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Reinstatement of *Ectocarpus crouaniorum* Thuret in Le Jolis as a third common species of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Western Europe, and its phenology at Roscoff, Brittany

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SUMMARY

Based on morphological characters, cross-fertility and molecular systematics, two species are currently recognized in the ubiquitous temperate brown algal genus *Ectocarpus*: the type species *E. siliculosus* (Dillwyn) Lyngbye and *E. fasciculatus* Harvey. We studied diversity, cross-fertility and ecology of *Ectocarpus* in megatidal areas in northwest France (Western Europe) and propose to reinstate a third species, *E. crouaniorum* Thuret in Le Jolis. Genotyping of 67 individuals from five localities, including the type locality of *E. crouaniorum*, using internal transcribed spacer 1 (ITS1) length as a marker, showed that the three species co-occurred whenever the habitat was suitable. Our survey also revealed a single putative field hybrid between *E. crouaniorum* and *E. siliculosus*, and a single individual of a further *Ectocarpus* genotype. In laboratory experiments, *E. crouaniorum* was crossed with *E. siliculosus* and *E. fasciculatus*. In 12 of 13 crosses, the zygotes did not develop (postzygotic sterility); in one experiment a viable hybrid was produced after crossing a female *E. crouaniorum* with a male *E. siliculosus*, but this hybrid was unable to form meiospores. Phylogenetic analysis of five molecular markers from the nuclear, mitochondrial and plastid genomes (in total 1818 bp) confirmed genetic separation of the three species. Ecologically, *E. crouaniorum* was confined to high intertidal pools and run-offs, where the gametophyte was common from spring to summer. Another characteristic was that it usually occurred as an epiphyte of up to 12 cm in length on erect thalli of *Scytosiphon lomentaria*. Sporophytes of *E. crouaniorum* were found all year long; they were <3 cm in size or microscopic and were epilithic in the same habitat. The presence of a third species of *Ectocarpus* in Western Europe suggests that species diversity in this genus is larger than recognized during the last 40 years.

Key words: biological species, *Ectocarpus*, genotyping, hybrid, internal transcribed spacer 1 length, phenology, phylogeny, post-zygotic sterility, taxonomy.

INTRODUCTION

Ectocarpus is a brown algal genus with a long research history (Peters *et al.* 2004a; Coelho *et al.* 2007; Charrier *et al.* 2008) and includes the first macroalga for which the genome has been entirely sequenced (http://www.genoscope.cns.fr/spip/Ectocarpus-siliculosus,740.html). However, species diversity within the genus is not yet resolved. Based on the number of taxa described, *Ectocarpus* is one of the most species-rich genera of the Phaeophyceae: AlgaeBase lists 410 species of which 101 are flagged as currently accepted taxonomically (Guiry & Guiry 2009). In the 19th and the first half of the 20th century, brown algae with uniseriate branched filaments were generally classified in *Ectocarpus*, leading to a proliferation of new species; currently two morphological characters are being used to separate members of the genus *Ectocarpus sensu stricto* from other small brown algae. First, plastids are extended ribbons bearing

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pedunculated pyrenoids, and are not discoid as in the majority of genera with similar thalli, such as *Hincksia*, *Pylaiella*, *Acinetospora* and *Feldmannia* (Kornmann & Sahling 1977; Peters & Ramírez 2001). Second, *Ectocarpus* has no genuine phaeophycean hairs, which are present in the sister genus, *Kuckuckia* (Hamel 1939; Kuckuck 1958; Pedersen 1989).

Species distinctions within *Ectocarpus sensu stricto* have differed greatly among investigators; morphological, biological and phylogenetic species concepts and chemotaxonomy have been used to investigate its diversity. The most comprehensive morphological studies were done on European representatives. A classification of five species into two major groups (section *siliculosi* and section *fasciculati*) based on branching pattern and sporangium shape was proposed by Hamel (1931–1939). Cardinal (1964) recognized four species, with seven varieties in *E. siliculosus* and three varieties in *E. fasciculatus*. Russell (1966, 1967) showed that sporangium morphology, formerly used to distinguish species in the *siliculosus* complex, was not informative. However, branching pattern consistently revealed a difference between thalli with conspicuous main axes and thinner, often fasciculate, laterals (*E. fasciculatus*) and thalli showing subdichotomous branching (*E. siliculosus*). Russell's proposal for a classification of European *Ectocarpus* in merely two species was generally accepted and was extended to all *Ectocarpus* world-wide. However, some authors continued to recognize additional species, such as *E. constanciae* Hariot, *E. acutus* Setchell et Gardner and *E. penicillatus* C. Agardh (Ricker 1987; Santelices 1989; Kim & Lee 1992; Stegenga *et al.* 1997).

Both *Ectocarpus siliculosus* and *E. fasciculatus* show an alternation of two generations. In culture studies Kornmann (1956), Müller (1964, 1966, 1967, 1972) and Kornmann and Sahling (1977) showed that the generations might differ in morphology, which to some extent would help explain the morphological diversity of field material noticed in previous studies. However, little is known about the distribution and phenology of the different generations in the field.

Crossing experiments (Müller & Eichenberger 1995) showed that there is intersterility between *E. siliculosus* and *E. fasciculatus* occurring sympatrically in Brittany: although male gametes of *E. fasciculatus* were able to fuse with female gametes of *E. siliculosus*, the resulting zygotes did not develop beyond a two-celled stage (post-zygotic intersterility). Gamete fusions were not observed in the reciprocal gamete combination (pre-zygotic intersterility). Within *E. siliculosus*, which is more widely distributed than *E. fasciculatus*, strains from different geographical origins were often but not always cross-fertile at the level of plasmogamy. However, post-zygotic sterility barriers were frequent: hybrids, particularly between isolates from different hemispheres, showed reduced development or normal growth but inhibition of meiosis (Müller 1977, 1979, 1988; Stache 1989, 1993); there were also cases of pre-zygotic reproductive isolation of *E. siliculosus* populations from NE America (Müller 1976a; cross-fertility data reviewed in Stache-Crain *et al.* 1997). Müller and Kawai (1991) concluded that *E. siliculosus* is a single species with a world-wide distribution consisting of many geographically separated populations that show full or slightly reduced interbreeding patterns.

Phylogeographic analysis of nuclear ribosomal internal transcribed spacer (ITS) and plastid Rubisco spacer sequences of 43 strains of *Ectocarpus* and seven of *Kuckuckia*, which had been isolated by Dieter Müller on all continents except Antarctica, supported a principal division of *Ectocarpus* into two major clades (Stache-Crain *et al.* 1997). This appeared to corroborate the recognition of only *E. siliculosus* and *E. fasciculatus*. However, cases of reproductive isolation between strains within the clade of *E. siliculosus* are documented. Moreover, sub-clades separated by considerable genetic distances (partly unalignable ITS sequences) suggest that in reality more species of *Ectocarpus* may exist.

The coast of Brittany in Western Europe, situated at the border of cold and warm-temperate biogeographic regions, is characterized by large tides and a mild stenotherm climate, which permit the presence of a diverse macroalgal flora (van den Hoek 1975). It was thus suitable for testing the hypothesis that only two species of *Ectocarpus* exist. For our study we collected and genotyped 67 isolates of *Ectocarpus* from five localities, crossed and sequenced a few of them and found evidence supporting recognition of a third species of *Ectocarpus*, which we found to best agree with *E. crouaniorum*, the name henceforth used in this paper.

MATERIALS AND METHODS

Field observations

A total of seven intertidal and upper subtidal habitats in northwestern France at four localities in Brittany and one in Normandy (Fig. 1) were examined for the presence of *Ectocarpus*. At the main study site, which lies close to the Roscoff Marine Station, observations were made in all seasons, the others were visited one to three times (Table 1). Thalli were placed in separate plastic bags during collection and processed immediately after transport to the laboratory.

Strain isolation and culturing

Replicate isolates of *Ectocarpus* were made from January 2002 onwards. Cultivation was done in autoclaved sea water from Roscoff, adding 10 mL Provasoli enrichment (prepared according to Starr & Zeikus

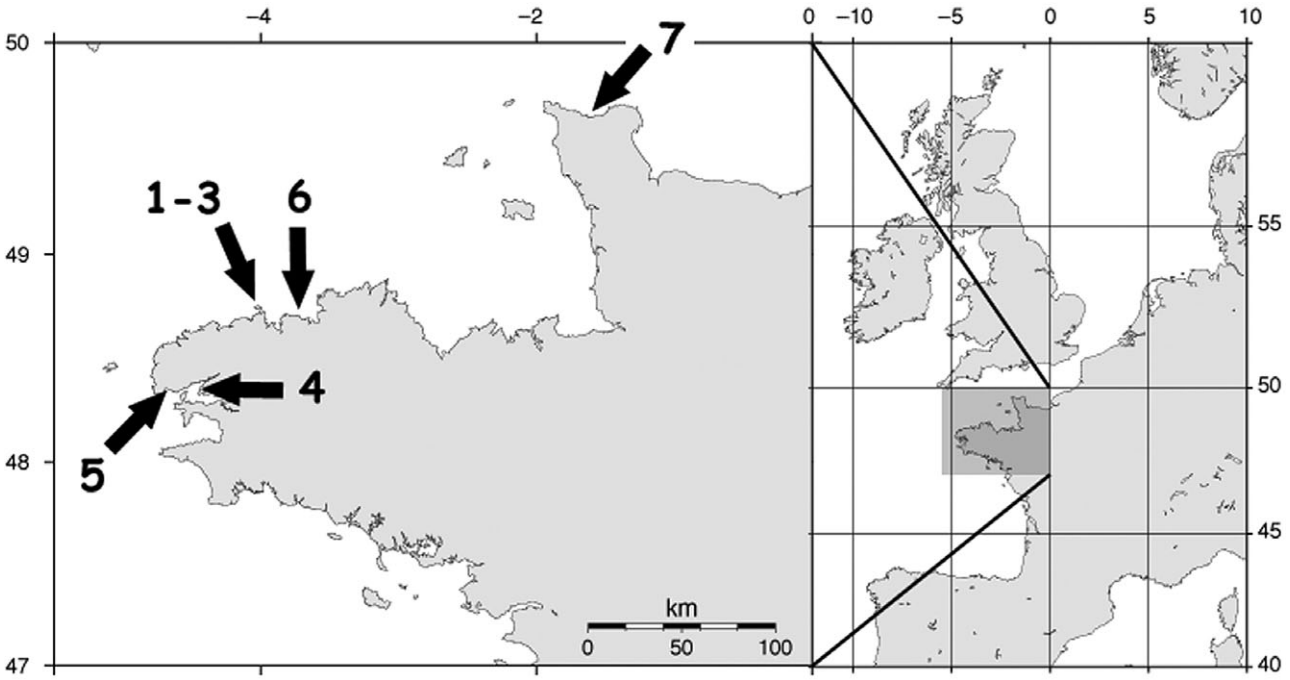


Fig. 1. Collecting sites of *Ectocarpus* in northwest France, Western Europe. See Table 1 for details.

1993) per liter, in non-aerated cultures at 14 ± 1°C in white light of 10–30 μmol photons m^{-2} s^{-1} and a day-length of 10 : 14 h LD (light : dark). For isolation, minute fragments of each field thallus were inoculated in four replicates in 2–3 mL culture medium with or without 6 mg L^{-1} GeO_2. After 2–6 weeks, the inoculates were microscopically examined for the presence of contaminants and for each sample a unialgal culture without eukaryotic contamination was transferred to culture medium without GeO_2.

Strain designations beginning with 'CCAP1310/' are from the Culture Collection of Algae and Protozoa, Oban, Scotland, numbers preceded by 'Ec' are isolates housed in the macroalgal culture collection at Roscoff maintained by the first author.

Genotyping and sequencing

Different sub-clades of *Ectocarpus* may show significantly differing ITS1 lengths, due to large indels in the first part of ITS1 (Stache-Crain *et al.* 1997). Length of ITS1 was therefore chosen for genotyping. DNA was extracted from culture material as described in Peters *et al.* (2004b), followed by polymerase chain reaction (PCR). Cultures were preferred over field material because in the latter a sample may contain more than a single individual. ITS1 length genotyping was carried out twice for each strain. We subsequently sequenced (method described in Peters *et al.* 2004b) nuclear and cytoplasmic markers (Table 2) in one to three different individuals of each ITS1 length genotype. Sequences were also generated in reference strains used for phylogenetic analyses (Table 2). Primer information is provided in Table 3.

Sexual crosses and raising of zygotes

Gametophytes of *E. crouaniorum* for experiments on cross-fertility were field thalli or they developed in culture from spores from unilocular sporangia of sporophytes. All originated from a population in the upper intertidal at site 1 growing on *Scytosiphon*, from which six thalli (including four involved in crosses) were genotyped and identified as *E. crouaniorum*. None of our new isolates of *E. siliculosus* was sexual; for crossing experiments we therefore used reference gametophyte strains from stock cultures. They were CCAP1310/330 (female) and CCAP1310/329 (male); these had been obtained from meiospores on a sporophyte (CCAP1310/331) resulting from a cross of a female strain from Roscoff (CCAP1310/178) with a male strain from Naples, Italy (CCAP1310/131), both isolated previously by Dieter Müller. The strains CCAP1310/178 and CCAP1310/131 are genetically similar (Peters *et al.* 2004b, as 'Ros f' and 'Na m') and belong to lineage 1a of *E. siliculosus* in Stache-Crain *et al.* (1997). Gametophytes of *E. fasciculatus* were obtained from a sporophyte growing endophytically in *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl *et* Saunders, which was isolated by the first author at site 1 on 20 January 2002. The specific identity of the *E. fasciculatus* reference strains was confirmed by their ITS1 length and *rps*14 sequences.

Table 1. Collecting sites and genotyped isolates

Site no.	Collecting dates	Region	Locality	Coordinates†	Site	Zones examined	Site properties	Reason for choice; comments	Species collected‡
1	Repeatedly 20-i-2002 to 21-viii-2009	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	*Ectocarpus siliculosus, Ectocarpus fasciculatus, Ectocarpus crouaniorum*, putative hybrid cro × sil
2	18-v-2002	Brittany	Roscoff	48.729N, 3.993W	Near Biological Station	Mid intertidal	Sandy beach with stones, tide pools, sheltered	Vicinity to laboratory	*E. siliculosus*
3	24-iv-2005, 25-v-2005	Brittany	Roscoff	48.725N, 3.973W	Old harbor, close to Chapel St Barbe	High intertidal	Sandy beach with stones, tide-pools, sheltered	Vicinity to laboratory	*E. crouaniorum*
4	12-iii-2005, 10-iv-2005	Brittany	Plougastel	48.343N, 4.434W	Le Caro	High intertidal to upper subtidal	Gravel beach, sheltered, no tide pools	Rich in small algae	*E. siliculosus, E. fasciculatus*
5	24-v-2005, 22-viii-2005, 27-v-2006	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 19th century§	*E. siliculosus, E. fasciculatus, E. crouaniorum*
6	24-vi-2006	Brittany	Plougasnou	48.708N, 3.749W	Beg an fry	High to mid intertidal	Rocky shore, wave-exposed	Exposure	*E. crouaniorum*
7	26-vi-2006	Normandy	Cherbourg	49.657N, 1.652W	Baie Ste Anne	High to mid intertidal	Rocky shore with tide pools, sheltered	Type locality of *E. crouaniorum*	*E. siliculosus, E. crouaniorum, Ectocarpus* sp.

†Determined using Google maps. ‡Identification based on ITS1 length genotype (see text) or DNA sequences. §Crouan and Crouan (1852).
cro, *E. crouaniorum*; sil, *E. siliculosus*.

Table 2. DNA sequences used in the present study (newly generated sequences in bold type)

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	*Ectocarpus siliculosus*	Site 1	**FN564440**	1806	**FN564440**	716	254	**FN564440**	1071	**FN564467**	1467	**FN564467**	515	**FN564512**	665	**FN564545**	288	145
Ec540	*E. siliculosus*	Site 5	ND	ND	**FN564448**	714	254	ND	ND	ND	ND	**FN564470**	515	**FN564517**	665	**FN564548**	288	145
Ec395	*Ectocarpus fasciculatus*	Site 1	**FN564441**	1806	**FN564441**	429	289	**FN564441**	1071	**FN564468**	1467	**FN564468**	515	**FN564513**	665	**FN564546**	288	140
Ec541	*E. fasciculatus*	Site 5	ND	ND	**FN564449**	440	289	ND	ND	ND	ND	**FN564471**	515	**FN564518**	665	**FN564549**	288	148
Ec471	*Ectocarpus crouaniorum*	Site 1	**FN564442**	1806	**FN564442**	867	263	**FN564442**	1072	**FN564469**	1467	**FN564469**	515	**FN564514**	665	**FN564547**	288	139
Ec496	*E. crouaniorum*	Site 3	ND	ND	**FN564451**	869	263	ND	ND	ND	ND	**FN564473**	515	**FN564515**	665	**FN564551**	288	141
Ec537	*E. crouaniorum*	Site 5	ND	ND	**FN564450**	863	259	ND	ND	ND	ND	**FN564472**	515	**FN564516**	665	**FN564550**	288	141
Ec318	*E. crouaniorum*	Site 7	ND	ND	**FN564443**	869	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ec319	*Ectocarpus sp.*	Site 7	ND	ND	**FN564452**	646	282	ND	ND	ND	ND	**FN564474**	515	**FN564519**	665	**FN564552**	288	206
CCAP1310/177	*E. siliculosus*	Roscoff, Brittany, France	ND	ND	U38760	714	255	ND	ND	ND	ND	U38747	515	**FN564520**	665	AJ550051	288	145
CCAP1310/12	*E. fasciculatus*	Plouescat, Brittany, France	ND	ND	U38824	431	294	ND	ND	ND	ND	U38711	515	**FN564521**	665	AJ550055‡	288	148
CCAP1310/144	*E. crouaniorum*	Isle of Man, United Kingdom	ND	ND	U38771	865	263	ND	ND	ND	ND	U38726	515	**FN564522**	665	ND	ND	ND
CCAP1310/47	*E. "siliculosus"*	Kaikoura, New Zealand	ND	ND	U38766	753	267	ND	ND	ND	ND	U38722	515	**FN564523**	665	AJ550052	288	126
CCAP1310/4	*E. siliculosus*	San Juan de Marcona, Peru	from genome project	1806	AJ550048	362	252	From genome project	xxx	FP102296§	1467	AJ550050	515	from genome project	665	AJ550053	288	138
KuckVF	*Kuckuckia sp.*	Villefranche, France	ND	ND	U38829	828	274	ND	ND	ND	ND	U38705	467	**FN564524**	665	**FN564553**	288	136
KuckJFer	*Kuckuckia sp.*	I. Robinson Crusoe, Chile	ND	ND	U38825	690	274	ND	ND	ND	ND	U38709	515	**FN564525**	665	**FN564554**	288	136

†Including flanking *rbc*L and *rbc*S 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.* 2009). Acc, DDBJ accession; bp, base pairs (length); LSU, large subunit; ND, no data; SSU, small subunit.

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

Organelle	Marker	Primer Name	Sequence 5′-3′	Reference
Nucleus	SSU part 1	TW1CF	GTAGTCATACGCTTGTCTC	White *et al.* (1990)
		TW5SR	TTCCGTCAATTCCTTTAAGTT	White *et al.* (1990)
	SSU part 2	TW5F	AACTTAAAGGAATTGACGGAAG	White *et al.* (1990)
		AFP1R	GGTAATGATCCTTCCGCAG	New primer
	ITS1	AFP4LF	CAATTATTGATCTTGAACGAGG	Peters *et al.* (2004b)
		5.8S1R	TGATGATTCACTGGATTCTG	Peters *et al.* (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 *et al.* (2008)
		cox3P2R	ACAAARTGCCAATACCAAGC	Ni-Ni-Win *et al.* (2008)
	rps14+spacer	nad3F1D	GGTAGYYTAGATTGGGAATG	Peters *et al.* (2004b)
		atp8R1D	AAAAAAGTCATTRTATCRAATTG	Peters *et al.* (2004b)
Plastid	rbcL part 1	rbcL3F	GGCACCGGAGAATCTATATG	Peters and Ramírez (2001)
		rbcL958R	ACACCACACATACGCATCCA	New primer
	rbcL part 2	rbcL461F	CTTACTTAAAAACTTTCCAAGG	Peters and Ramírez (2001)
		rbcL1087R	CCATATCAAAGAATAAACCTTC	New primer
	rbcL part 3	rbcL461F	CTTACTTAAAAACTTTCCAAGG	Peters and Ramírez (2001)
		rbcL1432R	GCAACTTCTACGAAATCAGG	New primer
	Rubisco spacer	rbcL1273F	GTGCGACAGCTAACCGTG	Peters *et al.* (2004b)
		rbcS139R	AGACCCCATAATTCCCAATA	Peters and Ramírez (2001)

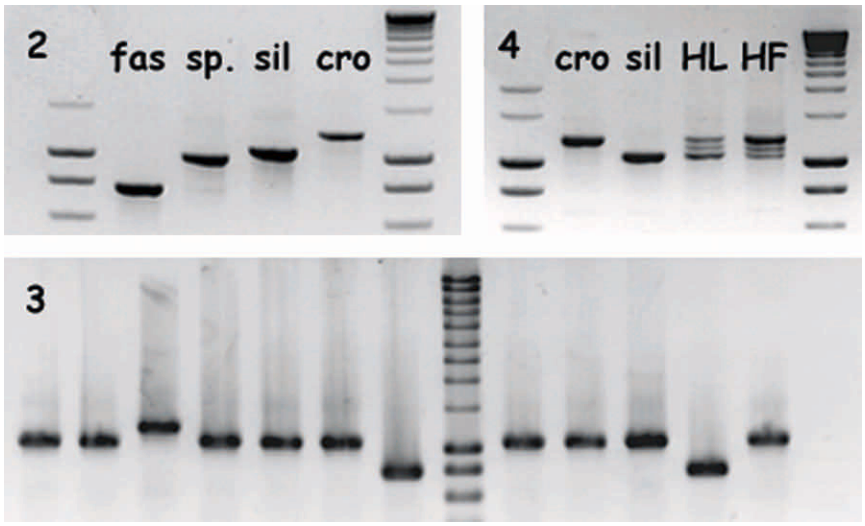
The fourth *Ectocarpus* species encountered (see Results) was not involved in crossing experiments because we did not obtain gametophytes from it.

Zygotes were produced by combining fertile gametophyte fragments in hanging-drop preparations in which gamete fusions could be observed under the microscope. Even without witnessing plasmogamy, putative zygotes were distinguishable from settled gametes by their double inventory of plastids and stigmata. Incompletely cleaved zoids ('Doppelschwärmer') may resemble zygotes, but they occur only rarely among gametes (Müller 1967: ≤0.2%). To raise zygotes, we chose cells in preparations containing numerous cells with double inventory of organelles. This made an erroneous selection of a 'Doppelschwärmer' unlikely. Zygotes were raised and isolated as described previously (Peters *et al.* 2004b).

Sequence comparisons and phylogenetic analyses

Sequences obtained were compared with *Ectocarpus* sequences in GenBank using blastn (Altschul *et al.* 1997) and to sequences of the genome-sequenced strain (Le Corguillé *et al.* 2009; Cock *et al.* unpublished).

To avoid erroneous aligning, most indel-rich parts of the ITS regions and the entire spacer between *rps*14 and *atp*8 were excluded before executing phylogenetic analyses. The alignment consisted of ITS1 (187 bp) and ITS2 (163 bp), Rubisco spacer plus adjacent sectors of *rbc*L and *rbc*S (515 bp), *cox*3 (665 bp) and *rps*14 (288 bp). Our taxon sampling comprised 15 strains including 10 *Ectocarpus* from France, one each from the Isle of Man, New Zealand and Peru, and two strains of *Kuckuckia* used as an outgroup. Phylogenetic trees were calculated according to maximum parsimony (MP), neighbor joining (NJ; Kimura-2-parameter genetic distance), maximum likelihood (ML) and Bayesian analyses. The best model for maximum likelihood analysis was traced under the Akaike Information Criterion (AIC) using ModelTest 3.08b (Posada & Crandall 1998). The ML analysis was carried out under the GTR + I + Γ model using PAUP* v4.0b10 (Swofford 2002). The analysis was carried out by heuristic searches with 100 random sequence additions, tree bisection-reconnection (TBR) branch swapping, and MulTrees options. Bootstrap analysis was made for 1000 replicates with 10 random sequence additions. Other options were the same as with the ML tree search. MP and NJ analyses were also made using PAUP. Bayesian analysis was carried out using the GTR + I + Γ model for the combined data using MrBayes vs. 3.1.2 (Huelsenbeck & Ronquist 2001). The analysis was conducted from a random starting tree, and the program was set to perform two independent runs with four chains of Markov chain Monte Carlo iterations simultaneously for 2 000 000 generations with trees sampled every 100th generation, respectively. We harvested trees after 500 000 generations after the average standard deviation of split frequencies reached a value below 0.005. A total of 15 000 trees were combined to produce a 50% majority rule tree.



Figs 2–4. Internal transcribed spacer 1 (ITS1) length genotypes in *Ectocarpus* from northwest France. DNA bands after polymerase chain reaction (PCR) with primers AFP4LF and 5.8S1R amplifying ITS1 + 340 bp flanking sequences. Agarose gel electrophoresis, length standards (from below) 400, 600, 800, 1000, 1500 bp. fas, *E. fasciculatus*; sp., *Ectocarpus* sp.; sil, *E. siliculosus*; cro, *E. crouaniorum*. 2. The four different ITS1 length genotypes found in the present study. 3. Genotyping of 12 isolates from site 4, including two fas, nine sil and one cro. Lane 8 (counted from left side) = length standard, lane 14 = PCR without template DNA. 4. ITS1 of *Ectocarpus* hybrids. HL, hybrid produced in laboratory by crossing cro female × sil male. HF, putative hybrid isolated from field. The central of the triple bands in the hybrids is a PCR artifact due to amplification of both sequences in the same tube.

RESULTS

Genotyping and sequencing

Diagnostic PCR in 67 strains revealed four different ITS1 lengths (Fig. 2). Thalli from sites 1–3 (28 samples) had three different ITS1 lengths. ITS1 of individuals from the high intertidal, which were in the course of the study identified as *E. crouaniorum*, was about 150 bp longer than that of *E. siliculosus* from the mid intertidal and subtidal and 430 bp longer than that of *E. fasciculatus* from the low intertidal and subtidal. The same three ITS1 lengths and the same distribution across the intertidal were obtained in thalli collected at site 5 (22 samples; Fig. 3) At site 4, only genotypes of *E. siliculosus* and *E. fasciculatus* were present (six samples). At site 6, which in contrast to all other sites has strong wave exposure, we collected only in the intertidal (four samples) and obtained only *E. crouaniorum*, both from high and mid intertidal pools. Sampling at site 7 (seven samples) was also done only in the intertidal and there, we found *E. crouaniorum*, *E. siliculosus*, and in addition, a thallus with an ITS1 length approximately 70 bp shorter than in *E. siliculosus*.

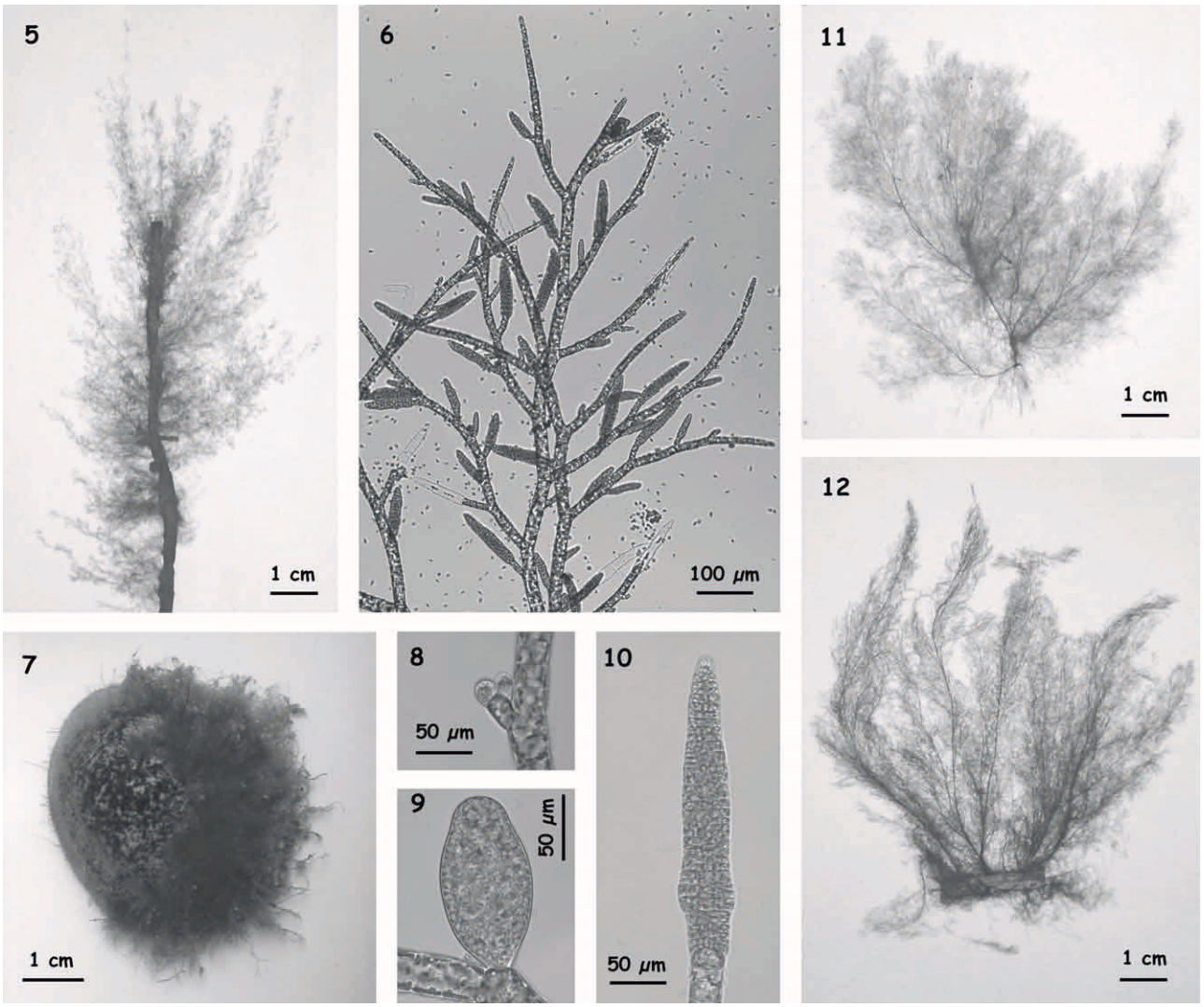
A single field isolate from site 1 showed a double band with the sizes of *E. crouaniorum* and *E. siliculosus*. The same bands were obtained in the hybrid produced in the laboratory described below (Fig. 4).

The entire small subunit (SSU) gene, approximately 1070 bp of 5′-large subunit (LSU), *rbc*L and the 5.8S gene (161 bp) were similar across species (Table 2). ITS 1 and 2, Rubisco spacer, *cox*3, *rps*14+spacer were generally near-identical in the replicate isolates of the same species; however, the two isolates of *E. fasciculatus* showed some differences in the second half of ITS1 and in the *rps*14-*atp*8 spacer. Across different species, Rubisco spacer, *cox*3 and *rps*14 showed different sequences but aligned perfectly; however, in the ITS regions and in the *rps*14-*atp*8 spacer, conserved regions were interrupted by unalignable sectors. The first part of ITS1 was particularly variable, comprising the large indels that are responsible for the differences in ITS1 length in the different species.

Field presence, phenology and morphology

At site 1, *Ectocarpus* was recorded all year round, occurring from intertidal pools in the *Fucus vesiculosus* Linnaeus zone (hereafter ‘high intertidal’) to the upper subtidal. Contrary to *Pylaiella littoralis* (Linnaeus) Kjellman, which was common at the same sites, often epiphytic on *Fucus* and regularly emersed at low tide, *Ectocarpus* apparently required constant submersion and in the mid and upper intertidal it was confined to pools or run-offs.

In the high intertidal, macroscopic *Ectocarpus* thalli up to 12 cm in length were observed from the end of March until the end of June; their substratum was the



Figs 5–12. *Ectocarpus* spp. at Roscoff, Brittany. 5–10. *Ectocarpus crouaniorum*. 5. Gametophytes epiphytic on the erect phase of *Scytosiphon*. 21 March 2004, high intertidal run-off. Herbarium specimen. 6. Mature field gametophyte, releasing gametes, 17 March 2004. 7. Thalli forming a felt on old *Patella* shell. 15 April 2007. Dark spots on left side of shell are brown crusts, possibly the prostrate phase of *Scytosiphon*. 8. Culture from material in Figure 7; two hemispherical unilocular sporangium mother cells are visible. 9. Mature unilocular sporangium in the same sporophyte as in Figure 8. 10. Plurilocular sporangium of sporophyte. Isolate from 24 May 2005, reproductive organ formed in culture. 11. A thallus of *E. siliculosus*, epiphytic on *Ulva* (host removed). 1 July 2003, mid-intertidal pool. Herbarium specimen. 12. Thalli of *E. fasciculatus*, epiphytic on *Zostera*. 1 July 2003, drift material. Herbarium specimen.

erect phase of *Scytosiphon* (Fig. 5) or occasionally thalli of *Asperococcus*. Genotyping of 18 thalli from this habitat at sites 1, 3 and 5–7 showed that they belonged to *E. crouaniorum*. They were gametophytes as they did not bear unilocular sporangia and on several occasions the zoids released from their plurilocular organs (Fig. 6) behaved as gametes (see below). Macroscopic field sporophytes of *E. crouaniorum* were only observed at sites 1 and 3. In March and April 2004 and 2005, four groups of thalli of up to 30 mm height were collected in high intertidal run-offs. They grew on a dead *Patella* shell, on a small stone and on old wood and bore both unilocular and plurilocular sporangia. An individual from each group was genotyped and identified as *E. crouaniorum*. The substratum around the *E. crouaniorum* gametophyte population at site 1 was in the following screened for small *Ectocarpus* thalli. They were observed at seven occasions, growing on dead mollusc shells or stones. They were microscopic, rarely attaining more than 10 mm in size (Fig. 7). Nevertheless they were fertile bearing unilocular sporangia (Figs 8,9) in March and April, accompanied by plurilocular sporangia (Fig. 10), which were the only reproductive organs on these minute field sporophytes in summer, autumn and winter. Two isolates made from such microscopic thalli on 15 April 2007 and 28 June 2007 were genotyped;

Table 4. Results of crossing experiments between *Ectocarpus siliculosus* (sil), *Ectocarpus fasciculatus* (fas) and *Ectocarpus crouaniorum* (cro) from Roscoff

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

†Experiment not carried out; 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). f, female, m, male.

the first was again *E. crouaniorum* but the second showed the double band mentioned above (Fig. 4).

In the mid intertidal (*Fucus serratus* Linnaeus zone), macroscopic *Ectocarpus* thalli up to 15 cm in length (Fig. 11) were found in spring, summer and autumn. They grew mainly on *Ulva* and *Sargassum*, occasionally on *Porphyra* or maerl. Genotyping of 24 samples from this zone at sites 1–3, 5 and 7 showed that 23 belonged to *E. siliculosus* and one to *E. fasciculatus*; at site 6, in contrast, two small thalli growing on *Corallina* were *E. crouaniorum*.

In the low intertidal and upper subtidal (*Laminaria* zone), *Ectocarpus* could be encountered all year long. Thalli were epiphytic on *Himanthalia*, *Laminaria*, *Saccharina, Saccorhiza*, *Gracilaria, Ulva* and *Zostera marina* Linnaeus (Fig. 12). At site 4, this was the only zone in which *Ectocarpus* was present. According to genotyping of 16 samples from sites 1, 4 and 5, seven thalli from this zone belonged to *E. fasciculatus*, eight to *E. siliculosus*, and one, which was epiphytic on *Bifurcaria*, to *E. crouaniorum*.

Overall thallus size and number of stalk cells of plurilocular organs were measured in *E. crouaniorum* gametophytes and *E. siliculosus* thalli at site 1. Individuals of *E. crouaniorum* were up to 12 cm long, but usually smaller (mean 25 ± 25 mm SD; $n = 24$), and often they were already fertile at a size of 10–20 mm. *Ectocarpus siliculosus* thalli were significantly larger (mean 74 ± 40 mm SD; $n = 29$; *t*-test: $P = 0.0001$). Stalk cells were counted at the base of 135 plurilocs in five thalli of *E. crouaniorum* and in 60 plurilocs in two thalli of *E. siliculosus*. The means (3.2 and 3.8, respectively) were insignificantly different (*t*-test: $P = 0.2$).

Crossing studies

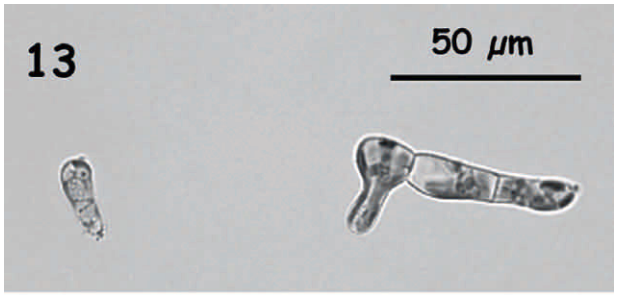
Gamete fusions were observed in all intraspecific crosses when fragments from fertile male and female gametophytes were placed in the same preparation (Table 4). In interspecific crosses, female and male gametes from the *E. crouaniorum* thalli underwent plasmogamy with male and female gametes of *E. siliculosus*, respectively. In crosses with *E. fasciculatus*, zygotes were formed between female gametes of *E. crouaniorum* and male gametes of *E. fasciculatus*. Female gametes of *E. fasciculatus* did not produce any zygotes with male gametes of *E. crouaniorum*, which were, however, fertile with the conspecific female gametes.

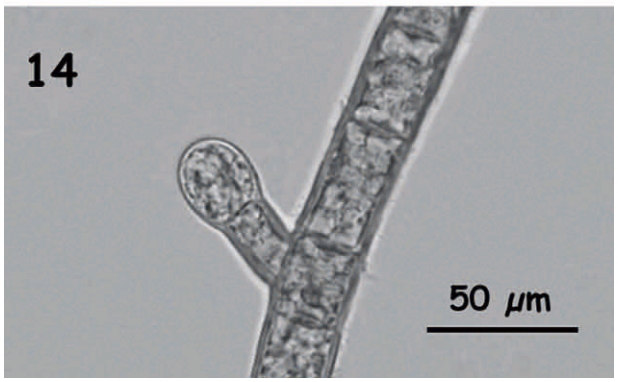
The development of 292 zygotes was followed (Table 4). Whereas zygotes from intraspecific crosses developed normally (not shown), those from interspecific crosses usually died within 3–4 days after plasmogamy, before the first cell division. In the same preparations, germlings from non-fused settled gametes displayed a normal development into parthenogenetic sporophytes (Fig. 13), showing that vitality of the gametes was unaffected. In addition, intraspecific control crosses involving material from the same gametophyte thalli, carried out for six of the 12 experiments with abortive zygote development, yielded viable zygotes.

In one experiment of the combination female *E. crouaniorum* × male *E. siliculosus*, all nine selected zygotes developed normally. One of the thalli was raised to maturity. It formed unilocular sporangium mother cells (the site of meiosis in normal sporophytes), which were characteristically dome-shaped to ovoid (Fig. 14). In the hybrid thallus, unilocular sporangia did not develop beyond this early stage, whereas in sporophytes of *E. crouaniorum*, both from the field and from raised zygotes, they increased in size (Figs 8,9) and finally released meiospores.

Sequence comparisons

A blastn search using ITS1 of *E. crouaniorum* as a query showed that highly similar sequences (98% iden-





Figs 13 and 14. Crossing experiments between *Ectocarpus crouaniorum* and *E. siliculosus*. 13. Culture 6 days after cross between a male *E. crouaniorum* and a female *E. siliculosus*; left, degenerating hybrid germling, right, germling from settled gamete, developing normally. 10. Viable hybrid from a cross between female *E. crouaniorum* × male *E. siliculosus*. Unilocular sporangium initial in terminal position on short lateral. Meiosporangia in the hybrid did not develop beyond this stage. Compare with regular development in *E. crouaniorum* sporophyte (Figs 8,9).

tity) had been found previously in isolates from the Isle of Man (accession U38771; CCAP1310/144) and the Aran Islands (U38770; CCAP1310/291). ITS1 of our isolates of *E. siliculosus* and *E. fasciculatus* agreed with known sequences of strains of the two species (e.g. U38760; CCAP1310/177; 97% and U38824; CCAP1310/12; 96% identity, respectively). ITS1 of the isolate from site 7, with an ITS1 length between *E. siliculosus* and *E. fasciculatus*, matched ITS1 of a previous isolate from Copenhagen (U38777; CCAP1310/100; 97% identity).

Phylogenetic analyses

Concatenated sequences from the five markers gave an alignment of 1818 bp, of which 302 were variable, thereof 211 parsimony-informative. All phylogenetic analyses provided essentially the same tree (Fig. 15). *Ectocarpus* was divided into two clades. The larger one contained *E. siliculosus* and *E. crouaniorum* in two well separated sub-clades. The genome-sequenced *Ectocarpus* from Peru clustered with *E. siliculosus* (≥93% bootstrap support) but the strain from New Zealand formed an independent lineage. The other clade contained *E. fasciculatus* and the unnamed isolate from Cherbourg. Analyses using data from single markers (results not shown) gave similar trees, always placing the samples of *E. crouaniorum* in a clade separated from the branch containing *E. siliculosus*.

DISCUSSION

The present study demonstrates that three biological species of *Ectocarpus* occur in Brittany. Pre- or post-zygotic incompatibility maintains their genetic separation. Differences in the DNA sequences of five genetic markers (nucleus: ITS1 and ITS2; plastid: *rbc*L + Rubisco spacer; mitochondrion: *cox*3, *rps*14-*atp*8 spacer) independently confirmed that there is no gene flow among the three species. Two of the species had been distinguished previously, based on morphology and lipid composition: *E. siliculosus* with pseudo-dichotomous branching and not containing diacylglycerylhydroxymethyltrimethyl-β-alanine (DGTA), and *E. fasciculatus* with prominent principal filaments and thinner, often fasciculate laterals, containing DGTA (Müller & Eichenberger 1995). These species were confirmed in our study; they were found in Brittany from mid-intertidal pools to the subtidal (*E. siliculosus*) or from the lower intertidal to the subtidal (*E. fasciculatus*). The habitat on *Scytosiphon* in high intertidal pools was the key to finding the appropriate name for the third species. Thuret (in Le Jolis 1863) described *E. crouaniorum* from exactly this habitat and we collected this species both at the type locality and at the site of a historical record (Crouan & Crouan 1852, as *E. fenestratus* Crouan). In the original description, the name was *E. crouani* but as it honored the two brothers Crouan it has to be corrected to the Latin genitive plural (Ergün Taskin, pers. comm., 2008). *Ectocarpus crouaniorum* had still been recognized as a distinct species by Hamel (1931–1939), before it was considered a variety or growth phase of *E. siliculosus* by later authors (e.g. Cardinal 1964, Russell 1966, Gallardo 1992).

Our observation that thalli of *E. crouaniorum* growing on *Scytosiphon* are dioecious gametophytes correlates with the fact that no unilocular sporangia have been described in this species (Cardinal 1964). The corresponding sporophytes were morphologically different, small thalli with a maximum size of 30 mm. Microscopic sporophytes were present on stones and shells throughout the year in the zone where the gametophytes occurred in spring. Meiosporangia were only found in spring, which would coincide with the appearance of the gametophytes in that season; in the other seasons, the sporophytes produced mitosporangia to replicate the same generation. Judging from laboratory cultures, other species of *Ectocarpus* may also possess

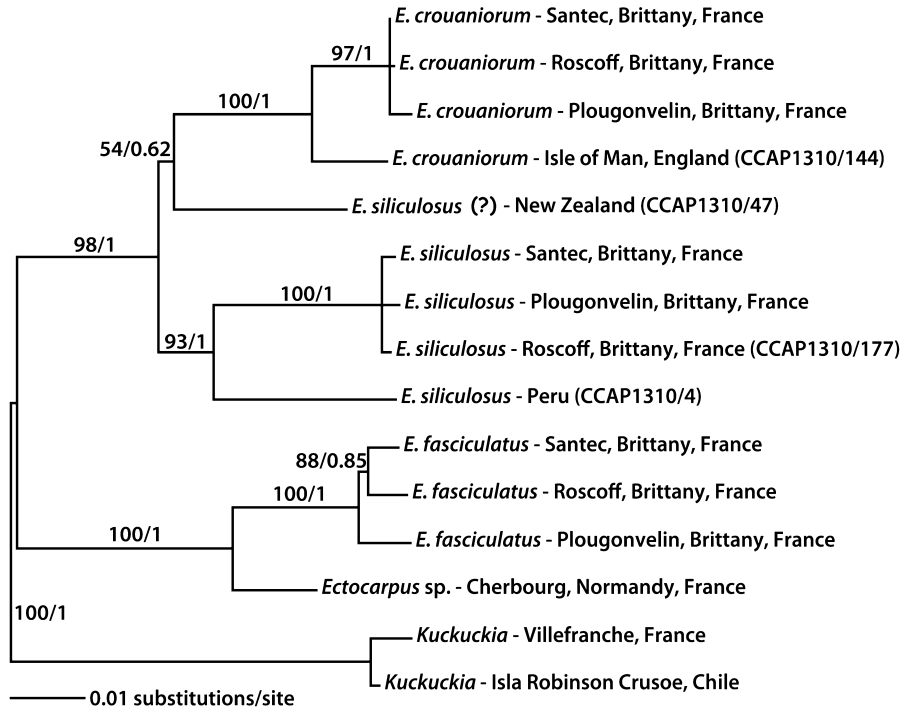


Fig. 15. Maximum likelihood (ML) tree (-ln *L* = 4764.7661) of 13 *Ectocarpus* and two *Kuckuckia* isolates based on combined sequences from ITS1 + ITS2 + Rubisco spacer region + *cox*3 + *rps*14. ML bootstrap (left) and Bayesian posterior probability (right) values are shown above the branches. The three main clades (from below) are *Kuckuckia* (outgroup), *Ectocarpus* section *fasciculati* and *Ectocarpus* section *siliculosi*. Within the latter, *E. crouaniorum* (above) forms a clade separated from *E. siliculosus* (below). Affiliation of the strain from New Zealand, which was referred to as *E. siliculosus* in Müller (1991) and Peters *et al.* (2004b), is uncertain as it does not cluster with significant support with *E. crouaniorum* or *E. siliculosus*.

dissimilar generations; the gametophytes are ephemeral erect thalli, whereas the sporophytes are persistent and raise on an initially prostrate base (Müller 1964, 1966, 1972; Peters *et al.* 2008). In *E. siliculosus*, however, the sporophyte may form large thalli as well. There are a few reports on the presence of fertile gametophytes of *E. siliculosus* in nature (Berthold 1881; Sauvageau 1896; Kuckuck 1912; Papenfuss 1935; Müller 1976b) but the present field observations on *E. crouaniorum* provide the first data on the phenology of both generations of any *Ectocarpus*.

Ectocarpus crouaniorum is easily accessible on the shore and may have been described earlier, for example, by Kützing (1843, 1845, 1849, 1855, 1860) who described 56 species of *Ectocarpus*; however, the types of these taxa are considered to be lost (Prud'homme van Reine & den Hartog 1973) and the descriptions and original figures are usually not informative enough, in particular concerning ecological data. Among the taxa described before 1863 we have not found any that would match the species as well as Thuret's *E. crouaniorum*. We have not found any older description with which the small sporophyte of *E. crouaniorum* would agree. Plurilocular sporangia in *E. subulatus* Kützing (including *E. amphibius* Harvey), which was also described from a high intertidal habitat, are significantly longer than the plurilocular organs of *E. crouaniorum*. We consider it most appropriate to propose reinstatement of *E. crouaniorum* for the entity in the high intertidal.

Morphological differences between *E. crouaniorum* and *E. siliculosus* are minute. Le Jolis (1863) and Hamel (1931–1939) mentioned that plurilocular organs in lower parts of *E. crouaniorum* were often borne on long pedicels. However, our count of stalk cells in individuals of both taxa from the same site did not reveal significant differences. Overall size of mature thalli, in contrast, may be informative as the gametophytes of *E. crouaniorum* were, despite some overlap, significantly smaller than the thalli of *E. siliculosus*.

Taking habitat and season into consideration may also help to distinguish the two species, at least on sheltered megatidal shores in Brittany where the different zones are well separated. The conspicuous *E. crouaniorum* gametophytes were only present in spring and usually confined to upper intertidal pools, where they were epiphytes on *Scytosiphon* and occasionally on *Asperococcus*; the small sporophytes occurred in the same zone on abiotic substrata. However, we also recorded the species once on *Bifurcaria* in the low

intertidal, and on an exposed shore, where *E. crouaniorum* was the only intertidal *Ectocarpus*, thalli were also found on *Corallina* in a mid-intertidal pool. *Ectocarpus siliculosus* occurred from mid-intertidal pools down to the upper subtidal, on *Sargassum, Ulva, Porphyra, Gracilaria, Saccharina* and *Himanthalia*, within a seagrass bed and on old *Zostera* leaves. A previously isolated strain of *E. siliculosus* from Roscoff (CCAP1310/177) was dredged from 10 m depth (Müller & Eichenberger 1995). Macrothalli of *E. siliculosus* were not only present in spring but also collected in August, September and November. Admittedly, individual *Ectocarpus* thalli may be difficult to identify in spring, and given the morphological similarity between *E. crouaniorum* and small *E. siliculosus*, these two may be regarded as cryptic species.

Occasionally, *E. crouaniorum* may form viable hybrids with *E. siliculosus*. This occurred in one out of our 11 crossing experiments involving the two species, but in the hybrids meiosis was not completed, similar to the abortive unilocular sporangia described in crosses between *Ectocarpus* strains from geographically distant origins (Müller 1988; Stache 1989). One out of 26 genotyped field thalli was a putative field hybrid showing a double band corresponding to ITS1 lengths of both *E. crouaniorum* and *E. siliculosus*. Judging from their rarity in the field the hybrids do not seem to show increased hybrid vigor (heterosis). Viable natural hybrids between closely related seaweed species have been described before (e.g. in *Fucus*, Coyer *et al.* 2002). More extensive sampling will be required to estimate the extent of hybridization between *E. crouaniorum* and *E. siliculosus* in the field.

On the shore, *Ectocarpus fasciculatus* is even more separated from *E. crouaniorum*, as it was found in the lower intertidal and the subtidal, where it was regularly encountered on *Laminaria, Saccharina, Saccorhiza, Himanthalia* and green leaves of *Zostera marina*. It could be collected all year round and was abundant in late summer and autumn. Its different morphology usually allows distinction from *E. siliculosus*, which may occur in the same habitat, as well as from *E. crouaniorum*.

Using the ITS1 sequences of *E. crouaniorum* as a barcode we found that strains of *Ectocarpus* collected by Dieter Müller in 1976 at the Isle of Man (Müller 1977) and in 1990 at the Aran Islands, Ireland, belonged to the same species. Both had been collected in intertidal pools, the Aran Island isolate was even epiphytic on *Scytosiphon* (cf. strain information on http://www.ccap.ac.uk). This shows that *E. crouaniorum* also occurs in the British Isles. Batters (1902), who recognized the species, mentioned records from Cornwall, Essex and southwest Scotland but considered it to be rare. Müller (1977) crossed gametophytes from the Isle of Man with Mediterranean *E. siliculosus* and regarded the observed zygotic compatibility as proof of conspecificity. However, apparently no attempts were made to raise the zygotes.

The Manx strains of *E. crouaniorum* collected by Dieter Müller survived up to 28°C in ecophysiological studies on temperature requirements of *E. siliculosus* (Bolton 1983). This result coincides well with the occurrence of *E. crouaniorum* in upper intertidal pools, which may warm up during low tide on sunny days. According to the phylogenetic tree of Dieter Müller's worldwide *Ectocarpus* strain collection (Stache-Crain *et al.* 1997), *E. crouaniorum* belongs to branch 2 containing also isolates from North Carolina, Florida, Texas and the Canary Islands: localities with elevated summer sea surface temperatures. Other strains in that branch are from Chile, South Africa, Helgoland and from a German river influenced by salt mining. It appears likely that *E. crouaniorum* is widely distributed.

Müller and Eichenberger (1994, 1995) introduced DGTA content to distinguish species in *Ectocarpus*. This was corroborated but modified in Stache-Crain *et al.* (1997) who showed that both *E. fasciculatus* (branch 5b) and members of branch 2abc (*E. crouaniorum*) contained DGTA. Of the two gametophytes of *E. crouaniorum* from the Isle of Man, however, only one contained DGTA, and the strain from Aran Island only traces. In addition, a case of lack of DGTA in *E. fasciculatus* (Müller & Eichenberger 1997) showed that the value of DGTA for chemotaxonomy of *Ectocarpus* is limited.

A single isolate of *Ectocarpus* in our collection did not belong to *E. siliculosus*, *E. fasciculatus* or *E. crouaniorum*; sequences showed that it is more closely related to *E. fasciculatus* than to the two other taxa but that it belongs to a lineage (branch 5a in Stache-Crain *et al.* 1997), which is separated from Roscovite *E. fasciculatus* (branch 5b). Our material, which originated from a minute *Ectocarpus* thallus epiphytic on an intertidal *Scytosiphon* at Cherbourg, Normandy, was genetically similar to an isolate collected by Dieter Müller at Hvidøre near Copenhagen in June 1979. According to his unpublished notes, field thalli were up to 40 cm in length and bore plurilocular and unilocular sporangia. He isolated male and female gametophytes and both were capable of plasmogamy with strains of *E. siliculosus* from Naples. Müller concluded that the Copenhagen isolate belonged to *E. siliculosus*, which is contradicted by the sequences. So far we are unable to provide a name for this entity.

Our phylogenetic analyses were based on a more limited taxon set than in Stache-Crain *et al.* (1997), but two additional mitochondrial markers (953 bp) provided higher resolution. Our results confirmed that within *Ectocarpus*, two main branches, corresponding to the sections *fasciculati* and *siliculosi*, can be recognized. However, the trees suggest there are at least five

different lineages within *Ectocarpus* separated by large genetic distances. *Ectocarpus crouaniorum* clustered within the *siliculosi* but was well separated from *E. siliculosus*. Phylogenetic analyses of the entire diversity of *Ectocarpus* from world-wide collections may show whether other lineages within the two sections also merit recognition as separate species.

Our study was restricted to *Ectocarpus* from intertidal habitats. The sublittoral offers a diversity of additional habitats and we do not exclude the possibility that more entities of *Ectocarpus* may be present in that zone. For instance, we have not collected any *Kuckuckia* although the genus had been reported in our study area by Cardinal (1964).

In conclusion, our results suggest that species diversity in *Ectocarpus* is higher than assumed during the last four decades but that morphology is not sufficient to distinguish all species. In future studies using *Ectocarpus*, care should be taken that the exact origin, habitat and collection date of the material are recorded, and that the identity is established without doubt, best by using molecular markers.

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REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A. *et al.* 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**: 3389–402.

Batters, E. A. L. 1902. A catalogue of the British marine algae. *J. Bot.* **40** (Suppl): 1–107.

Berthold, G. 1881. Die geschlechtliche Fortpflanzung der eigentlichen Phaeosporeen. *Mitt. Zool. Stat. Neapel* **2**: 401–13.

Bolton, J. J. 1983. Ecoclinal variation in *Ectocarpus siliculosus* (Phaeophyceae) with respect to temperature growth optima and survival limits. *Mar. Biol.* **73**: 131–8.

Cardinal, A. 1964. Étude sur les Ectocarpacées de la Manche. *Nova Hedwigia, Beih.* **15**: 1–86.

Charrier, B., Coelho, S. M., Le Bail, A. *et al.* 2008. Development and physiology of the brown alga *Ectocarpus siliculosus*: two centuries of research. *New Phytol.* **177**: 319–32.

Coelho, S. M., Peters, A. F., Charrier, B. *et al.* 2007. Complex life cycles of multicellular eukaryotes: new approaches based on the use of model organisms. *Gene* **406**: 152–70.

Coyer, J. A., Peters, A. F., Hoarau, G., Stam, W. T. and Olsen, J. L. 2002. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (Heterokontophyta: Phaeophyceae) in a 100-year-old zone of secondary contact. *Proc. R. Soc. Lond. B* **269**: 1829–34.

Crouan, P. L. and Crouan, H. M. 1852. *Algues Marines du Finistère*. Brest.

Gallardo, T. 1992. Nomenclatural notes on some Mediterranean algae, I: Phaeophyceae. *Taxon* **41**: 324–5.

Guiry, M. D. and Guiry, G. M. 2009. AlgaeBase. World-wide Electronic Publication. National University of Ireland, Galway. http://www.algaebase.org.

Hamel, G. 1931–1939. *Phéophycées de France*. Wolf, Paris.

Hamel, G. 1939. Sur la classification des Ectocarpales. *Bot. Notiser* **1939**: 65–70.

van den Hoek, C. 1975. Phytogeographic provinces along the coasts of the Northern Atlantic Ocean. *Phycologia* **14**: 317–30.

Huelsenbeck, J. P. and Ronquist, F. 2001. MRBAYES: bayesian inference of phylogeny. *Bioinformatics* **17**: 754–5.

Kim, H. S. and Lee, I. K. 1992. Morphotaxonomic studies on the Korean Ectocarpaceae (Phaeophyta). I. Genus *Ectocarpus* Lyngbye. *Korean J. Phycol.* **7**: 225–42.

Kornmann, P. 1956. Über die Entwicklung einer *Ectocarpus confervoides*-Form. *Pub. Sta. Zool. Napoli* **28**: 32–43.

Kornmann, P. and Sahling, P.-H. 1977. Meeresalgen von Helgoland. Benthische Grün-, Braun- und Rotalgen. *Helgoländer wiss. Meeresunters.* **29**: 1–289.

Kuckuck, P. 1912. Beiträge zur Kenntnis der Meeresalgen. 11. Zur Fortpflanzung der Phaeosporeen. *Wiss. Meeresunters. Abt. Helgol.* **5**: 155–84.

Kuckuck, P. 1958. Ectocarpaceen-studien. V. *Kuckuckia, Feldmannia*. *Helgol. Wiss. Meeresunters.* **6**: 171–92.

Kützing, F. T. 1843. *Phycologia Generalis*. Brockhaus, Leipzig.

Kützing, F. T. 1845. *Phycologia Germanica*. Nordhausen.

Kützing, F. T. 1849. *Species Algarum*. Brockhaus, Leipzig.

Kützing, F. T. 1855. *Tabulae Phycologicae 5*. Nordhausen.

Kützing, F. T. 1860. *Tabulae Phycologicae 10*. Nordhausen.

Le Corguillé, G., Pearson, G., Valente, M. *et al.* 2009. Plastid genomes of two brown algae, *Ectocarpus siliculosus* and *Fucus vesiculosus*: further insights on the evolution of red-algal derived plastids. *BMC Evol. Biol.* **9**: 253–266

Le Jolis, A. 1863. *Liste des Algues Marines de Cherbourg*. J.B. Bailliere et Fils, Paris.

Müller, D. G. 1964. Life-cycle of *Ectocarpus siliculosus* from Naples, Italy. *Nature* **203**: 1402.

Müller, D. G. 1966. Untersuchungen zur Entwicklungsgeschichte der Braunalge *Ectocarpus siliculosus* aus Neapel. *Planta* **68**: 57–68.

Müller, D. G. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge *Ectocarpus siliculosus* im Kulturversuch. *Planta* **75**: 39–54.

Müller, D. G. 1972. Life cycle of the brown alga *Ectocarpus fasciculatus* var. refractus (Kütz.) Ardis. (Phaeophyceae, Ectocarpales) in culture. *Phycologia* **11**: 11–13.

Müller, D. G. 1976a. Sexual isolation between a European and an American population of *Ectocarpus siliculosus* (Phaeophyta). *J. Phycol.* **12**: 252–4.

Müller, D. G. 1976b. Relative sexuality in *Ectocarpus siliculosus*. A scientific error. *Arch. Microbiol.* **109**: 89–94.

Müller, D. G. 1977. Sexual reproduction in British *Ectocarpus siliculosus* (Phaeophyta). *Br. Phycol. J.* **12**: 131–6.

Müller, D. G. 1979. Genetic affinity of *Ectocarpus siliculosus* (Dillw.) Lyngb. from the Mediterranean, North Atlantic and Australia. *Phycologia* **18**: 312–18.

Müller, D. G. 1988. Studies on sexual compatibility between *Ectocarpus siliculosus* (Phaeophyceae) from Chile and the Mediterranean Sea. *Helgol. Meeresun.* **42**: 469–76.

Müller, D. G. 1991. Mendelian segregation of a virus genome during host meiosis in the marine brown alga *Ectocarpus siliculosus*. *J. Plant Physiol.* **137**: 739–43.

Müller, D. G. and Eichenberger, W. 1994. Betaine lipid content and species delimitation in *Ectocarpus*, *Feldmannia* and *Hincksia* (Ectocarpales, Phaeophyceae). *Eur. J. Phycol.* **29**: 219–25.

Müller, D. G. and Eichenberger, W. 1995. Crossing experiments, lipid composition, and the species concept in *Ectocarpus siliculosus* and *E. fasciculatus* (Phaeophyceae, Ectocarpales). *J. Phycol.* **31**: 173–6.

Müller, D. G. and Eichenberger, W. 1997. Mendelian genetics in brown algae: inheritance of a lipid defect mutation and sex alleles in *Ectocarpus fasciculatus* (Ectocarpales, Phaeophyceae). *Phycologia* **36**: 79–81.

Müller, D. G. and Kawai, H. 1991. Sexual reproduction of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) in Japan. *Jpn. J. Phycol.* **39**: 151–5.

Ni-Ni-Win, Hanyuda, T., Arai, S., Uchimura, M., Abbott, I. A. and Kawai, H. 2008. Three new records of *Padina* in Japan based on morphological and molecular markers. *Phycol. Res.* **56**: 288–300.

Papenfuss, G. F. 1935. Alternation of generations in *Ectocarpus siliculosus*. *Bot. Gaz.* **96**: 421–46.

Pedersen, P. M. 1989. Studies on *Kuckuckia spinosa* (Fucophyceae, Sorocarpaceae): life history, temperature gradient experiments, and synonymy. *Nord. J. Bot.* **9**: 443–7.

Peters, A. F. and Ramírez, M. E. 2001. Molecular phylogeny of small brown algae, with special reference to the systematic position of *Caepidium antarcticum* (Adenocystaceae, Ectocarpales). *Cryptogam., Algol.* **22**: 187–200.

Peters, A. F., Marie, D., Scornet, D., Kloareg, B. and Cock, J. M. 2004a. Proposal of *Ectocarpus siliculosus* as a model organism for brown algal genetics and genomics. *J. Phycol.* **40**: 1079–88.

Peters, A. F., Scornet, D., Müller, D. G., Kloareg, B. and Cock, J. M. 2004b. Inheritance of organelles in artificial hybrids of the isogamous multicellular chromist alga *Ectocarpus siliculosus* (Phaeophyceae). *Eur. J. Phycol.* **39**: 235–42.

Peters, A. F., Scornet, D., Ratin, M. *et al.* 2008. Life-cycle-generation-specific developmental processes are modified in the immediate upright mutant of the brown alga *Ectocarpus siliculosus*. *Development* **135**: 1503–12.

Posada, D. and Crandall, K. A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817–18.

Prud'homme van Reine, W. F. and den Hartog, C. 1973. The types of Kützing's new species of *Corticularia, Ectocarpus* and *Spongonema*. *Taxon* **22**: 93–6.

Ricker, R. W. 1987. *Taxonomy and Biogeography of Macquarie Island Seaweeds*. British Museum (Natural History), London.

Russell, G. 1966. The genus *Ectocarpus* in Britain. I. The attached forms. *J. Mar. Biol. Assoc. UK* **46**: 267–94.

Russell, G. 1967. The genus *Ectocarpus* in Britain. II. The free-living forms. *J. Mar. Biol. Assoc. UK* **47**: 233–50.

Santelices, B. 1989. *Algas Marinas de Chile. Distribución, Ecología, Utilización, Diversidad*. Editiones Universidad Católica de Chile, Santiago.

Sauvageau, C. 1896. Sur la conjugaison des zoospores de l'*Ectocarpus siliculosus*. *C. R. Acad. Sci. Paris* **123**: 431–3.

Stache, B. 1989. Sexual compatibility and species concept in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) from Italy, North Carolina, Chile, and New Zealand. *In* Garbary, D. J. and South, R. G. (Eds) *Evolutionary Biogeography of the Marine Algae of the North Atlantic.* Springer, Berlin, pp. 173–86.

Stache, B. 1993. *Kreuzungsexperimente bei Braunalgen. Vergleich von Lokalpopulationen des Kosmopoliten* Ectocarpus siliculosus. Hartung-Gorre, Konstanz.

Stache-Crain, B., Müller, D. G. and Goff, L. J. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear and plastid-encoded DNA sequences. *J. Phycol.* **33**: 152–68.

Starr, R. C. and Zeikus, J. A. 1993. UTEX-The culture collection of algae at the University of Texas at Austin. *J. Phycol.* **29** (Suppl): 1–106.

Stegenga, H., Bolton, J. J. and Anderson, R. J. 1997. *Seaweeds of the South African West Coast*. Bolus Herbarium, Cape Town.

Swofford, D. L. 2002. *PAUP: Phylogenetic Analysis Using Parsimony, Version 4.0b10*. Sinauer Associates, Sunderland.

White, T. J., Bruns, T. D., Lee, S. B. and Taylor, J. W. 1990. Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal genes. *In* Innis, M. A., Geleand, D. H., Sninsky, J. J. and White, T. J. (Eds) *PCR Protocols*. Academic Press, New York, pp. 315–22.