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Phylogeography of the Red Algal *Laurencia* Complex in the Macaronesia Region and Nearby Coastal Areas: Recent Advances and Future Perspectives

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Abstract: Since the conception of the genus *Laurencia* by Lamouroux in 1813, several red macroalgal species have been included in it. In recent decades, the development of modern molecular tools has resulted in multiple taxonomic modifications, and presently, eight related genera are recognized in the so-called *Laurencia* complex. In the Macaronesian Region (Central East Atlantic Ocean), species from the *Laurencia* complex are keystone elements of the benthic communities, especially in the intertidal zone. In this review, we consolidate the existing knowledge about the *Laurencia* complex within the Macaronesian archipelagos and nearby areas. Morphological descriptions and phylogeographic remarks of the 16 currently accepted species—whose records were molecular or morphologically confirmed—are included together with an identification key for the Macaronesian Region. The phylogeographic data allowed us to re-visit the role of the Macaronesian archipelagos as a bridge area for the marine flora of the Mediterranean and Caribbean Seas (remnants of the former Thetyan Sea) or contemplate their marine flora as the result of successive processes of recolonization after the Quaternary glaciations from those donor areas. Finally, some comments about the frontiers of the research in the *Laurencia* complex in the Macaronesian Region and nearby coastal areas are included.

Keywords: molecular systematic; *Laurencia*; *Laurenciella*; *Palisada*; *Osmundea*; Azores; Madeira; Selvagens; Canary Islands; Cape Verde; biogeographic relationships

1. Introduction

The genus *Laurencia* (Rhodomelaceae, Ceramiales) was established by Lamouroux [1] with only eight red macroalgal species. During the last century, Yamada [2] made a detailed taxonomic treatment of diverse type materials whereas Y. Saito and co-workers made important contributions to the morphological and reproductive characteristics of several species, mainly from the Pacific coasts [3–9]. Thus, the number of species assigned to *Laurencia* has been growing, and with the arrival of diverse molecular tools in the last decades [10,11], this genus has been subjected to multiple taxonomic modifications. Currently, eight related genera constitute the so-called *Laurencia* complex: *Laurencia sensu stricto* Lamouroux [1], *Osmundea* Stackhouse [8], *Chondrophycus* (Tokida & Saito) Garbary & Harper [12], *Palisada* (Yamada) Nam [13,14], *Yuzurua* (Nam) Martin-Lescanne [15], *Laurenciella* Cassano, Gil-Rodríguez, Sentíes, Díaz-Larrea, M.C. Oliveira & M.T. Fujii [16], *Coronaphycus* Metti [17] and, more recently, *Olehopapa* Rousseau, Martin-Lescanne & Le Gall [18]. Members of these genera are common elements of benthic habitats growing in the intertidal and subtidal zones of tropical, subtropical, and temperate regions [3,4,19–22].

A total of 530 species have been included in the *Laurencia* complex; however, at present time only 213 are accepted taxonomically [22]. The large number of specific names that are considered synonymous (homotypic or heterotypic) or with uncertain taxonomic status is clear evidence of the taxonomic complexity of this red algal group, which has undergone numerous changes throughout its history [18,23,24].

While the genera of the *Laurencia* complex are relatively easy to recognize by their morphological or molecular features, the delimitation at species level presents great difficulty [12,25]. The remarkable morphological plasticity of individuals, the difficulty in observing certain vegetative or reproductive characteristics, and the existence of incomplete and non-uniform species descriptions have contributed to increased un-certainties about the identification, classification, and nomenclature of organisms putatively included in this complex [26,27]. During the last two decades, molecular tools and data have been integrated in several species' delimitation to overcome difficulties associated with classical morphological characterizations. On one hand, molecular taxonomy [28] based on sequences of the plastid-encoded large subunit of RuBisCO (*rbc*L) have been used to identify specimens, describe new species, and infer intra- and inter-specific relationships inside the *Laurencia* complex [15,16,25,29–34]. On the other hand, the DNA-barcoding routine, based on a standardized sequence of the mitochondrial cytochrome c oxidase subunit I gene (COI-5P) [35] has proved to be an effective taxonomic tool to identify and analyse species diversity within the *Laurencia* complex, substantiating identification based on morphological characters [36].

Species within the *Laurencia* complex are common in all Macaronesian archipelagos in the Central East Atlantic Ocean as ecologically important elements, especially in the rocky intertidal zones [16,19,20,33,37,38]. Individuals can dominate the communities at mid and low littoral levels along the coastline, located mainly in dim-light environments, puddles, and cracks of rocky habitats, mainly in coasts exposed to strong–medium wave action.

Macaronesia, from the Greek words *makarion* (fortunate) and *neosi* (islands), is the geographical name given to the group of five archipelagos located in the central-east north Atlantic Ocean: the Azores, Madeira, the Selvagens, the Canary Islands, and the Cape Verde Islands [39] (Figure 1). These archipelagos are composed of oceanic islands of volcanic origin, distributed between the parallels 14.8° N (Brava island, Cape Verde Islands) and 39.7° N (Corvo, Azores) and the meridians 13.4° E (Roque del Este, Canaries) and 30.9° E (Flores, Azores). All these islands and the associated seamounts are the expression of several hotspots of a remarkable volcanic belt off of north-western Africa. The Canary Islands constitute the largest archipelago (744 km^2) and nearest to a continent (97 km from Fuerteventura to Punta Stafford, Western Sahara, Africa), while the Selvagens Islands are the smallest archipelago (4–5 km^2) and oldest (27 Ma), indicating that they are in the last stages of their erosive dismantling process. The Azores is undoubtedly the most isolated archipelago in the region, with 1370 km between the island of San Miguel and Cabo da Rocha in Portugal [40].

The Canary Current is a branch of the Gulf Stream, which normally moves from the Azores through Madeira down to the Canary Islands and the Cape Verde Islands [41]. This oceanic surface current is especially important in the establishment of a connection within the marine biota of the Lusitanian Macaronesian archipelagos and beyond [42]. Differences between the composition and diversity of marine taxa could be explained by the combination of several factors, including the degree of geographical isolation of the islands, which would clarify the similarity between the insular and continental marine flora [43,44]. The Azores, for example, shares elements with the continental coasts of the North Atlantic, Eastern Mediterranean, or Eastern America [45], while the Canary Islands show a greater affinity with the marine flora present in the western Mediterranean and tropical eastern Atlantic shores [46]. Moreover, patterns of species richness and composition of algal assemblages is considerably affected by the insular building size (the space and diversity of potential substrata that can be occupied), as well as by the isolation degree of each archipelago with respect to potential donor areas [44].

As defined by Avise [47], phylogeography is "a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species.” Inside that research field, the overall goal of this review is to complete and consolidate the existing knowledge about the representatives of the *Laurencia* complex within the geographical context of the Macaronesia Region and nearby areas, taking into consideration recent molecular and taxonomic advances. The distributional pattern of those species may contribute to re-visit the role of the Macaronesian archipelagos as a bridge area for the marine flora of the Mediterranean and Caribbean Seas (remnants of the former Thetyan Sea) or as the result of successive processes of recolonization after the Quaternary glaciations from those donor areas. This paper also provides a practical and updated resource which facilitates future tasks, such as checklists of species from Macaronesia and nearby coastal areas, the correction and updating of valid species, or the construction of artificial analytical identification keys.

2. Materials and Methods

The results presented in this review are drawn from the critical analysis of data from: (1) morphological studies of vegetative and reproductive characters of samples collected from the Azores, Madeira, the Selvagens, and the Canary Islands; (2) molecular and morphological analysis of the herbarium material from these archipelagos deposited in TFC, BCM, L, PC and BM (Herbarium abbreviations follow the on-line Index Herbariorum: http://sweetgum.nybg.org/science/ih/ [48], continuously updated) including the study of Type material wherever it was possible; and (3) the review of literature on all taxa in the *Laurencia* complex reported from the Macaronesian Region.

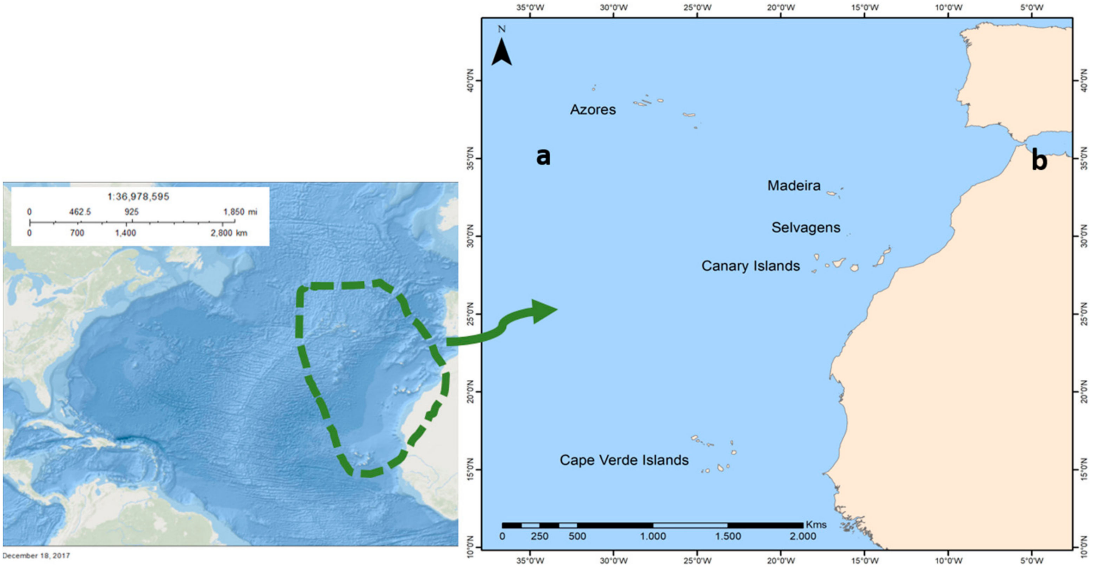

Figure 1. (**a**) Location of the Macaronesian Region in the Central East Atlantic Ocean (modified from European Atlas of the Sea, © European Union 1995–2016); (**b**) Geographic positions of the Macaronesian Archipelagos.

In this sense, the different taxa of the *Laurencia* complex included in Table 1 are based on the information extracted from the literature review, as well as from unpublished results obtained by the authors inside the research project CGL 2010-14881, entitled “*The Laurencia complex in the Atlantic Ocean*” funded by the Ministry of Education (Spanish Government). The accepted species in the different Macaronesian archipelagos is in accordance with an integrative analytical approach done by the authors, taking account of both morphological and molecular data, wherever available for the concerned species. The discrimination criteria used are included in the short description paragraphs of each species.

3. Results

3.1. Checklist of Species Included in the Laurencia Complex from the Azores, Madeira, the Selvagens, and the Canary Islands

The following list of species (Table 1) was created from the information included in the monograph on genera *Laurencia*, *Laurenciella*, and *Palisada* by Gil-Rodriguez et al. [49], from data in Machín-Sánchez et al. [27,36,50], as well as from unpublished results obtained during the research project CGL 2010-14881, entitled "*The Laurencia complex in the Atlantic Ocean*" funded by the Ministry of Education (Spanish Government), where the two first authors were deeply involved.

Thirty-two species of *Laurencia sensu lato* have been included in the floristic lists of the Macaronesian archipelagos; this number varies according to the author to be consulted (see references quoted in Table 1). However, taking account of the current knowledge (including molecular and/or morphological evidence) and the extensive review of the scientific literature, we consider that only 16 species—appearing in bold letters—must be retained within the *Laurencia* complex. On the other hand, records whose presence in the Macaronesia region could not be confirmed by molecular or morphological evidence are appropriately classified as *taxa inquirenda* or *taxa excludenda*.

Taxa inquirenda refers to a taxon whose taxonomic status is uncertain due to: (1) the specific herbarium material referring to the taxon has not been found or could not be reviewed; (2) when the herbarium material was located, it was insufficient or in bad condition for taxonomic confirmation; (3) after several visits to the locality where the taxon was recorded, it was not located because of changes and disturbances in the coast, or not sampling in a suitable period or habitat (i.e., unexplored subtidal habitats) for the taxon.

Taxa excludenda refers to a taxon that should be removed from the list or catalogue of species in the region, since after the revision of herbarium material, it was confirmed that it corresponds to an incorrect identification.

Table 1. Checklist of the *Laurencia* complex species reported from the Macaronesian archipelagos: the Azores (*A*), Madeira (*M*), the Selvagens (*S*) and the Canary Islands (*C*), with **accepted species**, *taxa inquirenda*, and *taxa excludenda*.

Chondrophycus glandulifer (Kützing) Lipkin & P.C. Silva	*S:* John et al. 2004 *Taxa inquirenda*.
	M: Neto et al. 2001 (as *L. glandulifera*); John et al. 2004. *Taxa inquirenda*.
	C: Haroun et al. 2002 (as *Laurencia glandulifera*); John et al. 2004; Gil-Rodríguez et al. 2012. *Taxa inquirenda*.
Laurencia brongniartii J. Agardh	*C:* Haroun et al. 2002. *Taxa inquirenda* by Gil-Rodríguez et al. 2012.
Laurencia caduciramulosa Masuda & Kawaguchi	*C:* Cassano et al. 2008; Gil-Rodríguez et al. 2012.
Laurencia canariensis Montagne ex Kützing	*C:* Afonso-Carrillo & Sansón 1999; John et al. 2004. *Taxa inquirenda* by Gil-Rodríguez et al. 2012.
Laurencia catarinensis Cordeiro-Marino & M.T. Fujii	*C:* Haroun et al. 2002 (as *L. intricata*); Machín-Sánchez et al. 2012; 2014; Gil-Rodríguez et al. 2012.
Laurencia chondrioides Børgesen	*C:* Haroun et al. 2002. *Taxa inquirenda* by Gil-Rodríguez et al. 2012.
Laurencia dendroidea J. Agardh	*C:* Haroun et al. 2002 (as *L. majuscula*); Cassano et al. 2012b; Machín-Sánchez et al. 2014.
Laurencia intricata J.V. Lamouroux	*S:* Parente et al. 2000. *Taxa inquirenda*.
	C: Afonso-Carrillo & Sansón 1999. *Taxa excludenda* by Machín-Sánchez et al. 2012.
Laurencia majuscula (Harvey) A.H.S. Lucas	*M:* Neto et al. 2001; *Taxa inquirenda*.
	S: John et al. 2004; *Taxa inquirenda*.
	C: Haroun et al. 2002. *Taxa excludenda* by Cassano et al. 2012a.
Laurencia microcladia Kützing	*A:* Tittley et al. 2009.
	M: Neto et al. 2001.
	S: Parente et al. 2000.
	C: Haroun et al. 2002; Aylagas et al. 2011; Gil-Rodríguez et al. 2012.

Table 1. Cont.

Laurencia minuta Vandermeulen, Garbary & Guiry	*C:* Haroun et al. 2002.
Laurencia nidifica J. Agardh	*M:* John et al. 2004 *Taxa inquirenda* by Gil-Rodríguez et al. 2012.
Laurencia obtusa (Hudson) J.V. Lamouroux	*A:* Neto 1994.
	M: Neto et al. 2001.*S:* John et al. 2004.
	C: Haroun et al. 2002; Gil-Rodríguez et al. 2012.
Laurencia papillosa (C. Agardh) Greville	*C:* Gil-Rodríguez & Haroun 1993. *Taxa excludenda* by Gil-Rodríguez et al. 2012.
Laurencia pyramidalis (C. Agardh) Greville	*A:* Machín-Sánchez et al. 2014.
	M: Machín-Sánchez et al. 2014.
	C: Gil-Rodríguez et al. 2012 (as *Laurencia* sp.1); Machín-Sánchez et al. 2014.
Laurencia tenera C.K. Tseng	*C:* Haroun et al. 2002. *Taxa excludenda* by Gil-Rodríguez et al. 2012.
Laurencia viridis Gil-Rodríguez & Haroun	*A:* Gil-Rodríguez & Haroun 1992; Machín-Sánchez et al. (2014).
	M: Gil-Rodríguez & Haroun 1992; Neto et al. 2001; Machín-Sánchez et al. 2014
	S: Gil-Rodríguez & Haroun 1992; Parente et al. 2000.
	C: Gil-Rodríguez & Haroun 1992; Haroun et al. 2002; Gil-Rodríguez et al. 2012; Machín-Sánchez et al. 2014.
Laurenciella marilzae (Gil-Rodríguez, Sentíes & M.T. Fujii) Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M.T. Fujii	*A:* Machín-Sánchez et al. 2014.
	C: Gil-Rodríguez et al. 2009 (as *Laurencia marilzae*); Gil-Rodríguez et al. 2012.
Olehopapa flexilis (Setchell) F. Rousseau, Martin-Lescanne, Payri & L. Le Gall	*M:* Neto et al. 2001 (as *Laurencia flexilis*). *Taxa inquirenda*.
	S: Parente et al. 2000 (as *L. flexilis*). *Taxa inquirenda*.
	C: Haroun et al. 2002 (as *L. flexilis*). *Taxa excludenda* by Gil-Rodríguez et al. 2012 (as *L. flexilis*).
Osmundea hybrida (De Candolle) K.W. Nam	*A:* Neto 1994 (as *Laurencia hybrida*). *Taxa inquirenda*.
	M: Levring 1974 (as *L. hybrida*); Neto et al. 2001. *Taxa inquirenda* by Machín-Sánchez et al. 2016.
	S: Audiffred & Weisscher 1984 (as *L. hybrida*); John et al. 2004. *Taxa inquirenda*.
	C: Gil-Rodríguez & Afonso-Carrillo 1980 (as *L. hybrida*); Haroun et al. 2002. *Taxa inquirenda* by Machín-Sánchez et al. 2012.
Osmundea oederi (Gunnerus) G. Furnari	*A:* Machín-Sánchez et al. 2016,
	C: Gil-Rodríguez et al. 2003 (as *Osmundea ramosissima*); *Taxa inquirenda* by Machín-Sánchez et al. 2016.
Osmundea osmunda (S. Gmelin) K.W. Nam & Maggs	*M:* Neto et al. 2001; John et al. 2004. *Taxa inquirenda* by Gil-Rodríguez et al. 2012.
Osmundea pinnatifida (Hudson) Stackhouse	*A:* Neto 1994 (as *Laurencia pinnatifida*); Machín-Sánchez et al. 2014.
	M: Neto et al. 2001; Machín-Sánchez et al. 2016
	S: John et al. 2004.
	C: Haroun et al. 2002; Machín-Sánchez et al. 2012b; Machín-Sánchez et al. 2016.
Osmundea silvae Machín-Sánchez & Gil-Rodríguez	*M:* Machín-Sánchez et al. 2016.
Osmundea truncata (Kützing) K.W. Nam & Maggs	*A:* Machín-Sánchez et al. 2016.
	M: Neto et al. 2001; Machín-Sánchez et al. 2016.
	S: Parente et al. 2000; John et al. 2004.
	C: Haroun et al. 2002; Machín-Sánchez et al. 2012b; Machín-Sánchez et al. 2016.
Osmundea prudhommevanreinei Machín-Sánchez & Gil-Rodríguez	*A:* Machín-Sánchez et al. 2016.
	M: Machín-Sánchez et al. 2016.
	C: Machín-Sánchez et al. 2012b; Machín-Sánchez et al. 2016.
Palisada corallopsis (Montagne) Sentíes, M.T. Fujii & Díaz-Larrea	*M:* Neto et al. 2001 (as *Chondrophycus corallopsis*).
	S: Parente et al. 2000 (as *C. corallopsis*); John et al. 2004 (as *C. corallopsis*).
	C: Haroun et al. 2002 (as *C. corallopsis*). *Taxa excludenda* by Gil-Rodríguez et al. 2012.

Table 1. Cont.

Palisada flagellifera (J. Agardh) K.W. Nam	*C:* Gil-Rodríguez et al. 2010; Gil-Rodríguez et al. 2012; Machín-Sánchez et al. 2014.
Palisada patentiramea (Montagne) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii	*S:* Parente et al. 2000 (as *Chondrophycus patentirameus*).
	C: Audiffred & Prud'homme van Reine 1985 (as *Laurencia paniculata*). *Taxa inquirenda* by Gil-Rodríguez et al. 2012.
Palisada perforata (Bory de Saint-Vincent) K.W. Nam	*M:* Neto et al. 2001 (as *Chondrophycus perforatus*).
	S: Parente et al. 2000 (as *C. perforatus*); John et al. 2004 (as *C. perforatus*).
	C: Haroun et al. 2002 (as *Chondrophycus papillosum* and as *C. perforatus*); Cassano et al. 2009; Gil-Rodríguez et al. 2012; Machín-Sánchez et al. 2014.
Palisada thuyoides (Kützing) Cassano, Sentíes, Gil-Rodríguez & M.T. Fujii	*C:* Gil-Rodríguez & Haroun 1993 (as *Laurencia paniculata*). *Taxa excludenda* by Gil-Rodríguez et al. 2012.
Yuzurua poiteaui (J.V. Lamouroux) Martin-Lescanne	*S:* Parente et al. 2000 (as *Chondrophycus poiteaui*); John et al. 2004 (as *C. poiteaui*).
	C: Haroun et al. 2002 (as *C. poiteaui*). *Taxa excludenda* by Gil-Rodríguez et al. 2012.

3.2. Phylogeographic Comments and Brief Morphological Descriptions of the Species Currently Accepted for the Macaronesia Region

3.2.1. *Laurencia caduciramulosa* Masuda & Kawaguchi

Cassano et al. [38] recorded *Laurencia caduciramulosa* for the first time for Macaronesia, based on anatomical studies on fresh specimens and by comparison with type material in the Herbarium of Sao Paulo, Brazil (SAP 062086).

The specimens were collected in 2006 and 2008 in Tenerife (Canary Islands), growing epilithically in the lower intertidal zone on moderately exposed rocky shores, with turfs of articulated *Corallinaceae* [38,49].

Laurencia caduciramulosa from the Canary Islands was characterized by reduced size, a well-developed stolon-like basal system, and erect axes with abundant deciduous branchlets that function as propagules, except on the upper-most portions of the branches, where they form crowns of branchlets. Epidermal cells with one *corp en cerise* per cell, secondarily pit-connected and projecting near branch apices [38]. As in previous reports of this species, gametangia and sporangia were not observed, but Cassano et al. [38] underlined that such reproductive structures seem to be efficiently replaced by the vegetative propagules formed by the deciduous branchlets, which are closely related with the dispersal of *L. caduciramulosa* [38,49,51–55].

This species is considered as an introduced species to the Canary Islands, with transport of *Laurencia catarinensis* propagules by ocean-going vessels from the Mediterranean Sea as the proposed introduction vector. The small size and the very low abundance of *L. caduciramulosa* in the Canaries [38] may suggest that this process was a recent introduction.

3.2.2. *Laurencia catarinensis* Cordeiro-Marino & M.T. Fujii

The presence of *Laurencia catarinensis* on the coasts of the Canary Islands was confirmed by molecular (*rbc*L phylogenies) and morphological analyses, which also proved that the herbarium samples from the Canary Islands previously referred to as *Laurencia intricata* must be identified as *Laurencia catarinensis* [27].

The application of molecular taxonomy [28] for delimiting *Laurencia catarinensis* and *Laurencia intricata* in the Canary Islands (given their morphological similarity) was based on the *rbc*L gene, detecting the degree of nucleotide variation between the sequences analysed and assuming that the interspecific divergence exceeded the intraspecific divergence. The boundaries between the two taxa were established according to: (1) the degree of monophyly of each clade (defined in the phylogenetic tress), and (2) the genetic distances calculated within and between clades. Based on the bibliography consulted about the *Laurencia* complex, it was established that values of genetic divergence less than 1% would be considered intraspecific (varying those obtained for *L. catarinensis* between 0–0.9%), while values above 2% would be considered as interspecific values (4.9–5.7% being the range observed between the clade of *L. catarinensis* and *L. intricata*). In this case, the use of a threshold value in the genetic distances previously established did not cause any inconvenience in the delimitation of both species, and therefore in the taxonomic correction that was finally proposed (supported both by the morphological description of the specimens sampled, as by the revision of the type material).

This species has an entangled habit, cushion-like turf formation, is pink-purple to greenish, usually pink-tipped, attaches to the substrate by a small holdfast, cortical cell walls slightly to markedly projecting near the apex, with 1 or 2 (−3) *corps en cerise*, and lacks lenticular thickenings in medullary cells.

From a phytogeographical perspective, its presence represents a dramatic increase of its distribution towards the eastern Atlantic Ocean, and converted *L. catarinensis* into an amphi-Atlantic species [27].

3.2.3. *Laurencia dendroidea* J. Agardh

Laurencia dendroidea was reported for the first time from the Canary Islands, as *Laurencia majuscula* (Harvey) Lucas by Gil-Rodríguez & Haroun [19]. Later, Masuda et al. [56] and Haroun et al. [57–59] also informed of the presence of *L. majuscula* in this archipelago. However, the combined use of morphological and molecular taxonomy studies, based on the *rbc*L region of the chloroplast genome, confirmed that the taxa previously identified as *L. majuscula* in the Canary Islands constituted erroneous identifications of *L. dendroidea,* which is an extremely plastic taxonomic entity [33].

The morphology of *Laurencia dendroidea* from the Canary Islands is characterized by thalli densely branched from the base to upper portions, pyramidal in outline, forming tufts from 4 to 12 cm high, brown-purple in colour. Erect axes arise from a single discoid holdfast or from an aggregation of discoid holdfasts, with stolon-like branches. In surface view, cortical cells are regularly arranged throughout the thalli in longitudinal rows and connected to each other by longitudinally secondary pit connections, with 2–3 *corps en cerise* per cell. Lenticular thickenings are abundant, rare, or absent. When present, they occur in the medullary cells walls of main axes [49].

3.2.4. *Laurencia microcladia* Kützing

Prud'homme van Reine et al. [60] carried out a reinvestigation of taxa collected by Piccone from the shores of the islands of Macaronesia. The study of these specimens allowed the authors to discuss and propose nomenclatural changes and new identifications. Based on this revision, the voucher specimens of *Laurencia obtusa* (L: 1884, 56) described by Piccone from Gran Canaria (Canary Islands) was identified as *Laurencia microcladia* Kützing, a new record for Macaronesia.

Afterwards, *Laurencia microcladia* was included on the list of records of marine algae of the Azores [61] the Selvagens [62], Madeira [63], and the Canary Islands [57]. However, information about the morphology and habitat of this species in the Macaronesian archipelagos was limited.

Aylagas et al. [64] described the distribution, morphology, and reproductive characteristics of *Laurencia microcladia* for the Canary Islands. Fresh collected specimens are identified by the presence of stolon-like branches, four pericentral cells, secondary pit-connections, and 1–3 *corps en cerise* in cortical cells, abundant lenticular thickening, sessile urceolate cystocarps, and tetrasporangial formation from third and fourth pericentral cells.

Additionally, specimens of *Laurencia microcladia* from Macaronesia, deposited in herbariums of the Canary Islands (TFC, BCM), the Azores (AZB), Málaga (MGC), and Barcelona (BCN) universities, were compared with specimens of *L. microcladia* from the type locality ("Indias Occidentales"). These material was in agreement with those presented from the Canary Islands [64].

This research allows for the identification and renaming as *Laurencia microcladia* of some specimens that were previously assigned as *Laurencia obtusa* or *Laurencia majuscula* (Harvey) Lucas, and confirms the presence of *L. microcladia* in the Azores and the Canary Islands.

3.2.5. *Laurencia minuta* Vandermeulen, Garbary & Guiry

Laurencia minuta was included on the checklist of the marine plants from the Canary Islands by Haroun et al. [57] based on previous reports [49]. However, following a conservative approach, Gil-Rodríguez et al. [49] addressed the taxon *L. minuta* from the Canary Islands as *taxa inquirenda*, since the last specimens sampled—which were included in TFC—had not been molecularly confirmed. However, Gallardo et al. [65] considered enough of the previous morphological evidence to refer *L. minuta* as taxa confirmed.

Laurencia minuta from the Canaries usually grows on the leaves of *Cymodocea nodosa* (Ucria) Ascherson as a small individual characterized by: thalli 0.7–2 cm high, pink or red colour, attached to the substratum by discoid holdfast from which one or two terete main axes; cortical cells with one *corps en cerise*, connected to each other with secondary pit connection, projecting in transverse section; absent lenticular thickenings in medullar cells [37,58].

3.2.6. *Laurencia obtusa* (Hudson) J.V. Lamouroux

Laurencia obtusa has historically been reported in Macaronesia from the Azores to Cape Verde [22], but Gil-Rodríguez et al. [49] urged caution in many of those identifications due to the extremely wide range of variation in external morphology displayed by *L. obtusa*, which has led to many mis-identifications.

Specimens from the Canary Islands are epilithic, living in the lower mid-intertidal to the upper subtidal zones, in semi-exposed coasts. Plants erect, 5 cm high, red purple to bright orange in colour, but usually yellow in the main axes and primary lateral branches. Thalli terete, cartilaginous and fairly soft in texture; with stoloniferous holdfast and secondary attached by smaller discoid holdfast; one *corps en cerise* in each cortical cell, never projecting; medullary cells without lenticular thickenings.

3.2.7. *Laurencia pyramidalis* (C. Agardh) Greville

An integrative approach using both morphological and molecular data (COI-5P + *rbc*L) was undertaken to assess the presence of *Laurencia pyramidalis* in the Macaronesian archipelagos. It was required to locate and study the type of *L. pyramidalis* and to include a specimen from the type locality (Granville, Normandy, France) in the molecular analyses [36].

The review of the Kützing's herbarium from the Herbarium of the Naturalis Biodiversity Center, the Netherlands, led to the designation of a lectotype and syntypes of *Laurencia pyramidalis* (Herbarium sheet L0820668) [36].

The sequences of the mitochondrial gene COI-5P obtained from the Azores and the Canary Islands were compared with the barcode sequence (COI-5P) generated for *L. pyramidalis* from the type locality. The standardized procedure for the application of the DNA-barcoding tool was carried out [10,35,66–68], and therefore a phylogram was constructed using neighbour-joining analyses (NJ) from COI-5P sequences. Specimens collected in Macaronesia belonged to a fully supported clade, containing identical or very similar sequences to samples coming from the type locality. This routine allowed the identification of most of the specimens analysed, revealing a new record for the Azores, Madeira, and the Canary Islands, *Laurencia pyramidalis*.

Macaronesian individuals of *Laurencia pyramidalis* were found growing either in mid-to-lower intertidal rock pools or in turfs covering rocks. Morphology of specimens studied was in accordance with the descriptions given by Kützing [69], Maggs and Hommersand [70], and Serio et al. [71]. Specimens from Macaronesia exhibited a similar habit to the type specimens and sample collected in the type locality deposited in the herbarium of the Muséum National d'Histoire Naturelle, Paris, France (PC0157557). Thalli 10–18 cm high, brownish red to purple in colour, attached to the substratum by stoloniferous holdfast, and pyramidal in outline; with three or four orders of branches arranged in three whorls; cortical cells with one *corps en cerise* connected to each other with secondary pit connection; presence variable of annular thickenings in medullar, pericentral, and axial cells.

3.2.8. *Laurencia viridis* Gil-Rodríguez & Haroun

Laurencia viridis was described as a new species from specimens collected around the Canary Islands and herbarium material with samples coming from the Azores, Madeira, the Selvagens, and the Cape Verde Islands, by Gil-Rodríguez and Haroun [37].

Morphologically, specimens from Macaronesia show a distinct green colour with pinkish apical regions; radially arranged terete branches; secondary pit-connections and one *corps en cerise* in cortical cells; medullary cells without lenticular thickening; cup-shape spermatangial pit; cystocarps without prominent ostiole; tetrasporangia in parallel abaxial rows [37,49].

Molecular identification (COI-5P) and phylogenetic analyses (*rbc*L) of *Laurencia viridis* from Macaronesia were established in Machín-Sánchez et al. [36]. They obtained the first COI-5P sequences generated for specimens of *L. viridis* (from the Azores, Madeira, and the Canary Islands, including a sequence from the type locality, Punta del Hidalgo). Phylogenetic analyses of *rbc*L of these specimens resolved *Laurencia viridis* as a distinct lineage from other representatives of the *Laurencia* complex from Macaronesia, close to a lineage encompassing specimens of *Laurencia pyramidalis*.

In the 1990s, *Laurencia viridis* was a common element of the marine flora of the Canary Islands. However, since 2005 some populations have been drastically reduced (mainly in Tenerife island), eventually being represented by few units. The cause of the decrease could be the alteration of the nearby coastline and/or coastal pollution [49]. Due to its significance in the Canaries, *L. viridis* has been included in the *Catalogue of Protected Species of the Canary Islands* (Regional Autonomous Law 4/2010 of June 4) as "Interesting for ecosystems of the Canary Islands" (BOC 112, 9 June 2010).

3.2.9. *Laurenciella marilzae* (Gil-Rodríguez, Sentíes & M.T. Fujii) Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano & M.T. Fujii

Laurenciella marilzae was described as *Laurencia marilzae* [34], based on morphological and molecular studies from specimens collected in the Canary Islands, and transferred to *Laurenciella* afterward [16].

The distribution of *Laurenciella marilzae* was increased to include the Azores archipelago, and proposed a preference of *L. marilzae* for tropical and warm temperate waters [36].

The combination of morphological, chemical, and molecular evidence has allowed this taxon to be delimited and for its position within the *Laurencia* complex to be clarified. Morphologically, *Laurenciella marilzae* from Macaronesia is characterized by its yellow-orange thalli in natural habits an irregularly pyramidal outline, discoid holdfast, being the only taxa within the *Laurencia* complex with a single *corps en cerise* in each cell of the thallus [34,36,49]. Chemically, *L. marilzae* is a producer of a large variety of exclusive diterpenes, derived from a dactylomelane skeleton [72] or from others such as: 12-epoxyobthalene IV, obtusaleno X, marilzaleno, (+)-4-acetoxymarilzalene, (−)-4-acetoxymarilzalene, Z and E-Adrienyno, obtusalene IV (C15 acetogenins), and marilzabicycloalene AD (non-terpenoid bromoalenes) [73]. In addition, *L. marilzae* appears genetically (COI-5P and *rbc*L) as an evolutionary lineage divergent and independent of the rest of taxa of the *Laurencia* complex [16,34,36].

3.2.10. *Osmundea oederi* (Gunnerus) G. Furnari

Recently, Machín-Sánchez et al. [50,74] published relevant data on some *Osmundea* species from the Macaronesian archipelagos. In this sense, the distribution of *Osmundea oederi* has been increased in the Macaronesia region including the Azores archipelago. Although molecular and morphological data have demonstrated its presence in the Azores, this taxon is considered as a dubious record for the Canary Islands, where has been historically reported as *Osmundea ramosissima* or *Laurencia platycephala*.

The Azorean specimens of *Osmundea oederi* are brown to reddish-brown in colour, subcartilaginous in texture, attached by discoid holdfast; erect axes compressed except near the base, with terete branchlets; cortical cells with secondary pit connections; medullary cells without lenticular thickenings; cup-shaped spermatangial pits with spermatangial filament branched or unbranched, terminating in a cluster of large vesicular sterile cell; cystocarps sessile, ovoid with no protruding ostiole [50].

Osmundea oederi, as well as the other species within *Osmundea* genus present in the Azores, are a noticeable component of the non-calcareous turf vegetation in the rocky intertidal with strong wave action of diverse islands, dominating the benthic communities at mid and low littoral [75,76].

3.2.11. *Osmundea pinnatifida* (Hudson) Stackhouse

Osmundea pinnatifida was included for the first time in a provisional key of the genus *Laurencia* from the Canary Islands as *Laurencia pinnatifida* based on previous reports as Børgesen [76], Gil-Rodríguez and Afonso-Carrillo [77], and Gil-Rodríguez and Haroun [19]. At present, it is largely distributed in the Macaronesia region: the Azores [78], Madeira [63], the Selvagens [79], and the Canary Islands [57], forming turf growing in the lower intertidal zone of exposed coasts.

Plants are characterized by being brownish-purple in colour, with strongly compressed thallus and pinnate branches; attached to substratum by stoloniferous holdfast; with pungent smell and peppery taste when fresh; cortical cells without secondary pit connection; lenticular thickenings present or absent; pocket-shaped spermatangial pits with spermatangia filaments terminating in a single, vesicular, sterile cell.

Interestingly, morphological (morphometric differences and the presence or absence of specific vegetative structures) and molecular (species delimitation in two sister lineages inferred by molecular data from COI-5P sequences) variability among specimens of *O. pinnatifida* from Macaronesia have been detected [50,74], pointing out the possibility of cryptic candidate species inside this taxon.

3.2.12. *Osmundea prudhommevanreinei* Machín-Sánchez & Gil-Rodríguez

The current distribution of *Osmundea prudhommevanreinei* only encompasses the Azores, Madeira, and the Canary Islands, and is generally present in rock crevices, in the lower intertidal zone of exposed coasts of islands [50].

Thalli of *O. prudhommevanreinei* are attached to substratum by basal crust lacking stolons, terete in the basal region and subterete in the middle of the axes, subcartilaginous in texture, axes alternately ramified with two orders of branches; cortical cells with secondary pit connections; thickened walls and lenticular thickenings present in medullary cells; cup-shaped spermatangial pits, spermatangial filaments ending in a cluster of several large vesicular sterile cells; sessile cystocarps with a non-protuberant ostiole [50].

Before the assignment of *O. prudhommevanreinei* as a new species within the genus *Osmundea*, previous studies thoroughly documented morphological variations in *Osmundea truncata* (Kützing) K.W. Nam & Maggs from the Canary Islands [74]. Specimens with non-classic morphotype were segregated from *O. truncata* into a separate sister species, based on diagnostic morphological features and molecular data (COI-5P sequences delimitation methods and *rbc*L phylogenies) [50].

3.2.13. *Osmundea silvae* Machín-Sánchez & Gil-Rodríguez

Presently, *Osmundea silvae* is only reported in Madeira Island, growing in the lower intertidal forming turfs covering rocks and crevices.

The most significant morphological features to distinguish this taxon from other Macaronesian species of *Osmundea* are the differential traits of the attaching system to the substratum. Plants are attached by a discoid holdfast and descending basal branches from which arise arched and decumbent terete branches, and are secondarily attached by smaller discoid holdfast and stolon-like branches; main axes subterete, cartilaginous, rigid in texture and irregularly ramified; cortical cells connected by secondary pit connections; medullary cells with thickened walls but without lenticular thickenings; reproductive features have not been observed [50].

Phylogenetic analyses of COI-5P sequences showed a sister lineage of *Osmundea silvae* in the Azorian samples, with a clear biogeographical pattern, since its cluster included samples with identical morphology, except by the sizes of thallus and branches, which were significantly different. These two sister lineages have been proposed as pseudocryptic species [50].

3.2.14. *Osmundea truncata* (Kützing) K.W. Nam & Maggs

The records of *Osmundea truncata* in the Azores, Madeira, and the Canary Islands are supported by morphological and phylogenetic studies [50,74], whereas its presence in the Selvagens [62,79] is not yet confirmed.

Specimens from Macaronesia share the common features of *Osmundea truncata* from the North Atlantic European coasts [29,70]: plants growing individually or in turf over rocks, reddish-brown in colour, cartilaginous in texture, attached by a discoid holdfast; erect axes, subterete to compressed; presence of secondary pit connection; medullary cells with thickened walls and lenticular thickenings; cup-shaped spermatangial pits, spermatangial filaments usually unbranched, ending in an apical spherical sterile cell, which contains bright yellow pigment when fresh; cystocarps sessile with a protruding ostiole, a reproductive feature which distinguishes from the other *Osmundea* reported in Macaronesia [50].

3.2.15. *Palisada flagellifera* (J. Agardh) K.W. Nam

Palisada flagellifera was originally described as *Laurencia flagellifera* from material collected in Indonesia by J. Agardh [80]. The type locality ("ad oras Indiae orientalis") was misquoted as India [81]. Nam [14,21] transferred first to *Chondrophycus* and later to *Palisada* [49].

It is now reported from Lusitanian Macaronesia only in the Canary Islands based on morphological and molecular (*rbc*L) evidence. Gil-Rodríguez et al. [82] collected specimens which were in agreement with the Holotype of *P. flagellifera* (LD 36604-36606) and phylogenetically close to *Palisada perforata*, as previously shown by Fujii et al. [83], Díaz-Larrea [84], and Cassano et al. [25].

The species is characterized by a cartilaginous thalli, rigid in texture, forming brown, violet-brown or dark tufts, attached to substratum by a discoid holdfast, descending basal branch and occasionally stolon-like branches; in transverse section, by the elongated cortical cells arranged in palisade, without secondary pit connections; two pericentral cells per vegetative axial segment; presence of a sterile cell in the tetrasporangial segment and tetrasporangia produced by three fertile pericentral cells, and arranged at right angles [21,82].

Specimens occupy lower intertidal and subtidal zones to 2 m depth, and are associated with articulated *Corallinaceae* and *Cystoseira abies-marina* (S.C. Gmelin) C. Agardh at exposed sites [82].

3.2.16. *Palisada perforata* (Bory de Saint-Vincent) K.W. Nam

Palisada perforata is a species with a wide distribution in tropical and subtropical waters [22]. In the Macaronesian Region it has been reported in the Azores, Madeira, the Selvagens, and the Canarian archipelago, Tenerife (Canary Islands) being its type locality (proposed as *Fucus perforatus* by Bory in 1803) [85].

Macaronesian plants of *Palisada perforata* grows as strongly compacted turf-like mats or isolated turfs in the intertidal zone and are exposed to high-energy wave action [86]. The plants are violet-brown or greenish brown in colour, terete rigid, and cartilaginous in texture; attached to the substratum by a discoid holdfast from which arises arcuate and decumbent branches with unilateral branchlets, and secondarily attached by rhizoids or smaller discoid holdfast [25,49].

3.3. A Key to Lusitanian Macaronesia Species of the Laurencia Complex

Since the first taxonomic key done by Gil-Rodríguez and Haroun [19] to discriminate the major *Laurencia* species encountered in the coastal areas of the Canary Islands, a large amount of research effort has been conducted by diverse marine researchers in the Macaronesian archipelagos, increasing the number of taxa and robust scientific knowledge about the *Laurencia* complex. In order to provide a basic tool that facilitates and simplifies the task of identifying specimens belonging to the *Laurencia* complex, we have constructed an identification key (Table 2) which includes all those species whose records were genetically or morphologically confirmed; in this sense, *Osmundea hybrida* and *O. osmunda* are included, since there is numerous evidence of their presence in some of the Macaronesian archipelagos. This is an artificial identification key, based on morphological and anatomical characters (including *corps en cerise* in living specimens) with diagnostic value and relatively easy to observe using a stereoscopic dissecting microscope or microscope. Here it is worth mentioning that further molecular analysis will be required to obtain a complete identification of the concerned species.

Table 2. Artificial identification key of the macroalgal species of the *Laurencia* complex currently accepted for the Macaronesian archipelagos.

1.	a.	Vegetative axial segment with four pericentral cells	2
	b.	Vegetative axial segment with two pericentral cells	10
2.	a.	Plants yellow-orange in colour, irregularly pyramidal outline, discoid holdfast. Presence of one (rarely two) *corps en cerise* (*cc*) scattered in all cells of the thallus	*Laurenciella marilzae*
	b.	Plants variable in colour, discoid or stoloniferous holdfast. Presence of *cc* only in trichoblasts and in cortical cells	3
3.	a.	Plants growing on leaves of the seagrass *Cymodocea nodosa*, not taller than 0.5–2.5 cm; with cortical cell walls projected near the apices	*Laurencia minuta*
	b.	Plants epiphytic or epilithic, taller than 0.5 cm	4
4.	a.	Small plants, forming cushion-like tufts; usually associated with geniculate coralline algae	5
	b.	Plants up to 15 cm high, pyramidal in outline, never forming cushion-like tufts	6
5.	a.	Presence of clavate and deciduous branchlets, branches with many scars; crowns of branchlets at the uppermost portions of the branches; one *cc* per cell; medullary cells with lenticular thickenings	*Laurencia caduciramulosa*
	b.	Absence of branchlets clavate and deciduous; one or two (rarely three) *cc* per cell; medullary cells without lenticular thickenings	*Laurencia catarinensis*
6.	a.	Axes arising from a single or an aggregation of discoid holdfast, with stolon-like branches; brown-purple or violet-greenish in colour; presence from two to four *cc* per cell; in longitudinal sections (LS), the outer cortical cell walls near the apices project slightly or markedly; medullary cells with lenticular thickenings	*Laurencia dendroidea*
	b.	Axes arising from stoloniferous holdfast; variable in colour; one or more *cc* per cell; in LS, the outer cortical cell walls near the apices projecting or not; medullary cells with or without lenticular thickenings	7
7.	a.	In LS, the outer cortical cell walls near the apices do not project; medullary cells without lenticular thickenings; presence of one (rarely two) *cc* per cell	8
	b.	In LS, the outer cortical cell walls near the apices project; medullary cells with or without lenticular thickenings; variable number of *cc* per cell	9
8	a.	Thallus cartilaginous, dark green; branches green with pinkish tips; branching alternate or helicoid only in the upper $^2/_3$ of the thallus	*Laurencia viridis*
	b.	Thallus soft in texture, green to yellow greenish; with spiral branching with three or four orders of whorled branches	*Laurencia pyramidalis*
9	a.	Presence of two or three (rarely one, four, or five) *cc* per cell; medullary cells with lenticular thickenings	*Laurencia microcladia*
	b.	Presence of one (rarely two) *cc* per cell; medullary cells without lenticular thickenings	*Laurencia obtusa*
10	a.	Tetrasporangial production from particular pericentral cells; spermatangia of the trichoblast type; outer cortical cells arranged in palisades in cross-section (CS); right-angle arrangement of tetrasporangia	11
	b.	Tetrasporangial production from random cortical cells; spermatangia of the filament type; outer cortical cells do not arrange in palisades in CS; parallel arrangement of tetrasporangia	12
11	a.	Plants forming compacted turf-like mats or isolated tufts, violet-brown or greenish brown in colour; attached by a discoid holdfast from which arise arcuate branches, secondarily attached by rhizoids or smaller discoid holdfast; erect branches unilaterally disposed or irregularly alternate; with wart-like branchlets; one additional pericentral fertile cell produced in the tetrasporangial axis (3rd)	*Palisada perforata*
	b.	Plants forming tufts, brown, violet-brown, or dark brown in colour; attached by a discoid holdfast, descending basal branches and occasionally stolon-like branches; erect branches disposed irregularly alternate; without wart-like branchlets; two additional pericentral fertile cells produced in the tetrasporangial axis (3rd, 4th)	*Palisada flagellifera*
12.	a.	Cortical cells connected by secondary pit connections	13
	b.	Cortical cells not connected by secondary pit connections	16

Table 2. Cont.

13.	a.	Medullary cells with lenticular thickenings	14
	b.	Medullary cells without lenticular thickenings	15
14.	a.	Cup-shaped spermatangial pit with spermatangial filaments branched, normally terminating in a cluster of several large vesicular sterile cells; cystocarps with a non-protuberant ostiole	*Osmundea prudhommevanreinei*
	b.	Cup-shaped spermatangial pit with spermatangial filaments generally unbranched, and terminating in an apical spherical sterile cell, which usually contains bright yellow pigment when fresh; cystocarps with a protuberant ostiole	*Osmundea truncata*
15.	a.	Thallus compressed except near the base and branchlets, subcartilaginous in texture, decay rapidly after collection; attached by discoid holdfast, lacking stolon-like branches	*Osmundea oederi*
	b.	Thallus subterete, cartilaginous and rigid in texture; attached by discoid holdfast, descending basal branches from which arise arched and decumbent terete branch, and secondarily attached by smaller discoid holdfast and stolon-like branches	*Osmundea silvae*
16.	a.	Cup-shaped spermatangial pit	*Osmundea hybrida*
	b.	Pocket-shaped spermatangial pit with an ostiole-like opening	17
17	a.	Attached by discoid holdfast	*Osmundea osmunda*
	b.	Attached by stoloniferous holdfast	*Osmundea pinnatifida*

4. Discussion

Recently, phylogenetic analyses using a multi-gene dataset approach have revealed their efficiency for the systematic study of the *Laurencia* complex and showing the existence of independent lineages, which corresponds with new taxa within the complex [18]. These results were in agreement with previous data presented by Machín-Sánchez et al. [87] to infer the phylogenetic relationship and biogeographic affinities of members of the complex from Macaronesia using three independent markers COI-5P, *rbc*L, and LSU (large subunit ribosomal DNA) as well as the combined data set, uncovering the existence of a potential new genus from the Canary Islands (Figure 2). This gene approach has demonstrated its utility in diverse phylogenetic studies of Rhodophyta [18,88–92].

In this sense, exploring the diversity of the genus *Osmundea* from Macaronesian archipelagos, multiple lines of evidence were presented [50] for constructing robust taxonomic hypotheses in an integrative framework [93–97]. Aside from morphological descriptions and morphometric analyses, authors employed different approaches for delimiting species within *Osmundea*, including phylogenetic analyses (*rbc*L), COI-5P sequence-based species delimitation methods [11] such as Automatic Barcode Gap Discovery (ABGD) [95] and General Mixed Yule Coalescent Model (GMYC) [98–100]. The number of taxa delimited changed according to the method employed, highlighting the possibility of an under- or overestimation of the diversity of *Osmundea* species (taking or not taking into account cryptic or pseudocryptic candidate species), and emphasized the importance of incorporating multiple lines of evidence for constructing robust taxonomic hypothesis in an integrative approach. Moreover, the phylogenetic analyses of the plastid and mitochondrial data of *Osmundea* showed a deep divergence between the North-East Atlantic coast including the Macaronesian archipelagos–Mediterranean clade and the American clade. These results were taken into account for the construction of a hypothetical scenario of *Osmundea* phylogeography [18].

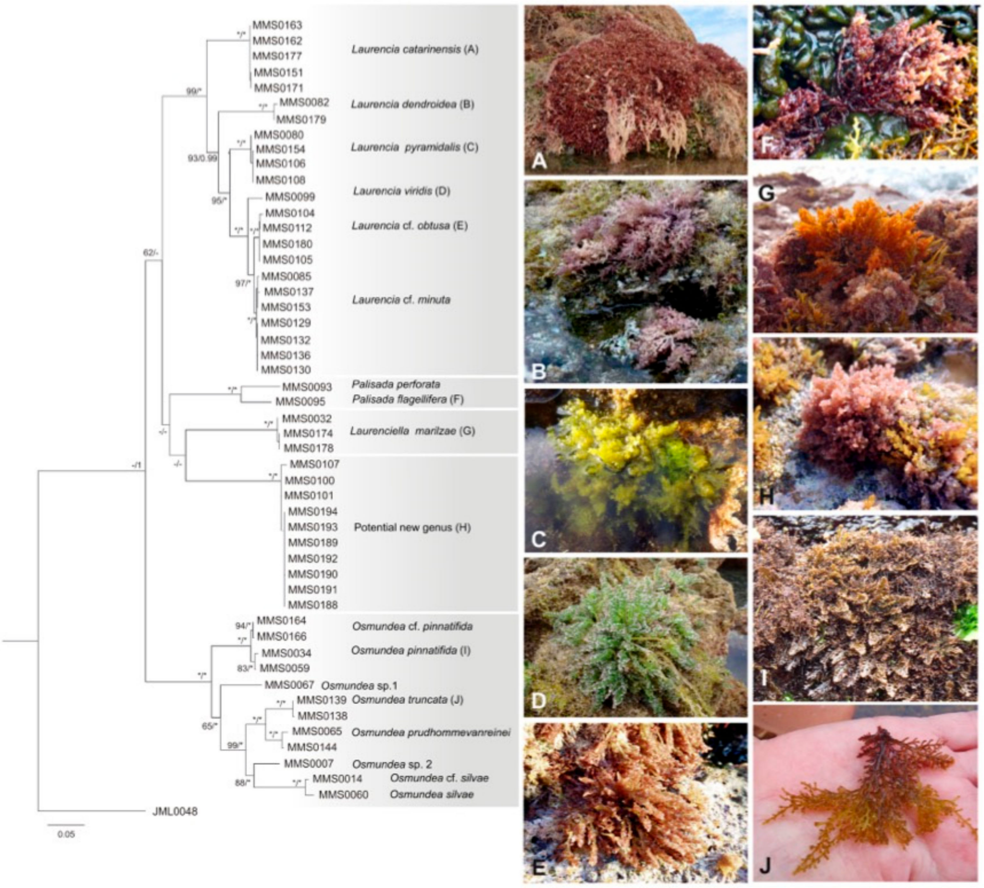

Figure 2. Maximum likelihood phylogenetic tree (ML) and morphological features of members of the *Laurencia* complex from the Macaronesian archipelagos obtained from the concatenation of the sequences of three molecular markers (LSU, COI-5P y *rbc*L). The numbers in the nodes indicate the support of the branches by means of ML bootstrap values (* = 100) and Bayesian posterior probability (* = 1); (-) indicates support values less than 60% or 0.95, respectively [87]. The new dataset from diverse species of this complex may bring support to previous studies [20,47,48,101] and further advance the knowledge about the affinities of the marine flora of the Eastern and Western Atlantic Ocean as potential remnants of the former Thetyan Sea. In this sense, *Laurencia caduciramulosa*, *L. catarinensis*, and *L. microcladia* are good examples of macroalgal species with an amphi-Atlantic distribution, as is also the case of *Laurenciella marilzae* and *Palisada perforata*. In a similar way, the results obtained by van den Strate et al. [102] on *Cladophoropsis membranacea* are congruent with this hypothesis, with material from the Canary Islands sharing genetic material with samples coming from the Caribbean and Mediterranean Seas. In another twist for this Thetyan remnant hypothesis, Pakker et al. [103] found no ecotype differentiation among four different amphiatlantic seaweeds from the Caribbean and the Canary Islands, suggesting that the populations at the Canary Islands became extinct during the Pleistocene glaciations and that the islands were re-populated later from tropical donor populations as a consequence of long-distance dispersal events.

DNA-barcoding data were congruent with the results displayed from phylogenetic analyses of *rbc*L sequences. The genus *Laurencia sensu stricto* was resolved as a monophyletic lineage, whereas relationships among species were only moderately or poorly supported [36,104]. From a biogeographical point of view, the current knowledge of the marine floristic diversity led to the inclusion of most of the Macaronesian archipelagos as part of the Lusitanian province [46]. Furthermore, these authors, depending on the specific macroalgal group composition—Chlorophyta, Phaeophyta, and Rhodophyta—have proposed the differentiation of two smaller biogeographic units: (a) the Canary

Islands, and (b) the Azores, Madeira, and the Selvagens. The larger number of macroalgal species in the Canary Islands seems to be the result of its closer proximity to donor areas and the existence of more diverse microclimates along its coastlines.

In the case of the Cape Verde archipelago, despite the low resolution of its marine biota database, its biogeographical relationship with the Lusitanian Macaronesian Region is being questioned by diverse marine researchers, who have demonstrated a higher affinity of its elements for nearby tropical regions. At present, the marine biota (including its marine flora) of this archipelago demands a stronger research effort from the international community to validate its biogeographical role and ecosystem services.

5. Future Perspectives

The *Laurencia* complex provides an interesting model for understanding the biogeography of the Macaronesian algal flora, taking advantage of the large latitudinal gradient from the Azores in the north to the Cape Verde Islands near the Tropic of Cancer. Moreover, the differential biogeographical settings of the concerned archipelagos—and consequently the variation of diverse abiotic (such as sea water temperature and nutrient concentrations) and biotic parameters (such as degree of isolation and herbivory pressure)—are evolutionary forces that have contributed to shaping the benthic flora of these islands [46,47,101,105–107]. In a similar way, the recent studies conducted with other marine researchers from the tropical and subtropical American coasts have been instrumental in providing diverse keystone data to understand the relationship and distributional pattern of diverse taxonomic entities of the *Laurencia* complex in both sides of the Atlantic Ocean [17,18,33,108,109].

Molecular studies based on taxon-rich datasets, as well as multi-locus datasets and maybe new candidate genes from the plastid genome from *Laurencia* species [18] would contribute to delimiting more precisely the taxonomic position of some members of the *Laurencia* complex, and will elucidate their evolutionary and biogeographical relationships in a worldwide context.

Despite the large research effort since the 1980s in most of the Macaronesian archipelagos, there are several geographic areas where higher sampling effort is still required, both inside the Macaronesia Region *sensu latu* (such as in the case of the Cape Verde Islands) as well as along the nearby continental coasts of Europe, Americas, and Africa, including the Mediterranean Sea. In fact, it is necessary to generate molecular data to build the backbone reference library of voucher-linked DNA barcodes for members of the *Laurencia* complex from the target oceanographic regions. The molecular analyses and collateral specimen data should conform with CBOL data standards [110] so that the specimens will be eligible for ranking as a reference barcode upon submission to GenBank. Such a strategy would increase the scientific and practical value of Macaronesian biological collections, and would ensure prolific international exchange.

It is urgent to carry out the assessment of the number of species for the *Laurencia* complex in this biogeographical area, employing species delimitation approaches (based on molecular and morphological data), and to elaborate taxonomic revisions of target species of the complex comparing with other related floras.

Moreover, multi-gene phylogenetic analyses of the *Laurencia* complex's species (including a comprehensive dataset) would provide robust insights into the complex from Macaronesia and nearby continental coast. Findings would supply insights on how current biogeographic patterns have been influenced by historical events (Tethyan Sea, Glaciations), and the extent to which patterns are related to particular dispersal strategies. The use of more samples from different regions around the world and additional molecular markers would strengthen the Macaronesian dataset.

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