

How was the ancient kelp?

– novel reproductive structure in *Aureophycus aleuticus* (Laminiariales) –

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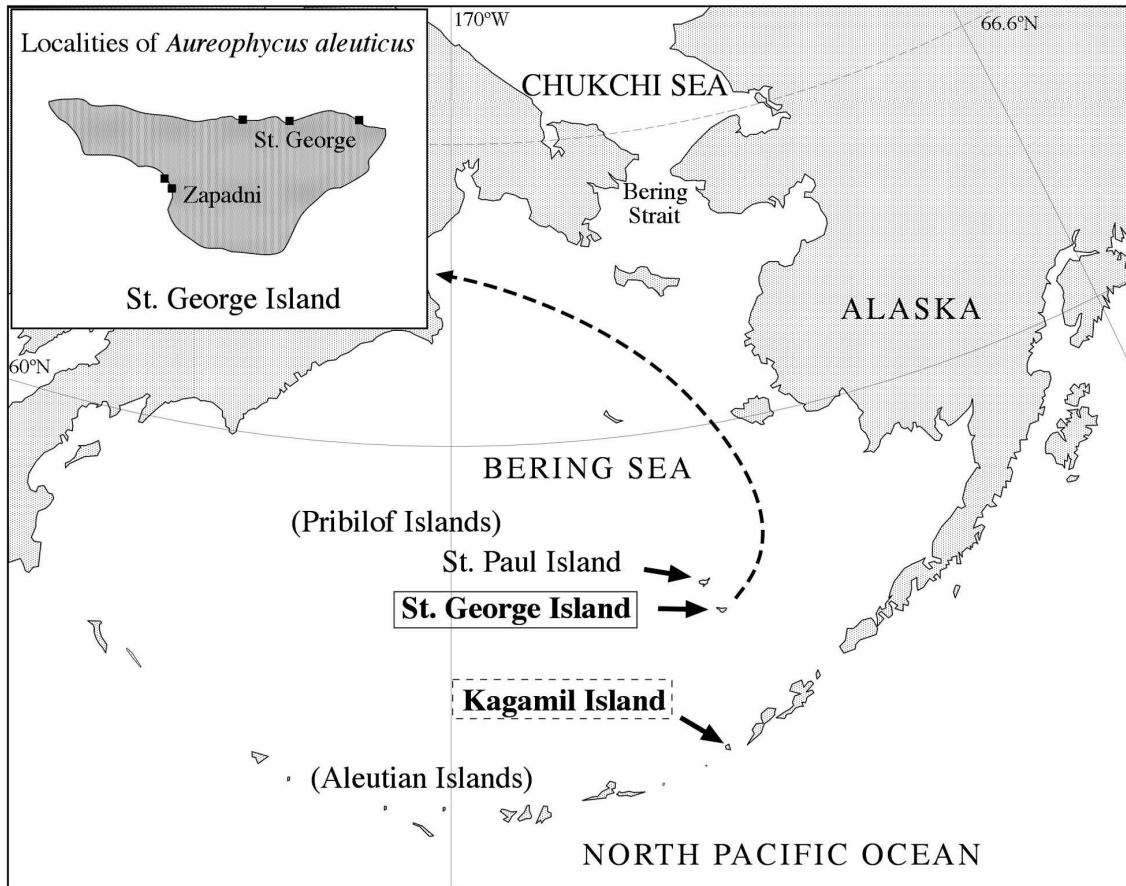
and

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**Supplementary Information 1–9**

**Supplementary Information 1.** Map showing the collections sites of *Aureophycus aleuticus*: Kagamil Island (type locality), and St. George Island, Pribilof Islands (this study). No populations have been found at the surrounding islands of Kagamil Island, and St. Paul Island. (Original drawing by H.K.)



**Supplementary Information 2.** List of primers used for polymerase chain reaction (PCR) and sequencing

Code	F/R	Sequence (5'-3')	Annealing position	Reference
ycf3-F1P	F	CAAGCDYTAAATAATATWGCTG	<i>ycf3</i> (319-340)	this study
atpB-F1P	F	GCWAAAGCNCATGGTGGTGT	<i>atpB</i> (535-554)	this study
atpB-F1.2P	F	AARGTMGTWGAYYTATTAGC	<i>atpB</i> (427-446)	this study
atpB-F1.3P	F	GTHMGHGCNATTGCNATGAGTGC	<i>atpB</i> (187-209)	this study
atpB-F2P	F	GCDGTRGAYCCHTTAGATTCA	<i>atpB</i> (1057-1077)	this study
atpB-2R	R	AGCTTGWACAAATCTAAAAATA	<i>atpB</i> (810-789)	1
atpB-R2P	R	GCWATAATATCTTGTAATTC	<i>atpB</i> (1184-1165)	this study
atpB-R2.2P	R	TCKACHACTAADCGRCTCTTC	<i>atpB</i> (1232-1213)	this study
atpB-R3P	R	CAYRTAYAAATCRTTTCCTTC	<i>atpB</i> (609-589)	this study
atpB-R4P	R	GCWGRKCKATGRATAGGTAA	<i>atpB</i> (374-355)	this study
atpB-R1P	R	TTTGCTTTAGMDATWGCTTC	<i>atpB</i> (1430-1411)	this study
psaA130F	F	AACWACWACTTGGATTTGGAA	<i>psaA</i> (126-146)	2
psaA-P2	R	NCCWGACCAAGMWARACAAC	<i>psaA</i> (639-620)	3
psaA-P2.2	R	TCDGCRTTYTGRAACCAAYTC	<i>psaA</i> (578-559)	this study
psaA-P3	R	GCTGGDTTRTARTCACGAACC	<i>psaA</i> (1253-1233)	3
psaA-P3.2	R	GCWGGRTTATARTCRCGDACC	<i>psaA</i> (1253-1233)	this study
psaA970R	R	GCYTCTARAATYTCTTTCA	<i>psaA</i> (977-959)	2
psaA970R2	R	TRCTATGDCCRATNCCCCAA	<i>psaA</i> (958-939)	this study
psaA1760R	R	CCTCTWCCWGGWCCATCRCAWGG	<i>psaA</i> (1736-1714)	2
psaA1760R2	R	CCRTCACAHGGRAAWCGGAA	<i>psaA</i> (1724-1705)	this study
psaA-F3P	F	CWGCWGAYTTTATGGTTCAYC	<i>psaA</i> (1583-1603)	this study
psaA-P4	R	NGATTCAATHARYTCTTGCC	<i>psaA</i> (2094-2075)	3
psaA-P5	F	CAYCATATHCATGCNTTYAC	<i>psaA</i> (1600-1619)	3
psaA-F1P	F	ATGCAATCNGAYRTTTGGGG	<i>psaA</i> (1834-1853)	this study
psaB-F1P	F	ATGCAATCNGAYRTTTGGGG	<i>psaA</i> (21-40)	this study
psaB-F3P	F	WGATGCWCAYMGWCCMCCTG	<i>psaA</i> (915-934)	this study
psaB-F3.2P	F	GSTATHGNCAYAAAYATGAAAGA	<i>psaA</i> (886-908)	this study
psaB-R2P	R	KAWTGCATACATATGYTGAG	<i>psaA</i> (1065-1046)	this study
psaB-R3P	R	CAATGCCAATARA AHGTAACCC	<i>psaA</i> (1787-1766)	this study
psaB-R3.2P	R	CAATARA AHGTAACCCAWCC	<i>psaA</i> (1781-1762)	this study
psaB-R1P	R	TTCCAGCHGTTGAWGCWATA	<i>psaB</i> (2194-2175)	this study
psaB-F4P	F	CCWTATGCHTTYATGGCAAAGAT	<i>psaB</i> (1069-1092)	this study
psbA-F	F	ATGACTGCTACTTTAGAAAGACG	<i>psbA</i> (1-23)	2

psbA-F1P	F	ACCGTTTATACATYGGTTGG	<i>psbA</i> (77-96)	this study
psbA500F	F	CTCTGATGGWATGCCWYTAGG	<i>psbA</i> (504-524)	2
psbA600R	R	CCAAATACACCAGCAACACC	<i>psbA</i> (620-601)	2
psbA600R2	R	AWACACCAGCAACACCAGCC	<i>psbA</i> (616-597)	this study
psbA-R1P	R	TACGYTCRTGCATTACTIONTCC	<i>psbA</i> (1003-984)	this study
psbA-R1	R	GCTAAATCTARWGGGAAGTTGTG	<i>psbA</i> (1031-1009)	2
psbC-P1.2	F	CCACGTGGAAACGCYCTTTA	<i>psbC</i> (33-52)	this study
psbC-P1	F	TAGCTCATGCAGGYWTAATGG	<i>psbC</i> (152-172)	this study
psbC-P3	R	CTTGCCAAGGTTGRATATCATT	<i>psbC</i> (1168-1147)	this study
psbC-P3.2	R	ATWCCTTTTTTCRAARCCAGC	<i>psbC</i> (1379-1360)	this study
psbC-P4	F	GCGTGAYAAAAAYAAAATGAC	<i>psbC</i> (444-464)	this study
psbC-P5	R	AARAATGGRAAWGAYTCTTC	<i>psbC</i> (431-412)	this study
psbC-P6	F	GAAGCWTCTCAAKCDCARGC	<i>psbC</i> (925-944)	this study
rbcL-P2	F	GAWCGRACCTCGAWTWAAAAGTG	<i>rbcL</i> (19-40)	4
rbc-R2.5	R	CCTTCATAAACAACACG	<i>rbcL</i> (587-571)	5
Ral-R952	R	CATACGCATCCATTTACA	<i>rbcL</i> (969-952)	4
rbcL-P5	F	CWTAYYTAAAAACWTTCCAAG	<i>rbcL</i> (440-460)	this study
rbcL-P4	R	AGKTGRTGCATYTGRCCACA	<i>rbcL</i> (1178-1159)	this study
rbcL-Rh3	F	TTAAYTCTCARCCDTTYATGCG	<i>rbcL</i> (629-650)	6
rbcL-P1	F	GKGTWATTTGTAARTGGATGCG	<i>rbcL</i> (944-965)	4
rbcL-P3	F	CARTTYGGWGGWGGTACDATTGG	<i>rbcL</i> (1210-1232)	5
rbcL-P3.2	F	GARGGTCCCTGADATTYTACGT	<i>rbcL</i> (1327-1347)	this study
rbcS-P1	R	GGATCATCTGYCCATTCTACAC	<i>rbcS</i> (122-101)	4
GazF2	F	CCAACCAYAAAGATATWGGTAC	<i>cox1</i> (104-125)	7
cox1-P1.2	F	GATHTTYTTTATGGTDATGCC	<i>cox1</i> (276-296)	this study
cox1-P2	R	GGDATAACGDCGHGGCATAACC	<i>cox1</i> (1400-1381)	this study
cox1-P3	R	CNGTAAACATRTGRTGVGCC	<i>cox1</i> (961-942)	this study
cox1-P4	F	DGCRGCNTTTACNATGTTTG	<i>cox1</i> (1230-1249)	this study
cox1-P5	R	TAATACCNCRCGYAAAACWGG	<i>cox1</i> (700-679)	this study
cox1-P5.2	R	GCHGTDATTAADACHGACCA	<i>cox1</i> (656-637)	this study
trnI-P1.2	R	GCTTATCAGGCGTACACTCT	<i>trnI</i> (20-39)	this study
trnI-P1	R	TTGAACGAWCGVCTTTACGC	<i>trnI</i> (38-57)	this study
trnY-P2	F	GKCAGATTGTAAATCTGTTGG	<i>trnY</i> (27-47)	3
trnY-P1	F	TCYATCRTAGGTTCTGAATCC	<i>trnY</i> (51-70)	8
cox3-P5.2	F	KCHCCHGTYTTTAATATTGG	<i>cox3</i> (340-359)	9
cox3-P6	R	CDACAATHGCATGATGAGCCC	<i>cox3</i> (478-458)	9

cox3-P2	R	ACAAARTGCCAATACCAAGC	<i>cox3</i> (755-736)	8
18F-1	F	AAGGTGAAGTCGTAACAAGG	18S rDNA (1768-1787)	10
5.8F-1	F	ACGCAGCGAAATGCGATACG	5.8S rDNA (47-66)	10
26R-1	R	GTTAGTTTCTTTTCCTCCGC	26S rDNA (70-51)	10

Annealing positions correspond to the sequences of *Ectocarpus siliculosus* (*ycf3*, *atpB*, *psaA*, *psaB*, *psbA*, *psbC*, *rbcL*, and *rbcS*, FP102296: Le Corguille et al. 2009; *cox1*, *trnI*, *trnY*, *cox3*, FP885846: Cock J.M. unpublished), *Scytosiphon lomentaria* (Lyngbye) Link (18S rDNA, 5.8S rDNA and 26S rDNA, D16558: Kawai et al. 1995).

**Supplementary Information 3.** Origin of samples and sequence data used for molecular analyses, including their (DDBJ/Genbank) database accession numbers. Specimen codes KU-d#####, silicagel-dried specimens in Kobe University Research Center for Inland Seas; KU-#####, unialgal culture strains in KU-MACC (Kobe University Macroalgal Culture Collections).

Species	Origin	Accession code								
		<i>coxI</i>	<i>cox3</i>	<i>atpB</i>	<i>psaA</i>	<i>psaB</i>	<i>psbA</i>	<i>psbC</i>	<i>rbcL</i>	ITS+5.8S
<b>Aureophyceae</b>										
<b>fam. nov.</b>										
<i>Aureophycus aleuticus</i> Kawai, Hanyuda, Lindeberg & Lindstrom	5	-	-	-	-	-	-	-	AB355025	AB355026
	Present study (KU-d3198)	AB775218	AB775233	AB775250	AB775266	AB775282	AB775298	AB775314	-	-
	Present study (KU-d4869)	-	AB775234	-	-	-	-	AB775315	-	AB776649
	Present study (KU-d4882)	-	AB775235	-	-	-	-	AB775316	-	AB776650
<b>Akkesiphycaceae</b>										
<i>Akkesiphycus lubricum</i> Yamada & Tanaka	Present study (KU-470)	AB775219	AB775236	AB775251	AB775267	AB775283	AB775299	AB775317	AB775330	-
<b>Alariaceae</b>										
<i>Alaria crassifolia</i> Kjellman	Present study (KU-1165)	AB775220	AB775237	AB775252	AB775268	AB775284	AB775300	AB775318	AB775331	-
<i>Pleurophycus gardneri</i> Setchell & Saunders ex Tilden	Present study (KU-799)	AB775221	AB775238	AB775253	AB775269	AB775285	AB775301	AB775319	AB775332	-
<i>Pterygophora californica</i> Ruprecht	Present study (KU-798)	AB775222	AB775239	AB775254	AB775270	AB775286	AB775302	AB775320	AB775333	-
<i>Undaria pinnatifida</i> (Harvey) Suringar	Present study (KU-206)	AB775223	AB775240	AB775255	AB775271	AB775287	AB775303	-	AB775334	-
<i>Undaria pinnatifida</i> (Harvey) Suringar	11	-	-	-	-	-	-	FM957120	-	-
<b>Chordaceae</b>										
<i>Chorda asiatica</i> H. Sasaki & H. Kawai	Present study (KU-503)	AB775224	AB775241	AB775256	AB775272	AB775288	AB775304	AB775321	AB775335	-
<b>Costariaceae</b>										
<i>Agarum fimbriatum</i> Harvey	Present study (KU-150)	AB775225	AB775242	AB775257	AB775273	AB775289	AB775300 5	AB775322	AB775336	-
<i>Costaria costata</i> (C.Agardh) D.A. Saunders	Present study (KU-679)	AB775226	AB775243	AB775258	AB775274	AB775290	AB775306	AB775323	AB775337	-
<b>Laminariaceae</b>										
<i>Laminaria digitata</i> (Hudson) J.V. Lamouroux	Present study (KU-d4680)	-	-	AB775259	AB775275	AB775291	AB775307	AB775324	AB775338	-
	12	AJ344328	AJ344328	-	-	-	-	-	-	-

<i>Macrocystis pyrifera</i> (Linnaeus) C. Agardh	Present study (KU-1277)	AB775227	AB775244	AB775260	AB775276	AB775292	AB775308	AB775325	AB775339	-
<i>Saccharina japonica</i> (Areschoug) Lane, Mayes, Druehl & Saunders	Present study (KU-508)	AB775228	AB775245	AB775261	AB775277	AB775293	AB775309	-	-	-
	11	-	-	-	-	-	-	FM957121	FN667660	-
<b>Lessoniaceae</b>										
<i>Ecklonia radiata</i> (C.Agardh) J. Agardh	Present study (KU-1134)	AB775229	AB775246	AB775262	AB775278	AB775294	AB775310	AB775326	AB775340	-
<i>Lessonia variegata</i> J.Agardh	Present study (KU-1271)	AB775230	AB775247	AB775263	AB775279	AB775295	AB775311	AB775327	AB775341	-
<b>Pseudochordaceae</b>										
<i>Pseudochorda nagaii</i> (Tokida) Inagaki	Present study (KU-735)	AB775231	AB775248	AB775264	AB775280	AB775296	AB775312	AB775328	AB775342	-
<b>Outgroup</b>										
<i>Ectocarpus siliculosus</i> (Dillwyn) Lyngbye	13	-	-	FP102296	FP102296	FP102296	FP102296	FP102296	FP102296	-
<i>Ectocarpus siliculosus</i> (Dillwyn) Lyngbye	Cock J. M. (unpublished)	FP885846	FP885846	-	-	-	-	-	-	-
<i>Stictyosiphon soriferus</i> (Reinke) Rosenvinge	Present study (KU-704)	AB775232	AB775249	AB775265	AB775281	AB775297	AB775313	AB775329	AB775343	-

## References

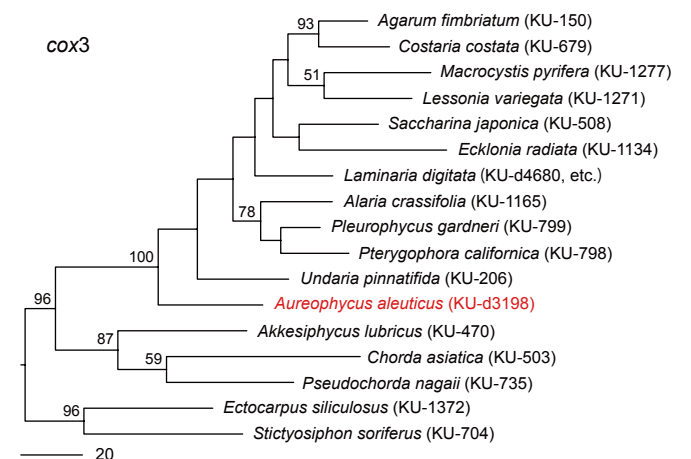
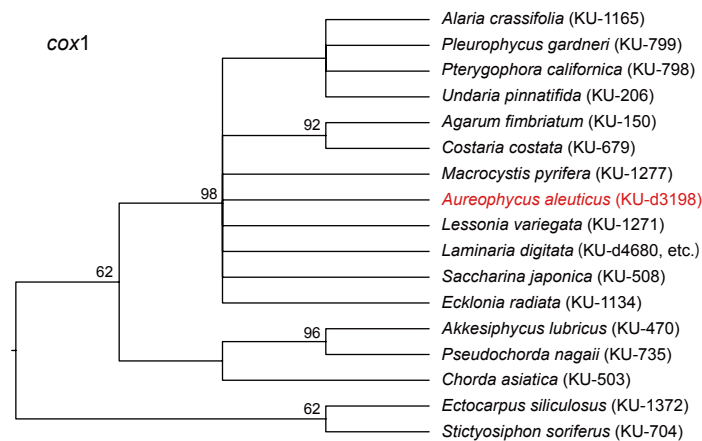
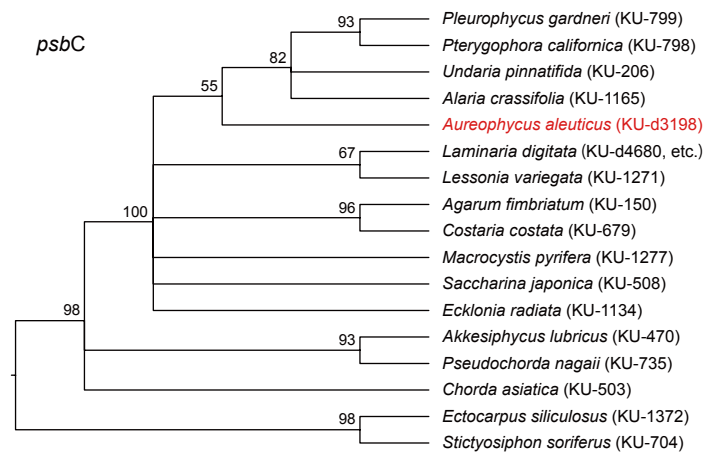
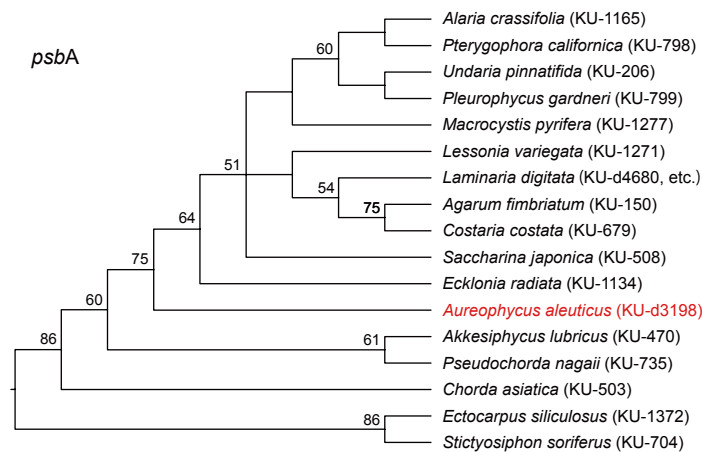
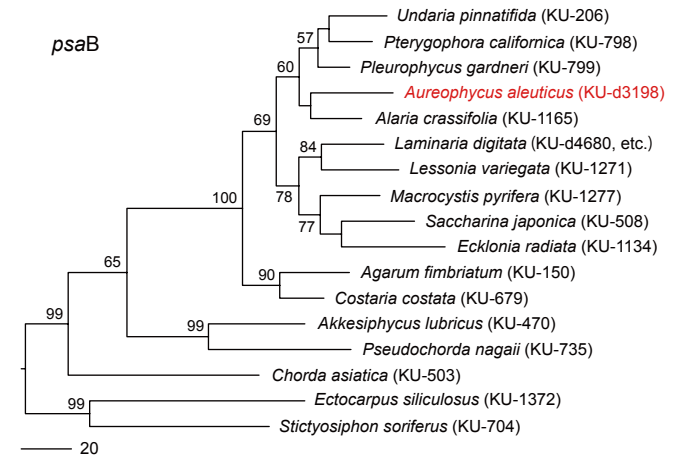
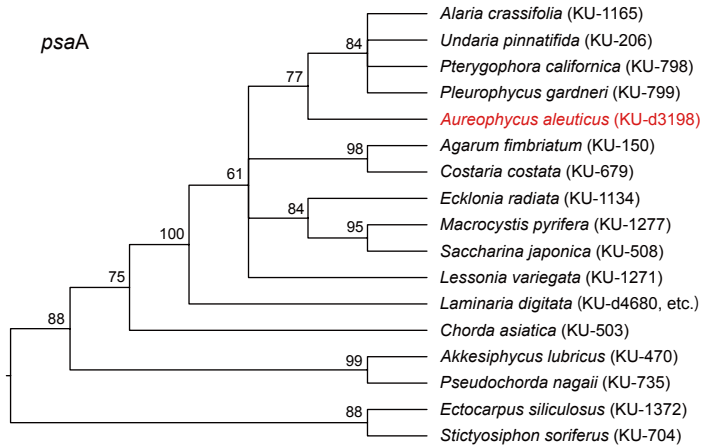
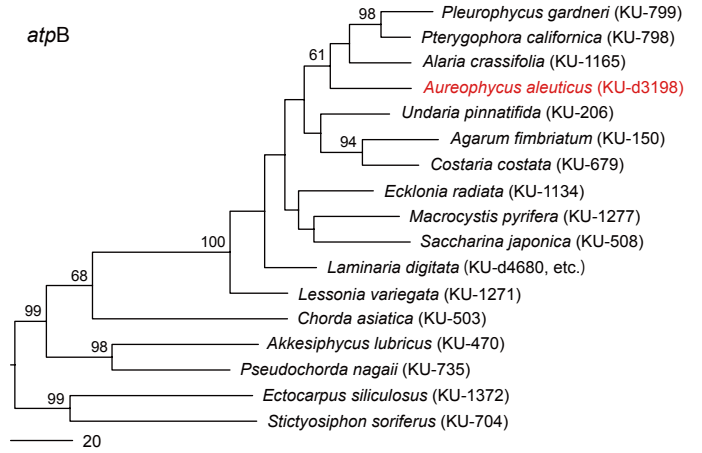
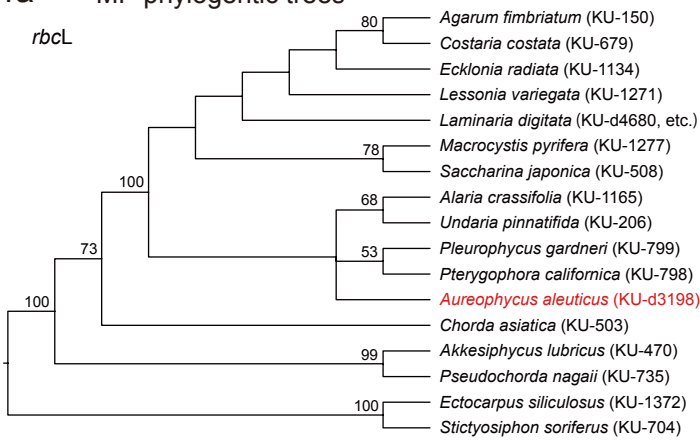
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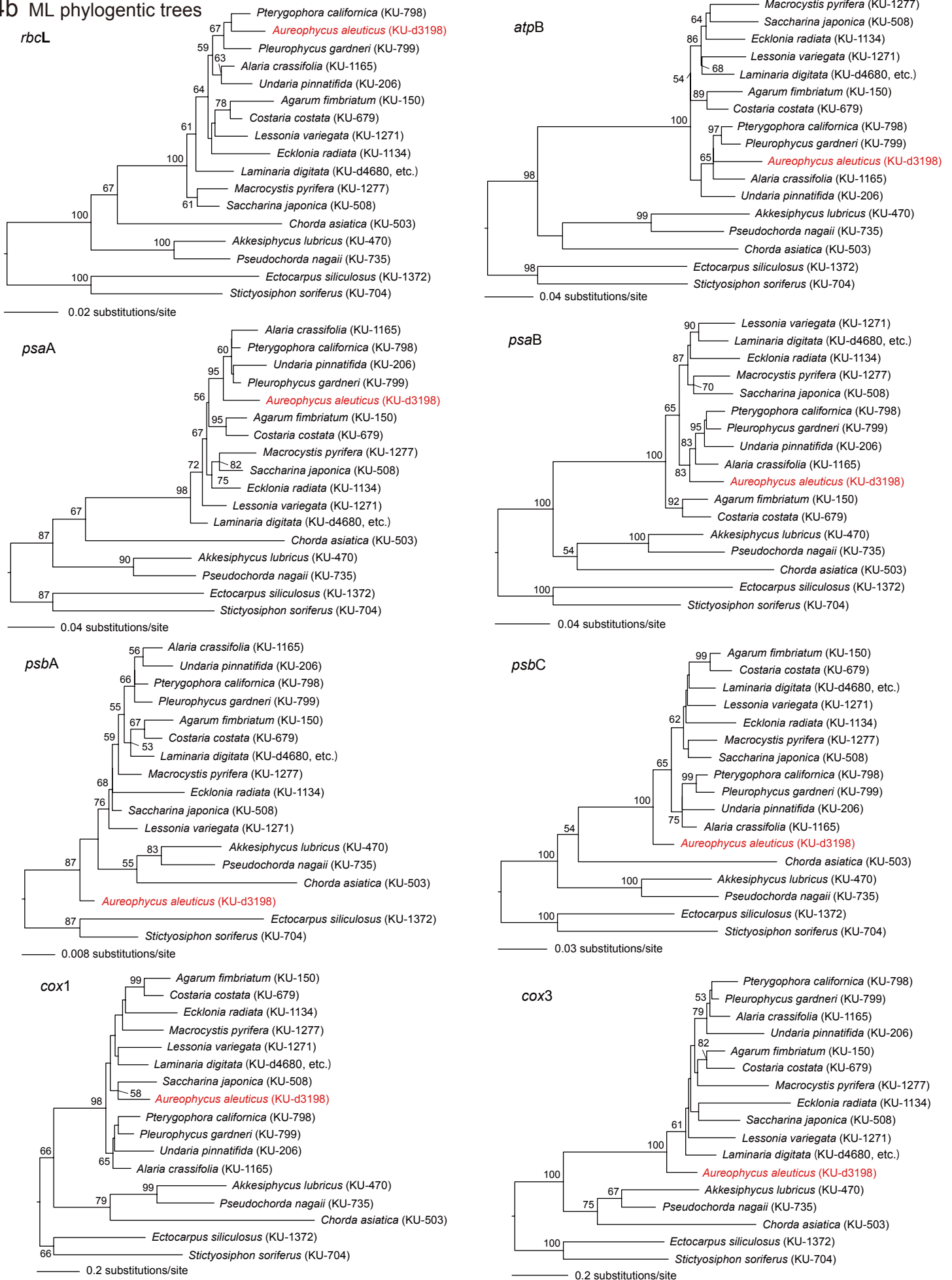


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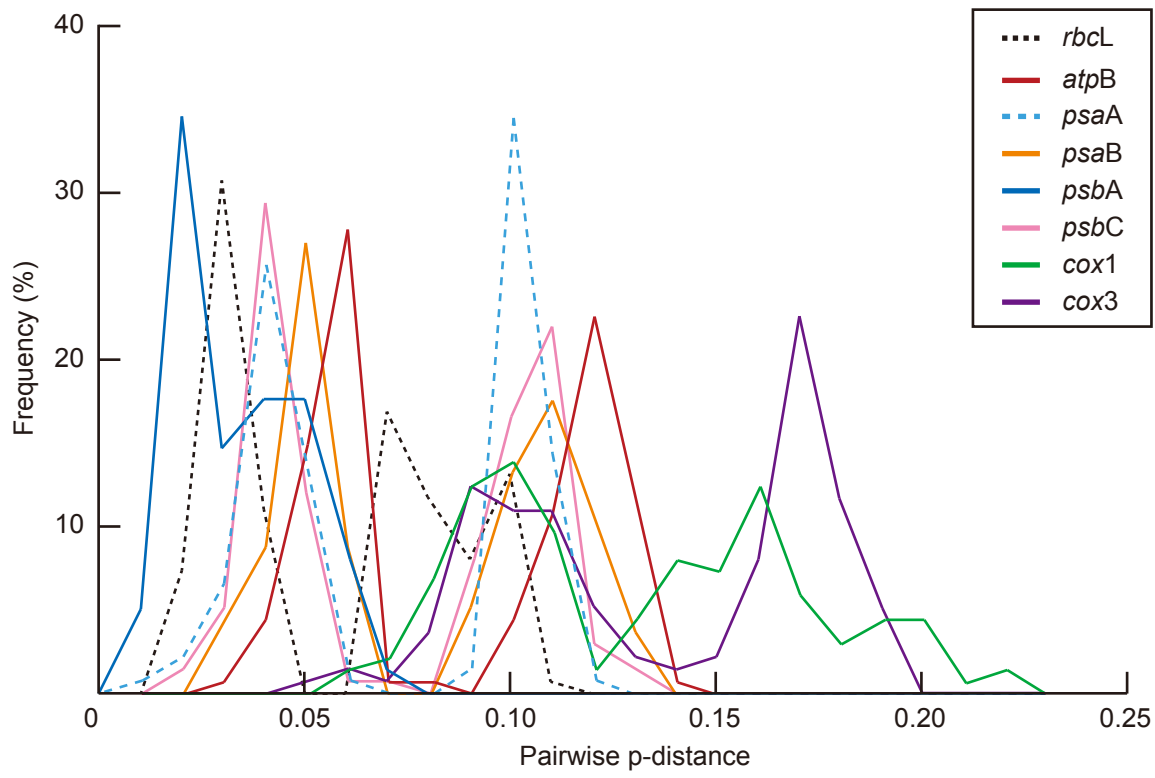
#### 4a MP phylogenetic trees



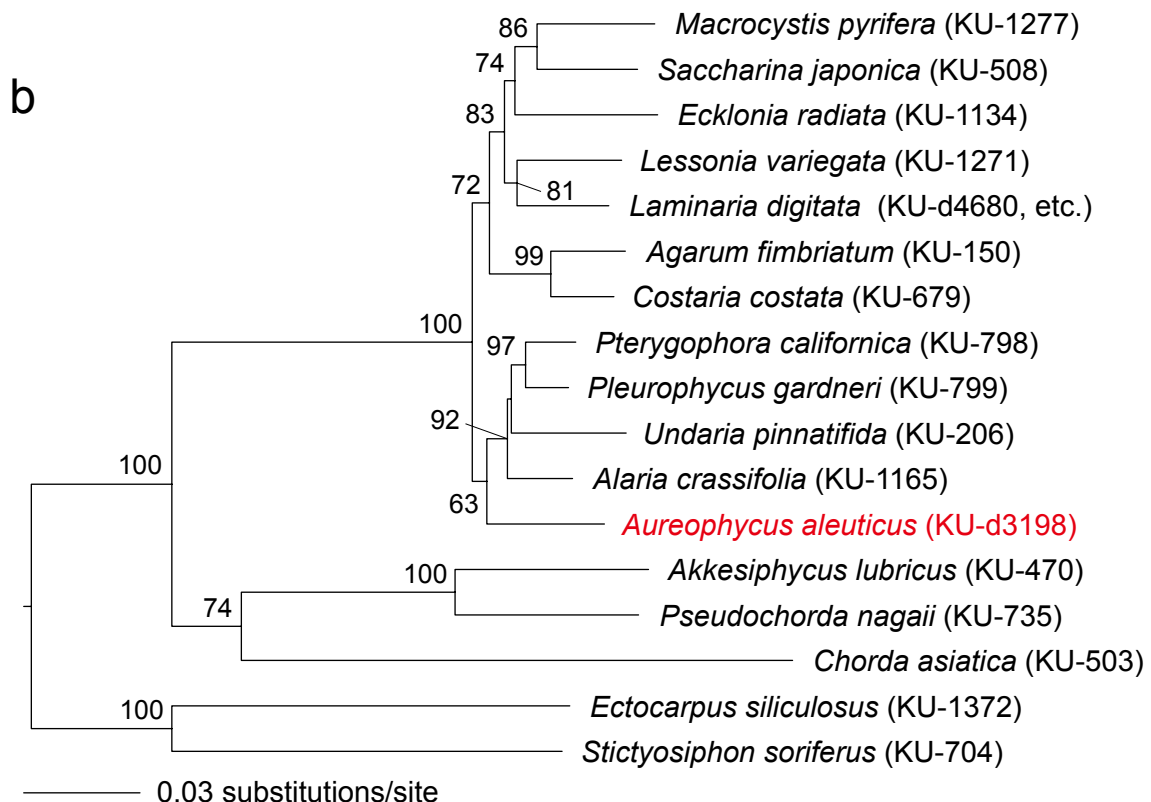
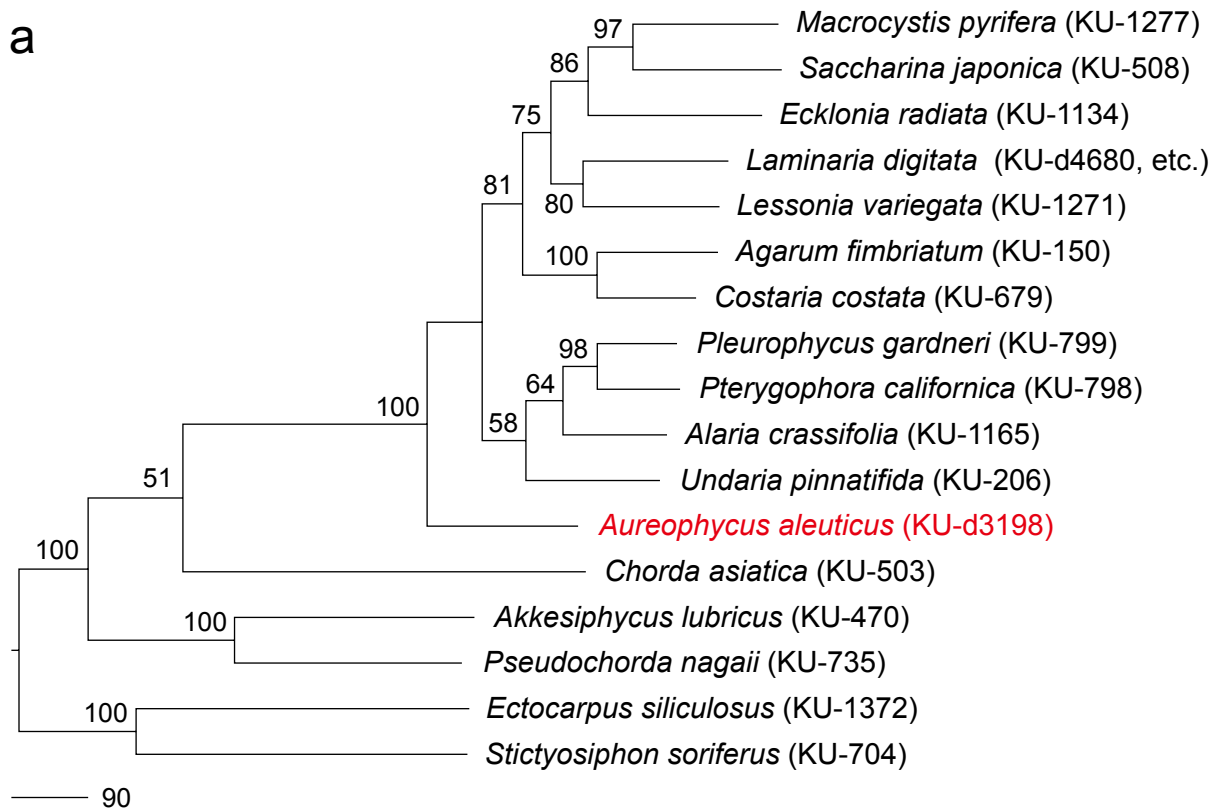
## 4b ML phylogenetic trees



**Supplementary Information 4.** a, Molecular phylogenetic trees based on 8 genes sequences (chloroplast *rbcL*, *atpB*, *psaA*, *psaB*, *psbA*, *psbC* and mitochondrial *cox1* and *cox3* genes) in MP analyses. b, Molecular phylogenetic trees based on 8 independent genes sequences (chloroplast *rbcL*, *atpB*, *psaA*, *psaB*, *psbA*, *psbC* and mitochondrial *cox1* and *cox3* genes) in ML analyses. Bootstrap values > 50% are shown on each node.

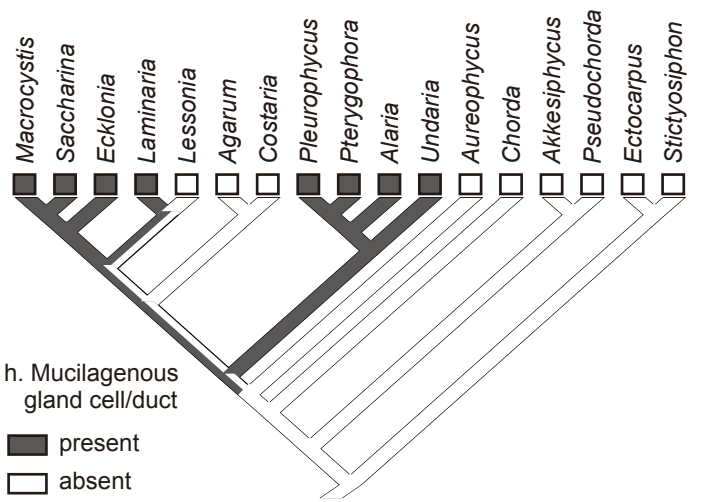
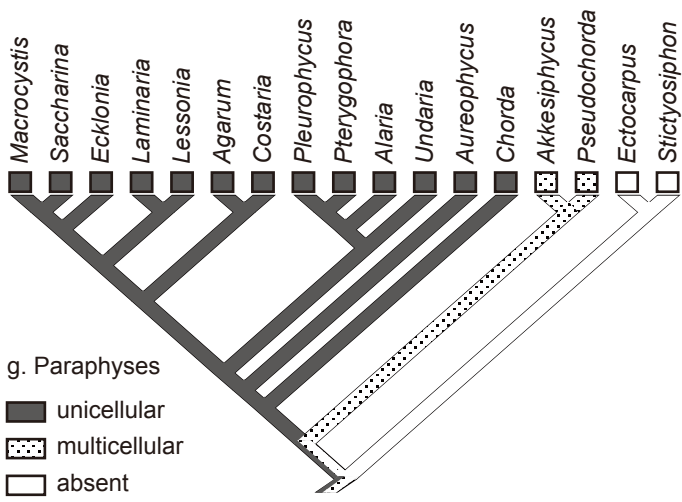
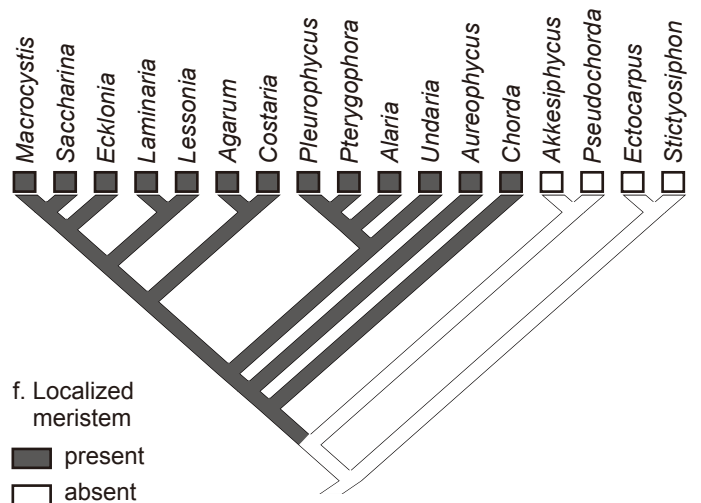
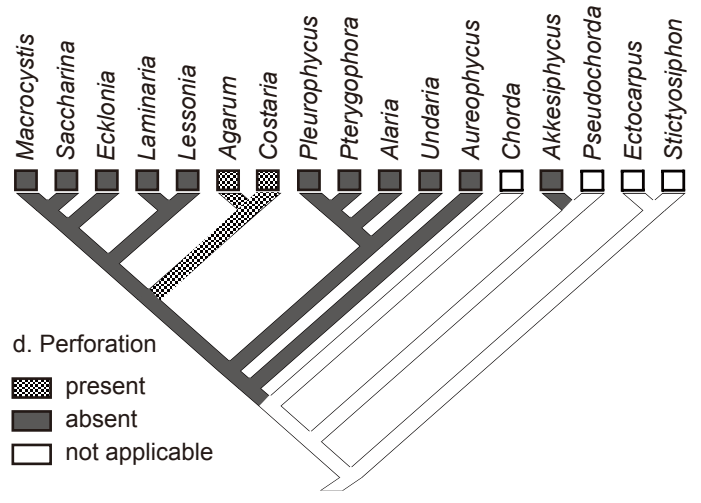
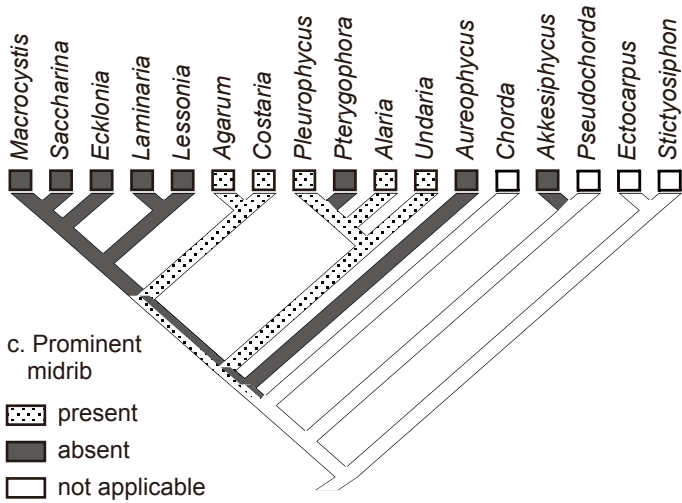
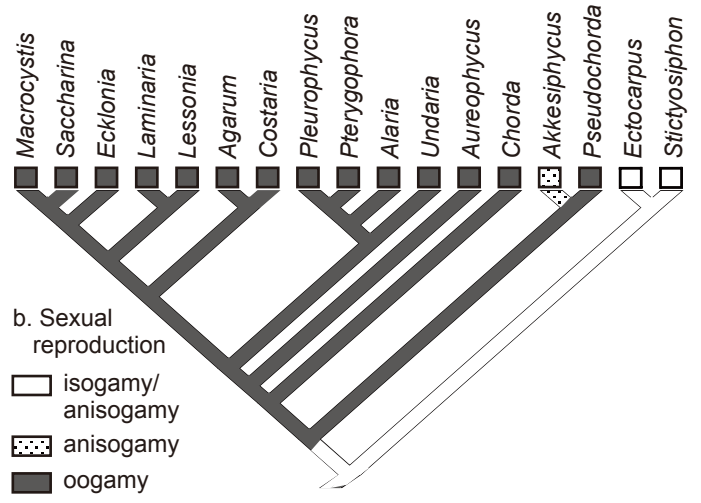
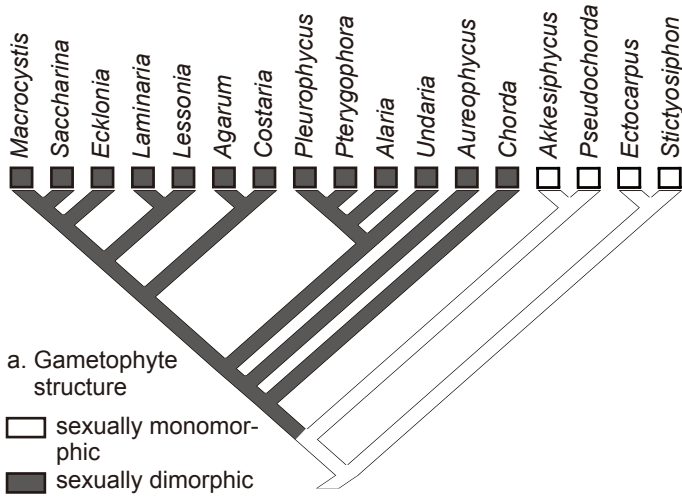


**Supplementary Information 5.** Comparisons of frequency of uncorrected p-distance for eight independent genes (*rbcL*, *atpB*, *psaA*, *psaB*, *psbA*, *psbC*, *cox1* and *cox3*).



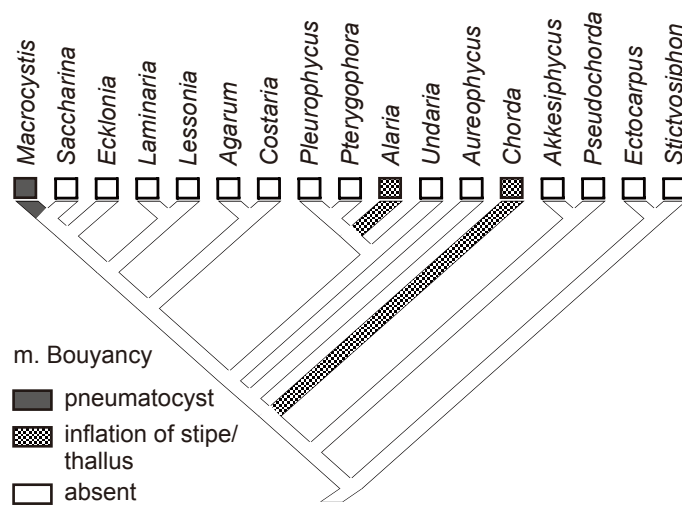
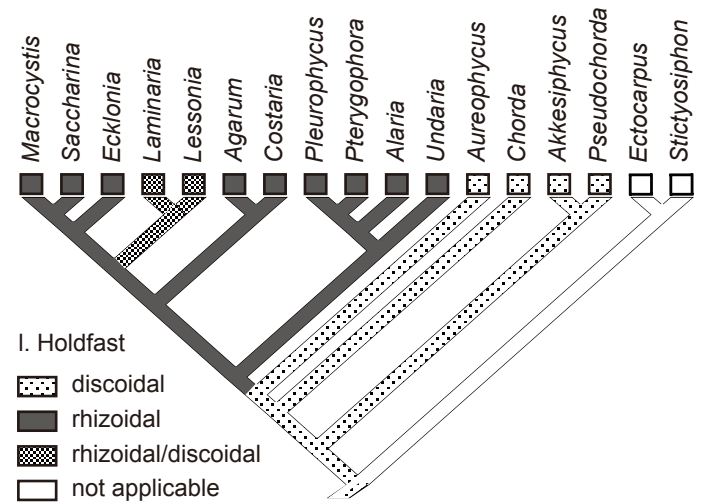
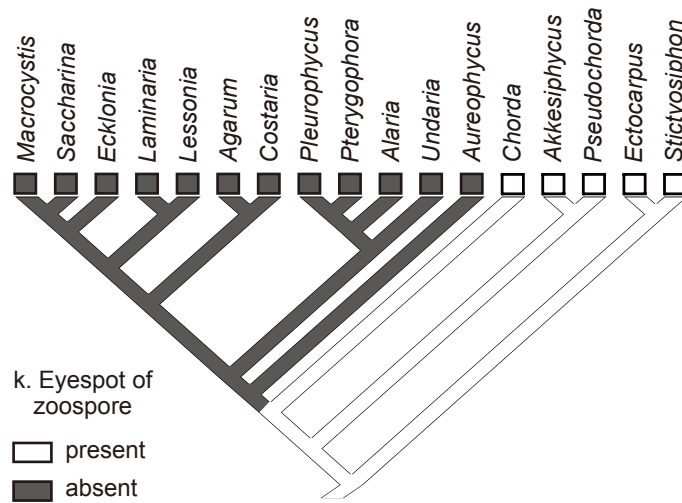
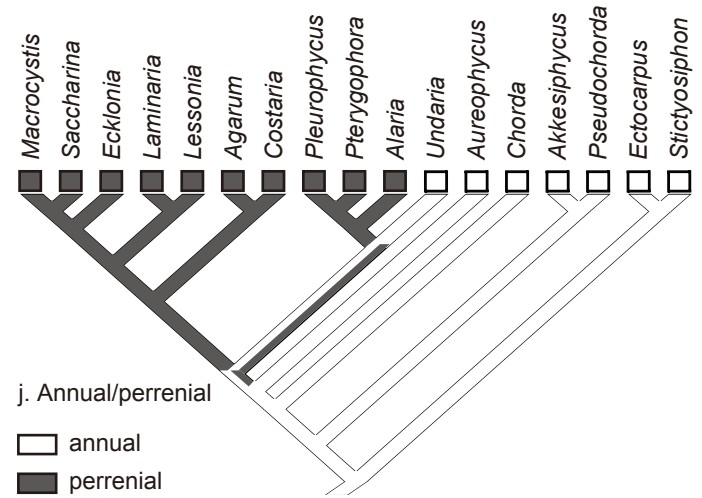
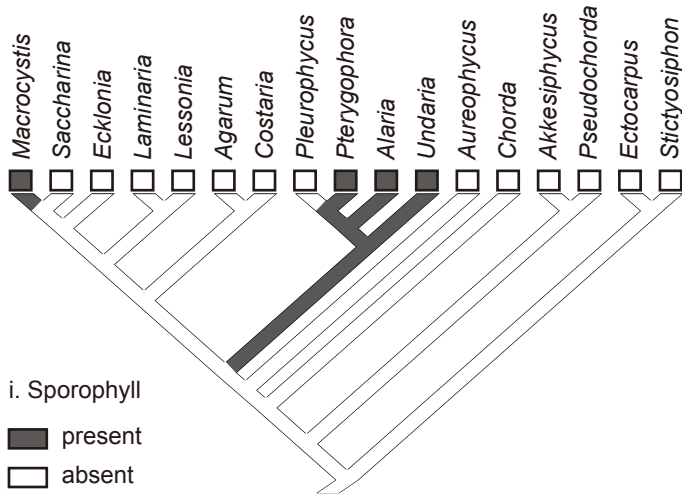
**Supplementary Information 6a, b.** Molecular phylogeny of representative laminarialean species including *Aureophycus aleuticus* based on the concatenated DNA sequences of chloroplast *rbcL*, *atpB*, *psaA*, *psaB*, *psbC* and mitochondrial *cox1* and *cox3* genes. a, Maximum Parsimony (MP) tree. Only bootstrap values > 50% are shown. b, Maximum Likelihood (ML) trees without constraint.

7a



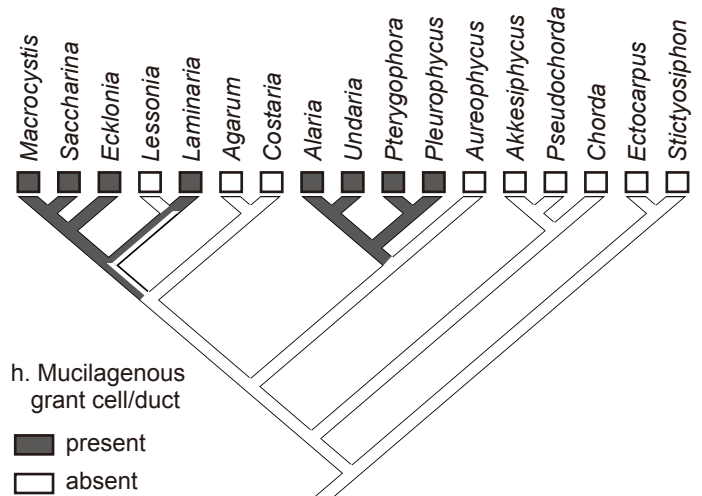
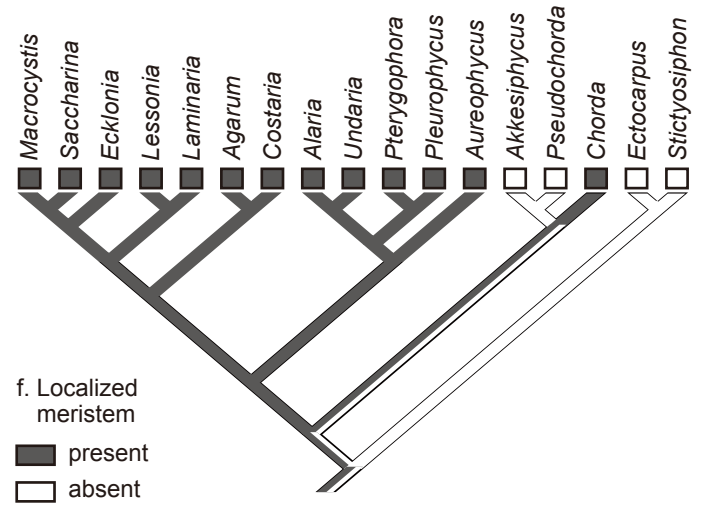
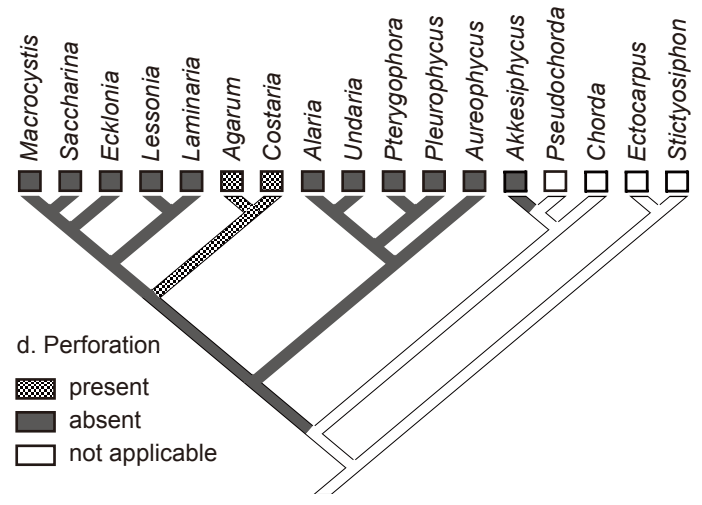
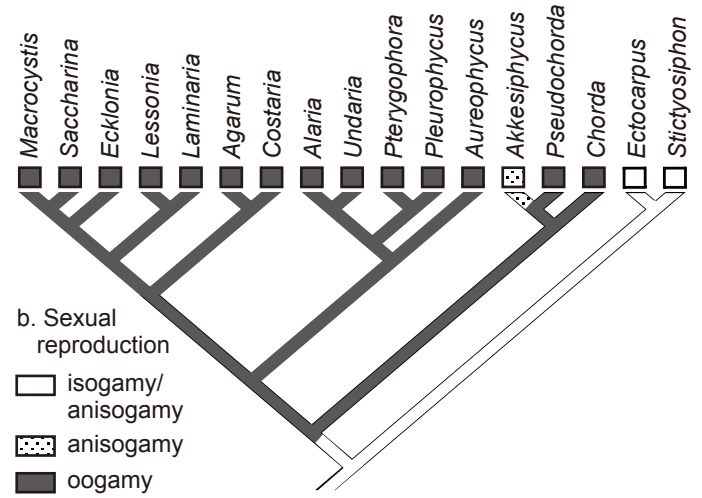
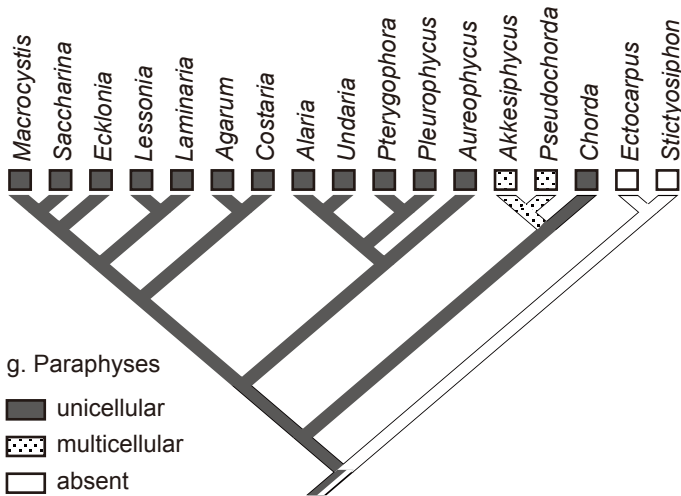
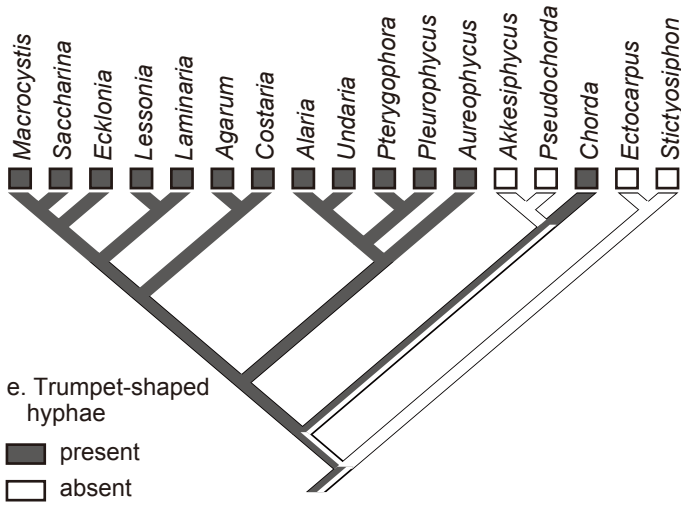
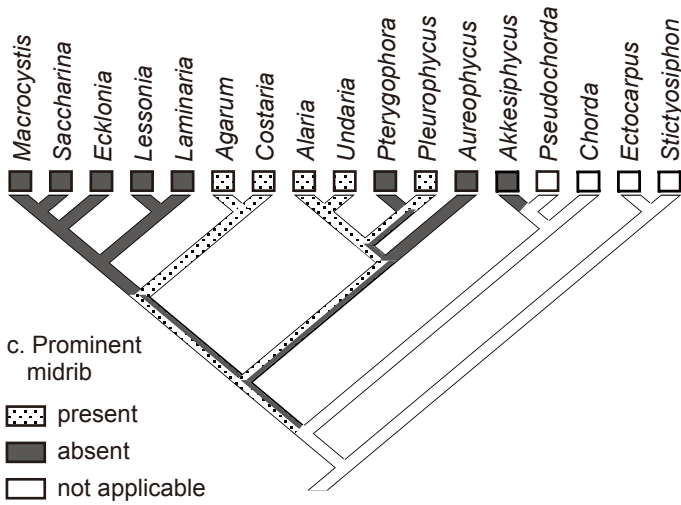
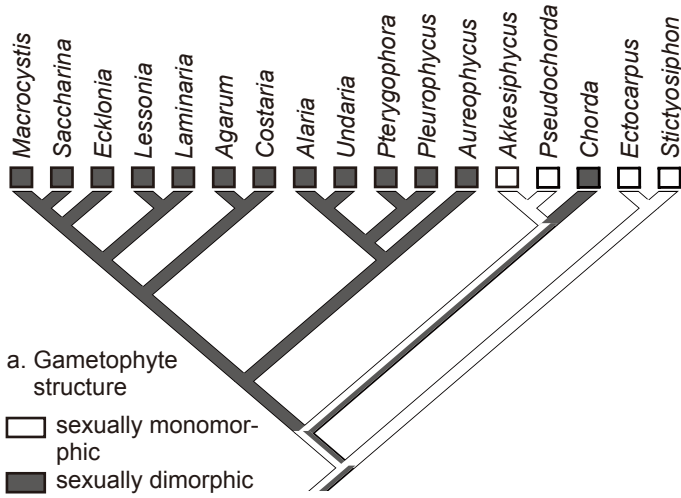


7b



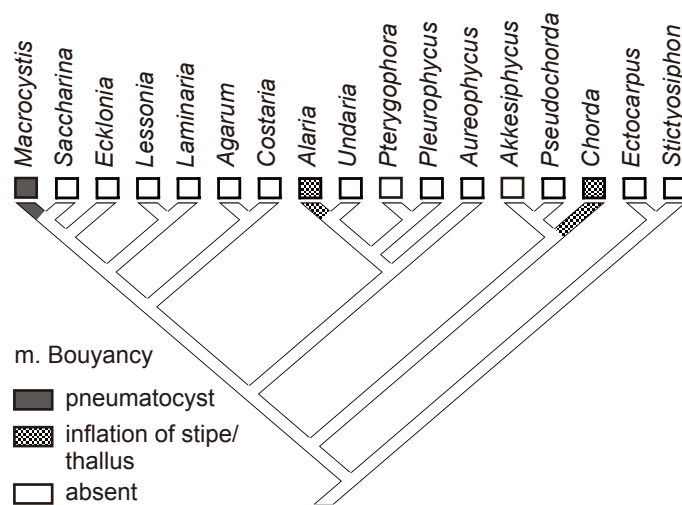
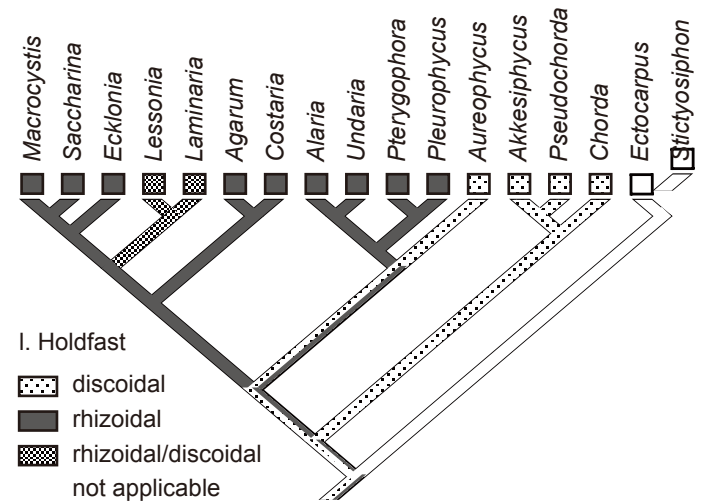
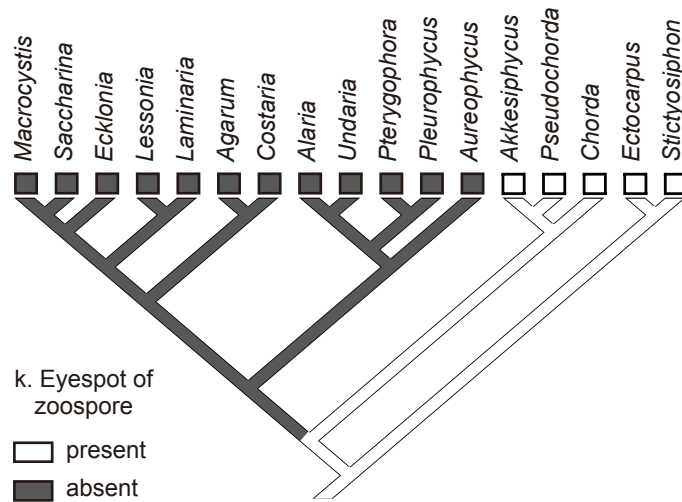
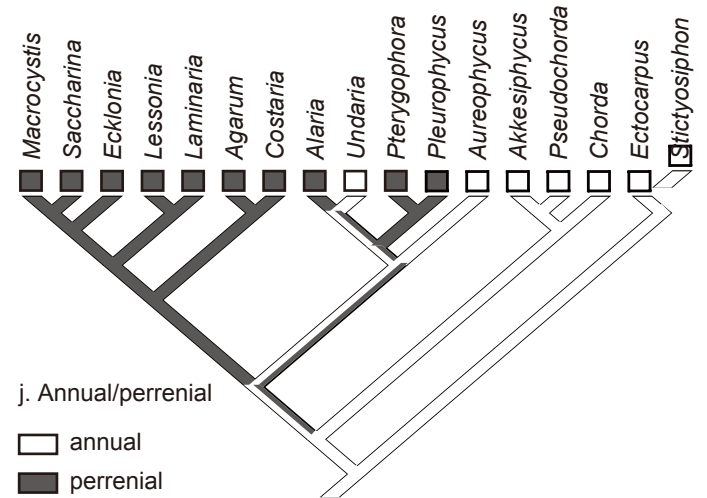
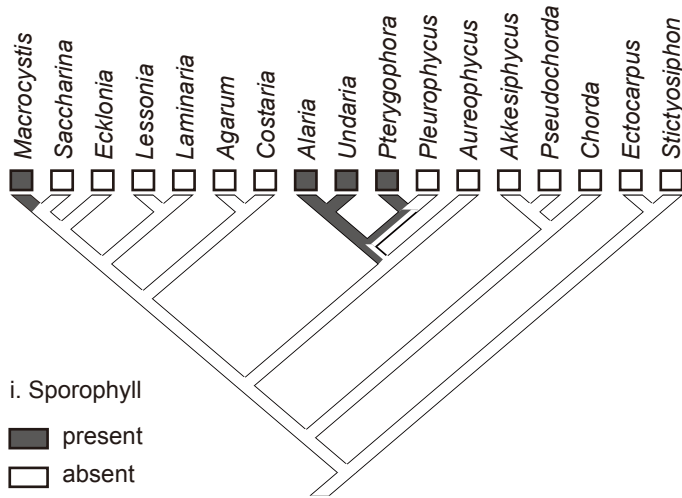
**Supplementary Information 7a,b.** a, Character mapping of representative taxonomic features of Laminariales on to the MP phylogenetic tree inferred from *rbcL*, *atpB*, *psaA*, *psaB*, *psbA*, *psbC*, *cox1* and *cox3* genes. The boxes under each terminal taxon name indicate the state known in that taxon: a, gametophyte structure; b, sexual reproduction; c, midrib; d, perforation; e, trumpet-shaped hyphae; f, localized meristem; g, paraphyses; h, mucilagenous grant cell/duct; i, sporophyll; j, annual/perennial; k, eyspot of zoospore; l, holdfast; m, bouyancy. [original drawings]

8a



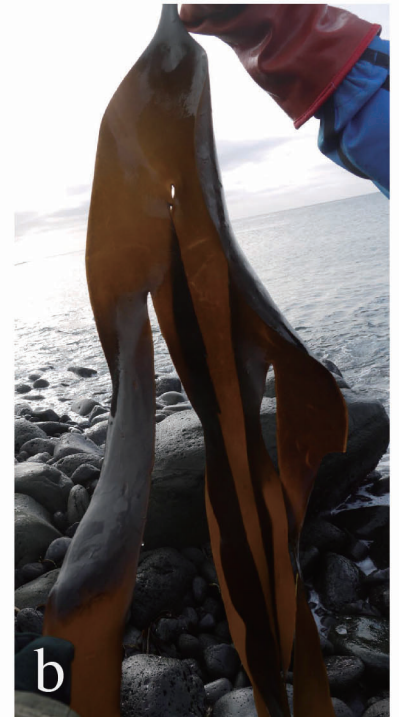


8b



**Supplementary Information 8a,b.** a, Character mapping of representative taxonomic features of Laminariales on to one of the ML phylogenetic trees inferred from *rbcL*, *atpB*, *psaA*, *psaB*, *psbA*, *psbC*, *cox1* and *cox3* genes. The boxes under each terminal taxon name indicate the state known in that taxon: a, gametophyte structure; b, sexual reproduction; c, midrib; d, perforation; e, trumpet-shaped hyphae; f, localized meristem; g, paraphyses; h, mucilaginous grant cell/duct; i, sporophyll; j, annual/perennial; k, eyspot of zoospore; l, holdfast; m, bouyancy. [original drawings]





**Supplementary Information 9.**  
Comparisons of *Laminaria yezoensis* (a, b) and *Aureoophycus auleus* (c-e) at St. George Is. e shows thalli damaged by herbivores (sea snails).  
[Photographs by H.Kawai]