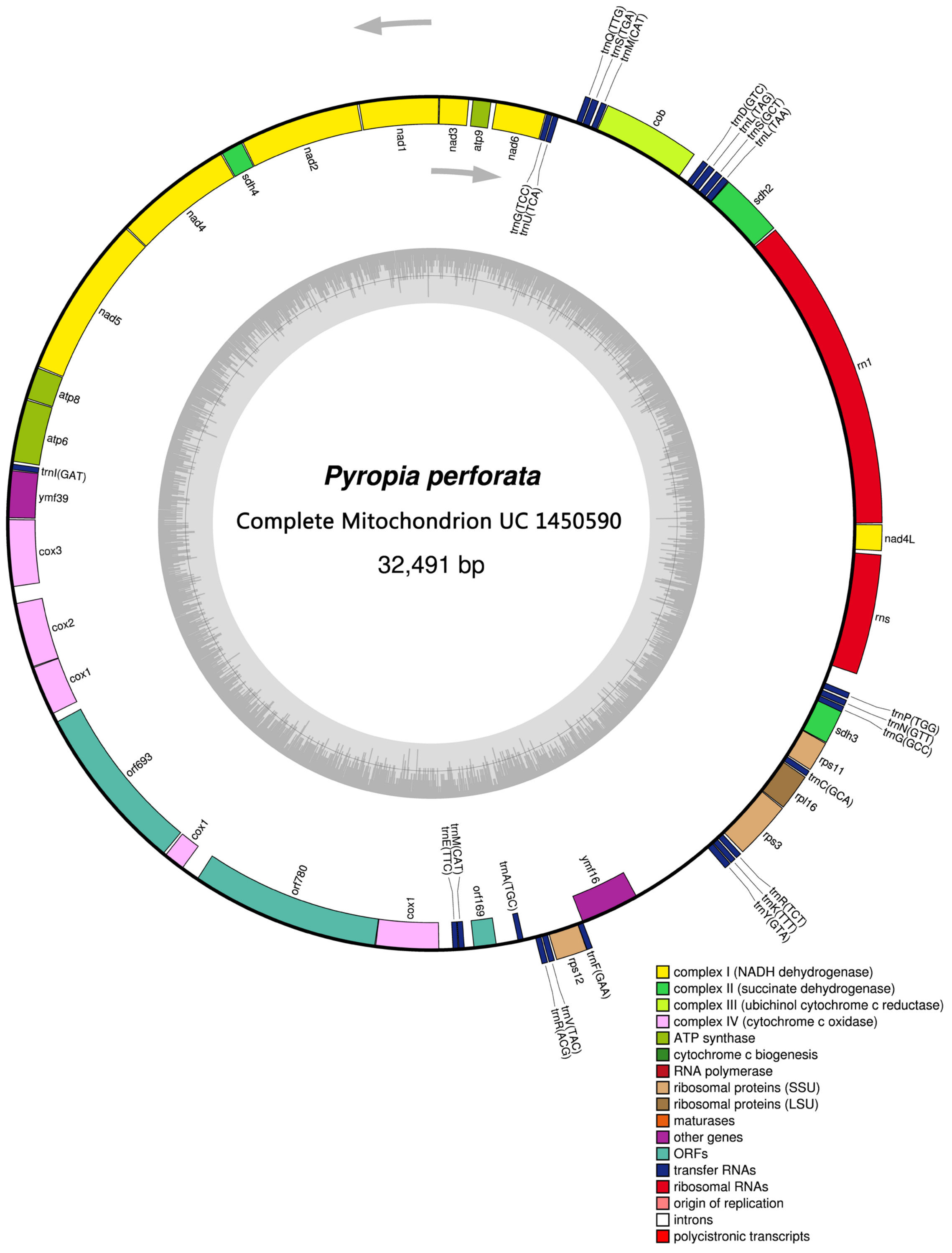
Supplementary Figure 8. The circular-mapping mitochondrial genome of the syntype specimen of *Porphyra perforata* f. *segregata* (UC 807662, La Jolla, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



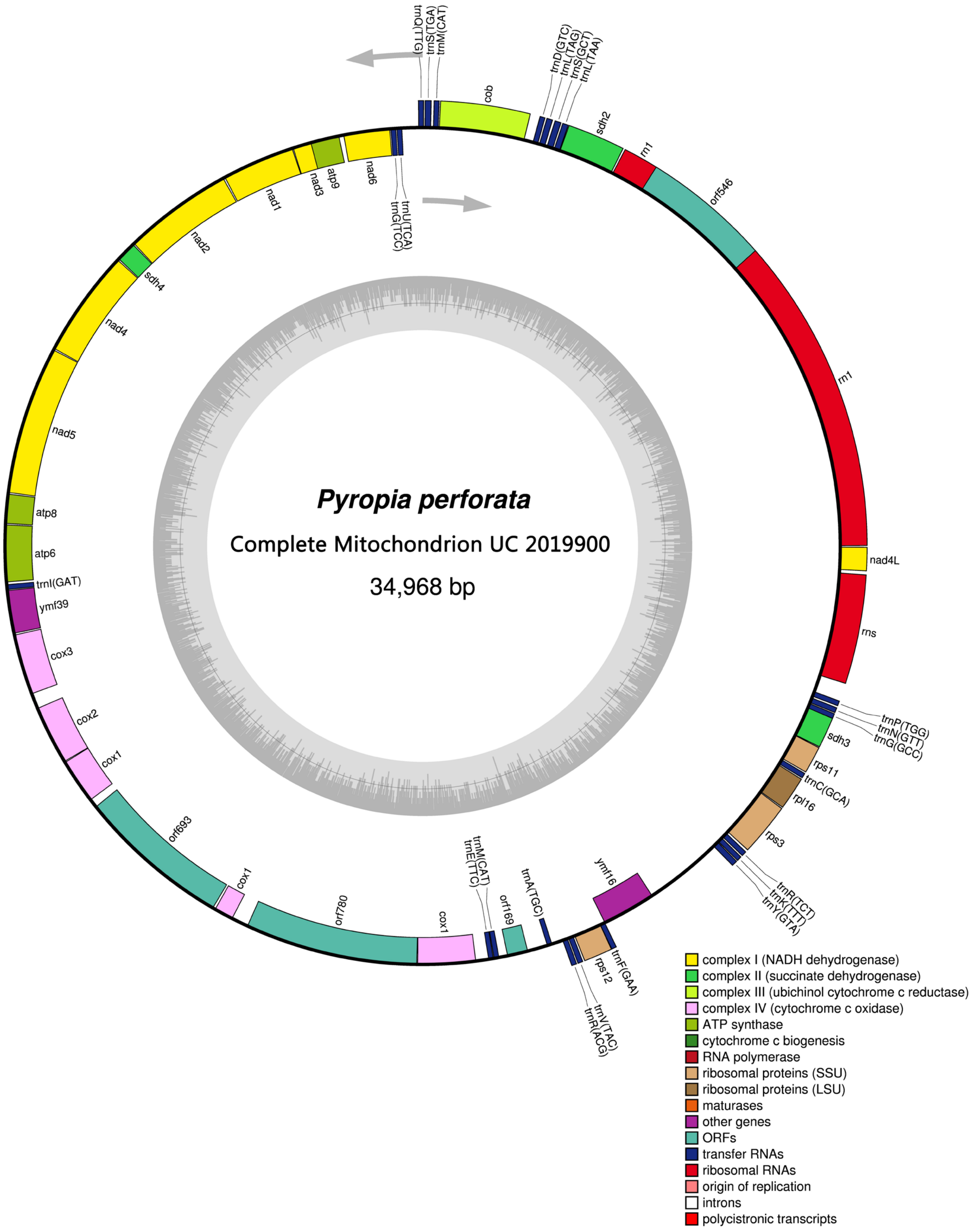
Supplementary Figure 9. The circular-mapping mitochondrial genome of the syntype specimen of *Pyropia perforata* (LD-Ag 13031, Santa Barbara, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



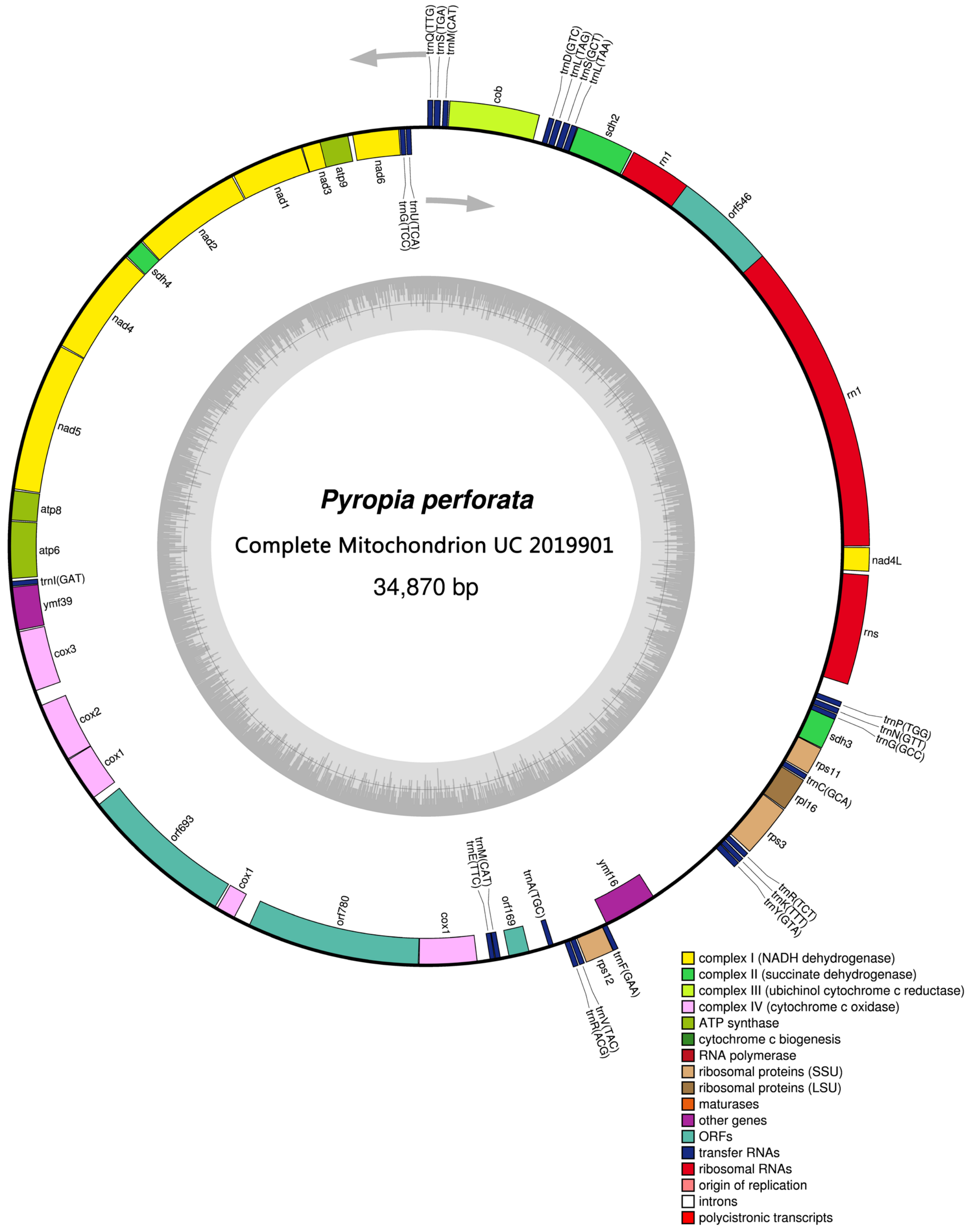
Supplementary Figure 10. The circular-mapping mitochondrial genome of *Pyropia perforata* (UC 95735, Punta San Roque, Baja California Sur). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



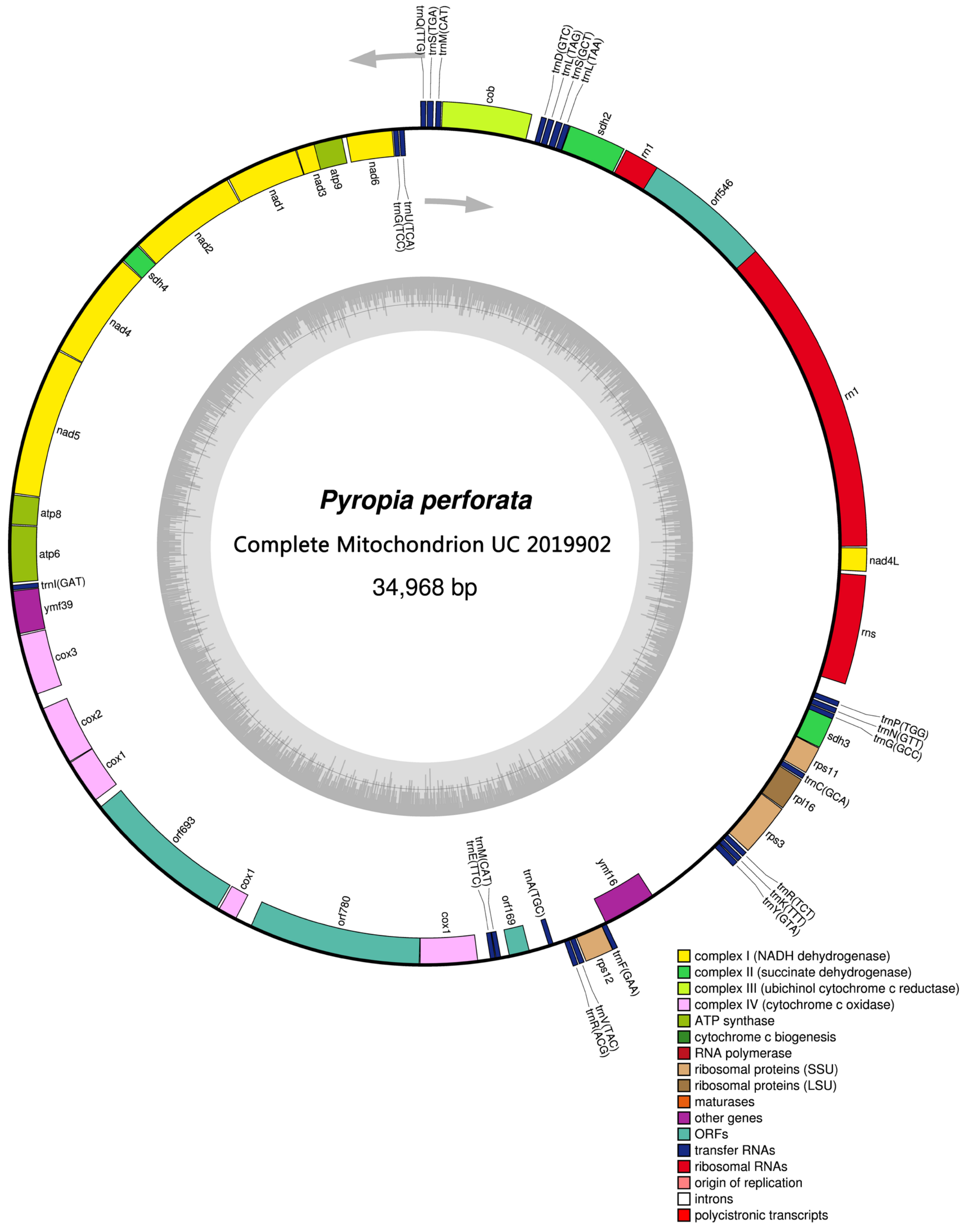
Supplementary Figure 11. The circular-mapping mitochondrial genome of *Pyropia perforata* (UC 1450590, Carmel, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



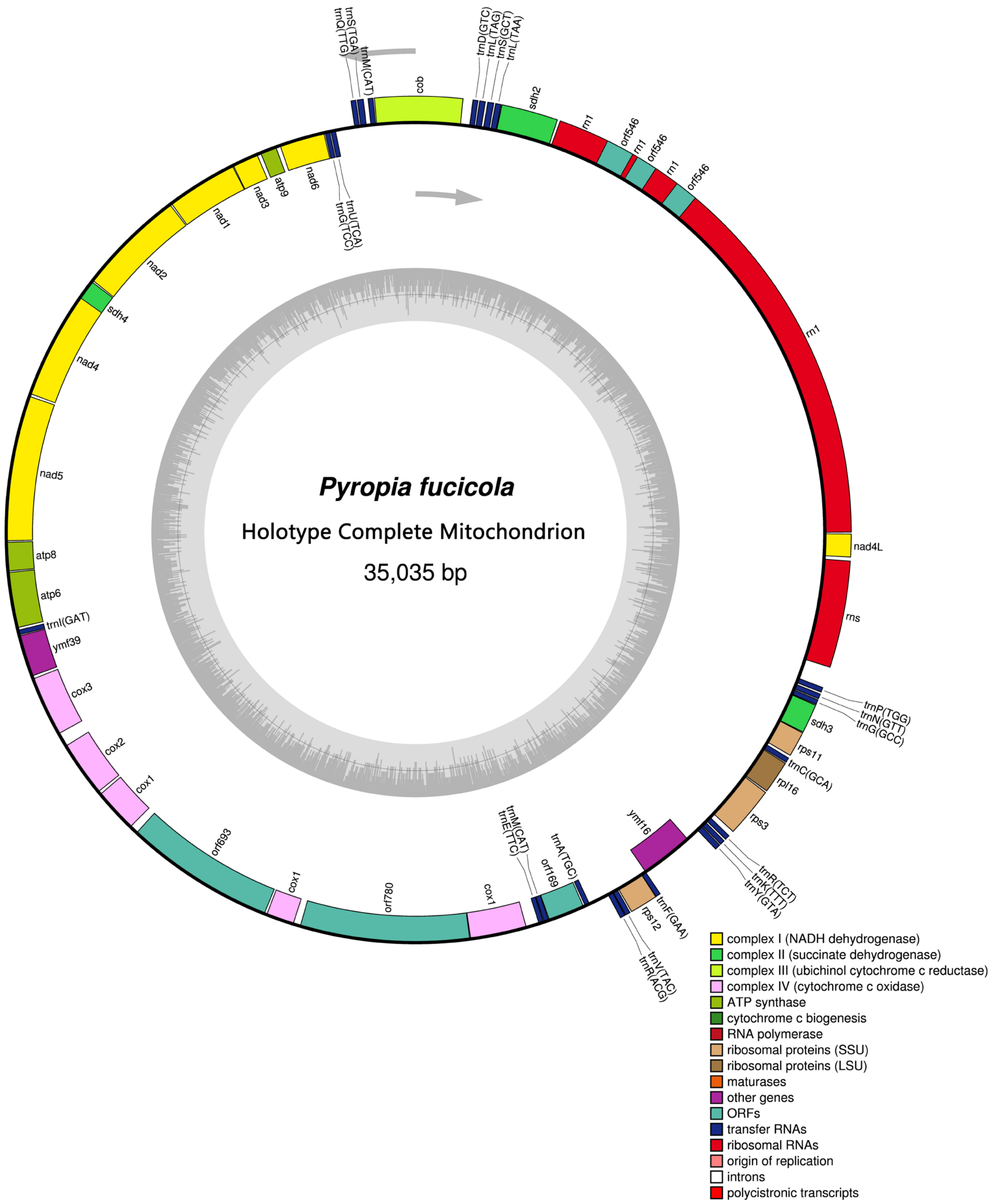
Supplementary Figure 12. The circular-mapping mitochondrial genome of *Pyropia perforata* (UC 2019900, Tomales Bay, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



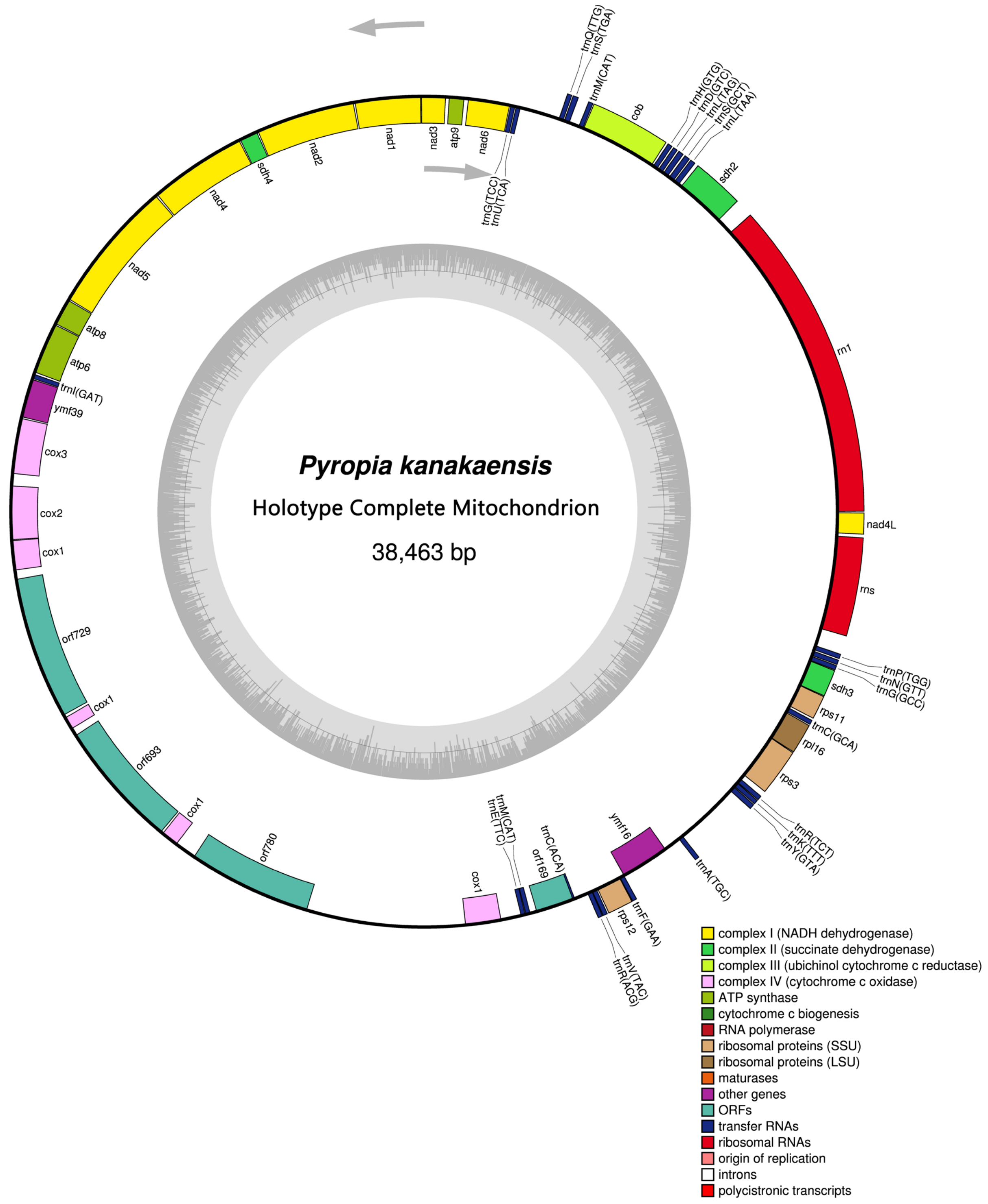
Supplementary Figure 13. The circular-mapping mitochondrial genome of *Pyropia perforata* (UC 2019901, Near Turn Island, San Juan Island, Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



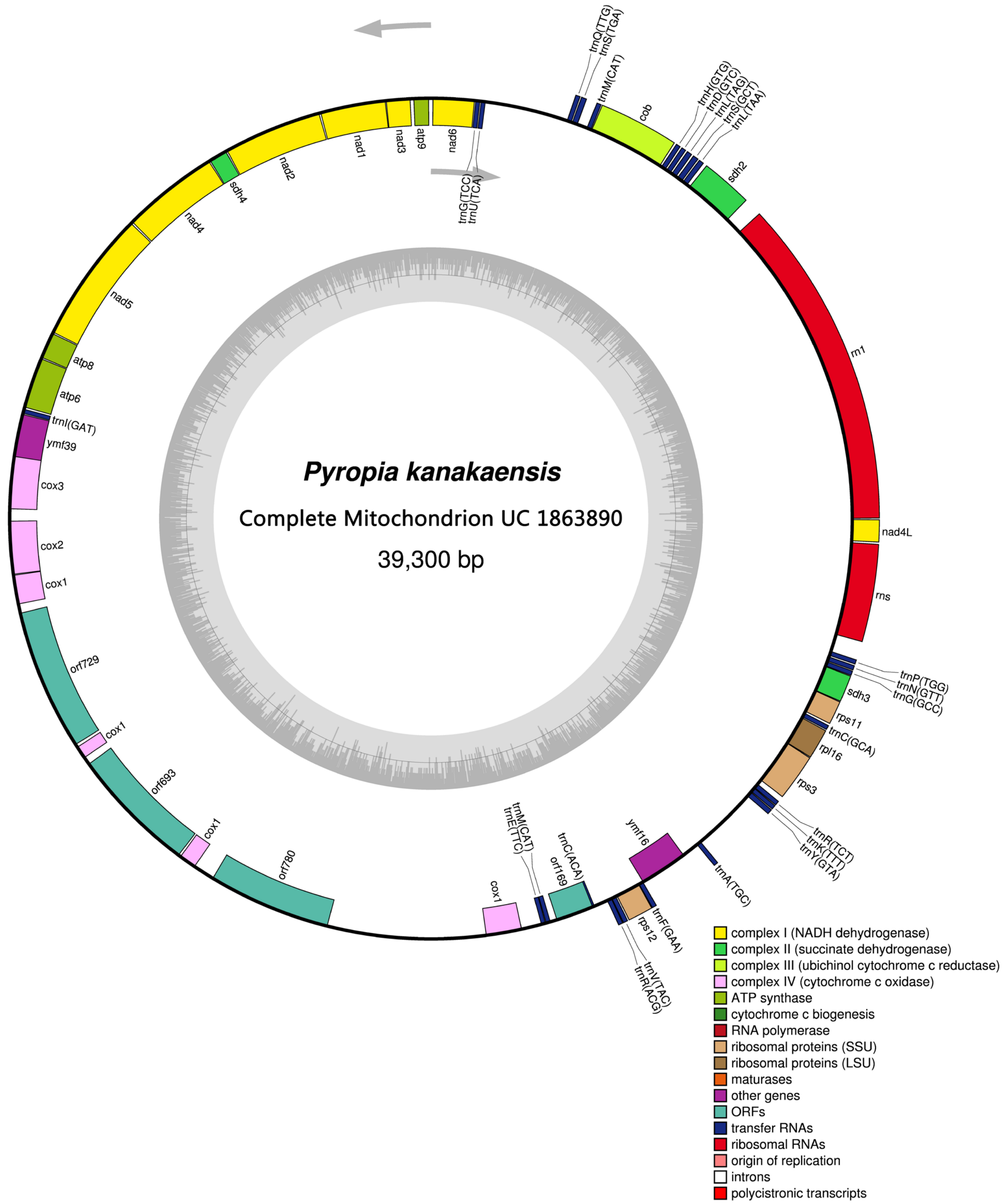
Supplementary Figure 14. The circular-mapping mitochondrial genome of *Pyropia perforata* (UC 2019902, Friday Harbor, San Juan Island, Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



Supplementary Figure 15. The circular-mapping mitochondrial genome of the holotype specimen of *Pyropia fucicola* (VK-11-00121, Makah Bay, Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



Supplementary Figure 16. The circular-mapping mitochondrial genome of the holotype specimen of *Pyropia kanakaensis* (Mumford #161, WTU 255136, Kanaka Bay, San Juan Is., Washington). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].



Supplementary Figure 17. The circular-mapping mitochondrial genome of *Pyropia kanakaensis* (UC 1863890, Land's End, San Francisco, California). Features transcribed clockwise are on the inside, while counter-clockwise transcriptions are on the outside, as indicated by the grey arrows. The color coding corresponds to genes of different groups as listed in the key to the bottom right. The GC content is shown in the inner grey circle. The map was drawn using OGDRAW[1].

Reference

1. Lohse, M., Drechsel, O. & Bock, R. Organellargenomedraw (ogdraw)– a tool for the easy generation of high-quality custom graphical maps of plastid and mitochondrial genomes. *Curr. Genet.* **52**, 267–274 (2007).

Supplementary Table 1 Chloroplast gene content for *Porphyra perforata*

Chloroplast Genomes

Gene Groups	**Genes**
Photosystem I	psaA, psaB, psaC, psaD, psaE, psaK, psaI, psaF, psaJ, psaL, psaM
Photosystem II	psbA, psbC, psbD, psbE, psbF, psbH, psbI, psbJ, psbK, psbL, psbN, psbT, psbV, psbW, psbX, psbY, psbZ
Protochlorophyllide reductase	chlL, chlN, chlB, chlI-2
Phycobiliproteins	apcA, apcB, apcD, apcE, apcF, cpeA, cpeB, cpcA, cpcB, cpcG
Cytochrome b/f complex	petA, petB, petD, petF, petG, petJ, petL, petN
ATP synthase	atpA, atpB, atpD, atpE, atpF, atpG, atpH, atpI
RNA polymerase	rpoA, rpoB, rpoC1, rpoC2
Ribosomal proteins (SSU)	rps1, rps2, rps3, rps4, rps5, rps6, rps7, rps8, rps9, rps10, rps11, rps12, rps13, rps14, rps16, rps17, rps18, rps19, rps20
Ribosomal proteins (LSU)	rpl1, rpl2, rpl3, rpl4, rpl5, rpl6, rpl9, rpl11, rpl12, rpl13, rpl14, rpl15, rpl16, rpl17, rpl18, rpl19, rpl20, rpl21, rpl22, rpl23, rpl24, rpl27, rpl28, rpl29, rpl31, rpl32, rpl33, rpl34, rpl35, rpl36
Hypothetical chloroplast orfs	ycf3, ycf4, ycf12, ycf17, ycf19, ycf20, ycf21, ycf22, ycf23, ycf26, ycf28, ycf29, ycf33, ycf34, ycf35, ycf36, ycf37, ycf38, ycf39, ycf46, ycf59, ycf61, ycf63, ycf65
Transfer RNAs	trnA-GGC, trnA-TGC, trnC-GCA, trnD-GTC, trnE-TTC, trnF-GAA, trnG-GCC, trnG-TCC, trnH-GTG, trnI-GAT, trnK-TTT, trnL-CAA, trnL-GAG, trnL-TAA, trnL-TAG, trnM-CAT(x3), trnN-GTT, trnP-TGG, trnQ-TTG, trnR-ACG, trnR-CCG, trnR-CCT, trnR-TCT, trnS-CGA, trnS-GCT, trnS-GGA, trnS-TGA, trnT-GGT, trnT-TGT, trnV-GAC, trnV-TAC, trnW-CCA, trnY-GTA
Ribosomal RNAs	rrsA, rrlA, rrfB
Other genes	accA, accD, acpP, argB, bas1, carA, cbbx, ccs1, ccsA, cemA, clpC, dnaK, fabH, ftsH, glnB, gltB, groEL, ilvB, ilvH, infB, infC, moeB, nb1A, odpA, odpB, ompR, pbsA, pgmA, preA, rbcL, rbcR, rbcS, rne, secA, secY, sufB, sufC, syfB, syh, tatC, thiG, trpA, trpG, trxA, tsf, tufa
Open reading frames	orf36, orf49, orf58, orf62, orf68, orf71(x2), orf75, orf107, orf108, orf111, orf114, orf121, orf148, orf149, orf174, orf198, orf203, orf238, orf240, orf263, orf287, orf320, orf327, orf382, orf450, orf565, orf621

Supplementary Table 2 Pairwise distances for chloroplast genomes analyzed in this study

	Sanjuanensis Holotype VK-11-00061	Perforata Lectotype LD-Ag 13037	Perforata Syntype LD-Ag 13038	Perforata Tomales Bay UC 2019900	Perforata San Juan Is. UC 2019901	Perforata Friday Harbor UC 2019902	Perforata Syntype LD-Ag 13031	Perforata Syntype LD-Ag 13032
Sanjuanensis Holotype VK-11-00061	0							
Perforata Lectotype LD-Ag 13037	0.000390	0						
Perforata Syntype LD-Ag 13038	0.000380	0.000011	0					
Perforata Tomales Bay UC 2019900	0.000527	0.000506	0.00050	0				
Perforata San Juan Is. UC 2019901	0.000627	0.000606	0.00060	0.000459	0			
Perforata Friday Harbor UC 2019902	0.000511	0.000490	0.00048	0.000322	0.000432	0		
Perforata Syntype LD-Ag 13031	0.000775	0.000754	0.00074	0.000606	0.000664	0.000590	0	
Perforata Syntype LD-Ag 13032	0.000786	0.000764	0.00075	0.000617	0.000675	0.000601	0.000021	0
Perforata Carmel UC 1450590	0.000743	0.000722	0.00071	0.000575	0.000633	0.000559	0.000580	0.000601
Segregata Syntype UC 807662	0.000923	0.000902	0.00089	0.000754	0.000812	0.000738	0.000664	0.000686
Segregata Syntype UC 95739	0.000928	0.000907	0.00090	0.000759	0.000817	0.000744	0.000670	0.000691
Perforata Baja California UC 95735	0.005331	0.005294	0.00528	0.005150	0.005209	0.005146	0.005081	0.005103
Haitanensis JQ736808.1	0.118000	0.118052	0.11804	0.117978	0.118050	0.117947	0.118012	0.117997
Yezoensis JQ736809.1	0.140834	0.140884	0.14087	0.140684	0.140756	0.140688	0.140664	0.140687
Fucicola Holotype WTU 255128	0.145310	0.145323	0.14531	0.145166	0.145251	0.145164	0.145213	0.145237
Kanakaensis Holotype WTU 255136	0.144224	0.144241	0.14423	0.144193	0.144240	0.144195	0.144144	0.144144
Porphyra purpurea AF114794.1	0.197095	0.197139	0.19712	0.197031	0.197074	0.196967	0.197132	0.197132
Porphyra umbilicalis JQ388471.1	0.192228	0.192288	0.19227	0.192123	0.193351	0.192891	0.192956	0.192955

Perforata Carmel UC 1450590	Segregata Syntype UC 807662	Segregata Syntype UC 95739	Perforata Baja California UC 95735	Haitanensis JQ736808.1	Yezoensis JQ736809.1	Fucicola Holotype WTU 255128	Kanakaensis Holotype WTU 255136	Porphyra purpurea AF114794.1	Porphyra umbilicalis JQ388471.1
0									
0.000717	0								
0.000722	0.000005	0							
0.005134	0.005067	0.005072	0						
0.118002	0.117781	0.117927	0.118544	0					
0.140750	0.140602	0.140620	0.140991	0.118193	0				
0.145247	0.145071	0.145089	0.145550	0.121335	0.033753	0			
0.144196	0.144091	0.144110	0.144516	0.117789	0.128832	0.128989	0		
0.196999	0.196854	0.196869	0.197360	0.179847	0.181160	0.187107	0.190509	0	
0.192818	0.203253	0.203253	0.203671	0.185190	0.181699	0.187910	0.191267	0.083348	0

Supplementary Table 3 Mitochondrial gene content for the type specimens of *Porphyra perforata*, *Porphyra perforata* f. *segregata*, *Porphyra sanjuanensis*, *Porphyra fucicola*, and *Porphyra kanakaensis*

Mitochondrial Genomes

Gene Groups	**Genes**
NADH dehydrogenase	nad1, nad2, nad3, nad4, nad4L, nad5, nad6
Succinate cytochrome c oxidoreductase	sdh2, sdh3, sdh4
Ubichinol cytochrome c reductase	cob
Cytochrome c oxidase	cox1(x2)[a], cox2, cox3
ATP synthase	atp6, atp8, atp9
ORFs	orf169, orf546[b], orf693[c], orf729[d], orf780, orf813[e]
Ribosomal proteins (SSU)	rps3, rps11, rps12
Ribosomal proteins (LSU)	rpl16
Other genes	ymf16, ymf39
Transfer RNAs	trnM-CAT(x2), trnS-TGA, trnS-GCT, trnL-TAA, trnL-TAG, trnQ-TTG, trnD-GTC, trnR-ACG, trnR-TCT, trnV-TAC, trnA-TGC, trnP-TGG, trnU-TCA, trnG-TCC, trnG-GCC, trnC-GCA[f], trnN-GTT, trnK-TTT, trnF-GAA, trnY-GTA, trnE-TTC, trnI-GAT, trnH-GTG[g].
Ribosomal RNAs	rrnl(x2), rns

[a] cox1 is represented by 3 exons in *P. fucicola* and 4 exons in *P. kanakaensis*

[b] orf546 is not present in *P. kanakaensis*, but is represented by 3 intronic orfs in *P. sanjuanensis* and *P. fucicola*, 2 in *P. segregata*, and 1 in *P. perforata*

[c] orf693 is not present in *P. segregata*

[d] orf729 is present in *P. kanakaensis*

[e] orf813 is present in *P. sanjuanensis*

[f] trnC-ACA in *P. kanakaensis*

[g] trnH-GTG is present in *P. segregata* and *P. kanakaensis*

Supplementary Table 4 Pairwise distances for mitochondrial genomes analyzed in this study

	Perforata Syntype LD-Ag 13038	Perforata Lectotype LD-Ag 13037	Perforata Carmel UC 1450590	Perforata Tomales Bay UC 2019900	Kanakaensis San Franc. UC 1863890	Kanakaensis Holotype WTU 255136
Perforata Syntype LD-Ag 13038	0					
Perforata Lectotype LD-Ag 13037	0.00005897	0				
Perforata Carmel UC 1450590	0.00326428	0.00323253	0			
Perforata Tomales Bay UC 2019900	0.02288324	0.02285323	0.00237473	0		
Kanakaensis San Franc. UC 1863890	0.28309746	0.28302199	0.2659347	0.27422198	0	
Kanakaensis Holotype WTU 255136	0.27752039	0.27744451	0.26249699	0.26862549	0.00388726	0
Fucicola Holotype WTU 255128	0.1963746	0.19633767	0.20428958	0.21640519	0.29263438	0.28715291
Tenera KC750917.1	0.26914495	0.26906905	0.27326727	0.28050192	0.28260101	0.27807815
Yezoensis JQ736809.1	0.31141588	0.3113731	0.29596773	0.27954675	0.34989783	0.34300299
Haitanensis JQ736808.1	0.29554889	0.29546391	0.28036215	0.26319045	0.2852639	0.28025561
Perforata Baja California UC 95735	0.06407257	0.06407408	0.04531013	0.04249629	0.25107255	0.24534104
Perforata Friday Harbor UC 2019902	0.02294085	0.02291085	0.00256032	0.00105911	0.27394036	0.26838354
Perforata San Juan Is. UC 2019901	0.02250169	0.02247167	0.00312627	0.00247152	0.27410176	0.26850049
Sanjuanensis Holotype VK-11-00061	0.02244734	0.02241636	0.00466476	0.00447662	0.26940396	0.26387907
Perforata Syntype LD-Ag 13032	0.00495917	0.00492774	0.00437461	0.02513519	0.24400344	0.2382571
Perforata Syntype LD-Ag 13031	0.00505447	0.00502303	0.00447473	0.02522944	0.24393482	0.23814641
Segregata Syntype UC 95739	0.02572674	0.02569496	0.0067327	0.00690032	0.23875846	0.23285066
Segregata Syntype UC 807662	0.0258259	0.02579413	0.00679975	0.0069933	0.23884217	0.23293472
Porphyra purpurea AF114794.1	0.36892329	0.36879224	0.38644704	0.35987908	0.41458829	0.3958339
Porphyra umbilicalis JQ388471.1	0.34084485	0.34070469	0.36002582	0.36051447	0.36477604	0.35792343

Fucicola Holotype WTU 255128	Tenera KC750917.1	Yezoensis JQ736809.1	Haitanensis JQ736808.1	Perforata Baja California UC 95735	Perforata Friday Harbor UC 2019902	Perforata San Juan Is. UC 2019901
0						
0.11128623	0					
0.14632734	0.12831018	0				
0.30120723	0.26975302	0.26826708	0			
0.26939315	0.24205605	0.24144558	0.22147197	0		
0.21594256	0.27983465	0.27896123	0.26266698	0.04242729	0	
0.21589245	0.27866551	0.28035668	0.26417153	0.04312727	0.00252911	0
0.21588138	0.28086398	0.32258394	0.2640384	0.04498024	0.00447645	0.00244297
0.20498989	0.23423737	0.27437262	0.25805037	0.0314175	0.02526107	0.02468681
0.20517545	0.23423003	0.27431808	0.25812802	0.031384	0.02535531	0.024781
0.2322716	0.26360975	0.25475137	0.23721793	0.01208651	0.00689993	0.00465903
0.23240079	0.2636486	0.25479264	0.23725876	0.01218357	0.00699292	0.00475201
0.3592354	0.39064764	0.40107181	0.38940047	0.33823856	0.35950956	0.36129795
0.33652915	0.36313187	0.38881423	0.39374179	0.33688359	0.36029128	0.36214572

Sanjuanensis Holotype VK-11-00061	Perforata Syntype LD-Ag 13032	Perforata Syntype LD-Ag 13031	Segregata Syntype UC 95739	Segregata Syntype UC 807662	Porphyra purpurea AF114794.1	Porphyra umbilicalis JQ388471.1
0						
0.02456192	0					
0.02465608	0.00009189	0				
0.01860783	0.02254028	0.02256322	0			
0.01869563	0.02263589	0.02265879	0.00008538	0		
0.3669525	0.38524783	0.38508974	0.36420718	0.36425691	0	
0.3682213	0.35963193	0.35953089	0.36580495	0.36579203	0.15665674	0

Supplementary Table 5 Comparison of circular genomes to sequences deposited in GenBank

Specimen	**Identical *rbc* L sequences (Locality/GenBank Accessions)**
LD-Ag 13037, *P. perforata* lectotype	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
LD-Ag 13038, *P. perforata* syntype	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
LD-Ag 13031, *P. perforata* syntype	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
LD-Ag 13032, *P. perforata* syntype	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
UC 807662, *P. perforata* f. *segregata* syntype	No exact matches in GenBank, 1 bp diverged from the lectotype of *P. perforata*
UC 95739, *P. perforata* f. *segregata* syntype	No exact matches in GenBank, 1 bp diverged from the lectotype of *P. perforata*
UC 95735 *P. perforata*	No exact matches, nearest match is 6 bp diverged
UC 1450590 *P. perforata*	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
UC 2019900 *P. perforata*	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
UC 2019901 *P. perforata*	No exact matches in GenBank, 1 bp diverged from the lectotype of *P. perforata*
UC 2019902 *P. perforata*	No exact matches in GenBank, 2 bp diverged from the lectotype of *P. perforata*
VK-11-00061, *P. sanjuanensis* holotype	Sitka, Alaska (AF452438), Afognak Is., Alaska (EU223132), Qualicum Bch., Brit. Col. (JN028996)
WTU 255136, *P. kanakaensis* holotype	Sooke Harbour, Vancouver Is., Brit. Col. (JN028976)
UC 1863890 *P. kanakaensis*	No exact matches in GenBank, 2 bp diverged from the holotype of *P. kanakaensis*
VK-11-00121, *P. fucicola* holotype	No exact matches in GenBank, 1 bp diverged from three specimens in GenBank

Specimen	**Identical CO1 (Locality/GenBank Accessions)**
LD-Ag 13037, *P. perforata* lectotype	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
LD-Ag 13038, *P. perforata* syntype	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
LD-Ag 13031, *P. perforata* syntype	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
LD-Ag 13032, *P. perforata* syntype	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
UC 807662, *P. perforata* f. *segregata* syntype	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
UC 95739, *P. perforata* f. *segregata* syntype	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
UC 95735 *P. perforata*	No exact matches, 3 bp diverged from the lectotype of *P. perforata*
UC 1450590 *P. perforata*	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
UC 2019900 *P. perforata*	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
UC 2019901 *P. perforata*	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
UC 2019902 *P. perforata*	12 exact matches all from Brit. Col., identical to lectotype of *P. perforata*
VK-11-00061, *P. sanjuanensis* holotype	Large ORF splitting COX1 gene, otherwise identical to *P. perforata*
WTU 255136, *P. kanakaensis* holotype	Species not present in GenBank
UC 1863890 *P. kanakaensis*	Species not present in GenBank, 2 bp diverged from the holotype of *P. kanakaensis*
VK-11-00121, *P. fucicola* holotype	7 exact matches all from British Columbia

Specimen	**Identical UPA (Locality/GenBank Accessions)**
LD-Ag 13037, *P. perforata* lectotype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029133)
LD-Ag 13038, *P. perforata* syntype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029133)
LD-Ag 13031, *P. perforata* syntype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
LD-Ag 13032, *P. perforata* syntype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 807662, *P. perforata* f. *segregata* syntype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 95739, *P. perforata* f. *segregata* syntype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 95735 *P. perforata*	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 1450590 *P. perforata*	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 2019900 *P. perforata*	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 2019901 *P. perforata*	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
UC 2019902 *P. perforata*	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
VK-11-00061, *P. sanjuanensis* holotype	Mazarredo Is., Brit. Col. (JN029132), Tswassen Ferry Terminal, Brit. Col. (JN029132)
WTU 255136, *P. kanakaensis* holotype	Pachena Beach, Brit. Col. (JN029107), Whiffen Spit, Vancouver Is., Brit. Col. (JN029108)
UC 1863890 *P. kanakaensis*	Pachena Beach, Brit. Col. (JN029107), Whiffen Spit, Vancouver Is., Brit. Col. (JN029108)
VK-11-00121, *P. fucicola* holotype	No exact matches in GenBank, 2 bp diverged from one specimen