Molecular phylogeny of the brown algal genus *Petrospongium* Nägeli *ex* Kütz. (Phaeophyceae) with evidence for Petrospongiaceae *fam. nov.*

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Résumé – Sur des bases morphologiques, la position systématique du genre *Petrospongium* et ses relations avec les genres *Leathesia* et *Corynophlaea*, ont été longtemps débattues. Dans la présente étude, la position systématique au sein de l'ordre des Ectocarpales de l'espèce type du genre *Petrospongium, Petrospongium berkeleyi* (Grev. *in* Berk.) Nägeli *ex* Kütz., a pu être établie grâce à l'analyse des séquences des gènes *rbc*L et *psa*A. *Petrospongium berkeleyi* et son espèce vicariante du Pacifique, *P. rugosum*, ne sont pas directement apparentées avec les membres de la famille des Chordariaceae, les genres *Leathesia* et *Corynophlaea* en particulier, ni avec aucune autre famille de l'ordre des Ectocarpales. Les deux espèces du genre *Petrospongium* forment un groupe monophylétique indépendant, frère du clade formé par les représentants de la famille des Ectocarpaceae. Les genres *Ectocarpus* et *Petrospongium* diffèrent notamment aux plans anatomique (thalle filamenteux et thalle en coussinet pseudoparenchymateux, respectivement), et cytologique (plastes rubanés et plastes discoïdes, respectivement). En conséquence des résultats des analyses phylogénétiques et en accord avec ces différences morphologiques, la famille nouvelle des Petrospongiaceae est proposée pour accueillir le genre *Petrospongium*.

***Corynophlaea* / Ectocarpales / Petrospongiaceae / *Petrospongium* / Phaeophyceae / phylogénie moléculaire / *psa*A / *rbc*L**

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Abstract — Based on morphological evidence, the systematic position of the genus *Petrospongium* and its relationship with the genera *Leathesia* and *Corynophlaea* have long been disputed. In the present study, the position within the order Ectocarpales of the type species, *Petrospongium berkeleyi* (Grev. *in* Berk.) Nägeli *ex* Kütz., has been investigated using molecular phylogenetic analysis based on *rbc*L and *psa*A sequences. *Petrospongium berkeleyi* and its Pacific vicariant *P. rugosum* appeared closely related and did not cluster with other members of the Chordariaceae, particulalrly the genera *Leathesia* and *Corynophlaea*, nor with any other currently recognised ectocarpalean family. The genus *Petrospongium* formed an independent monophyletic group which was a sister clade of the family Ectocarpaceae. The genera *Ectocarpus* and *Petrospongium* differ in their mode of thallus construction (filamentous thallus and pseudoparenchymatous cushion-like thallus, respectively), and in the type of plastids (ribbon-shape and discoid, respectively). These morphological differences agree with our phylogenetic analyses and lead us to propose the family Petrospongiaceae *fam. nov.* to accommodate the genus *Petrospongium*.

Corynophlaea / Ectocarpales / molecular phylogeny / Petrospongiaceae / *Petrospongium* / Phaeophyceae / *psa*A / *rbc*L

INTRODUCTION

The genus *Petrospongium* Nägeli *ex* Kütz. (1858) was described based on the following combination of characters: spongy and cushion-like; pseudoparenchymatous in construction, with a well-developed inner medulla and outer cortex embedded in a gelatinous matrix; hairs with a basal meristem and sheath; unilocular sporangia laterally inserted at the base of the cortical filaments. The type species *Petrospongium berkeleyi* (Grev. *in* Berk.) Nägeli *ex* Kütz. and its Pacific vicariant *Petrospongium rugosum* (Okamura) Setch. *et* N.L.Gardner are the only two members of the genus, and have commonly been classified either within the family Corynophlaeaceae (Kuckuck, 1929; Hamel, 1935; Smith, 1951; Gayral, 1966; Russell & Fletcher, 1975; Abbott & Hollenberg, 1976; Giaccone, 1978; Fletcher, 198; Ribera *et al.*, 1992; Christensen, 1994; Haroun *et al.*, 2002: Table 1), or within the family Leathesiaceae (Setchell & Gardner, 1925; Segawa, 1968; Womersley, 1987; Neto, 1994; Yoshida *et al.*, 1990: Table 1), usually along with genera such as *Corynophlaea* Kütz. (1843), *Leathesia* Gray (1821), *Myriactula* Kuntze (1898), and *Microcoryne* Strömf. (1888), to which they bear striking morphological and anatomical similarities. All five genera possess cushion-like thalli and a basal and mid medulla-like region of erect, closely-packed, branched filaments of large, colourless cells, which give rise terminally to an outer cortex of multicellular, pigmented filaments (termed sometimes paraphyses). Based on a detailed examination of these anatomical characters, Womersley (1987) distinguished the three genera *Corynophlaea*, *Leathesia* and *Petrospongium*. The genus *Corynophlaea* differs from the genera *Leathesia* and *Petrospongium* by the length of its determinate cortical filaments, which are 20-60 cells long in *Corynophlaea* compared to 3-10 cells long in the two other genera. The genus *Leathesia* has medullary cells which produce anastomoses, and has simple, terminal paraphyses-like filaments, with an inflated apical cell and unilocular sporangia arising at their base. In contrast, *Petrospongium* lacks anastomoses in the medullary cells, and has branched, terminal, paraphyses-like filaments, with unilocular sporangia laterally attached (Womersley, 1987). However, these diagnostic characters did not seem satisfactory to Harvey who classified *P. berkeleyi* as *Leathesia berkeleyi* (Grev. *in* Berk.) Harv. (1846 [1846-1851, Vol. I], pl. CLXXVI). Later, *Petrospongium*

Table 1. Non-exhaustive historical review of the ordinal (in square brackets) and familial classification of the genus *Petrospongium*. The classification of closely related genera *Corynophlaea*, *Cylindrocarpus*, *Petrospongium* (sometimes referred as a synonym of *Petrospongium*), *Leathesia*, *Microcoryne* and *Myriactula* is also presented.

Studies / Genera	Setchell & Gardner 1925	Kuckuck 1929	Hamel 1935	Smith 1951	Gayral 1966	Segawa 1968	Russell & Fletcher 1975	Abbott & Hollenberg 1976	Giaccone 1978	Fletcher 1987	Womersley 1987	Yoshida et al. 1990	Ribera et al. 1992	Christensen 1994	Neto 1994	Haroun et al. 2002	Present study
Corynophlaea	–	Cory. [–]	Cory. [Ecto.]	–	Cory. [Chor.]	–	Cory. [Ecto.]	–	Cory. [Chor.]	Cory. [–]	Leat. [Chor.]	–	Cory. [Chor.]	–	–	Cory. [Chor.]	Chor. [Ecto.]
Cylindrocarpus	–	Cory. [–]	Cory. [Ecto.]	–	Cory. [Chor.]	–	Cory. [Ecto]	Cory. [Chor.]	Cory. [Chor.]	–	–	–	–	Cory. [Ecto.]	–	–	–
Leathesia	Leat. [Ecto]	Cory. [–]	Cory. [Ecto.]	Cory. [Chor.]	Cory. [Chor.]	Leat. [Chor.]	Cory. [Ecto.]	Cory. [Chor.]	Cory. [Chor.]	Cory. [–]	Leat. [Chor.]	Leat. [Chor.]	–	–	Leat. [Chor.]	–	Chor. [Ecto.]
Microcoryne	–	Cory. [–]	–	–	–	–	Cory. [Ecto.]	–	–	Cory. [–]	–	–	–	Chor. [Ecto.]	–	Cory. [Chor.]	–
Myriactula	–	–	–	–	Cory. [Chor.]	–	Cory. [Ecto.]	–	Cory. [Chor.]	Cory. [–]	Leat. [Chor.]	Leat. [Chor.]	Cory. [Chor.]	Cory. [Ecto.]	Leat. [Chor.]	Cory. [Chor.]	–
Petrospongium	Leat. [Ecto.]	–	–	Cory. [Chor.]	–	Leat. [Chor.]	–	–	–	Cory. [–]	Leat. [Chor.]	Leat. [Chor.]	Cory. [Chor.]	–	Leat. [Chor.]	Leat. [Chor.]	Petr. [Ecto.]

Abbreviations used for families: Cory. = Corynophlaeaceae; Leat. = Leathesiaceae; Petr. = Petrospongiaceae. Abbreviations used for orders: Chor. = Chordariales; Ecto. = Ectocarpales.
– : the genus is not included in the authors' classification. [–] : the ordinal classification is not available.

berkeleyi was named as *Cylindrocarpus berkeleyi* by H.Crouan & P.Crouan (1851), who erected the genus *Cylindrocarpus* based on the observation of slightly swollen cells in the basal and mid-regions of the erect filaments of the endophytic type species *C. microscopicus*. *Petrospongium rugosum* was reported for the first time in 1903 by Okamura as *Cylindrocarpus rugosus*. The genus *Cylindrocarpus,* typified by *Cylindrocarpus microscopicus* H.Crouan & P.Crouan (1851), was criticised by Setchell & Gardner (1925), who strongly disagreed with the place-ment of *Petrospongium* species within the genus *Cylindrocarpus*, because of its distinct habit (*i.e.* minute tufts with an endophytic base) and structure (*i.e.* absence of a well-developed medulla). Thus, *C. berkeleyi* and *C. rugosus* were re-classified as *Petrospongium berkeleyi* and *P. rugosum* by Setchell & Gardner (1924, 1925). However, this classification was not accepted by some authors such as Hamel (1935) and Abbott & Hollenberg (1976), who kept including *Petrospongium* species under *Cylindrocarpus* (see Table 1 for an historical summary).

A recent molecular phylogenetic study on the systematic position of *P. rugosum* and *Leathesia difformis* (L.) Aresch. (1847) by Cho & Boo (2006) confirmed their classification within the order Ectocarpales (*sensu* Rousseau & Reviers, 1999). Although the position of *L. difformis* was clearly resolved within the family Chordariaceae (*sensu* Peters & Ramírez, 2001), *P. rugosum* formed an independent clade in the order Ectocarpales, which includes the five families Ectocarpaceae (*sensu* Siemer *et al.*, 1998), Scytosiphonaceae (*sensu* Kogame *et al.*, 1999), Adenocystaceae (Rousseau *et al.*, 2000), Acinetosporaceae (*sensu* Peters & Ramírez, 2001) and Chordariaceae (*sensu* Peters & Ramírez, 2001). If distinct cytological and life history characters define the Ectocarpaceae and the Scytosiphonaceae, the delimitation of the Acinetosporaceae, Adenocystaceae, and Chordariaceae is not the result of diagnosed morphological characters but the consequence of molecular phylogeny. Members of the Ectocarpaceae have a ribbon-shaped plastid and a sub-isomorphic life history. In the family Scytosiphonaceae, species feature a single plate-like plastid per cell and a heteromorphic life history (with exceptions demonstrated so far for the two species *Myelophycus simplex* (Harv.) Papenf. (1967) and *Chnoospora implexa* J.Agardh (1848), which have an isomorphic life history (Kogame *et al.*, 1999)). Members of the Adenocystaceae (*sensu* Peters & Ramírez, 2001) have discoid plastids and a heteromorphic life history (Rousseau *et al.*, 2000). Members of the Acinetosporaceae (*sensu* Peters & Ramírez, 2001) possess discoid plastids, but sexuality seems to be lost or is difficult to detect in most taxa. Finally, members of the Chordariaceae (*sensu* Peters & Ramírez, 2001), possess discoid plastids and include both heteromorphic and isomorphic life histories. This later family possesses probably the highest number of genera in the order Ectocarpales, and presents the richest diversity of morphological forms ranging from microscopic, branched filaments to macroscopic parenchymatous plants.

The present study investigates the systematic positions of the species *Corynophlaea crispa* (Harv.) Kuck. (1929) and *Petrospongium berkeleyi* within the order Ectocarpales (*sensu* Rousseau & Reviers, 1999). Inclusion of the only two representatives of the genus *Petrospongium*, the type species *P. berkeleyi* and *P. rugosum*, in the present phylogenetic analysis enable us to test the monophyly of the genus. We show that the genus *Petrospongium* is monophyletic and confirm its phylogenetic position as an independent clade within the order Ectocarpales, as was first suggested by Cho & Boo (2006). We propose to reposition the genus *Petrospongium* in an independent family, Petrospongiaceae fam. nov., within the order Ectocarpales based on DNA sequences of the Rubisco Large subunit (*rbc*L) and the photosystem I P700 chl *a* apoprotein A1 (*psa*A).

MATERIALS AND METHODS

Specimens of *Petrospongium berkeleyi* and *Corynophlaea crispa* collected from a number of sites on the European coast of the North Atlantic were used for molecular analyses of the plastid genes, *rbc*L and *psa*A (Tab. 2). Genomic DNA was extracted from freshly collected specimens, which had been rapidly desiccated in silica gel powder, using the NucleoSpin Plant Standard kit Nucleospin, Macherey-Nagel (MN), GmbH, Germany following the manufacturer's instructions. Double-stranded amplifications of the *rbc*L were obtained as two or three fragments with the following sets of primers: *rbc*L3F and *rbc*L616R from Peters & Ramírez (2001); *rbc*L68F, *rbc*L543F, *rbc*L573F, *rbc*L708R, *rbc*L1124R and *rbc*L1393R designed in this study (Tab. 3); *rbc*L1124F and *rbc*S3R from Siemer *et al.* (1998). The *psa*A gene was partially amplified with the primers *psa*A130F, *psa*A870F, *psa*A970R and *psa*A1760R from Yoon *et al.* (2002). PCR amplifications were performed with a GeneAmp PCR Cycler 2400 or 9700 (Applied Biosystems, Warrington, UK). Carry-over of amplified DNA sequences was prevented using uracil-DNA-glycosylase (UNG; Longo *et al.*, 1990) together with substitution of dUTPs for dTTPs in all reaction mixtures. The 25-μl reaction volume consisted of 1X reaction Buffer (Promega, Madison, USA), 2 mM of $MgCl_2$ (Promega), 0.2 mM of each dATPs, dCTPs, dGTPs (Applied Biosystems), 0.4 mM of dUTPs (Applied Biosystems), 0.5 mM of each primers (Invitrogen, Carlsbad, USA), 1 unit of UNG (Applied Biosystems), 1 unit of Taq Polymerase (Promega), and 1-2 ng/μl of DNA. The profile of the PCR conditions started with a short glycosylase incubation at 34°C for 10 min, then an initial denaturation step at 94°C for 10 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min, primer extension at 74°C for 1 min, and a final primer extension at 72°C for 10 min. PCR products were sequenced commercially on an automated sequencer using the same primers as for the PCR amplification. The final consensus sequences were collated using Sequencher (version 4.5, 1991-1999, Genes codes corporation, Ann Arbor, USA).

The sequences of each *rbc*L and *psa*A gene were aligned using the Clustal W program (Thompson *et al.*, 1994) and subsequently refined manually. In these coding regions, no gaps or stop codon were observed and, therefore, the alignment was unambiguous. Sequences of *rbc*L and *psa*A were combined for 22 ectocarpalean taxa (*sensu* Rousseau & Reviers, 1999) from which four were newly sequenced in the present study and 17 were retrieved from the GenBank Nucleotide Sequence database (Tab. 2). The outgroup taxa was selected as in Cho & Boo (2006) and included *Laminaria digitata*, *Sporochnus radiciformis*, and *Scytothamnus australis*. Congruence between the two genes was shown by Cho *et al.* (2004) on a data set of 32 Phaeophycean taxa using the incongruence length difference test of Farris *et al.* (1994) with the parsimony criterion. Phylogenetic reconstructions were implemented by Maximum Parsimony (MP) with PAUP* version 4.0b10 (Swofford, 2000) and Bayesian Inference (BI) using MrBayes 3.1b2 (Huelsenbeck & Ronquist, 2001). MP analyses were performed on the the *rbc*L-*psa*A dataset with incomplete gaps treated as missing data, in a general heuristic search with the TBR branch-swapping algorithm, the MulTrees option, the branches with a maximum length of zero collapsed (*i.e.* creating polytomies), and 1000 random-additions replicates. The robustness and stability of the resulting MP tree topologies were assessed with bootstrap (BS) analyses (Felsenstein, 1985) using 100 replicates, each with 10 random sequence-addition.

Table 2. Species included in the phylogenetic analyses with taxonomic authorships, data source and Genbank accession numbers for *rbcL* and *psaA*.

Order, family, and species with taxonomic authorship	Reference or locality and collector of rbcL/psaA	GenBank accession number	
		rbcL sequences	*psaA sequences*
Ectocarpales			
Acinetosporaceae			
Pylaiella littoralis (L.) Kjellman	Assali *et al.* (1990)/Yoon *et al.* (2002)	X55372 (1467 nt)	AY119724 (1394 nt)
Adenocystaceae			
Adenocystis utricularis (Bory) Skottsberg	Peters & Ramírez (2001)/Cho *et al.* (2004)	AJ295823 (1467 nt)	AY372939 (1488 nt)
Chordariaceae			
Asperococcus fistulosus (Hudson) Hooker	Cho *et al.* (2003)/Cho *et al.* (2004)	AY095321 (1467 nt)	AY372940 (1488 nt)
Chordaria flagelliformis (O.F. Müller) C. Argardh	Cho *et al.* (2003)/Cho *et al.* (2004)	AY095324 (1467 nt)	AY372941 (1488 nt)
Corynophlaea crispa (Harvey) Kuckuck	Dorset, UK (R.L. Fletcher and M.-F. Racault)	EU850277 (1450 nt)	EU850281 (1488 nt)
Delamarea attenuata (Kjellman) Rosenvinge	Siemer *et al.* (1998)/Cho *et al.* (2004)	AF055396 (1467 nt)	AY372942 (1488 nt)
Dictyosiphon foeniculaceus (Hudson) Greville	Cho *et al.* (2004)/Cho *et al.* (2004)	AY372973 (1467 nt)	AY372943 (1488 nt)
Leathesia difformis (Linnaeus) J.E. Areschoug	Cho & Boo (2006)/Cho & Boo (2006)	AY996365 (1467 nt)	AY996371 (1488 nt)
Punctaria latifolia Greville	Cho *et al.* (2003)/Cho *et al.* (2004)	AY095322 (1467 nt)	AY372948 (1488 nt)
Spermatochnus paradoxus (Roth) Kützing	Cho & Boo (2006)/Cho & Boo (2006)	AY996366 (1467 nt)	AY996372 (1488 nt)
Ectocarpaceae			
Ectocarpus sp SMB2003	Cho *et al.* (2004)/Cho *et al.* (2004)	AY372978 (1467 nt)	AY372949 (1488 nt)
Scytosiphonaceae			
Colpomenia sinuosa (Mertens ex Roth) Derbès et Solier	Kogame *et al.* (1999)/Cho *et al.* (2004)	AB022234 (1467 nt)	AY372950 (1488 nt)
Hydroclathrus clathratus (Bory ex Agardh) Howe	Kogame *et al.* (1999)/Cho *et al.* (2004)	AB022233 (1467 nt)	AY372951 (1488 nt)
Myelophycus simplex (Harvey) Papenfuss	Cho *et al.* (2003)/Cho *et al.* (2004)	AY095320 (1467 nt)	AY372952 (1488 nt)
Petalonia fascia (O.F. Müller) O. Kuntze	Kogame *et al.* (1999)/Cho *et al.* (2004)	AB022243 (1467 nt)	AY372953 (1488 nt)
Scytosiphon lomentaria (Lyngbye) Link	Kogame *et al.* (1999)/Cho *et al.* (2004)	AB022238 (1467 nt)	AY372954 (1488 nt)
Petrospongiaceae			
Petrospongium berkeleyi (Greville) Nägeli & Kützing - PB1	Devon, UK (R.L. Fletcher and M.-F. Racault)	EU850273 (1467 nt)	EU850278 (1488 nt)
Petrospongium berkeleyi (Greville) Nägeli & Kützing - PB2	Brittany, France (B. de Reviers)	EU850274 (1299 nt)	EU850279 (1488 nt)
Petrospongium berkeleyi (Greville) Nägeli & Kützing - PB3	Sao Miguel, Azores (M.I. Parente)	EU850275 (776 nt)	EU850280 (1488 nt)
Petrospongium rugosum (Okamura) Setchell & N.L. Gardner - PE141	Cho & Boo (2006)/Cho & Boo (2006)	AY996361 (1467 nt)	AY996367 (1488 nt)
Petrospongium rugosum (Okamura) Setchell & N.L. Gardner - PE236	Cho & Boo (2006)/Cho & Boo (2006)	AY996362 (1467 nt)	AY996368 (1488 nt)
Petrospongium rugosum (Okamura) Setchell & N.L. Gardner - PE163	Cho & Boo (2006)/Cho & Boo (2006)	AY996363 (1467 nt)	AY996369 (1488 nt)
Scytothamnales			
Scytothamnus australis (J. Agardh) J.D. Hooker & Harvey	Peters & Ramírez (2001)/Cho *et al.* (2004)	AJ295833 (1371 nt)	AY372967 (1488 nt)
Laminariales			
Laminaria digitata (Hudson) J.V. Lamouroux	Cho *et al.* (2004)/Cho *et al.* (2004)	AY372984 (1467 nt)	AY372964 (1488 nt)
Sporochnales			
Sporochnus radiciformis (R.Brown ex Turner) C. Agardh	Kawai & Sasaki (2002) as *S. scoparius* Harvey/Cho *et al.* (2004)	AB037142 (1409 nt)	AY528861 (1469 nt)

Table 3. Sequences of the primers used for DNA amplification and sequencing (provided as pairs).

Primer Name	Length (nt)	Position	Sequence 5' ↔ 3'	Reference
rbcL3F	20	*rbc*L (-17 – 3)	GGCACCGGAGAATCTATATG	Peters & Ramirez (2001)
rbcL616R	25	*rbc*L (640 – 616)	GTTGTGAGTTAATATTTTCATCATC	Peters & Ramirez (2001)
rbcL68F	23	*rbc*L (46 – 68)	GCNAAAATGGGNWAYTGGGATGC	This study
rbcL708R	23	*rbc*L (730 – 708)	TTAAGNTAWGAACCYTTAACTTC	This study
rbcL573F	25	*rbc*L (549 – 573)	GTCTTTCTGGTAAAAACTACGGACG	This study
rbcL1124R	25	*rbc*L (1148 – 1124)	CCAGAAGCTACAGGAACACATTTTC	This study
rbcL543F	26	*rbc*L (518 – 543)	CCWAAATTAGGTCTTTCWGGWAAAAA	This study
rbcL1393R	23	*rbc*L (1415 – 1393)	TANTCAAANGTNATATCTTTCCA	This study
rbcL1124F	20	*rbc*L (1105 – 1124)	ATGGATTGGGCAGCTCTTAG	Siemer *et al.* (1998)
rbcS3R	21	*rbc*S (23 – 3)	AAACATCCTTGTGTAACTCTC	Siemer *et al.* (1998)
psaA130F	21	*psa*A (110 – 130)	AACWACWACTTGGATTTGGAA	Yoon *et al.* (2002)
psaA970R	19	*psa*A (988 – 970)	GCYTCTARAATYTCTTTCA	Yoon *et al.* (2002)
psaA870F	20	*psa*A (851 – 870)	GGNGGWYTATGGTTAAGTGA	Yoon *et al.* (2002)
psaA1760R	23	*psa*A (1782 – 1760)	CCTCTWCCWGGWCCATCRCAWGG	Yoon *et al.* (2002)

Prior to BI analyses, the Akaike Information Criterion (Akaike, 1974) was applied using the program Modeltest v.3.07 (Posada & Grandall, 1998) to determine the best-fit model of sequence evolution. The general-time-reversible (GTR) model of DNA substitution (Rodríguez *et al.*, 1990) with a gamma correction for among-site variation (G) and a proportion of invariable sites (I) was the most appropriate for the present dataset (A↔C = 1.6109, A↔G = 3.5340, A↔T = 0.8489, C↔G = 0.8607, C↔T = 8.5657, G↔T = 1.0000; gamma distribution shape parameter = 0.9792; proportion of invariable sites = 0.5886). The selected model and parameter estimates were then used for tree searches with a substitution rate (Nst) of 6 and a gamma shape parameter from the respective dataset. BI was inferred running four simultaneous Monte-Carlo Markov chains for 2,000,000 generations, sampling every 100 generations, resulting in 20,000 trees. The first 600 trees generated before the chain reached stationary ("burn-in") were discarded, and the remaining trees were used to construct a majority-rule consensus tree showing relative occurrences (*i.e.* posterior probabilities) of all nodes in the tree.

RESULTS

Partial *psa*A sequences determined in this study for three specimens of *Petrospongium berkeleyi* and one specimen of *Corynophlaea crispa* totalled 1488 nucleotides (nt). The complete *rbc*L sequence of 1467 nt was obtained for

the strain *Petrospongium berkeleyi* PB1 and partial *rbc*L sequences were determined for the strain *P. berkeleyi* PB2 and PB3 as well as for *Corynophlaea crispa*. The *rcb*L and *psa*A sequences were completely identical between the strains PB1 and PB3 and only one substitution for each gene was observed with the strain PB2. Within the 1488 nt and 1467 nt of the *psa*A and *rbc*L alignments respectively, parsimony analyses of 25 taxa including the three outgroup species revealed 475 and 413 variable sites respectively, of which 321 and 254 were parsimoniously informative sites respectively. The MP heuristic search found three most-parsimonious trees of 2299 steps with a consistency index (CI) of 0.5294 and a retention index (RI) of 0.5990. The MP and Bayesian analysis produced similar tree topologies and only the Bayesian tree is presented in Figure 1 along with Bootstrap values (BS) and Bayesian Posterior Probabilities (PP).

        In all phylogenetic trees, the genus *Petrospongium* composed of the type species *P. berkeleyi* and of *P. rugosum* appeared monophyletic and fully supported (Fig. 1; BS = 100% and PP = 1.0). Three different specimens of *Petrospongium berkeleyi* collected from Devon (UK, PB1), Brittany (France, PB2) and the Azores (Portugal, PB3) formed monophyly (BS = 100% and PP = 1.0) and they clustered with the species *Petrospongium rugosum* collected from three different localities in Korea by Cho & Boo (2006). The newly sequenced type species *Corynophlaea crispa* showed a close relationship with the type species *Leathesia difformis* (BS = 100% and PP = 1.0) and clustered within the Chordariaceae (*sensu* Peters & Ramírez, 2001). From these two *rbc*L-*psa*A based trees, it was possible to identify the five families proposed by Peters & Ramírez (2001) within the Ectocarpales (*sensu* Rousseau & Reviers, 1999): the Ectocarpaceae family *sensu* Siemer *et al.* (1998); the Scytosiphonaceae family (BS = 100% and PP = 1.0) *sensu* Kogame *et al.* (1999); the Adenocystaceae family (Rousseau *et al.* 2000); the Acinetosporaceae family *sensu* Peters & Ramírez (2001); and the Chordariaceae family (BS = 100% and PP = 1.0) *sensu* Peters & Ramírez (2001); plus one additional independent clade corresponding to both representative of the genus *Petrospongium*.

DISCUSSION

        The DNA sequences of the *rbc*L and *psa*A chloroplastic genes were determined in the present study for the specimens *Petrospongium berkeleyi* and *Corynophlaea crispa*. Inclusion of the two representatives of the genus *Petrospongium*, the type species *P. berkeleyi* and *P. rugosum* in a molecular phylogenetic analysis on some members of the order Ectocarpales confirms the preliminary results of Cho & Boo (2006). The genus *Petrospongium* is monophyletic and forms an independent clade, unrelated to the genera *Corynophlaea* and *Leathesia*, which cluster together within the Chordariaceae *sensu* Peters & Ramírez (2001).

        We propose a new family, Petrospongiaceae, within the order Ectocarpales (*sensu* Rousseau & Reviers, 1999) to include the two taxa *Petrospongium berkeleyi* and *P. rugosum*. The similarities shared between species of the Petrospongiaceae, Corynophlaeaceae, and Leathesiaceae (*i.e.* a differentiation into a medulla and cortex) are the result of convergent evolution,
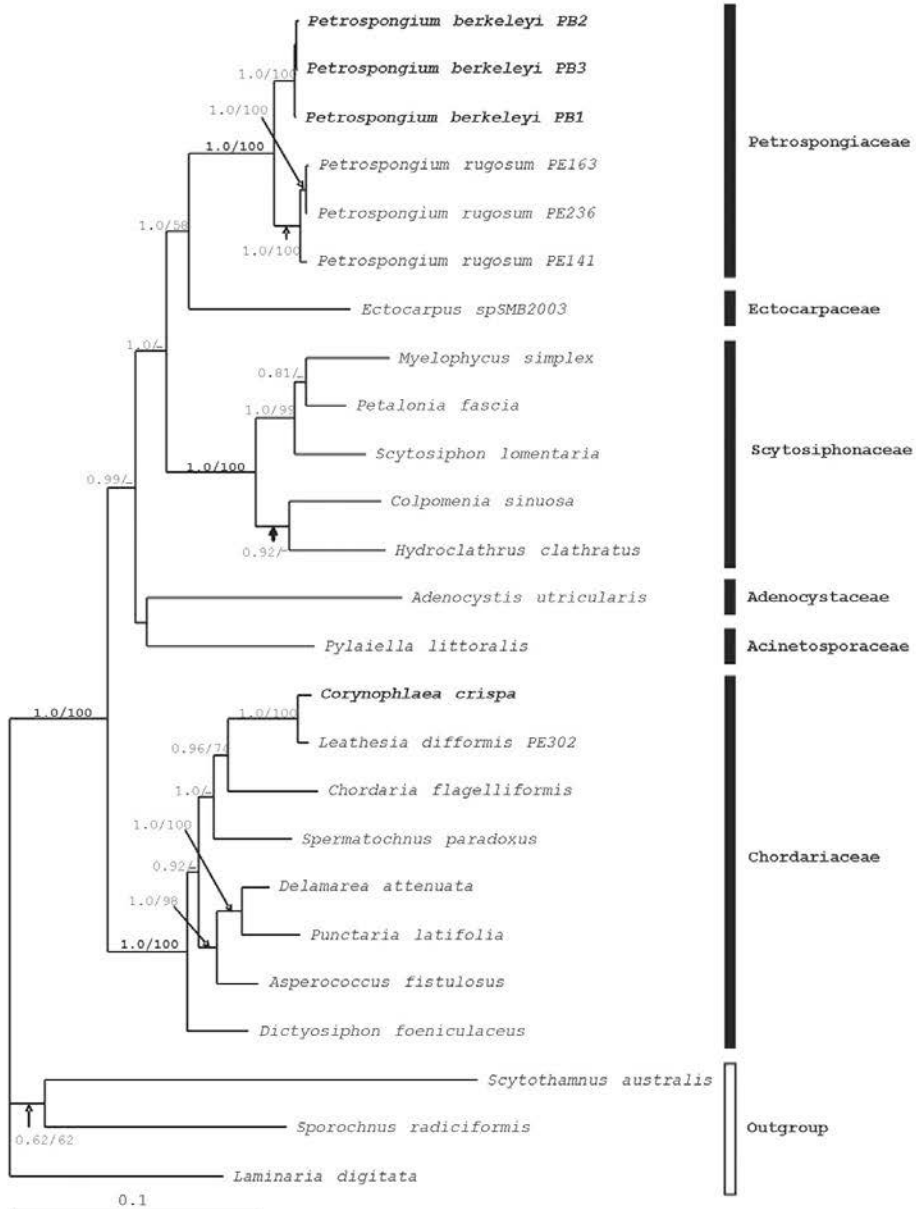
Fig. 1. Bayesian tree for 22 ectocarpalean taxa estimated from combined *rbc*L+*psa*A sequence data. Newly sequenced species are represented as boldface. Numbers above the branches indicate Bayesian PP (left) and MP bootstrap proportions (right) (values ≤ 0.5/50% shown only).

and their analogous features are homoplasic. The genera *Microcoryne* and *Cylindrocarpus* have never been sequenced and consequently it remains difficult to decide on their potential close relationships with the other members of the Corynophlaeaceae, the Petrospongiaceae or the Ectocarpaceae. Fletcher (1987) described the unlikely relationship between the taxa *P.berkeleyi* and *C. microscopicus* from three main features: *C. microscopicus* grows endophytic filaments which then emerge as erect, microscopic, tufts of branched filaments rather than gelatinous cushions, the erect filaments are not closely packed into a pseudoparenchyma and they do not differentiate into a very obvious medulla and cortex-like region. These features seem more characteristic of ectocarpacean taxa, and this link was proposed by Setchell & Gardner (1925) and Newton (1931). Such a suggestion is very interesting as in the present study the Petrospongiaceae and the Ectocarpaceae appeared as sister families, supported by Bayesian posterior probabilities of 0.99 and Bootstrap values of 58%. However, we disagree with the proposal of Cho & Boo (2006) that the species included in these two families look similar, as they have very few common characters, e.g. *Ectocarpus*/ *Kuckuckia* species are branched filamentous, whilst *Petrospongium* is pseudoparenchymatous.

More specifically, Draisma *et al.* (2003) have proposed two molecular and five non-molecular characters to distinguish orders and families of brown algae: the 11 nucleotides at the end sequence of the Rubisco spacer and the start codon of the *rbc*S, life history, type of plastid, absence/presence and number of pyrenoids, structure of the most complex generation, and pheromones. Except for the kind of pheromones (for which no information is available for the genus *Petrospongium*), all characters have been reported by Cho & Boo (2006) for *P. rugosum*, and are in accordance with *P. berkeleyi* for most of them. Thus, in members of the Petrospongiaceae, the sequence of the end of the Rubisco spacer is TT TGA ATA GTG and the start codon of the *rbc*S is ATG (Rubisco spacer sequences available on GenBank for *P. berkeleyi* - EU850276 and *P. rugosum* – EU807980). They possess one plate-like or 1-2 discoid plastids per cell, with exserted pedunculated pyrenoids. The most complex generations display haplostichous thalli. The life history of *P. rugosum* was described as being heteromorphic with an alternation between a microscopic, filamentous gametophyte and a macroscopic, gelatinous sporophyte (Tatewaki *in* Hori, 1993), on which only unilocular sporangia have been observed. In *P. berkeleyi*, an isomorphic life history was reported by Caram (1957), who proposed that meiotic divisions occurred within the unilocular sporangia, releasing spores that fused to form an identical diploid plant. She also observed a "direct life history" with the spores from the unilocular sporangia directly germinating into an identical parental phase. However, she never obtained any plurilocular sporangia during the culture of this species. This feature is usually always recognised for *P. berkeleyi*, except more than a century ago Hanna (1899) observed and illustrated plurilocular sporangia from specimens collected in Ireland. The possibility of endophytic contamination was suggested by Hanna and eliminated according to her observations, and this record remains very intriguing. Indeed, either the plurilocular sporangia are extremely rare in *P. berkeleyi,* and perhaps produced only under certain environmental conditions, and no one, so far, has had a second chance to observe them, or they have become extinct. It is possible also that Hanna (1899) misidentified the material with a species of *Corynophlaea* or *Leathesia*, which both bear plurilocular sporangia, and this reinforces the proposal of Caram (1957). More culture studies need to be undertaken on this species to confirm the complete life cycle of *Petrospongium berkeleyi*.

Petrospongiaceae *fam. nov.*

Thallus epiphytic or epilithic, forming a hemispherical, spongy, cushion-like plant, with broad radiating lobes; comprising a prostrate system of radially spreading branched filaments which give rise above to an extensive medullary region of erect, di-trichotomously branched filaments of large, elongate-cylindrical to globular cells, giving rise below (if epiphytic) to downwardly growing rhizoidal filaments, and above to a determinate cortical region of filaments comprising smaller, more barrel-shaped cells, each containing one plate-like or 1-2 discoid phaeoplasts, each with an exserted pedunculated pyrenoid. Hairs arising from the cortical cells, with basal meristem and sheath. Unilocular sporangia common, laterally attached at the base of the terminal cortical filaments. The family Petrospongiaceae is represented by a single genus, *Petrospongium.*

Petrospongiaceae *fam. nov.*

Thallus epiphytus vel in rupibus destinatus, qui parvos in hemispherii formam pulvinos atque spongiae firmitate confingit. Thallus summam fert filorum quae serpunt diffunduntque, patentque in modo radii; quae fila gignunt, in regione inferiore, rhizoidea, cum epiphytus est, superiore autem in regione, medullam erectis atque in ramorum formam diffusis, dichotonis vel trichotomis, quae componuntur magnis et proceris cellulis sive cylindratis sive globulosis. Qua super medulla, corticalis minorum filorum regio versatur, compositorum cellulis quae dolii figuram praebent, e quibus quaeque complectitur aut pheoplastum ferculi figuram praebens, aut unum vel duo pheoplasta orbis figuram praebentia, cum pyrenoide cuique exserta pedunculataque. Corticales cellulae filos emittunt vagina meristemateque basalibus instructos. Multa sporangia unilocularia in latere posita, ab infimis filiis corticalibus basa sunt.

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