African Journal of Marine Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tams20

# Morphological and Molecular Evidences Within Osmundea (Ceramiales, Rhodophyta) from the Canary Islands, Eastern Atlantic Ocean

M Machín-Sánchez [a] , J Díaz-Larrea [b] , MT Fujii [c] , A Sentíes [b] , V Cassano [d] & MC Gil-Rodríguez [a]

[a] Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, 38071, La Laguna, Tenerife, Islas Canarias, Spain

[b] Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa, AP 55-535, México, DF, 09340, México

[c] Instituto de Botânica, Avenida Miguel Estéfano, 3687, 04301-012, São Paulo, Brazil

[d] Departamento de Botânica, Universidade de São Paulo, Rua do Matão 277, São Paulo, 05508-900, Brazil

**To cite this article:** M Machín-Sánchez, J Díaz-Larrea, MT Fujii, A Sentíes, V Cassano & MC Gil-Rodríguez (2012): Morphological and Molecular Evidences Within Osmundea (Ceramiales, Rhodophyta) from the Canary Islands, Eastern Atlantic Ocean, African Journal of Marine Science, 34:1, 27-42

**To link to this article:** http://dx.doi.org/10.2989/1814232X.2012.673269

African Journal of Marine Science 2012, 34(1): 27–42
Printed in South Africa — All rights reserved

AFRICAN JOURNAL OF
MARINE SCIENCE
ISSN 1814-232X EISSN 1814-2338
http://dx.doi.org/10.2989/1814232X.2012.673269

# Morphological and molecular evidences within *Osmundea* (Ceramiales, Rhodophyta) from the Canary Islands, eastern Atlantic Ocean

**M Machín-Sánchez[1]*, J Díaz-Larrea[2], MT Fujii[3], A Sentíes[2], V Cassano[4] and MC Gil-Rodríguez[1]**

[1] *Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, 38071 La Laguna, Tenerife, Islas Canarias, Spain*
[2] *Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa, AP 55-535, México, DF 09340, México*
[3] *Instituto de Botânica, Avenida Miguel Estéfano, 3687, 04301-012 São Paulo, Brazil*
[4] *Departamento de Botânica, Universidade de São Paulo, Rua do Matão 277, São Paulo 05508-900, Brazil*
** Corresponding author, e-mail: mmachin@ull.es*

**The genus *Osmundea* is a strongly supported monophyletic group within the *Laurencia* complex and shows a disjunct distribution occurring in the North-East and South-West Pacific, the Indian and Atlantic oceans and the Mediterranean Sea. Its phenotypic plasticity on the Canary Islands may be the result of the high ecological variability partially due to the particular oceanographic characteristics in this region. The combination of molecular analyses based on the comparison of the chloroplast-encoded *rbc*L sequences and morphological data allowed us to delimit three distinct taxa from the coasts of the Canarian Archipelago: *Osmundea pinnatifida*, *Osmundea truncata* and an unidentified species, *Osmundea* sp. Moreover, the high value of genetic divergence between *Osmundea* sp. and the rest of the *Osmundea* species suggests that this taxon should be assigned to a new species within the *Osmundea* genus. Occurrence of *O. hybrida* and *O. oederi* (synonym: *O. ramosissima*) has not been confirmed. Our results also suggest a possibly questionable record of the taxa *O. hybrida* and *O. oederi* on the Canary Islands.**

**Keywords**: *Laurencia* complex, phylogeny, *rbc*L, taxonomy

## Introduction

The genus *Osmundea* Stackhouse, resurrected by Nam et al. (1994), is segregated from the other four genera of the *Laurencia* complex [*Laurencia sensu stricto* J.V. Lamouroux, *Chondrophycus* (Tokida and Saito) Garbary and Harper, *Palisada* (Yamada) KW Nam, and *Yuzurua* (KW Nam) Martin-Lescanne] by a set of vegetative and reproductive characters. Some of these characters are unique to the genus *Osmundea*, not being shared with any other genera of the complex, such as the tetrasporangial production from random cortical cells and filament-type spermatangial development (Nam et al. 1994). *Osmundea* is further distinguished from *Laurencia s.s.* by presenting two pericentral cells in each vegetative axial segment, whereas *Laurencia s.s.* possesses four pericentral cells per axial segment (Nam et al. 1994, Garbary and Harper 1998, Nam 1999).

*Osmundea* has been considered the only genus of the *Laurencia* complex that has a disjunct distribution (McIvor et al. 2002). Previous phylogenetic studies based on *rbc*L gene sequencing showed a clear divergence between the *Spectabilis* group from the North-East Pacific formed by *O. blinksii* (Hollenberg and I.A. Abbott) Nam, *O. sinicola* (Setchell and Garnder) Nam, *O. spectabilis* and *O. splendens*, and the other one from the North Atlantic and the Mediterranean coasts formed by *O. hybrida* (A.P. de Candolle) Nam, *O. osmunda* (S.G. Gmelin) Nam and Maggs, the type species of the genus, *O. pinnatifida* (Hudson) Stackhouse, *O. oederi* (Gunnerus) G. Furnari [as *O. ramosissima* (Oeder) Athanasiadis] and *O. truncata* (Kützing) Nam and Maggs (McIvor et al. 2002, Abe et al. 2006, Fujii et al. 2006, Martin-Lescanne et al. 2010).

Four species of the genus *Osmundea* have been described for the Canarian Archipelago: *O. hybrida, O. oederi, O. pinnatifida* and *O. truncata* (Gil-Rodríguez et al. 2003). Nonetheless, due its phenotypic plasticity, the occurrence of the genus *Osmundea* in the Canary Islands has been most likely overestimated.

To date, the combination of molecular techniques based on the comparison of the chloroplast-encoded *rbc*L sequences with morphological studies have permitted to better delimit the *Laurencia* complex, deduce phylogenetic relationships and validate the nomenclature adopted for distinct species (Nam et al. 2000, McIvor et al. 2002, Abe et al. 2006, Fujii et al. 2006, Díaz-Larrea et al. 2007, Lewis et al. 2008, Cassano et al. 2009, Gil-Rodríguez et al. 2009, 2010, Martin-Lescanne et al. 2010, Sentíes et al. 2011).

For the purpose of completing the taxonomic and phylogenetic outlook on the genus *Osmundea* on the Canary Islands, the present study had the following three objectives: (1) identify the actual representation of the genus *Osmundea* on the Canaries; (2) assess its distribution; and (3) define the morphological and phylogenetic characteristics of its members (inferred from their *rbc*L sequences).

## Material and methods

Between 2007 and 2011, samples of *Osmundea* were collected from 12 intertidal locations on the Islands of La Palma (La Fajana de Barlovento 28R 0227347/3193712, Las Salinas-Cancajos 28R 0230568/3171968, Bco. de La Herradura 28R 0229635/3191032), La Gomera (Charco del Conde 28R 0270286/3108917), Tenerife (Punta del Hidalgo 28R 0369850/3161577, Puerto de la Cruz 28R 0348346/3144602, La Barranquera 28R 0363281/3157675, Playa Paraíso 28R 0325227/3111777), Gran Canaria (Quintanilla 28R 0446622/3113614, Punta de Gáldar 28R 0437424/3116437), and Fuerteventura (Corralejo 28R 0607498/3181158, El Viejo Rey-La Pared 28R 0575584/3120754) (Canary Islands, Spain) (Figure 1).

### *Morphological observations*

Anatomical studies were carried out on both fresh specimens and plants were fixed in 4% formalin seawater. A minimum of 20 specimens of *O. truncata* and *O. pinnatifida*, and five specimens of *Osmundea* sp. were studied. Sections for microscopic observations were made by hand using a stainless steel razor blade under a Leica MZ 12.5 stereoscopic dissection microscope (Wetzlar, Germany) and stained with 0.5% aqueous aniline blue solution acidified with 1 N HCl (Tsuda and Abbott 1985). Photomicrographs were taken with a Leica DFC290 digital camera coupled to a Leica DM 2000 microscope (Wetzlar, Germany). Voucher specimens were deposited in the herbarium TFC (University of La Laguna). For the purpose of comparison, additional specimens from the Canary Islands provided by the Netherlands Centre for Biodiversity Naturalis (section NHN) (L) and the herbarium of the University of Las Palmas de Gran Canaria (BCM) were examined. Herbarium abbreviations follow the online Index Herbariorum (Thiers [continuously updated]). Specimens examined are listed in the Appendix.

### *DNA analyses*

DNA and phylogenetic analyses were carried out using the methodologies described in Gil-Rodríguez et al. (2009). Samples used for molecular analyses were dried in silica gel. Specimens including their GenBank access numbers are shown in Table 1. Total DNA was extracted by using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, California) according to the manufacturer's instructions. A total of 1 467 base pairs of the *rbc*L gene was amplified in three fragments with the primer pairs FrbcLstart-R753, F577-R1150 and F753-RrbcS (Freshwater and Rueness 1994) by using the Taq PCR Core Kit (Quiagen). All PCR products were analysed by electrophoresis in 1% agarose to confirm product size. The PCR products were purified with the Qiagen QIAquick Purification Kit according to the manufacturer's instructions. Cycle sequencing was carried out using the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, Princeton, New Jersey) on an 'ABI PRISM 3100 Genetic Analyzer' (Applied Biosystems). The primers used for PCR amplification were also used for the sequencing reactions. Sequences were analysed with the Sequence Navigator software (Applied Biosystems).

### *Phylogenetic analyses*

Phylogenetic relationships were inferred with PAUP* version 4.0b10 (Swofford 2002) and MrBayes version 3.0 beta 4 (Huelsenbeck and Ronquist 2001). Maximum parsimony (MP) trees were constructed by using the heuristic search option, tree-bisection-reconnection (TBR) branch swapping, unordered and unweighted characters. Branch length was optimised by using delayed transformation (DELTRAN), which favours parallelisms over reversals. Support values for the relationships discovered in this analysis were calculated by performing bootstrap (Felsenstein 1985) analysis, as implemented in PAUP*. Ten thousand heuristic search replicates were executed by using the TBR branch-swapping algorithm. The model used in the Bayesian analysis was the general time-reversible model of nucleotide substitution with invariant sites and gamma-distributed rates for the variable sites (GTR+I+G). This model was selected based on maximum likelihood (ML) ratio test implemented by the software Modeltest version 3.06 (Posada and Crandall 1998) with a significance level of 0.01. For Bayesian analysis, we ran four chains of the Markov chain Monte Carlo, sampling one tree every one thousand generations for three million generations starting with a random tree. A 50% consensus tree (majority rule as implemented by PAUP*) was computed after the 'burn in' point. The range of *rbc*L divergence values within and among species was calculated using uncorrected '*p*' distances resulting from PAUP*.

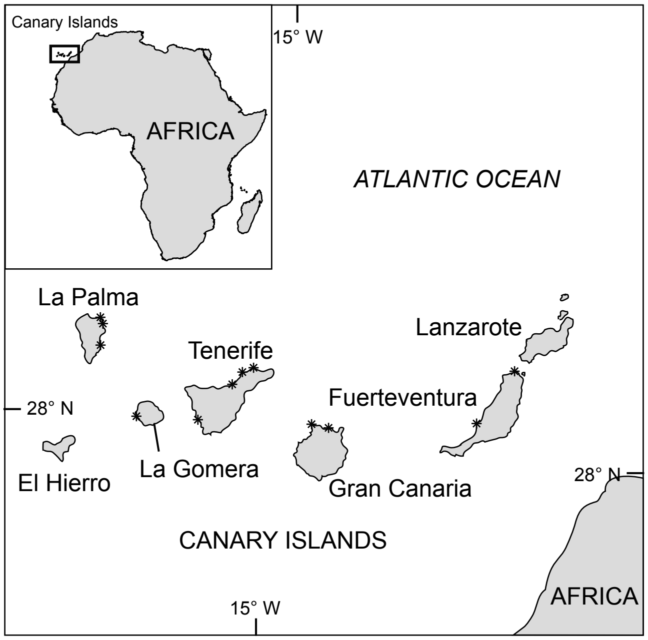

**Figure 1:** Map of the Canarian Archipelago, showing the sampling sites where the genus *Osmundea* was found, 2007–2011

## Results

### *Morphological analyses*

***Osmundea truncata*** (Kützing) Nam et Maggs (Nam et al. 1994:393).

Basionym: *Laurencia truncata* Kützing (1865:19, pl.51c).

Holotype: Pius Titius; L 941.99.271 (Maggs and Hommersand 1993).

Type locality: Pirano, Croatia (Maggs and Hommersand 1993).

**Table 1:** List of species used for phylogenetic analyses, with their collection data and GenBank accession numbers

| Species | Collection data/reference | GenBank accession number |
|---|---|---|
| *Centroceras* sp. [as *C. clavatum* (C. Agardh) Montagne] | USA, Texas, Port Aransas, Redfish Bay, 18 May 1998 (Lin et al. 2001) | AF259490 |
| *Ceramium brevizonatum* H. E. Petersen | Mexico, Campeche Bay, laguna de Yulcapeten, 12 Feb. 1998 (Lin et al. 2001) | AF259491 |
| *Chondrophycus* cf. *undulatus* | New Caledonia, Loyalty, Marè, 22 March 2005 (Martin-Lescane et al. 2010) | FJ785307 |
| *Chondrophycus* cf. *undulatus* | New Caledonia, Loyalty, Marè, 22 March 2005 (Martin-Lescane et al. 2010) | FJ785308 |
| *Chondrophycus* sp.1 | New Caledonia, Loyalty, Lifou, 26 March 2005 (Martin-Lescane et al. 2010) | FJ785309 |
| *Chondrophycus* sp.2 | New Caledonia, Loyalty, Marè, 21 March 2005 (Martin-Lescane et al. 2010) | FJ785310 |
| *Chondrophycus* sp.3 | New Caledonia, Loyalty, Beautemps/Beaupré, 6 April 2005 (Martin-Lescane et al. 2010) | FJ785311 |
| *Laurencia caduciramulosa* Masuda et Kawaguchi | Spain, Canary Islands, Tenerife, Punta del Hidalgo,6 May 2008 (this study) | JF781525 |
| *Laurencia dendroidea* J. Agardh | Brazil, Rio de Janeiro, Areias Negras, Rio das Ostras, 16 December 2009 (Unpublished) | GU330237 |
| *Laurencia intricata* Lamouroux | USA, Florida, Long Key, Channel 5, 10 Dec ember 1998 (Fujii et al. 2006) | AY588410 |
| *Laurencia obtusa* (Hudson) Lamouroux | Ireland, County Donegal, Fanad Head, 6 July 1998 (Nam et al. 2000) | AF281881 |
| *Laurencia viridis* Gil-Rodríguez et Haroun | Spain, Canary Islands, Tenerife, Punta del Hidalgo, Roca Negra, 6 October 2005 (Gil-Rodríguez et al. 2009) | EF685999 |
| *Osmundea blinksii* (Hollenberg et Abbott) Nam | USA, California, San Mateo Co., Año Nuevo, Greyhound Rock, 17 July 1996 (McIvor et al. 2002) | AY172575 |
| *Osmundea hybrida* (A.P. de Candolle) Nam | Ireland, County Donegal, Fanad Head, 7 November 1999 (Nam et al. 2000) | AF281878 |
| *Osmundea oederi* (Gunnerus) G. Furnari [as *O. ramosissima* (Oeder) Athanasiadis] | Ireland, County Donegal, St Jhon's Point, 12 October 1999 (Nam et al. 2000) | AF281880 |
| *Osmundea osmunda* (S.G. Gmelin) Nam et Maggs | France, Brittany, Roscoff, 5 December 2002 (Martin-Lescane et al. 2010) | FJ785318 |
| *Osmundea osmunda* | Ireland, County Donegal, St. John's Point (McIvor et al. 2002) | AF281877 |
| *Osmundea pinnatifida* (Hudson) Stackhouse | Ireland, County Donegal, St. John's Point (McIvor et al. 2002) | AF281875 |
| *Osmundea pinnatifida* | France, Brittany, Penmarch (Lin et al. 2001) | AF259495 |
| *Osmundea pinnatifida* | Spain, Canary Islands, Tenerife, San Telmo, Puerto de la Cruz, 7 October 2005 (Cassano et al. 2009) | EF686005 |
| *Osmundea pinnatifida* | Spain, Asturias, Vidiago, 2 March 2010 (this study) | JF781518 |
| *Osmundea pinnatifida* | Spain, Asturias, Oleiros, 3 March 2010 (this study) | JF781516 |
| *Osmundea pinnatifida* | Spain, Canary Islands, Gran Canaria, Quintanilla, Arucas, 28 February 2010 (this study) | JF781514 |
| *Osmundea sinicola* (Setchell et Gardner) Nam | USA, California, Orange Co., Crescent Beach, 28 May 2002 (Fujii et al. 2006) | AY588407 |
| *Osmundea spectabilis* var. *spectabilis* (Postell et Ruprecht) Nam | Mexico, Baja California, Punta Santo Thomas (McIvor et al. 2002) | AY172574 |
| *Osmundea splendens* (Hollenberg) Nam | Mexico, Baja California, Bahia Colnett, Drift, 2 July 1996 (McIvor et al. 2002) | AY172576 |
| *Osmundea truncata* (Kützing) Nam et Maggs | Ireland, Lough Hyne, C. Cork (McIvor et al. 2002) | AF281879 |
| *Osmundea truncata* | Spain, Canary Islands, Tenerife, Barranquera, 5 May 2008 (this study) | JF781523 |
| *Osmundea truncata* | Spain, Canary Islands, Tenerife, San Telmo, Puerto de la Cruz, 3 May 2008 (this study) | JF781524 |
| *Osmundea truncata* | Spain, Canary Islands, La Palma, La Fajana de Barlovento, 24 January 2008 (this study) | JF781517 |
| *Osmundea truncata* | Spain, Canary Islands, La Palma, Las Salinas, Los Cancajos, 25 September 2008 (this study) | JF781521 |
| *Osmundea truncata* | Spain, Canary Islands, La Palma, La Fajana de Barlovento, 24 January 2008 (this study) | JF781522 |
| *Osmundea truncata* | Spain, Canary Islands, Tenerife, Playa Paraíso, 7 May 2008 (this study) | JF781515 |

**Table 1** (cont.)

| Species | Collection data/reference | GenBank accession number |
|---|---|---|
| *Osmundea truncata* | Spain, Canary Islands, Fuerteventura, Veril Blanco, Corralejo, 26 June 2009 (this study) | JF781520 |
| *Osmundea truncata* | Spain, Canary Islands, La Gomera, Charco del Conde, Valle Gran Rey, 21 February 2011 (this study) | JF781526 |
| *Palisada corallopsis* (Montagne) Sentíes, Fujii et Díaz | Mexico, Quintana Roo, Puerto Morelos, Punta Brava, 18 April 2004 (Díaz-Larrea et al. 2007) | EF061646 |
| *Palisada* cf. c*ruciata* | New Caledonia, Ile des Pins, 4 Decamber 2005 (Martin-Lescane et al. 2010) | FJ785319 |
| *Palisada flagellifera* (J. Agardh) Nam | Spain, Canary Islands, Tenerife, Playa Paraíso, 16 July 2006 (Gil-Rodríguez et al. 2010) | EF685998 |
| *Palisada perforata* (Bory) Nam | Spain, Canary Islands, Tenerife, San Telmo, 14 July 2006 (Cassano et al. 2009) | EU256329 |
| *Palisada* cf. *robusta* | New Caledonia, Lifou, 23 March 2005 (Martin-Lescane et al. 2010) | FJ785321 |
| *Yuzurua poiteaui* (J.V. Lamouroux) Martin-Lescanne var. *gemmifera* (Harvey) M.J. Wynne [as *Palisada poiteaui* (J.V. Lamouroux) K.W. Nam var. *gemmifera* (Harvey) Sentíes, Fujii et Díaz] | Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, 16 April 2004 (Díaz-Larrea et al. 2007) | EF061648 |
| *Yuzurua poiteaui* | Mexico, Quintana Roo, Playa del Carmen, 17 April 2004 (Díaz-Larrea et al. 2007) | EF061649 |
| *Yuzurua poiteaui* | Cuba, La Habana, Rincon de Guanabo 29 July 2005 (Díaz-Larrea et al. 2007) | EF061650 |
| *Yuzurua poiteaui* [as *Palisada poiteaui* (Lamouroux) Nam var. *poitaui*] | USA, Florida, Long Key, Ocean Side (Díaz-Larrea et al. 2007) | EF061652 |
| *Yuzurua poiteaui* | Mexico, Quintana Roo, Playa del Carmen, 15 March 2005 (Díaz-Larrea et al. 2007) | EF061653 |
| *Yuzurua poiteaui* (as *Palisada papillosa*-3) | USA, Florida, Content Key, 12 March 1997 (McIvor et al. 2002) | AY172577 |

*Morphology* — Thallus erect, up to 5 cm high, terete in the basal region, slightly or strongly compressed in the middle and apical regions of the axes, ultimate branches cylindrical, truncated at the apices (Figure 2a). Reddish-brown in colour, cartilaginous in texture and main axis in the middle is 25–55 mm long × 0.3–0.5 mm wide. It grows individually or in tufts over rocks, attached by discoid holdfast (Figure 2b). Thallus irregularly ramified with four orders of branches, 0.4–1.5 mm long × 0.2–0.3 mm wide, branches irregularly alternate. Branchlets with sub-dichotomy (Figure 2a). Presence of few, dense hyaline trichoblasts in the apical depressions located in the pit of the branchlets. Alternately arranged buds can be frequently observed on the main axis (Figure 2c).

*Vegetative structures* — In surface view, cortical cells in the middle region of the thalli are polygonal to elongated, 11–24 μm long × 10–17 μm wide, and connected to one another by numerous secondary pit connections (Figure 3a). *Corps en cerise* were absent in living material. Transverse sections of the thallus (Figure 3b) show two cortical layers of heavily pigmented cells. Outer cortical cells are rectangular, 14–19 μm long × 8–14 μm wide, not arranged in palisades. Secondary pit connections are present between adjacent outer cortical cells (Figure 3d). Inner cortical cells are rounded. Medullary cells are 28–47 μm long × 25–53 μm wide. Annular (Figure 3e) and lenticular (Figure 3f) thickenings are present. Discernible axial segments are found in the apical pit of branchlets. Each vegetative axial segment cuts off two periaxial cells, 26–52 μm long × 16–44 μm wide (Figure 3c). In longitudinal sections of the ultimate branches, outer cortical cells walls near apices do not project beyond the surface, but expose secondary pit connections between adjacent cortical cells (Figure 3g).

*Reproductive structures* — Fertile male branches with cup-shaped spermatangial pits, which are located in the apex and/or at the bifurcation of the branchlets (Figure 4a). Spermatangial branches of filament-type. Spermatangial branches are unbranched, 43–85 μm long, and usually terminate in an apical, vesicular, sterile cell of 20–42 × 20–33 μm (Figure 4b). Clusters of 2–3 or even 4–6 apical, vesicular, sterile cells can also be found (Figure 4c). Ovoid spermatangia, 8–12 μm long × 3–6 μm wide, each with a single apical nucleus. Female gametophyte with cystocarps located sub-apical in the fertile branches. Sessile and ovoid cystocarps, 0.6 mm long × 0.7 mm wide, with a protruding ostiole (Figure 4d). Carposporangia are ovoid, 71 μm long × 41 μm wide (Figure 4e). Tetrasporophyte with simple cylindrical branches, 0.8–1.7 mm long × 0.4–0.5 mm wide, with mature tetrasporangia present in the last two orders of branching, arranged parallel to the stichidial axis (Figure 4f). Tetrasporangia are randomly born on cortical cells. The two presporangial cover cells are arranged parallel to the stichidial axis in surface view. The postsporangial cover cell divides and thus contributes to cortex formation around the tetrasporangia (Figure 4g, h, i).

*Distribution and habitat in the Canary Islands* — La Palma, Tenerife, Lanzarote (Haroun et al. 2002), La Gomera, and Fuerteventura as new records (Figure 1); in the borderline of mid-intertidal rock pools, hidden among species of *Cystoseira* C. Agardh. Specimens were also collected from crevices and large boulders in the lower intertidal.

***Osmundea pinnatifida*** (Hudson) Stackhouse (1809:79)
Basionym: *Fucus pinnatifidus* Hudson (1762:473).
Lectotype: No. 405, Herb. Petiver, British Museum.
Nomenclatural synonyms: *Laurencia pinnatifida* (Hudson) J.V. Lamouroux (1813:42), *Gelidium pinnatifidum* (Hudson) Lyngbye (1819:40), *Chondria pinnatifida* (Hudson) C. Agardh (1822:337).
Type locality: Harwich, Essex, England (Maggs and Hommersand 1993)

*Morphology* — Thallus erect, up to 4 cm high, strongly compressed, cartilaginous in texture, brownish-purple colour, main axis 0.3–0.7 mm wide in the middle (Figure 5a). Erect fronds pinnate, attached to substratum by stoloniferous branches and basal crust (Figure 5b). Thallus has three orders of branches, branching irregularly alternate-distichous (Figure 5a). Branches compressed, 0.1–1.5 mm long × 0.3–0.5 mm wide, ultimate branchlets terete. Fresh plants have a strong chemical smell and flavour.

*Vegetative structures* — In surface view, morphology of the outer cortical cells in the middle of the thalli are polygonal, pentagonal or hexagonal, 10–24 µm long × 7–19 µm wide, not connected by secondary pit connections (Figure 5c). *Corps en cerise* were absent in living material. Transverse sections of the thallus show two different layers of cortical cells (Figure 5e). Outer cortical cells are hyaline, rectangular, small, 15–23 µm long × 12–20 µm wide, not arranged in palisades. Secondary pit connections are absent between adjacent outer cortical cells (Figure 5e). Inner cortical cells are pigmented, larger than external cells, 16–25 µm long × 16–18 µm wide. Medullary cells are rounded, large, 26–43 µm long × 27–42 µm wide and with thickened walls. Annular and lenticular thickenings can be present or absent (Figure 5f, g). Axial segments are discernible in the apical pit of the branchlets. Each vegetative axial segment cuts off two pericentral cells, 24–35 µm long × 17–24 µm wide (Figure 5d). In longitudinal sections of the ultimate branches, outer cortical cells walls near apices do not project beyond the surface.

*Reproductive structures* — Fertile male branches with pocket-shaped spermatangial pits, located at the bifurcations of the ultimate branchlets or laterally in series, 0.3–0.4 mm long × 0.1–0.2 mm wide (Figure 6a). Pocket-shaped has a narrow apical pore (Figure 6b). Spermatangial branches of filament-type, terminating in a vesicular, sterile cell, 18 µm × 12 µm. Ovoid spermatangia, 5–11 µm long × 2–5 µm wide, each with a single apical nucleus. Female gametophyte with cystocarps laterally located, mainly in the second-order branches, sessile, slightly ovoid, 0.5 mm long × 0.4 mm wide, with a non-protuberant ostiole (Figure 6c). Tetrasporophyte has simple or composed cylindrical branches, 0.8–0.9 mm long × 0.2–0.4 mm wide. Tetrasporangia are randomly born on cortical cells. In surface view, the two presporangial cover cells are

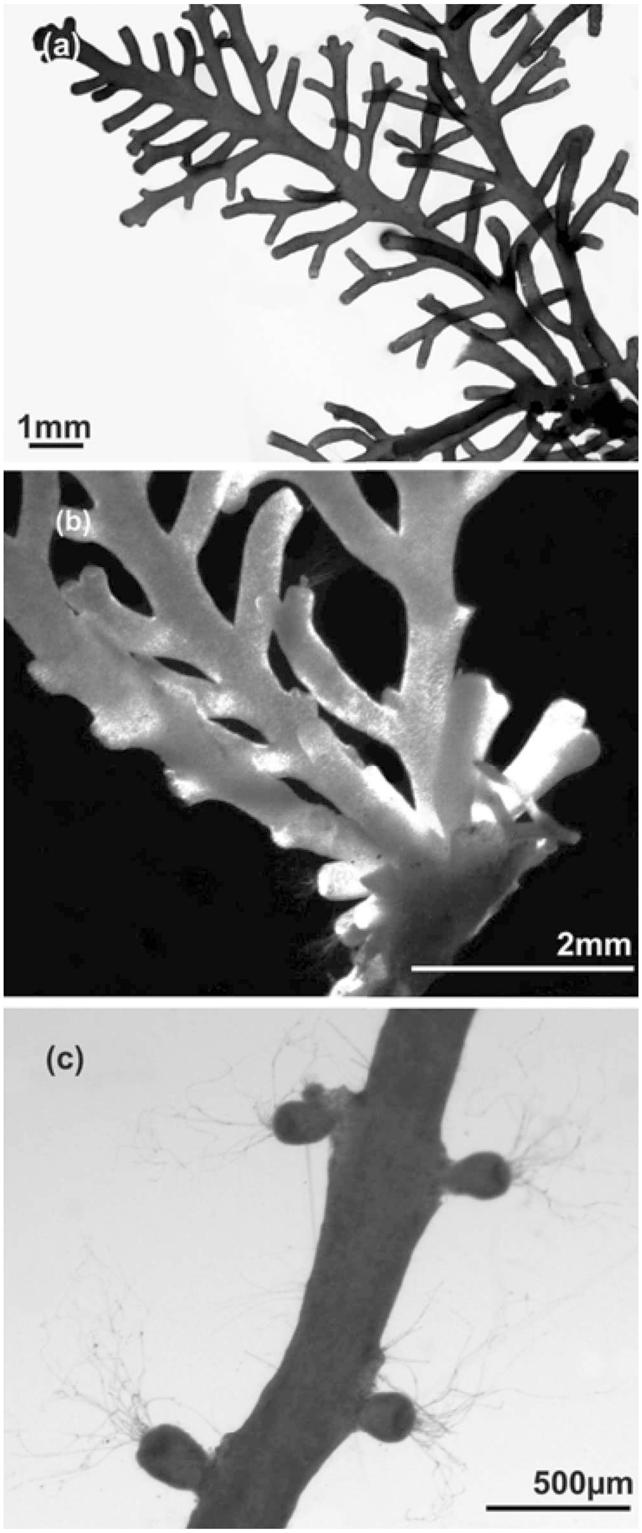

**Figure 2:** *Osmundea truncata* from the Canary Islands: (a) habit of a plant; (b) detail of dicoid holdfast; (c) detail of buds alternately arranged in main axis, new branches originating from damages produced by loss of old branches

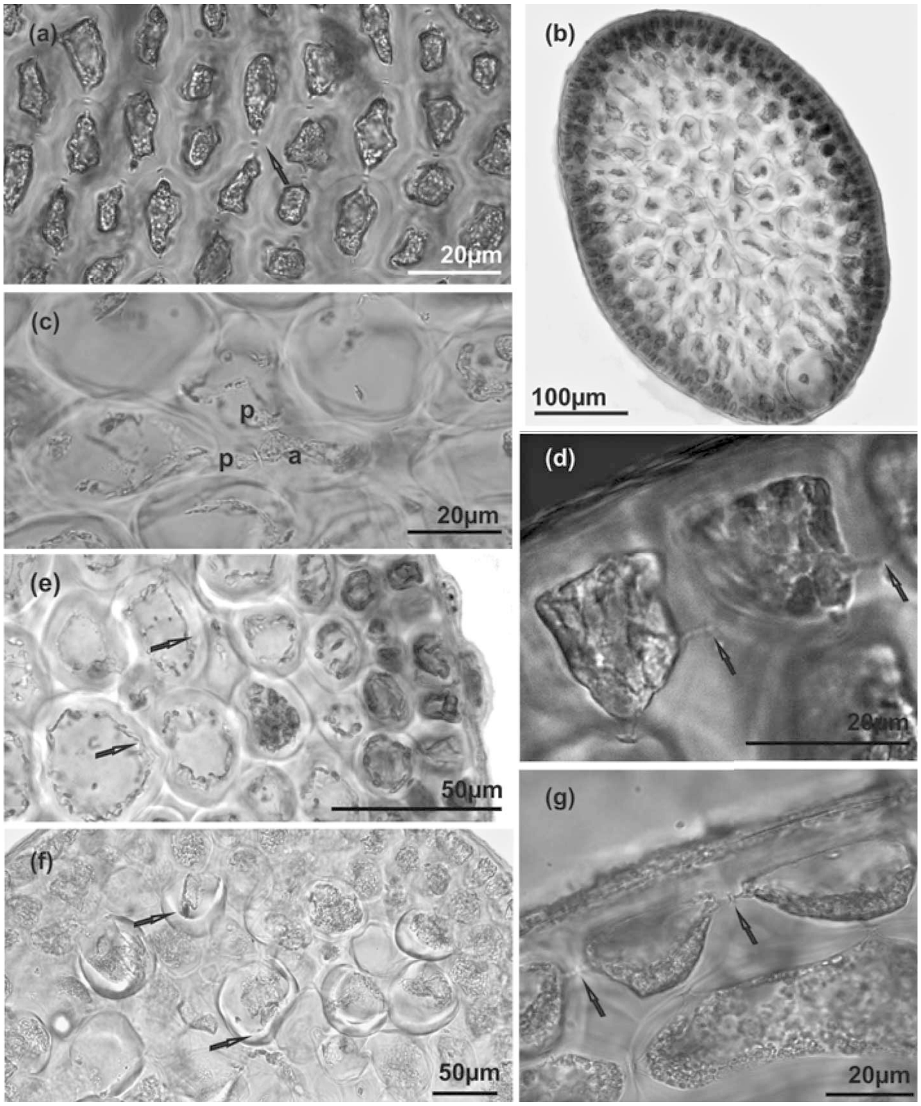

**Figure 3:** Vegetative structures of *Osmundea truncata*: (a) external cortical cells with secondary pit connections (arrow) in surface view; (b–f) transverse sections through the apical region of the main axis — (b) overall view, (c) an axial cell with two pericentral cells, (d) secondary pit connections between outer cortical cells (arrows), (e) evidence of annular thickenings in the medullary cells (arrows), (f) evidence of numerous lenticular thickenings in the medullary cells (arrows); (g) longitudinal section — note secondary pit connections between adjacent cells of outermost cortical layer (arrows)

arranged parallel to the stichidial axis. The postsporangial cover cell divides and thus contributes to cortex formation around the tetrasporangia (Figure 6d, e, f).

*Distribution and habitat in the Canary Islands* — El Hierro, La Palma, La Gomera, Tenerife, Gran Canaria, Fuerteventura, and Lanzarote (Haroun et al. 2002) (Figure 1), growing on basaltic substratum in the lower intertidal zone of exposed coasts.

### *Osmundea* sp.

*Morphology* — Thallus erect, up to 3 cm high, main axes cylindrical in the basal and the middle region, slightly compressed in the apical region. Reddish-pink in colour, cartilaginous in texture, main axis in the middle is 0.3–0.8 mm wide. It is growing isolated, not tufted, attached by a discoid holdfast. Branching is irregularly alternate. Axes denuded in lower part and with 1(–2) orders of branches in the upper third of the main axis. Branches 0.2–2 mm long × 0.3–0.6 mm wide, with truncated apices in which dense tufts of hyaline trichoblasts are inserted (Figure 7a).

*Vegetative structures* — In surface view, cortical cells in the middle region of the thalli are polygonal, pentagonal or hexagonal, 11–22 µm long × 7–14 µm wide, with secondary pit connections between adjacent cells (Figure 7e). *Corps en cerise* were absent in living specimens. Transverse sections of the thallus have two layers of pigmented cortical cells (Figure 7b). Outer cortical cells are rectangular, 20 µm long × 14 µm wide, not arranged in palisades. Secondary pit connections are present between adjacent outer cortical cells. Inner cortical cells are rounded and larger than external cells, 19–33 µm long × 16–28 µm wide. Medullary cells are round,

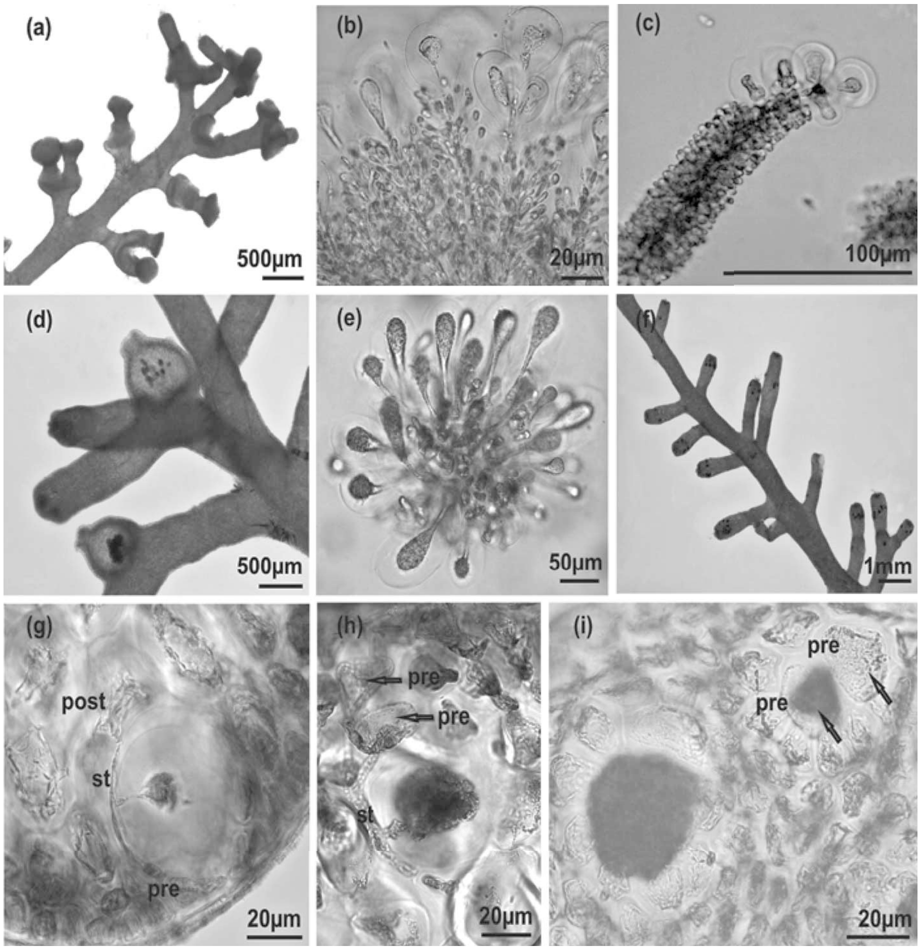

**Figure 4:** Reproductive structures of *Osmundea truncata* from the Canary Islands: (a–c) male gametophyte — (a) cup-shaped spermatangial receptacles located in the apical region of the branches and/or at the bifurcations of the branchlets, (b) spermatangial filaments terminating in by 1–3 sterile, vesicular cells, (c) spermatangial filaments terminating in 4–6 sterile, vesicular cells; (d–e) female gametophyte — (d) sessile cystocarps with protruding ostioles, (e) large clavate carposporangia; (f–i) tetrasporophyte with conspicuous tetrasporangia — (f) tetrasporangial branchlets showing parallel arrangement of tetrasporangia, (g–h) transverse section of a tetrasporangial branchlet — note a post-sporangial cell [post], a stiquidial cell [st] and two presporangial cover cells [pre] arranged parallel (arrows) to the stichidial axis and (i) presporangial cover cells in surface view (arrows)

38–62 µm in diameter, with thick walls, annular thickenings are presents (Figure 7b). Axial segments are discernible in the apical pits of the branchlets. Each vegetative axial segment cuts off two periaxial cells, 43–56 µm long × 39–43 µm wide (Figure 7c).

*Reproductive structures* — Tetrasporophyte with simple cylindrical branchlets, 0.4–0.6 mm long × 0.3–0.5 mm wide, with mature tetrasporangia arranged parallel to the axial axis. Tetrasporangia are randomly born on cortical cells. The two presporangial cover cells are arranged parallel to the stichidial axis in surface view. The postsporangial cover cell divides to contribute to the formation of the cortex around the tetrasporangia. (Figure 7e, f). Male and female gametophytes were not observed.

*Distribution and habitat in the Canary Islands — Osmundea* sp. was collected in La Punta de Gáldar, a very exposed coast north-west of the Island of Gran Canaria (Figure 1), growing epilithically in crevices of basaltic platforms in the lower intertidal zone.

### *Molecular analyses*

*Variation in the* rbc*L sequences* — We sequenced the *rbc*L gene for 13 specimens of the *Laurencia* complex: one specimen of *Laurencia caduciramulosa* Masuda and Kawaguchi, three of *O. pinnatifida*, eight of *O. truncata* and one of *Osmundea* sp. A total of 47 sequences was analysed, including *Ceramium brevizonatum* H.E. Petersen and *Centroceras* sp. [as *C. clavatum* (C. Agardh in Kunth) Montagne in Durieu de Maisonneuve] as outgroups. Intergeneric divergence variations are shown in Table 2.

*Phylogeny* — The dataset consisted of 802 constant characters and 90 parsimony non-informative sites. Maximum parsimony produced two trees of 1 060 steps (CI = 0.8, HI = 0.2). The topology of the majority rule Bayesian tree with bootstrap and posterior probability values is shown in Figure 8. The analysis shows a monophyletic *Laurencia* complex with high bootstrap values in relation to members of the outgroups. The *Laurencia* complex separated into five monophyletic

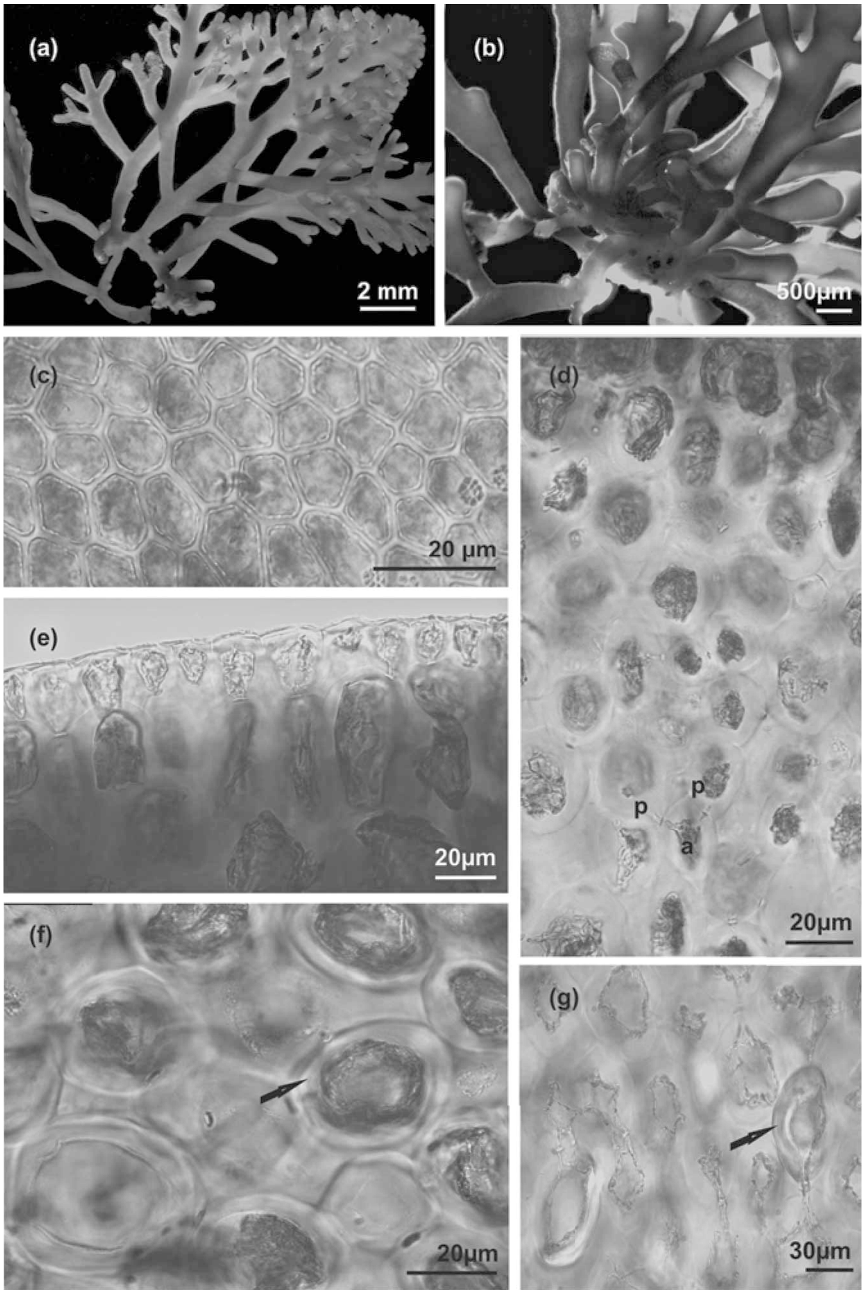

**Figure 5:** Habit and vegetative structures of *Osmundea pinnatifida*: (a) habit of a plant showing stoloniferous branches; (b) basal anchorage crust present in some specimens; (c) surface view of external cortical cells — note absence of secondary pit connections; (d) transverse section of the upper portion of a branch showing an axial cell [a] with two pericentral cells [p]; (e) transverse section of main axis showing hyaline external cortical cells without secondary pit connections; (f) annular thickenings in medullary cells in transverse section of the thallus (arrow); (g) transverse section of main axis showing scarce lenticular thickening in the medullary cells (arrow)

clades with high bootstrap support, corresponding to the genera that form the group: *Chondrophycus*, *Laurencia*, *Osmundea*, *Palisada* and *Yuzurua*. The earliest diverging clade, the genus *Laurencia* included five taxa: *L. viridis*, *L. obtusa*, *L. intricata*, *L. dendroidea* and *L. caduciramulosa.* The monophyletic clade that corresponded to the genus *Palisada* also included five taxa: *P. perforata*, *P. flagellifera*, *P.* cf. *cruciata*, *P.* cf. *robusta* and *P. corallopsis.* The genera *Palisada* and *Yuzurua* turned out to be sister groups forming a clade with high bootstrap support and posterior probability values. The monophyletic clade corresponding to the genus *Yuzurua* included one taxon: *Y. poiteaui.* The *Chondrophycus* clade included four taxa: *C.* cf. *undulatus, C.* sp.1*, C.* sp. 2 and *C.* sp. 3. Finally, the genera *Osmundea* assemblage included two sister groups. The earliest diverging clade included four taxa from the North-East Pacific region: *O. spectabilis* var. *spectabilis, O. blinsksii, O. splendens* and *O. sinicola*. The

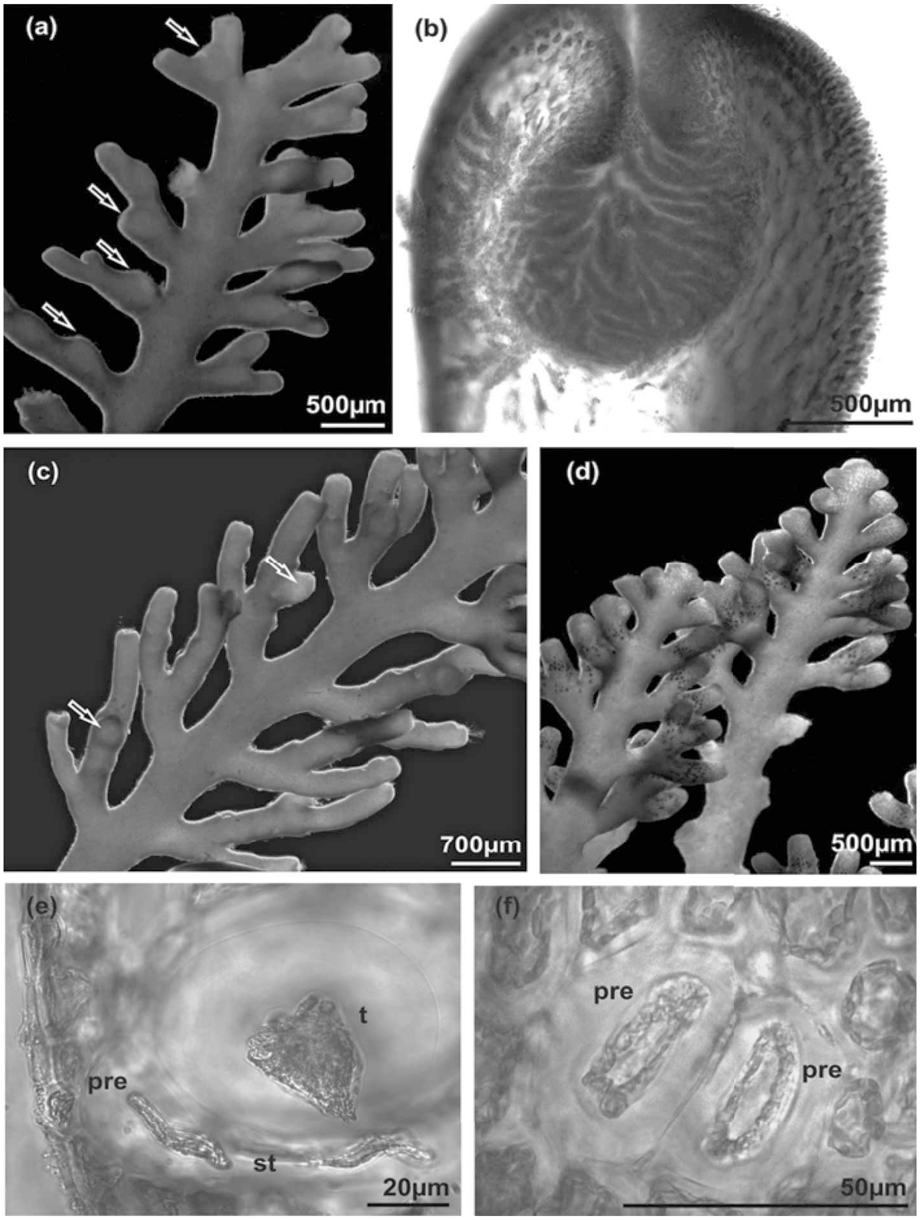

**Figure 6:** Reproductive structures of *Osmundea pinnatifida*: (a–b) male gametophyte — (a) pocket-shaped spermatangial receptacles (arrows) localised at the bifurcations of the ultimate branchlets or laterally in series and (b) longitudinal section of pocket-shaped spermatangial receptacle with narrow pore, containing densely packed spermatangial filaments; (c) female gametophyte with sessile cystocarps lacking protruding ostiole (arrows); (d–f) tetrasporophyte with simple and compound branches with conspicuous tetrasporangia — (d) tetrasporangial branchlets showing parallel arrangement of tetrasporangia, (e) transverse section of a tetrasporangial branchlet showing tetrasporangia [t], stichidial cell [st] and presporangial cover cells [t], (f) surface view showing presporangial cover cells [pre] in parallel arrangement with respect to stichidial cell

other sister clade was resolved into two sister lineages that included several taxa from the North Atlantic coast. One subclade included three taxa: *O. truncata, O.* sp. and *O. oederi;* the other subclade included three taxa: *O. osmunda, O. hybrida* and *O. pinnatifida*.

## Discussion

Similar to the other species of the marine habitat of the Canary Islands, the genus *Osmundea* is characterised by its diversification, high spatial variability, and by constituting generally small populations, both in biomass and in distribution. These features are likely the result of the particular oceanographic characteristics at the meso- as well as macro-scale, which usually occur in the bodies of water surrounding the islands (Haroun et al. 2009). The species referred to in this study populate a great variety of habitats in the intertidal zone, generally located in the north, north-west and north-east of the archipelago, all of them being exposed to strong, high-energy waves. Specimens were

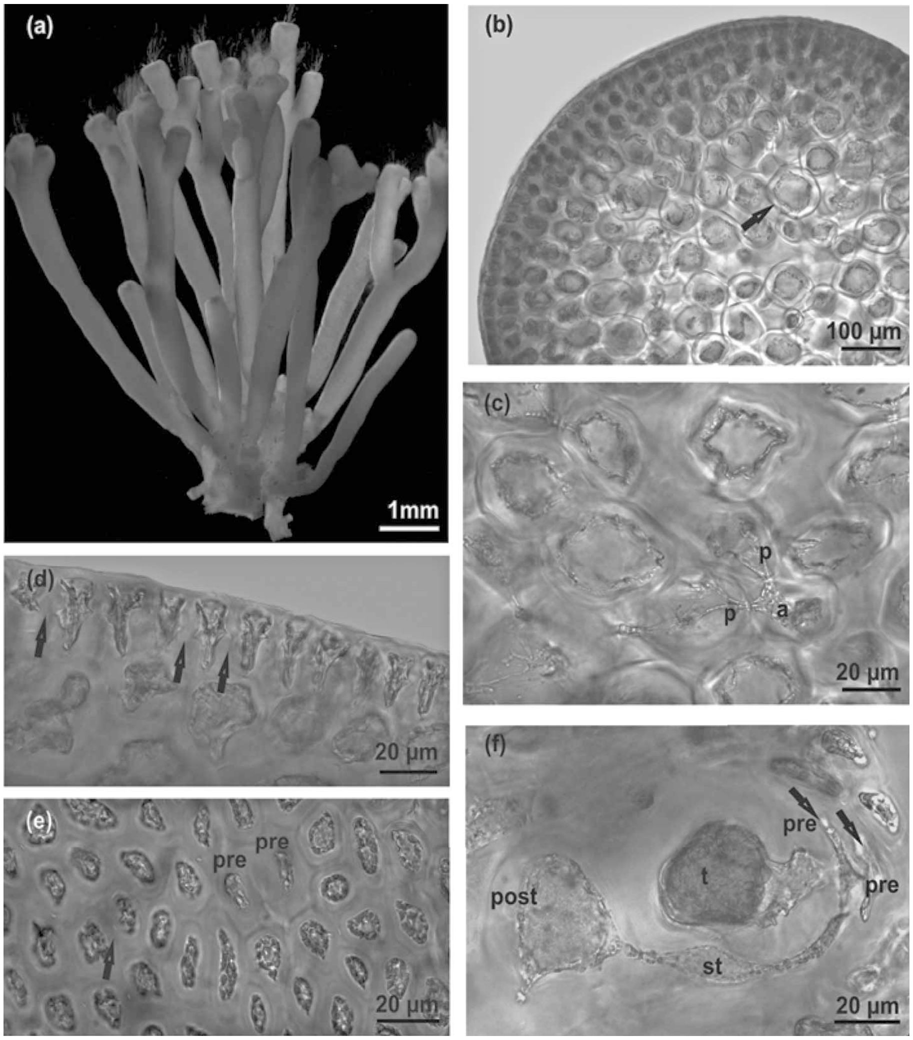

**Figure 7:** Habit, vegetative and reproductive structures of *Osmundea* sp.: (a) habit, showing erect axes borne on discoid holdfast — note 1–2 orders of branches in the upper third of the main axis; (b–c) transverse section of the upper portion of a branch — (b) medullary cells with annular thickenings (arrow), (c) vegetative axial segment with an axial cell [a] and two pericentral cells [p]; (d) longitudinal section of cortex showing two layers of pigmented cortical cells — note outer cortical cells with secondary pit connections (arrows); (e) surface view of tetrasporophyte — detail of cortical cells with secondary pit connections (arrow) and two presporangial cover cells (pre)arranged parallel to the stichidial axis; (f) transverse section of a tetrasporangial branchlet, showing the post-sporangial cell [post], the stichidial cell [st] and the two presporangial cover cells [pre] (arrows)

normally collected in shady zones of intertidal rock pools, basaltic, littoral-platform surfaces, and in deep crevices of rocks located in the lower intertidal zone.

Morphological comparisons among the species of *Osmundea* are shown in Table 3. Collected specimens of *O. truncata* revealed two morphotypes. In the Canary Islands, specimens of *O. truncata* were generally located in pools of the mid-intertidal zone, hidden between thalli of the genus *Cystoseira* C. Agardh; some specimens, however, were found in large crevices of the lower intertidal zone. These samples exhibited only two orders of branches instead of four or five, prevalent in *O. truncata* (Maggs and Hommersand 1993). Also, they exhibited male gametophytes with spermatangial filaments terminating in a cluster of sterile, apical cells and not in a single one, which is commonly observed in *O. truncata* (Nam et al. 2000). The two located morphotypes are most likely the result of the ecological conditions which characterise the habitat.

In the Canary Islands, the occurrence of *O. truncata* had been reported for La Palma, Tenerife and Lanzarote (Haroun et al. 2002). Our morphological findings, however, extended the distribution area of the taxon, including, for the first time, the islands of La Gomera and Fuerteventura. Our results additionally confirmed the occurrence of *O. pinnatifida* in the intertidal zone of particularly exposed rocky coasts and also revealed several differences. The population of *O. pinnatifida* assessed in Puerto de la Cruz (Tenerife) exhibited smaller and less robust thalli than the specimens collected in Quintanilla, Arucas, Island of Gran Canaria. Moreover, the samples from Tenerife typically had very few and sometimes no lenticular thickenings. Again, these differences in morphology could be the result of the habitat they occupied.

**Table 2:** The intergeneric divergence and interspecific divergence values obtained for *rbc*L sequences in this work and values reported by others workers for the *Laurencia* complex

| Taxa | Intergeneric divergence values for *rbc*L sequences (%) | Values reported by other authors (%) |
|---|---|---|
| *Chondrophycus-Laurencia* | 9.0–11.3 | – |
| *Chondrophycus-Osmundea* | 9.2–11.9 | 11.28–12.85 (McIvor et al. 2002) |
| *Chondrophycus-Palisada* | 9.2–11.1 | – |
| *Chondrophycus-Yuzurua* | 10–11.8 | – |
| *Laurencia-Osmundea* | 8.6–12.8 | 10.56–10.59 (McIvor et al. 2002) |
| *Laurencia-Palisada* | 9.1–11.7 | 10–12 [(*Palisada* as *Chondrophycus*) Díaz-Larrea et al. 2007]; 6–10 (Cassano et al. 2009) |
| *Laurencia-Yuzurua* | 7.9–10.8 | 9–11 (Martin-Lescanne et al. 2010) |
| *Osmundea-Palisada* | 9.6–12.8 | 10–13 [(*Palisada* as *Chondrophycus*) Díaz-Larrea et al. 2007]; 9–12 (Cassano et al. 2009) |
| *Osmundea-Yuzurua* | 9.5–13.5 | – |
| *Palisada-Yuzurua* | 9.2–11.2 | 10–11 (Martin-Lescanne et al. 2010) |
| *Chondrophycus* | 3.1–6.9 | – |
| *Laurencia* | 3.3–5.9 | 6–8 (Díaz-Larrea et al. 2007); 3–8 (Cassano et al. 2009) |
| *Osmundea* | 4.0–9.8 | 4.6–8.5 (Nam et al. 2000); 2.04–8.9 (McIvor et al. 2002) |
| *Palisada* | 2.8–5.8 | 6–8 [(*Palisada* as *Chondrophycus*) Díaz-Larrea et al. 2007]; 4–10 (Cassano et al. 2009) |
| *Yuzurua* | 2.3–5.9 | 0.01–0.02 [(*Yuzurua* as *C. gemmiferus* and *C. poiteaui*) Díaz-Larrea et al. 2007]; 0.2 (Martin-Lescanne et al. 2010) |
| *Osmundea* sp.-*O. truncata* | 6.8–7.7 | – |
| *Osmundea* sp.-*O. pinnatifida* | 7.7–9.0 | – |
| *Osmundea* sp.-*O. oederi* | 4.5 | – |

In Tenerife, the population was found on small surfaces, in rocky, abrupt zones, semi-protected from the impact of the strong waves. In contrast, the population of *O. pinnatifida* is widely spread in Gran Canaria, inhabiting the vertical, highly wave-exposed areas of large clumps of rock. The observed morphotypes could represent the result of adaptation to diverse ecological conditions.

Our results indicate a new taxonomic entity for the Canary Islands, *Osmundea* sp. However, taxonomic determination was not possible as all of the collected specimens turned out to be sterile or tetrasporophytes. The fact that no gametophyte was found in the collections restricted identification to morphologic characterisation.

*Osmundea hybrida* and *O. oederi* have been reported for the Canary Islands by Gil-Rodríguez et al. (2003). The record of these two taxa in the islands was based on the before-mentioned citations of *Laurencia hybrida* and *Osmundea ramosissima* (as *L. platycephala*) (Gil-Rodríguez and Haroun 1993).

In the past, there have been many errors in species identification in the genus *Osmundea* on the European coasts, particularly with *O. hybrida*, *O. pinnatifida*, *O. oederi* and *O. truncata,* which has led to taxonomic confusions (Maggs and Hommersand 1993, Nam et al. 1994, 2000).

*Osmundea hybrida* and *O. oederi* were not collected in our study, although the sites of previous records of both species, such as Gran Canaria, Tenerife and La Palma, were sampled. However, specimens of these two taxa previously collected in the Canaries and deposited in the herbaria TFC, BCM and L were examined for comparison. Our analyses revealed that this material had been misidentified. In some cases, the study specimens showed morphological characters of *O. truncata* or *O. pinnatifida*, others exhibited morphological features of the genus *Laurencia.* Thus, all of this material is considered to be incorrectly determined.

The intergeneric divergence values obtained in our work for *rbc*L sequences are comparable to those reported by other workers for the *Laurencia* complex (Table 2). The interspecific divergence values reported here are also comparable with those reported previously for the genus *Osmundea* (Table 2).

Our phylogenetic analyses confirmed the results obtained by Martin-Lescanne et al. (2010), inferring the *Laurencia* complex as a monophyletic group that includes five genera. Bayesian topology (Figure 8) demonstrated that the samples identified as *O. truncata* for seven Canarian localities (Los Cancajos and La Fajana de Barlovento, island of La Palma; Charco del Conde, island of La Gomera; La Barranquera, Puerto de la Cruz and Playa Paraíso, island of Tenerife; Corralejo, island of Fuerteventura) were grouped in the clade that included the *O. truncata* sequence from GenBank (Ireland). Our results confirmed the presence of the taxonomic entity *O. truncata* in the Canarian Archipelago and concur with records obtained by Haroun et al. (2002) who published on the islands of La Palma and Tenerife. Also, the presence of *O. truncata* for La Gomera and Fuerteventura in our study is the first record of this species for these islands.

The sample from the locality of Quintanilla, Gran Canaria, identified as *O. pinnatifida,* was grouped in the same clade as the *O. pinnatifida* sequences from GenBank. Our results are in agreement with Haroun et al. (2002) who recorded *O. pinnatifida* for the island of Gran Canaria based on morphological evidence. The sequences of the species of *O. truncata* and O. *pinnatifida* from the Canary Islands were resolved into two subclades which correspond to the observed morphotypes. This could be the result of speciation

**Table 3:** Comparative morphological data of *Osmundea* spp.

| Species | Vegetative structures | | | | Male structures | | Female structures | Geographic distribution | Source |
|---|---|---|---|---|---|---|---|---|---|
| | Thallus | Type of attachment | Secondary pit connection | Lenticular thickening | Spermatangial pit | No. sterile cells | Mature cystocarps | | |
| *O. blinksii* (Hollenberg et Abbott) Nam | Compressed | Spreading crust | Absent | Absent | Pocket-shaped | … | … | Pacific North America* | Serio et al. (1999), *Yoneshigue-Valentin et al. (2003) |
| *O. crispa* (Hollenberg) Nam | Angular to terete | … | Absent | Absent | Pocket-shaped | Cluster | Ovoid to subconical, slightly constricted at base, with non-protuberant ostiole | Pacific North America | Nam and Choi (1999) |
| *O. hybrida* (A.P. de Candolle) Nam | Terete | Discoid holdfasts | Absent | Absent | Cuplike | Cluster | Remarkably constricted at the base | Atlantic Europe | Nam and Saito (1994) (as *L. hybrida*) |
| *O. lata* (M. Howe & W.R.Taylor) Yoneshigue-Valentin, M.T.Fujii & Gurgel | Compressed | Discoid holdfasts | Present | Absent | Cuplike | Cluster | Subconical with non-constricted bases | Atlantic South America | Yoneshigue-Valentin et al. (2003) |
| *O. maggsiana* Serio, Cormaci et G. Furnari | Terete | Discoid holdfasts | Absent | Absent | Cuplike | 1,2 (3) | Cystocarps unknown | Mediterranean | Serio et al. (1999) |
| *O. multibulba* (Dawson, Neushul et Wildman) Nam | Compressed | … | Absent | Absent | Pocket-shaped | … | … | Pacific North America | Yoneshigue-Valentin et al. (2003) |
| *O. osmunda* (S.G. Gmelin) Nam et Maggs | Compressed | Discoid holdfasts | Absent | Present | Pocket-shaped | Cluster | Ovoid with non-protuberant ostiole | Atlantic Europe | Nam and Choi (2000) |
| *O. pelagiensis* G. Furnari | Compressed | Basal crust | Absent | Absent | Cuplike | … | Sessile and ovoid | Mediterranean | Cormaci et al. (1994) (as *L. pelagiensis*) |
| *O. pelagosae* (Schiffner) Nam | … | Discoid holdfasts | Present | Present | Pocket-shaped | 1* | Ovoid | Mediterranean | Furnari et al. (2001), *Furnari and Serio (1993) (as *L. pelagosa*) |
| *O. pinnatifida* (Hudson) Stackhouse | Compressed | Stolonous | Absent | Absent/ present | Pocket-shaped | Cluster of several | With non-protuberant ostiole | Atlantic Europe | Nam et al. (2000) |
| *O. oederi* (Gunnerus) G. Furnari | Subterete | Discoid holdfasts | Present | Absent | Cuplike | Cluster of several | Ovoid to subconical with a non-protuberant ostiole | Atlantic Europe, Mediterranean* | Nam et al. (2000) (as *O. ramosissima*), *Serio et al. (2008) |
| *O. sinicola* (Setchell et Gardner) Nam | Compressed | … | ? | Present | Pocket-shaped | … | … | Pacific North and Central America* | Yoneshigue-Valentin et al. (2003), *Fernández-García et al. (2011) |
| *Osmundea* sp. | Subterete | Discoid holdfasts | Present | Absent | ? | ? | ? | Canary Islands | This study |
| *O. spectabilis* (Postels et Ruprecht) Nam | … | … | Present | Absent | Pocket-shaped | Several | Ovoid to subconical with a non-protuberant ostiole | Northern South-East Alaska to Baja California, Mexico, Pacific North America | Nam et al. (1994) |
| *O. splendens* (Hollenberg) Nam | Compressed | … | Absent | Absent | Pocket-shaped | … | … | Pacific North America | Yoneshigue-Valentin et al. (2003) |
| *O. truncata* (Kützing) Nam et Maggs | Subterete to compressed | Discoid holdfasts | Present | Present | Cuplike | 1, (4–6)* | Protuberant ostiole | Atlantic Europe, Mediterranean | Nam et al. (2000), *this study |
| *O. verlaquei* G. Furnari | Compressed | Basal crust | Present | Absent | Cuplike | ? | Not observed | Mediterranean | Cormaci et al. (1994) (as *L. pelagiensis*) |

* Denotes a specific reference for the character, which is detailed in the Source column

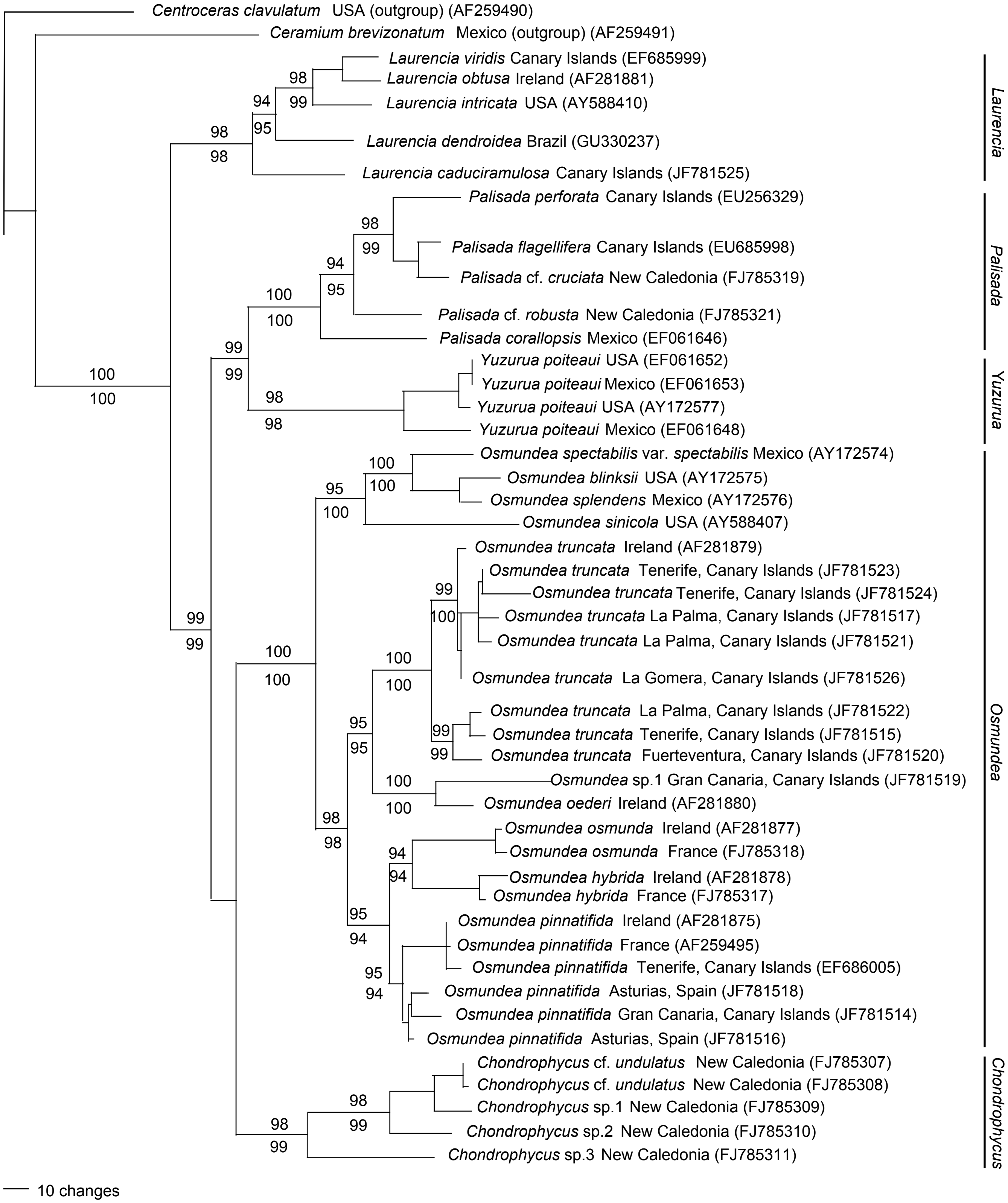

**Figure 8:** Phylogenetic relationships of the *Laurencia* complex based on Bayesian analysis of *rbc*L DNA sequences. Pairs of numbers at the nodes reflect the bootstrap (top) and Bayesian posterior probability (bottom) values

events, which are common in islands' ecosystems (Whittaker and Fernández-Palacios 2007).

*Osmundea* sp. diverged from *O. oederi* in highly uncorrected *p*-distance values (4.5%). The sister clade, which includes *O. truncata* samples, also diverged from *Osmundea* sp. with *p*-distance values of 6.8%–7.7%, as well as *O. pinnatifida* samples (7.7%–9%). These data suggest that *Osmundea* sp. constitutes a distinct entity and should be assigned to a new taxon within the genus *Osmundea*.

In summary, this work provides the first citations of *O. truncata* on the islands of La Gomera and Fuerteventura and confirms the presence of *O. pinnatifida* in Gran Canaria. These two species both exhibit distinct morphotypes which could be the result of adaptation to ecological factors of their particular environment. Furthermore, *O. hybrida* and *O. oederi* appear to be dubious records for the Canary Islands. Finally, the high degree of genetic variation between *Osmundea* sp. from the rest of the *Osmundea* species suggests that this species should be assigned to a new taxon within the *Osmundea* genus from the Atlantic region (Canary Islands).

*Acknowledgements* — We thank E Aylagas, C Fernandez, R Haroun, J Leal Pérez, A Losada Lima, E Soler, M Stroobant and M A Viera for help with field collection and the Instituto Universitario de Enfermedades Tropicales (ULL) and M Hdez-Ferrer for kindly providing laboratory facilities to MM-S. We are indebted to Prud'homme van Reine for providing the specimens from the Netherlands Centre for Biodiversity Naturalis (section NHN), Leiden, and the curator of the Herbarium Biología Ciencias del Mar, Universidad de Las Palmas de Gran Canaria (BCM), for his support. We thank Martina K Pec for assistance with manuscript preparation. MM-S was financed by a grant from the Canarian Government. This study was partly supported by project grants MEC (CGL 2007-60635/BOS) and MICINN (CGL 2010-14881).

## References

Abe T, Kurihara A, Kawaguchi S, Terada R, Masuda M. 2006. Preliminary report on the molecular phylogeny of the *Laurencia* complex (Rhodomelaceae). *Coastal Marine Science* 30: 209–213.

Agardh CA. 1822. *Species algarum* 1 (2). Gryphiswaliae, reprint by A Asher & Co., Amsterdam.

Cassano V, Díaz-Larrea J, Sentíes A, Oliveira MC, Gil-Rodríguez MC, Fujii MT. 2009. Evidence for the conspecificity of *Palisada papillosa* with *P. perforata* (Ceramiales, Rhodophyta) from the western and eastern Atlantic Ocean on the basis of morphological and molecular analyses. *Phycologia* 48: 86–100.

Cormaci M, Furnari G, Serio D. 1994. Two new species of *Laurencia* (Ceramiales, Rhodophyta) from the Mediterranean Sea: *Laurencia pelagiensis* sp. nov. and *Laurencia verlaquei* sp. nov. *Japanese Journal of Phycology* 42: 365–375.

Díaz-Larrea J, Sentíes A, Fujii MT, Pedroche FF, Oliveira MC. 2007. Molecular evidence for *Chondrophycus poiteaui* var. *gemmiferus* comb. and stat. nov. (Ceramiales, Rhodophyta) from the Mexican Caribbean Sea: implications for the taxonomy of the *Laurencia* complex. *Botanica Marina* 50: 250–256.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Fernández-García C, Riosmena-Rodríguez R, Wysor B, Tejada OL, Cortéz J. 2011. Checklist of the Pacific marine macroalgae of Central America. *Botanica Marina* 54: 53–73.

Freshwater DW, Rueness J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbc*L nucleotide sequence analysis. *Phycologia* 33: 187–194.

Fujii MT, Guimarães SMPB, Gurgel CFD, Fredericq S. 2006. Characterization and phylogenetic affinities of the red alga *Chondrophycus flagelliferus* (Rhodomelaceae, Ceramiales) from Brazil on the basis of morphological and molecular evidence. *Phycologia* 45: 432–441.

Furnari GM, Comarci M, Serio D. 2001. The *Laurencia complex* (Rhodophyta, Rhodomelaceae) in the Mediterranean Sea: an overview. *Cryptogamie Algologie* 22: 331–373.

Furnari G[M], Serio D. 1993. The reproductive structures of the Mediterranean alga *Laurencia pelagosae* (Ceramiales, Rhodophyta). *European Journal of Phycology* 28: 141–143.

Garbary DJ, Harper JT. 1998. A phylogenetic analysis of the *Laurencia* complex (Rhodomelaceae) of the red algae. *Cryptogamie Algologie* 19: 185–200.

Gil-Rodríguez MC, Cassano V, Aylagas E, Sentíes A, Díaz-Larrea J, Oliveira MC, Fujii MT. 2010. *Palisada flagellifera* (Ceramiales, Rhodophyta) from the Canary Islands, Spain: a new record for the eastern Atlantic Ocean based on morphological and molecular evidence. *Botanica Marina* 53: 31–40.

Gil-Rodríguez MC, Haroun RJ. 1993. The genus *Laurencia* (Rhodomelaceae, Rhodophyta) in the Canary Islands. *Courier Forschungsinstitut Senckenberg* 159: 113–117.

Gil-Rodríguez MC, Haroun R, Ojeda Rodríguez A, Berecibar Zugasti E, Domínguez Santana P, Herrera Morán B. 2003. Div. Protoctistas. In: Moro L, Martín JL, Garrido MJ, Izquierdo I (eds), *Lista de especies marinas de Canarias (algas, hongos, plantas y animales)* (1st edn). Tenerife: Consejería de Política Territorial y Medio Ambiente del Gobierno de Canarias. pp 5–30.

Gil-Rodríguez MC, Sentíes A, Díaz-Larrea J, Cassano V, Fujii MT. 2009. *Laurencia marilzae* sp. nov. (Ceramiales, Rhodophyta) from the Canary Islands, Spain, based on morphological and molecular evidence. *Journal of Phycology* 45: 264–271.

Haroun RJ, Gil-Rodríguez MC, Díaz de Castro J, Prud'homme van Reine WF. 2002. A checklist of the marine plants from the Canary Islands (Central Eastern Atlantic Ocean). *Botanica Marina* 45: 139–169.

Haroun RJ, Gil-Rodríguez MC, Wildpret de la Torre W, Prud'homme van Reine WF. 2009. *Marine plants of the Canary Islands*. Las Palmas de Gran Canaria: BlaBla Ediciones.

Hudson W. 1762. *Flora anglica*. London: J Nourse and C Moran.

Huelsenbeck JP, Ronquist FR. 2001. MrBayes. Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

Kützing FT. 1865. *Tabulae phycologicae*. Vol. 15. Nordhausen: W. Koehne.

Lamouroux JVF. 1813. Essai sur les genres de la famille des Thallassiophytes non articulées. *Annales Muséum National D'Histoire Naturelle* 20: 21–293.

Lewis S, Gacesa P, Gil-Rodríguez MC, Valdés F, Frías I. 2008. Molecular systematics of the genera *Laurencia, Osmundea* and *Palisada* (Rhodophyta) from Canary Islands, analysis of rDNA and RUBISCO spacer sequences. *Anales del Jardin Botánico de Madrid* 65: 97–109.

Lin SM, Fredericq S, Hommersand MH. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbc*L sequences, including the Phycodryoideae, subfam. nov. *Journal of Phycology* 37: 881–899.

Lyngbye HC. 1819. *Tentamen hydrophytologiae danicae continens omnia hydrophyta cryptogama Daniae, Holsatiae, Faeroae, Islandiae, Groenlandiae hucusque cognita, systematice disposita, descripta and iconibus illustrata, adjectis simul speciebus norvegicis.* Hafniae [Copenhagen]: typis Schultzianis, in commissis Librariae Gyldendaliae.

Maggs CA, Hommersand MH. 1993. *Seaweeds of the British Isles*. London: British Museum (Natural History).

Martin-Lescanne J, Rousseau F, De Reviers B, Payri C, Couloux A, Cruaud C, Le Gall L. 2010. Phylogenetic analyses of the *Laurencia* complex (Rhodomelaceae, Ceramiales) support recognition of five genera: *Chondrophycus, Laurencia, Osmundea, Palisada* and *Yuzurua* stat. nov. *European Journal of Phycology* 45: 51–61 doi:10.1080/09670260903314292.

McIvor L, Maggs CA, Guiry MD, Hommersand MH. 2002. Phylogenetic analysis of the geographically disjunct genus *Osmundea* Stackhouse (Rhodomelaceae, Rhodophyta). *Constancea.* 83.9 [online publication of the Jepson Herbarium, UC, Berkeley]. Available at http://ucjeps.berkeley.edu/constancea/83/mcivor_andal/osmundea.html [accessed 11 March 2011].

Nam KW. 1999. Morphology of *Chodrophycus undulata* and *C. parvipapillata* and its implications for the taxonomy of the *Laurencia* (Ceramiales, Rhodophyta) complex. *European Journal of Phycology* 34: 455–468.

Nam KW, Choi HG. 1999. Developmental morphology of *Osmundea crispa* (Ceramiales, Rhodophyta) from California. *Journal of Fisheries Science and Technology* 2: 25–31.

Nam KW, Choi HG. 2000. A detailed morphological study of the type species of *Osmundea* (Rhodomelaceae, Rhodophyta). *Botanica Marina* 43: 291–297.

Nam KW, Maggs CA, Garbary DJ. 1994. Resurrection of the genus *Osmundea* with an emendation of the generic delineation of *Laurencia* (Ceramiales, Rhodophyta). *Phycologia* 33: 384–395.

Nam KW, Maggs CA, McIvor L, Stanhope MJ. 2000. Taxonomy and phylogeny of *Osmundea* (Rhodomelaceae, Rhodophyta) in Atlantic Europe. *Journal of Phycology* 36: 759–772.

Nam KW, Saito Y. 1994. A re-examination of *Laurencia hybrida* (Ceramiales, Rhodophyta) from the British Isles: vegetative and reproductive morphology. *Phycologia* 33: 34–41.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Sentíes A, Díaz-Larrea J, Cassano V, Gil-Rodríguez MC, Fujii MT. 2011. *Laurencia marilzae* (Ceramiales, Rhodophyta) from the Mexican Caribbean: a new record for the tropical western Atlantic. *Bulletin of Marine Science* 87: 681–686. doi:10.5343/bms.2010.1094.

Serio D, Cormaci M, Furnari G. 1999. *Osmundea maggsiana* sp. nov. (Ceramiales, Rhodophyta) from the Mediterranean Sea. *Phycologia* 38: 277–282.

Serio D, Pandrocelli A, Cormaci M, Cecere E, Furnari G. 2008. First record of *Osmundea oederi* (Gunnerus) G. Furnari comb. nov. (Rhodomelacea, Rhodophyta) from the Mediterranean Sea. *Cryptogamie Algology* 29: 119–127.

Stackhouse J. 1809. Tentamen marino-cryptogamicum, ordinem novum, in genera and species distributum, in Classe XXIVta Linnaei sistens. *Mémoires de la Societé Impériale des Naturalistes de Moscou* 2: 50–97.

Swofford DL. 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods).* Version 4. Sunderland, Massachusetts: Sinauer Associates.

Thiers B. [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available at http://www.nybg.org/bsci/ih/ih.html [accessed 11 March 2011].

Tsuda RT, Abbott IA. 1985. Collecting, handling, preservation and logistics. In*:* Littler MM, Littler DS (eds), *Handbook of phycological methods. Ecological field methods: macroalgae.* Cambridge, New York: Cambridge University Press. pp 67–86.

Whittaker RJ, Fernández-Palacios JM. 2007. *Island biogeography: ecology, evolution, and conservation* (2nd edn). Oxford: Oxford University Press.

Yoneshigue-Valentin Y, Fujii MT, Gurgel CFD. 2003. *Osmundea lata* (M. Howe & W.R. Taylor) comb. nov. (Ceramiales, Rhodophyta) from the Brazilian south-eastern continental shelf. *Phycologia* 42: 301–307.

Manuscript received May 2011; accepted August 2011

## **Appendix:** Taxa used for morphological study

***Osmundea truncata.*** Fresh specimens. La Palma island: La Fajana de Barlovento (TFC Phyc. 14016, TFC Phyc. 14017, TFC Phyc. 14019), Las Salinas-Cancajos (TFC Phyc. 14258, TFC Phyc. 14259, TFC Phyc. 14260, TFC Phyc. 14262, TFC Phyc. 14263, TFC Phyc. 14264, TFC Phyc. 14367, TFC Phyc. 14368), Bco. de La Herradura (TFC Phyc. 14255, TFC Phyc. 14256). La Gomera island: Charco del Conde (TFC Phyc. 14625). Tenerife island: Punta del Hidalgo (TFC Phyc. 13166), Puerto de la Cruz (TFC Phyc. 14078), La Barranquera (TFC Phyc. 14087), Playa Paraíso (TFC Phyc.14068, TFC Phyc.14069). Fuerteventura island: Veril Blanco-Corralejo (TFC Phyc. 14539), Viejo Rey-La Pared (TFC Phyc. 14540). Herbarium material. La Palma island: Varadero-Los Sauces (TFC Phyc. 11310, TFC Phyc. 11419). Tenerife island: Punta del Hidalgo (TFC Phyc. 9948, TFC Phyc. 9949).

***Osmundea pinnatifida.*** Fresh specimens. Tenerife island: Puerto de la Cruz (TFC Phyc. 13151, TFC Phyc. 14116, TFC Phyc. 14533). Gran Canaria island: Quintanilla (TFC Phyc. 14026, TFC Phyc. 14513, TFC Phyc. 14514, TFC Phyc. 14515, TFC Phyc. 14516, TFC Phyc. 14517, TFC Phyc. 14518, TFC Phyc. 14519, TFC Phyc. 14520, TFC Phyc. 14521, TFC Phyc. 14522, TFC Phyc. 14523, TFC Phyc. 14524, TFC Phyc. 14525). Herbarium material. La Gomera island: Los Órganos (TFC Phyc. 2829). La Palma island: La Fajana de Barlovento (TFC Phyc. 3052). Tenerife island: Puerto de la Cruz (TFC Phyc. 14236). Gran Canaria island: Punta de Gáldar (BCM 3754, BCM 4038), Charco de San Felipe (BCM 4039), Agaete (BCM 1599).

***Osmundea* sp.** Fresh specimens. Gran Canaria island: Punta de Gáldar (TFC Phyc. 14025).

***Osmundea hybrida.*** Herbarium material. La Palma Island: Bco. de La Herradura (TFC Phyc. 10319). Gran Canaria island: Bañaderos (BCM 6655). ***Osmundea hybrida*** (as *Laurencia hybrida*). Herbarium material. El Hierro island: La Caleta (TFC Phyc. 714). Tenerife island: Las Caletillas (TFC Phyc. 1789), Punta del Hidalgo (TFC Phyc. 732, TFC Phyc. 7004), Puerto de la Cruz (TFC Phyc. 5819). Lanzarote island: Sur de Lanzarote (L 2681).

***Osmundea oederi*** (as *Laurencia platycephala*). Herbarium material. Tenerife island: Punta del Hidalgo (TFC Phyc. 7003). Gran Canaria island: Las Canteras (TFC Phyc. 5464, TFC Phyc. 5491). Lanzarote island: La Graciosa (TFC Phyc. 2675).