

Table 1. Collecting sites and genotyped isolates; sil = *E. siliculosus*, cro = *E. crouaniorum*

Site no	Collecting dates	Region	Locality	Coordinates†	Site	Zones examined	Site properties	Reason for choice; comments	Species collected‡
1	Repeatedly 20-01-02 to 21-08-09	Brittany	Santec	48.721N, 4.012W	Western shore of Perharidy peninsula	High intertidal to upper subtidal	Rocky shore with sandy beaches, tide- pools, sheltered	Vicinity to laboratory; main study site	<i>E. siliculosus</i> , <i>E. fasciculatus</i> , <i>E. crouaniorum</i> , putative hybrid cro x sil
2	18-05-02	Brittany	Roscoff	48.729N, 3.993W	Near Biological Station	Mid intertidal	sheltered	Vicinity to laboratory	<i>E. siliculosus</i>
3	24-04-05, 25-05-05	Brittany	Roscoff	48.725N, 3.973W	Old harbour, close to Chapel St Barbe	High intertidal	sheltered	Vicinity to laboratory	<i>E. crouaniorum</i>
4	12-03-05, 10-04-05	Brittany	Plougastel	48.343N, 4.434W	Le Caro	High intertidal to upper subtidal	sheltered, no tide pools	Rich in small algae	<i>E. siliculosus</i> , <i>E. fasciculatus</i>
5	24-05-05, 22-08-05, 27-05-06	Brittany	Plougonvelin	48.345N, 4.700W	Traezh Hir	High intertidal to upper subtidal	Rocky shore with tide pools, sheltered	Collecting site of P.L. and H.M. Crouan in the 19 th century [§]	<i>E. siliculosus</i> , <i>E. fasciculatus</i> , <i>E. crouaniorum</i>
6	24-06-06	Brittany	Plougasnou	48.708N, 3.749W	Beg an fry	High to mid intertidal	Rocky shore, wave- exposed	Exposure	<i>E. crouaniorum</i>
7	26-06-06	Normandy	Cherbourg	49.657N, 1.652W	Baie Ste Anne	High to mid intertidal	Rocky shore with tide pools, sheltered	Type locality of <i>E. crouaniorum</i>	<i>E. siliculosus</i> , <i>E. crouaniorum</i>

E. sp.

† Determined using google maps

‡ Identification based on ITS1 length genotype (see text) or DNA sequences

§ Crouan and Crouan (1852)

Table 2. DNA sequences utilised in the present study; newly generated sequences in bold. Acc=DDBJ accession; bp=base pairs (length)

Strain	Species	Origin	SSU		ITS1		ITS2	5'-LSU		rbcL		Rubisco spacer†		cox3		rps14		rps14- atp8 spacer
			Acc	bp	Acc	bp	bp	Acc	bp	Acc	bp	Acc	bp	Acc	bp	Acc	bp	bp
Ec393	<i>E. siliculosus</i>	Site 1	FN564440	1806	FN564440	716	254	FN564440	1071	FN564467	1467	FN564467	515	FN564512	665	FN564545	288	145
Ec540	<i>E. siliculosus</i>	Site 5	---	---	FN564448	714	254	---	---	---	---	FN564470	515	FN564517	665	FN564548	288	145
Ec395	<i>E. fasciculatus</i>	Site 1	FN564441	1806	FN564441	429	289	FN564441	1071	FN564468	1467	FN564468	515	FN564513	665	FN564546	288	140
Ec541	<i>E. fasciculatus</i>	Site 5	---	---	FN564449	440	289	---	---	---	---	FN564471	515	FN564518	665	FN564549	288	148
Ec471	<i>E. crouaniorum</i>	Site 1	FN564442	1806	FN564442	867	263	FN564442	1072	FN564469	1467	FN564469	515	FN564514	665	FN564547	288	139
Ec496	<i>E. crouaniorum</i>	Site 3	---	---	FN564451	869	263	---	---	---	---	FN564473	515	FN564515	665	FN564551	288	141
Ec537	<i>E. crouaniorum</i>	Site 5	---	---	FN564450	863	259	---	---	---	---	FN564472	515	FN564516	665	FN564550	288	141
Ec318	<i>E. crouaniorum</i>	Site 7	---	---	FN564443	869	---	---	---	---	---	---	---	---	---	---	---	---
Ec319	<i>E. sp</i>	Site 7	---	---	FN564452	646	282	---	---	---	---	FN564474	515	FN564519	665	FN564552	288	206
CCAP1310/177	<i>E. siliculosus</i>	Roscoff, Brittany, France Plouescat, Brittany,	---	---	U38760	714	255	---	---	---	---	U38747	515	FN564520	665	AJ550051	288	145
CCAP1310/12	<i>E. fasciculatus</i>	France Isle of Man,	---	---	U38824	431	294	---	---	---	---	U38711	515	FN564521	665	AJ550055+	288	148
CCAP1310/144	<i>E. crouaniorum</i>	United	---	---	U38771	865	263	---	---	---	---	U38726	515	FN564522	665	---	---	---

		Kingdom																
		Kaikoura,																
		New																
CCAP1310/47	<i>E. "siliculosus"</i>	Zealand	---	---	U38766	753	267	---	---	---	---	U38722	515	FN564523	665	AJ550052	288	126
		San Juan de Marcona,	from					from						from				
			genome					genome						genome				
CCAP1310/4	<i>E. "siliculosus"</i>	Peru	project	1806	AJ550048	362	252	project	xxx	FP102296 [§]	1467	AJ550050	515	project	665	AJ550053	288	138
		Villefranche,																
KuckVF	<i>Kuckuckia sp.</i>	France	---	---	U38829	828	274	---	---	---	---	U38705	467	FN564524	665	FN564553	288	136
		I. Robinson																
		Crusoe,																
KuckJFer	<i>Kuckuckia sp.</i>	Chile	---	---	U38825	690	274	---	---	---	---	U38709	515	FN564525	665	FN564554	288	136

† Including flanking *rbcL* and *rbcS* gene sequences

‡ Sequence for an *E. fasciculatus* strain from Roscoff used for crossing; no sequence of CCAP1310/12 available for this marker

§ plastid sequence (Le Corguillé *et al.* 2010)

Table 3. Primers used for polymerase chain reaction (PCR)

Organelle	Marker	Primer Name	Sequence 5'-3'	Reference
Nucleus	SSU part 1	TW1CF	GTAGTCATACGCTTGTCTC	White <i>et al.</i> (1990)
		TW5SR	TTCCGTCAATTCTTTAAGTT	White <i>et al.</i> (1990)
	SSU part 2	TW5F	AACTTAAAGGAATTGACGGAAG	White <i>et al.</i> (1990)
		AFP1R	GGTAATGATCCTTCCGCAG	new primer
	ITS1	AFP4LF	CAATTATTGATCTTGAACGAGG	Peters <i>et al.</i> (2004b)
		5.8S1R	TGATGATTCACTGGATTCTG	Peters <i>et al.</i> (2004b)
	ITS2	5.8S3F	CGACGGATGTCTTGGCTC	new primer
		LSU410R	TCCTTCGCTTCCCTTTCAG	new primer
	5'-LSU	LSU-16F	CCGATCAAGCAAGAGGACC	Peters and Ramírez (2001)
		LSU1046R	TGGCCCACTAGCAACCTTC	Peters and Ramírez (2001)
Mitochondrion	cox3	cox3P1F	GAYCCWAGTCCMTGGCCWTTAG	Ni-Ni-Win <i>et al.</i> (2008)
		cox3P2R	ACAAARTGCCAATACCAAGC	Ni-Ni-Win <i>et al.</i> (2008)
	rps14+spacer	nad3F1D	GGTAGYYTAGATTGGGAATG	Peters <i>et al.</i> (2004b)
		atp8R1D	AAAAAAGTCATTRTATCRAATTG	Peters <i>et al.</i> (2004b)
Plastid	rbcL part 1	rbcL3F	GGCACCGGAGAATCTATATG	Peters and Ramírez (2001)
		rbcL958R	ACACCACACATACGCATCCA	new primer
	rbcL part 2	rbcL461F	CTTACTTAAAACTTTCCAAGG	Peters and Ramírez (2001)
		rbcL1087R	CCATATCAAAGAATAAACCTTC	new primer
	rbcL part 3	rbcL461F	CTTACTTAAAACTTTCCAAGG	Peters and Ramírez (2001)
		rbcL1432R	GCAACTTCTACGAAATCAGG	new primer
	Rubisco spacer	rbcL1273F	GTGCGACAGCTAACCGTG	Peters <i>et al.</i> (2004b)

rbcS139R

AGACCCATAATTCCCAATA

Peters and Ramírez (2001)

Table 4. Results of crossing experiments between *E. siliculosus* (sil), *E. fasciculatus* (fas) and *E. croauaniorum* (cro) from Roscoff. f=female, m=male, +=present, -=absent

Gamete combination	Zygote development	Replicate experiments	Zygotes examined
sil f x sil m	Sporophyte	3	35
fas f x fas m	Sporophyte	2	55
cro f x cro m	Sporophyte	4	37
sil f x fas m†	Abortive	0	0
fas f x sil m†	No gamete fusion	0	0
sil f x cro m	Abortive	5	71
cro f x sil m	Abortive/+‡	6	56
fas f x cro m	No gamete fusion	2	0
cro f x fas m	Abortive	2	38

† Experiment not performed; result taken from Müller and Eichenberger (1995)

‡ In one out of six experiments hybrid sporophytes developed; they were unable to form meiospores (see text for details)

Table 5. Field records of the generations of *E. crouaniorum* at Sites 1 and 3.

P = plurilocular, U = unilocular, veg = vegetative, - = not applicable

	Spring	Repro- duction	Summer	Repro- duction	Autumn and winter	Repro- duction
Gametophyte	End of March	P	25-06-09	P	searched	-
(epiphytic on	to June	P	28-06-07	P	but not	
<i>Scytosiphon</i> or	(nine visits	P	22-07-09	P	seen	
<i>Asperococcus</i>)	in 2003- 2009): always encountered	P				
Sporophyte	21-03-04	U+P	28-06-07	P	27-11-07	P
(on shells,	06-04-08	veg	21-08-09	P	20-01-08	P
stones or old	15-04-07	U+P	30-08-07	P		
wood)	24-04-05	U+P				