Genetic diversity of Ectocarpus (Ectocarpales, Phaeophyceae) in Peru and northern Chile, the area of origin of the genome-sequenced strain

Akira F. Peters, Aaron D. Mann, César A. Córdova, Juliet Brodie, Juan A. Correa, Declan C. Schroeder, J. Mark Cock

Genetic

Diversity of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Peru and northern Chile, the area of origin of the genome-sequenced strain

Akira F. Peters[1,2,3], Aaron D. Mann[4], César A. Córdova[5], Juliet Brodie[6], Juan A. Correa[4], Declan C. Schroeder[1] and J. Mark Cock[3]

[1] Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, UK

[2] Bezhin Rosko, 28 route de Perharidy, 29680 Roscoff, France

[3] UMR7139, Station Biologique, Centre National de la Recherche Scientifique et Université Pierre & Marie Curie Paris VI, Place Georges Teissier, 29682 Roscoff cedex, France

[4]Departamento de Ecología and Center for Advanced Studies in Ecology and Biodiversity, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile

[5]Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Ciudad Universitaria, Lima, Perú

[6]Natural History Museum, Department of Botany, Cromwell Road, London SW7 5BD, UK

Author for correspondence:

Akira F. Peters

Email: akirapeters@gmail.com

Received: xx

Accepted: xx

Summary

- The origin of the *Ectocarpus* strain used for genome sequencing (the "genome strain") was Peru whence no *Ectocarpus* was recorded previously. To study the genetic diversity in the region and to increase the number of individuals from this area available for genetic experiments, 119 new *Ectocarpus* strains were isolated at eight localities along the 3000 km coastline from central Peru to central Chile.
- ITS1 genotyping revealed nine different genotypes, four of which were endemic to the area studied and two were so far unknown.
- Individuals of the same genotype as the genome strain occurred from Peru to northernmost Chile, representing 61% of the samples in this area from which five more genotypes were isolated. Further south, down to central Chile, most individuals matched European *E. siliculosus*, *E. fasciculatus* and *E. crouaniorum*. In sexual crosses, the genome strain and our new isolates of the same genotype were fully compatible.
- Sequences from four nuclear and cytoplasmic genetic markers (ITS1, ITS2, Rubisco spacer, *cox*3) separated the genome strain from the European species of *Ectocarpus,* but it grouped with one of the other South American endemic genotypes. This clade may in future be recognised as a separate species.

Key words: Genetic diversity, *Ectocarpus, Kuckuckia*, Chile, Peru

word count: summary: 197 words [200 permitted]; all text (excluding Title and Summary): 4604 words [6500 permitted]; tables: 1152 words; supporting material: 451 words

Introduction

The *Ectocarpus* strain chosen for genome sequencing (henceforth referred to as the "genome strain") is a male gametophyte which has the designation "Ec32" in the *Ectocarpus* strain collection at Roscoff. It was selected because it displays an alternation of two morphologically distinguishable generations (Peters *et al.,* 2008) and produces unilocular sporangia on parthenogenetic sporophytes, both of which facilitate studies on the complex brown algal life history (for more reasons and terminology of morphology and life history see Peters *et al.,* 2004a; Coelho *et al.,* 2007; Charrier *et al.,* 2008). The strain was obtained from a meiospore of a field sporophyte collected by the first author in November 1988 at San Juan de Marcona, Peru (Site 3 in Fig. 1). Identified as *E. siliculosus* (Dillwyn) Lyngbye in a first molecular phylogeny (Stache-Crain *et al.,* 1997, designation "SAm120h"; lineage 1c), it later showed post-zygotic incompatibility (Peters *et al.,* 2004b) when crossed with strains from Europe regarded as genuine *E. siliculosus* (i.e. genetically close to those used by Müller (1967) for the description of the life history; lineage 1a in Stache-Crain *et al.,* 1997) or with a strain of *Ectocarpus* from New Zealand previously used in research on virus inheritance (Müller, 1991; lineage 4 in Stache-Crain *et al.,* 1997): hybrids possessed a normal morphology, but were unable to form meiospores. Further crosses of the genome strain with an *Ectocarpus* from northern Chile, as well as with the recently reinstated *E. crouaniorum* Thuret in Le Jolis from Europe (Peters *et al.,* 2010), gave similar results (unpublished data). Although sisters of the genome strain (female gametophytes from the same field sporophyte from Peru) were available for genetic experiments, for instance to test sex-linkage of a mutation (Peters *et al.,* 2008), no female gametophyte of sufficient genetic distance was available for experiments requiring outcrossing (e.g. to produce a genetic map, Heesch *et al.,* 2010).

Apart from the genome strain there was no unambiguous published record of *Ectocarpus* from Peru. Howe (1914: p. 50) mentioned "small specimens of *Ectocarpus*" on *Desmarestia peruviana* Montagne at Ancón (13 February 1907) and on *Lessonia nigrescens* Bory de Saint-Vincent at Chincha Island (18 June 1907); however, he regarded the material as "too meager to justify an attempt at determination or description". The nearest published record of *Ectocarpus* was from Iquique, northern Chile, at ca 800 km distance from Site 3 (Ramírez & Santelices, 1991).

To isolate additional strains of *Ectocarpus* showing full compatibility with the genome strain, we collected more individuals from localities along the South American Pacific coast from central Peru to central Chile. They were identified based on comparisons with nuclear ribosomal internal transcribed spacer 1 (ITS1) sequences available for 43 strains of *Ectocarpus* and seven of *Kuckuckia* (the sister genus of *Ectocarpus*) from all continents except Antarctica (Stache-Crain *et al.,* 1997). In the studied area, *Ectocarpus* presented a surprisingly high genetic diversity described in the present paper. Isolates of one genotype were genetically similar to the genome strain and proved to be fully interfertile with it.

Materials and Methods

Field collection and isolation of cultures. Nine localities (four in Peru, five in Chile) spanning a distance of approximately 3000 km were visited for collection, most during one visit in February-March 2006 (Fig. 1, Table 1). Sampling at Site 8 was done for studies on the response of *Ectocarpus* to copper pollution (Mann *et al.,* unpublished). From each field thallus macroscopically resembling *Ectocarpus*, filaments were inoculated in a 2 ml Eppendorff tube containing autoclaved sea water. After transfer to the Biological Station at Roscoff, isolation and cultivation of clean unilalgal clonal cultures were undertaken as described previously (Peters *et al.,* 2010). A number of thalli from Site 8 were isolated by filtering nearshore surface seawater and cultivating filaments that developed on filter paper, or by inoculating field macrothalli of *Scytosiphon* and isolating all *Ectocarpus* thalli developing on them in culture. Only thalli possessing ribbon-shaped plastids were retained for further study.

Strain designations beginning with "CCAP1310/" are from the Culture Collection of Algae and Protozoa; numbers or numbers preceded by "Ec" are isolates housed in the *Ectocarpus* strain collection at Roscoff, and are maintained by the first author.

Molecular methods. DNA was extracted from living cultures as described (Peters *et al.,* 2004b). Initial identification was based on ITS1 length (see Peters *et al.*, 2010 for rationale), followed by sequencing of ITS1 or by a diagnostic PCR using a primer specific for the ITS1 sequence of the genome strain (for details and oligonucleotide primers used see supporting information). Sequences were aligned manually using Se-Al v. 2.0a11 (Rambaut, 2002). Sequences similar over the full length of ITS1 and not requiring indels of more than 20bp were

considered to belong to the same ITS1 genotype (GT). For at least one member of each ITS1 genotype discovered, three additional markers (ITS2, 3'-*rbc*L+Rubisco spacer, *cox*3) were sequenced to provide data for phylogenetic analyses. For the genome strain, the corresponding data were taken from the genome. Sequences were deposited in the EMBL/Genbank/DDBJ database (Table 2).

Data analyses. The ITS1 sequences obtained were compared to *Ectocarpus* and *Kuckuckia* sequences available in GenBank using blast (Altschul *et al.,* 1997). ITS1 was preferred over Rubisco spacer sequences because ITS1 is more variable, particularly in its first part (Stache-Crain *et al.,* 1997).

Alignable sequences of the four markers were concatenated, sequences of reference strains (nine from Europe, one each from South America and New Zealand; Table 2) were then added, and phylogenetic analyses undertaken. Phylogenetic trees were constructed using the various programs in PHYLIP version 3.69 (Felsenstein, 1995), and the robustness of the alignments was tested with the bootstrapping option (SeqBoot). Genetic distances, applicable for distance matrix phylogenetic inference, were calculated using the Dnadist program in the PHYLIP package. Phylogenetic inferences based on the distance matrix (Neighbor), maximum likelihood (Dnaml) and parsimony (Dnapars) algorithms were applied to the alignments. In all cases, the best tree or majority rule consensus tree was selected using the consensus program (Consense). The trees were visualized and drawn using the TREEVIEW software version 2.1 (Page, 1998).

Results

In the Museo de Historia Natural, Lima, seven herbarium specimens were thalli of *Ectocarpus* collected by César Acleto in central Peru around Lima between September and February from 1964 to 1976. In the field, *Ectocarpus* occurred at eight out of the nine localities visited, usually as an epiphyte on other macroalgae such as *Macrocystis, Lessonia, Desmarestia* and *Gracilaria*, and only occasionally on rocks. It was present from sheltered to exposed conditions but was not found at a highly exposed locality (Table 1). Although some individuals were up to 14 cm in size others were minute felts or dark spots; they appeared in culture after inoculation with their substratum or they developed on paper that was used to filter sea water (Figs 2-4; Table 3). A total of 120 strains, including strain CCAP1310/40 isolated previously at Site 9, were available for molecular identification.

Determination of the length of the nuclear ribosomal ITS1 in all strains identified 5-6 ITS1 length types which were distinguishable on agarose gels, and one putative hybrid (Fig. 5). Sequencing of 36 individuals revealed that two of the length types were heterogeneous and contained more than one ITS1 genotype (GT). In total we obtained nine different GTs representing seven *Ectocarpus*, one *Kuckuckia* and one hybrid between two of the *Ectocarpus* genotypes (Table 3).

Individuals with the same GT as the genome strain (GT4) occurred from Site 1 where they were the only *Ectocarpus* collected, to Site 5 at the northern border of Chile (Tables 1,2). The ITS1 regions of the seven sequenced strains of this GT were identical to that of the genome strain (n=2, Sites 2 and 3), differed by a single nucleotide substitution (n=4, three from Site 1 and one

from Site 5; see Table 2 for sequence accession of the latter strain), or had an ambiguity at the same position (n=1, Site 5).

There were eight additional genotypes in the study area (Table 3). GT1, which has not been sequenced previously (no blast hit with 100% coverage), was collected at Sites 2, 5 and 8. With 302-306 bp it had the shortest ITS1 so far found in any *Ectocarpus*. GT2 was common at Site 3, but also present at the most southern Site 10. It showed no perfect match with any published ITS1; the best blast hit was a previous isolate from Puerto Deseado, Patagonia, Argentina (93% identity). However, when we sequenced ITS1 of CCAP1310/40 from Site 9 (for which only the Rubisco spacer sequence had hitherto been available; U38736) its ITS1 closely resembled that of our isolates of GT2; CCAP1310/40 only differed by nine substitutions and four small indels of, in total, 10bp length. GT3 was collected once (at Site 6); its ITS1 was alignable with that of GT2 but differed by 22 substitutions and four indels of in total seven bp length. It was considered just different enough to justify recognition as a separate GT. It more strongly (95% identity) resembled that of the isolate from Argentina mentioned above which again gave the best blast hit. GT5 (*E. fasciculatus*) was present from Site 3 to Site 10. It strongly resembled previous sequences from Isla Robinson Crusoe (off central Chile) and from Europe. GT6 (*E. siliculosus*) was the only *Ectocarpus* collected at Site 7, and was also common at Site 8. GT7, obtained in a single isolate from Site 5, was a genotype that had not been sequenced previously; the most similar sequence available was from a *Kuckuckia,* but it showed only 77% identity. In culture the isolate of GT7 developed phaeophycean hairs which are characteristic for *Kuckuckia* and absent in *Ectocarpus* (Hamel, 1939). As the ITS1 of GT7 had the same length as that of *E. siliculosus*, all isolates of this ITS1 length were examined for the presence of hairs, but they were only observed in the isolate from Site 5. ITS1 of two isolates of this ITS1 length from Site 7 and three from Site 8 (including strain CCAP1310/333 from Site 8 which showed high copper tolerance;

Ritter *et al.,* 2010; Dittami *et al.*, 2010) were sequenced and their ITS1 matched that of European *E. siliculosus*, e.g. of well-studied strains from Naples and Roscoff. We concluded that all isolates with an ITS1 length of 714-716 bp, apart from the one from Site 5, belonged to *E. siliculosus*. GT8 was present only at Site 7; it was identified as *E. crouaniorum* because its long ITS1 was similar (>90% identity) with strains of this species from Europe. The best blast hits were isolates from the salt-polluted German Werra river and from Niebla, Valdivia (southern Chile) (96 and 95% identity, respectively). GT9 presented a double band with lengths that corresponded to *E. siliculosus* and *E. crouaniorum*, two species which in Europe are known to occasionally produce viable hybrids; these are, however, unable to form meiospores (Peters *et al.*, 2010). The two strains of this putative hybrid genotype were isolated from Site 7 where they co-occurred with *E. siliculosus* and *E. crouaniorum*.

The concatenated alignable sequences from ITS1, ITS2, Rubisco spacer and *cox*3 (Table 2) had a length of 1533 bp. The different calculation methods of phylogenetic trees gave basically the same topology, and only one is shown (Fig. 6). The three isolates of *Kuckuckia,* including the isolate of GT7 from Site 5, formed the sister group to *Ectocarpus* which itself was split into two major lineages. The first consisted of *E. fasciculatus* and an additional European genotype, and the second comprised the remaining isolates. Within this latter clade, there were four sub-clades with strong and one sub-clade with moderate (>80%) statistical support but the hierarchy among the sub-clades varied and none of the hierarchies had strong statistic support in any analysis. In all trees, the genome strain clustered strongly with GT1, but was separated from genuine *E. siliculosus, E. crouaniorum*, the strain from New Zealand, and a lineage formed by the other South American endemic genotypes 2 and 3.

Discussion

Our study revealed a diverse *Ectocarpus* flora in Peru and northern Chile (Fig. 7). Three species recognised for Western Europe, including the recently reinstated *E. crouaniorum* (Peters *et al.,* 2010), were present. In addition there was a *Kuckuckia* and four genotypes of *Ectocarpus* which have not been recorded outside South America, and for which we can only propose provisional designations, i.e. genotypes (GT) 1-4. *Kuckuckia* had been reported previously from Isla Robinson Crusoe, but according to sequences, our isolate was significantly different from both that strain and from six other previously sequenced isolates from Europe and South Africa which form two lineages within *Kuckuckia* (Stache-Crain *et al.,* 1997). Our new strain from northern Chile adds a third lineage. Based on culture studies, all *Kuckuckia* were merged into a single species, *K. spinosa* (Kützing) Kuckuck (Pedersen, 1979); the molecular data and our new isolate suggest the systematics of the genus may need to be re-assessed.

Our data suggest a separation of the study area into a northern half from Ancón, central Peru to Pisagua, northern Chile (Sites 1-6) dominated by the genotype of the genome strain (GT4; 61% of the strains isolated in the northern half but absent in the South) and a southern half from Antofagasta to Quintay (Sites 7-10) dominated by *E. siliculosus*, *E. crouaniorum* and their hybrids (together representing 65% of the samples in the southern half but missing in the North). This apparent split may reflect adaptation to different oceanographic environments (see below), but we cannot exclude that it is an artefact due to the different sampling methods: in the North we collected at the end of summer from drift or subtidal material, in the South we collected in spring and summer in the intertidal and from spores in the water column. The sampling zone and season certainly influence which species of *Ectocarpus* are encountered; for instance, in Western

Europe, *E. crouaniorum* occurs only in the high intertidal and macrothalli are seasonal in spring and early summer (Peters *et al.,* 2010). More complete sampling would be required for a genuine quantification of the occurrence of the different genotypes in the area.

Isolates similar to the genome strain were the northernmost samples (Table 1) and represented two thirds of the isolates from Peru. The distribution area of this genotype is characterised by continuous upwelling of cool water (16-20ºC at the sea surface) irregularly interrupted by El Niño events which may result in several weeks of >10ºC higher sea surface temperatures due to the southward incursion of warm waters (Peters & Breeman, 1993). So far no *Ectocarpus* has been collected from similar low latitudes. It remains to be examined whether it extends even further north, perhaps up to the deep-water kelp forests around Galápagos where its frequent host, *Desmarestia*, is present (Graham *et al.,* 2007). We have not found GT4 south of Arica. Apart from the two individuals from Arica, which were collected *in situ* at 3-5m depth, all samples of this genotype were from drift material, and their original habitat and depth distribution are not known. There are very limited data on seasonality of this genotype. All our new collections were from late summer, and it was present as a minute epiphyte or endophyte in *Desmarestia peruviana* in November 1988. The Museo de Historia Natural, Lima, houses seven herbarium specimens which are thalli of *Ectocarpus*, collected by César Acleto around Lima between September and February from 1964 to 1976. If it is assumed they belong to the same genotype, macroscopic thalli appear to be present from September to March.

GT1 and GT2 were present in both parts of the study area. Whereas GT1 formed minute dark spots on *Macrocystis* and *Lessonia*, thalli of GT2 were large (Fig. 4) and grew on rock or on different red and brown algae, such as *Gracilaria* and *Desmarestia*. Both genotypes are endemic

to the Pacific coast of South America. GT3, found once at an exposed site, was previously known from Atlantic South America; it also resembles *Ectocarpus* strains collected in New Zealand (U38761) and at Isla Robinson Crusoe (U38763) (Stache-Crain *et al.,* 1997). This genotype may be widely distributed in the southern hemisphere.

Our phylogenetic analyses, based on the genetic markers used in Stache-Crain *et al.* (1997) plus *cox*3, confirmed most lineages obtained in that pioneer study. However, the lineages 2d and 3 in Stache-Crain *et al.* (1997) were not represented in our taxon set. Our analyses confirmed that GT4 groups within the *Ectocarpus* subclade "*siliculosi*" (Fig. 6). In a previous analysis involving fewer taxa (Peters *et al.,* 2010), it had clustered with Stache-Crain's lineage 1a which is genuine *E. siliculosus*. The addition of more South American strains led to its separation from that lineage. A post-zygotic sterility barrier between the genome strain and genuine *E. siliculosus* has been reported (Peters *et al.*, 2004b). The genome strain was also genetically distant from *E. crouaniorum* and from an *Ectocarpus* from New Zealand referred to as *E. siliculosus* in previous studies (Stache, 1989; Müller, 1991; Peters *et al.,* 2004b). It was likewise separated from the lineage formed by GT2 and GT3. GT2 and GT4 co-occurred at Site 3; a cross between the genome strain and a member of GT2 from Site 8 produced viable hybrids with weakly-growing erect thalli which did not form meiosporangia (unpublished results). GT1 and GT3 were not yet involved in crossing studies.

In summary, the genome-sequenced *Ectocarpus* appears to be genetically separated from all genotypes with recognised names and also from all but one (GT1) of the genotypes endemic to South America or the southern hemisphere; it could therefore be regarded as a further species of *Ectocarpus*. The problem is to find its correct name.

A proportion of the seaweeds that are reported from Peru and Chile also occur in the NE Pacific (Santelices, 1989); this is the case for *Ectocarpus acutus* Setchell et Gardner reported in Chile from Coquimbo and Cartagena (Santelices, 1989; Ramírez & Santelices, 1991). It is possible that the lineages GT1+4 and 2+3 belong to this or another one of the species of *Ectocarpus* described from the Pacific coast of North America (Setchell & Gardner, 1922, 1925) or NE Asia (Yoshida *et al.,* 1995; Yoshida, 1998). So far four isolates of *Ectocarpus* from this vast region have been sequenced. Strains from San Francisco and Santa Barbara (California, USA) and from Kanagawa, Enoshima Prefecture, central Japan, were genuine *E. siliculosus*, a strain from Akkeshi, NE Hokkaido, Northern Japan, in contrast, was more closely related to GT4 (Stache-Crain *et al.*, 1997; Tanaka *et al.*, unpublished). The Akkeshi strain was nevertheless in crosses fully compatible with genuine *E. siliculosus*, but it has not been tested against GT4 (Müller & Kawai, 1991). More comprehensive sampling in the North Pacific may be helpful for the taxonomic revision of the genus and the nomenclature of South American *Ectocarpus*.

Our work also aimed at finding strains that were sexually compatible with the genome strain. Seven out of our 36 new strains of GT4 produced unilocular sporangia in culture; female gametophytes obtained from meiospores of two sporophytes from Sites 2 and 5 were used for crosses with the genome strain. In both combinations the zygotes developed into sporophytes capable of meiosis, and one of these sporophytes was selected for further experiments leading to the production of the genetic map of the *Ectocarpus* genome (Heesch *at al.,* 2010).

Acknowledgements

We thank César Acleto for providing access to the phycological herbarium at the Museo de Historia Natural, Lima, and Matt Hall for advice during the molecular experiments. AFP was supported by a travel grant from Université Pierre et Marie Curie for the collections and by a Ray Lankester Investigatorship by the Marine Biological Association of the United Kingdom for the molecular work. Collections and isolations in Chile were supported by FONDAP 1501 0001 funded by CONICYT, to the Center for Advanced Studies in Ecology & Biodiversity (CASEB) Program 7. Additional support was provided by ICA grant (to JAC).

References

Altschul, SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25:** 3389-3402.

Andrade S, Moffett J, Correa JA. 2006. Distribution of dissolved species and suspended particulate copper in an intertidal ecosystem affected by copper mine tailings in Northern Chile. *Marine Chemistry* **101:** 203-212. [this paper is only cited in footnote to Table 1]

Charrier B, Coelho SM, Le Bail A, Tonon T, Michel G, Potin P, Kloareg, B, Boyen C, Peters AF, Cock JM. 2007. Development and physiology of the brown alga *Ectocarpus siliculosus*: two centuries of research. *New Phytologist* **177:** 319-332.

Cock JM *et al*. 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in the brown algae. *Nature*, submitted.

Coelho SM, Peters AF, Charrier B, Roze D, Destombe C, Valéro M, Cock JM. 2007. Complex life cycles of multicellular eukaryotes: new approaches based on the use of model organisms. *Gene* **406:** 152-170.

Dittami SM, Rousvoal S, Coppée J-Y, Boyen C, Tonon T. 2010. Comparative genome hybridizations of *Ectocarpus* strains and ecotypes. *New Phytologist* **xx**: xxx-xxx. [this is another companion paper, to appear in the same volume of NP]

Felsenstein J. 1995. PHYLIP (Phylogeny Inference Package) Version 3.57c. University of Washington, Seattle.

Graham MH, Kinlan BP, Druehl LD, Garske LE, Banks S. 2007. Deep-water kelp refugia as potential hotspots of tropical marine diversity and productivity. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 16576-16580.

Hamel G. 1939. Sur la classification des Ectocarpales. *Botaniska Notiser* **1939:** 65-70.

Heesch S, Cho GY, Peters AF, Le Corguillé G, Falentin C, Boutet G, Cöedel S, Corre E, Coelho SM, Cock JM. 2010. A sequence-tagged genetic map for the brown alga *Ectocarpus siliculosus* provides large-scale assembly of the genome sequence. *New Phytologist* **xx:** xxx-xxx. [this is another companion paper, to appear in the same volume of NP]

Müller DG. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge *Ectocarpus siliculosus* im Kulturversuch. *Planta (Berlin)* **75**: 39-54.

Müller DG. 1991. Mendelian segregation of a virus genome during host meiosis in the marine brown alga *Ectocarpus siliculosus*. *Journal of Plant Physiology* **137:** 739-743.

Müller DG, Kawai H. 1991. Sexual reproduction of *Ectocarpus siliculosus* (Ectocarpales Phaeophyceae) in Japan. *Japanese Journal of Phycology* **39:** 151-155.

Page, RDM. 1998. TREEVIEW software version 2.1 (http://taxonomy.zoology.gla.za).

Pedersen PM. 1989. Studies on *Kuckuckia spinosa* (Fucophyceae, Sorocarpaceae): life history, temperature gradient experiments, and synonymy. *Nordic Journal of Botany* **9:** 443-447.

Peters AF, Breeman AM. 1993. Temperature tolerance and latitudinal range of brown algae from temperate Pacific South America. *Marine Biology* **115:** 143-150.

Peters AF, Marie D, Scornet D, Kloareg B, Cock JM. 2004a. Proposal of *Ectocarpus siliculosus* as a model organism for brown algal genetics and genomics. *Journal of Phycology* **40:** 1079-1088.

Peters AF, Scornet D, Müller DG, Kloareg B, Cock JM. 2004b. Inheritance of organelles in artificial hybrids of the isogamous multicellular chromist alga *Ectocarpus siliculosus* (Phaeophyceae). *European Journal of Phycology* **39:** 235-242.

Peters AF, Scornet D, Ratin M, Charrier B, Monnier A, Merrien Y, Corre E, Coelho SM, Cock JM. 2008. Life-cycle-generation-specific developmental processes are modified in the

immediate upright mutant of the brown alga *Ectocarpus siliculosus*. *Development* **135:** 1503-1512.

Peters AF, van Wijk S, Cho GY, Scornet D, Hanyuda T, Kawai H, Schroeder DC, Cock JM, Boo SM. 2010. Reinstatement of *E. crouaniorum* Thuret in Le Jolis as a third common species of *Ectocarpus* (Ectocarpales, Phaeophyceae) in western Europe, and its phenology at Roscoff, Brittany. *Phycological Research* **58** (in press)

Rambaut A. 2002. *Se-Al v2.0a11*. http://tree.bio.ed.ac.uk/software/seal/.

Ramírez ME, Santelices B. 1991. *Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica*. Santiago de Chile: Universidad Católica de Chile.

Ritter A, Umbertini M, Romac S, Gaillard F, Delage L, Mann A, Cock JM, Tonon T, Correa JA, Potin P. 2010. Copper stress proteomics highlights local adaptation of two strains of the model brown alga *Ectocarpus siliculosus*. *Proteomics*, in press.

Santelices, B. 1989. *Algas marinas de Chile.* Santiago de Chile: Universidad Católica de Chile.

Stache B. 1989. Sexual compatibility and species concept in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) from Italy, North Carolina, Chile, and New Zealand. In: Garbary DJ., South RG, eds. *Evolutionary biogeography of the marine algae of the North Atlantic*. Berlin: Springer Verlag, 173-186.

Stache-Crain B, Müller DG, Goff LJ. 1997. Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear and plastid-encoded DNA sequences. *Journal of Phycology* **33:** 152-168.

Setchell WA, Gardner NL. 1922. Phycological contributions. VI. New species of *Ectocarpus*. *University of California Publications in Botany* **11**: 403-426.

Setchell WA, Gardner NL. 1925. The marine algae of the Pacific coast of North America. Part III. Melanophyceae. *University of California Publications in Botany* **8:** 383-898.

Yoshida T. 1998. *Marine algae of Japan.* Tokyo: Uchida Rokakahu Publishing Co.

Yoshida T, Yoshinaga K, Nakajima Y. 1995. Check list of marine algae of Japan (revised in 1995). *Japanese Journal of Phycology* **43:** 115-171.

Supporting information

Details of molecular strain identification, PCR and sequencing

Table S1. Primer information

Figure Legends

Fig. 1. Origin of isolates for the present study. See Table 1 for details on collecting sites. Diameters of closed circles represent the number of isolates from each site. No *Ectocarpus* was found at the highly exposed Site 4 (open circle). The genome strain was collected in 1988 at Site 3. The isolate from Site 9 was available from CCAP. Map generated using www.aquarius.ifm-geomar.de/omc/.

Figs 2-4. Herbarium specimens of *Ectocarpus* from Peru, all collected from drift material. 2. GT4, Site 1. 3. Several thalli of GT4 epiphytic on *Desmarestia firma* Skottsberg, Site 2. 4. GT2, Site 3.

Fig. 5. ITS1 lengths in *Ectocarpus* and *Kuckuckia* from Peru and Chile. 2% agarose gel run at low voltage, stained with ethidium bromide. Flanking lanes contain length standards (600, 800, 1000, 1500 bp from the bottom). Lanes 1-9: genotypes (GT). 1. GT1, 2. GT2, 3. GT3, 4. GT4 (=genome-sequenced strain), 5. *E. fasciculatus*, 6. *E. siliculosus*, 7. *Kuckuckia* sp., 8. *E. crouaniorum*, 9. Putative hybrid between *E. siliculosus* and *E. crouaniorum*; the middle band is a PCR artefact. Note that lanes 2-4 and 6-7 have lengths that are hardly (if at all) distinguishable in an ordinary 1% agarose gel. See Table 3 for precise ITS1 lengths.

Fig. 6. Molecular phylogeny of *Ectocarpus* and *Kuckuckia* inferred from genetic distance analysis based on concatenated alignable sequences from ITS1, ITS2, Rubisco spacer and *cox*3. Maximum likelihood and maximum parsimony analyses gave similar results. Thick lines represent branches with 100% bootstrap support in all analyses. Minimum bootstrap values (in

one of the three different analyses) for two branches with moderate support are provided under the respective branch. All other branches had below 60% bootstrap support. Taxon labels in red (grey in printed version) are isolates from the study area, including the genome strain CCAP1310/4. The three boxes illustrate the principal split into *Kuckuckia* (bottom), *Ectocarpus* section *fasciculati* (top) and section *siliculosi* (centre). cro Br, fas Br and sil Br are reference strains of *E. crouaniorum*, *E. fasciculatus* and genuine *E. siliculosus*, respectively, all from Brittany, France (Peters *et al.*, 2010). Names starting with "1310" are CCAP strain numbers, those with "Ec" are accessions at the Roscoff *Ectocarpus* strain collection, those with "Kck" are strain designations in Stache-Crain *et al.* (1997). Genotypes obtained in the present study (GT1-8) are provided to the right of the tree, as well as the lineage numbers (1a-6b) of strains included in Stache-Crain *et al.* (1997). Genuine *E. siliculosus* was in our analyses only represented by European isolates because individuals from Chile had highly similar ITS1 sequences (99% identity in blast; Table 3) and sequences of additional markers were not generated for them. Ec319 represents Stache-Crain lineage 5a instead of strain CCAP1310/100 originally employed in Stache-Crain *et al.* (1997) for which *cox*3 is not yet available. Accessions of sequences utilised are provided in Table 2.

Fig. 7. Diversity of *Ectocarpus* and *Kuckuckia* in Peru and northern Chile (n=120 samples). sil = *E. siliculosus*, fas = *E. fasciculatus*, cro = *E. crouaniorum*, sil+cro = putative hybrid between *E. siliculosus* and *E. crouaniorum*, Kuck = *Kuckuckia* sp. GT1-4 = other genotypes, including that of the genome strain (GT4). For details see Table 3.

Table 1. Origin and number of isolates. Kuck = *Kuckuckia*; sil = *E. siliculosus*; fas = *E. fasciculatus*; cro = *E. crouaniorum*; ND = no data

Site no	Locality	Date	Coordinates	No of isolates	Genotypes present (number of isolates)	ITS1 sequenced (n)	Site details†		Collection details	Strains‡
							Playa de Ancón	Sheltered		
1	Ancón	07-03-06	-11.7 -77.2	4	GT4 (4)	3	Playa San Francisco	Sheltered	Drift, on *Gracilaria*	311-314
										201-202, 246-247,
										266-267, 269-271,
	Bahía Mendieta									274-285, 289-293,
2	(South of Paracas)	08-03-06	-14.1 -76.3		GT4 (28)	1	Playa Mendieta	Sheltered	Drift, on various macroalgae	315-316
									Upper subtidal, on phylloid of	
				31	GT1 (3)	2	Cueva de la Zorra	Mid-exposed	*Macrocystis*	286-288
3	San Juan de Marcona	09-03-06	-15.4 -75.2		GT4 (2)	1	Playa Hermosa	Sheltered	Drift, on *Desmarestia peruviana*	301, 303
										294-300, 302, 304-
					GT2 (12)	7	Playa Hermosa	Sheltered	Drift, on various macroalgae	307
									Upper subtidal, on phylloid of	
				16	fas (2)	1	Reserva Punta San Juan	Mid-exposed	*Macrocystis*	308-309
4	Ilo	10-03-06	-17.6 -71.3		none	---	Playa Boca del Río	Highly	---	---
				0	none	---	Punta Coles	exposed	---	---
									Subtidal 3-5m, on *Lessonia*	
5	Arica	01-03-06	-18.5 -70.3		GT4 (2)	2	South end of town	Mid-exposed	*trabeculata* and red macroalga	161, 721
									Subtidal 3-5m, on *Lessonia*	
					fas (1)	1	South end of town	Mid-exposed	*trabeculata*	310
									Upper subtidal, on *Lessonia*	
					fas (1)	1	Yacht harbour	Sheltered	*trabeculata*	165
				5	Kuck (1)	1	Yacht harbour	Sheltered	Upper subtidal, on rope	160

6	Pisagua	02-03-06	-19.6	-70.2		GT1 (1)	0	Cementerio	Exposed	Drift, on *Lessonia nigrescens*	159
						GT1 (1)	1	Shore near wharf	Exposed	Intertidal, on *Lessonia*	157
					3	GT3 (1)	1	Shore near wharf	Exposed	*nigrescens*	156
7	Antofagasta	28-02-06	-23.6	-70.4		sil (5)	1	Bolsico (N of town); beach near *Gracilaria* bed	Sheltered	Drift	150-151, 153-155
					6	sil (1)	1	Coloso (S of town): artificial rocky shore	Sheltered	Intertidal	147
8	Chañaral	feb 2005	-26.3	-70.7		GT1 (1)	1	Soldado (N of copper discharge point)§	Sheltered	Subtidal, from spore suspension	521
		jan 2004				sil (16)	2	La Lancha and Palito (close to copper discharge point)§	Exposed	Intertidal or developed in culture on *Scytosiphon*	466, 509-512, 524-525, 606, 614-615, 621-623, 630-632
		feb 2005				fas (12)	2	Different sites, all without copper pollution	Exposed	Subtidal, from spore suspension, or intertidal	523, 526, 528, 607, 611, 620, 624-629
		jan 2004				cro (16)	3	La Lancha and Palito (close to copper discharge point)§	Exposed	Intertidal or developed in culture on *Scytosiphon*	514-520, 527, 608, 610, 612-613, 616-619
		jan 2004			47	sil+cro (2)	0	La Lancha and Palito (close to copper discharge point)§	Exposed	Intertidal or developed in culture on *Scytosiphon*	513, 609
9	Caldera¶	02-10-90	-27.1	-70.8	1	GT2 (1)	1	*Gracilaria* farm	Sheltered	On *Gracilaria*	CCAP1310/40
10	Quintay	05-10-06	-33.2	-71.7		GT2 (1)	1	Marine Station of			639
					7	fas (6)	2	Universidad Andrés Bello	Sheltered	Large tide pool, on rock	633-638
	Total				120		36				

†Exposure was estimated during collection

‡Designation in *Ectocarpus* strain collection at Roscoff or CCAP

§For a description of the site see Andrade *et al.* (2006)

¶Strain collected by Mariela Gonzales and isolated by Dieter G. Müller

Table 2. DNA sequences utilised in the present study; newly generated sequences in bold. Acc = GenBank/EBI/DDBJ accession; bp=base pairs (length); -=not applicable

	Species/genotype	Strain	Origin	ITS1+2	ITS1†	ITS2†	Rubisco spacer‡ (515bp)	*cox*3 (665 bp)
				Acc	bp	bp	Acc	Acc
1	GT1	Ec157	Site 5	**FN564453**	306	247	**FN564475**	**FN564526**
2	GT1	Ec286	Site 2	**FN564454**	302	247	**FN564476**	**FN564527**
3	GT2	Ec298	Site 3	**FN564456**	344	293	**FN564477**	**FN564528**
4	GT2	CCAP1310/40	Site 9	**FN564455**	352	292	U38736	**FN564529**
5	GT3	Ec156	Site 6	**FN564457**	347	264	**FN564478**	**FN564530**
6	GT4	Genome strain Ec32=CCAP1310/4	Site 3	AJ550048 and genome sequence	362	252	AJ550050	from genome project
7	GT4	Ec721	Site 5	**FN564446**∫	362	-	-	-
8	GT5 = *E. fasciculatus*	Ec310	Site 3	**FN564458**	433	292	**FN564479**	**FN564531**
9	GT6 = *E. siliculosus*	Ec147	Site 7	**FN564466**∫	716	-	-	-
10	GT7 = *Kuckuckia* sp.	Ec160	Site 5	**FN564460**	714	290	**FN564480**	**FN564532**
11	GT8 = *E. crouaniorum*	Ec608	Site 8	**FN564459**	855	253	**FN564481**	**FN564533**
12	*E. fasciculatus*	1310/12	Plouescat, Brittany, France	U38824	429	286	U38711	FN564521
13	*E. fasciculatus*	fas R1 = Ec395	Santec,	FN564441	428	282	FN564468	FN564513

			Brittany					
			Plougonvelin,					
14	*E. fasciculatus*	fas R2 = Ec541	Brittany	FN564449	439	282	FN564471	FN564518
			Santec,					
15	*E. siliculosus*	sil R1 = Ec393	Brittany	FN564440	714	247	FN564467	FN564512
			Plougonvelin,					
16	*E. siliculosus*	sil R2 = Ec540	Brittany	FN564448	712	247	FN564470	FN564517
			Kaikoura,					
17	*E. "siliculosus"*	1310/47§	New Zealand	U38766	749	260	U38722	FN564523
			Santec,					
18	*E. crouaniorum*	cro R = Ec471	Brittany	FN564442	865	256	FN564469	FN564514
			Isle of Man,					
			United					
19	*E. crouaniorum*	1310/144	Kingdom	U38771	863	256	U38726	FN564522
			Cherbourg,					
20	*E. sp*	Ec319¶	France	FN564452	644	275	FN564474	FN564519
			Villefranche,					
21	*Kuckuckia sp.*	KuckVF	France	U38829	827	266	U38705	FN564524
			Isla Robinson					
22	*Kuckuckia sp.*	KuckJFer	Crusoe, Chile	U38825	689	267	U38709	FN564525

† Limits: ITS1: start atcattaCCGA, end GTTGTAaaacttt; ITS2: start gtctgttGACACC, end TTTCGTTcggacct (Stache-Crain *et al.*, 1997)

‡ Including flanking rbcL and rbcS gene sequences

§ Strain used in Müller (1991) and Peters *et al.* 2004b; lineage 4 in Stache-Crain *et al.* (1997)

¶ Strain representing lineage 5a of Stache-Crain *et al.* (1997)

∫ Only ITS1; in addition to the sequence of strain 147, the slightly different ITS1 sequence of a particularly copper-resistant strain from Site 8 (CCAP1310/333; Ritter *et al.*, 2010) was deposited (FN564444)

Table 3. Genotypes (GT) and ITS1 length types (LT) of *Ectocarpus/Kuckuckia* from Peru and northern Chile; sil = *E. siliculosus*; cro = *E. crouaniorum*; ND = no data; -=not applicable. Figures in bold face in last two columns indicate that there was no close blast hit

GT	Designation/ species	Field morphology¶	ITS1 length (bp)†	Length of PCR product‡	LT‡	Examples	Sites in Peru	Sites in Chile	Elsewhere§	Best hits in blast of ITS1 sequence	Coverage	Identity
1	GT1	Minute dark spots on hosts	302-6	526-30	1	Ec157	2	6, 8	Isle of Man (United Kingdom)	U38771	**75%**	93%
2	GT2	Large thalli (Fig. 4)	344-52	568-76	2	Ec298	3	9, 10	Pto. Deseado (Argentina)	U38762	100%	93%
3	GT3	Minute dark spot on host	347	571	2	Ec156	-	6	Pto. Deseado (Argentina)	U38762	100%	95%
4	GT4 (the genome-sequenced *Ectocarpus*)	Small to large thalli (Figs 2-3)	362	586	2	Ec721	1-3	5	-	AJ550048	100%	99%
5	*E. fasciculatus* Harvey	Minute dark spots on hosts or	431-3	655-7	3	Ec310	3	5, 8, 10	Isla Robinson Crusoe (Chile); Europe	U38780 U38781 U38759	100%	98% 97% 99%
6	*E. siliculosus* (Dillwyn) Lyngbye	From thin felt on rock or invertebrates to large drifting thalli	714-6	938	4	Ec147	-	7, 8	Cosmopolitan	U38760	100%	99%
7	*Kuckuckia* sp.	Minute thallus on rope	714	938	4	Ec160	-	5	Isla Robinson Crusoe (Chile	U38825	100%	**77%**
8	*E. crouaniorum* Thuret	Minute thalli on rock and invertebrates	855	1079	5	Ec608	-	8	Europe (Werra river); Valdivia (Chile)	U38774, U38772	98%	96%
9	Putative hybrid sil+cro	Minute thalli on rock and invertebrates	ND	ND	4+5	Ec513	-	8	Brittany (France))	-	-	-

¶Note that for strains isolated from filtered water or developed in culture on inoculated substratum the field morphology is unknown

†ITS1 start atcattaCCGA, end GTTGTAaaacttt (Stache-Crain *et al.*, 1997)

‡Compare with Fig. 5

§Origin of strains giving best blast hits

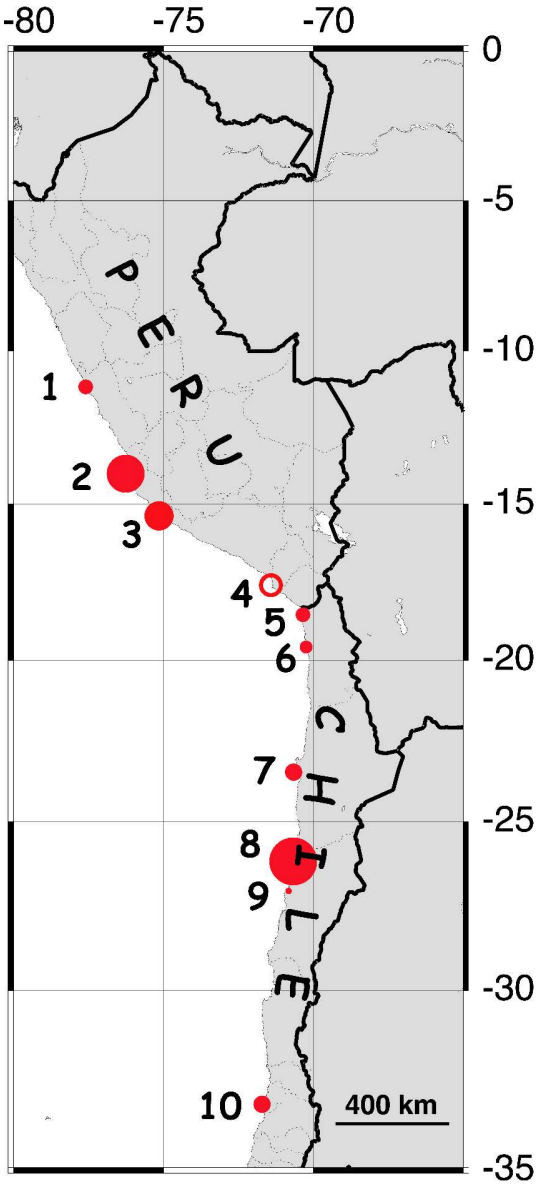

Fig. 1. Origin of isolates for the present study. See Table 1 for details on collecting sites. Diameters of closed circles represent the number of isolates from each site. No Ectocarpus was found at the highly exposed Site 4 (open circle). The genome strain was collected in 1988 at Site 3. The isolate from Site 9 was available from CCAP. Map generated using www.aquarius.ifm-geomar.de/omc/.
161x325mm (600 x 600 DPI)

Figs 2-4. Herbarium specimens of Ectocarpus from Peru, all collected from drift material. 2. GT4, Site 1. 3. Several thalli of GT4 epiphytic on Desmarestia firma Skottsberg, Site 2. 4. GT2, Site 3.

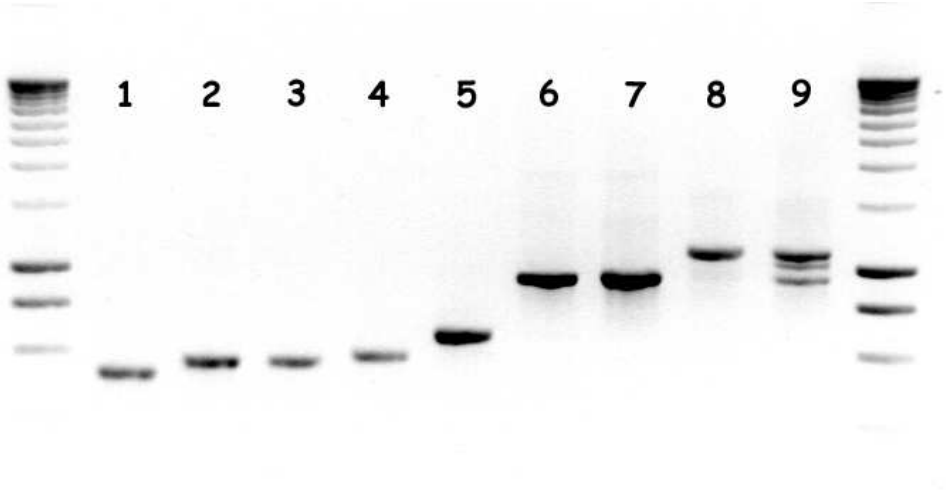

Fig. 5. ITS1 lengths in Ectocarpus and Kuckuckia from Peru and Chile. 2% agarose gel run at low voltage, stained with ethidium bromide. Flanking lanes contain length standards (600, 800, 1000, 1500 bp from the bottom). Lanes 1-9: genotypes (GT). 1. GT1, 2. GT2, 3. GT3, 4. GT4 (=genome-sequenced strain), 5. E. fasciculatus, 6. E. siliculosus, 7. Kuckuckia sp., 8. E. crouaniorum, 9. Putative hybrid between E. siliculosus and E. crouaniorum; the middle band is a PCR artefact. Note that lanes 2-4 and 6-7 have lengths that are hardly (if at all) distinguishable in an ordinary 1% agarose gel. See Table 3 for precise ITS1 lengths.
182x93mm (98 x 98 DPI)

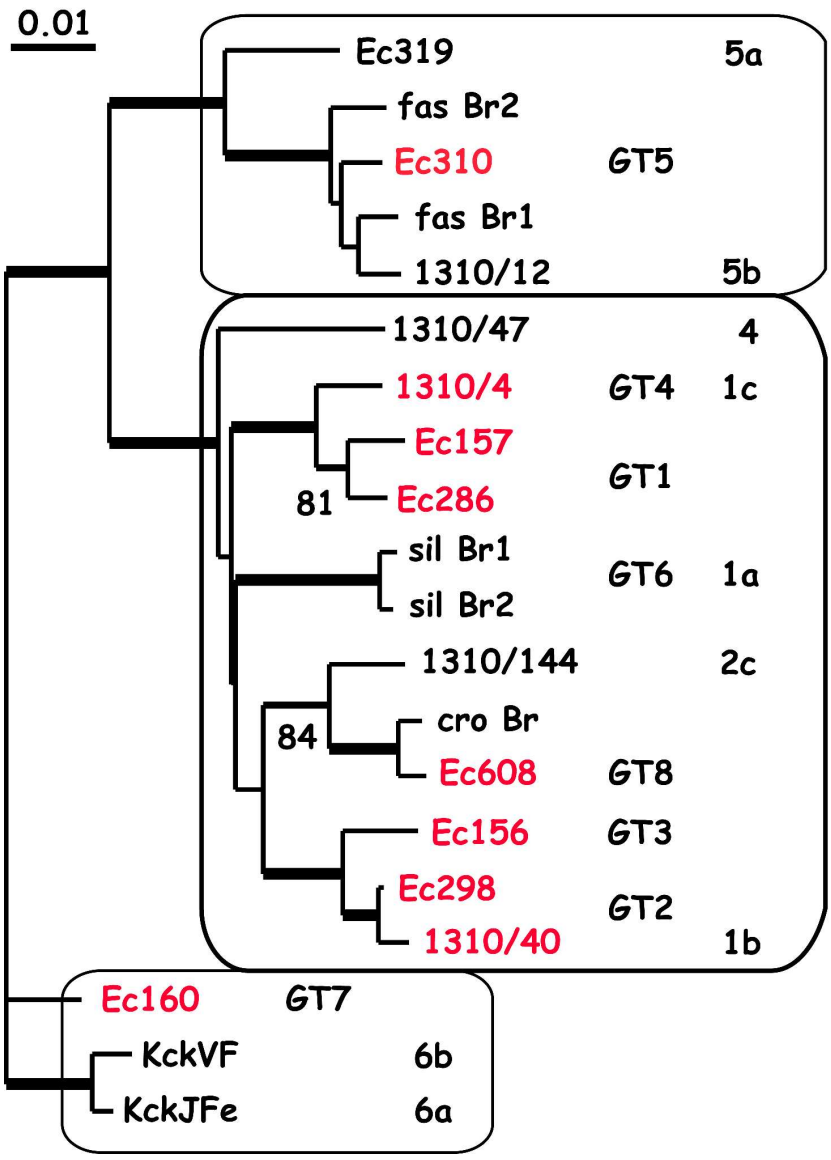

Fig. 6. Molecular phylogeny of Ectocarpus and Kuckuckia inferred from genetic distance analysis based on concatenated alignable sequences from ITS1, ITS2, Rubisco spacer and cox3. Maximum likelihood and maximum parsimony analyses gave similar results. Thick lines represent branches with 100% bootstrap support in all analyses. Minimum bootstrap values (in one of the three different analyses) for two branches with moderate support are provided under the respective branch. All other branches had below 60% bootstrap support. Taxon labels in red (grey in printed version) are isolates from the study area, including the genome strain CCAP1310/4. The three boxes illustrate the principal split into Kuckuckia (bottom), Ectocarpus section fasciculati (top) and section siliculosi (centre). cro Br, fas Br and sil Br are reference strains of E. crouaniorum, E. fasciculatus and genuine E. siliculosus, respectively, all from Brittany, France (Peters et al., 2010). Names starting with "1310" are CCAP strain numbers, those with "Ec" are accessions at the Roscoff Ectocarpus strain collection, those with "Kck" are strain designations in Stache-Crain et al. (1997).

Genotypes obtained in the present study (GT1-8) are provided to the right of the tree, as well as the lineage numbers (1a-6b) of strains included in Stache-Crain et al. (1997). Genuine E. siliculosus was in our analyses only represented by European isolates because individuals from Chile had highly similar ITS1 sequences (99% identity in blast; Table 3) and sequences of additional markers were not generated for them. Ec319 represents Stache-Crain lineage 5a instead of strain CCAP1310/100 originally employed in Stache-Crain et al. (1997) for which cox3 is not yet available. Accessions of sequences utilised are provided in Table 2.
135x179mm (600 x 600 DPI)

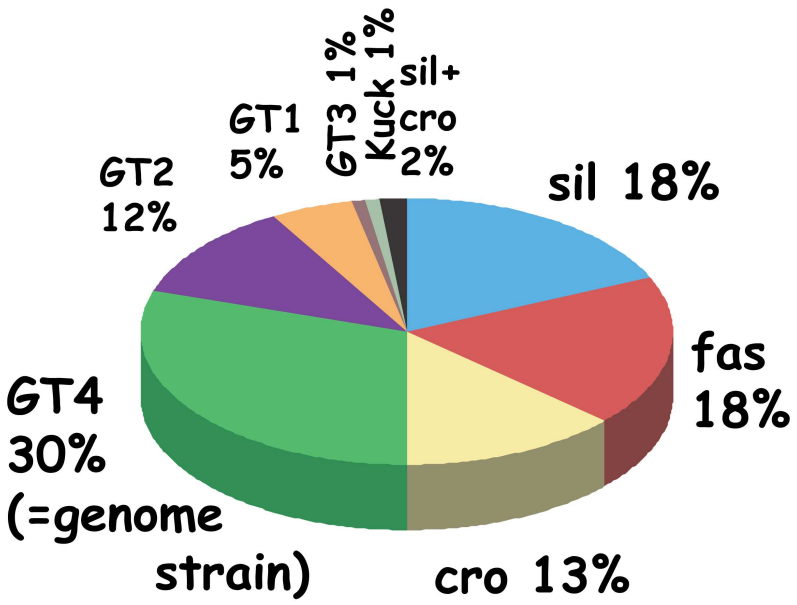

Fig. 7. Diversity of Ectocarpus and Kuckuckia in Peru and northern Chile (n=120 samples). sil = E. siliculosus, fas = E. fasciculatus, cro = E. crouaniorum, sil+cro = putative hybrid between E. siliculosus and E. crouaniorum, Kuck = Kuckuckia sp. GT1-4 = other genotypes, including that of the genome strain (GT4). For details see Table 3.
130x99mm (600 x 600 DPI)