

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

VALÉRIA CASSANO¹*, JHOANA DÍAZ-LARREA², ABEL SENTÍES², MARIANA C. OLIVEIRA³, M. CANDELARIA GIL-RODRÍGUEZ⁴ AND MUTUE T. FUJII⁵

¹*Departamento de Biologia Vegetal, Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, Maracanã, Rio de Janeiro 20550-013, Brazil*

²*Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa, AP. 55-535, Mexico D.F. 09340, Mexico*

³*Departamento de Botânica, Universidade de São Paulo, Rua do Matão 277, São Paulo 05508-900, Brazil*

⁴*Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, Canary Islands, Spain*

⁵*Seção Ficologia, Instituto de Botânica, Av. Miguel Estéfano, 3687, 04301-902 São Paulo, Brazil*

V. CASSANO, J. DÍAZ-LARREA, A. SENTÍES, M.C. OLIVEIRA, M. CANDELARIA GIL-RODRÍGUEZ AND M.T. FUJII. 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. DOI: 10.2216/08-22.1.

Morphological and molecular studies were carried out on *Palisada papillosa* and *P. perforata* from the Canary Islands (type locality of *P. perforata*), Mexico and Brazil. The two species have been distinguished by features of their external morphology such as size and degree of compactness of the thalli, presence or absence of arcuate branches, branching pattern and basal system. A detailed morphological comparison between these taxa showed that none of the vegetative anatomical or reproductive characters was sufficient to separate these species. The presence or absence of cortical cells in a palisade-like arrangement, also previously used to distinguish these species, is not applicable. The species present all characters typical of the genus, and both share production of the first pericentral cell underneath the basal cell of the trichoblast, production of two fertile pericentral cells (the second and the third additional, the first remaining sterile), spermatangial branches produced from one of two laterals on the suprabasal cell of trichoblasts, and the procarp-bearing segment with four pericentral cells. Details of the procarp are described for the species for the first time. The phylogenetic position of these species was inferred by analysis of the chloroplast-encoded *rbcL* gene sequences from 39 taxa, using one other Rhodomelacean taxon and two Ceramiales as outgroups. Relationships within the clade formed by *P. papillosa* and *P. perforata* have not been resolved due to the low level of genetic variation in their *rbcL* sequences (0–0.4%). Considering this and the morphological similarities, we conclude that *P. papillosa* is a taxonomic synonym of *P. perforata*. The phylogenetic analyses also supported the nomenclatural transfer of two species of *Chondrophycus* to *Palisada*, namely, *P. patentiramea* (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov. and *P. thuyoides* (Kützinger) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.

KEY WORDS: *Palisada papillosa*, *P. perforata*, Phylogeny, *rbcL*, Rhodomelaceae, Taxonomy

INTRODUCTION

In recent years, the taxonomy of the *Laurencia* J.V. Lamouroux complex has undergone several changes initiated by the use of new characters and cladistic analyses to segregate genera, subgenera and sections (Garbary & Harper 1998; Nam 1999, 2006, 2007; Nam & Saito 1995). These changes include the resurrection of *Osmundea* Stackhouse (Nam *et al.* 1994), the elevation of Tokida and Saito's subgenus *Chondrophycus* (in Saito 1967) to generic rank (Garbary & Harper 1998), the proposal of the genus *Palisada* K.W. Nam based on Yamada's (1931) section *Palisadae* (Nam 2006) and its later validation (Nam 2007). Therefore, the *Laurencia* complex is currently divided into four genera: *Laurencia sensu stricto*, *Osmundea*, *Chondrophycus* and *Palisada*. Molecular phylogenies have been used to clarify the relationships within the complex

and have corroborated its current classification system (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).

The taxonomic status of *Palisada perforata* (Bory) K.W. Nam and *P. papillosa* (C. Agardh) K.W. Nam was established by Nam (2006, 2007). These species are closely related and morphologically similar, being distinguished by their external morphology. *Palisada perforata* forms strongly compacted turf-like mats attached by a discoid holdfast and arcuate and decumbent branches unilaterally ramified with secondary attachments at their apices. *Palisada papillosa* possesses taller thalli with branches radially ramified, and is attached by a discoid holdfast and stolon-like branches (Haroun & Gil-Rodríguez 1995; Masuda *et al.* 1998; Fujii & Senties 2005; Wynne *et al.* 2005). In addition to size of mature thalli, Nam (2006) utilized the character of presence of a palisade-like arrangement of cortical cells in *P. perforata* and its absence in *P. papillosa* to segregate the species.

*Corresponding author (valcassano@yahoo.com.br).

In this study, arguments for the conspecificity of *P. papillosa* with *P. perforata* are presented and discussed based on morphological characters and chloroplast-encoded *rbcL* gene sequences. The phylogenetic analysis also supported the transfer of *Chondrophycus patentirameus* (Montagne) K.W. Nam and *C. thuyoides* (Kützinger) G. Furnari to the genus *Palisada*.

MATERIAL AND METHODS

Samples of *Palisada perforata* and *P. papillosa* were collected from Spain (Canary Islands), Atlantic Mexico and Brazil. Voucher specimens and material for morphological study were fixed in 4% formalin/seawater or pressed as herbarium sheets. Transverse and longitudinal hand sections were made with a stainless-steel razor blade and stained with 0.5% aqueous aniline blue solution, acidified with 1N HCl (Tsuda & Abbott 1985). Living specimens were also examined to check for presence of *corps en cerise*. Vouchers are deposited in HRJ, SP, UAMIZ and TFC Phyc. For comparison purposes, additional specimens provided by the National Herbarium Netherlands, Leiden University branch (L); from the Herbarium of the Department of Botany, University of Catania, Italy (CAT-Sectio Algae); and from the Botanical Museum Herbarium, Lund, Sweden (LD) were analyzed. Line drawings were prepared using a camera lucida mounted on a Nikon Eclipse E200 microscope (Tokyo, Japan), and photomicrographs were taken with a Sony W5 digital camera (Tokyo, Japan) coupled to a Nikon microscope. Herbarium abbreviations follow the online *Index Herbariorum* (<http://www.nybg.org/bsci/ih/ih.html>). Samples used for molecular analysis were dried in silica gel.

Total DNA was extracted, after grinding in liquid nitrogen, using the Dneasy Plant Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. A total of 1467 base pairs of the *rbcL* gene were amplified in three overlapping parts with the primers pairs: FrbcLstart × R753, F577 × R1150 and F753 × RrbcS (Freshwater & Rueness 1994) using the master mix of the Bioneer (Daedeok-Gu, Daejeon, Korea) Premix. All PCR products were analyzed by electrophoresis in 1% agarose to check product size. The PCR products were purified with the Qiagen QIAquick Purification Kit in accordance with the manufacturer's instructions.

Sequencing was carried out with the BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, NJ, USA) on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The primers used for the sequencing were those used for the PCR amplification. The full sequence was obtained from both DNA strands. The analysis of the sequences was done with the computer program Sequence Navigator (Applied Biosystems) and aligned with the CLUSTAL algorithm (Thompson *et al.* 1994). The specimens sequenced in this study, plus the other available sequences used in phylogenetic analysis, are shown in Table 1, including their GenBank accession numbers (NCBI GenBank 2003).

Phylogenetic relationships were inferred with PAUP 4.0b10 (Swofford 2002) and MrBayes v.3.0 beta 4

(Huelsenbeck & Ronquist 2001). Maximum-parsimony trees (MP) were constructed using the heuristic search option, tree-bisection-reconnection branch swapping, unordered and unweighted characters, and gaps treated as missing data. Branch length was optimized using delayed transformation (DELTRAN), which favors parallelisms over reversals. Support values for the relationships discovered in each analyses were calculated by performing bootstrap analyses (Felsenstein 1985), as implemented in PAUP. Ten thousand heuristic search replicates were executed using the TBR branch-swapping algorithm. Consistency (CI), homoplasy (HI) and retention (RI) indexes were computed to evaluate the level of homoplasy in the most parsimonious tree. 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 ratio tests implemented by the software Modeltest version 3.06 (Posada & Crandall 1998) with a significance level of 0.01. For the Bayesian analysis, we ran four chains of the Markov chain Monte Carlo (one hot and three cold), sampling one tree every 10 generations for 1,000,000 generations starting with a random tree. The 17,680 generations were discarded as 'burn in'. A 50% consensus tree (majority rule as implemented by PAUP) was computed after the 'burn in' point. The range of *rbcL* divergence values within and among species was calculated using uncorrected 'p' distances using PAUP.

RESULTS

Morphological analyses

Palisada perforata (Bory) K.W. Nam (2007, 54)

Figs 1–27

BASIONYM: *Fucus perforatus* Bory (1803, 505, pl. 5 fig. 1).

HOLOTYPE: PC!

TYPE LOCALITY: Tenerife, Canary Islands, Spain.

NOMENCLATURAL SYNONYMS: *Laurencia perforata* (Bory) Montagne (1840, 155); *Chondrophycus perforatus* (Bory) K.W. Nam (1999, 463); *Fucus papillosus* Forsskål (1775, 190), *nom. illeg.* (later homonym of *Fucus papillosus* S.G. Gmelin 1768); *Chondria papillosa* C. Agardh (1822, 344); *Laurencia papillosa* (C. Agardh) Greville (1830, lii); *Chondrophycus papillosus* (C. Agardh) Garbary & J.T. Harper (1998, 195); *Palisada papillosa* (C. Agardh) K.W. Nam (2007, 54).

HABIT: Plants forming strongly compacted turf-like mats, up to 5 cm high, or isolated tufts, up to 10 cm high, violet-brown or greenish brown in color, terete, rigid, cartilaginous in texture, not adhering to herbarium paper when dried (Figs 1–8). Thalli attached to the substratum by a discoid holdfast from which arise arcuate and decumbent branches, 0.5–1.4 mm in diameter, and secondarily attached by rhizoids or smaller discoid holdfasts (Fig. 9). Stolon-like branches can be produced from the lower

Table 1. Species used in this study for phylogenetic analysis.

Species	Collection data/reference	GenBank accession numbers	Percentage of the <i>rbcL</i> gene sequenced
<i>Bostrychia radicans</i> (Montagne)	USA, Mississippi, St. Louis Bay, leg. C.F.D. Gurgel, 11 Feb. 1998 (Lin <i>et al.</i> 2001)	AF259497	90
<i>Centroceras</i> sp. (as <i>C. clavulatum</i> (C. Agardh in Kunth)	USA, Texas, Port Aransas, Redfish Bay, leg. S. Fredericq & C.F.D. Gurgel, 18 May 1998 (Lin <i>et al.</i> 2001)	AF259490	97
<i>Ceramium brevizonatum</i> H.E. Petersen	Mexico, Yucatán, Campeche Bay, Laguna de Yulcapeten, leg. C.F.D. Gurgel, 12 Feb. 1998 (Lin <i>et al.</i> 2001)	AF259491	97.2
<i>Laurencia filiformis</i> (C. Agardh) Montagne (as <i>L. arbuscula</i> Sonder)	Brazil, São Paulo, Ubatuba, Ilha das Couves, leg. M.T. Fujii, 31 Aug. 2000 (Fujii <i>et al.</i> 2006)	AF465810	95.6
<i>L. cf. brongniartii</i>	Taiwan, Makang Harbour, leg. S. Fredericq, 11 Jul. 1993 (Fujii <i>et al.</i> 2006)	AF465814	95.6
<i>L. complanata</i> (Suhr) Kützing	South Africa, KwaZulu-Natal, Port Edward, leg. S. Fredericq & O. De Clerck, 8 Jul. 2001 (Fujii <i>et al.</i> 2006)	AF465813	97.7
<i>L. intricata</i> J.V. Lamouroux	Mexico, Yucatán, Campeche Bay, leg. C.F.D. Gurgel, 14 Feb. 1999 (Fujii <i>et al.</i> 2006)	AF465809	96.9
<i>L. intricata</i>	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, leg. J. Díaz-Larrea & A. Senties, 16 Apr. 2004, UAMIZ 1008 (this study)	EF658644	99.8
<i>L. natalensis</i> Kylin	South Africa, KwaZulu-Natal, Palm Beach, leg. S. Fredericq, 7 Feb. 2001 (Fujii <i>et al.</i> 2006)	AF465816	100
<i>L. obtusa</i> (Hudson) J.V. Lamouroux	Ireland, County Donegal, Fanad Head (McIvor <i>et al.</i> 2002)	AF281881	84.7
<i>L. translucida</i> M.T. Fujii & Cordeiro-Marino	Brazil, Espírito Santo, Marataízes, leg. M.T. Fujii, 15 Sep. 2001 (Fujii <i>et al.</i> 2006)	AY588408	98.9
<i>Palisada corallopsis</i> (Montagne) Senties, M.T. Fujii & Díaz-Larrea	Mexico, Quintana Roo, Cancún, Chaac-Mol Beach, leg. J. Díaz-Larrea & A. Senties, 21 Aug. 2005 (Díaz-Larrea <i>et al.</i> 2007)	EF061646	98.6
<i>P. flagellifera</i> (C. Agardh) K.W. Nam	Brazil, Espírito Santo, Marataízes, leg. M.T. Fujii, 15 Sep. 2001 (Díaz-Larrea <i>et al.</i> 2007)	EF061647	98.6
<i>P. papillosa</i> (C. Agardh) K.W. Nam	Spain, Canary Islands, Tenerife, Playa Paraíso, leg. M.C. Gil-Rodríguez, A. Senties & M.T. Fujii, 14 Jul. 2006, TFC Phyc 13134 (this study)	EU256325	100
<i>P. papillosa</i>	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras, leg. V. Cassano & M.B. Barros-Barreto, 3 Aug. 2005, HRJ 10838 (this study)	EU256326	100
<i>P. papillosa</i>	Mexico, Baja California, Todos Santos, leg. S. Fredericq, 24 Oct. 1999 (Fujii <i>et al.</i> 2006)	AY588409	99.1
<i>P. papillosa</i>	Brazil, São Paulo, Ubatuba, Praia do Lamberto, leg. M.T. Fujii, 31 Aug. 2000 (Fujii <i>et al.</i> 2006)	AF465806	99.1
<i>P. papillosa</i>	Mexico, Quintana Roo, Puerto Morelos, CRIP, leg. J. Díaz-Larrea & A. Senties, 16 Apr. 2004 (Díaz-Larrea <i>et al.</i> 2007)	EF061651	100
<i>P. papillosa</i>	USA, Florida, Old Dan's Reef., leg. S. Fredericq, 8 Apr. 1988 (Fujii <i>et al.</i> 2006)	AF465807	96.7
<i>P. patentiramea</i> (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.	Philippines (Lluisma, unpublished)	AF489862	89
<i>P. perforata</i> (Bory) K.W. Nam	Mexico, Quintana Roo, Cancún, Isla Mujeres, leg. A Senties & M.C. Gil-Rodríguez, 2 Mar. 2007, UAMIZ 1041 (this study)	EF658641	100
<i>P. perforata</i>	Spain, Canary Islands, Tenerife, Pta del Hidalgo, Faro, Bahía izquierda, leg. M.C. Gil-Rodríguez, 6 Oct. 2005, TFC Phyc 13068 (this study)	EU256327	100
<i>P. perforata</i>	Spain, Canary Islands, Tenerife, Pta del Hidalgo, Faro, Bahía izquierda, leg. M.C. Gil-Rodríguez, 6 Oct. 2005, TFC Phyc 13072 (this study)	EU256328	100
<i>P. perforata</i>	Spain, Canary Islands, Tenerife, San Telmo, Pto de la Cruz, leg. M.C. Gil-Rodríguez, A. Senties & M.T. Fujii, 14 Jul. 2006, TFC Phyc 13132 (this study)	EU256329	100
<i>P. perforata</i>	Brazil, Rio de Janeiro, Rio das Ostras, Areias Negras, leg. V. Cassano & M.B. Barros-Barreto, 3 Aug. 2005, HRJ 10839 (this study)	EU256330	100
<i>P. perforata</i>	Brazil, Rio de Janeiro, Parati, Praia Vermelha, leg. V. Cassano, 30 Dec. 2005, HRJ 10840 (this study)	EU256331	100
<i>Palisada poiteaui</i> (J.V. Lamouroux) K.W. Nam var. <i>gemmaifera</i> (Harvey) Senties, M.T. Fujii & Díaz-Larrea	Mexico, Quintana Roo, Puerto Morelos, Ojo de Agua, leg. J. Díaz-Larrea & A. Senties, 16 Apr. 2004 (Díaz-Larrea <i>et al.</i> 2007)	EF061648	96.5
<i>P. poiteaui</i> var. <i>gemmaifera</i>	Mexico, Quintana Roo, Playa del Carmen, leg. J. Díaz-Larrea & A. Senties, 17 Apr. 2004 (Díaz-Larrea <i>et al.</i> 2007)	EF061649	100

Table 1. Continued

Species	Collection data/reference	GenBank accession numbers	Percentage of the <i>rbcL</i> gene sequenced
<i>P. poiteaui</i> var. <i>gemmifera</i>	Cuba, La Havana, Rincon de Guanabo, leg. J. Díaz-Larrea & A. A. Mallea, 29 Jul. 2005 (Díaz-Larrea <i>et al.</i> 2007)	EF061650	98.6
<i>P. poiteaui</i> (J.V. Lamour.) K.W. Nam var. <i>poiteaui</i>	USA, Florida, Long Key, Ocean Side, leg. S. Fredericq (Díaz-Larrea <i>et al.</i> 2007)	EF061652	100
<i>P. poiteaui</i> var. <i>poiteaui</i>	Mexico, Quintana Roo, Playa del Carmen, leg. J. Díaz-Larrea & A. Senties, 15 Mar. 2005 (Díaz-Larrea <i>et al.</i> 2007)	EF061653	100
<i>P. thuyoides</i> (Kützinger) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.	Philippines (Lluisma, unpublished)	AF489863	95
<i>Palisada</i> sp.	Spain, Canary Islands, Tenerife, Playa Paraíso, leg. M.C. Gil-Rodríguez, A. Senties & M.T. Fujii, 14 Jul. 2006 (Gil-Rodríguez <i>et al.</i> unpublished)	EF685998	98.2
<i>Osmundea osmunda</i> (S.G. Gmelin) K.W. Nam	Ireland, County Donegal, St. John's Point (McIvor <i>et al.</i> 2002)	AF281877	84.8
<i>O. pinnatifida</i> (Hudson) Stackhouse	Ireland, County Donegal, St. John's Point (McIvor <i>et al.</i> 2002)	AF281875	84.8
<i>O. pinnatifida</i>	France, Brittany, Penmarch (Lin <i>et al.</i> 2001)	AF259495	97
<i>O. pinnatifida</i>	Spain, Canary Islands, Tenerife, San Telmo, Pto. de la Cruz, leg. M.C. Gil-Rodríguez, 7 Oct. 2005, TFC Phyc 13077 (this study)	EF686005	98.9
<i>O. speckabilis</i> (Postels & Ruprecht) K.W. Nam var. <i>spectabilis</i>	Mexico, Baja California, Pta Santo Thomas (McIvor <i>et al.</i> 2002)	AY172574	82.9
<i>O. truncata</i> (Kützinger) K.W. Nam & Maggs	Ireland, County Cork, Lough Hyne (McIvor <i>et al.</i> 2002)	AF281879	84.8

portion of axes (Fig. 10). Erect branches unilaterally disposed or irregularly alternate and spirally arranged, usually with 2–3 (–4) orders of branches, measuring 0.8–1.6 mm in diameter at the middle portions of the thalli. Erect branches densely placed at the uppermost portions with numerous wart-like ultimate branchlets. Ultimate branchlets cylindrical or clavate to papillate, 0.8–3.6 mm long and 0.4–1.1 mm in diameter.

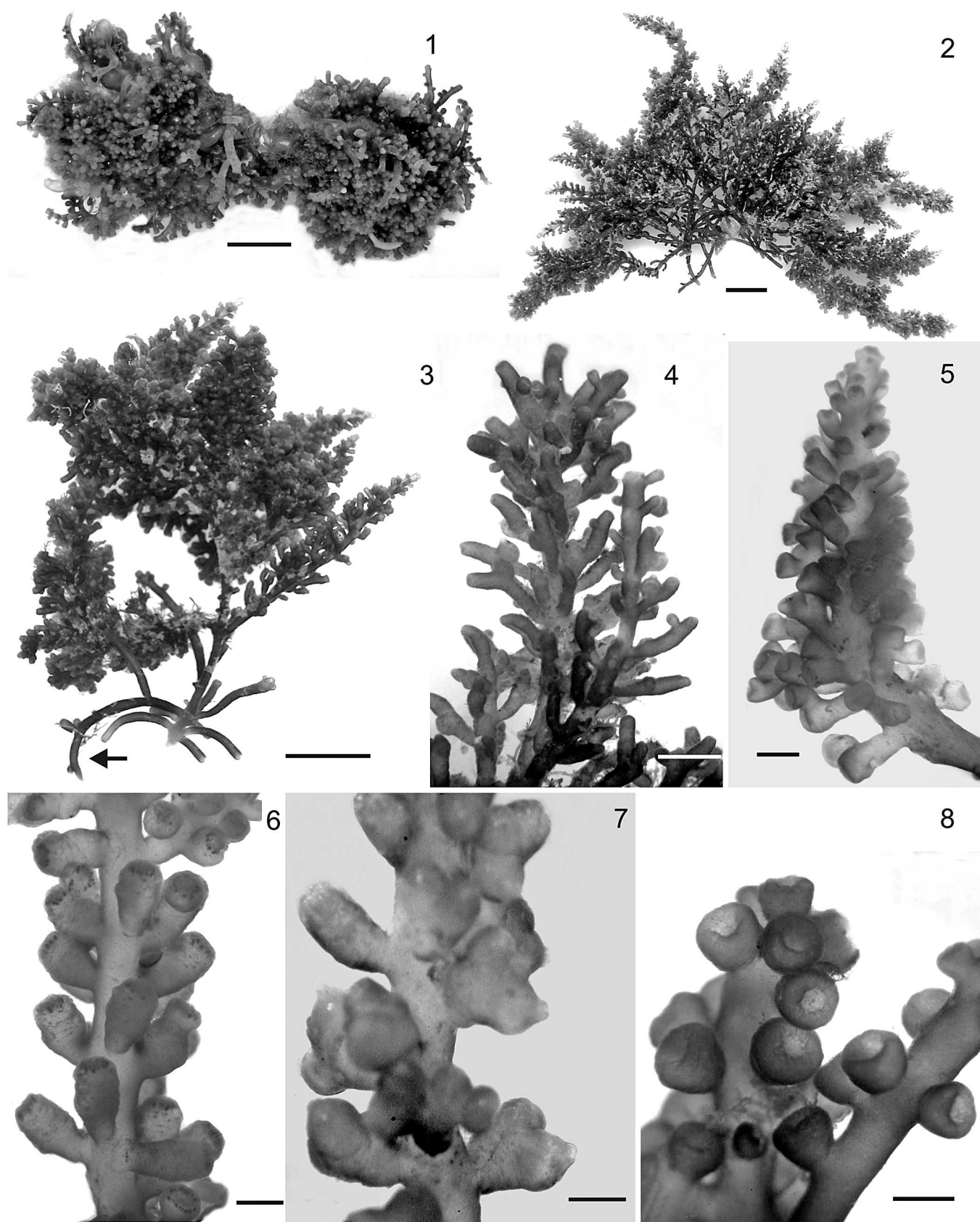
VEGETATIVE STRUCTURES: In surface view, cortical cells are regularly arranged throughout the thalli, slightly radially elongated in the upper portions, isodiametric-polygonal in the middle portions, 17.5–52.5 µm long and 15–47.5 µm wide, and elongate-polygonal in the lower portions. Secondary pit connections between cortical cells absent (Fig. 11). *Corps en cerise* absent. In transverse section, thalli with one or two layers of pigmented cortical cells; surface cortical cells quadratic to radially elongated and arranged as palisade, 20–45 µm long and 12.5–32.5 µm wide in the middle portions of the main axes (Figs 12, 13–16). Palisade-like arrangement may be lacking in the ultimate branchlets. Medullary region with four or five layers of colorless cells, rounded or slightly radially elongated, 50–155 µm long and 40–122.5 µm wide in the middle portions of the main axes. Medullary cells walls uniformly thickened, but lenticular thickenings are absent. Each vegetative axial segment cuts off two pericentral cells (Fig. 17); the first pericentral cell is produced underneath the basal cell of the trichoblast (Fig. 18). In median longitudinal section through a branchlet, outer cortical cells walls near apices not projecting beyond the surface.

REPRODUCTIVE STRUCTURES: Tetrasporangial branchlets are cylindrical or papillate, simple or compound, 0.7–3.0 mm long and 0.6–1.2 mm in diameter (Fig. 6). At the

apex of fertile branches, each axial segment produces one additional pericentral cell situated oppositely to the position to the existing two normal pericentral cells. The third additional and the second pericentral cells become fertile and the first pericentral cell remains vegetative (Figs 19, 20). The second and third fertile pericentral cells cut off two presporangial cover cells distally and abaxially the tetrasporangial initial (Fig. 21). Subsequently, one postsporangial cover cell is produced and continues dividing and contributes to produce cortication around the tetrasporangium. The presporangial cover cells do not divide and display a transverse-type alignment in relation to the fertile axis in surface view. Tetrasporangial maturation is in a clockwise spiral, and the final arrangement is a right-angle pattern in relation to fertile branchlets (Fig. 22). Mature tetrasporangia are tetrahedrally divided, 50–117.5 µm in diameter.

In female thalli, each procarp-bearing segment produces four pericentral cells (Fig. 23), the fourth of which becomes the supporting cell of a four-celled carpogonial branch with two groups of sterile cells (Fig. 24). Fully developed cystocarps are conical with protuberant carpostomes, 0.7–1.2 mm in diameter, subapically arranged with the lower half immersed within the parent branch (Figs 7, 25). Carposporangia are clavate, 105–200 µm long and 25–55 µm in diameter. Details of the postfertilization stages are basically identical to those described by Nam & Sohn (1994) and Nam & Saito (1995).

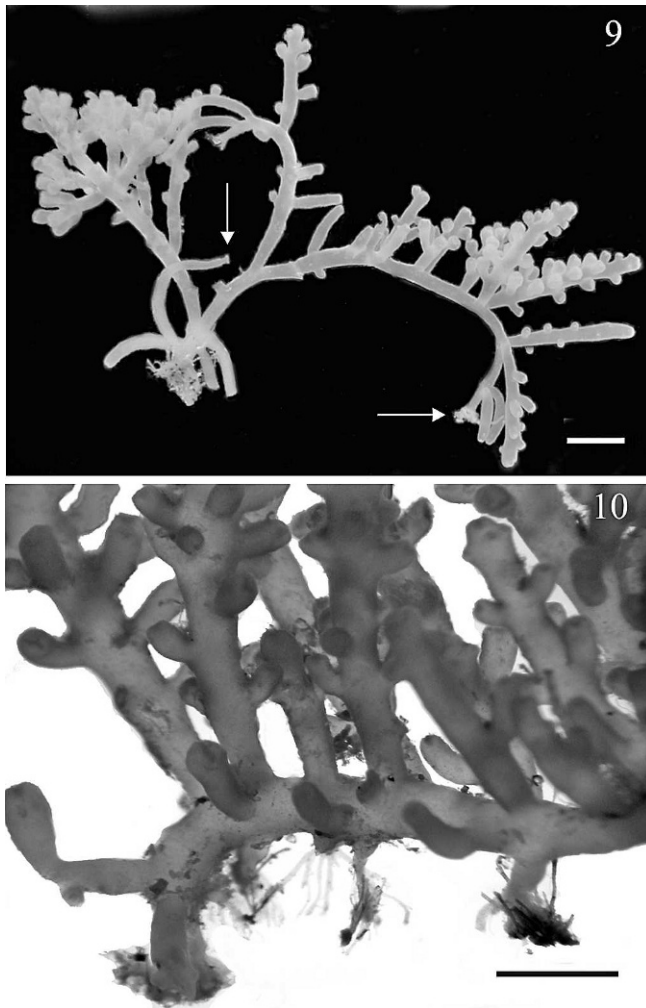
Male branches are characteristically swollen, 0.8–1.6 mm in diameter (Figs 5, 8). In longitudinal section through a fertile branchlet, the spermatangial pits are cup shaped, and an axial cell row is discernible at the base (Fig. 26). Spermatangial trichoblasts arise from axial cells, consisting of fertile and sterile branches (Fig. 27); the fertile branches



Figs 1–8. *Palisada perforata*. (Fig. 1, TFC Phyc 13170; Figs 2, 5, 6, HRJ 10901; Figs 3, 4, HRJ 10836; Figs 7, 8, HRJ 10867).

Figs 1, 2. Habit of plants showing compacted turf-like mats and isolated tufts, respectively. Scale = 1 cm.

Fig. 3. Detail of a specimen showing lower portion with stolon-like branches (arrow). Scale = 1 cm.



Figs 9, 10. *Palisada perforata*. (Fig. 9, HRJ 10868; Fig. 10, HRJ 10901).

Fig. 9. Specimen showing arcuate branches with secondary attachment (arrows). Note unilateral branching. Scale = 5 mm.

Fig. 10. Basal portion of a thallus showing stolon-like branch. Scale = 2 mm.

produce many ovoid spermatangia, 6.5–12.5 μm long and 5.0–7.5 μm in diameter, and terminate in vesicular sterile cells, 22.5–37.5 μm long and 17.5–35 μm in diameter; each spermatium possess an apical nucleus (Fig. 28).

HABITAT: The specimens were collected from the lower intertidal zone to subtidal zone at exposed or protected from wave action sites.

SPECIMENS EXAMINED MORPHOLOGICALLY: *Palisada perforata* (Bory) K.W. Nam – **Spain**, Canary Islands, **Tenerife**. (leg. Montagne, L 11540/8, Herb. Lugb. Bat. no. 941.99 ...

104) (leg. Kranse, L 11540/7, Herb. Lugb. Bat. no. 941.99 ... 103); Punta del Hidalgo (leg. M.C. Gil-Rodríguez, 06 Oct. 2005, TFC Phyc 13068); Playa Paraíso (leg. M.C. Gil-Rodríguez, A. Senties & M.T. Fujii, 14 Jul. 2006, TFC Phyc 13132); Puerto de la Cruz, San Telmo (leg. M.C. Gil-Rodríguez, A. Senties, & M.T. Fujii, 13 Jul. 2006, TFC Phyc 13170); Las Galletas (leg. CANCAP 3, no. 1276, 26 Oct. 1978, L 11540/11, Herb. Lugb. Bat. no. 987.012 ... 132); Tacoronte, El Pris (leg. A. Bleckwenn & M.C. Gil-Rodríguez, 10 Feb. 1998, TFC Phyc 9951); **Gran Canaria**. Guía, Punta Ancha (leg. CANCAP 3, no. 1600, 09 Feb. 1978, L 11540/6, Herb. Lugb. Bat. n° 987.012 ... 164); Punta Camello (leg. F. Espino, Oct. 1998, BCM 4837); **Fuerteventura**. Castillo de Fuste (leg. M.C. Gil-Rodríguez & R. Haroun, 5 May 1980, TFC Phyc 2319); Punta Pesebre (leg. M.C. Gil-Rodríguez, 15 Mar. 2005, TFC Phyc 13209); **Lanzarote**. Arrecife (leg. M.E. Guadalupe, M.C. Gil-Rodríguez & M.C. Hernández-González, 25 Oct. 1991, TFC Phyc 8680); Alegranza, Punta de los Mosquitos (leg. R. Haroun *et al.*, 23 Aug. 1994, BCM 2017-2123) (HEINCKE Exped. St. 11, leg. R. Haroun *et al.*, 29 Nov. 1991, BCM 1221); Montaña Clara, Morro del Agüjero (leg. GRAMONAL'83 31 Mar. 1983, L 11540/1, Herb. Lugb. Bat. no. 986.324 ... 092, TFC Phyc 5666); **El Hierro**. La Restinga (leg. M.C. Gil Rodríguez, 15 Apr. 2005, TFC Phyc 13210), Faro de Orchilla (leg. CANCAP 2, no. 462, 07 Nov. 1977, L 11540/12, Herb. Lugb. Bat. no. 366.120); **La Palma**. Fajana de Barlovento (leg. M.C. Gil-Rodríguez & R. Haroun, 15 Jun. 1983, TFC Phyc 3072), Tijarafe (leg. CANCAP IV 04/05, Apr. 1980, L 11540/17, Herb. Lugb. Bat. no. 987.252 ... 963), San Andrés y Sauces, El Charco Azul (leg. F. Espino & R. Herrera, 29 Nov. 2002, BCM 6046); **La Gomera**. Los Órganos (leg. J. Afonso, R. Haroun & W. Wildpret, 03 Apr. 1982, TFC Phyc 2830); **Mexico**. **Quintana Roo**. Isla Mujeres (leg. A. Senties, 19 Feb. 2006, UAMIZ 1040); (leg. A. Senties & M.C. Gil-Rodríguez, 02 Mar. 2007, UAMIZ 1041); **Brazil**. **Rio de Janeiro**. Rio das Ostras, Areias Negras (leg. V. Cassano & M.B. Barros-Barreto, 03 Aug. 2005, HRJ 10839); Armação dos Búzios, Praia Rasa (leg. V. Cassano & J.C. De-Paula, 21 Jul. 2005, HRJ 10866); Parati, Praia Vermelha (leg. V. Cassano, 30 Dec. 2005, HRJ 10840), Praia da Lula (leg. V. Cassano, 09 Feb. 2005, HRJ 10868); Baía da Ilha Grande, Angra dos Reis, Ilha Grande, Praia da Feiticeira (leg. V. Cassano & J.C. De-Paula, 29 Jul. 2005, HRJ 10867); **as Palisada papillosa** (C. Agardh) K.W. Nam – **Spain**, Canary Islands, **Tenerife**. Adeje, Playa Paraíso (leg. M.C. Gil-Rodríguez, A. Senties & M.T. Fujii, 14 Jul. 2006, TFC Phyc 13134); El Médano (leg. J. Reyes-Hernández & M. Sansón, 29 Oct. 1991, TFC Phyc 7609); **Mexico**. **Quintana Roo**. Puerto Morelos (leg. A. Senties, 29 Apr. 1995, UAMIZ 850); Playa del Carmen (leg. A. Senties & M.C. Gil-Rodríguez, 27 Feb.

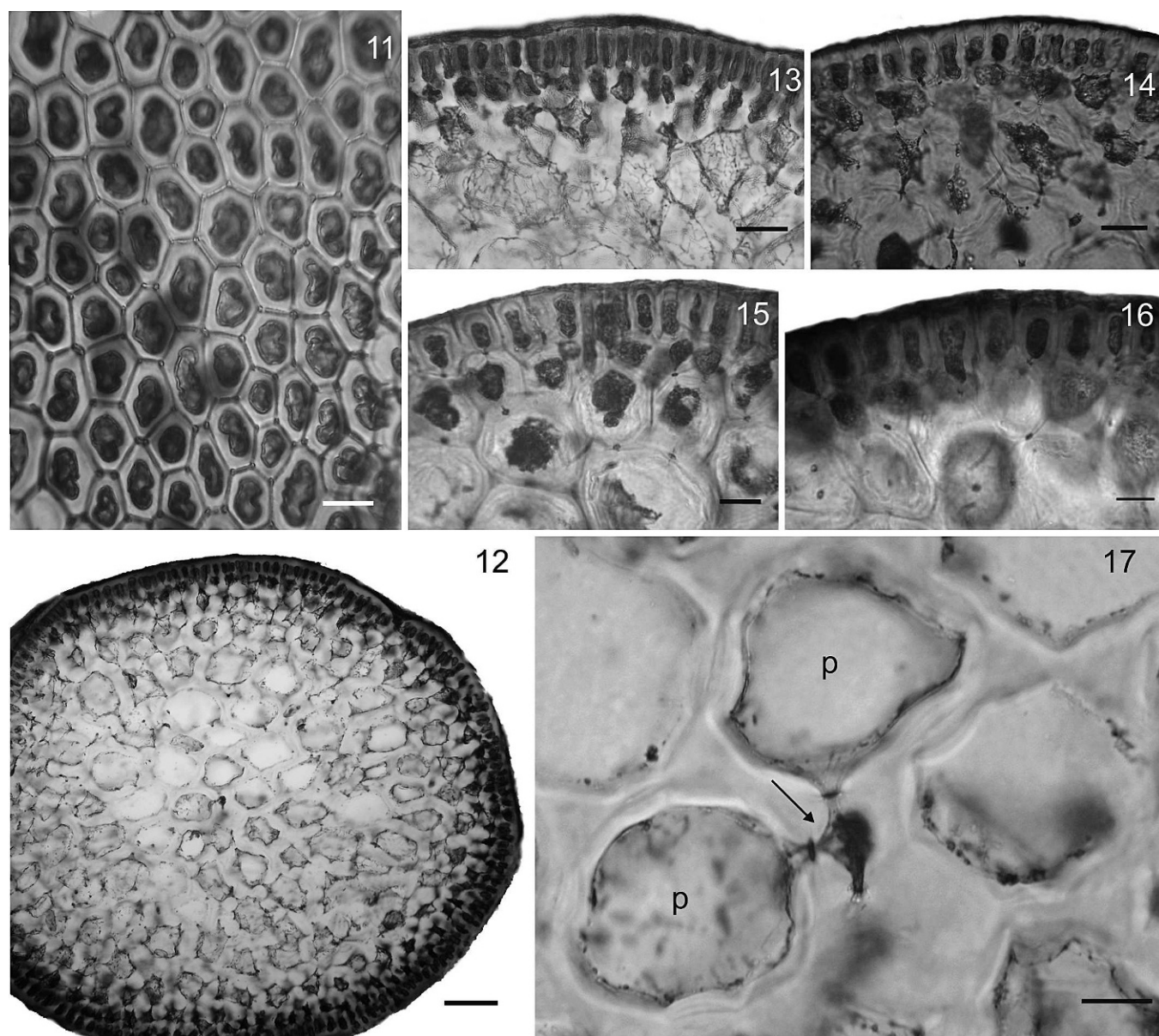
Fig. 4. Female branches. Scale = 2 mm.

Fig. 5. Male branches. Scale = 1 mm.

Fig. 6. Tetrasporangial branches. Scale = 1 mm.

Fig. 7. Detail of female branches with cystocarps. Scale = 1 mm.

Fig. 8. Detail of male branches. Scale = 1 mm.



Figs 11–17. *Palisada perforata*. (Fig. 11, HRJ 10839; Figs 12, 17, HRJ 10836; Fig. 13, 14, TFC Phyc 13170; Figs 15, 16, HRJ 10837).

Fig. 11. Cortical cells in surface view without secondary pit connections. Scale = 20 μ m.

Fig. 12. Transverse section of the thallus. Scale = 100 μ m.

Figs 13, 14. Transverse sections of main axis and ultimate branchlet, respectively, showing palisade-like arrangement of cortical cells. Scale = 50 μ m and 40 μ m, respectively.

Figs 15, 16. Transverse sections of main axis and ultimate branchlet, respectively, of a plant previously identified as *P. papillosa* showing palisade-like arrangement of cortical cells. Scale = 25 μ m.

Fig. 17. Transverse section of the upper portion of a branch showing an axial cell (arrow) with two pericentral cells (p). Scale = 25 μ m.

2007, UAMIZ 1021); **Brazil**. Rio de Janeiro. Rio das Ostras, Areias Negras (leg. V. Cassano & M.B. Barros-Barreto, 03 Aug. 2005, HRJ 10865); Armação dos Búzios, Praia Rasa (leg. V. Cassano & J.C. De-Paula, 13 Jan. 2005, HRJ 10901); Parati, Trindade, Praia do Meio (leg. V. Cassano & José B. de Carvalho, 08 Feb. 2005, HRJ 10903); Ilha do Mantimento (leg. V. Cassano, 25 Feb. 2007, HRJ 10836); Baía da Ilha Grande, Angra dos Reis, Praia Brava (leg. V. Cassano, 23 Oct. 2004, HRJ 10837); Saco de Piraquara de Fora, Ponta do Arame (leg. V. Cassano, M.T.M. Széchy & M.T. Fujii, 12 Dec. 2006, HRJ 10822); Ilha do Bonfim (leg.

V. Cassano, M.T.M. Széchy & M.T. Fujii, 13 Dec. 2006, HRJ 10904).

ADDITIONAL MATERIAL EXAMINED: Isotype: *Fucus papillosus* Forsskål (LD 36784). As *Chondrophycus papillosus* (C. Agardh) Garbary & J. T. Harper – **Italy**. Sicily, Syracuse, Brucoli (leg. D. Serio, 29 May 1993, CAT-Sectio Algae 1698).

MATERIAL OF OTHER SPECIES EXAMINED FOR COMPARISON: *Chondrophycus thuyoides* (Kützinger) G. Furnari – **Italy**. Sicily, Apulia, Gargano (leg. Fanelli, autumn 1997, 5 m

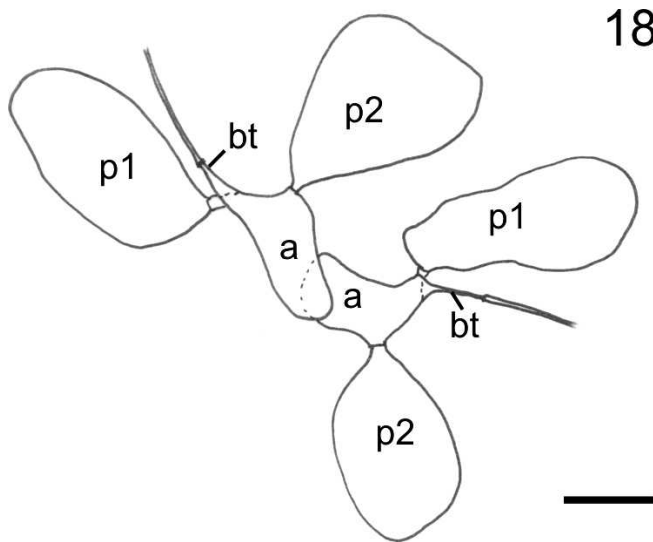


Fig. 18. *Palisada perforata*. (HRJ 10868). Transverse section near the apex of branchlet showing two superimposed vegetative axial segments with an axial cell (a) and two pericentral cells (p1, p2). Note the first pericentral cell (p1) produced underneath the basal cell of the trichoblast (bt). Scale = 10 μ m.

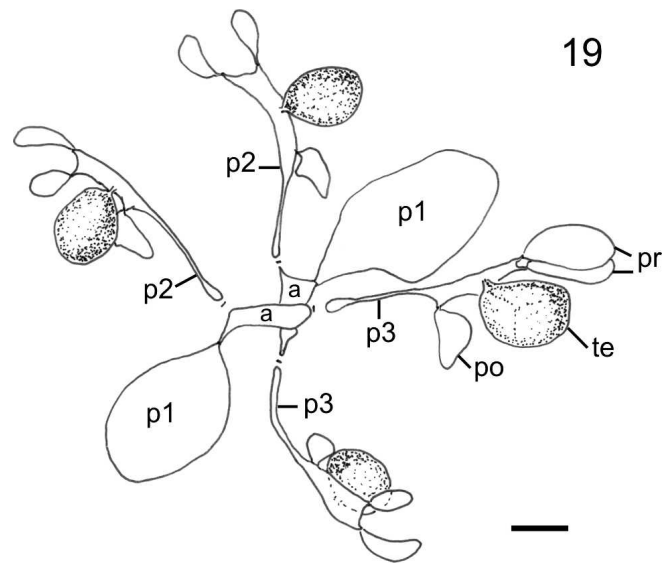


Fig. 19. *Palisada perforata*. (HRJ 10868). Diagrammatic representation. Transverse section near the apex of branchlet showing tetrasporangial axial segments. Each axial segment (a) with one vegetative pericentral cell (p1) and two fertile pericentral cells, the existing (p2) and the additional (p3). Presporangial cover cells (pr), tetrasporangial initial (te); postsporangial cover cell (po). Scale = 25 μ m.

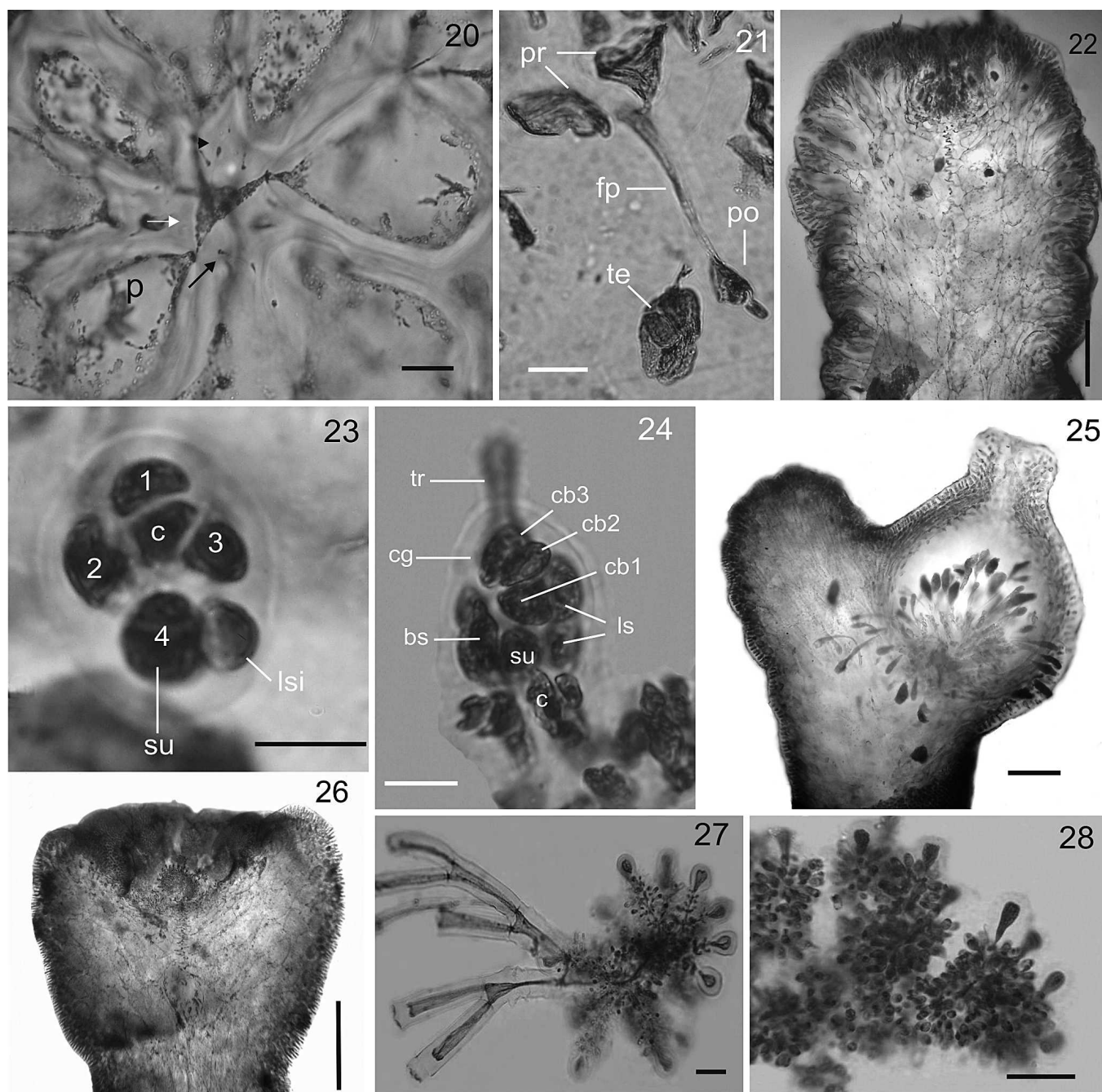
depth CAT-Sectio Algae 1635); *Chondrophycus patentirameus* (Montagne) K.W. Nam – **Spain**. Terragona, Delta del Ebro (leg. F. Boisset, 20 Aug. 1996, CAT-Sectio Algae 1594).

Molecular analyses

VARIATION IN THE *rbcL* SEQUENCES: We sequenced the *rbcL* gene for 10 specimens of the *Laurencia* complex: six specimens of *Palisada perforata*, two of *P. papillosa*, one of *Laurencia intricata* J.V. Lamouroux and one of *Osmundea pinnatifida* (Hudson) Stackhouse. A total of 39 sequences were analyzed including the outgroups *Bostrychia radicans* (Montagne) Montagne, *Centroceras* sp. [as *Centroceras clavulatum* (C. Agardh) Montagne] and *Ceramium brevizonatum* E. H. Petersen (Table 1). A total of 250 nucleotides were removed from all *rbcL* sequences at the beginning or end of the sequences as many sequences from the GenBank were incomplete, producing a data set of 1217 base pairs. Intergeneric divergence varied from 6 to 10% between *Palisada* and *Laurencia* and from 9 to 12% between *Palisada* and *Osmundea*. Interspecific divergences obtained for the species of *Laurencia* varied from 3 to 8%; whereas, for those of *Palisada* varied from 4 to 10%. In the case of *P. papillosa* and *P. perforata*, the observed *rbcL* nucleotide divergences between Atlantic specimens were very low, 0–0.4%.

PHYLOGENY: The data set consisted of 802 constant characters, 321 parsimony-informative sites and 94 parsimony noninformative sites. MP produced two trees of 1069 steps (CI = 0.755, RI = 0.554, HI = 0.245). The topology of the 50% consensus tree (majority rule as implemented by PAUP) with corresponding bootstrap values is shown in Fig. 29. The topology of Bayesian tree with corresponding

Bayesian posterior probabilities values is shown in Fig. 30. The MP and Bayesian tree topologies were very similar. The analysis shows a monophyletic *Laurencia* complex with high bootstrap support in relation to the members of the outgroup. The *Laurencia* complex separated into three clades with high bootstrap support, corresponding to the genera: *Osmundea*, *Laurencia* and *Palisada*. In all analyses, the earliest diverging clade was the genus *Osmundea* with its species *O. pinnatifida* (Hudson) Stackhouse, *O. osmunda* (S.G. Gmelin) K.W. Nam, *O. truncata* (Kützinger) K.W. Nam & Maggs and *O. spectabilis* (Postels & Ruprecht) K.W. Nam var. *spectabilis*. The genera *Laurencia* and *Palisada* were sister groups forming a clade with high bootstrap support and with a posterior probability of 100%. The monophyletic clade that corresponded to the genus *Laurencia* included seven species: *Laurencia filiformis* (C. Agardh) Montagne (as *L. arbuscula* Sonder), *L. cf. brongniartii* J. Agardh, *L. intricata* J.V. Lamouroux, *L. complanata* (Suhr) Kützinger, *L. natalensis* Kylin, *L. translucida* M.T. Fujii & Cordeiro-Marino and *L. obtusa* (Hudson) J.V. Lamouroux. The monophyletic clade that corresponded to the genus *Palisada* included seven species. Within the *Palisada* assemblage, *P. poiteaui* (J.V. Lamouroux) K.W. Nam var. *poiteaui* and *P. poiteaui* var. *gemmifera* (Harvey) Senties, M. T. Fujii & Díaz-Larrea were segregated from others forming a distinct clade. The species *P. flagellifera* (J. Agardh) K.W. Nam, *P. papillosa*, *P. perforata*, *P. corallopsis* (Montagne) Senties, M.T. Fujii & Díaz-Larrea, *Chondrophycus patentirameus* and *C. thuyoides* formed another well-supported clade. Therefore, considering the criteria established by Nam (2006) for the genus *Palisada* and the evidence presented in phylogenetic analysis, the following new combinations are proposed.



Figs 20–28. *Palisada perforata*. (Figs 20, 22, 25, HRJ 10836; Figs 21, 23, TFC Phyc 13134; Fig. 24, HRJ 10901; Fig. 26, HRJ 10866; Figs 27–28, HRJ 10868).

Fig. 20. Transverse section of tetrasporangial axial segments showing an axial cell (white arrow) and two fertile pericentral cells. The second pericentral cell (black arrow) becomes fertile; an additional third fertile pericentral cell is formed in the opposite position (arrowhead), and the first pericentral cell remains vegetative (p). Scale = 25 μ m.

Fig. 21. Detail of a fertile pericentral cell (fp) with two presporangial cover cells (pr), tetrahedrally divided tetrasporangium (te) and one postsporangial cover cell (po). Scale = 20 μ m.

Fig. 22. Longitudinal section through tetrasporangial branchlet showing right-angle arrangement of the tetrasporangia. Scale = 500 μ m.

Fig. 23. Procarp-bearing segment with four pericentral cells, the fourth becoming the supporting cell (su). Central cell of procarp-bearing segment (c), lateral sterile group initial (lsi). Scale = 10 μ m.

Fig. 24. Procarp before fertilization with four-celled carpogonial branch (cb), lateral sterile group (ls) and basal sterile group (bs). Carpogonium (cg), trichogyne (tr), supporting cell (su), central cell of procarp-bearing segment (c). Scale = 10 μ m.

Fig. 25. Longitudinal section through female branchlet with partly immersed cystocarp and protuberant carpostome. Scale = 500 μ m.

Fig. 26. Longitudinal section through male branchlet showing spermatangial branches in cup-shaped tips. Scale = 400 μ m.

Fig. 27. Detail of trichoblast type spermatangial branches. Note trichoblast with two laterals (sterile and spermatangial branches). Scale = 40 μ m.

Fig. 28. Detail of spermatangial branches with terminal vesicular sterile cells and spermatia with an apical nucleus. Scale = 40 μ m.

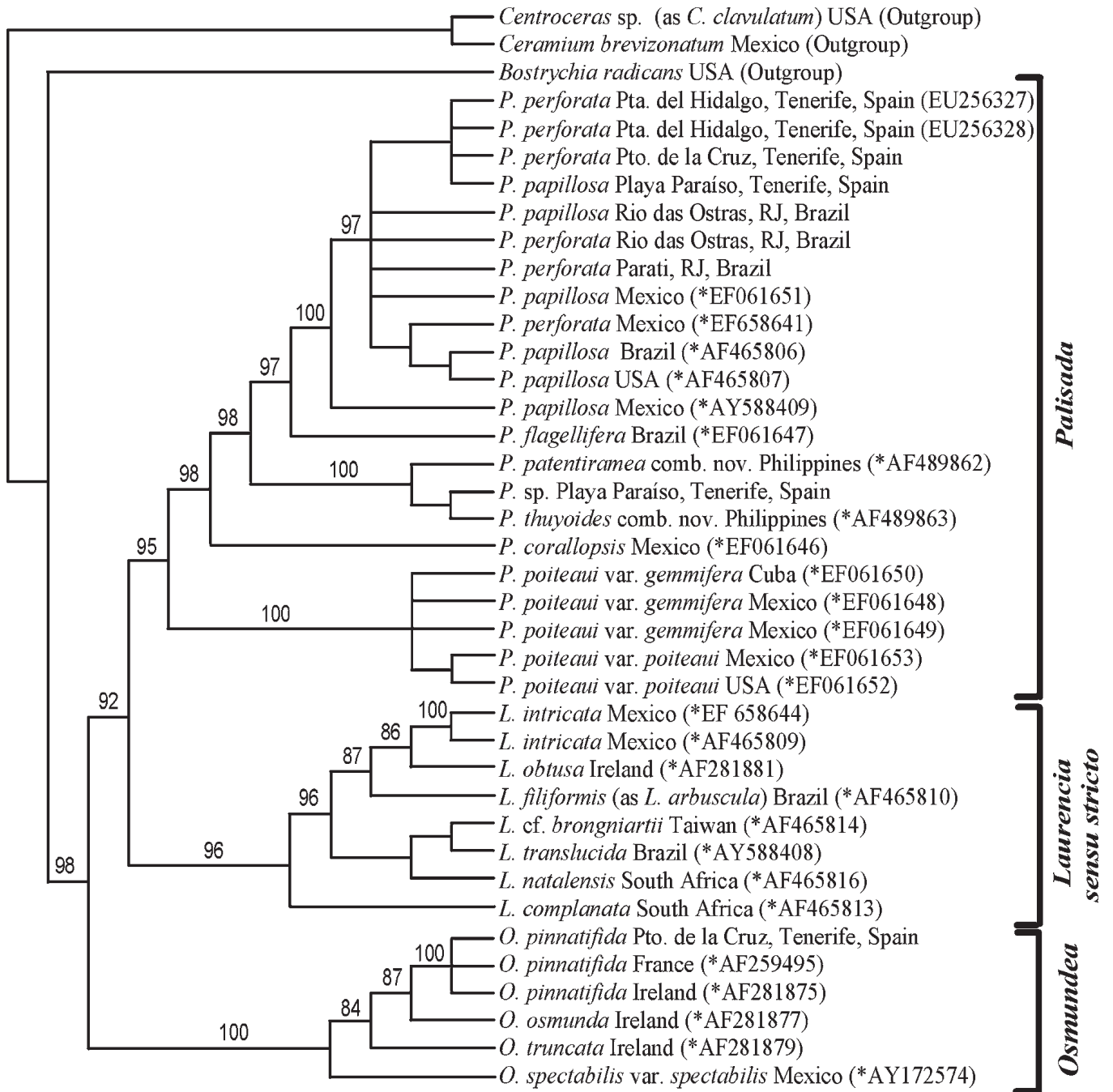


Fig. 29. Consensus majority rule tree of two maximum parsimony trees for *rbcL* sequences of the *Laurencia* complex species, rooted with *Bostrychia radicans*, *Centroceras* sp. and *Ceramium brevizonatum* as outgroups. Bootstrap values indicated at the nodes. CI = 0.755, RI = 0.554, HI = 0.245.

Palisada patentiramea (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.

BASIONYM: *Chondria obtusa* (Hudson) C. Agardh var. *patentiramea* Montagne (1836, vol. 6, 322, pl. 18, fig. 3).

SYNONYMS: *Chondrophycus patentirameus* [patentiramea] (Montagne) K.W. Nam (1999, 463); *Laurencia patentiramea* (Montagne) Kützing (1849, 854); *Laurencia obtusa* var. *patentiramea* (Montagne) Rabenhorst (1847, 155).

Palisada thuyoides (Kützing) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.

BASIONYM: *Laurencia thuyoides* Kützing (1865, vol. 15, 26, pl. 74, figs a, b).

SYNONYMS: *Chondrophycus thuyoides* (Kützing) G. Furnari (in Furnari et al. 2001, 341, figs 1–3); *Chondrophycus paniculatus* (C. Agardh) G. Furnari (in Boisset et al. 2000, 392, figs 18–23); *Chondria obtusa* var. *paniculata* C. Agardh (1822, 343); *Laurencia obtusa* var. *paniculata* (C. Agardh)

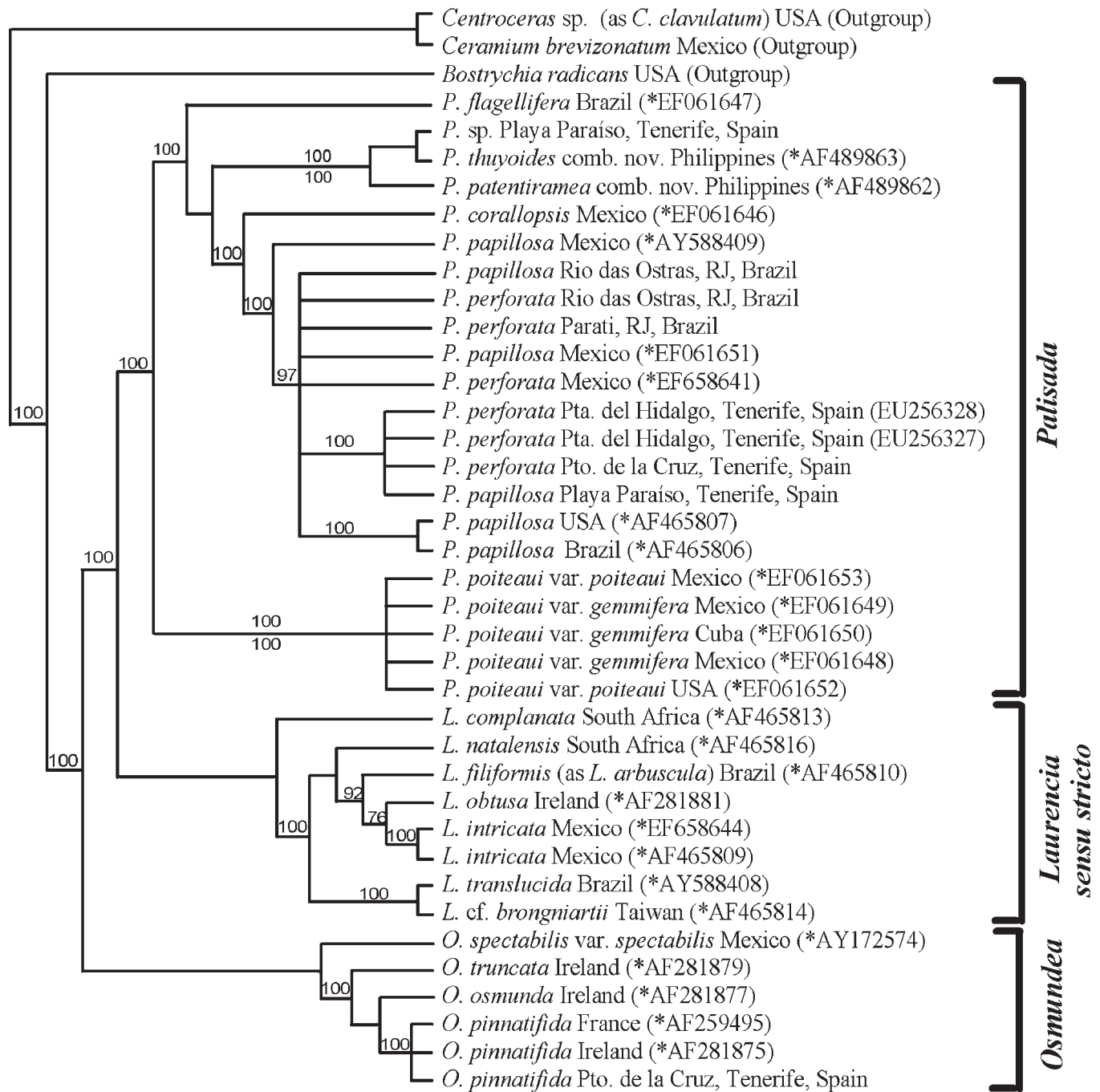


Fig. 30. Bayesian analysis of *rbcL* sequence. Fifty percent majority-rule consensus tree was computed after the 'burn-in' point. Evolutionary model used in the Bayesian analysis was the GTR+I+G, selected by a maximum likelihood ratio test. Bayesian posterior probabilities values are indicated at the nodes.

Zanardini (1847, 20); *Laurencia paniculata* (C. Agardh) J. Agardh (1852, 755) *nom. illeg.* [later homonym of *L. paniculata* Kützinger (1849, 855)].

Palisada papillosa-*P. perforata* were monophyletic with high support in all analyses with *P. papillosa* from Baja California in the Pacific Ocean basal to the Atlantic samples. The eastern Atlantic *P. papillosa* and *P. perforata* from Tenerife, Canary Islands, form a monophyletic clade with a high posterior probability, segregating from the

western Atlantic *P. papillosa* and *P. perforata* from Florida (USA), Mexico, and southern Brazil.

DISCUSSION

Palisada perforata was first proposed as *Fucus perforatus* by Bory (1803) from Tenerife, Canary Islands; whereas,

Table 2. Morphological comparison of *Palisada* species considered in this study.

Species	Attachment	Arcuate branches	Position of the first pericentral cell relative to trichoblast	Secondary pit connections	Epidermal cells in TS	Cell wall projections	Spermatangial branches	Procarp-bearing segments	Tetrasporangial pericentral cells	Tetrasporangial axis	References
<i>Palisada patentiramea</i> (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.	Disc and stolon-like branches	Absent	Underneath ¹	Absent	NPD	Absent	SOL	4P	Second	1SP	Boisset <i>et al.</i> (2000), Furnari <i>et al.</i> (2001)
<i>P. thuyoides</i> (Kützinger) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.	Disc	Absent	Underneath ¹	Absent	PD	Absent	?	?	Second	1SP	Boisset <i>et al.</i> (2000), Furnari <i>et al.</i> (2001)
<i>P. corallopsis</i> (Montagne) Senties, M.T. Fujii & Diaz-Larrea	Disc	Absent	?	Absent	PD	Absent	?	?	Second, third, fourth*	1SP	Fujii & Senties (2005), Senties & Diaz-Larrea (2008)
<i>P. flagellifera</i> (J. Agardh) K.W. Nam	Disc	Absent	?	Absent	PD	Absent	?	5P	Second, third, fourth*	1SP	Fujii <i>et al.</i> (2006), Wynne <i>et al.</i> (2005), Nam (2006), this study
<i>P. papillosa</i> (C. Agardh) K.W. Nam	Disc and stolon-like branches	Absent (rarely present)	Underneath	Absent	PD	Absent	SOL	4P	Second, third*	1SP	Wynne <i>et al.</i> (2005), Nam (2006), this study
<i>P. perforata</i> (Bory) K.W. Nam	Disc and secondary attachment in arcuate branches	Present	Underneath	Absent	PD	Absent	SOL	4P	Second, third*	1SP	Wynne <i>et al.</i> (2005), Nam (2006), this study
<i>P. poiteaui</i> (J.V. Lamouroux) K.W. Nam var. <i>poiteaui</i>	Disc	Absent	?	Present	NPD	Absent	?	5P	Second, third, fourth*	1SP	Fujii <i>et al.</i> (1996), Fujii & Senties (2005)
<i>P. poiteaui</i> (J.V. Lamouroux) K.W. Nam var. <i>geminifera</i> (Harvey) Senties, M.T. Fujii & Diaz-Larrea	Disc	Absent	?	Present	NPD	Apiculate	?	5P	Second, third, fourth*	1SP	Fujii <i>et al.</i> (1996), Fujii & Senties (2005), Senties & Diaz-Larrea (2008)

Modified from Nam (1999).

¹ Interpreted by Boisset *et al.*'s (2000) illustrations; NPD, nonpalisade; PD, palisade; P, pericentral cells; SP, sterile pericentral cells; SOL, spermatangial branches from one of the two laterals; TS, transverse section; ?, unknown; *, produced additionally.

Palisada papillosa was originally described as *Fucus papillosus* by Forsskål (1775) from the Red Sea. However, according to Silva *et al.* (1996), *Fucus papillosus*, the intended basionym of *L. papillosa*, lacks priority since it is a later homonym of *Fucus papillosus* Gmelin (1768), a name of uncertain application. *Chondria papillosa* C. Agardh (1822) is treated as a *nomen novum* and the legitimate basionym of *L. papillosa*. Later, both species were transferred to the genus *Laurencia*: *Laurencia perforata* (Bory) Montagne and *L. papillosa* (C. Agardh) Greville, following Lamouroux's (1813) circumscription of the genus: red-purple plants, little gelatinous, included in the order 'Floridéés' that presented globose cystocarps situated at the tips of branches and branchlets.

Garbary & Harper (1998), on the basis of a cladistic analysis of morphological characters, segregated members of the *Laurencia* complex originally included in the subgenus *Chondrophycus* (Saito 1967; Nam & Sohn 1994) and elevated that subgenus to generic rank. They characterized these species by having the presence of two pericentral cells in vegetative axes, secondary pit connections between cortical cells mostly absent, no *corps en cerise*, spermatangial development of the trichoblast type, tetrasporangia development from pericentral cells with fertile segments producing additional fertile pericentral cells and tetrasporangia typically arranged in a right-angle pattern. Later, Nam (1999) redefined the genus *Chondrophycus* and characterized it by its possession of only two pericentral cells in vegetative axes, the trichoblast type development of the spermatangia and the production of tetrasporangia from a particular pericentral cell. In light of this circumscription, *Laurencia perforata* and *L. papillosa* were transferred to *Chondrophycus*.

Nam (2006, 2007) transferred *Chondrophycus papillosus* and *C. perforatus* to the genus *Palisada* and segregated it from *Chondrophycus*, based on cladistic analysis, using the following morphological features: the first pericentral cell is produced underneath the basal cell of the trichoblast as in *Laurencia*, the second pericentral cell in the tetrasporangial axis is always fertile and the resultant axis has one sterile pericentral cell, the spermatangial branches are produced from one of two laterals on the suprabasal cell of trichoblasts, the auxiliary cells in the postfertilization process show a normal developmental pattern after presumed fertilization and the procarp-bearing segments have four pericentral cells, except in *Palisada poiteaui* (J.V. Lamouroux) K.W. Nam var. *poiteaui* and *P. poiteaui* var. *gemmifera* (Harvey) Senties, M.T. Fujii & Díaz-Larrea, which possess five pericentral cells [Fujii *et al.* 1996, as *Laurencia poiteaui* (J.V. Lamouroux) M. Howe and as *L. gemmifera* Harvey].

Yamada (1931) included *Palisada perforata* [as *Laurencia perforata*] and *Palisada papillosa* [as *Laurencia papillosa*] in the section *Palisadae* Yamada by the presence of palisade-like arrangement of cortical cells, confirmed by the examination of both holotypes [*Fucus perforatus*, Thuret herbarium, Paris and *F. papillosus* Forsskål herbarium, Copenhagen]. Nam & Saito (1991), who re-examined the holotype of *P. papillosa*, pointed out that the cortical cells of ultimate branchlets are never elongated radially, but are elongated longitudinally, and the palisade layer is not clear

in transverse section. Thus, Nam & Saito (1991) argued that the palisade structure is not a useful criterion because it is not always evident in *P. papillosa*. Later, Nam (1999) elevated the section *Palisadae* to subgeneric rank [subgenus *Palisada* K.W. Nam] and transferred *P. papillosa* to the section *Papillosae* J. Agardh *emend.* K.W. Nam by the absence of palisade-like arrangement of cortical cells in transverse section. On the other hand, Masuda *et al.* (1997) and Furnari *et al.* (2001) argued that main axes, indeterminate branches, and also ultimate branchlets possess a palisade-like arrangement of cortical cells in transverse section; although, it may be absent in some ultimate branchlets. Despite the arguments above, Nam (2006) maintained this character as a distinguishing difference between *P. papillosa* and *P. perforata*. Our study confirmed the presence of a palisade arrangement of cortical cells in both species; although, it occasionally may be lacking in ultimate branchlets as pointed out by Masuda *et al.* (1997) and Furnari *et al.* (2001). The examination of the isotype of *Fucus papillosus* in LD showed that it is in agreement with the material from the Canary Islands, Mexico and Brazil analyzed in this study.

The habit difference observed in these species seems to be related to environmental conditions. The species are known to be polymorphic and usually assume the turf morphology when they grow in the intertidal zone and are exposed to high-energy wave action (Fujii & Senties 2005). The basal system used to segregate the species is variable. The stolon-like branches may be lacking in *P. papillosa*, and the basal branches arising from the lower portions of the erect branches may assume an arcuate habit and can also be attached by smaller holdfasts as in *P. perforata*. Fujii & Senties (2005), after comparative studies of these species, already had suggested that the specimens of *P. perforata* and *P. papillosa* from Brazil were morphological variants of the same species.

Morphological comparisons among the species of the *Palisada* clade considered in this study are shown in Table 2. *Palisada perforata* and *P. papillosa* share the following diagnostic characters: the first pericentral cell is produced underneath the basal cell of the trichoblast, a palisade-like arrangement of cortical cells, the tetrasporangial axes produce two fertile pericentral cells per segment (the second vegetative which becomes fertile and a third additional), the first pericentral cell remaining vegetative, the spermatangial branches are produced from one of two laterals on the suprabasal cell of trichoblasts and the procarp-bearing segment possesses four pericentral cells. Although the species have been widely reported, details of the procarp are described here for the first time.

The intergeneric divergence values obtained in this work (6–12%) for *rbcL* sequences are comparable to those reported by other authors for the *Laurencia* complex. Nam *et al.* (2000) recorded intergeneric divergence values between 11% (*Laurencia obtusa* vs. *Osmundea pinnatifida*) and 13% [*L. obtusa* vs. *O. hybrida* (A.P. de Candolle) W.K. Nam], and McIvor *et al.* (2002) recorded values of 11% (*O. pinnatifida* vs. *L. obtusa*) to 13% [*O. blinksii* vs. *P. papillosa* (as *C. papillosus*)]. Díaz-Larrea *et al.* (2007) obtained intergeneric divergence values from 10 to 12% for *Palisada*

(as *Chondrophycus*) and *Laurencia* and from 10 to 13% for *Palisada* (as *Chondrophycus*) and *Osmundea*.

The interspecific divergence values reported in this work (3–10%) for *rbcL* sequences are comparable to those reported by other authors for the genus *Osmundea*. Nam *et al.* (2000) estimated divergence percentages that varied from 5% (*O. osmunda* vs. *O. pinnatifida*) to 9% (*O. hybrida* vs. *O. truncata*), and McIvor *et al.* (2002) recorded values of 2% (*O. blinksii* vs. *O. splendens*) to 9% (*O. blinksii* vs. *O. truncata*). Díaz-Larrea *et al.* (2007) obtained interspecific divergence values for the species of *Laurencia* that varied from 6 to 8%, whereas for those of *Palisada* (as *Chondrophycus*) varied from 6 to 9%.

The molecular phylogeny shows that the clade formed by the five populations of *P. perforata* (Bahía Izquierda and San Telmo, Canary Islands; Isla Mujeres, Mexico; Rio das Ostras and Parati, Brazil) and four populations of *P. papillosa* (Playa Paraíso, Canary Islands; Puerto Morelos, Mexico; Rio das Ostras, Brazil; Old Dan's Reef, Florida, USA) are poorly resolved as a result of the low level of genetic variation between their *rbcL* sequences (0–0.4%). This divergence value is comparable to that reported by Díaz-Larrea *et al.* (2007) for *P. poiteaui*–*P. gemmifera* (0.01–0.02%) and for three samples of *O. pinnatifida* analyzed in this work (0.4%). Within the *P. papillosa*–*P. perforata* assemblage, eastern Atlantic samples from Tenerife, Canary Islands, form a monophyletic clade, segregating from the western Atlantic *P. papillosa* and *P. perforata* from Florida (USA), Mexico, and southern Brazil. The taxon named as *Palisada papillosa* from Baja California, Mexican Pacific, may be a distinct species from the Atlantic entities, as its *rbcL* sequence divergence from the rest of the *P. papillosa* and *P. perforata* specimens is 1%. Therefore, the entity known as *P. papillosa* from Baja California needs to be critically re-examined as suggested previously by Fujii *et al.* (2006). The morphological similarities and the molecular data support the conspecificity of *C. papillosus* with *C. perforatus*, the latter epithet having priority over the former. The phylogenetic analyses also supported the nomenclatural changes for two species of *Chondrophycus* to *Palisada*, namely, *P. patentiramea* (Montagne) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov. and *P. thuyoides* (Kützinger) Cassano, Senties, Gil-Rodríguez & M.T. Fujii comb. nov.

ACKNOWLEDGEMENTS

We are very grateful to Giovanni Furnari for providing the specimens from the Herbarium of the Department of Botany, University of Catania, Italy, and for kindly receiving the first author in his laboratory. We also thank Michael Wynne, Wilson Freshwater and Eurico C. Oliveira for their critical comments on the manuscript, K.W. Nam for his helpful comments, the curators of the herbaria cited in this study and our colleagues listed in the section titled Specimens Examined Morphologically for help with field material. This study was partly supported by Ministry of Education and Sciences of the Spanish Government (CGL 2007-60635/BOS), the Coordenação de Aperfeiçoamento de

Pessoal de Nível Superior (Capes) to V.C. and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Proc. 4546592006-7 to M.T.F., 470417/2006-4 to M.C.O. A.S. thanks SEP PROMEP (P/CA-117 2006-35-52). M.T.F. and M.C.O. thank CNPq for a Research Productivity Fellowship (Proc. 308426/2006-1, 301217/2007-6).

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 Sciences* 30: 209–213.
- AGARDH C.A. 1822. *Species algarum*, vol.1, part 2a. Berling, Lund, Sweden. pp. 169–398.
- AGARDH J.G. 1852. *Species genera et ordines algarum. Volumen secundum: algas florideas complectens*, part 3. Gleerup, Lund, Sweden. pp. 701–786.
- BOISSET F., FURNARI G., CORMACI M. & SERIO D. 2000. The distinction between *Chondrophycus patentirameus* and *C. paniculatus* (Ceramiales, Rhodophyta). *European Journal of Phycology* 35: 387–395.
- BORY DE SAINT-VINCENT J.B.G.M. 1803. *Essais sur les îles Fortunées et l'antique Atlantide, ou précis de l'histoire général de l'archipel des Canaries*. Baudouin, Paris. 522 pp.
- DÍAZ-LARREA J., SENTIES A., FUJII M.T., PEDROCHE F.F. & OLIVEIRA M.C. 2007. Molecular evidence for *Chondrophycus poiteaui* var. *gemmiferus* comb. et 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.
- FORSSKÅL P. 1775. *Flora Aegyptiaco-Arabica*. Copenhagen. 219 pp.
- FRESHWATER D.W. & RUENESS J. 1994. Phylogenetic relationships of some European *Gelidium* (Gelidiales, Rhodophyta) species, based on *rbcL* nucleotide sequence analysis. *Phycologia* 33: 187–194.
- FUJII M.T. & SENTIES A. 2005. Taxonomia do complexo *Laurencia* (Rhodomelaceae, Rhodophyta) do Brasil, com ênfase nas espécies dos estados de São Paulo e do Espírito Santo. In: *Monografias Ficológicas*. II (Ed. by A. Senties & K.M. Dreckmann), pp. 69–135. Universidad Autónoma Metropolitana – Iztapalapa, México, Instituto de Botânica de São Paulo, Brasil.
- FUJII M.T., COLLADO-VIDES L. & CORDEIRO-MARINO M. 1996. Morphological studies of *Laurencia gemmifera* and *Laurencia poiteaui* (Rhodomelaceae, Rhodophyta) from the Nichupté Lagoon System, Quintana Roo, México. *Botanica Marina* 39: 317–326.
- FUJII M.T., GUIMARÃES S.M.P.B., GURGEL C.F.D. & FREDERICO S. 2006. Characterization and phylogenetic affinities of the red alga *Chondrophycus flagelliferus* (Rhodomelaceae, Ceramiales) from Brazil based on morphological and molecular evidence. *Phycologia* 45: 432–441.
- FURNARI G., CORMACI M. & SERIO D. 2001. The *Laurencia* complex (Rhodophyta, Rhodomelaceae) in the Mediterranean Sea: an overview. *Cryptogamie, Algologie* 22: 331–373.
- GARBARY D.J. & HARPER J.T. 1998. A phylogenetic analysis of the *Laurencia* complex (Rhodomelaceae) of the red algae. *Cryptogamie, Algologie* 19: 185–200.
- GMELIN S.G. 1768. *Historia fucorum*. Academia Scientiarum Petropoli, St. Petersburg. 239 pp.
- GREVILLE R.K. 1830. *Algae Britannicae*. MacLachlan & Stewart, Edinburgh. 218 pp.
- HAROUN R.J. & GIL-RODRIGUEZ M.C. 1995. Reproductive strategies of *Laurencia perforata* (Bory) Montagne (Ceramiales, Rhodomelaceae) in the Canary Islands. *Nova Hedwigia* 61: 269–274.

- HUELSENBECK J.P. & RONQUIST F.R. 2001. MrBayes: Bayesian inference of phylogeny. *Biometrics* 17: 754–755.
- KÜTZING F.T. 1849. *Species algarum*, vol. 6. Lipsiae. 922 pp.
- KÜTZING F.T. 1865. *Tabulae Phycologicae*, vol. 15. Nordhausen. 36 pp.
- LAMOUROUX J.V.F. 1813. Essai sur les genres de la famille des Thallasiophytes non articulées. *Annales du Muséum d'Histoire Naturelle* (Paris) 20: 21–47, 115–139, 267–293.
- LEWIS S., GACESA P., GIL-RODRÍGUEZ M.C., VALDÉS F. & FRÍAS I. 2008. Molecular systematics of the genus *Laurencia*, *Osmundea* and *Palisada* (Rhodophyta) from the Canary Islands – analysis of rDNA and RUBISCO spacer sequences. *Anales del Jardín Botánico de Madrid* 65: 97–109.
- LIN S.M., FREDERICQ S. & HOMMERSAND M.H. 2001. Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on LSU rDNA and *rbcL* sequences, including the Phycodryioideae, subfam. nov. *Journal of Phycology* 37: 881–899.
- MASUDA M., KAWAGUCHI S. & PHANG S.M. 1997. Taxonomic notes on *Laurencia similis* and *L. papillosa* (Ceramiales, Rhodophyta) from the Western Pacific. *Botanica Marina* 40: 229–239.
- MASUDA M., KOGAME K., ARISAWA S. & SUZUKI M. 1998. Morphology and halogenated secondary metabolites of three Gran Canaria species of *Laurencia* (Ceramiales, Rhodophyta). *Botanica Marina* 41: 265–277.
- MCIVOR L., MAGGS C.A., GUIRY M.D. & HOMMERSAND M.H. 2002. Phylogenetic analysis of the geographically disjunct genus *Osmundea* Stackhouse (Rhodomelaceae, Rhodophyta). *Constancea* 83.9. Online publication of the Jepson Herbarium, University of California, Berkeley: http://ucjeps.berkeley.edu/constancea/83/mcivor_etal/osmundea.html.
- MONTAGNE C. 1836. Notice sur les plantes cryptogames récemment découvertes en France, contenant aussi l'indication précise des localités de quelques espèces les plus rares de la flore française. *Annales des Sciences Naturelles, Botanique Sér. 2*, 6: 28–36, 321–339.
- MONTAGNE C. 1840. Plantae Cellulaires, Section 4. In: *Histoire Naturelle des Iles Canaries* (Ed. by P.B. Webb & S. Berthelot), vol. 3(2): pp. 1–208. Béthune, Paris.
- NAM K.W. 1999. Morphology of *Chondrophycus undulata* and *C. parvipapillata* and its implications for the taxonomy of the *Laurencia* (Ceramiales, Rhodophyta) complex. *European Journal of Phycology* 34: 455–468.
- NAM K.W. 2006. Phylogenetic re-evaluation of the *Laurencia* complex (Rhodophyta) with a description of *L. succulenta* sp. nov. from Korea. *Journal of Applied Phycology* 18: 679–697.
- NAM K.W. 2007. Validation of the generic name *Palisada* (Rhodomelaceae, Rhodophyta). *Algae* 22: 53–55.
- NAM K.W. & SAITO Y. 1991. Anatomical characteristics of *Laurencia papillosa* (Rhodomelaceae, Rhodophyta) from Guam and Palau. *Micronesica* 24: 87–94.
- NAM K.W. & SAITO Y. 1995. Vegetative and reproductive anatomy of some *Laurencia* (Ceramiales, Rhodophyta) species with a description of *L. maris-rubri* sp. nov. from the Red Sea. *Phycologia* 34: 157–165.
- NAM K.W. & SOHN C.H. 1994. *Laurencia kangjaewonii* sp. nov. (Ceramiales, Rhodophyta) from Korea. *Phycologia* 33: 397–403.
- NAM K.W., MAGGS C.A. & GARBARY D.J. 1994. Resurrection of the genus *Osmundea* with an emendation of the generic delineation of *Laurencia* (Ceramiales, Rhodophyta). *Phycologia* 33: 384–395.
- NAM K.W., MAGGS C.A., MCIVOR L. & STANHOPE M.J. 2000. Taxonomy and phylogeny of *Osmundea* (Rhodomelaceae, Rhodophyta) in Atlantic Europe. *Journal of Phycology* 36: 759–772.
- NCBI GENBANK. 2003. <http://www.ncbi.nlm.nih.gov> (1 October 2005).
- POSADA D. & CRANDALL K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- RABENHORST L. 1847. *Deutschlands Kryptogamen Flora. Zweiter Band. Zweiter Abteilung: Algen*. Leipzig. 216 pp.
- SAITO Y. 1967. Studies on Japanese species of *Laurencia*, with special reference to their comparative morphology. *Memoirs of the Faculty of Fisheries, Hokkaido University* 15: 1–81.
- SENTÍES A. & DÍAZ-LARREA J. 2008. Proposals of *Palisada poiteaui* var. *scemmifera* comb. nov. and *Palisada corallopsis* comb. nov. (Rhodomelaceae, Rhodophyta). *Botanica Marina* 51: 69–70.
- SILVA P., BASSON P.W. & MOE R.L. 1996. *Catalogue of the benthic marine algae of the Indian Ocean*, vol. 79. University of California Press, Berkeley. 1259 pp.
- SWOFFORD D.L. 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, version 4. Sinauer Associates, Sunderland, MA, USA.
- THOMPSON J.D., HIGGINS D.G. & GIBSON T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.
- TSUDA R.T. & ABBOTT I.A. 1985. Collecting, handling, preservation, and logistics. In: *Handbook of phycological methods*, Vol. 4. *Ecological field methods: macroalgae* (Ed. by M.M. Littler & D.S. Littler), pp. 67–86. Cambridge University Press, Cambridge, UK.
- WYNNE M.J., SERIO D., CORMACI M. & FURNARI G. 2005. The species of *Chondrophycus* and *Laurencia* (Rhodomelaceae, Ceramiales) occurring in Dhofar, the Sultanate of Oman. *Phycologia* 44: 497–509.
- YAMADA Y. 1931. Notes on *Laurencia*, with special reference to the Japanese species. *University of California Publications in Botany* 16: 185–310.
- ZANARDINI G. 1847. Notizie intorno alle cellulari marine delle lagune e de' litorali di Venezia. *Atti del Reale Istituto Veneto di Scienze, Lettere ed Arti* 6: 185–262.

Received 1 April 2008; accepted 31 October 2008

Associate editor: David Ballantine