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## Comparative analysis of the *corps en cerise* in several species of *Laurencia* (Ceramiales, Rhodophyta) from the Atlantic Ocean

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### Article

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**Abstract:** Different species of *Laurencia* have proven to be a rich source of natural products yielding interesting bioactive halogenated secondary metabolites, such as terpenoids and acetogenins. It is shown that such compounds are accumulated in the spherical, reniform to claviform refractive inclusions called *corps en cerise* (CC), which are intensively osmiophilic and located mainly in the cortical cells of the thalli and also in trichoblast cells. Up to now, it was believed that CC were present only in these two kinds of cells. Recently, however, a species of *Laurencia*, *L. marilzae*, with CC in all cells of the thallus, *i.e.*, cortical, medullary, including the pericentral and axial cells, as well as in the trichoblasts, was described from the Canary Islands, and subsequently also reported to Brazil and Mexico. Within the *Laurencia* complex, only *Laurencia* species produce CC. Since the species of *Laurencia* are targets of interest for the prospection of bioactive substances due to their potential antibacterial, antifungal, anticholinesterasic, antileishmanial, cytotoxic, and antioxidant activities, the present paper carries out a comparative analysis of the *corps en cerise* in several species of *Laurencia* from the Atlantic Ocean to obtain basic information that can support natural product bioprospection projects. Our results show that the number and size of the CC are constant within a species, independent of the geographical distribution, corroborating their use for taxonomical purposes to differentiate groups of species that present a lower number from those that have a higher number. In this regard, there was a tendency for the number of CC to be higher in some species of *Laurencia* from the Canary Islands. The presence of CC can also be used to distinguish species in which these organelles are present in all cells of the thallus from those in which CC are restricted to the cortical cells. Among the species analyzed, *L. viridis* displayed the most varied secondary metabolites composition, such as sesquiterpenes, diterpenes, triterpenes, all of which showed potent antiviral, cytotoxic, and antitumoral activities, including protein phosphatase type 2A (PP2A) inhibitory effects.

### Keywords:

biological activities

*corps en cerise*

*Laurencia*

Rhodophyta

secondary metabolites

### Introduction

The species of *Laurencia* J.V. Lamouroux *sensu lato* (Ceramiales, Rhodophyta) are an extremely rich source of halogenated secondary metabolites with diverse structural features (Fenical & Norris, 1975; Martín & Darias, 1978; Erickson, 1983; Pereira & Teixeira, 1999) that can be divided into two groups according to their biogenetic origin. The first one is the nonterpenoid group, which contains the acetogenins derived from the

metabolism of fatty acids. The other one is the terpenoid group, in which the sesquiterpenes are the most abundant, but also containing diterpenes and triterpenes (Pereira & Teixeira, 1999; Fernández et al., 2005; Cen-Pacheco et al., 2011a).

The prominent bioactivity of marine terpenes is evident in their ecological role and makes them interesting as potential drugs. Many marine algae, especially those from tropical and subtropical seas, are able to produce a wide range of compounds, many of which exhibit at least

some degree of bioactivity (Fernández et al., 1998, 2005; Da Gama et al., 2002; Cassano et al., 2008a; Lhullier et al., 2009; Santos et al., 2010; Machado et al., 2011; Stein et al., 2011a, b).

*Laurencia sensu lato* is characterized by its uniaxial construction, with two to four pericentral cells, depending on the genus. *Laurencia* presents four pericentral cells per vegetative axial segment, while *Chondrophycus* (Tokida & Saito) Garbary & J.T. Harper, *Osmundea* Stackhouse, *Palisada* (Yamada) K.W. Nam, and *Yuzurua* (K.W. Nam) Martin-Lescanne have two. The thalli can be terete or have a certain degree of flatness up to completely flattened, entirely corticated, and with the apical cell always immersed at the bottom of the apical pit, from which dichotomously branched colourless trichoblasts develop. The cortical cells contain photosynthetic pigments arranged in the thylakoids, while other internal cells are colourless. The *Laurencia* species are differentiated from all other members of the *Laurencia* complex by the presence of refractive specific intra-cellular organelles called *corps en cerise* (CC) (Feldmann & Feldmann, 1950). These organelles occur as intracellular membrane-bound vesicles in trichoblasts and in the outermost cortical cells. In Brazil, they were observed for the first time in *Laurencia dendroidea* J. Agardh (as *L. obtusa*) and *L. oliveirana* Yoneshigue from the southeastern Brazilian coast (Yoneshigue, 1985). Their shape varies from spherical, reniform to claviform, and a connection to the cell wall is frequently observed. These organelles were confirmed as the main storage site for halogenated compounds (Young et al., 1980; Salgado et al., 2008; Paradas et al., 2010).

Dworjany et al. (1999) confirmed the localization of bioactive secondary metabolites in the red alga *Dilsea pulchra* (Greville) Montagne in the central vesicle of gland cells using a combination of fluorescence microscopy, culture studies and quantitative chemical analyses. Paul et al. (2006) also confirmed the localization of natural products in the gland cells of tetrasporophytes of *Asparagopsis armata* Harvey using different microscopy techniques. Salgado et al. (2008) used time-lapse videomicroscopy to observe exocytosis of vesicles in the cortical cells of *L. dendroidea* (as *L. obtusa*). These authors suggested that exocytosis specifically transports the halogenated compounds to the surface and that variations in this mechanism could result in different concentrations of the compounds on the algal surface. Paradas et al. (2010) have also demonstrated that variations in temperature, irradiance level, and desiccation can influence the transport of vesicles from the CC to the cell wall region in clones of *L. dendroidea* (as *L. obtusa*).

The morphology of the CC varies depending on the availability of bromine (Br) in seawater. It was also shown that Br depletion resulted in a reduction in

halogenated compounds and an increase in bacterial density over the surface of the algal thallus (Paul et al., 2006). However, the number of CC present per cell within the same species is usually constant (Nam et al., 1994; Furnari et al., 2001; Fujii & Senties, 2005; Fujii et al., 2011). Among the *Laurencia* species reported from Brazil (Fujii et al., 2011), only *L. translucida* M.T. Fujii & Cordeiro-Marino does not possess CC in its thallus; instead, the outermost cortical cells are small and translucent with few plastids in them, while the inner cells are larger and present photosynthetic pigments and other organelles as in all red algae (Fujii & Cordeiro-Marino, 1996). The authors suggested that the main function of translucent cells is to store still unidentified volatile substances, responsible for the sweet smell characteristic of the species.

Recently, Gil-Rodríguez et al. (2009) proposed a new species, *L. marilzae* Gil-Rodríguez, Senties, Díaz-Larrea, Cassano & M.T. Fujii, from the Canary Islands (Spain), that differs from all other species of the genus by presenting CC (one per cell) in all cells of the thallus (cortical, medullary, including pericentral and axial cells, and trichoblasts). The species also produces unique secondary metabolites, not found in any other species of *Laurencia*, such as dactylomelane (diterpene), 12-epoxyobtusallene IV, obtusallene X, marilzallene, (+)-4-acetoxymarilzallene, (-)-4-acetoxymarilzallene, Z- and E-Adrienyne, obtusallene IV (C15 acetogenins), and marilzabicycloallenes A-D (nonterpenoid bromoallenes) (Fernandez et al., 2005; Gutiérrez-Cepeda et al., 2011a, b). Rocha-Jorge et al. (2010) and Senties et al. (2011) have subsequently found *L. marilzae* in the western Atlantic Ocean in the Laje de Santos Marine State Park, São Paulo (Brazil), and in the Mexican Caribbean at Isla Mujeres, Quintana Roo, respectively. The taxonomic identification was corroborated by using the plastid-encoded *rbcL* gene as molecular marker.

Since species of *Laurencia* are targets of interest for the prospection of bioactive substances with potential as antibacterial, antifungal, anticolinesterasical, antileishmanial, cytotoxic, and antioxidant agents (Machado et al., 2011; Cen-Pacheco et al., 2011b; Fujii et al., 2011; Gressler et al., 2011; Stein et al., 2011a, b) and since the number of CC in the cortical cells can be useful as taxonomical criterion for distinguishing species of *Laurencia* (Masuda & Abe, 1993; Nam et al., 1994; Abe et al., 1998), the present paper carries out a comparative analysis of the number, shape and location of the CC in several species of *Laurencia* from the Atlantic Ocean to obtain basic information in support of natural product bioprospection projects.

## Materials and Methods

For this study, five species of *Laurencia* were

selected: *L. caduciramulosa* Masuda & Kawaguchi, *L. catarinensis* Cordeiro-Marino & M.T. Fujii, *L. dendroidea* J. Agardh, *L. marilzae* and *L. viridis* Gil-Rodríguez & Haroun. The samples were collected in Brazil, the Canary Islands and the Mexican Caribbean in 2006-2009. The analysis of the number, size, shape and location of the CC in the thalli, as well as different strategies to accumulate natural defense compounds, were observed in living material. The CC were analyzed in surface view and in transversal and/or longitudinal hand-sections of the thalli. The photographs were taken with a digital camera coupled to a Zeiss microscope (Göttingen, Germany) and/or by photomicrography on a Zeiss Axioskop 2 microscope (Göttingen, Germany).

#### Specimens examined

*Laurencia marilzae*: Spain, Canary Islands, Tenerife, Punta del Hidalgo, 12.vii.2006, leg. M.T. Fujii, A. Senties and M.C. Gil-Rodríguez (SP 365661; TFC Phyc. 13129), Brazil, São Paulo, Laje de Santos Marine State Park, Parcel do Sul, 25.iii.2007, leg. R. Rocha-Jorge (SP 399814), 18.viii.2008, leg. R. Rocha-Jorge (SP 399826), Mexico, Quintana Roo, Isla Mujeres, 16.xi.2008, leg. A. Senties and M.T. Fujii (UAMIZ 1019), 10.vi.2009, leg. A. Senties and K.M. Dreckmann (UAMIZ 1020); *L. dendroidea*: Spain, Canary Islands, Tenerife, Puerto de la Cruz, 13.vii.2006, leg. M.T. Fujii, A. Senties and M.C. Gil-Rodríguez (SP 365.663; TFC Phyc. 13131), Brasil, Rio de Janeiro, Baía da Ilha Grande, Angra dos Reis, Saco de Piraquara de Fora, Praia do Velho, 22.i.2006, leg. V. Cassano and J.C. De-Paula (SP 399882), Armação dos Búzios, Praia Rasa, 01.ii.2006, leg. V. Cassano (SP 399802); *Laurencia viridis*: Spain, Canary Islands, Tenerife, Playa Paraiso, 14.vii.2006, leg. M.T. Fujii, A. Senties and M.C. Gil-Rodríguez (SP 365662; TFC Phyc. 13130); *L. caduciramulosa*: Spain, Canary Islands, Tenerife, Punta del Hidalgo, 12.vii.2006, leg. M.T. Fujii, A. Senties and M.C. Gil-Rodríguez (TFC Phyc 13205), Brazil, Rio de Janeiro, Baía da Ilha Grande, Angra dos Reis, Saco de Piraquara de Fora, Praia do Velho, 22.i.2006, leg. V. Cassano and J.C. De-Paula (SP 371292), Ponta do Arame, 22.i.2006, leg. V. Cassano and J.C. De-Paula (SP 399918); *L. catarinensis*: Brazil, Espírito Santo, Anchieta, Ponta dos Castelhanos, 05.x.2006, leg. M.T. Fujii and V. Cassano (SP 399937), Rio de Janeiro, Angra dos Reis, Baía da Ribeira, Saco de Piraquara de Dentro, Ponta da Fortaleza, 14.xii.2006, leg. V. Cassano, M.T.M. de Széchy and M.T. Fujii (SP 399890).

#### Results and Discussion

A comparative study of the number, size, shape and location of the CC for *Laurencia caduciramulosa*, *L.*

*catarinensis*, *L. dendroidea*, *L. marilzae* and *L. viridis*, as well as the major chemical compounds produced by each species and their main biological activities is shown in Table 1. Except for *L. caduciramulosa*, all the other species are known only from the Atlantic Ocean (Cordeiro-Marino & Fujii, 1985; Gil-Rodríguez & Haroun, 1992; Cassano et al., 2006, 2008a, b; Gil-Rodríguez et al., 2009; Rocha-Jorge et al., 2010; Senties et al., 2010, 2011). Detailed morphological and molecular studies performed recently with species previously identified as *L. intricata* J.V. Lamouroux and *L. majuscula* (Harvey) A.H.S. Lucas from the Canary Islands demonstrated that the former corresponds to *L. catarinensis* (Machín-Sánchez et al., 2012) and the latter to *L. dendroidea* (Cassano et al., 2012).

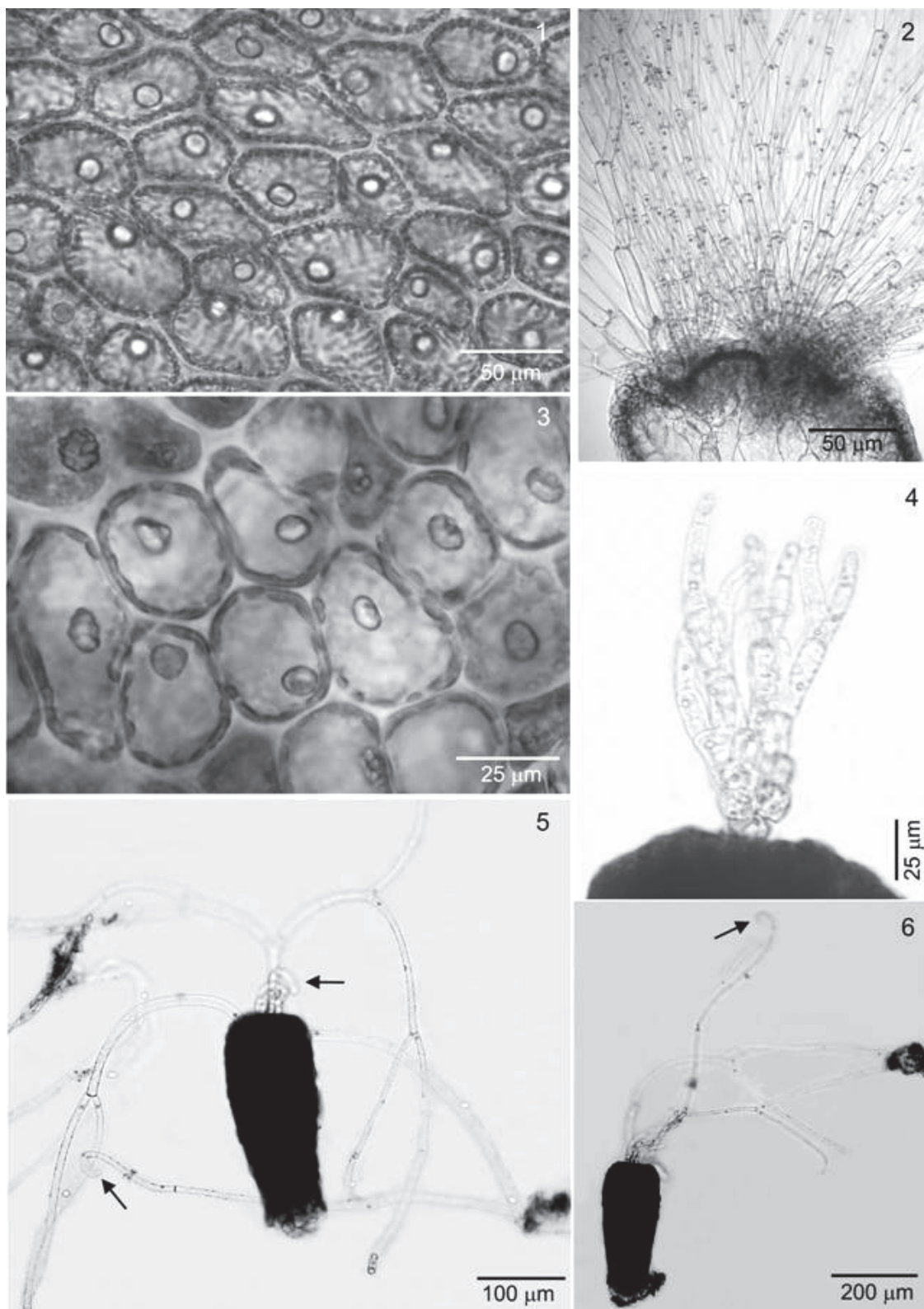
The variations of the CC observed in the cells of the thalli and trichoblasts are shown in Figures 1-13. In cortical cells, the number of CC ranged from 1 to 4 among species, varying from only 1 in *L. viridis* (Figure 1) to 1, rarely 2 in *L. caduciramulosa* (Figure 3) and *L. marilzae* (Figure 7), and (1) 2-3 (4) in *L. catarinensis* and *L. dendroidea* (Figures 11-13). Although the range of variation was constant within a species, there was a tendency of the numbers of CC to be higher in specimens of *L. catarinensis* and *L. dendroidea* from the Canary Islands. What factors may be influencing the slight differences observed in the number of CC in these specimens is difficult to predict, but it is believed that different strategies for secondary metabolite accumulation are related to defense against herbivores in the competitive environment, characteristic of the tropical and subtropical regions. The number of CC per cortical cell also seems to be a useful character for separating groups of species within the *Laurencia* genus. It was considered to be a critical taxonomic feature, segregating many species that possess a single CC from the few species that have several CC per cortical cell (Masuda & Abe, 1993; Masuda et al., 1998). The shape of the CC also varied from spherical, reniform to claviform (Figures 1, 3, 7 and 11-13). The size of the CC varied and overlaps among the species studied, however *L. caduciramulosa*, *L. marilzae* and *L. viridis* showed the smallest size (5.0-10.0 µm), and *L. catarinensis* and *L. dendroidea* the largest size (8.0-13.0 µm) (Table 1).

Regarding the number of CC in the trichoblasts, there is invariably one per cell in all species analyzed, but the shape can vary slightly from spherical, reniform to claviform. The functional roles of algal hairs and trichoblasts have not been clearly established, but various functions have been attributed to them such as aiding resistance against desiccation, nutrient uptake, metabolite secretion, shading and, in the case of the *Laurencia* complex, except *Osmundea*, reproduction by the production of female and male gametangia associated

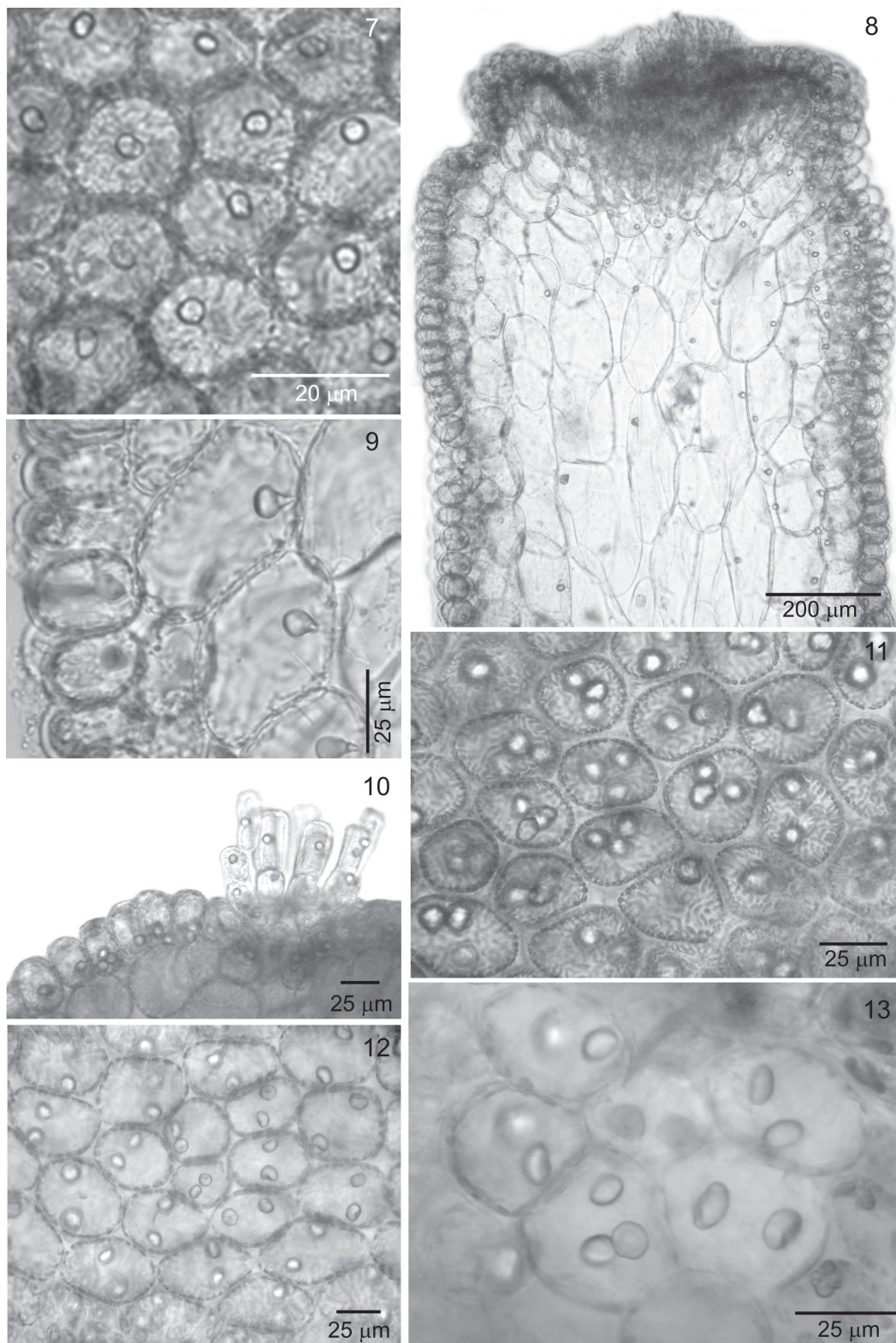
**Table 1.** Comparison of the *corps en cerise*, major chemical compounds produced and their main bioactivities in several species of *Laurencia* from Brazil (BR), Canary Islands (CI), Mexican Caribbean (MC) and Vietnam (VI). (Na=not available).

Characteristics	<i>L. caduciramulosa</i>		<i>L. catarinensis</i>		<i>L. dendroidea</i>		<i>L. marilzae</i>		<i>L. viridis</i>		
	VI Type locality	BR	CI	BR Type locality	CI	BR Type locality	CI	MC	BR	CI	
CC in the trichoblast/ number	1	1	1	1	1	1	1	1	1	1	
CC in the cortical cell/ number	1	1 (2)	1 (2)	2-3	2-4	(1) 2-3	2-4	1	1 (2)	1 (2)	
shape in superficial view	Na	reniform	reniform	reniform	reniform	spherical	spherical	claviform	claviform	spherical	
size in superficial view	Na	5-8.8	7.3-9.3	8.8-12.5	8.0-13.0	8.8-11.3	8.0-12.0	5.5- 8.0	7.0-10.0	6.0-7.0	
CC in all cells of the thallus	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present	Absent	
Sesquiterpene	<sup>1</sup> deoxypre-pacifenol (Chamigrane)	<sup>2</sup> pacifenol (major) (Chamigrane)	Na	<sup>3</sup> caespitol (Bisabolane)	<sup>4</sup> caespitol (Bisabolane)	<sup>5</sup> elatol, obtusane, obtusol, <sup>8</sup> cartilagineol <sup>9</sup> scopariol (Chamigrane)	<sup>4</sup> elatol, isobutisol (Chamigrane)		Present	Present	<sup>15</sup> viridanol
Secondary metabolites		filiformin, debromofiliformin ( $\delta$ -Bisabolene)									
Diterpene	<sup>1</sup> aplysiadiol	allolaurinterol, debromoallo-laurinterol (Laurane)							Na	<sup>16</sup> viridiols (Cyclophytane)	
Triterpene										<sup>17,18,19,20,21,22,23,24,25,26</sup> (Squalene derivatives)	
C <sub>15</sub> acetogenin	laurenynyne A and B									<sup>14</sup> marilzabicyclopallene (Bromoallene) <sup>15</sup> (Obusallene)	
Bioactivities	Na	antifouling	Na	<sup>3,6</sup> cytotoxic activities against human tumor cell	Na	<sup>3</sup> biofouling <sup>5,10,11</sup> antibioactivity <sup>9</sup> antihelminthic <sup>8,12</sup> antileishmanial <sup>6</sup> cytotoxic activities	Na	inative to some human tumor cell lines	Na	antitumoral, cytotoxic, antiviral activities, <sup>24</sup> PP2A inhibitory effects	

<sup>1</sup>Masuda et al. (1997), <sup>2</sup>Cassano et al. (2008a), <sup>3</sup>Lhullier et al. (2010), <sup>4</sup>Masuda et al. (1998 as *L. intricata*, <sup>4\*</sup> as *L. majuscula*), <sup>5</sup>Perreira et al. (2003 as *L. obtusa*), <sup>6</sup>Stein et al. (2011a), <sup>7</sup>Stein et al. (2011b), <sup>8</sup>Machado et al. (2011), <sup>9</sup>Davyt et al. (2001 as *L. scoparia*), <sup>10</sup>Da Gama et al. (2002, as *L. obtusa*), <sup>11</sup>Lhullier et al. (2009 as *L. microcladia*), <sup>12</sup>Santos et al. (2010), <sup>13</sup>Fernández et al. (2005), <sup>14</sup>Gutiérrez-Cepeda (2011a), <sup>15</sup>Gutiérrez-Cepeda (2011b), <sup>16</sup>Norte et al. (1994), <sup>17</sup>Norte et al. (1996a), <sup>18</sup>Norte et al. (1996b), <sup>19</sup>Norte et al. (1997a), <sup>20</sup>Norte et al. (1997b), <sup>21</sup>Fernández et al. (1998), <sup>22</sup>Manriquez et al. (2001), <sup>23</sup>Souto et al. (2002), <sup>24</sup>Souto et al. (2003), <sup>25</sup>Cen-Pacheco et al. (2011a), <sup>26</sup>Cen-Pacheco et al. (2011b).



**Figures 1-6.** Details of *corps en cerise* (CC). 1-2. *Laurencia viridis*. 1. Cortical cells in surface view showing a single CC per cell. 2. Apical region of the branchlet showing abundant trichoblasts; each cell with one CC. 3-6. *Laurencia caduciramulosa*. 3. Cortical cells in surface view showing one CC per cell. 4. Apical region of the branchlet not released showing short trichoblasts; each cell with one CC. 5-6. Details of deciduous branchlets (propagules) with long trichoblasts, terminated by hooks (arrows); each cell with one CC.



**Figures 7-13.** Details of corps en cerise (CC). 7-9 *Laurencia marilzae*. 7. Cortical cells in surface view showing one CC per cell. 8. Longitudinal section of a branchlet showing one CC in all cells of the thallus. 9. Detail of the longitudinal section showing CC in cortical and medullary cells. 10-11. *Laurencia dendroidea*. 10. Longitudinal section of a branchlet showing one CC per trichoblast cell and up to three CC in cortical cell. 11. Cortical cells in surface view showing one (two-three) CC per cell. 12-13. *Laurencia catarinensis*. 12. Cortical cells in surface view showing two CC per cell. 13. Cortical cells in surface view showing two-three CC per cell.

with the trichoblasts (Fritsch, 1945; Bold & Wynne, 1985; Delivopoulos, 2002). The similar functions attributed to both, such as nutrient uptake, are reinforced by several morphological similarities such as the presence of few chloroplasts and their position around the apices of the branches (DeBoer & Whoriskey, 1983; Garbary & Clarke, 2001). *Laurencia viridis* displays a single CC in each cortical cell and in the trichoblast cells. However, the trichoblasts are extremely long (Figure 2) and cover completely the thalli, suggesting that they may act against desiccation and may also confer efficient protection against herbivores, considering that they are chemically defended by the presence of the CC.

A different function for the trichoblasts was verified in *Laurencia caduciramulosa*; numerous long trichoblasts terminated by strongly recurved hooks were observed in deciduous branchlets (Cassano, 2009) (Figures 5-6), that differ from those branchlets not yet detached from the thalli (Figure 4). These branchlets, which function as vegetative propagules, are its only way of reproduction, since neither gametangia nor sporangia are produced by this species. The development of hooks in trichoblasts has never been described before for any species of the *Laurencia* complex. The hooks, entangled with each other or with the thalli of other macroalgae, aid in the dispersal of propagules. The formation of rhizoids was verified after mobilization of propagules by hooks. This suggests that the hooks assume the function of assisting the initial fixation of propagules. This is the first time that such a dispersal strategy has been observed. The existence of propagules as an alternative way of reproduction is important to understand the spread of *L. caduciramulosa*, a species introduced into the Mediterranean Sea (Klein & Verlaque, 2005) and Brazil (Cassano et al., 2006). *Laurencia caduciramulosa* from the type locality (Vietnam) was chemically characterized by the following halogenated secondary metabolites: aplysiadiol (diterpene), deoxyrepacifenol (sesquiterpene), and laurenenyne A and B (C15 acetogenins) (Masuda et al., 1997), while the Brazilian specimens produced only sesquiterpenes that differ from the mixture of sesquiterpenes and diterpenes reported for specimens from Vietnam. The main compound identified in *L. caduciramulosa* from Brazil is pacifenol, while filiformin, debromofiliformin, allolaurinterol and debromoallolaurinterol were observed as minority components of the crude extract (Cassano et al., 2008a). The mixture of sesquiterpenes and diterpenes reported for this species from Vietnam has drawn attention to a possible mixture of material in *L. caduciramulosa* from Vietnam, since the simultaneous production of sesquiterpenes and diterpenes is an uncommon feature in the *Laurencia* complex (Cassano et al., 2008a).

The crude extract obtained from Brazilian *L. caduciramulosa* was assayed for its antifouling

properties using the mussel *Perna perna* Linnaeus, 1778, as described by Epifanio et al. (2006) which exhibited more than 70% inhibition (Cassano et al., 2008a). Interestingly, the major component of the crude extract of *Laurencia caduciramulosa* from Brazil was pacifenol, a compound that has been reported for *Laurencia* species from the Pacific Ocean and Mediterranean Sea (Sims et al., 1971; Caccamese et al., 1986; Argandoña et al., 1993) and that was isolated for the first time from *Laurencia* species introduced into Brazilian waters by transoceanic shipping.

Only *Laurencia marilzae* possesses CC in every cell of the thallus (Figures 8-9) and, in this case, the shape and size are homogeneous, regardless of their location in cortical and medullary cells or trichoblasts. A similar situation was observed in *L. majuscula* from Dhofar, the Sultanate of Oman, by Wynne et al. (2005) and from the Mediterranean Sea by Serio et al. (2000) and Furnari et al. (2001). However, the similarities between *L. marilzae* and *L. majuscula* from Italy and Oman regarding the color of the thalli, and number and location of CC (one in all cells of the thallus) led Rocha-Jorge et al. (2010) to suggest that they should be the same taxonomic entity, viz., *L. marilzae*. This fact supports the use of the CC as a criterion for taxonomic recognition of *L. marilzae* for which the presence of CC in all cells of the thallus seems to be a feature unique to this species.

On the other hand, in *Laurencia dendroidea* from the Canary Islands, previously identified as *L. majuscula* by Masuda et al. (1998), up to six CC per each cortical cell were observed, although three-four is more usual. This condition also differs from the specimens from Oman identified by Wynne et al. (2005) and from the Mediterranean Sea identified by Serio et al. (2000) and Furnari et al. (2001) as *L. majuscula*, which displayed a single CC in all cells of the thallus.

Of the species studied, *L. viridis* is the most prolific synthesizer of secondary metabolites, producing sesquiterpene, diterpene and a variety of squalene-derived polyether compounds with important pharmacological properties, such as potent cytotoxic effects, protein phosphatase type 2A (PP2A) inhibition and integrin antagonist activity (Norte et al., 1996b, 1997a, 1997b; Fernández et al., 1998; Manriquez et al., 2001; Souto et al., 2002, 2003; Cen-Pacheco et al., 2011a, b). Such a large chemical arsenal produced by a single species is unknown for other species of *Laurencia*, showing the great biotechnological potential to be explored.

Salgado et al. (2008), studying the localization of bromo-containing products in *Laurencia dendroidea* (as *L. obtusa*) from Brazil, confirmed that CC are the main brominated substance accumulation sites. Other species of this study should also work in the same way, since all species without exception, produce some kind of halogenated secondary metabolite and all of them have



CC in the cortical cells and in the trichoblasts and in all cells of the thallus in the case of *L. marilzae*.

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