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Osmundea sanctarum sp. nov. (Ceramiales, Rhodophyta) from the southwestern Atlantic Ocean

RENATO ROCHA-JORGE[1,6], VALÉRIA CASSANO[2], MARIA BEATRIZ BARROS-BARRETO[3], JHOANA DÍAZ-LARREA[4], ABEL SENTÍES[4], MARIA CANDELARIA GIL-RODRÍGUEZ[5] & MUTUE TOYOTA FUJII[6,7]

[1]*Post-Graduate Program "Biodiversidade Vegetal e Meio Ambiente", Instituto de Botânica, Av. Miguel Estéfano, 3687, 04301-902 São Paulo, SP, Brazil.*
[2] *Departamento de Botânica, Universidade de São Paulo, Rua do Matão 277, 05508-900 São Paulo, SP, Brazil.*
[3]*Departamento de Botânica, Universidade Federal do Rio de Janeiro, Av. Prof. Rodolpho Rocco 211, CCS, bloco A, subsolo, sala 99, 21941-902 Rio de Janeiro, RJ, Brazil.*
[4]*Departamento de Hidrobiología, Universidad Autónoma Metropolitana-Iztapalapa, A.P. 55-535, 09340 Mexico, D.F.*
[5]*Departamento de Biología Vegetal (Botánica), Universidad de La Laguna, 38071. La Laguna, Tenerife, Islas Canarias, Spain.*
[6]*Núcleo de Pesquisa em Ficologia, Instituto de Botânica, Av. Miguel Estéfano, 3687, 04301-902 São Paulo, SP, Brazil.*
[7]*Author for correspondence. E-mail: mutue.fujii@pq.cnpq.br*

Abstract

An ongoing phycological survey in the Laje de Santos Marine State Park (LSMSP) of São Paulo in southeastern Brazil revealed a previously undescribed species of *Osmundea* (Rhodophyta, Rhodomelaceae), which was found in the subtidal zone at a depth of 7 to 20 m. Morphological studies conducted on *Osmundea* specimens collected in the LSMSP revealed characteristics typical of the genus *Osmundea*, including two pericentral cells per each axial segment and tetrasporangia cut off randomly from cortical cells. The phylogenetic position of this species was inferred by analysis of chloroplast-encoded *rbc*L gene sequences from 37 taxa, including one Rhodomelaceae and one Ceramiaceae as outgroups. The Brazilian species of *Osmundea* formed a well-supported clade with the 'Spectabilis' group from the coast of Pacific North America, composed of *O. spectabilis*, *O. blinksii*, *O. splendens* and *O. sinicola*. The present species, however, diverged greatly from the 'Spectabilis' group representatives (5.4–7.1%), which confirms that it constitutes a different taxonomic entity, herein proposed as the new species *Osmundea sanctarum* M.T. Fujii & Rocha-Jorge. This is the first report of a member of *Osmundea* related to the 'Spectabilis' group in the South Atlantic Ocean.

Key words: Laje de Santos, *Laurencia* complex, phylogeny

Introduction

The genus *Osmundea* Stackhouse (1809: 56, 79–80), which had been treated as a *nomen rejiciendum* and as an earlier facultative synonym of *Laurencia* J.V. Lamouroux (1813: 130) (Papenfuss 1947), was resurrected to accommodate members of *Laurencia sensu lato* that demonstrated morphological characteristics not shared by other members of the *Laurencia* complex, such as the production of filament-type rather than trichoblast-type spermatangial branches and the production of tetrasporangia from random cortical cells rather than particular pericentral cells. Furthermore, *Osmundea* is distinguished from *Laurencia sensu stricto* because it possesses two rather than four pericentral cells in each vegetative axial segment (Nam *et al*. 1994).

Currently, the *Laurencia* complex includes six genera: *Laurencia*, *Osmundea*, *Chondrophycus* (Tokida & Y. Saito in Saito 1967: 72) Garbary & J.T. Harper (1998: 194), *Palisada* K.W. Nam (2007: 53), *Yuzurua* (K.W. Nam 1999: 467) Martin-Lescanne (2010: 59) (Martin-Lescanne *et al*. 2010) and the most recently established

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genus *Laurenciella* Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira *&* M.T. Fujii (2012a: 354). Several morphological, vegetative and reproductive characteristics used in discriminating members of the complex have been shown to have diagnostic value only at the generic level (Saito 1967, Nam *et al*. 1994, Garbary & Harper 1998, Nam 1999, 2006). Most species have extensive morphological variation, which makes taxonomic delimitation difficult. In this context, the use of molecular markers has proven to be useful in delimiting taxa and inferring their phylogenetic relationships. In particular, *rbc*L sequence analyses have clearly corroborated the taxonomic distinction of genera within the *Laurencia* complex. The genus *Osmundea* is strongly supported as a monophyletic clade. Recent studies, including the present one, have been able to deduce phylogenetic relationships, describe new taxa and solve several taxonomic problems previously existing for the complex (Nam *et al*. 2000, McIvor *et al*. 2002, Abe *et al.* 2006, Fujii *et al*. 2006, Díaz-Larrea *et al.* 2007, Cassano *et al*. 2009, 2012a, b, Gil-Rodríguez *et al*. 2009, Martin-Lescanne *et al*. 2010, Rocha-Jorge *et al*. 2010).

Osmundea has been considered the only genus of the *Laurencia* complex that has a geographically disjunct distribution, as it occurs in the North American Pacific (Saito 1969, Nam *et al*. 1994, Nam & Choi 1999), Asia (Atmadja & Prud'homme van Reine 2012), Atlantic Europe (Maggs & Hommersand 1993, Nam *et al*. 1994, 2000, Nam & Choi 2000), Mediterranean Sea (Furnari & Serio 1993a, b, Maggs & Hommersand 1993, Cormaci *et al*. 1994, Nam *et al*. 1994, 2000, Serio *et al*. 1999, 2008, Furnari *et al*. 2001, McIvor *et al*. 2002, Taskin *et al*. 2008), Indian Ocean (Sahoo *et al.* 2001, Wynne *et al.* 2005), Australia and surrounding islands (Lewis 1984, Millar *et al.* 1999, Bostock & Holland 2010), eastern Atlantic Islands (Gil-Rodríguez *et al*. 2003) and Atlantic South America (Yoneshigue-Valentin *et al*. 2003, García-Ortiz 2006), whereas all registers of *Osmundea* are in Brazil: *Osmundea lata* (M.A.Howe & W.R.Taylor 1931: 21) Y.Yoneshigue-Valentin, M.T.Fujii, & C.F.Gurgel (2003: 301–304), *O. hybrida* (A.P. de Candolle in Lamarck & De Candolle 1805: 30) K.W.Nam in Nam, Maggs & Garbary (1994: 393) and *O. pinnatifida* (Hudson 1778: 473) Stackhouse (1809: 79).

The Laje de Santos Marine State Park (LSMSP) in Santos, southeastern Brazil, is the only marine park in the state of São Paulo and was established in 1993 as a protected area of high biological diversity (Neves 1997, Amado Filho *et al.* 2006, Rocha-Jorge *et al*. 2012). The park is situated in a transition zone between tropical and sub-tropical regions and occupies an area of c. 5,000 ha formed by a consolidated substrate, Laje de Santos, a large-rock formation (33 m high, 550 m long and 185 m wide), a pair of cliffs called Calhaus and four reefs (Brilhante, Bandolim, Sul and Novo) (Fig. 1). The hard substrate available for macroalgal development extends to a depth of 40 m and is an important habitat that supports a high density and diversity of marine life (Neves 1997, Rocha-Jorge *et al*. 2012). The LSMSP is under the influence of the South Atlantic Central Water (SACW), which is a cold water mass. In the summer season, a local upwelling current results in the intrusion of the SACW into shallow waters rich in nutrients, thus generating a strong thermocline (Machado *et al.* 2003, Miranda *et al.* 2003). It is therefore possible to find in the park some genera that predominantly occur in colder waters (Amado Filho *et al.* 2006, Rocha-Jorge *et al*. 2012).

Since the 1990s, the phycological flora of the park have been inventoried, and 184 taxa have been recorded (eight new occurrences for Brazil, two for the South Atlantic and one for western Atlantic), which represents 52% of the taxa described for the state of São Paulo and 25% of the taxa described for the whole Brazilian coast, thereby confirming the richness of macroalgae in the area (Amado Filho *et al.* 2006, Rocha-Jorge *et al.* 2012).

In this paper, a new species of *Osmundea* from the southwestern Atlantic Ocean is described based on morphological characteristics and chloroplast-encoded *rbc*L gene sequences.

Materials and methods

Specimens were collected at Laje de Santos Marine State Park, c. 32 km from the city of Santos, which has the largest port in Latin America. Samples were collected using SCUBA from the Sul reef (24° 19' 36.6" S, 46° 11'

07.8” W) at a depth of 7 to 15 m and in the Laje de Santos (24° 19’ 2.5” S, 46° 10’ 54.8” W) at a depth of 7 to 20 m. The geographical positions were recorded by GPS using WGS84 datum.

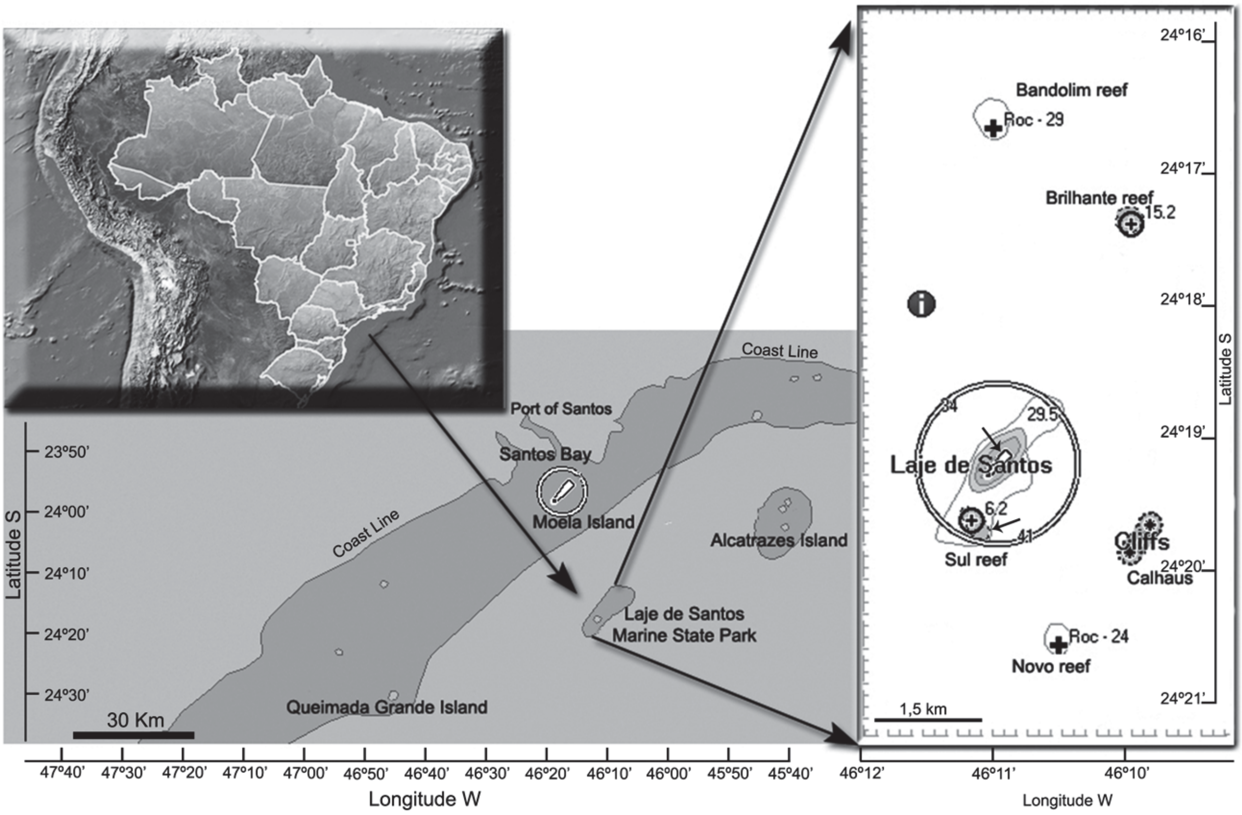


FIGURE 1. Southern coast of São Paulo State, showing the Laje de Santos Marine State Park, including bathymetric lines (20 m depth), Laje de Santos, Calhaus and four reefs (Brilhante, Bandolim, Sul and Novo). The arrows show the collection sites.

Morphological study: Voucher specimens and material for morphological studies were fixed in 4% formalin/seawater or pressed as herbarium sheets. Transverse and longitudinal hand-sections were obtained with a razor blade and stained with 0.5% aqueous aniline blue solution acidified with 1 N HCl. The microscopic measurements were obtained using a calibrated ocular micrometer. Photomicrographs were obtained using a Zeiss Axiocan ERC–5S digital camera (Göttingen, Germany) coupled to an Axioskop 2 Zeiss microscope (Göttingen, Germany) and Stemi SV6 Zeiss stereomicroscope (Göttingen, Germany). The vouchers were deposited in MICH, SP, SPF, TFC and UAMIZ. The herbarium abbreviations follow Holmgren & Holmgren (2012).

Molecular analysis: The samples used for molecular analysis were dried in silica gel. The total DNA was extracted, after the material was ground in Precellys tissue homogenizers (Bertin Technologies, France), by using a DNeasy Plant Mini Kit (Qiagen, Valencia—California, USA) according to the manufacturer’s instructions. In total, 37 sequences were analyzed, including two newly generated *rbc*L sequences and the rest obtained from GenBank, with one species of Ceramiaceae and one Rhodomelaceae as outgroups (Table 1). In total, 1,467 bp (base pairs) of the *rbc*L gene was amplified in three parts, i.e., FrbcLstart-R753, F577-R1150 and F753-RrbcS (Freshwater & Rueness 1994) by using a polymerase chain reaction (PCR) master mix (Bioneer Premix, Daedeok-Gu, Daejeon-Korea). All PCR products were run on a 1% agarose gel to check product size. The PCR products were purified with MicroSpinTMS-300 HR Columns (GE Healthcare Life Sciences, Piscataway, NJ, USA) in accordance with the manufacturer’s instructions. Sequencing was conducted using a BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, New Jersey-USA) on an ABI PRISM3100 Genetic Analyzer (Applied Biosystems, Branchburg, NJ, USA). The primers used for sequencing were the same as the primers used for the amplification.

TABLE 1. List of species used for phylogenetic analyses, with their collection data and GenBank accession numbers.

Species	Collection data	GenBank no.	Reference
Out groups			
Bostrychia radicans (Montagne 1840: 198) Montagne (1842a: 661)	St. Louis Bay, Massachusetts, USA; 11 February 1998	AF259497	Lin *et al.* (2001)
Ceramium brevizonatum H.E. Petersen (1918: 14)	Laguna de Yulcapeten, Campeche Bay, Mexico; 12 February 1998	AF259491	Lin *et al.* (2001)
Chondrophycus			
Chondrophycus cf. *undulatus*	New Caledonia, Loyalty, Marè; 22 March 2005	FJ785307	Martin-Lescanne *et al.* (2010)
Chondrophycus sp.1	New Caledonia, Loyalty, Lifou; 26 March 2005	FJ785309	Martin-Lescanne *et al.* (2010)
Chondrophycus sp.2	New Caledonia, Loyalty, Marè; 21 March 2005	FJ785310	Martin-Lescanne *et al.* (2010)
Chondrophycus sp.3	New Caledonia, Loyalty, Beautemps/ Beauprè; 6 April 2005	FJ785311	Martin-Lescanne *et al.* (2010)
Laurencia			
L. obtusa (Hudson 1778: 586) J.V. Lamouroux (1813: 130)	Fanad Head, Donegal, Ireland; 6 December 1998	AF281881	Nam *et al.* (2000)
L. intricata J.V.Lamouroux (1813: 131)	Cayo Coco, Ciego de Ávila, Cuba; 25 September 2005	GU330238	Cassano *et al.* (2012b)
L. dendroidea J. Agardh (1852: 753)	Praia do Velho, Angra dos Reis, Rio de Janeiro, Brazil, 20 July 2006	GU330232	Cassano *et al.* (2012b)
L. natalensis Kylin (1938: 24)	Palm Beach, Kwa-Zulu Natal, South Africa; 7 February 2001	AF465816	Fujii *et al.* (2006)
L. oliveirana Y. Yoneshigue-Valentin (1985: 329)	Ponta da Cabeça, Arraial do Cabo, Rio de Janeiro, Brazil; 7 July 2008	JF810352	Cassano *et al.* (2012a)
Laurenciella			
L. marilzae (Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M.T. Fujii 2009: 265) Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M.T. Fujii in Cassano, Oliveira, Gil-Rodríguez, Sentíes, Díaz-Larrea & Fujii (2012: 354)	Pta. del Hidalgo, Tenerife, Canary Islands, Spain; 12 July 2006	EF686002	Gil-Rodríguez *et al.* (2009)
L. marilzae	Laje de Santos Marine State Park, Parcel do Sul, São Paulo, Brazil; 25 March 2007	GU938189	Rocha-Jorge *et al.* (2010)
L. marilzae	Isla Mujeres, Quintana Roo, Mexico; 16 November 2008	HQ115065	Sentíes *et al.* (2011)
Osmundea			
O. spectabilis var. *spectabilis*	Punta Santo Thomas, Baja California, Mexico; 2 July 1996	AY172574	McIvor *et al.* (2002)
O. spectabilis var. *diegoensis* (E.Y. Dawson 1944: 236) K.W. Nam in Nam, Maggs & Garbary (1994: 393)	Point Loma, California, USA; 7 July 1999	AY172572	McIvor *et al.* (2002)
O. blinksii	Año Nuevo, California, USA; 17 July 1996	AY172575	McIvor *et al.* (2002)
O. splendens	Bahia Colnett, Baja California, Mexico; 2 July 1996	AY172576	McIvor *et al.* (2002)
O. sinicola	Crescent Beach, California, USA; 20 May 2002	AY588407	Fujii *et al.* (2006)
O. truncata (Kützing 1865: 19) K.W. Nam & Maggs in Nam, Maggs & Garbary (1994: 393)	Long Hyne, Cork, Ireland; 11 November 1999	AF281879	Nam *et al.* (2000)

...... *continued on the next page*

TABLE 1 (continued)

Species	Collection data	GenBank no.	Reference
O. oederi (Gunnerus 1772: 100) G.Furnari in Serio, Petrocelli, Cormaci, Cecere & G. Furnari (2008: 124)	St. John's Point, Donegal, Ireland; 12 October 1999	AF281880	Nam *et al.* (2000)
O. osmunda (S.G. Gmelin 1768: 155) K.W. Nam & Maggs in Nam, Maggs & Garbary (1994: 393)	St. John's Point, Donegal, Ireland; 12 October 1999	AF281877	Nam *et al.* (2000)
O. hybrida	St. Lunaire, Brittany, France; 20 March 1999	FJ785317	Martin-Lescanne *et al.* (2010)
O. pinnatifida	Penmarch, Brittany, France	AF259495	Lin *et al.* (2001)
O. pinnatifida	St. John's Point, Donegal, Ireland; 12 October 1999	AF281875	Nam *et al.* (2000)
O. pinnatifida	St. John's Point, Donegal, Ireland; 12 October 1999	AF281876	Nam *et al.* (2000)
O. pinnatifida	Roscoff, France; 22 July 1999	AY172571	McIvor *et al.* (2002)
O. sanctarum sp. nov.	Laje de Santos Marine State Park, Laje de Santos, São Paulo, Brazil; 19 April 2012	KC012600	This study
O. sanctarum sp. nov.	Laje de Santos Marine State Park, Parcel do Sul, São Paulo, Brazil; 19 August 2008	KC012601	This study
Palisada			
P. perforata (Bory de Saint-Vincent 1803: 305) K.W. Nam (2007: 54)	Isla Mujeres, Cancún, Quintana Roo, Mexico; 2 March 2007	EF658641	Cassano *et al.* (2009)
P. patentiramea (Montagne 1836: 322) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii (2009: 95)	Phillippines	AF489862	A.O. Luisma (unpublished)
P. corallopsis (Montagne 1842b: 9) Sentíes, Fujii & Díaz in Sentíes & Díaz-Larrea (2008: 69)	Punta Brava, Puerto Morales, Quintana Roo, Mexico; 18 April 2004	EF061646	Díaz-Larrea *et al.* (2007)
P. flagellifera (J. Agardh 1852: 747) K.W. Nam (2007: 54)	Playa Paraíso, Tenerife, Canary Islands, Spain; 16 July 2006	EF685998	Gil-Rodríguez *et al.* (2010)
P. flagellifera	Praia Brava, Ubatuba, São Paulo, Brazil; 25 May 2001	AF465804	Fujii *et al.* (2006)
Yuzurua			
Y. poiteaui var. *gemmifera* (Harvey 1853: 73) M.J. Wynne (2011: 98)	Rincón de Guanabo, La Habana, Cuba; 29 July 2005	EF061650	Díaz-Larrea *et al.* (2007)
Y. poiteaui var. *gemmifera*	Puerto Morelos, Quintana Roo, Mexico; 16 April 2004	EF061648	Díaz-Larrea *et al.* (2007)
Y. poiteaui (J.V. Lamoroux 1805: 63–64) Martin-Lescanne in Martin-Lescanne e*t al.* (2010: 59) var. *poiteaui*	Long Key, Ocean Side, Florida, USA; 1998	EF061652	Díaz-Larrea *et al.* (2007)

Sequence alignments and phylogenetic analysis: Sequences were obtained for both DNA strands, assembled and corrected using Sequence Navigator (Applied Biosystems, Branchburg, NJ, USA). The sequences were aligned according to the CLUSTAL algorithm (Thompson *et al.* 1994). Maximum parsimony (MP) analyses were performed using PAUP* version 4.0b10 (Swofford 2002) and a heuristic search with 1,000 random additions, unordered and unweighted characters, with tree bisection-reconnection branch swapping in effect. The model used in the Bayesian analysis (GTR + I + G) was selected based on maximum likelihood (ML) ratio tests implemented in Modeltest version 3.7 (Posada & Crandall 1998) with a significance level of 0.01 by the Akaike Information Criterion. ML analysis was conducted with TOPALi v2 (Milne *et al*. 2009) using the parameter values estimated during the run. ML and MP analyses were subjected to bootstrap resampling (1,000 replicates and 1,000 replicates with 10 random additions, respectively) to estimate robustness (Felsenstein 1985). MrBayes version 3.1 (Huelsenbeck & Ronquist 2001) was used to complete Bayesian inference. The analyses were run with four heated Monte–Carlo Markov Chains for 2,000,000 generations. Output trees and data were sampled every 100 generations. Appropriate burn-in for each run was determined by plotting the overall likelihood against generations prior to estimation of the posterior probability distribution. In all analyses, likelihood values were stable after the first 150,000 generations.

Results

Osmundea sanctarum M.T. Fujii & Rocha-Jorge, *sp. nov.* (Figs. 2–12, Table 2)

Thalli are red–purple with axes terete, up to 2.5 cm high, cartilaginous in texture and attached to the substratum by a discoid holdfast. Secondary attachments are present. Stoloniferous branches are absent. Thalli are subterete to compressed, 0.92–1.40 mm wide in the middle portions and cylindrical at the bases with a diameter of 0.96–1.30 mm, and the ultimate branches have a diameter of 0.36–0.43 mm. Branching is sparse, alternate to irregular, and not pinnate. Anastomoses are occasionally present (Figs. 2, 3). First-order branches have a diameter of 0.36–0.63 mm at their bases, 0.55–0.80 mm wide at the middle portions and 0.30–0.40 mm at the apices. Fresh specimens have a strong chemical odor. Transverse sections of the thallus display two cortical layers (Figs. 4–7). The outer layer is composed of translucent (hyaline) cells smaller (diameter of 6–10 μm) than the cells in the inner layer, which has rounded pigmented cells with a diameter of 12–18 μm. (Figs. 6–8). Cortical cells do not form a palisade-like layer, and the secondary pit connections are absent in the outermost cortical cells. Medullary cells are rounded and have a diameter of 15–25 μm. Each vegetative axial segment cuts off two pericentral cells; the first pericentral cell is produced on the side of the trichoblast basal cell (Fig. 5). 'Corps en cerise' and lenticular thickenings are absent in living material. Longitudinal sections present cortical cell walls near apices that do not project beyond the surface and apical cells located in depressions from which translucent trichoblasts arise. Tetrasporophytes are composed of simple cylindrical branchlets with a length of 0.3–1.0 mm long and a diameter of 0.1–0.5 mm (Figs. 9, 10). Tetrasporangia are cut off randomly from the innermost cortical cells and have a diameter of 50–80 μm (Figs. 11, 12). Gametophytes were not found.

Type:—BRAZIL. São Paulo: Laje de Santos Marine State Park, 24° 19' 2.5" S, 46° 10' 54.8" W, 18 m depth, coll. *R. Rocha-Jorge, M.B. Barros-Barreto, I.B. Silva & M.T. Fujii*, 19 April 2012 (holotype SP! 427820. Isotypes MICH!, SPF!, TFC!, UAMIZ!).

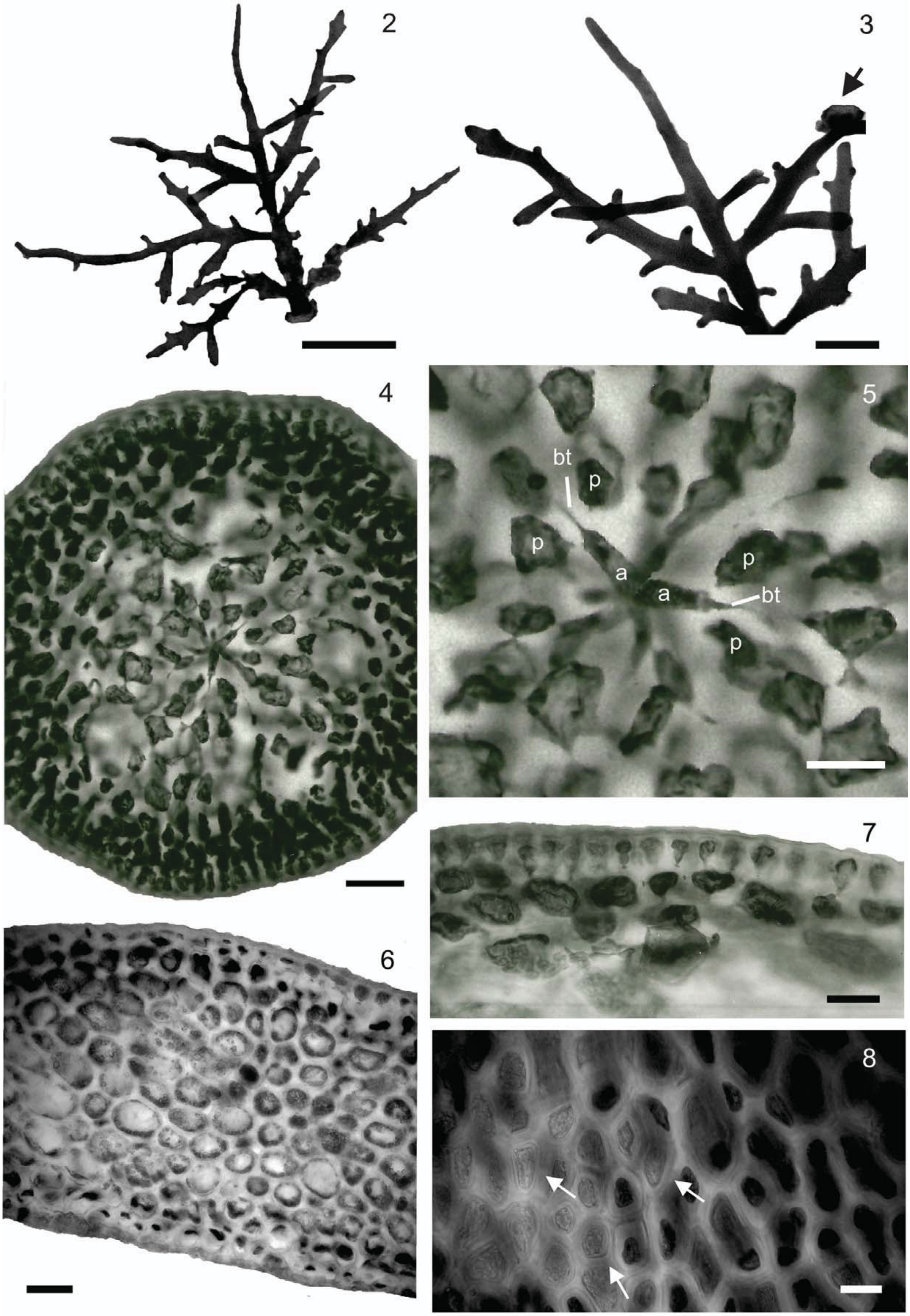
Paratypes:—Laje de Santos Marine State Park, Sul reef (24° 19' 36.6" S and 46° 11' 7.8" W) SP! 400134 (as *Osmundea* sp.); Laje de Santos Marine State Park, Laje de Santos (24° 19' 2.5" S and 46° 10' 54.8" W) SP! 400137, 400547, 427821 (all as *Osmundea* sp.).

Distribution and habitat:—Laje de Santos Marine State Park, in Sul reef and Laje de Santos (large rock formation), growing in the subtidal zone (depth of 7–20 m) on rocky substrates associated with other macroalgae species.

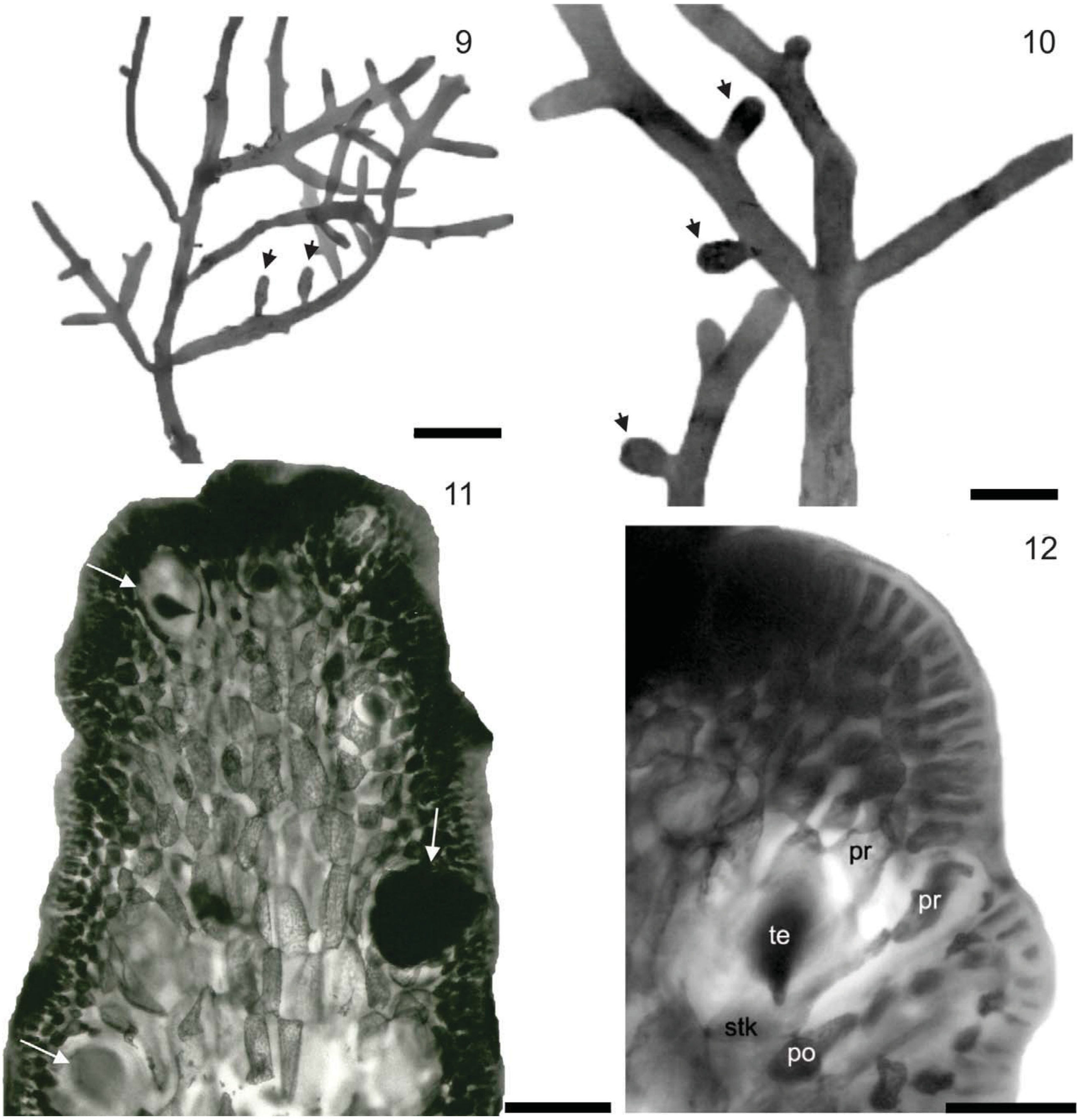
Etymology:—The specific epithet *sanctarum*, Latin for "of the saints," is derived from the type locality, *i.e.*, Laje de Santos Marine State Park, São Paulo, Brazil.

Phylogenetic analyses:—Parsimony analysis of our dataset produced two equally parsimonious trees, which were 153 steps long and had a consistency index of 0.6. The dataset for *rbc*L sequences consisted of 700 constant characteristics and 417 parsimony-informative sites.

The topology of the Bayesian tree (Fig. 13) indicated that the Brazilian *Osmundea* samples formed a distinctive and highly supported monophyletic clade with a sister clade that included samples of *O. blinksii* (Hollenberg & I.A. Abbott 1965: 1186) K.W. Nam in Nam, Maggs & Garbary (1994: 393), *O. sinicola* (Setchell & N.L. Gardner 1924: 764) K.W. Nam in Nam, Maggs & Garbary (1994: 393), *O. splendens* (Hollenberg in Smith & Hollenberg 1943: 219) K.W. Nam in Nam, Maggs & Garbary (1994: 393) and *O. spectabilis* (Postels & Ruprecht 1840: 16) K.W. Nam in Nam, Maggs & Garbary (1994: 393). The specimens of *Osmundea* from Laje de Santos and Parcel do Sul (São Paulo) are molecularly and morphologically identical, which confirms that these samples constitute the same taxonomic entity. The mentioned samples diverged at a high level of genetic variation from the *Osmundea spectabilis* group (5.4–7.1%), which supports their recognition as a new taxon within the genus *Osmundea.*



FIGURES 2–8. *Osmundea sanctarum*: vegetative morphology. Fig. 2. Representative plant. Holotype (SP! 427820) (5 mm). Fig. 3. Detail of a branch with secondary small holdfast (arrow) (2 mm). Fig. 4. Transverse section of the upper portion of a branch (100 µm). Fig. 5. Transverse section showing two axial cells (a), each of which has two pericentral cells (p) and a basal trichoblast cell (bt) (50 µm). Fig. 6. Transverse section of main axes (100 µm). Fig. 7. Detail of transverse section of a branch showing external cortical layer with translucent cells (25 µm). Fig. 8. Translucent cortical cells in surface view (arrows) (25 µm).



FIGURES 9–12. Reproductive morphology of tetrasporangial *Osmundea sanctarum* plants (SP! 427821). Fig. 9. Tetrasporangial plant showing fertile branchlets (arrows) (3 mm). Fig. 10. Detail of tetrasporangial branchlets (arrows) (1 mm). Fig. 11. Longitudinal section through an apical portion of tetrasporangial branchlet showing tetrasporangia (arrows) originating from cortical cells (100 µm). Fig. 12. Detail of a stalk cell (stk) with two pre-sporangial cover cells (pr), tetrasporangium (te) and one post-sporangial cover cell (po) (250 µm).

Remarks:—Morphological comparisons of *O. sanctarum* with other species of *Osmundea* are shown in Table 2. The interspecific divergence values obtained in this study were similar to those previously reported for the *Laurencia* complex (Nam *et al*. 2000, McIvor *et al*. 2002, Díaz-Larrea *et al*. 2007, Cassano *et al*. 2009, 2012a, b, Martin-Lescanne *et al*. 2010, Rocha-Jorge *et al*. 2010).

Discussion

To date, 17 species and one variety of *Osmundea* have been recognized globally (Guiry & Guiry 2013), but only three species, *i.e.*, *O. lata*, *O. hybrida* and *O. pinnatifida* have been reported from the Brazilian coast, as mentioned above. *Osmundea hybrida* and *O. pinnatifida* were recorded in Brazil in the 19th century and, since then, no other specimens of these taxa have been collected again, and there is no mention of herbaria voucher where these specimens can be checked; therefore, these early reports must be treated as uncertain records (Taylor 1960, Oliveira Filho 1977, Fujii & Sentíes 2005, Fujii *et al.* 2011). The new species described in the present study diverges, genetically, by 6.5% from *O. pinnatifida* and 6.4% from *O. hybrida*.

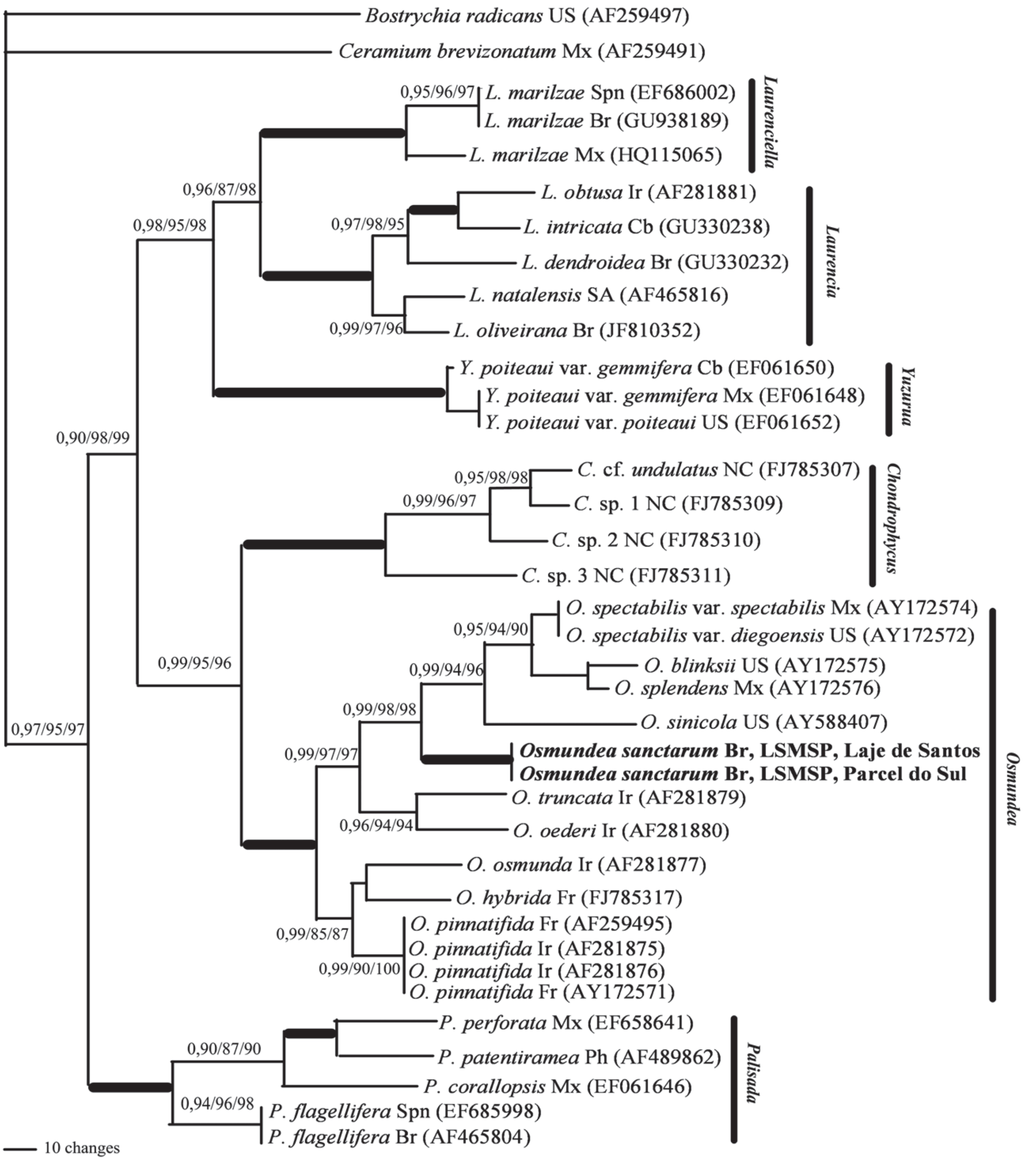


FIGURE 13. Bayesian phylogram inferred from analyses of *rbc*L sequences for 35 *Laurencia* complex taxa and two outgroup species. Numbers above branches correspond to support values for Bayesian inference posterior probability/maximum likelihood bootstrap/ and maximum parsimony bootstrap, respectively. Bold lines indicate a fully supported node in all three analyses. Taxa marked in bold indicate newly determined sequences.

Morphologically, *Osmundea sanctarum* has no radial branching and thus differs from *O. hybrida*, and it has non-pinnate fronds, lacks stoloniferous branches and lacks annular and lenticular thickenings. In contrast, *O. pinnatifida* has pinnate fronds attached to the substratum by stoloniferous branches and may contain annular and lenticular thickenings. It is possible that the *O. pinnatifida* specimens reported previously in Brazil were, in fact, specimens of *O. sanctarum* because in morphological analyses, such as those performed in the 19th century according to knowledge available at the time, these taxa could have been confused. None of the authors (Saint-Hilaire 1833, Martius 1833 or Montagne 1846) provided a detailed description or any illustrations for *O. pinnatifida* from Brazil (Table 2).

Osmundea lata was originally described as *Laurencia lata* by Howe & Taylor (1931) from material collected during the Hassler Expedition near the Cabo Frio, north of Rio de Janeiro state. Later, in a scientific kelp expedition on the Brazilian coast, fertile specimens of *O. lata* (as *Laurencia lata*) were found growing as epiphytes on rhizoids of *Laminaria abyssalis* A.B. Joly & E.C. Oliveira Filho (1967: 7) dredged from a 60 m depth on the continental shelf. The morphological features of the specimens allowed the transfer of the species from *Laurencia* to *Osmundea* (Yoneshigue-Valentin *et al*. 2003). *Osmundea lata* differs from *O. sanctarum* in its possession of secondary pit connections between outermost cortical cells, absence of translucent cortical cells and dense, alternate/distichous branching (Table 2).

Compared with other *Osmundea* species mentioned for the Atlantic Ocean (Nam *et al.* 2000, Nam & Choi 2000, Gil-Rodríguez *et al*. 2003, Taskin *et al.* 2008), only *O. crispa* (Hollenberg in Smith & Hollenberg 1943: 219) K.W. Nam in Nam, Maggs & Garbary (1994: 393), *O. pelagiensis* G. Furnari in Cormaci, Furnari & Serio (1994: 366), *O. maggsiana* Serio, Cormaci & G. Furnari (1999: 277) and *O. splendens* have cortical cells without secondary pit connections in the outermost layer, and lenticular thickening is absent. McIvor *et al.* (2002) mentioned that pit connections were rare for *O. splendens*, but Saito (1969), Abbott & Hollenberg (1976) and Nam *et al.* (2000) described that pit connections were absent for this taxon. *Osmundea maggsiana* and *O. crispa* have entirely cylindrical thalli and radial branching that forms dense compact masses and are thus different from *O. sanctarum,* which has compressed thalli (cylindrical only at the base) and sparse branching. *Osmundea maggsiana* has cortical cells arranged in palisade-like manner, which is not observed in *O. sanctarum*. *Osmundea pelagiensis* has stoloniferous portions, whereas *O. sanctarum* lacks this characteristic. Finally, *O. splendens*, similar to the other three taxa mentioned above, has no translucent cortical cells (Table 2).

Within the genus *Osmundea,* there are two types of spermatangial structures: cup-shaped and pocket-shaped (Nam *et al.* 1994). The Pacific North American species of *Osmundea* in the 'Spectabilis' group, *i.e.*, *O. spectabilis*, *O. blinksii, O. sinicola, O. splendens*, *O. multibulba* (E.Y. Dawson, Neushul & Wildman 1960: 28) K.W. Nam in Nam, Maggs & Garbary (1994: 393) and *O. crispa*, possess only the pocket-shaped spermatangial pits, whereas the species from the Atlantic and Mediterranean coasts display either cup-shaped or pocket-shaped spermatangial pits (Nam *et al.* 2000, Yoneshigue-Valentin *et al*. 2003). Previous phylogenetic studies based on the *rbc*L gene demonstrated a clear divergence between these two groups (McIvor *et al*. 2002, Abe *et al*. 2006, Fujii *et al*. 2006, Martin-Lescanne *et al*. 2010). Tetrasporangial reproductive structures of *O. sanctarum* have rarely been observed, and female and male specimens have never been found since the first collection of this species in 2001. Consequently, we are unable to describe the spermatangial pit type.

The present report represents the first time that an *Osmundea* related to the 'Spectabilis' group has been reported for the South Atlantic Ocean. This finding supports that genus *Osmundea* has a geographically disjunct distribution, as mentioned above.

The high levels of genetic variation and morphological differences between the *Osmundea* samples from Brazil and the rest of the *Osmundea* species suggest that this species should be assigned to a new taxon within the genus *Osmundea*, herein designated as *Osmundea sanctarum* M.T. Fujii & Rocha-Jorge.

TABLE 2: Comparative morphological data on *Osmundea* spp.

Species	Thallus	Type of attachment	Branching	Secondary pit connection	Lenticular thickening	Cortical cells (arrangement)	Translucent outermost cortical cells	Spermatangial receptacles	References
O. spectabilis	Compressed	Discoid holdfast	Alternate	Present	Absent	Non-palisade	Absent	Pocket	Nam *et al.* (1994)
O. blinksii	Compressed	Stoloniferous holdfast	Alternate-distichous	Absent/ Rare*	Absent	Non-palisade	Absent	Pocket	Saito (1969), Abbott & Hollenberg (1976), Nam *et al.* (1994, as *L. blinksii*), Nam *et al.* 2000; *McIvor *et al.* 2002
O. splendens	Compressed	Discoid holdfast	Alternate	Absent/ Rare*	Absent	Non-palisade	Absent	Pocket	Saito (1969), Abbott & Hollenberg (1976, as *L. splendens*), Nam *et al.* 2000; *McIvor *et al.* 2002
O. sinicola	Compressed	?	Alternate	Present	Present	Non-palisade	Absent	Pocket	Dawson (1963, as *L. sinicola*), Nam *et al.* (1994)
O. truncata	Compressed/ terete	Discoid holdfast	Alternate	Present	Present	Non-palisade	Absent	Cup	Furnari & Serio (1993a), Maggs & Hommersand (1993, as *L. truncata*), Nam *et al.* (1994)
O. oederi	Subterete to compressed	Discoid holdfast	Alternate	Present	Absent	Non-palisade	Absent	Cup	Nam *et al.* (2000, as *O. ramosissima*), Serio *et al.* (2008)
O. osmunda	Compressed	Discoid holdfast	Alternate-distichous	Absent	Present	Non-palisade	Absent	Pocket	Maggs & Hommersand (1993, as *L. osmunda*), Nam & Choi (2000)
O. hybrida	Terete	Discoid holdfast	Radial/ alternate	Absent	Absent	Non-palisade	Present	Cup	Maggs & Hommersand (1993), Nam & Saito (1994, as *L. hybrida*)
O. pinnatifida	Compressed	Stoloniferous holdfast	Alternate-distichous	Absent	Present	Non-palisade	Present	Pocket	Nam *et al.* (1994), Machín-Sánchez *et al.* (2012)
O. crispa	Terete	?	Radial	Absent	Absent	Non-palisade	?	Pocket	Abbott & Hollenberg (1976), Nam *et al.* (1994), Nam & Choi (1999)
O. lata	Compressed	Discoid holdfast	Alternate-distichous	Present	Absent	Non-palisade	Absent	Cup	Yoneshigue-Valentin *et al.* (2003)
O. maggsiana	Terete	Discoid holdfast	Radial/ alternate	Absent	Absent	Palisade	Absent	Cup	Serio *et al.* (1999)
O. multibulba	Compressed	?	Alternate-distichous	Absent	Absent	Non-palisade	Absent	Pocket	Dawson (1963), Saito (1969), Nam *et al.* (1994, as *L. multibulba*)
O. pelagiensis	Compressed	Stoloniferous holdfast	Alternate	Absent	Absent	Palisade	Absent	Cup	Cormaci *et al.* (1994, as *L. pelagiensis*)
O. pelagosae (Schiffner 1931: 149) K.W. Nam in K.W. Nam, Maggs & Garbary (1994: 393)	Compressed	Discoid holdfast	Alternate-distichous	Present	Present	Non-palisade	Absent	Pocket	Furnari & Serio (1993b, as *L. pelagosae*)
O. verlaquei G.Furnari in Cormaci, Furnari & Serio (1994: 368)	Compressed	Stoloniferous holdfast	Alternate-distichous	Present	Absent	Non-palisade	Absent	Cup	Cormaci *et al.* (1994, as *L. verlaquei*)
O. pedicularioides (Børgesen 1933: 136) G.Furnari, Serio & Cormaci (2004: 455)	Compressed	Discoid holdfast	Alternate-distichous	Absent	Absent	Non-palisade	Absent	?	Furnari *et al.* (2004)
O. sanctarum sp. nov.	Compressed	Discoid holdfast	Alternate	Absent	Absent	Non-palisade	Present	Unknown	Present study

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