

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE BIOCÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

Tese de Doutorado

**Estudos taxonômicos em espécies de *Ramalina* Ach. (*Ascomycota*
liquenizados, *Ramalinaceae*)**

Emerson Luiz Gumboski

PORTO ALEGRE, 2016

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Tese apresentada ao Programa de Pós-Graduação em Botânica, Área de Concentração em Taxonomia Vegetal (Ficologia e Micologia), da Universidade Federal do Rio Grande do Sul (UFRGS), como parte dos requisitos para a obtenção do título de Doutor em Ciências (Botânica).

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PORTO ALEGRE, 2016

*There was a time when my life was easy
Stretched out in the sun
Everything was clover
The world was off my shoulders for awhile
Then the sky turned a bonfire shade
And hit me like a gun
It passed with flying colors
There's no flying over*

*The storm
We will dance as it breaks
The storm
It will give as it takes
And all of our pain is washed away
Don't cry or be afraid
Some things only can be made
In the storm*

FLYING COLORS – The Storm (2012)

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RESUMO

Estudos taxonômicos em espécies de *Ramalina* Ach. (*Ascomycota* liquenizados, *Ramalinaceae*)

O gênero *Ramalina* Ach. possui cerca de 250 espécies aceitas e está distribuído por todo o mundo. Desde a circunscrição inicial do gênero em 1809, aceitava-se que boa parte das espécies possuía distintos graus de plasticidade morfológica e até química, resultando em centenas de nomes na literatura e certa confusão a respeito da sistemática do mesmo. Estudos recentes revelaram uma diversidade oculta para o grupo e que, possivelmente, algumas características estavam sendo negligenciadas. Com o objetivo de contribuir para o entendimento da sistemática de *Ramalina*, o presente estudo utilizou-se de análises morfológicas, anatômicas, químicas e moleculares. No total foram examinados 2415 espécimes de *Ramalina*, dos quais 332 representam espécimes tipo. Foram geradas 278 novas sequências relativas a 24 espécies, sendo 195 sequências de ITS, 59 de IGS e 24 de rpb2. O estudo identificou 27 espécies distribuídas por doze estados brasileiros e em diversos ambientes, tais como restingas, mata de araucária, campos de altitude, Cerrado e Caatinga. Duas espécies são novas para a ciência. *Ramalina anceps* foi confirmada como espécie distinta de *R. usnea*. A espécie *R. subfraxinea* foi excluída da micota brasileira. Foram registradas 33 novas ocorrências em vários Estados brasileiros, o que amplia consideravelmente a distribuição das espécies no país. A importância de ter descrições detalhadas sobre a morfologia e anatomia foi comprovada através dos estudos tendo respaldo das análises moleculares. O presente estudo contribui muito para o conhecimento a cerca dos problemas taxonômicos e sistemáticos das espécies de *Ramalina*, não apenas em nível nacional, mas mundial. Sobe para 38 o número de espécies de *Ramalina* conhecidas para o Brasil. A Região Sul teve substancial ganho no conhecimento a respeito das espécies presentes, bem como parte da Região Sudeste e Nordeste. As Regiões Centro-Oeste e Norte ainda carecem de coleções suficientes mesmo em herbários nacionais. Descrições, comentários, ilustrações e chaves de identificação são apresentadas.

Palavras-chave: Análises moleculares, circunscrição de espécies, fungos liquenizados, taxonomia, talo fruticoso.

ABSTRACT

Taxonomic studies in species of *Ramalina* Ach. (Lichenized *Ascomycota*, *Ramalinaceae*)

Ramalina Ach. has ca. 250 species accepted and is distributed worldwide. Since the initial division of the genus in 1809, it was accepted that many of the species had different degrees of morphological and even chemical plasticity, resulting in hundreds of names in literature and some confusion about the systematic of the group. Recent studies have revealed a hidden diversity to the group and possibly some characteristics were being neglected. In aim to contribute to the understanding of systematic of *Ramalina*, this study used morphological, anatomical, chemical and molecular analyzes. 2415 specimens of *Ramalina* were examined, of which 332 represent type specimens. 278 new sequences related to 24 species were generated: 195 sequences of ITS, 59 of IGS and 24 of rpb2. The study identified 27 species distributed on twelve Brazilian states and in different environments, such as coastal vegetation, Araucaria forest, high altitude grasslands, savannah and Caatinga vegetation. Two species are new to science. *Ramalina anceps* was confirmed as a distinct species of *R. usnea*. The species *R. subfraxinea* was excluded from the Brazilian mycota. Thirty three new records were found in some Brazilian states, which considerably expand the distribution of species in the country. The importance of having detailed descriptions of morphology and anatomy has been proven through the studies and supported by the molecular analysis. This study contributes to the knowledge about the taxonomic and systematic problems of the species of *Ramalina*, not only nationally, but worldwide. The number of *Ramalina* species known to Brazil increases to 38. The South region had substantial gain to the knowledge about the species present, and part of the Southeast and Northeast regions. The North and Midwest regions still lack of sufficient collections even in national herbaria. Descriptions, comments, illustrations and identification keys are given.

Key words: fruticose thallus, lichenized fungi, molecular analysis, species circumscription, taxonomy.

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1. INTRODUÇÃO

Ramalina Ach. in Luyken (1809), pertencente a família *Ramalinaceae*, é um gênero de fungos liquenizados cosmopolita que ocorre desde regiões litorâneas até áreas montanhosas (Stevens 1987; Marcano & Méndez 1994; Kashiwadani & Nash 2004), de áreas quentes tropicais ao frio antártico (e.g., Kashiwadani & Nash 2004; Øvstedal & Smith 2001).

O gênero é relativamente bem representado na maior parte das micotas de diversos ambientes, tanto em número de espécies quanto em biomassa (e.g. Krog & Swinscow 1976; Brodo *et al.* 2001; Aptroot & Bungartz 2007). Devido a ampla distribuição e a sensibilidade frente a mudanças ambientais, frequentemente as espécies de *Ramalina* são utilizadas em estudos que enfocam alterações ambientais (e.g. Boonpragob & Nash 1991; Cercasov *et al.* 2002; Augusto *et al.* 2007).

De modo geral, *Ramalina* pode ser caracterizado pelo talo fruticoso geralmente com pseudocifelas. Frequentemente possui um tecido de suporte prosoplectenquimático (condroideo). O fotobionte é do gênero *Trebouxia* Puymaly. Possui ascósporos hialinos, 1(-3)-septados (Kashiwadani & Kalb 1993; Brodo *et al.* 2001) e produz ácido úsnico presente na região cortical (Krog & James 1977; Krog & Østhagen 1980).

Atualmente são reconhecidas cerca de 150 a 250 espécies (Kashiwadani & Nash 2004; Kirk *et al.* 2001; Sérusiaux *et al.* 2010), entretanto na literatura são encontrados mais de 1200 nomes (mais de 480 espécies!), incluindo as espécies válidas, sinonímias, nomes e combinações supérfluas. A sistemática do gênero é por vezes confusa e reflete as distintas interpretações a seu respeito que surgiram ao longo do tempo.

1.1. Breve histórico do gênero

Ramalina possui amplo histórico sistemático, tendo seus principais fatos relatados de forma objetiva por Stevens (1987, p. 115-117). Contudo, embora Stevens (1987) tenha mencionado que Acharius descreveu o gênero *Ramalina* pela primeira vez em 1810, o liquenólogo sueco já o havia realizado no trabalho de Luyken, em 1809 [nome conservado de acordo com o art. 14 do Código Internacional de Nomenclatura para Algas, Fungos e Plantas] (Hawksworth *et al.* 2007).

Cerca de 50 anos depois do estabelecimento de *Ramalina* como gênero, Montagne (1852) propôs o gênero *Desmazieria* Mont. baseado em características anatômicas do córtex de *R. homalea* Ach., e Massalongo (1854) propôs *Cenozosia* Massal. focando na morfologia do talo de *R. inanis* Mont. Stizenberger (1862) não concordou com os novos gêneros e, utilizando características dos picnídios aliado a morfologia dos ramos, o autor dividiu *Ramalina* em três grupos utilizando os nomes *Cenozosia* (Massal.) Stizenb., *Desmazieria* (Mont.) Stizenb. e *Euramalina* Stizenb.

Nylander (1870) não seguiu a classificação genérica dos autores anteriormente citados e, em sua monografia sobre *Ramalina*, dividiu o gênero em três grupos informais, não nomeados, fundamentado apenas na coloração do picnídio.

Vainio (1890) também não seguiu, para *Ramalina*, os gêneros propostos por Montagne (1852) e Massalongo (1854), aceitando como seções *Euramalina* Stizenb., *Desmazieria* (Mont.) Vain. e *Cenozosia* (Massal.) Vain. Vainio (1890) então subdividiu *Euramalina* em *Fistularia* Vain. e *Myelopoea* Vain., sendo esta última subdividida novamente nas séries *Teretiusculae* Vain. e *Compressiusculae* Vain. de acordo com a morfologia dos ramos das espécies.

Hue (1901) dividiu *Ramalina* em três grupos, com base na morfologia dos ramos utilizou os nomes *Compressiusculae* Vain., *Fistulosae* Hue e *Teretiusculae* Vain. Em 1904, Steiner dividiu *Ramalina* em três seções propostas de acordo com a morfologia do tecido externo à camada algal, sendo seção *Corticatae* Stein., *Bitactae* Stein. e *Ecorticatae* Stein. Pouco tempo depois, Zahlbruckner (1907) dividiu *Ramalina* também em três seções, sendo elas: *Ecorticatae* Stein., *Corticatae* Stein. e *Euramalina* Stizenb. Essa última seção ainda foi dividida nas subseções *Fistularia* Vain., *Myelopoea* Vain., *Teretiusculae* (Vain.) Zahlbr. e *Compressiusculae* (Vain.) Zahlbr.

Howe (1913; 1914) não seguiu as classificações anteriormente propostas e, buscando uma classificação mais natural, utilizou características dos ascósporos para separar as espécies de *Ramalina* em três seções: Seção *Elliposporae* R. Howe, *Fusisporae* R. Howe e *Bistortae* R. Howe. Ao passo que Du Rietz (1926) utilizou *Desmazieria* (Mont.) Du Rietz e *Euramalina* (Stizenb.) Du Rietz como subgêneros, sendo este último dividido nas seções *Tenuicorticatae* R. Howe e *Bitectae* Stein. Na seção *Bitectae* o autor utilizou duas subseções: *Myelopoea* Vain. e *Fistularia* Vain., enquanto na seção *Tenuicorticatae*, Du Rietz (1926) dividiu as espécies entre as

subseções *Solidae* Du Rietz e *Tubulosae* Du Rietz. Em 1931, Choisy propôs o gênero *Dievernina* Choisy com base nas espécies da subseção *Solidae* Du Rietz.

Magnusson & Zahlbruckner (1945) dividiram o gênero em quatro seções, sendo elas: *Ramalinopsis* Zahlbr. (incluía a única espécie de *Ramalina* que apresentava talo parmelióide), *Ecorticatae* Stein., *Desmaziera* (Mont.) Stizenb. e *Euramalina* Stizenb. Em 1969, Follmann & Huneck (1969) elevaram a gênero *Ramalinopsis* (Zahlbr.) Follmann & Huneck.

Rundel & Bowler (1974) descreveram *Trichoramalina* Rundel & Bowler, baseados em *Ramalina crinita* Tuck. Em 1977, Bowler & Rundel propuseram o gênero *Fistulariella* Bowler & Rundel incluindo neste as espécies pertencentes ao grupo *Fistularia* de Vainio (1890), espécies essas com talo oco, inflado, entretanto, tal característica pode ser encontrada de modo inconstante em muitas espécies de *Ramalina*. Um ano depois, Rundel & Bowler (1978) propuseram o gênero *Niebla* Rundel & Bowler, contemplando as espécies inclusas em *Desmaziera* Mont. [nome não utilizado por ser homonímia de *Desmazeria* Dumort., *Poaceae*, Art. 53.3 do Código Internacional de Nomenclatura para Algas, Fungos e Plantas].

Muitas contribuições para a sistemática de *Ramalina* foram feitas pela Dra. Hildur Krog e colaboradores (e.g., Krog & Swinscow 1974, 1976; Krog & James 1977; Krog & Østhagen 1978, 1980; Krog 1983, 1990, 1994), entretanto, os trabalhos não apresentavam divisões infragenéricas. O principal comentário a respeito das divisões diz respeito a rejeição do gênero *Fistulariella* em Krog & Østhagen (1980, p. 262).

Vinte e quatro anos depois, Kashiwadani & Nash (2004) reduziram *Trichoramalina* a sinonímia de *Ramalina*, considerando também sinonímias do gênero os nomes *Fistulariella* Bowler & Rundel e *Dievernina* Choisy.

Interessante notar que mesmo sendo um gênero com muitas espécies e distribuído pelo mundo todo, pouquíssimos trabalhos incluindo análises filogenéticas foram realizados até então. LaGreca (1999) foi o primeiro trabalho a utilizar dados moleculares para definir a circunscrição de espécies do complexo ‘*Ramalina americana*’, revelando, a exemplo, uma nova espécie dentre os quimiotipos estudados.

O primeiro (e único!) trabalho utilizando dados e análises genéticas para demonstrar as relações filogenéticas (intra e infragenéricas) em *Ramalina* foi realizado por Sérusiaux *et al.* (2010). Os autores concluem, resumidamente, que *Niebla* e *Ramalina* são gêneros distintos e que nenhuma das classificações infragenéricas

propostas outrora possui suporte filogenético. Embora não tenha sido o foco do trabalho, é interessante notar a presença de nomes (espécies) polifiléticos, como o caso de *R. subfarinacea* (Nyl. ex Cromb.) Nyl., *R. requienii* (De Not.) Jatta, *R. subwebbiana* (Nyl.) Hue e *R. bourgeana* Mont. ex Nyl., apontando para a necessidade de estudos mais aprofundados sobre a real diversidade contida no gênero. Importante também apontar que o referido estudo utilizou essencialmente espécimes europeus, norte americanos e da Macaronésia (conjunto de ilhas do noroeste africano).

Os demais trabalhos, poucos foram anteriores a 2010, focaram nas relações filogenéticas de algumas espécies, ou grupos de espécies, visando resolver problemas de circunscrição em nível específico (Stocker-Wörgötter *et al.* 2004; Ohmura *et al.* 2008; Timsina *et al.* 2012; Pérez-Vargas & Pérez-Ortega 2014; Oh *et al.* 2014; Hayward *et al.* 2014).

1.2. Morfologia e anatomia

Historicamente *Ramalina* têm sido conhecido por apresentar espécies com notável plasticidade fenotípica (e.g., Stevens 1987; Krog & Swinscow 1976). Entretanto, estudos recentes indicam que tal plasticidade deve ser vista com mais cautela, e que determinadas características antes tidas como ‘plásticas’ podem ser úteis para a distinção de espécies (Ohmura *et al.* 2008; Pérez-Vargas & Pérez-Ortega 2014; Hayward *et al.* 2014).

As espécies de *Ramalina* apresentam talo fruticoso, variando de poucos centímetros, com característica cespitosa a longos talos pendentes, que podem atingir mais de dois metros de comprimento (Krog & Østhagen 1980; Aptroot & Bungartz 2007). Os talos podem apresentar a parte interna completamente preenchida por uma camada medular e então são considerados sólidos, ou apresentar a parte interna completamente oca, formando assim talos fistulosos (e.g., Krog & Swinscow 1974). Contudo, essa característica deve ser vista com cautela, pois algumas espécies podem apresentar dilatações em alguns pontos dos ramos, o que pode levar a confusão de interpretação sobre se os talos são sólidos ou não (Krog & Østhagen 1980).

Os ramos variam de achatados a cilíndricos, apresentando diversos padrões ao longo desse gradiente. Podem, inclusive, apresentar-se de modo distinto em um mesmo talo. Espécimes de *Ramalina peruviana* Ach. podem, por exemplo, apresentar os ramos

principais claramente achatados, ao passo que os ramos secundários e terminais frequentemente são cilíndricos ou irregularmente cilíndricos (Krog & Swinscow 1976; Stevens 1987). Algumas espécies podem apresentar um padrão bem mais definido, com ramos unicamente achatados ou então unicamente cilíndricos. Dentre as espécies com ramos achatados, duas formas merecem atenção. A primeira, embora poucas apresentem o talo desse modo, é o formato palmado tal como *R. unifolia* J.W. Thomson. E a segunda diz respeito aos ramos lanceolados que se caracterizam por serem achatados e com estreitamento da base até a parte terminal dos ramos, tal qual ocorre em uma lança. Os ramos ainda podem ser simples (únicos), ou apresentar divisão desde irregular a claramente dicotômica, às vezes irregularmente dicotômica ou tricotômica (mais rara) (Kashiwadani & Nash 2004).

A superfície dos ramos também é bastante variável entre as espécies, ao passo que a variação intraespecífica deve ser observada com cautela. Podem ser encontradas espécies com a superfície dos ramos claramente lisa ao longo de todo o talo, em outras há certa rugosidade principalmente em ramos principais. Algumas espécies podem apresentar a superfície estriada, escrobiculada ou foveolada. A presença de rachaduras não é incomum em espécimes recém-coletados, mas deve ser vista com cuidado principalmente quando se analisar espécimes antigos de herbários (Krog & Swinscow 1976; Krog & James 1977).

Uma das características das espécies de *Ramalina* é a presença de pseudocifelas na superfície. Poucas espécies não apresentam pseudocifelas, dentre elas, comumente as que possuem talos ocos. As pseudocifelas variam de puntiformes, orbiculares, elipsoides até longo lineares. Elas podem estar presentes em todo o talo ou restritas a margem. Distribuídas de modo conspícuo com muitas pseudocifelas ao longo do ramo, ou inconspícuo com pseudocifelas diminutas em poucos locais dos ramos. Algumas podem formar sulcos, outras permanecem ao nível da superfície, contíguas, ou ainda podem se apresentar de forma saliente, tratadas como tuberculoides (e.g., Krog & Østhaugen 1980; Stevens 1987; Kashiwadani & Kalb 1993).

Os propágulos simbióticos em *Ramalina* dizem respeito essencialmente a produção de sorédios, raras espécies apresentam isídios granulares (i.e., Kashiwadani 1987), entretanto, estes últimos parecem mais com um empilhamento de sorédios do que com um isídio propriamente dito. Cerca de metade das espécies conhecidas do gênero produzem sorédios. A produção se dá nos sorais ou diretamente na medula

exposta. Os sorais podem ocorrer de modo uniforme em todo o talo ou então em locais delimitados, como por exemplo, nas margens ou ápice dos ramos secundários (Stevens 1987). Algumas espécies produzem uma dilatação terminal no ramo (cócleas) que ao se romper passa a produzir sorédios diretamente na medula exposta, a exemplo de *Ramalina cochlearis* Zahlbr (Kashiwadani & Kalb 1993). Os sorédios variam em tamanho entre as espécies, contudo, em muitos trabalhos (principalmente anteriores a 1950) era frequente definir os sorédios apenas como farinosos ou granulares e sem a devida mensuração de diâmetro. Ohmura *et al.* (2008) demonstraram a importância taxonômica da mensuração do diâmetro dos sorédios ao comparar três espécies muito semelhantes morfologicamente. Os autores observaram diferenças significativas no tamanho dos sorédios para todas elas, portanto, sendo um bom caractere para distinção das mesmas.

Embora tenha sido utilizado outrora como característica para classificar inter e intragenericamente o gênero (*e.g.*, Nylander 1890), a presença de picnídios em espécimes de *Ramalina* não é frequente (Stevens 1987; Kashiwadani & Nash 2004). Os picnídios variam em coloração, de pálida quase incolor a negra, de esférico a hemiesférico, de 0,1 mm a 0,2 mm de diâmetro. O ostíolo também pode variar de uma coloração pálida a negra, acompanhando ou não a coloração geral do picnídio. Distribuem-se essencialmente pelos ramos, geralmente na porção terminal do mesmo. Os conídios são hialinos, baciliformes, variando de 3,5 a 6,0 μm de comprimento por 0,5 a 1,0 μm de largura (Krog & James 1977; Stevens 1987; Kashiwadani & Nash 2004).

A presença de apotécios é comum em diversas espécies de *Ramalina*, em especial àquelas que não produzem sorédios (Kashiwadani & Kalb 1993). Podem ser curto-pedicelados ou sésseis. A posição dos apotécios nos ramos bem como o formato e dimensões dos ascósporos têm sido considerados importantes taxonomicamente. (*e.g.*, Krog & Swinscow 1974, Stevens 1987; Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004). Os apotécios podem distribuir-se sobre todo o ramo [laminal], apenas na margem [marginal/lateral], apenas no ápice dos ramos [terminal] ou pouco abaixo do ápice [subterminal] (Stevens 1987; Kashiwadani & Kalb 1993). Em algumas espécies, principalmente as que possuem ramos mais finos, a presença do apotécio gera um desvio no sentido de crescimento do ramo, ou seja, o ramo apresenta aspecto geniculado na inserção do apotécio (Kashiwadani & Kalb 1993).

Em algumas espécies, na margem dos apotécios podem ocorrer pequenas projeções isidioides, similares a esporões (*e.g.*, Krog & Swinscow 1974; Kashiwadani & Kalb 1993). O formato dos apotécios pode variar de côncavos, planos a convexos. Pode também ocorrer variação no decorrer do desenvolvimento do mesmo, ou seja, inicialmente o apotécio é côncavo e vai se tornando convexo com a maturação. A margem é frequentemente distinta e inteira, às vezes apresentando quebras principalmente na maturidade. O diâmetro varia de espécie para espécie, mas geralmente mede de 0,2 mm a 5,0 mm, raramente atingindo 10,0 mm (Stevens 1987).

A coloração do disco himenial pode variar de verde esbranquiçado a suavemente alaranjado. Quando em herbário a coloração pode ser intensificada, adquirindo inclusive tonalidades amarronzadas. A presença de pruína é constante em algumas espécies, discreta ou ausente em outras (Kashiwadani 1987; Stevens 1987). O himênio, formado por ascos e paráfises, é incolor. Os ascos são clavados a subcilíndricos e as paráfises são esparsamente ramificadas. O hipotécio é geralmente escurecido, variando de amarelo amarronzado a amarronzado (Stevens 1987). Uma camada, regular a irregular, de tecido condroideo pode ou não estar presente logo abaixo do hipotécio.

De acordo com Kashiwadani & Nash (1993), a anatomia dos apotécios e ramos tem sido bem estudada devido a sua importância taxonômica. O primeiro estudo anatômico mais detalhado dos ramos foi realizado por Krog & Østhagen (1980), considerando importante a disposição do tecido condroideo e medular. Contudo, foi apenas com os estudos do líquenólogo Dr. Hiroyuki Kashiwadani (*e.g.*, Kashiwadani 1986, 1987, 1988, 1990; Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004; Kashiwadani *et al.* 2007) que características do próprio tecido condroideo [presença ou não de fragmentação] tiveram sua importância demonstrada. Ou seja, muitas espécies de *Ramalina* carecem de informações detalhadas a respeito de características anatômicas, o que ressalta a urgente necessidade de revisão de tipos nomenclaturais.

Com relação aos ramos dos talos, as espécies podem ou não apresentar uma camada cortical externa prosoplectenquimática. Logo abaixo pode ou não estar presente o tecido condroideo, de aspecto cartilaginoso e bastante resistente, dando sustentação ao talo. O tecido condroideo pode ou não apresentar fragmentação e sua disposição pode ser logo abaixo do córtex, disposto de forma contínua, descontínua, em feixes cilíndricos ou então de modo aleatório (*e.g.*, Krog & Østhagen 1980; Kashiwadani & Kalb 1993). A medula pode estar presente abaixo do córtex ou então abaixo do tecido

condroideo. Em espécies fistulosas, a medula fica exposta na parte interior do ramo. Dependendo da espécie, a medula pode se apresentar de forma frouxa ou densa (e.g., Krog & Swinscow 1974, 1976; Aptroot & Bungartz 2007).

Kashiwadani & Kalb (1993) enfatizam que as características anatômicas também são importantes na distinção de espécies de *Ramalina*, mas essas informações foram em sua grande maioria desconsideradas em estudos antigos (Nylander 1870; Howe 1913; Wade 1961; Krog & James 1977) e até mesmo em trabalhos recentes (Aptroot & Bungartz 2007; Sipman 2011). Isso acaba por dificultar a comparação de espécimes com as descrições disponíveis na literatura.

1.3. Química secundária

De acordo com Krog & Østhagen (1980), o ácido úsnico varia em concentração e ocorre na região cortical, ou externa, em provavelmente todas as espécies de *Ramalina*. A presença de atranorina na região cortical é mais rara (Krog & Swinscow 1976). A produção de compostos secundários no gênero é bastante rica, sendo relatados ácidos graxos, triterpenoides, orcinol depsídeos, β -orcinol depsídeos e β -orcinol depsídonas (e.g., Culberson 1965, 1966, 1967, 1970; Krog & Swinscow 1976; Krog & Østhagen 1980; Kashiwadani & Nash 2004).

As características morfológicas e químicas têm sido a base para a distinção das espécies deste gênero (Wade 1961; Krog & Swinscow 1976; Stevens 1987). Na parte química, a aceitação de quimiotipos [exemplares com química distinta, mas considerados sob a mesma espécie por não apresentarem diferenças morfológicas] é frequente (Kashiwadani & Kalb 1993). Entretanto, tal fato tem sido visto com mais cautela atualmente.

Ohmura *et al.* (2008) publicaram um estudo que reforça a necessidade de revisão de exemplares tipos das espécies de *Ramalina* seguindo princípios taxonômicos mais detalhados. Os autores estudaram três quimiotipos sorediados muito similares morfológicamente, mas que em algum momento foram considerados por alguns autores como espécies distintas. Com a utilização de análises morfo-anatômicas e moleculares, Ohmura *et al.* (2008) concluíram que os quimiotipos eram espécies distintas, sendo que as diferenças químicas, de propágulo e de habitat se revelaram boas para tal separação.

1.4. Estudos sobre *Ramalina* no Brasil

Eschweiler (1833) foi o primeiro pesquisador a citar, e descrever, uma *Ramalina* para o Brasil. O autor descreveu *Parmelia denticulata* Eschw. [*Ramalina denticulata* (Eschw.) Nyl.] em 1833 (*in* Martius 1833) baseado em um espécime coletado sobre ramos de plantas as margens do rio Amazonas.

Dez anos depois, Meyen & Flotow (1843) descreveram *R. costata* e *R. costata* var. *compressa* para o Rio de Janeiro. Nylander (1870) descreveu três novas espécies [*R. lanceolata* Nyl., *R. rectangularis* Nyl. e *R. subpollinaria* Nyl.], e citou mais oito ocorrentes no país. Müller Argoviensis (1879) descreveu *R. tenella* Müll. Arg. e, dois anos depois, o mesmo autor (Müller Argoviensis 1881) também descreveu *R. puiggarii* Müll. Arg., ambas para São Paulo.

Vainio (1890) descreveu uma nova espécie [*R. flagellifera* Vain.] e dois novos táxons infraespecíficos [*R. complanata* f. *reagens* Vain., *R. denticulata* var. *subolivacea* Vain.], bem como relatou a ocorrência de mais cinco espécies para o Brasil. Importante ressaltar que o mesmo autor ainda distribuiu 16 táxons de *Ramalina* brasileiras através do *Lichenes Brasiliensis Exsiccati*.

Zahlbruckner (1902) descreveu *R. denticulata* var. *stephanophora* Zahlbr. e citou a ocorrência de mais três espécies para o país. Três anos depois, o mesmo autor (Zahlbruckner 1905) descreveu *R. yemensis* var. *minima* Zahlbr. e *R. cochlearis* Zahlbr., incluindo *R. yemensis* var. *eckloni* para Minas Gerais.

Malme (1934), no primeiro trabalho exclusivo de *Ramalinae* coletadas no Brasil e Paraguai, descreveu *R. ecklonii* f. *lobuligera* Malme e citou a ocorrência de outras sete espécies para o país.

Durante cerca de 40 anos não houve mais registros de espécies de *Ramalina* para o Brasil. A partir de 1977, o líquenólogo Héctor S. Osório citou diversas espécies (e.g., Osório 1977a,b, 1981, 1992; Osório & Homrich 1978; Osório & Fleig 1986a,b; Osório *et al.* 1981) para várias localidades do Brasil, mas principalmente para o Rio Grande do Sul (Spielmann 2006; Spielmann & Marcelli 2009).

Kashiwadani (1987) descreveu *R. grumosa* Kashiw. para o Peru, mas citou um espécime proveniente do Brasil. Seis anos depois, Kashiwadani & Kalb (1993) realizaram um trabalho exclusivo sobre as espécies brasileiras de *Ramalina*. Os autores descreveram 22 espécies, sete táxons foram reduzidos a sinônimas, e cinco espécies

foram registradas pela primeira vez para o país. O trabalho conta principalmente com espécimes coletados pelo líquenólogo Dr. Klaus Kalb e principalmente da Região Sudeste.

Quase 15 anos depois, Kashiwadani *et al.* (2007) descreveram *R. gallowayi* Kashiw., T.H. Nash & K.H. Moon para o Brasil baseados em espécimes oriundos de Minas Gerais. E recentemente, Gumboski *et al.* (2014) citaram a ocorrência de *R. lacera* (With.) J.R. Laundon para o país.

Atualmente, existem 37 espécies de *Ramalina* registradas para o Brasil, sendo 17 para a Região Sul, 30 para o Sudeste, nove para o Nordeste, quatro para o Centro-Oeste e apenas duas para o Norte (Figura 1). Os Estados onde foram registrados maior número de espécies são: Rio de Janeiro com 21 espécies, São Paulo com 18 e Minas Gerais com 16. Em quatorze Estados não foi registrada nenhuma espécie de *Ramalina* (Figura 2), o que deixa claro a carência de estudos sobre o gênero no país.

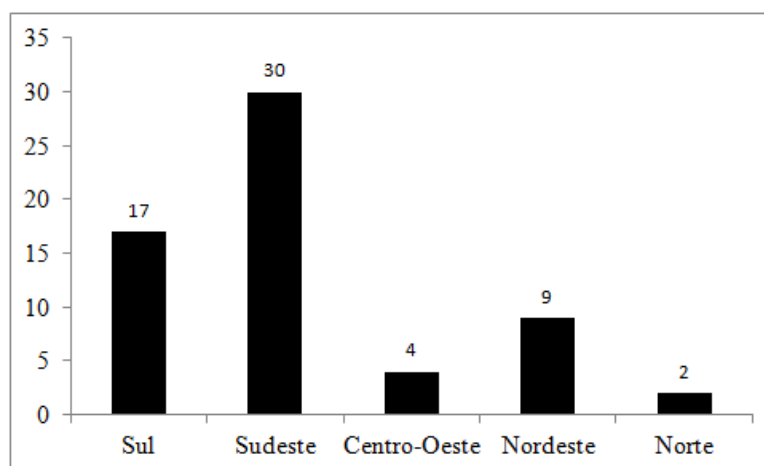


Fig. 1: Número de espécies de *Ramalina* registradas por Região.

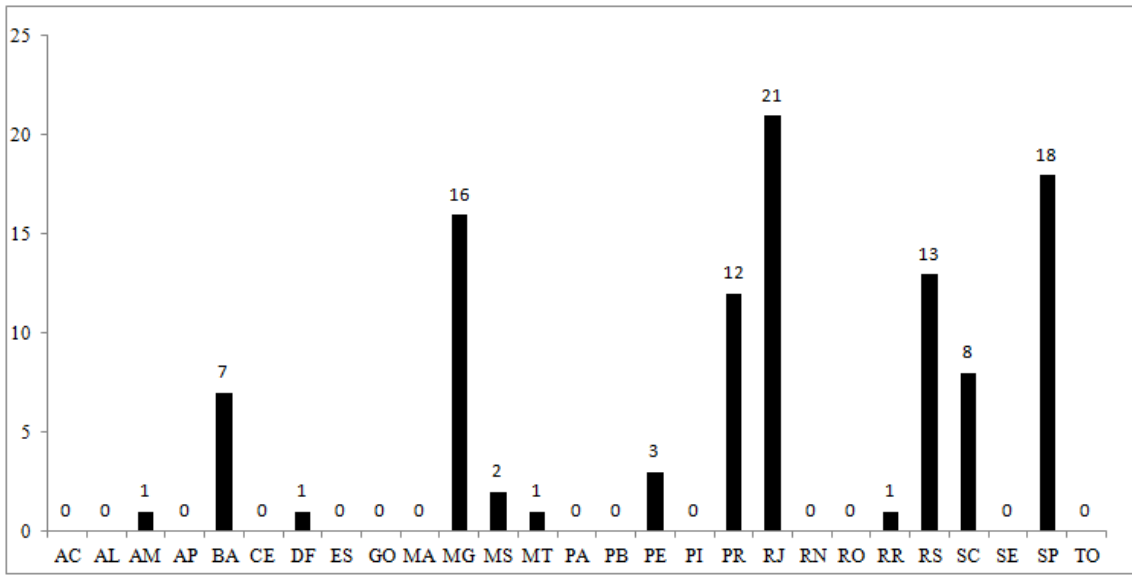


Fig. 2: Número de espécies de *Ramalina* registradas por Estado.

2. OBJETIVOS

O objetivo principal do trabalho é resolver os problemas taxonômicos acerca das espécies brasileiras de *Ramalina*, por meio de uma abordagem taxonômica baseada em análise morfológica, química e molecular.

Estão inclusos como objetivos específicos: realizar um estudo taxonômico detalhado de todos os tipos de *Ramalina*; caracterizar morfológica, anatômica e quimicamente as espécies estudadas; propor novas circunscrições de táxons problemáticos com base em estudos morfológicos, químicos e moleculares; proporcionar meios para a identificação das mesmas através de chaves, ilustrações e descrições.

3. MATERIAIS E MÉTODOS

3.1. Áreas de coleta

Foram visitados 29 municípios (49 localidades) entre os meses de abril de 2012 e janeiro de 2015. Contudo, espécimes previamente coletados pelo autor desde 2008 também foram incluídos no trabalho tendo, portanto, espécimes de 43 municípios (Tabela 1). Maior atenção foi dada a Santa Catarina e Paraná devido a escassez de exemplares em herbário e facilidade de locomoção. O Rio Grande do Sul possui vasta coleção depositada no herbário ICN essencialmente de espécimes coletados pela líquenóloga Dra. Mariana Fleig.

Tab. 1. Municípios onde foram coletados espécimes de *Ramalina*.

	Mato Grosso do Sul		Santa Catarina	30	Monte Castelo
1	Porto Murtinho	14	Balneário Gaivota	31	Palhoça
	Minas Gerais	15	Bom Jardim da Serra	32	Penha
2	Catas Altas	16	Bombinhas	33	Rio Negrinho
	Paraná	17	Caçador	34	Santa Cecília
3	Campo Largo	18	Campo Alegre	35	Santo Amaro da Imperatriz
4	Matinhos	19	Canoinhas	36	São Bento do Sul
5	Guaraqueçaba	20	Florianópolis	37	São Francisco do Sul
6	Guaratuba	21	Fraiburgo	38	Três Barras
7	Paranaguá	22	Garuva		São Paulo
8	Piraquara	23	Imbituba	39	Apiáí
9	Rio Negro	24	Itaiópolis	40	Itapetininga
	Rio Grande do Sul	25	Joinville	41	Botucatu
10	São Francisco de Paula	26	Laguna		Sergipe
11	Torres	27	Lebon Régis	42	Areia Branca
12	São José dos Ausentes	28	Mafra	43	Nossa Senhora da Glória
13	Jaquirana	29	Major Vieira		

No presente estudo foram visitadas diversas formações vegetacionais, dentre elas: Pampa, Floresta Ombrófila Densa, Floresta Ombrófila Mista, Floresta Estacional Semidecidual, Cerrado, Chaco, Caatinga e Brejos de Altitude.

3.2. Coletas e conservação dos espécimes

Os espécimes foram coletados com auxílio de faca ou canivete e acondicionados em sacos de papel. Dados ecológicos foram anotados para cada exemplar e posteriormente transferidos para tabela de coleta e etiqueta definitiva. Os espécimes coletados foram secos a temperatura ambiente, raramente, quando algum exemplar estava muito úmido o mesmo era seco com auxílio de estufa a baixa temperatura (inferior a 40°C)

Após secagem foram acondicionados em envelope padrão. Os exemplares montados e identificados foram incorporados ao herbário ICN (Universidade Federal do Rio Grande do Sul). Duplicatas foram encaminhadas ao herbário JOI (Universidade da Região de Joinville).

3.3. Análise de espécimes de herbários

Foram encaminhadas 82 solicitações de empréstimos a herbários nacionais (12) e internacionais (70), dos quais 36 (Tabela 2) encaminharam a grande maioria dos materiais solicitados. Foram realizadas visitas aos seguintes herbários: ICN e HAS do Rio Grande do Sul, UPCB do Paraná, JOI de Santa Catarina e CGMS do Mato Grosso do Sul. Infelizmente os espécimes enviados pelo herbário CDS (Equador: Galápagos) foram perdidos pelo serviço postal brasileiro.

Tab. 2. Herbários que enviaram espécimes de *Ramalina*.

Acrônimo	Instituição	Localização
AK	Auckland War Memorial Museum	Nova Zelândia. Auckland.
B	Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin	Alemanha. Berlin.
BHCB	Universidade Federal de Minas Gerais	Brasil. Minas Gerais.
BISH	Bishop Museum	E.U.A. Hawaii.
BM	The Natural History Museum	Reino Unido. England.
BRI	Queensland Herbarium	Austrália. Queensland.

CESJ	Universidade Federal de Juiz de Fora	Brasil. Minas Gerais.
CHR	Landcare Research New Zealand Limited	Nova Zelândia. Lincoln.
COLO	University of Colorado Museum	E.U.A. Colorado.
CTES	Instituto de Botânica del Nordeste	Argentina. Corrientes.
DUKE	Duke University	E.U.A. North Carolina.
E	Royal Botanic Garden Edinburgh	Reino Unido. Scotland.
F	Field Museum of Natural History	E.U.A. Illinois.
FLOR	Universidade Federal de Santa Catarina	Brasil. Santa Catarina.
G	Conservatoire et Jardin botaniques de la Ville de Genève	Suíça. Genève.
GB	University of Gothenburg	Suécia. Göteborg.
H	University of Helsinki	Finlândia. Helsinki.
HO	Tasmanian Museum and Art Gallery	Austrália. Tasmania.
M	Botanische Staatssammlung München	Alemanha. München.
MEL	Royal Botanic Gardens Victoria	Austrália. Victoria.
MICH	University of Michigan	E.U.A. Michigan.
MIN	University of Minnesota	E.U.A. Minnesota.
MPUC	Pontificia Universidade Católica do Rio Grande do Sul	Brasil. Rio Grande do Sul.
MSC	Michigan State University	E.U.A. Michigan.
NSW	Botanic Gardens and Centennial Parklands	Austrália. New South Wales.
O	Botanical Museum	Noruega. Oslo.
RB	Jardim Botânico do Rio de Janeiro	Brasil. Rio de Janeiro.
SP	Instituto de Botânica	Brasil. São Paulo.
TUR	University of Turku	Finlândia. Turku.
UB	Universidade de Brasília	Brasil. Distrito Federal.
UFP	Universidade Federal de Pernambuco	Brasil. Pernambuco.
UPS	Uppsala University	Suécia. Uppsala.
W	Naturhistorisches Museum Wien	Áustria. Wien.
WELT	Museum of New Zealand Te Papa Tongarewa	Nova Zelândia. Wellington.
WIS	University of Wisconsin	E.U.A. Wisconsin.
WRS�	Wroclaw University	Polônia. Wroclaw.

3.4. Análise morfológica e química

Em laboratório, foi realizada análise morfológica com auxílio de microscópio estereoscópico (20-40X) para observação de características externas dos talos, a exemplo: padrão de ramificação, superfície dos ramos, presença e padrão das pseudocifelas e propágulos simbióticos; e interna tal como presença e compactação da medula. Para as análises anatômicas do talo e de estruturas reprodutivas do micobionte foram feitos cortes à mão livre, as lâminas foram montadas com água e analisadas em microscópio óptico (100-1000X), posteriormente foram realizadas as medições das estruturas.

Os metabólitos secundários de importância taxonômica foram identificados através de testes de coloração de córtex e medula; observação do talo sob lâmpada UV; e cromatografia em camada delgada de acordo com Huneck & Yoshimura (1996) e Orange *et al.* (2001).

3.5. Análises moleculares

As extrações e análises moleculares foram realizadas no Laboratório de Evolução e Biodiversidade da Universidade Federal do Mato Grosso do Sul, campus Campo Grande, com a orientação da Profª. Dra. Aline Pedroso Lorenz Lemke.

As amostras foram imersas em acetona durante 12 horas e depois foram submetidas a extração de DNA através do protocolo descrito em Cubero *et al.* (1999). As amplificações por PCR de dois locos nucleares (Tabela 3) foram realizadas utilizando os seguintes *primers*: ITS1F (Gardes & Bruns, 1993) e ITS4 (White *et al.* 1990) para a região ITS (espaçadores internos transcritos do DNA ribossomal) e IGSf e IGSr (Wirtz *et al.* 2008) para a região IGS (espaçadores intergênicos do DNA ribossomal), utilizando Termociclador Veriti (Applied Biosystems). Cada reação de PCR continha: 5,0 µl de tampão (Promega) (5X), 5,0 µl de dNTP (2mM), 5,0 µl de cada primer (2µM), 5,0 µl de MgCl₂ (25 mM), 1µl de DNA Polimerase (Promega) (5u/µl) e 2 µL de DNA total (20 - 50 ng/ µl).

Tab. 3. Sequências dos *primers* utilizados neste estudo.

Primer	Sequencia de nucleotídeos	Referência
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	White <i>et al.</i> (1990)
ITS4	5'-TCCTCCGCTTATTGATATGC-3'	White <i>et al.</i> (1990)
IGSf	5'-TAGTGGCCGWTRGCTATCATT-3'	Wirtz <i>et al.</i> (2008)
IGSr	5'-TGCATGGCTTAATCTTTGAG-3'	Wirtz <i>et al.</i> (2008)

O programa de amplificação para ITS foi o seguinte: 2 min a 95°C para desnaturação inicial, seguido de 30 ciclos de 30 s a 95°C, 1 min a 50°C, 1 min a 72°C, com extensão final de 10 min a 72°C. Para IGS foi: 2 min a 95°C para desnaturação inicial, seguido de 30 ciclos de 30s a 95°C, 45s a 46°C, 1 min a 72°C, com extensão final de 10 min a 72°C. Os produtos de PCR foram purificados com solução de acetato de amônio e etanol segundo o protocolo modificado de modificado de Moore & Dowhan (2002). O sequenciamento de DNA foi realizado por empresa especializada em sequenciador automático ABIPRISM-3500 (Applied Biosystems).

Os programas MEGA6 (Tamura *et al.* 2013) e CHROMAS 2.0 (Technelysium Pty Ltd, Queensland, Australia) foram utilizados para a verificação e leitura dos cromatogramas. As sequências foram alinhadas utilizando o algoritmo MAFFT disponível no *web server* Guidance [<http://guidance.tau.ac.il/ver2/>].

Foram obtidos alinhamentos de ITS, IGS e de ambos os marcadores concatenados com sequências obtidas e sequências oriundas do GenBank (informações detalhadas disponíveis em cada capítulo da tese). As reconstruções filogenéticas foram realizadas através de análises de Máxima Verossimilhança (ML) e abordagem Bayesiana (BA). Os modelos de substituição nucleotídica foram obtidos com o programa jModeltest2 (Guindon & Gascuel 2003; Darriba *et al.* 2012). As análises foram realizadas em ambiente virtual por meio da plataforma CIPRES (<https://www.phylo.org/portal2/>). Sequências de *Cliostomum griffithii*, espécie do gênero irmão de *Ramalina*, foram utilizadas como grupo externo conforme indicado por estudos anteriores (Ekman 2001; Sérusiaux *et al.* 2010; Pérez-Vargas & Pérez-Ortega 2014).

As análises de ML foram realizadas no programa RAxML 8.2.4 (Stamatakis 2006) com 1000 replicações de bootstrap para cálculo de suporte dos ramos. As análises bayesianas foram realizadas no programa BEAST 1.8.2 (Drummond *et al.* 2012). A

reconstrução filogenética foi realizada utilizando a opção do relógio molecular relaxado (*uncorrelated lognormal relaxed molecular clock*). A *tree prior* utilizada foi o Speciation - Yule Process. O software Tracer 1.6 (Rambaut *et al.* 2014) foi utilizado para checar a convergência das Cadeias de Monte Carlo (MCMC). Foram realizadas duas corridas paralelas de MCMC, ambas utilizando quatro cadeias de 15.000.000 de gerações, amostrando árvores a cada 1000 gerações. Foi utilizado um *burn-in* de 25%. As árvores filogenéticas foram editadas utilizando o programa FigTree v1.4 (disponível em <http://tree.bio.ed.ac.uk/software/figtree/>).

4. RESULTADOS E DISCUSSÃO

Durante o estudo foram coletados 566 espécimes de *Ramalina* de diversas localidades e ambientes brasileiros. Os herbários que gentilmente cederam empréstimos de exemplares contribuíram sobremaneira com a condução do estudo, tendo sido analisados 1246 espécimes, dos quais 332 eram espécimes tipo (Tabela 4). Foram visitados quatro herbários para análise de espécimes depositados, sendo eles: herbários ICN (321 exemplares) e HAS (74 exemplares); UPCB (207 exemplares) e CGMS (01 exemplar). Portanto, no presente foram analisados 2415 espécimes.

Tab. 4. Lista de herbários e número de espécimes enviados.

Acrônimo	Número de espécimes	Número de tipos	Acrônimo	Número de espécimes	Número de tipos
AK	2	1	M	7	7
B	5	5	MEL	6	6
BHCB	96	0	MICH	13	7
BISH	27	0	MIN	35	3
BM	78	60	MPUC	168	0
BRI	13	1	MSC	157	0
CESJ	65	0	NSW	1	1
CHR	4	4	O	24	24
COLO	2	2	RB	144	0
CTES	45	0	SP	72	0
DUKE	37	31	TUR	14	5
E	14	9	UB	9	9
F	22	2	UFP	58	58
FLOR	6	0	UPS	12	6
G	21	21	W	7	7
GB	1	1	WELT	2	2
H	67	53	WIS	5	3
HO	1	1	WRSL	6	3

Os espécimes coletados que não apresentavam contaminação aparente foram encaminhados para extração de material genético. Foram realizadas tentativas para sequenciamento de quatro marcadores moleculares, nuITS, nuIGS, rpb2 e LSU. Porém, não obtivemos sucesso nas tentativas com o marcador LSU. Os demais, nuITS, nuIGS, rpb2 deram resultado positivo.

Foram geradas 314 novas sequências relativas a 24 espécies, sendo que 16 (5,10% do total de sequências obtidas) não apresentaram leitura satisfatória e 20 representavam contaminação (6,37% do total de sequências obtidas). Considerando apenas as 278 sequências utilizáveis: 195 são sequências de ITS, 59 de IGS e 24 de rpb2. As análises moleculares realizadas essencialmente com ITS deram um bom panorama sobre os problemas a respeito da sistemática do gênero bem como da necessidade de recircunscrição de diversas espécies.

Foram identificadas 27 espécies distribuídas por doze Estados brasileiros (Tabela 4) em diversos ambientes, tais como restingas, costões rochosos, mata de araucária, campos de altitude, Pampa, Floresta Ombrófila Estacional, Cerrado e Caatinga. Até mesmo em áreas com muita influência antrópica, como regiões urbanas e rurais.

Duas espécies são novas para a ciência, sendo elas: *Ramalina fleigiae*, de hábito ripário e saxícola, encontrada nos Campos de Cima da Serra no Rio Grande do Sul; e *R. kashiwadanii*, espécie corticícola encontrada desde a região litorânea até o planalto em altitude próxima a 900 metros nos três Estados da Região Sul Brasileira.

Foram registradas 33 novas ocorrências, ampliando consideravelmente a distribuição das espécies de *Ramalina* no Brasil bem como o conhecimento sobre suas preferências de ambientes e habitat. Para o Estado do Espírito Santo foram registradas sete novas ocorrências, para o Mato Grosso do Sul foram duas, para Pernambuco foram três, para o Paraná duas, para o Rio Grande do Sul seis, para Santa Catarina nove, para o Sergipe quatro, e para São Paulo foi registrada uma nova ocorrência (Tabela 5).

Tratando-se das regiões brasileiras, foram identificadas 24 espécies para a Região Sul, 16 para a Região Sudeste, sete para o Nordeste e apenas duas para a Região Centro-Oeste. Infelizmente mesmo em herbários não havia espécimes originários da Região Norte do país, permanecendo assim bastante desconhecida não apenas com relação a *Ramalina*, mas a líquenologia como um todo.

Tab. 5. Lista de espécies encontradas nos estados brasileiros (siglas oficiais). Ocorrências sinalizadas com asterisco (*) sinalizam novo registro para o Estado.

Espécies	BA	ES	MG	MS	MT	PE	PR	RJ	RS	SC	SE	SP
<i>R. anceps</i>									X*	X*		X
<i>R. asahinae</i>										X*		X
<i>R. aspera</i>			X	X*		X*			X	X*		X
<i>R. calcarata</i>		X*	X									X
<i>R. camptospora</i>		X*	X				X*		X*	X*		
<i>R. canalicularis</i>							X*					
<i>R. celastri</i>		X*	X				X	X	X	X		X
<i>R. cochlearis</i>		X*	X						X*			
<i>R. complanata</i>	X	X*				X*	X		X	X	X*	
<i>R. continentalis</i>				X*	X							
<i>R. dendriscooides</i>							X		X*	X		
<i>R. dendroides</i>			X				X			X		
<i>R. exiguella</i>									X	X*		
<i>R. fleigiae</i>									X			
<i>R. gracilis</i>		X*					X	X		X		X
<i>R. kashiwadani</i>							X		X	X		
<i>R. lacera</i>									X*			
<i>R. laevigata</i>									X			
<i>R. peruviana</i>						X	X	X	X	X	X*	X
<i>R. prolifera</i>									X	X		
<i>R. puiggarii</i>			X						X	X*		X
<i>R. pusiola</i>							X		X*	X*		
<i>R. rectangularis</i>	X											
<i>R. solediosa</i>						X	X			X	X*	X
<i>R. sprengelii</i>							X		X	X*		X
<i>R. subpollinaria</i>						X				X*	X	X*
<i>R. usnea</i>		X*				X*	X		X	X		

Parte do grande número de espécies registradas para as Regiões Sul e Sudeste se deve a um maior esforço de coleta, não apenas pelo autor e colaboradores, mas também de diversos espécimes depositados em herbários. Contudo, é importante salientar que a ocorrência de espécies de *Ramalina* de modo geral é maior em regiões com umidade atmosférica elevada (tal qual Sul e Sudeste brasileiros), sendo que a biomassa das espécies aumenta consideravelmente em regiões onde a presença de neblina é frequente. Fato esse também observado por Rundel *et al.* (1972) na Baja Califórnia, México.

Através da análise de dados moleculares, *Ramalina anceps* Nyl. foi confirmada como espécie distinta de *R. usnea* (L.) R. Howe, não corroborando com a hipótese de ser um quimiotipo de *R. usnea* conforme Weber (1986) e Aptroot & Bungartz (2007).

Ramalina subfraxinea Nyl. foi excluída da micota do Rio Grande do Sul. Os espécimes identificados outrora (Fleig 1988) sob esse nome são exemplares de *R. celastri* (Spreng.) Krog & Swinscow, a última diferindo da primeira pela química e formato das pseudocifelas.

Os resultados estão apresentados a seguir no formato de manuscritos que serão submetidos a revistas científicas. O Manuscrito I (já publicado) diz respeito à nova ocorrência de *R. lacera* para o Brasil. O Manuscrito II trata da nova espécie, de hábito ripário, encontrada apenas nos Campos de Cima da Serra gaúcha. O Manuscrito III considera a circunscrição de espécies do grupo *R. usnea*, com a descrição de uma nova espécie. O Manuscrito IV diz respeito a novidades sobre as espécies de *Ramalina* encontradas no Nordeste brasileiro. O Manuscrito V trata de várias novas ocorrências para os Estados da Região Sul do Brasil. E o Manuscrito VI apresenta uma revisão a respeito das espécies de *Ramalina* encontradas no Brasil.

4.1. Manuscrito I

Gumboski, E.L.; Eliasaro, S. & Borges da Silveira, R.M. 2014. Extending the distribution of *Ramalina lacera* (With.) J. R. Laundon (Ramalinaceae, lichen forming Ascomycota): First record from eastern South America. **Check List** 10(1): 195–198.

Extending the distribution of *Ramalina lacera* (With.) J. R. Laundon (Ramalinaceae, lichen forming Ascomycota): First record from eastern South America

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ABSTRACT: The lichen forming fungus *Ramalina lacera* is reported for the first time from Brazil. The specimens were collected in the State of Rio Grande do Sul (Southern of Brazil), only on rocks and between 210 m to 350 m altitude. A distribution map, figure and comments on the species are provided.

Ramalina Ach. is a cosmopolitan genus with ca. 150 to 200 species (Kashiwadani and Nash 2004; Sérusiaux *et al.* 2010). Generally the species have a greenish gray to yellowish-green fruticose thallus, always producing usnic acid. The branches may vary from flat to cylindrical, from solid to hollow, and often produces pseudocyphellae (*e.g.* Krog and James 1977; Krog and Østhagen 1980; Kashiwadani and Kalb 1993). The anatomy of the branches is also variable, which can often present a cortical layer followed by a chondroid tissue and a medulla (Kashiwadani and Kalb 1993; Kashiwadani and Nash 2004). The ascospores are hyaline, usually 2-celled and varying from ellipsoid to fusiform (*e.g.* Krog and James 1977; Kashiwadani and Nash 2004).

Ramalina lacera (With.) J. R. Laundon is a remarkable

member of the genus due to the absence of a supportive hyphal layer called chondroid tissue (Kashiwadani and Kalb 1993, Kashiwadani and Nash 2004). This layer is present in the majority of species of *Ramalina*. The species is also unusual within the genus because of the production of bourgeanic acid in the medulla (Kashiwadani and Nash 2004), a compound that is rarely produced in *Ramalina*.

Although Laundon (1984) considered *Ramalina duriaei* (De Not.) Bagl. to be a synonym of *R. lacera*, many authors have continued using the name *R. duriaei* (*e.g.* Canaani *et al.* 1984; Epstein *et al.* 1986; Garty 1987; Lurie and Garty 1991; Garty *et al.* 1993; 1997; Silberstein *et al.* 1996); thus, most records of *R. lacera* remain under the synonym.

Ramalina lacera has a broad but somewhat spotted distribution (Figure 1). It is known from Europe (*e.g.* Krog

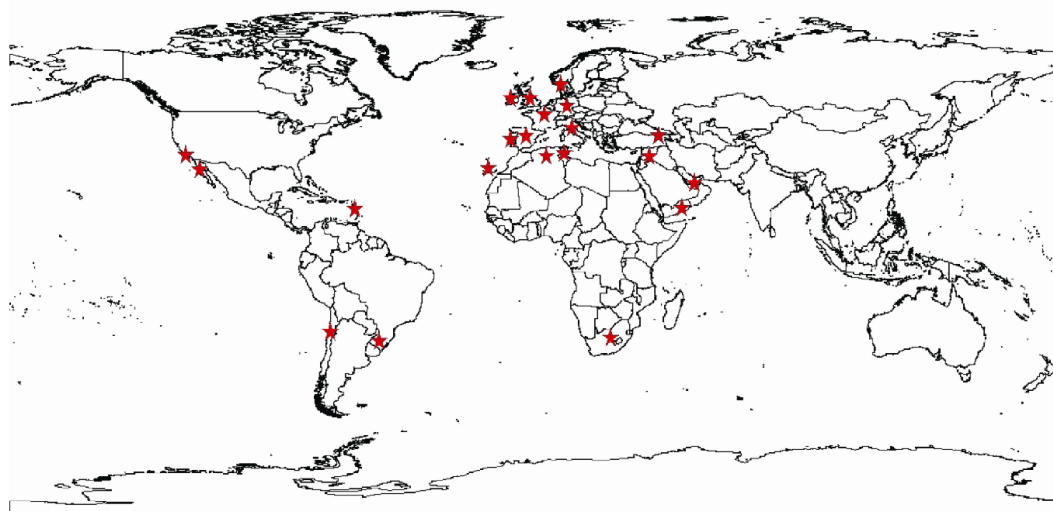


FIGURE 1. Worldwide distribution map of *Ramalina lacera* (red stars) based on previous publications and the new records. Source: Modified from Software DIVA-GIS, Version 7.5.

and James 1977; Jatta 1889, 1911; Follmann 1980; Jones 1980; Bruyn 2000; Alvarez *et al.* 2001), west of Asia (*e.g.* Canaani *et al.* 1984; Epstein *et al.* 1986; Sipman 2002; Yazıcı *et al.* 2011), Africa (*e.g.* De Notaris 1846; Baglietto 1879; Doidge 1950; Krog and Østthagen 1980; Follmann and Mies 1986; Follmann and Sanchez-Pinto 1987; Van Haluwyn *et al.* 1994; Egea 1996; Aptroot 2012), southwest of North America (Howe 1913; Rundel *et al.* 1972; Kashiwadani and Nash 2004) and Central America (Howe 1913; Rundel *et al.* 1972). In South America it was reported only from northern Chile (Rundel 1978). In this study, we report the occurrence of *R. lacera* in the most southern state of Brazil, Rio Grande do Sul, in two sites located at approximately the same latitude as the Chilean specimen, and extending its distribution to the east of South America.

The study is based on unidentified specimens collected by Dr. Mariana Fleig between 1989 and 1998 housed in the Herbarium of Universidade Federal do Rio Grande do Sul (ICN). They were examined using standard stereoscopic (20–40×) and light microscopic (400–1000×) techniques. Sections of thallus and apothecia were mounted in water. Chemical constituents were identified by spot tests, under UV light and thin layer chromatography (TLC) using solvent system C (Orange *et al.* 2001).

Ramalina lacera (With.) J. R. Laundon, *Lichenologist* 16: 220. 1984. Figure 2

Basionym: *Lichen lacerus* With., *Bot. Arr. Veg. Gr. Brit.*:

716. 1776.

Lectotype (designated by Laundon, 1984): Dillenius (1742: 163): tab. 21, fig. 57B (OXF).

Description: thallus solid, shrubby, up to 6.0 cm tall, pale stramineous in herbaria, growing from a common holdfast, irregularly palmate branched, dichotomous in narrow branches, branches flattened, bifacial, main branches up to 1.3 cm wide and terminal branches up to 1.0 mm wide, 200–500 µm thick, surface weakly to clearly scrobiculate; pseudocyphellae very rare, orbicular, flat, up to 0.1 mm in diam.; soralia at first marginal then becoming laminal, mainly in upper parts of thallus, laminal soralia growing mainly on top of the wrinkle, soredia (300–) 400–600 µm in diam. Cortex dirty yellow, continuous, 25–45 µm thick, chondroid tissue absent, medulla white, dense, 120–380 µm thick, algal layer almost continuous, 50–60 µm thick. Apothecia orbicular, concave to mainly flat, up to 5.0 mm in diam., without pruina, with marginal to rarely submarginal soralia, up to 0.6 mm long. Epihymenium brownish, 10–15 µm thick, hymenium hyaline, 35–40 µm thick, subhymenium hyaline to weakly grayish, 25–35 µm thick, algal layer under the hymenium present, continuous, 50–55 µm thick; ascospores hyaline, 1-septate, weakly curved to straight, 10–14 × 4–5 µm. Pycnidia not found.

Spot tests: cortex K-, C-, KC+ yellow, UV-; medulla K-, C-, KC-, UV-. TLC: bourgeanic acid (in medulla) and usnic acid.

Substrate and ecology: According to Kashiwadani and Nash (2004) this species occurs mainly on branches of

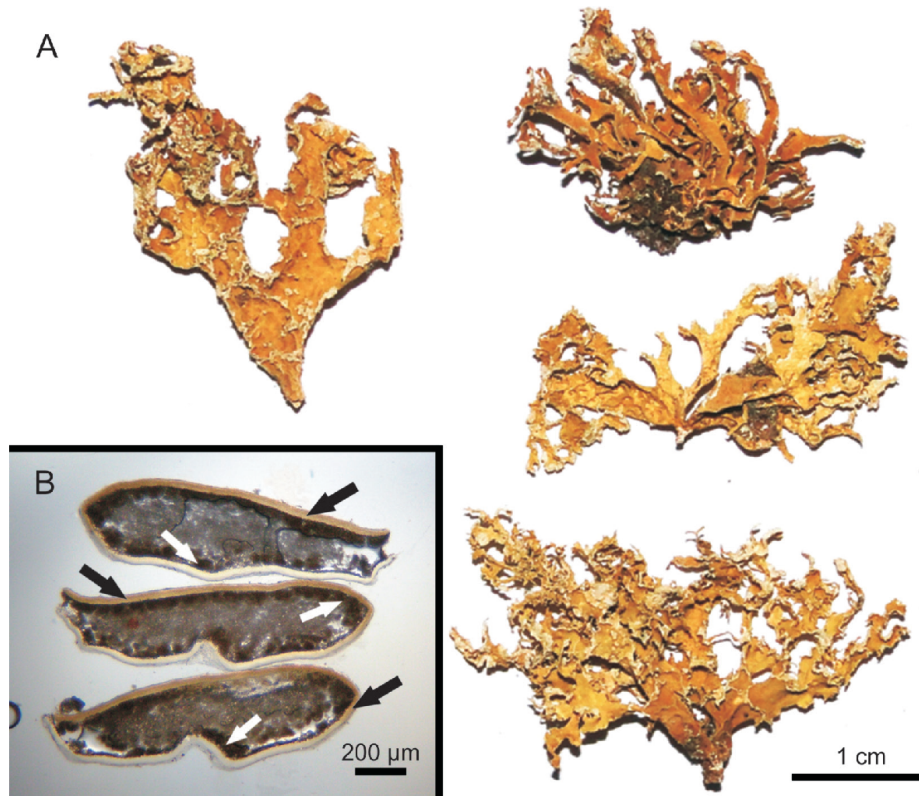


FIGURE 2. Thallus of *Ramalina lacera* (M. Fleig 7247). B. Anatomical section of branches. Black arrows indicate the cortex. White arrows indicate the algal layer. The gray central area in the thallus is the dense medulla.



various trees and rarely on rock, and Krog and Østthagen (1980) stated it can be corticolous or saxicolous, occurring in rather dry, well lit sites and often in man-made habitats. The specimens from Brazil were growing only on rocks, between 210 m to 350 m alt.

Material examined: BRAZIL. State of Rio Grande do Sul: Municipality of Bagé, Casa de Pedra, 14 Km in parallel road to highway BR 153, 210 m alt., 03.XI.1989, *M. Fleig* 3907 (ICN), *ibid.*, 04.XI.1989, *M. Fleig* 4001 (ICN), *ibid.*, 350 m alt., 15.XII.1989, *M. Fleig* 4144 (ICN); Municipality of Caçapava do Sul, Pedra do Segredo, undisturbed place, 27.XI.1993, *M. Fleig* 5948 (ICN), *ibid.*, on rock protected by shrubs, 300 m alt., 15.IX.1998, *M. Fleig* 7247 (ICN).

Additional species examined: *Ramalina canariensis* J. Steiner: GRAN CANARIA, Tafira, 400 m, 22 May 1900, *J. Bornmüller*, Pl. Canar. No. 3500 (W, isolectotype). *Ramalina mollis* Krog and Østh.: THE CANARY ISLANDS, Lanzarote, La Geria, ca. 5 Km ENE of Yaiza, 320 m, 13 Apr. 1975, *Krog and Østthagen* 1055 (O, UPS, isotypes).

Notes: The presence of fertile apothecia (*i.e.*, with mature asci and ascospores) in *R. lacera* is evidently very rare (Krog and James 1977; Krog and Østthagen 1980). In specimens from Canary Islands, Krog and Østthagen (1980) found apothecia, but without mature ascospores; whereas Kashiwadani and Nash (2004) did not find specimens with apothecia in the Greater Sonoran Desert Region. Krog and James (1977) mentioned the presence of mature ascospores in European material, giving the dimensions as 10–15 × 3–5 µm. This size range matches the dimensions of the ascospores found in Brazilian specimens.

According to the literature and our analyses, *Ramalina mollis* is very similar to *R. lacera* in their chemistry, ecological features and overall morphology. Krog and Østthagen (1980) distinguished *R. lacera* from *R. mollis* by the presence of soralia and rare presence of apothecia in the former, and by the absence of soralia and to usual presence of apothecia in the latter species. Both are identical in anatomical features too, however, molecular studies are needed to clarify the relationships between these two species (*i.e.*, whether they represent a monophyletic group) and whether they represent a pair of monophyletic clades that correspond to the presence and absence of soralia. While *R. lacera* has been reported from various localities around the world, *R. mollis* is known only from the Canary Islands and parts of Spain (Krog and Østthagen 1980; Stolley and Kappen 2002).

According to Kashiwadani and Nash (2004), *Ramalina lacera* might also be confused with *R. canariensis*. However, *R. lacera* has monophyllous main laciniae with marginal secondary branches, no chondroid tissue, a single-layered cortex and produces bourgeanic acid in the medulla. Whereas *R. canariensis* has palmately or irregularly branched laciniae, chondroid tissue present, a two-layered cortex and produces divaricatic acid in the medulla.

As conclusion, considering the previous studies (*e.g.*, Nylander 1870; Vainio 1890; Zahlbruckner 1902; Fleig 1988; Kashiwadani & Kalb 1993; Kashiwadani *et al.* 2007), the number of species of *Ramalina* known from Brazil is raised to 25.

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4.2. Manuscrito II

Gumboski, E.L.; Eliasaro, S.; Scur, M.C.; Lorenz-Lemke, A.P. & Borges da Silveira, R.M. A new riparian species of *Ramalina* (Ramalinaceae, lichenized Ascomycota) from Brazil, with a key to the Neotropical saxicolous species. **The Lichenologist**: a ser submetido.

A new riparian species of *Ramalina* (Ramalinaceae, lichenized Ascomycota) from Brazil, with a key to the Neotropical saxicolous species

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Abstract: The new species *Ramalina fleigiae* is here described from Brazil. It was found only on rocks in riverbeds, between the high altitude (above 1000 m) grasslands of Southern Brazil, known as ‘Campos de Cima da Serra’, belonging to the Atlantic Forest Biome. The thalli were found in areas with constant water flow, sometimes almost immersed and always in exposed habitat. A description with ecological data, molecular data, figures, and a comparison with closely associated species is provided, and a key to the saxicolous species of *Ramalina* from the Neotropical region is presented. Included are new data (based on examination of type specimens) on the anatomy of morphologically similar species: *R. australiensis*, *R. cuspidata*, *R. elegantula*, *R. geniculatella*, *R. hamulosa*, and *R. pluviariae*.

Keywords: fruticose thallus, lichenized fungi, rock, southern Brazil, taxonomy.

Introduction

Ramalina Ach. is a cosmopolitan genus with c. 150 to 200 species (Kashiwadani & Nash 2004; Fletcher *et al.* 2009; Sérusiaux *et al.* 2010), that has already been known to be morphologically and chemically quite variable at the species level (e.g., Culberson 1967; Sheard & James 1976; Rundel 1978; Krog & Østhagen 1980; Stevens 1987). However, recent studies, some including genetic analysis, have shown that different morphological and/or chemical characteristics, once thought to be possibly caused by environmental factors, can also indicate distinct species (Kashiwadani *et al.* 2007; Ohmura *et al.* 2008; Hayward *et al.* 2014; Pérez-Vargas & Pérez-Ortega 2014).

Few authors described anatomical characteristics of *Ramalina* species, and because of that, several species remain without this information in the literature. However, due to the efforts of Dr. H. Kashiwadani and collaborators (e.g., Kashiwadani 1986, 1987, 1992; Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004; Kashiwadani *et al.* 2006, 2007), the taxonomic significance of the cortex and the chondroid tissue was recognized.

Important contributions to the taxonomy of *Ramalina* already provided a better comprehension of the taxonomy and biology of this genus and its species: in Australia (Stevens 1987), East Africa (Krog & Swinscow 1976), Canary Islands (Krog & Østhagen 1980), Brazil (Kashiwadani & Kalb 1993), the Greater Sonoran Desert region of North America (Kashiwadani & Nash 2004), and in Galapagos Islands (Aptroot & Bungartz 2007). Although Kashiwadani & Kalb (1993) made a good overview of the Brazilian species, many regions and environments still lack adequate information about *Ramalina* species.

The aims of this work is to present a description of a new *Ramalina* species using morphological, anatomical, chemical and molecular data, and provide a key to the Neotropical saxicolous species.

Material & Methods

Study region

The study region is located in the Southern Brazilian highlands (>800 m above sea level), characterized by mosaics of grasslands (called ‘campos de cima da serra’) and an Atlantic mixed forest (known as the Araucaria forest because of the dominant presence of *Araucaria angustifolia*) (e.g.; Rambo 1953; Boldrini 1997; Behling *et al.* 2004; IBGE 2004). Collection points are situated into waterfalls areas: ‘Cachoeirão dos Rodrigues’ (Silveira River; 28°35'59.85"S, 49°59'19.89"W; Municipality of São José dos Ausentes) and ‘Cachoeira dos Venâncios’ (Camisas River; 29°01'03.72"S, 50°15'31.55"W; Municipality of Cambará do Sul) (Figs. 1 - 2). According to Köppen (1936), the climate can be categorized as Cfa (humid subtropical). The annual average temperature is 14.8°C and the relative humidity about 80% (Moreno, 1961). In the winter, the temperature reaches - 8°C, with frequent frost and, occasionally, snow formation. The annual rainfall varies from 1500 to 2000 mm, and rainfall is spread throughout the year (Boldrini, 1997).

Morphological, anatomical and chemical analyses

The specimens examined were collected using a knife and dried at room temperature. Specimens from herbaria ICN and UFP previously collected were also studied, allowing the recognition of the collection site and obtainment of fresh specimens.

Specimens were examined using standard techniques with stereoscopic (20–40×) and light microscopes (400–1000×). Freehand sections of the thalli and apothecia were mounted in water. Spot tests were conducted according to Huneck and Yoshimura (1996) and Orange *et al.* (2001), including observation under UV light and thin layer chromatography (TLC) using solvent C.

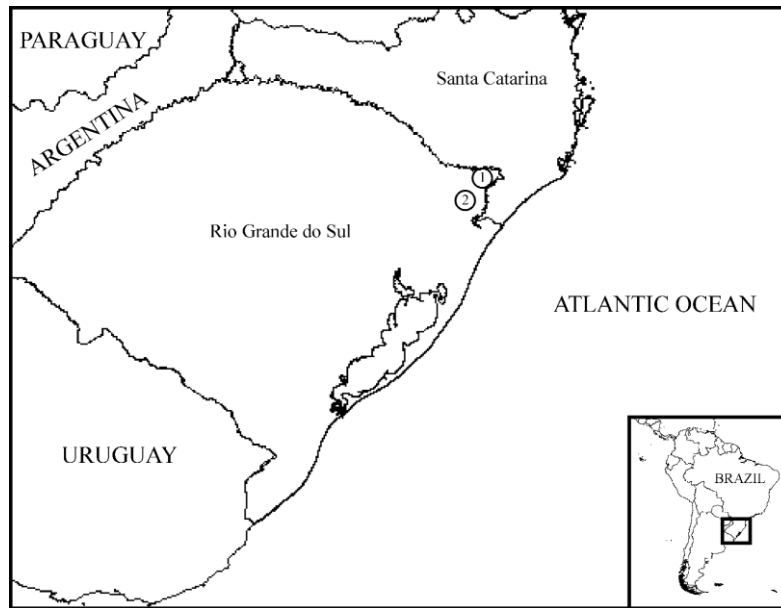


FIG. 1.: Representative map focusing on Rio Grande do Sul State, southern Brazil. 1, locality of ‘Cachoeirão dos Rodrigues’ in Municipality of São José dos Ausentes; 2, locality of ‘Cachoeira dos Venâncios’ between municipalities of Cambará do Sul and Jaquirana.

The key for the saxicolous species of *Ramalina* from the Neotropical region was partly made based on literature (e.g. Nylander 1870; Malme 1934; Grassi 1950; Kashiwadani 1987; Kashiwadani & Kalb 1993; Marcano & Morales Méndez 1994; Aptroot & Bungartz 2007; Kashiwadani *et al.* 2007) and includes species which only rarely occur on rocks, and also with doubtful records from South America, such as *R. siliquosa* (Huds.) A.L. Sm., and partly based on specimens examined from the following regions and herbaria: Argentina (CTES, MIN, MPUC, MSC), Bahamas (MSC), Brazil (BM, BHCB, CGMS, CTES, F, G, H, HAS, ICN, MPUC, SP, UFP, UPCB, TUR, W); Chile (BM, CTES, G, H, MIN, MSC, O, WRSL), Colombia (H), Cuba (G, H, MICH, MSC), Dominican Republic (MSC), Ecuador (MSC), Falkland Island (MSC), Guadalupe (MICH, MIN), Guatemala (F, MSC), Guyana (BM), Haiti (MSC), Honduras (BM), Jamaica (H, MIN, MSC), Mexico (WIS, WRSL), Panama (F),

Paraguay (CTES, H), Peru (DUKE, H, M, W), Porto Rico (F, MICH, MSC), Uruguay (DUKE, ICN, MPUC), and Venezuela (MIN).

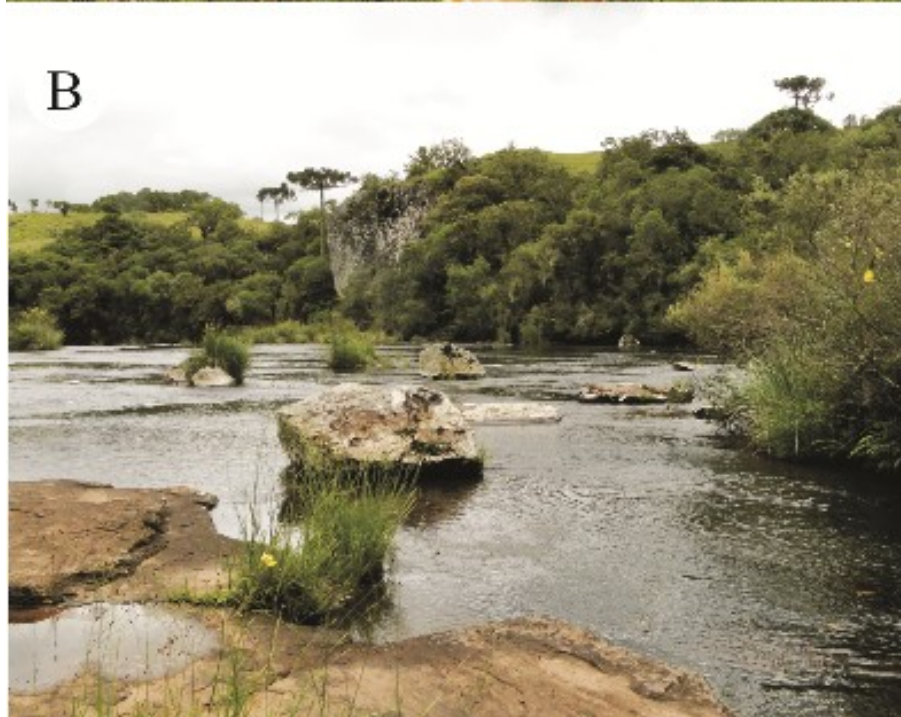


FIG. 2. A–B: *Ramalina fleigiae* collection points: A, ‘Cachoeirão dos Rodrigues’ (Silveira river), located on Municipality of São José dos Ausentes; B, ‘Cachoeira dos Venâncios’ (Camisas river), located between the municipalities of Cambará do Sul and Jaquirana.

Genetic data

The species were selected based on morphological, chemistry and genetic similarity between the studied taxa. However, as there are relatively few sequences available in GenBank with the molecular markers used in this study, were selected especially those containing more markers, and the greater morphological, anatomical and chemical similarities as possible.

Small samples of 17 fresh collected *Ramalina* thalli (Table 1) were soaked in acetone overnight and then submitted to DNA extraction using the protocol described in Cubero *et al.* (1999).

PCR amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems) using the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990) for ITS rDNA region and IGSf and IGSr (Wirtz *et al.* 2008) for IGS. Each 25 µl reaction contained: 1x Taq buffer (Promega), 2mM of dNTP set, 2µM of each primer, 25 mM of MgCl₂, 1U of DNA Polymerase (Promega) and ca. 20–50 ng of DNA. The PCR conditions for ITS amplification were 2 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C, 1 min at 50°C, 1 min at 72°C, with a final elongation of 10 min at 72°C. For IGS amplification the conditions used were: 2 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C, 45 s at 46°C, 1 min at 72°C, with a final elongation of 10 min at 72°C. PCR products were purified with ammonium acetate and ethanol and sequenced in a ABI PRISM 3500 Genetic Analyzer (Applied Biosystems).

The forward and reverse sequencing reads were analyzed using MEGA 6 (Tamura *et al.* 2013). The sequences were aligned using MAFFT algorithm available at Guidance web server (<http://guidance.tau.ac.il/ver2/>). ITS and IGS regions were analyzed together (concatenated) and separately (ITS) using a Maximum Likelihood (ML) and a Bayesian (BA) approaches using CIPRES platform (<https://www.phylo.org/portal2/>). The best model for each analysis was obtained using the jModeltest2 program (Guindon & Gascuel 2003; Darriba *et al.* 2012). Maximum Likelihood analyses were performed in RAxML 8.2.4 (Stamatakis 2006) with 1000 bootstrap replicates for calculation of the nodal support, using the GTRGAMMA substitution model. Phylogenetic trees (BA) were estimated using the software BEAST 1.8.2 (Drummond *et al.*, 2012). The tree searches were performed with the model TrNef+G with 1000 replicates for the ITS data set, and with the model TrN+G with 1000 replicates to the combined data set. Two parallel MCMC runs were performed both using four chains and 15 000 000 generations, sampling trees every 1000th generation, after discarding the first 25% as burn-in. The files generated were checked in Tracer v1.6 (Rambaut *et al.* 2014). For the generation of the tree combining the ML and BA approach, only a 50% majority-rule consensus tree were maintained. Conflicts were assumed significant if two different relationships for the same taxa were supported with ML bootstrap values above 75%. Phylogenetic trees were drawn using FigTree v1.4 (available at <http://tree.bio.ed.ac.uk/software/figtree/>). Sequences of *Cliostomum griffithii* obtained in the GenBank were used as outgroup, according to Ekman (2001), Sérusiaux *et al.* (2010), and Pérez-Vargas & Pérez-Ortega (2014).

Results & Discussion

In this study, 17 ITS and 13 IGS sequences of four *Ramalina* species were generated: *R. exiguella* Stirt. [3 specimens], *R. fleigiiae* [9], *R. gracilis* (Pers.) Nyl. [2], and *R. laevigata*

Fr. [3]. Added, 12 sequences of five species were obtained from GenBank (Table 1). The matrix of the ITS and IGS data set had 535 and 434 unambiguously aligned nucleotide position characters, respectively. A total of 969 positions characters were constant in the combined data set.

Phylogenetic trees generated by ML and BA analyses of the concatenated and ITS data sets did not show incongruences (only the ML tree is shown; Fig. 3-4).

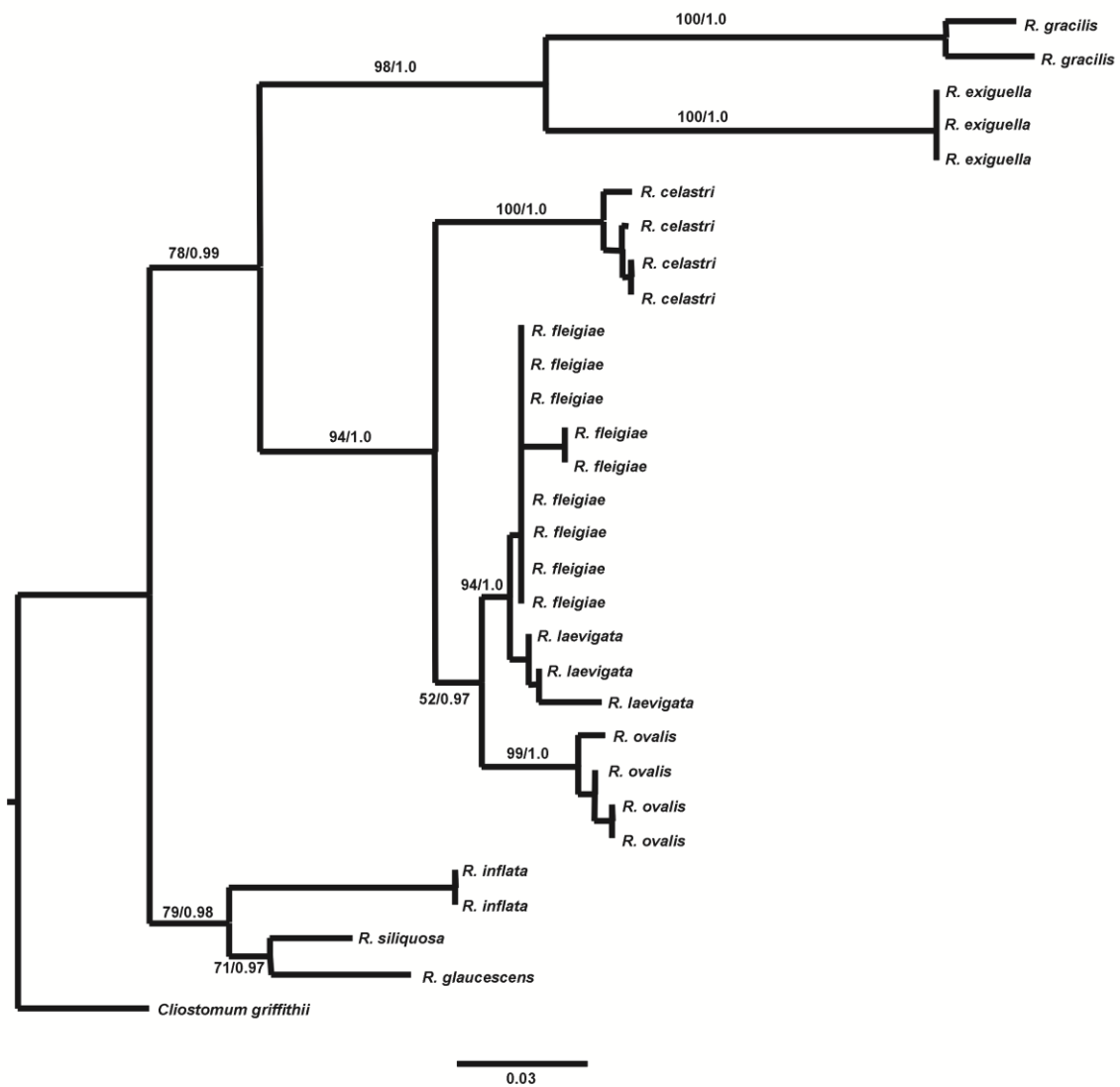


FIG. 3. Phylogenetic relationships of *Ramalina fleigia* specimens based on a maximum-likelihood analysis using ITS data set. The ML-bootstrap support (BS) are indicated first and

posterior probabilities (PP) of the Bayesian analysis as the second number above the branches.

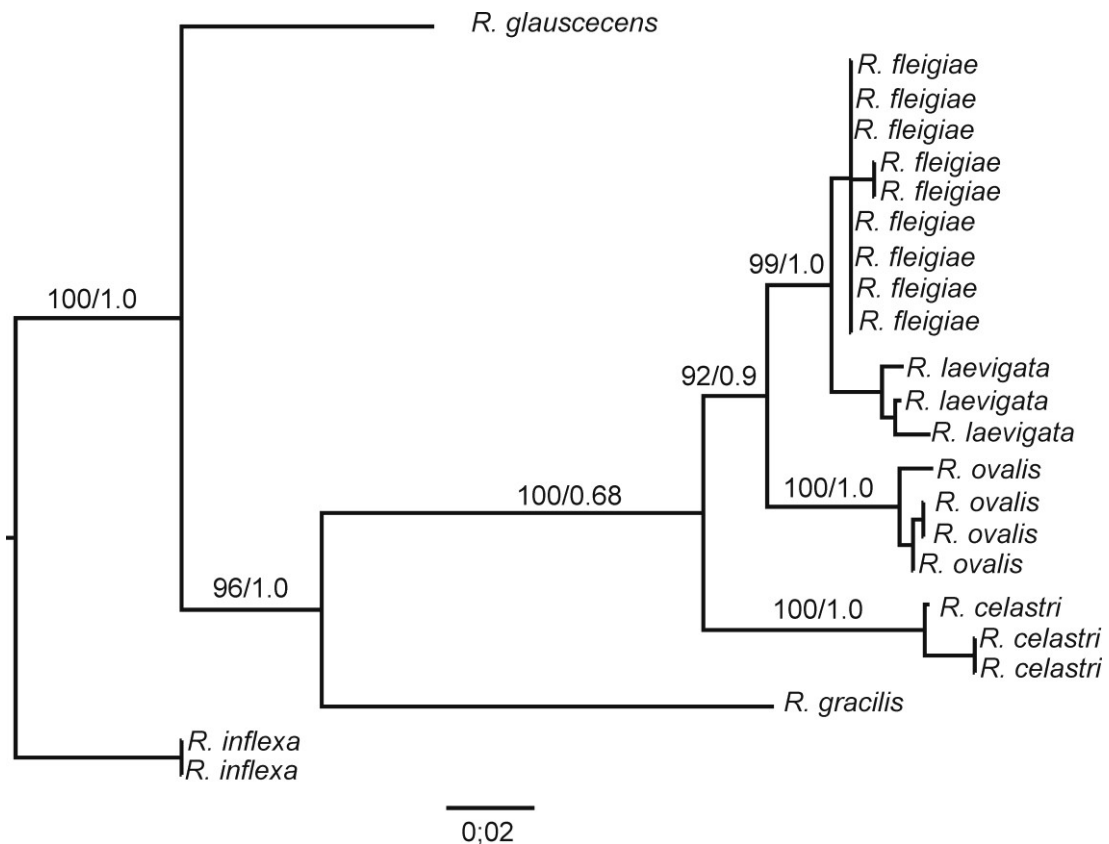


FIG. 4. Phylogenetic relationships of *Ramalina fleigiae* specimens based on a maximum-likelihood analysis using ITS and IGS data set. The ML-bootstrap support (BS) are indicated first and posterior probabilities (PP) of the Bayesian analysis as the second number above the branches.

The sequences of *R. fleigiae* specimens clustered in well-supported clades in both, ITS and combined data sets. The most genetic related species with *R. fleigiae* is *R. laevigata*, the latter has a clearly distinct morphology and anatomy with each other, however, both occur in similar habitat (riparian and saxicolous). *Ramalina fleigiae* has the branches densely branched in upper half, and the branches are irregularly flat to subterete, up to 1.5 mm wide. While *R.*

laevigata has the branches few to moderately branched, and have distinctly dorsiventral branches, up to 2.0(–4.0) mm wide (Fries 1825; Malme 1934).

Ramalina exiguella and *R. gracilis* remind *R. fleigiae* morphologically and chemically. They have shrubby thallus, irregularly flat to subterete branches, absence of symbiotic propagules, absence of medullar acids, and occur over rocks (occasionally for *R. exiguella* and *R. gracilis*). However, *R. exiguella* and *R. gracilis* are strictly coastal species, and differ from *R. fleigiae* for presenting linear to long linear pseudocyphellae. Added, *R. gracilis* has cracked chondroid tissue (Stevens 1987; Fleig 1988; Kashiwadani & Kalb 1993). While *R. fleigiae* have mainly irregularly ellipsoid to short linear pseudocyphellae. Genetically, *R. exiguella* and *R. gracilis* are sister species, but both are distant from *R. fleigiae*.

***Ramalina fleigiae* Gumboski, Eliasaro & R. M. Silveira, sp. nov.**

Mycobank No.: MB XXXXX

Barcoding GenBank No.: XXXXXX

Differing from *Ramalina exiguella* Stirt. by the thallus with branches originating from a single holdfast, most densely branched in the upper half of the thallus, further distinguished by numerous irregular ellipsoid to short linear pseudocyphellae on the surface and margin of its branches.

Typus: BRAZIL. Rio Grande do Sul State. Municipality of São José dos Ausentes, locality of ‘Cachoeirão dos Rodrigues’, on rock in the middle of Silveira River, ca. 1150 m alt., 28°35'59.85"S, 49°59'19.89"W, 20.I.2015, *E. Gumboski 5050* (ICN—holotype; UPCB, SP, H, F—iso-types).

(Fig. 5)

Thallus saxicolous, shrubby, up to 5.0 cm tall and 6.0 cm wide, whitish green in the field, becoming stramineous in the herbarium; densely branched in their upper half in best

developed thalli; branches originating from a single holdfast, consisting of dense groups of 2–7 branches rooting in a common, necrotic base of up to 0.7 cm high; branching dichotomously anisotomic; main branches ramify mainly from the upper half; branches solid, rigid, irregularly flat to subterete, somewhat inflated, but not hollow, distinctly flattened mainly at their tips; main branches 0.4–1.5 × 0.2–1.1 mm broad; secondary branches 0.15–0.6 × 0.1–0.5 mm wide; apex somewhat truncated to slightly acute, sometimes curved as a hook-shape; surface opaque, irregular, ±striate due the presence of abundant pseudocyphellae resembling maculae; symbiotic propagules absent; pseudocyphellae numerous on lamina and margins, present across both the main and secondary branches, depressed to on level surface, irregular ellipsoid to shortly linear, 0.15–1.0 × 0.05–0.15 mm, rarely orbicular, 0.1–0.2 mm diameter; cortex distinct, 10–35 µm thick; chondroid tissue not cracked, discontinuous, in irregular bundles intermixed with medulla (but sometimes the majority portion of the branches); main branches 80–620(–700) µm thick; secondary branches usually with one ±circular to irregular bundle, 30–210 µm thick; medulla compact; algal layer below the cortex and around the chondroid tissue, 15–70 µm thick. Ascomata and conidiomata not found.

Chemistry. Cortex and medulla: K-, C-, KC-, PD-, UV-. TLC: usnic acid.

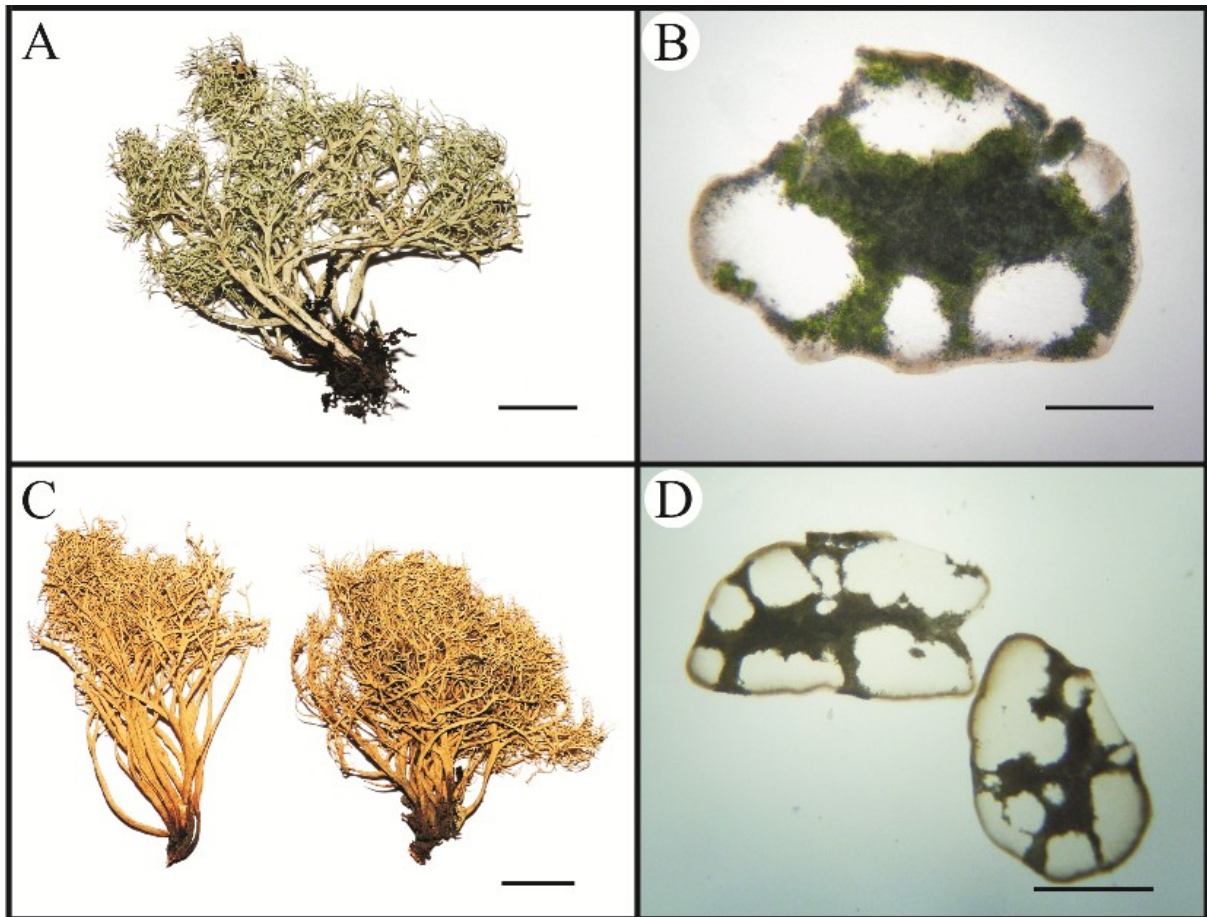


FIG. 5. A–D: *Ramalina fleigiae*; A, Thallus (holotype); B, Transversal section of the main branch (holotype); C, Thallus (*M. Fleig* 6660); D, Transversal sections of the main branch (*M. Fleig* 6660). Scales: A and C = 1 cm; B and D = 200 μ m.

Distribution and habitat. The species described here is known only from two localities in the northeastern plateau of the Rio Grande do Sul State (Fig. 1). *Ramalina fleigiae* colonizes only rocks in the riverbed and on waterfalls rocks, exposed to direct sunlight; some thalli were almost submersed in the water (Fig. 6), at c. 1150 m elevation. All thalli are probably temporarily submerged during occasional floods. No thallus was found on rocks that are more distant from the river. The species probably occurs also in nearby rivers, but as it occurs in very small populations it might easily have been overlooked.

The first author visited similar environments in the adjacent states of Santa Catarina and Paraná where the species was not found.



FIG. 6.: Thalli of *Ramalina fleigiae* almost in the water on Silveira river.

Etymology. This new species is dedicated to Dr Mariana Fleig, who devoted much of her life to the study of lichens and contributed enormously to the knowledge of Brazilian lichenology. Fleig also collected the earliest known herbarium specimens (in ICN) of this new species.

Discussion. *Ramalina fleigiae* is a distinct species, mainly by the saxicolous habitat, the branches originating from the same base and the densely branched upper half. Its branches are solid, irregularly flat to subterete, up to 1.5 mm wide, with numerous irregular ellipsoid to short linear pseudocyphellae on the lamina and on the margins. Anatomically, a distinct cortex with a discontinuous and not cracked chondroid tissue characterizes the species. There are no secondary metabolites in the medulla.

Similar species are *R. gracilis* and *R. exiguella*, but both have thalli growing from a delimited holdfast, their branches are angular in cross section, they have long linear pseudocyphellae, and no distinct cortex; also these two species are strictly coastal (Fleig 1988; Kashiwadani & Kalb 1993).

Ramalina osorioi Kashiw., T.H. Nash & K.H. Moon is a similar saxicolous species, and has shrubby thallus with solid branches, but is moderately branched, has a distinctly dorsiventral branches, and produces psoromic acid (Kashiwadani *et al.* 2007).

Ramalina continentalis Malme is a saxicolous species that inhabits rocks along rivers in Brazil, Paraguay, and Uruguay (*e.g.* Malme 1934; Osorio 1972). However, it has distinctly flattened, and much larger branches of 5–10–(–20) mm wide (Malme 1934).

Ramalina furcellangulida Aptroot, endemic to the Galapagos Islands, has no distinct holdfast. Its branches are irregularly angular in section, typically not flattened, often with rounded, irregular, laminal warts, linear pseudocyphellae, rarely also with few punctiform tuberculate pseudocyphellae. It produces divaricatic or salazinic and/or sekikaic acid (Aptroot & Bungartz 2007).

There are other species, not or only doubtfully recorded from Brazil or South America, that have features similar to *R. fleigiae*. *Ramalina siliquosa* is shrubby to pendulous, its main branches more or less flattened, rarely entirely terete, but its secondary branches are mostly furcated, rarely terete, and the surface is more or less matt, rugose or frequently even foveolate, occasionally with well-developed furrows (Krog & James 1977). In this species the chondroid tissue is clearly to moderately cracked (Kashiwadani 1992). *Ramalina cuspidata* (Ach.) Nyl. has simple or sparingly furcate branches, which are rarely becoming densely branched towards the apices. Its branches are mostly terete towards the base, remaining so above or gradually becoming flattened (Krog & James 1977). The holotype (H!) has a distinct

8–10 μm thick cortex, and the chondroid tissue is clearly cracked, continuous, and 50–220 μm thick.

Ramalina geniculatella Aptroot, endemic to the Galapagos Islands, is initially shrubby, becoming pendant with age, the branches are partly terete, but mostly flattened, most thallus parts with conspicuous whitish linear pseudocyphellae (Aptroot 2008). An examination of the holotype (B!) showed that the cortex is absent, the chondroid tissue is not cracked and forms almost regular bundles mixed with medulla.

Ramalina elegantula P.M. Jørg. has a caespitous thallus with linear thin branches, up to 0.5 mm wide. The pseudocyphellae are linear and marginal (Jørgensen 1977). The cortex is distinct with 10–20 μm , the chondroid tissue is continuous and not cracked, 60–105 μm thick (O—holotype!).

Ramalina leptocarpha Tuck. has the thallus shrubby to subpendulous. The branches are lanceolate, plane to somewhat canaliculated, the pseudocyphellae are linear to ellipsoid, laminal or rarely marginal. *Ramalina bajacalifornica* Bowler & Rundel has a shrubby to subpendulous thallus, flat or weakly canaliculated branches. Its pseudocyphellae are laminal or rarely marginal, short linear, somewhat elevated. The species produces salazinic acid in medulla (Kashiwadani & Nash 2004).

Two Azorean saxicolous species are morphologically similar with *R. fleigiae*. They differ by their branching and pseudocyphellae pattern, and by the production of secondary metabolites. *Ramalina azorica* Aptroot & Schumm has few antler-like branches or dense branches that become terete towards the tips. The pseudocyphellae are linear. It produces divaricatic acid together with an unknown pigment. *Ramalina wirthii* Aptroot & Schumm has branches that are mostly flattened, or in parts irregularly rounded, several of the branches usually becoming terete toward the tips. It produces salazinic acid, usually together with protocetraric acid (Aptroot & Schumm 2008).

Ramalina jamesii Krog, *R. portosantana* Krog and *R. timdaliana* Krog, all endemics from Porto Santo Island near Madeira, are saxicolous and have shrubby thalli. However, *R. jamesii* has subterete, uneven, branches, and fragmentation areas are common. The pseudocyphellae are shortly linear to ellipsoid. It produces divaricatic acid, salazinic acid and triterpenoids. *Ramalina portosantana* has subterete or more or less flat branches, the surface is rugulose with irregularly reticulate ridges which can develop pseudocyphellae. The cortex is very thick, up to 80 μm . The chondroid tissue is occasionally adjoining the cortex, but mostly forming numerous strands intermixed with the medulla. It produces salazinic acid. *Ramalina timdaliana* is richly branched, the branches are subterete to somewhat flat, often with shortly linear pseudocyphellae. Produces divaricatic acid and triterpenoids (Krog 1990).

Ramalina pluviariae Krog & Østh. is saxicolous and has subterete branches, and the pseudocyphellae are mainly linear, more or less anastomosing (Krog & Østhagen 1980). It has no cortex and the chondroid tissue is discontinuous and clearly cracked, 70–350 μm thick (O—holotype!). *Ramalina hamulosa* Krog & Østh. has subterete, angular to flattened branches, with numerous short branchlets terminating in nodules or hookshaped structures. The pseudocyphellae are linear, restricted mostly to the margin of flattened branches (Krog & Østhagen 1980). The cortex is distinct, 30–40 μm thick, the chondroid tissue is discontinuous, cracked, 10–275 μm thick (BM—isotype!).

Ramalina australiensis Nyl., a rarely saxicolous species, has thalli that are 3–10–(–20) cm long, with spars branching in the basal region of the thallus which becomes densely branched at the tips. The primary branches are usually subterete and thin, rarely thick, compressed and coarse (Stevens 1987). The chondroid tissue is clearly cracked, discontinuous, 140–300 μm thick (H-NYL—holotype!). *Ramalina tropica* G.N. Stevens has branches arising from the base with common lateral branchlets. The pseudocyphellae are linear, wherein tuberculate

extensions are sometimes present. Salazinic and protocetraric acids are characteristic for this species (Stevens 1987).

Additional specimens examined. **Brazil:** *Rio Grande do Sul State:* Municipality of Cambará do Sul, Cachoeira dos Venâncios, on rock, 08.I.2000, *W.B. Sanders* 00108.3B (UFP 29592); Municipality of Jaquirana, locality of Cachoeira dos Venâncios, on rock, ca. 850 m alt., 29°01'03.72"S, 50°15'31.55"W, 20.I.2015, *E. Gumboski* 5054, 5055 (ICN), 5056, 5057 (SP); Municipality of São José dos Ausentes, source of Rio do Marco, riverbed, on rocks, 09.XII.1994, *M. Fleig* 6643, 6660 (ICN 99838), locality of 'Cachoeirão dos Rodrigues', on rock in the middle of the river (Silveira river), ca. 1150 m alt., 28°35'59.85"S, 49°59'19.89"W, 20.I.2015, *E. Gumboski* 5049, 5051, 5052 (ICN), 5053 (JOI).

Key to saxicolous species of *Ramalina* from the Neotropical region (including the Galapagos but excluding the South Atlantic islands)

- | | | |
|------|---|--|
| 1 | Branches hollow..... | 2 |
| | Branches solid (even with a lax medulla)..... | 3 |
| 2(1) | Branches 0.1–1.0(–2.0) mm wide, sorediod granules produced at the apices of the branches..... | R. roesleri (Hochst. ex Schaer.) Nyl. |
| | Branches up to 4.0 mm wide, without sorediod granules on branches..... | R. turgida Kremp. |
| 3(1) | Soredia present..... | 4 |
| | Soredia absent..... | 19 |
| 4(3) | Branches mainly terete to subterete, or irregular in thickness but not distinctly dorsiventral..... | 5 |
| | Branches flat, canaliculate, lanceolate or palmate..... | 9 |
| 5(4) | Branches mainly terete..... | 6 |

	Branches mainly irregular in thickness	8
6(5)	Medulla K- or K+ pinkish	R. fragilis Aptroot & Bungartz
	Medulla K+ yellow → red	7
7(6)	Cortex distinct, chondroid tissue cracked	R. rigida Ach.
	Cortex indistinct, chondroid tissue not cracked	R. soresiosa (B. de Lesd.) Landrón
8(5)	Branched from a spreading holdfast; soredia subgranular to granular	
	R. subfarinacea (Nyl. ex Cromb.) Nyl.
	Branched from a narrow holdfast; soredia farinose	R. farinacea (L.) Ach.
9(4)	Branches flat, dorsiventral, 3.0–6.0(–12.0) mm wide.....	
	R. terebrata Hook. f. & Taylor
	Branches variable flat, 0.2–3.0 mm wide.....	10
10(9)	Medulla K+ yellow → red	11
	Medulla K-, K+ yellow or K+ pinkish.....	13
11(10)	Branches distinctly canaliculate.....	R. subpollinaria Nyl.
	Branches not canaliculate.....	12
12(11)	Branches with short conspicuous laminal striae along their entire length; soralia lateral, subapical to apical, capitate or labriform (Venezuela).....	
	R. microphylla V. Marcano & A. Morales
	Branches irregularly striate from cartilaginous cortical strands; soralia mostly apical, rather discrete but occasionally confluent, capitate or irregular (Galapagos).....	
	R. polyforma Aptroot
13(10)	Medulla K+ pinkish (homosekikaic acid and sekikaic acid)	14
	Medulla K- or K+ yellow	15
14(13)	Surface of branches shiny, smooth	R. intermedia (Delise ex Nyl.) Nyl.
	Surface of branches wrinkled, irregularly striate (Galapagos).....	R. polyforma

15(13)	Medulla PD+ golden yellow (psoromic acid)	R. dissecta Kashiw.	
	Medulla PD-		16
16(15)	Soredia marginal and/or terminal		17
	Soredia laminal, sometimes also marginal and/or terminal		18
17(16)	Soredia marginal only; unbranched branches	R. incana Kashiw.	
	Soredia mostly terminal; branches with variable branching pattern (Galapagos)		
	R. polyforma	
18(16)	Branches flattened to partly terete usually towards the apices, sometimes palmate; medulla with evernic acid agg (UV+ bluish white).	R. pollinaria (Westr.) Ach.	
	Branches usually palmately to irregularly branched; medulla with bourgeanic acid (UV-)	R. lacera (With.) J.R. Laundon	
19(3)	Branches terete to subterete, angular in cross section or irregularly flat		20
	Branches clearly flat		25
20(19)	Branches mainly irregularly flat, usually angular in cross section		21
	Branches terete to subterete		23
21(20)	Branches irregularly angular in cross section, surface often with laminal warts; pseudocyphellae linear	R. furcellangulida	
	Branches terete to flat, surface smooth, rugose or furrowed but without warts; pseudocyphellae orbicular to ellipsoid		22
22(21)	Main branches irregularly flat to subterete, somewhat inflated, at their tips usually distinctly flattened; chondroid tissue not cracked	R. fleigiae	
	Main branches somewhat flattened and secondary branches subterete to terete; chondroid tissue cracked	R. siliquosa	
23(20)	Medulla K- (protocetraric acid)	R. gallowayi Kashiw., T.H. Nash & K.H. Moon	
	Medulla K+ yellow → red (salazinic acid)		24

24(23)	Branches with many pale warty patches at the apical portion	R. dasypoga Tuck.	
	Branches without warty patches	R. santanensis V. Marcano & A. Morales	
25(19)	Branches mainly to distinctly canaliculate.....		26
	Branches flat, usually dorsiventral but not distinctly canaliculate.....		27
26(25)	Pseudocyphellae marginal, linear; ascospores 18–30 × 3–6 μm		
	R. puiggarii Müll. Arg.	
	Pseudocyphellae marginal, punctiform to sublinear; ascospores 10–12 × 4–5 μm.....		
	R. darwiniana Aptroot & Bungartz	
27(25)	Medulla K+ yellow → red (salazinic acid or norstictic acid)		28
	Medulla K-, K+ yellow or K+ pinkish.....		30
28(27)	Branches lanceolate; norstictic acid present	R. fasciata Kremp.	
	Branches flat but not lanceolate; salazinic acid present.....		29
29(28)	Surface of branches smooth to rugose; pseudocyphellae tuberculate to punctiform		
	R. complanata (Sw.) Ach.	
	Surface of branches conspicuously striate; pseudocyphellae linear		
	R. sideriza Zahlbr.	
30(27)	Medulla K+ pinkish (sekikaic acid)	R. fasciata Kremp.	
	Medulla K- or K+ yellow.....		31
31(30)	Medulla PD+ yellow (psoromic acid)	R. osorioi	
	Medulla PD-.....		32
32(31)	Thallus with a narrow holdfast; branches up to 15 mm wide	R. continentalis	
	Thallus with a common holdfast; branches up to 4 mm wide		33
33(32)	Branches 2.0–4.0 mm wide; ascospores 13–16 × 3–4 μm.....	R. laevigata	
	Branches 0.5–2.0 mm wide; ascospores 11–14(–17) × 4–5 μm	R. pilulifera Taylor	

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TABLE 1. Information about the *Ramalina* sequences analyzed.

Species	Voucher information	Reference	GenBank Nr.	
			ITS	IGS
<i>Cliostomum griffithii</i>	UNITED KINGDOM	Kelly <i>et al.</i> (2011)	FR799177	-
<i>R. celastri</i>	RWANDA	Sérusiaux <i>et al.</i> (2010)	GU827295	-
<i>R. celastri</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583540	KF594450
<i>R. celastri</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583542	KF594452
<i>R. celastri</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583544	KF594454
<i>R. exiguella</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 4068</i> (ICN)	This study.	XXXXXXXXXX	-
<i>R. exiguella</i>	BRAZIL. Santa Catarina State, <i>A. Gerlach 875b</i> (ICN)	This study.	XXXXXXXXXX	-
<i>R. exiguella</i>	BRAZIL. Santa Catarina State,	This study.	XXXXXXXXXX	-

	<i>A. Gerlach 873</i> (ICN)			
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5049</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5050</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5051</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5052</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5053</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5054</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5055</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5056</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. fleigiae</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5057</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. glaucescens</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583545	KF594455
<i>R. gracilis</i>	BRAZIL. Santa Catarina State, <i>A. Gerlach et al. 238</i> (ICN)	This study.	XXXXXXXXXX	-
<i>R. gracilis</i>	BRAZIL. Paraná State, <i>A. Gerlach 780b</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. inflexa</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583546	KF594456
<i>R. inflexa</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583547	KF594457

<i>R. laevigata</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5033</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. laevigata</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5046</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. laevigata</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski 5047</i> (ICN)	This study.	XXXXXXXXXX	XXXXXXXXXX
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583554	KF594464
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583556	KF594466
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583557	KF594467
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583558	KF594468
<i>R. siliquosa</i>	UNITED KINGDOM	Groner & LaGreca (1997)	U84587	-

4.3. Manuscrito III

Gumboski, E.L.; Lorenz-Lemke, A.P.; Eliasaro, S. & Borges da Silveira, R.M. Species circumscription of the Brazilian *Ramalina usnea* morphological group (Ramalinaceae, lichenized Ascomycota). **Fungal Diversity**: a ser submetido.

Species circumscription of the Brazilian *Ramalina usnea* morphological group (Ramalinaceae, lichenized Ascomycota)

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Abstract. The *Ramalina usnea* morphological group has species with long and pendulous thallus, solid branches and without soredia. This study presents a review of the Brazilian species belonging to that group: *R. anceps*, *R. rectangularis*, *R. sprengelii* and *R. usnea*. Specimens were collected in several localities, and specimens stored in herbaria were revised. Using morphological, anatomical, chemical and molecular data were recognized five species, one of which is new to science: *R. kashiwadanii*. Morphological and anatomical patterns proved to be good characteristics to distinguish the new species. *Ramalina anceps* was confirmed as a distinct species of *R. usnea* and not as a chemotype.

Keywords: Fruticose thallus, lichen, Kashiwadani, systematic, taxonomy.

Introduction

Ramalina Ach. is a cosmopolitan lichen genus that are known to be morphologically and chemically variable at the species level (e.g., Culberson 1967; Sheard & James 1976; Krog & Østhagen 1980; Stevens 1987). There are very small species, with thallus smaller than 3.0 cm long, whereas others present a very long

thallus with over 30 cm in length (Müller Argoviensis 1890; Krog & Swinscow 1976; Rundel 1978).

The species of *Ramalina* with long and pendulous thallus and without soredia can be considered as belonging to the *R. usnea* morphological group. This group was studied by Rundel (1978) that recognized four species for American continent: *R. anceps* Nyl., *R. chilensis* Bertero ex Nyl., *R. sharpii* Rundel and *R. usnea* (L.) R. Howe.

According to Kashiwadani & Kalb (1993), among the Brazilian species of *Ramalina*, *R. anceps*, *R. rectangularis* Nyl., *R. sprengelii* Krog & Swinscow and *R. usnea* can be considered as part of this group. *Ramalina peranceps* Nyl. recorded by Vainio (1890) to Rio de Janeiro was considered as a variety of *R. anceps* by Rundel (1978) and it was not treated by Kashiwadani & Kalb (1993).

Another interesting taxonomical issue of this group is the possibility of the species *R. anceps* to be a chemotype of *R. usnea*, as suggested by some authors (Weber 1986; Aptroot & Bungartz 2007), due their similarities on morphology, anatomy and habitat.

Investigating the species of *Ramalina* from Southern Brazil, we found some morphological/anatomical inconsistencies regarding the available literature (e.g., Krog & Swinscow 1976; Rundel 1978; Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004; Aptroot & Bungartz 2007). The objective of this study was to investigate the phylogenetic relationships within the *R. usnea* group using morphological, chemical, anatomical and molecular analyses.

Material and Methods

This study includes the analysis of fresh specimens collected mainly in Southern Brazil, from the coastal plain up to 900 m in the plateau area. The specimens were preserved in herbaria. Collections from the herbaria H, HAS, ICN, JOI, MPUC, RB, SP, UFP and UPCB were also analyzed.

The fresh specimens were collected using a knife and dried at room temperature. Specimens were examined using standard techniques with stereoscopic (20–40×) and light microscopes (400–1000×). Freehand sections of the thalli and apothecia were mounted in water. The chemical analyses were conducted according to Huneck & Yoshimura (1996) and Orange *et al.* (2001) and included: spot tests, observation under UV light, and thin layer chromatography (TLC) using solvent C.

The species were selected based on morphological, chemistry and genetic similarity. Sequences available in GenBank with the *loci* used in this study were included in the phylogenetic analyses.

Small samples of 28 fresh collected *Ramalina* thalli (Table 1) were soaked in acetone overnight and then submitted to DNA extraction using the protocol described in Cubero *et al.* (1999).

The molecular markers used were the internal transcribed spacers (ITS) and the intergenic spacer (IGS), both of the nuclear ribosomal DNA. Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems) using the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.* 1990) for ITS and IGSf and IGSr (Wirtz *et al.* 2008) for IGS. Each 25 μ l reaction contained: 1X Taq buffer (Promega), 2mM of dNTP set, 2 μ M of each primer, 25 mM of MgCl₂, 1U of DNA Polymerase (Promega) and ca. 20–50 ng of DNA. The PCR conditions for ITS amplification were 2 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C, 1 min at 50°C, 1 min at 72°C, with a final elongation of 10 min at 72°C. For IGS amplification the conditions used were: 2 min at 95°C for initial denaturation, followed by 30 cycles of 30 s at 95°C, 45 s at 46°C, 1 min at 72°C, with a final elongation of 10 min at 72°C. The PCR products were purified with ammonium acetate and ethanol and sequenced in a ABI PRISM 3500 Genetic Analyzer (Applied Biosystems).

Forward and reverse sequencing reads were analyzed using MEGA 6 (Tamura *et al.* 2013). Sequences were aligned using MAFFT algorithm available at Guidance web server (<http://guidance.tau.ac.il/ver2/>). The ITS and IGS regions were analyzed together (concatenated) and separately (ITS) using a Maximum Likelihood (ML) and a Bayesian (BA) approaches in the CIPRES platform (<https://www.phylo.org/portal2/>). The best model for each analysis was obtained using the jModeltest2 program (Guindon & Gascuel 2003; Darriba *et al.* 2012). Maximum Likelihood analyses were performed in RAxML 8.2.4 (Stamatakis 2006) with 1000 bootstrap replicates for calculation of the nodal support, using the GTRGAMMA substitution model. Bayesian phylogenetic trees were estimated using the software BEAST 1.8.2 (Drummond *et al.* 2012). The tree searches were performed with the model TrNef+G with 1000 replicates for the ITS data set, and with the model TrN+G with 1000 replicates to the combined data set. Two parallel MCMC runs were performed using four chains of 20 000 000 generations, sampling trees every 1000 generations, after discarding the first 25% as burn-in. The

files generated were checked in Tracer v1.6 (Rambaut *et al.* 2014). Conflicts between ML and BA analyses were assumed significant if two different relationships for the same taxa were supported with ML bootstrap values above 75%, and BA posterior probabilities above 95%. Phylogenetic trees were drawn using FigTree v1.4 (available at <http://tree.bio.ed.ac.uk/software/figtree/>). The sequences of *Cliostomum griffithii* obtained in the GenBank were used as outgroup, according to Ekman (2001), Sérusiaux *et al.* (2010), and Pérez-Vargas & Pérez-Ortega (2014).

Results and Discussion

We examined 184 Brazilian specimens of the *Ramalina usnea* morphological group concerning their morphology, anatomy, and chemistry: *R. anceps* [9 specimens], *R. kashiwadanii* [17], *R. rectangularis* [1], *R. sprengelii* [60], and *R. usnea* [97]. The chemical compounds found were: divaricatic, homosekikaic, sekikaic, norstictic, connorstictic, salazinic, and usnic acids.

Twenty-two sequences of ITS and 12 of IGS were generated in this study, representing four out of the five species morphologically recognized (Table 1). The alignment of ITS and IGS sequences presented 522 and 392, respectively, unambiguously aligned nucleotides. A total of 914 positions were constant in the combined data set [variable sites = 303; parsim-informative sites = 275]. Unfortunately, fresh specimens of *R. peranceps* and *R. rectangularis* were not found and, therefore, not included in the genetic analyses.

The ML and BA analyses with ITS showed only one incongruence only between the relationship of the species *R. inflexa*, *R. leptocarpha*, *R. menziesii*, *R. siliquosa* and *R. usnea*. The other clades in generated trees had an identical topology (Figs. 1-2). The ML and BA analyses with concatenated data set not show any incongruence, so only the ML tree is shown (Fig. 3).

The *Ramalina usnea* group species analyzed grouped into four well-supported clades, corroborating the morphological, anatomical and chemical data. However, although *R. usnea* group has morpho-anatomical similarities, it is not a phylogenetic complex. The species *R. usnea* is in a distant clade of the other studied species, and only *R. kashiwadanii* and *R. sprengelii* showed a closer phylogenetic relationship.

The classification of *R. anceps* as a chemotype of *R. usnea* was not confirmed since they were positioned in distant clades. Therefore, *R. anceps* was considered as a distinct species characterized by morphological, anatomical and ecological features that resemble *R. usnea*. The presence of salazinic acid in medulla is the only *R. anceps* distinctive characteristic.

The status of *Ramalina kashiwadanii* as a distinct species of *R. sprengelii* was confirmed. Both species formed well-supported clades and were confirmed as closely related species (Figs. 1–3). The difference in the anatomical pattern of the branches between the two species proved to be a good distinctive character (see more taxonomic discussion under the new species). These species share habitat and substrate preferences and often could be found in sympatry.

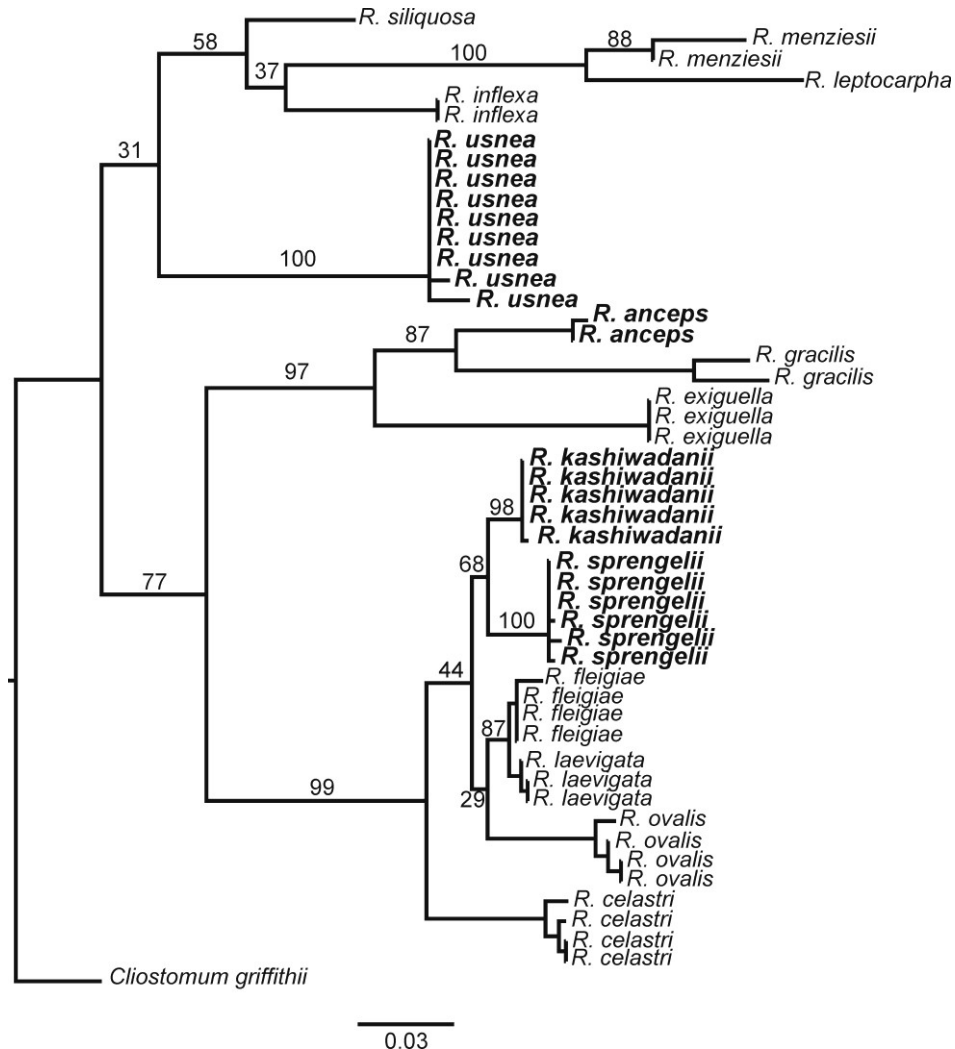


Fig. 1. Phylogenetic relationships of *Ramalina usnea* group based on maximum-likelihood analyses using ITS sequences. Bootstrap support is indicated above the branches.

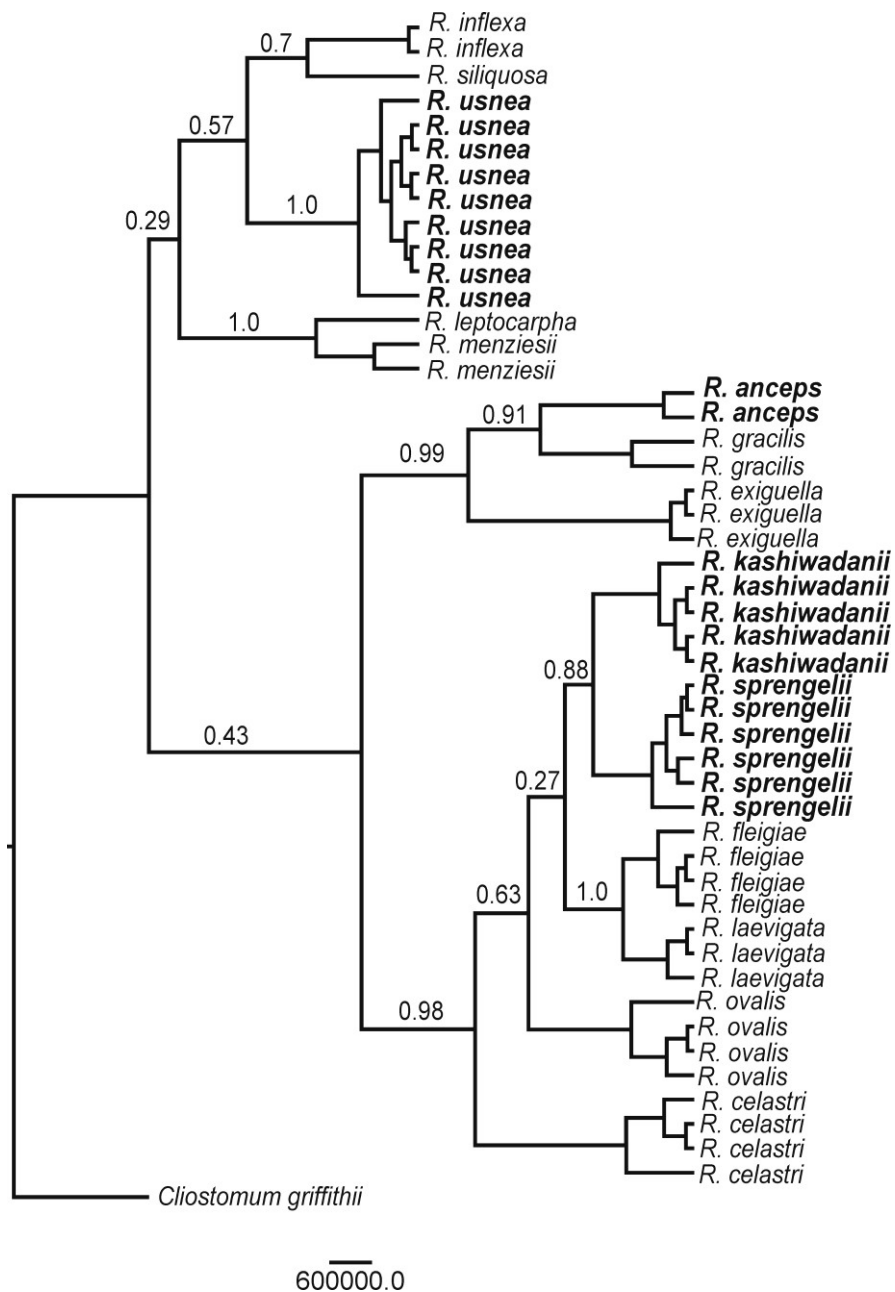


Fig. 2. Phylogenetic relationships of the *Ramalina usnea* group based on Bayesian analyses using ITS sequences. Posterior probability values are indicated above the branches.

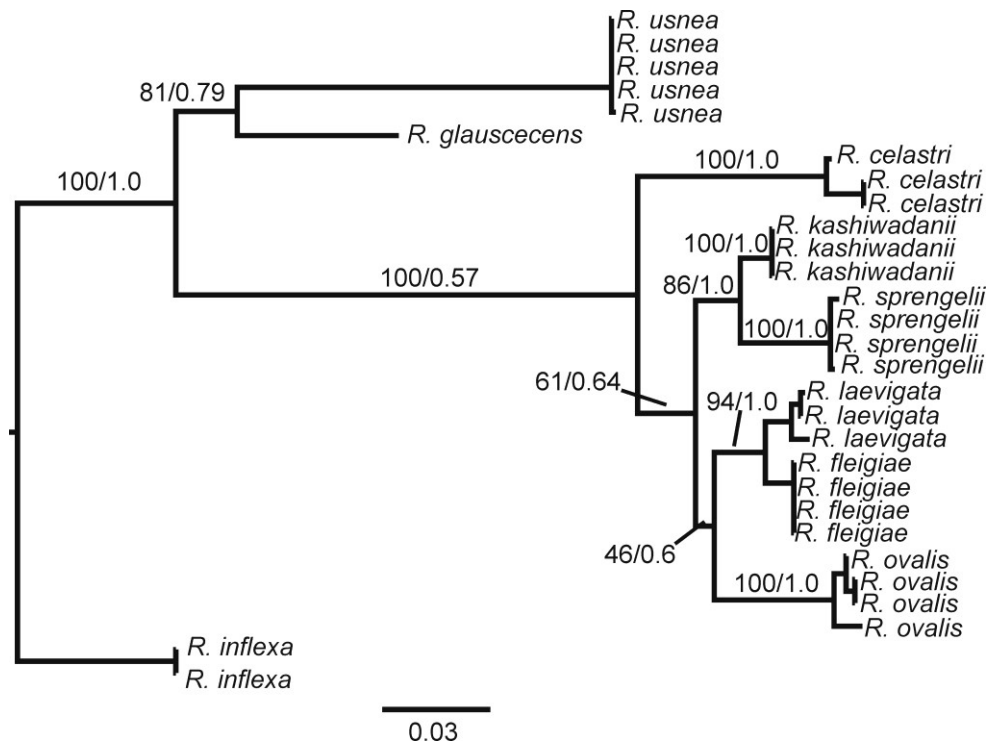


Fig. 3. Phylogenetic relationships of the *Ramalina usnea* group based on a maximum-likelihood analysis using ITS and IGS combined data set. Bootstrap support (ML) and posterior probabilities (BA) values are indicated above the branches.

Ramalina kashiwadanii Gumboski, Eliasaro & R. M. Silveira, *sp. nov.*

Mycobank No.: MB XXXXX

Barcoding GenBank No.: XXXXXX

Differing from *Ramalina sprengelii* by the continuous chondroid tissue and by the mainly marginal pseudocyphellae.

Typus: BRAZIL. State of Santa Catarina, Municipality of Rio Negrinho, locality of Rio dos Bugres, Araucaria Forest, corticolous, 07.IX.2012, *E. Gumboski 3946* (Holotype – ICN; Isotype – JOI, SP, G, H).

Fig. 4.

Description: Thallus pendulous, corticolous, up to 35 cm long, green whitish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 4.0 mm in diameter; branching dichotomously, anisotomic, solid branches, main branches flattened to irregularly flat at the basal part, rarely weakly canaliculated, 0.5–1.0 mm wide, secondary branches flattened to irregularly cylindrical, 0.1–0.3 mm wide, not contorted, surface weak to clearly striated transversely; symbiotic

propagules absent; in flat branches pseudocyphellae marginal and linear, flat to weakly depressed, 0.05–0.15 mm wide, rarely in irregular cylindrical branches pseudocyphellae are ellipsoid to short linear, 0.2–0.3 mm long and 0.05–0.10 mm wide. Cortex weakly distinguish, hyaline, 3–5 μm thick, chondroid tissue not cracked, continuous, 50–80 μm , algal layer discontinuous, up to 25 μm thick, medulla white, loose. Apothecia rounded, marginal, up to 1.5 mm in diameter, at first concave, flat when mature, then convex with age, branches weakly geniculate, anfracta smooth, without pseudocyphellae, margin entire, concolorous with the thallus, disc beige weakly pruinous; epihymenium hyaline, 4–6 μm thick, hymenium hyaline, 40–53 μm thick, hypohymenium yellowish, 50–70 μm , without algal layer under the hypohymenium and at margins of apothecia; ascospores 1-septate, fusiform, straight, 13–16 \times 3–5 μm . Pycnidia not found.

Chemistry: Spot test: Thallus K-, C-, KC- or KC+ dirty yellow and UV-; Medulla K-, C-, KC- and UV-. TLC: usnic acid.

Etimology: This new species is dedicated to Dr. Hiroyuki Kashiwadani, who enormously contributed to the knowledge of the species of *Ramalina*.

Distribution: *Ramalina kashiwadani* is known to South of Brazil, to sea level up to 900 m alt. on Araucaria forest. This corticolous species occurs on open places, from slightly shaded to direct sunlight habitat. It usually occurs intermixed with thalli of *R. sprengelii* and *R. usnea*.

Ramalina kashiwadani is morphologically similar to *R. sprengelii* in chemistry and thallus morphology. However, *R. sprengelii* have branches with continuous linear pseudocyphellae on lamina and margins and the chondroid tissue form almost cylindrical bundles of hyphae (Kashiwadani & Kalb 1993)

Ramalina anceps and *R. usnea* have branches with distinctly cracked chondroid tissue. Addicted, *R. anceps* produces norstictic acid, and *R. usnea* produces homosekikaic acid together sekikaic acid, and divaricatic acid and minors related compounds (Rundel 1978; Kashiwadani & Kalb 1993).

R. australiensis has laminal linear pseudocyphellae and a distinctly cracked and discontinuous chondroid tissue (Holotype – H!).

R. hoehneliana grows up to 60 cm long, with clear flat main branches, up to 1 cm wide (Krog & Swinscow 1976), and has a discontinuous not cracked chondroid tissue (Holotype – G!) as with *R. sprengelii*.

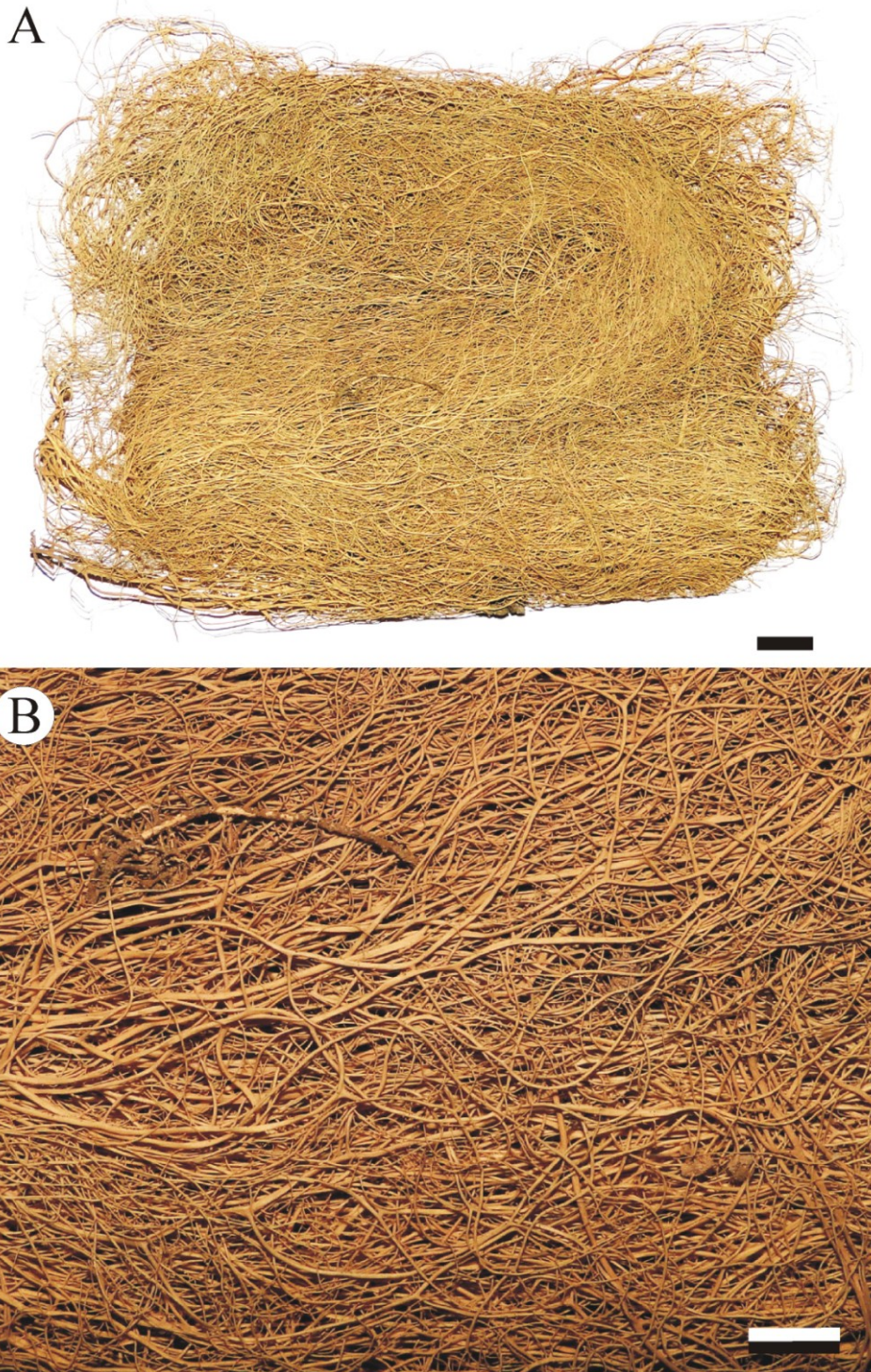


Fig. 4. Holotype of *Ramalina kashiwadanii*. A. Pendulous thallus. B. Details of branches. Scale bars = 1 cm.

Selected specimens examined: BRASIL: State of Paraná, Estrada antiga da Graciosa, margins of the road, near the bridge, corticolous, 03.VIII.1999, *W.B. Sanders* 99803.19A, 99803.18 (UFP). State of Rio Grande do Sul, Municipality of Rio Grande, Estação Ecológica do Taim, 3 Km em direção a mata do Albardão, corticolous, 10 m alt., 11.XII.1985, *M. Fleig* 2720 (ICN); Municipality of Amaral Ferrador, margin at Camaquã river, corticolous, 30 m alt., 12.VIII.1993, *M. Fleig* 5874 (ICN); Municipality of São Francisco de Paula, road to Cambará, corticolous, 900 m alt., 27.VI.1985, *R. Balbuena s.n.* (ICN), Aratinga, Atlantic forest, corticolous on *Araucaria angustifolia*, 800 m alt., 29.IV.1994, *M. Fleig* 6352 (ICN), Alpes de São Francisco, 13.VI.2009, *C.S.A. Martins s.n.* (MPUC 11873); Municipality of Encruzilhada do Sul, Passo dos Coqueiros, Cerro dos Mouros, Faz. Xafri, corticolous, 08.IX.1995, *M. Fleig* 7041 (ICN); Municipality of Triunfo, Copesul, corticolous, 29.VI.2004, *S. M. Mazzitelli & M. Käffer s.n.* (HAS 43371); Municipality of Candiota, corticolous, 13-15.XI.2009, *M. Käffer* 674 (HAS). State of Santa Catarina, Municipality of Rio Negrinho, Fazenda Velha, Araucaria forest, corticolous, 21.VIII.2010, *E. Gumboski* 2199 (JOI), locality of Volta Grande, Araucaria Forest, corticolous, 20.I.2012, *E. Gumboski* 3410 (ICN); Municipality of Campo Alegre, near to 'Engenho', floresta ombrófila densa montana, corticolous, 26°08'27"S, 49°17'36"W, 28.I.2012, *E. Gumboski* 3263 (ICN).

Ramalina anceps Nyl., Syn. meth. lich. 1(2): 290. 1860.

MB#357046

Typus: Guadalupe, *L'Herminier et al.* (Holotype – PC; Syntype – H-NYL 370128!).

Thallus pendulous, corticolous or saxicolous, up to 15 cm long, greenish in field, dark stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching dichotomously, almost isotomic; solid branches, subterete to almost flat, 0.2–0.5 mm wide, sometimes weakly contorted, surface smooth; symbiotic propagules absent; pseudocyphellae mainly marginal, rarely laminal, linear, flat, 0.04–0.07(–0.20) wide. Cortex distinct, hyaline to yellowish, 12–20 µm thick, chondroid tissue distinctly cracked, continuous, 50–170 µm thick, algal cells not forming a distinct layer, medulla white, compact. Apothecia rare, marginal, shortly stipitate, up to 0.7 mm in diameter; disc flat to slightly convex, anfithecia smooth, without pseudocyphellae, margin entire, concolorous with the thallus, disc beige not to weakly pruinous; epihymenium darkened, 4–6 µm thick, hymenium hyaline, 40–55 µm

thick, subhymenium hypothecium yellowish, 55–70 µm thick, without algal layer under the hypothecium and at margins of apothecia; ascospores 1-septate, short-fusiform, 11–16 × 5–8 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ bright yellow. Medulla: K+ yellow → red, C-, KC-. TLC: usnic acid, norstictic acid and (±) connorstictic acid.

Distribution: *Ramalina anceps* is known to Central and South America, from sea level up to 800 m alt. (Nylander 1890; Kashiwadani & Kalb 1993; Aptroot & Bungartz 2007).

This species differs from *R. usnea* by the mainly marginal pseudocyphellae and by the production of norstictic acid. Some authors (e.g., Weber 1986; Aptroot & Bungartz 2007) hypothesized that *R. anceps* could be a chemotype of *R. usnea* because the morphological and anatomical similarity. However, the molecular data showed that both are distant phylogenetically.

Ramalina anceps differ from *R. kashiwadanii* and *R. sprengelii* by the distinctly cracked chondroid tissue and by presence of norstictic acid in the medulla (Kashiwadani & Kalb 1993).

Specimens examined: BRAZIL. State of Paraná, Municipality of Guaraqueçaba, Ilha das Peças, mangrove, corticolous, 26.X.2003, *C. G. Donha 1289* (UPCB), Reserva Natural de Itaquí, mangrove, corticolous, 17.II.2005, *R. Reis 195* (UPCB); Municipality of Paranaguá, Ilha do Mel, corticolous, 05.II.2004, *R. Reis 30* (UPCB), on mangrove, corticolous, 25°32'50.26"S, 48°18'17.24"W, 27.VIII.2009, *E. Gumboski & S. Eliasaro IM121* (JOI). State of Rio Grande do Sul, Municipality of São Francisco de Paula, Loteamento Colina de São Francisco, corticolous, on *Araucaria angustifolia*, 19.IV.1982, *Osorio & M. Fleig SF2* (ICN 54772); Municipality of Cambará do Sul, Itaimbezinho, near the Hotel, 20.IV.1982, *Osorio & M. Fleig T34* (ICN 54842). São Paulo State, Municipality of Cananéia, Near Cananéia, on the Atlantic Coast S of São Paulo, Ilha Comprida, near road to Cananéia ferry, c. 10 m tall shrubby forest on sand, near the beach, 16.VII.1979, H.J.M. Sipman 14148 (SP).

Ramalina rectangularis Nyl., Bull. Soc. linn. Normandie, sér. 2 4(2): 121. 1870.

MB#403814

Typus: Brazil, Bahia, Blanchet (Syntypes – G!, H-NYL 37509!, PC, UPS, W!).

Thallus pendulous, stramineous to almost weakly brown in herbaria, branching dichotomic anisotomic, solid branches, flat, distinctly rectangular in cross-section, 1.4–3.0 mm wide, not contorted, surface smooth to rarely weakly rough; symbiotic propagules absent; pseudocyphellae linear, continuous, marginal, depressed to weakly elevated, (0.4–)0.6–5.0(–10.0) × 0.2–0.7 mm, the protuberances at margins are verruciformis, sometimes reminding an stalked apothecia, 0.2–1.3 mm tall × 0.5–1.8 mm wide, it seems to have originated in pseudocyphellae, mainly marginal, rarely submarginal. Cortex distinct, yellowish, 15–20 µm thick, chondroid tissue distinctly cracked, continuous, 200–550 µm thick, algae dispersed throughout the medulla, medulla whitish, loose. Apothecia not found. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K+ yellow→red, C-, KC-. TLC: usnic acid, salazinic acid (*fide* Kashiwadani & Kalb 1993).

Distribution: According to Kashiwadani & Kalb (1993), *R. rectangularis* is a poorly understood species known only from the type collection from Bahia State, Northeastern Brazil.

Ramalina rectangularis is a distinguish species in *R. usnea* group by the distinctly rectangular branches in cross-section, allied with the chondroid tissue distinctly cracked and by the production of salazinic acid in medulla. Unfortunately, neither specimens deposited in herbarium nor fresh specimens were found to contribute with more information about this species.

Specimens examined: Syntypes.

Ramalina sprengei Krog & Swinscow, Norwegian Journal of Botany 23 (3): 172. 1976.

MB#343520

Typus: South Africa, Uitenhagen, *Ecklon 212* (Holotype – S: Photo!).

Thallus pendulous, corticolous, up to 30 cm long, greenish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching main dichotomously to irregularly, solid branches, branches mainly flat and usually cylindrical to terete in distal parts, 0.2–0.7(–1.2) mm wide, not contorted, surface smooth in cylindrical to terete branches and striated in flat branches; symbiotic propagules absent; linear pseudocyphellae, marginal and laminal, flat, 0.05–

0.2 mm wide. Cortex distinct, hyaline to yellowish, 12–18 μm thick, chondroid tissue not cracked, mainly discontinuous in cylindrical bundles, and discontinuous but not in bundles in branches wider than 0.6 mm; medulla white, loose. Apothecia marginal or lateral, up to 1.2 mm in diameter, flat to convex, anfrithecia smooth without pseudocypbellae, margin entire, concolorous with the thallus, disc beige; epihymenium hyaline, 2–4 μm thick, hymenium hyaline, 40–60 μm thick, subhymenium hyaline, 20–30 μm thick, hypothecium hyaline to yellowish, 25–40 μm thick, without algal layer under the hypothecium and at margins of apothecia; ascospores 1-septate, fusiform, 12–15 \times 3–5 μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K-, C-, KC-. TLC: usnic acid.

Distribution: *Ramalina sprengelii* is known to Africa and South America, from sea level up to 900 m alt. (Krog & Swinscow 1976; Kashiwadani & Kalb 1993). In Brazil was found only in plateau regions. It occurs on tree trunks and branches, on open places and forest edges (Kashiwadani & Kalb 1993), mainly in places with frequent occurrence of fog.

Similar in morphology, chemistry and habitat with *R. kashiwadanii* (see discussion under that species).

Selected specimens examined: BRAZIL. State of Paraná, Municipality of Curitiba, Centro Politécnico, corticolous, 23.V.2001, *C. R. Jonck et al. s.n.* (UPCB), corticolous, 27.VII.1993, *S. Eliasaro 1058* (UPCB), Umbará, corticolous, 22.XI.1998, *L. T. Maranhão 24* (UPCB), Parque Barigui, 08.III.1994, *S. Eliasaro 1242* (UPCB); Municipality of Colônia Matos, Sítio Água Fresca, corticolous 17.VII.1999, *D. M. Barbosa et al. s.n.* (UPCB); Municipality of Quatro Barras, Morro do Anhangava, Borda do Campo, corticolous, 04.VI.1994, *L. A. Juliatto s.n.* (UPCB 35438), corticolous, VIII.2001, *L.M.C. Cordeiro s.n.* (UPCB 46431); Municipality of Piraquara, Mananciais da Serra - transição F.O.M e F.O.D., 08.II.2004, *R. Reis 106* (UPCB); Municipality of Piraquara, Morro do Canal, corticolous, open place, 24.V.2002, *S. Eliasaro 2338* (UPCB), Margem da BR 277 (Paranaguá-Curitiba), entre marcadores km 52 e km 53, corticolous, 01.VIII.1999, *W.B. Sanders 99801.12C* (UFP), Estrada antiga da Graciosa, margin of the Road, near the bridge, corticolous, 03.VIII.1999, *W.B. Sanders 99803.6, 99803.9, 99803.8, 99803.5, 99803.4, 99803.2B* (UFP). State of Rio

Grande do Sul, Municipality of Cambará do Sul, Cachoeira dos Venâncios, Rio passando por lajedos, corticolous, 08.I.2000, *W.B. Sanders 00108.4* (UFP); Municipality of Vacaria, 31.V.1978, *G. Hilt s.n.* (MPUC 4050); Municipality of Esmeralda, 10.X.2010, *R. Halinski s.n.* (MPUC 4084). São Paulo State, Municipality of Pirituba, 28.X.1893, *A. Loefgren s.n.* (SP 34267).

Ramalina usnea (L.) R. Howe, *The Bryologist* 17: 81, pl. 12, figs 1-2. 1914.

Basionym: *Lichen usnea* L., *Mantissa* 1: 131-132 (1767).

Typus: (Lectotype – LINN 1273-278: Photo!).

MB#403866

Thallus pendulous, corticolous, up to 50 cm long, greenish in field, stramineous in herbaria, growing from a narrow holdfast, concolours with the thallus to blackish, up to 3.0 mm in diameter; branching irregularly to dichotomously (anisotomic), solid branches, branches mainly flattened, but often with secondary branches irregularly cylindrical to subterete, often contorted, 0.1–2.0(–2.5) mm wide, surface mainly smooth to weakly striate; symbiotic propagules absent; pseudocyphellae linear, marginal and laminal, flat to weakly depressed, rarely sharply depressed, 0.05–0.1 mm wide. Cortex distinct, hyaline to dirty yellow, 08–12 μm thick, chondroid tissue distinctly cracked, continuous, 50–150 μm thick, algal layer discontinuous, 20–30 μm thick, medulla white, loose. Apothecia common, rounded, marginal or laminal, up to 1.3 mm diameter, flat, becoming convex with age, anfithecium smooth, without pseudocyphellae, margin entire to rarely cropped, concolorous with the thallus, disc beige weakly pruinous; epihymenium darkened, 8–12 μm thick, hymenium hyaline, 40–55 μm thick, subhymenium hyaline, 20–25 μm thick, hypothecium hyaline, continuous, 20–55 μm thick, with agglomerates of algae dispersed in medulla under the hypothecium; ascospores 1-septate, fusiform, 18–22(–25) \times 4–7 μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K+ pinkish or K-, C-, KC-. TLC: Race 1. usnic acid, homosekikaic and sekikaic acid. Race 2. Usnic acid, divaricatic acid.

Distribution. *Ramalina usnea* is known to American continent. It occurs from sea level to 1000 m alt. (e.g., Rundel 1978; Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004; Aptroot & Bungartz 2007). According to Stevens (1987), the records with the name *R. usnea* to Australia are related to *R. australiensis* Nyl.

Similar in morphology, anatomy and ecology with *R. anceps* (see discussion under that species).

Ramalina usnea is similar in morphology with *R. australiensis*, however the former has flattened branches, usually contorted and it usually produces homosekikaik, sekikaic or divaricatic acids (Rundel 1987; Kashiwadany & Kalb 1993), and the latter has mainly terete branches, never contorted and it produces only usnic acid (Stevens 1987).

Selected specimens examined: BRAZIL. State of Paraná, Municipality of Guaraqueçaba, Reserva Natural de Itaqui, magrove, 06.XI.2003, *C. G. Donha 1552* (UPCB), near the sea, corticolous, 07.VIII.2003, *C. G. Donha et al. 1001* (UPCB); Municipality of Curitiba, Parque Iguaçu, 17.VIII.1994, *D. Ramirez s.n.* (UPCB 35319), zoo, corticolous, 16.XII.1998, *S. Eliasaro & C. G. Donha 322* (UPCB); Municipality of Paranaguá, Ilha do Mel, corticolous, 27.VIII.2009, *S. Eliasaro & E. Gumboski 3133* (UPCB). State of Santa Catarina, Municipality of São Francisco do Sul, Morro da Enseada, saxicolous, on rocky shore, 26°13'11,8"S, 48°29'40,4"W, 19.VI.2008, *E. Gumboski 479, 597* (JOI), 26°13'44"S, 48°29'54"W, 04.IX.2009, *E. Gumboski 1663, 1664* (JOI); Municipality of Garuva, Monte Crista, Floresta Ombrófila Densa, corticolous, 26°04'53,9'S, 48°55'15,3"W, 15.V.2010, *E. Gumboski & F. Beilke 1969* (JOI); Municipality of São Bento do Sul, Estrada Sertãozinho, próx. a igrejinha da Vila Piltz, rural area, corticolous, 20.VII.2012, *E. Gumboski 3689* (JOI); Municipality of Fraiburgo, margins of road SC 453 (302), corticolous on *Syagrus sp.*, Araucaria Forest, 18.VII.2013, *E. Gumboski 4743* (JOI). State of Rio Grande do Sul, Municipality of Torres, Morro do Farol, saxicolous, on rocky shore, 11.XI.2011, *E. Gumboski; S. Eliasaro & F. Beilke 3038* (JOI), margens da Estrada do Cemitério, litoral, corticolous, 30.IX.2012, *E. Gumboski 4059, 4060, 4066* (JOI); Municipality of São Francisco de Paula, Pro-Mata, Araucaria Forest, corticolous, 18.IV.2012, *E. Gumboski 3557* (JOI). State of Rio de Janeiro, Municipality of Rio de Janeiro, Jardim Botânico, *L.Damazio s.n.* (RB 220161); Municipality of Maricá, restinga, pendente em galho, exposto ao sol, 05.III.1985, *M.A.A. Santos et al. 132* (RB), perto de Maricá, Lagoa do Padre, restinga, arbustiva, epífita, em briofita, 08.IX.1975, *D.Araújo 768 et A.Luna Peixoto 574* (RB).

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Tab.1. Information about the *Ramalina* sequences analyzed.

Species	Voucher information	Reference	GenBank No.	
			ITS	IGS
<i>Cliostomum griffithii</i>	UNITED KINGDOM	Kelly <i>et al.</i> (2011)	FR799177	-
<i>R. anceps</i>	BRAZIL. Paraná State, <i>E. Gumboski & S. Eliasaro IM121</i> (ICN)	This study	XXXXXXX	-
<i>R. anceps</i>	BRAZIL. Rio Grande do Sul State, <i>A. Gerlach 1485</i> (ICN)	This study	XXXXXXX	-
<i>R. celastri</i>	RWANDA	Sérusiaux <i>et al.</i> (2010)	GU827295	-
<i>R. celastri</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583540	KF594450
<i>R. celastri</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583542	KF594452
<i>R. celastri</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583544	KF594454

	BRAZIL. Rio Grande do			
<i>R. exiguella</i>	Sul State, <i>E. Gumboski</i> 4068 (ICN)	This study	XXXXXXXXXX	-
	BRAZIL. Santa Catarina			
<i>R. exiguella</i>	State, <i>A. Gerlach 875b</i> (ICN)	This study	XXXXXXXXXX	-
	BRAZIL. Santa Catarina			
<i>R. exiguella</i>	State, <i>A. Gerlach 873</i> (ICN)	This study	XXXXXXXXXX	-
	BRAZIL. Rio Grande do			
<i>R. fleigiae</i>	Sul State, <i>E. Gumboski</i> 5055 (ICN)	This study	XXXXXXXXXX	XXXXXXXXXX
	BRAZIL. Rio Grande do			
<i>R. fleigiae</i>	Sul State, <i>E. Gumboski</i> 5056 (ICN)	This study	XXXXXXXXXX	XXXXXXXXXX
	BRAZIL. Rio Grande do			
<i>R. fleigiae</i>	Sul State, <i>E. Gumboski</i> 5049 (ICN)	This study	XXXXXXXXXX	XXXXXXXXXX
	BRAZIL. Rio Grande do			
<i>R. fleigiae</i>	Sul State, <i>E. Gumboski</i> 5050 (ICN)	This study	XXXXXXXXXX	XXXXXXXXXX
	BRAZIL. Santa Catarina			
<i>R. gracilis</i>	State, <i>A. Gerlach et al. 238</i> (ICN)	This study	XXXXXXXXXX	XXXXXXXXXX
	BRAZIL. Paraná State, <i>A.</i>			
<i>R. gracilis</i>	<i>Gerlach 780b</i> (ICN)	This study	XXXXXXXXXX	XXXXXXXXXX
	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583546	KF594456
<i>R. inflexa</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583547	KF594457

<i>R. kashiwadanii</i>	BRAZIL. Paraná State, S. C. Feuerstein 583 (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. kashiwadanii</i>	BRAZIL. Santa Catarina State, E. Gumboski 3263 (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. kashiwadanii</i>	BRAZIL. Santa Catarina State, E. Gumboski 3945 (ICN)	This study	XXXXXXX	-
<i>R. kashiwadanii</i>	BRAZIL. Santa Catarina State, E. Gumboski 3946 (ICN)	This study	XXXXXXX	-
<i>R. kashiwadanii</i>	BRAZIL. Rio Grande do Sul State, M. Kaffer #1 (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. laevigata</i>	BRAZIL. Rio Grande do Sul State, E. Gumboski 5033 (ICN)	This study	XXXXXXXXX	XXXXXXXXX
<i>R. laevigata</i>	BRAZIL. Rio Grande do Sul State, E. Gumboski 5046 (ICN)	This study	XXXXXXXXX	XXXXXXXXX
<i>R. laevigata</i>	BRAZIL. Rio Grande do Sul State, E. Gumboski 5047 (ICN)	This study	XXXXXXXXX	XXXXXXXXX
<i>R. leptocarpha</i>	U.S.A.	LaGreca & Lumbsch (2001)	AF249908	-
<i>R. menziesii</i>	U.S.A.	LaGreca & Lumbsch (2001)	AF249909	-
<i>R. menziesii</i>	U.S.A.	LaGreca & Lumbsch (2001)	AF249910	-
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583554	KF594464
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583556	KF594466

<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583557	KF594467
<i>R. ovalis</i>	NEW ZEALAND	Hayward <i>et al.</i> (2014)	KF583558	KF594468
<i>R. siliquosa</i>	UNITED KINGDOM	Groner & LaGreca (1997)	U84587	-
<i>R. sprengelii</i>	BRAZIL. Santa Catarina State, <i>E. Gumboski 4009</i> (ICN)	This study	XXXXXXX	-
<i>R. sprengelii</i>	BRAZIL. Santa Catarina State, <i>E. Gumboski 4050</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. sprengelii</i>	BRAZIL. Santa Catarina State, <i>E. Gumboski 4054</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. sprengelii</i>	BRAZIL. Santa Catarina State, <i>A. Gerlach 539a</i> (ICN)	This study	XXXXXXX	-
<i>R. sprengelii</i>	BRAZIL. Santa Catarina State, <i>F. Beilke s.n.</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. sprengelii</i>	BRAZIL. Santa Catarina State, <i>A. Gerlach 1280</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. usnea</i>	BRAZIL. Paraná State, <i>A.</i> <i>Gerlach 780a</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. usnea</i>	BRAZIL. Santa Catarina State, <i>A. Gerlach 396</i> (ICN)	This study	XXXXXXX	-
<i>R. usnea</i>	BRAZIL. Paraná State, <i>E.</i> <i>Gumboski & S. Eliasaro</i> <i>IM62</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. usnea</i>	BRAZIL. Santa Catarina State, <i>E. Gumboski & F.</i> <i>Beilke 1765</i> (ICN)	This study	XXXXXXX	-
<i>R. usnea</i>	BRAZIL. Rio Grande do Sul State, <i>E. Gumboski</i> <i>3984</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. usnea</i>	BRAZIL. Rio Grande do	This study	XXXXXXX	-

	Sul State, <i>E. Gumboski</i> 4060 (ICN)			
	BRAZIL. Rio Grande do			
<i>R. usnea</i>	Sul State, <i>Fazolino e Rosa</i> <i>s.n.</i> (ICN)	This study	XXXXXXX	-
<i>R. usnea</i>	Uruguay. <i>C. A. Ribeiro s.n.</i> (ICN)	This study	XXXXXXX	XXXXXXX
<i>R. usnea</i>	BRAZIL. Espírito Santo State, <i>C. Vidigal s.n.</i> (ICN)	This study	XXXXXXX	XXXXXXX

4.4. Manuscrito IV

Gumboski, E.L.; Eliasaro, S. & Borges da Silveira, R.M. Notes on species of *Ramalina* Ach. (Ramalinaceae, lichenized Ascomycota) from Northeastern Brazil. **Acta Botanica Brasilica**: a ser submetido.

Notes on species of *Ramalina* Ach. (Ramalinaceae, lichenized Ascomycota) from Northeastern Brazil

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Abstract.

The Brazilian Northeastern region has ca. 1.5 million km² area and contains important ecosystems; however, the occurrence of *Ramalina* species is very scarce. Only ten species are known from two States, Bahia and Pernambuco, of nine States that form the Northeastern Region. Specimens were collected from State of Sergipe, and studied specimens from five Brazilian herbaria. The aim of this work is contribute with the knowledge of *Ramalina* from the Brazilian Northeastern region.

Keywords. Biodiversity, Caatinga vegetation, Cerrado vegetation, lichen, taxonomy.

Introduction

The lichen genus *Ramalina* Ach. has been record from Brazil since 1833, when Eschweiler (1833) described *Parmelia denticulata* Eschw. [*Ramalina denticulata* (Eschw.) Nyl.] based on corticolous specimens from Amazonas River margins. However, after that, few records of *Ramalina* were made from North and Northeastern regions of Brazil.

The Brazilian Northeastern region has ca. 1.5 million km² area and contains important ecosystems as Rainforest, Caatinga, Cerrado, ‘Mata dos Cocais’, and coastal vegetation like dunes, restinga and mangroves (IBGE 1992). Even with this great area and containing nine States, the records of *Ramalina* species from Northeastern region

were made essentially by Kashiwadani & Kalb (1993) that cited the species *R. aspera* Räsänen, *R. cochlearis* Zahlbr., *R. complanata* (Sw.) Ach., *R. gracilis* (Pers.) Nyl., *R. solediosa* (B. de Lesd.) Landrón and *R. usnea* (L.) R. Howe from State of Bahia, and *R. peruviana* Ach., *R. solediosa*, and *R. subpollinaria* Nyl. from State of Pernambuco. Before the work of Kashiwadani & Kalb (1993), the only records were made by Nylander (1870) that described *R. rectangularis* Nyl. from State of Bahia, Müller Argoviensis (1891) reported *R. dendriscoides* Nyl., *R. solediantha* Nyl., and *R. subpollinaria* from Pernambuco, and Malme (1934) that cited *R. bicolor* Müll. Arg. [= *R. gracilis*] from Bahia.

The aim of this paper is to present new distributional information about the species of *Ramalina* in the Brazilian Northeastern Region.

Material and Methods

Specimens were collected in State of Sergipe, Northeastern Brazil, from municipalities of Areia Branca (Serra de Itabaiana National Park, and Fazenda Cafuz) and Nossa Senhora da Glória (Assentamento Nossa Senhora Aparecida), from 165 to 655 m altitude. Historical collections from the herbaria G, H, ICN, JOI, MEL, RB, SP and UFP were analyzed.

The specimens examined were collected using a knife and dried at room temperature. Specimens were examined using standard techniques with stereoscopic microscope (20–40×) and light microscope (400–1000×). Freehand sections of the thalli and apothecia were mounted in water. The chemical analysis: spot tests, observation under UV light, and thin layer chromatography (TLC) using solvent C; were conducted according to Huneck & Yoshimura (1996) and Orange *et al.* (2001).

Results and Discussion

Six species were found from four States of Northeastern Brazil: Alagoas, Bahia, Sergipe, and Pernambuco. All species grow on trunks or twigs, five species occur in Cerrado vegetation (*R. aspera*, *R. complanata*, *R. peruviana*, *R. solediosa*, and *R. subpollinaria*) and five in Caatinga vegetation (*R. aspera*, *R. complanata*, *R. peruviana*, *R. subpollinaria*, and *R. usnea*).

Five species are new records to State of Sergipe (*R. aspera*, *R. complanata*, *R. peruviana*, *R. solediosa*, and *R. subpollinaria*), two to State of Pernambuco (*R. aspera* and *R. complanata*), and one to State of Alagoas (*R. usnea*).

Ramalina solediantha recorded by Müller Argoviensis (1891) from Pernambuco needs a reassessment, because it is possible that those specimens could be samples of *R. subpollinaria* due the morphological similarity.

Ramalina aspera Räsänen, Ann. Bot. Soc. Zool. Bot. Fenn. Vanamo 20: 5. 1944.

Type: Paraguay, Chaco, Rio Verde, Villa Hayes, *W. G. Herter 3* (H!—holotype).

MB#369881

Fig. 1A.

Description: Thallus caespitose, corticolous, up to 4 cm long, stramineous whitish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 5.0 mm in diameter; branching irregularly, rarely almost palmate, solid branches, mainly flat, dorsiventral, 0.5–4.0 mm wide, surface rugose, reticulately to irregularly ridged; symbiotic propagules absent; pseudocyphellae laminal and marginal, raised, tuberculate, 0.1–0.3 mm wide, 0.05–0.2 mm tall. Cortex distinct, hyaline to yellowish, 10–22 μ m thick, chondroid tissue not cracked, discontinuous, almost in irregular bundles, 50–110 μ m thick, algal cells dispersed in medulla, medulla white, loose. Apothecia rounded, subterminal to terminal, up to 4.5 mm in diameter, concave to flat, anfithecium with tuberculate pseudocyphellae, margin usually indented, concolorous with the thallus, disc whitish to beige, weakly pruinous; epihymenium greenish, 15–20 μ m thick, hymenium hyaline to grayish, 20–25 μ m thick, hipohymenium yellowish, 20–35 μ m thick, with chondroid tissue under de hypothecium, 10–70 μ m thick, algal layer continuous, 40–70 μ m thick; ascospores 1-septate, straight to slightly curved, 12–15 \times 3–5 μ m. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC-, UV-. Medulla K- or K+rose, C-, KC-, UV-. TLC: (Race 1) Usnic acid and divaricatic acid. (Race 2) Usnic acid and cryptochlorophaeic acid.

Distribution: Tropical in America and Africa (Krog & Swinscow 1976; Kashiwadani & Nash 2004).

Ramalina aspera is characterized mainly by the caespitose thallus growing from a narrow holdfast, by the flat branches with tuberculate pseudocyphellae, by the not

cracked chondroid tissue, and by the production of divaricatic acid or cryptochlorophaeic acids.

In Brazil, this species could be confused with *R. complanata* by the morphologically similar thallus and habitat preferences. However, *R. complanata* have a flat to almost canaliculate branches with a cracked chondroid tissue, and produces salazinic acid or divaricatic acid, the later usually together with salazinic acid.

Specimens examined: BRAZIL. State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, *E. Gumboski* 4757, 4765, 4781 (ICN), 10°44'50.8"S, 37°20'29.6"W, 18.IX.2013, *E. Gumboski* 4845, 4848 (ICN); Municipality of Nossa Senhora da Glória, locality of Povoado Retiro1, Assentamento Nossa Senhora Aparecida, 175 m alt., Caatinga, corticolous, 10°06'13.5"S, 37°25'12.9"W, 19.IX.2013, *E. Gumboski* 4854, 4858, 4860, 4870, 4883 (ICN). State of Pernambuco, Municipality of Alagoinha, locality of Sítio Lagoa Seca, sobre ramos de algaroba, perto da casa, em Caatinga, corticolous, 14.XII.1998, *W.B. Sanders* 98d14.19, 98d14.6 (UFP).

Ramalina complanata (Sw.) Ach., Lichenographia Universalis: 599. 1810.

Basionym: *Lichen complanatus* Sw. in Ach., K. Vet. Nya. Handl. 18: 290. 1797.

Type: India occidentalis, Jamaica, Swartz (S—holotype; H-ACH 1821!—isotype).

MB#403682

Fig. 1B.

Description: Thallus caespitose, corticolous, up to 7 cm long, stramineous whitish in field, dark stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 4.0 mm in diameter; branching irregularly, almost densely branched, solid branches, mainly flat, dorsiventral, almost but not clearly canaliculate, 1.0–3.0 mm wide, surface smooth to rugose, sometimes sulcate, almost scrobiculate; symbiotic propagules absent; pseudocyphellae laminal and marginal, raised, weakly to clearly tuberculate, 0.05–0.2 mm wide, 0.05–0.2 mm tall. Cortex distinct, hyaline, 10–18 μ m thick, chondroid tissue weakly to distinctly cracked, discontinuous, (50–)100–220 μ m thick, algal cells dispersed in medulla, medulla whitish, loose. Apothecia rounded, subterminal to terminal, up to 4.0 mm in diameter, mainly concave, anfithecia usually with tuberculate pseudocyphellae, margin usually entire, concolorous with the thallus,

disc beige, weakly pruinous; epihymenium stramineous to brownish, 10–14 µm thick, hymenium hyaline, 25–30 µm thick, hipohymenium yellowish, 30–50 µm thick, with chondroid tissue under de hypothecium, 25–35 µm thick, algal layer discontinuous, 20–50 µm thick; ascospores 1-septate, straight to slightly curved, 12–16 × 4–5 µm. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC-, UV-. Medulla K- or K+ yellow→red, C-, KC-, UV-. TLC: (Race 1) Usnic acid and salazinic acid. (Race 2) Usnic acid and divaricatic acid.

Distribution: Tropical in America (e.g., Kashiwadani & Kalb 1993).

Ramalina complanata is characterized by the flat to subcanaliculate branches with tuberculate pseudocyphellae, by the cracked chondroid tissue, and by the production of divaricatic acid and/or salazinic acid.

Similar with *R. aspera* (see comments under that species).

In Brazil could be confused with *R. canalicularis* (Nyl.) Kashiw. by the morphologically similar thallus and pseudocyphellae, and by the production of salazinic acid. However, *R. canalicularis* have a clearly canaliculate thallus with semi-globose pseudocyphellae, and does not produce divaricatic acid (Nylander 1870; Kashiwadani & Nash 2004).

Specimens examined: BRAZIL. State of Bahia, Municipality of Euclides da Cunha, Along road from Canudos to Euclides da Cunha, about 45 km south of Canudos, dense moist Caatinga shrubs 1-4m tall, 10°16-17'S, 39°4'W, 18.IV.1962, *G. Eiten & L.T. Eiten 5010* (SP). State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, *E. Gumboski 4784* (ICN); Municipality of Nossa Senhora da Glória, locality of Povoado Retiro 1, Assentamento Nossa Senhora Aparecida, 175 m alt., Caatinga, corticolous, 10°06'13.5"S, 37°25'12.9"W, 19.IX.2013, *E. Gumboski 4874, 4879, 4888* (ICN). State of Pernambuco, Municipality of Caruaru, Estação Experimental da Empresa Pernambucana de Pesquisa Agropecuária; Caatinga; em ramos, com Tillandsia, corticolous, 08.XII.1999, *W.B. Sanders 99D08.1, 99D08.4B, 99D08.4B* (UFP); Municipality of Alagoinha, Fazenda Cajueiro Seco, sobre ramos de Croton, em Caatinga, corticolous, 14.XII.1998, *W.B. Sanders 98d14.3* (UFP), Caatinga, pendulous,

corticolous, 27.VI.1998, *S. Ribeiro & E. Pereira s.n.* (UFP 28294); Municipality of Poção, Mata de altitude, VIII.1994, *E. Pereira s.n.* (UFP 28299).

Ramalina peruviana Ach., Lichenographia Universalis: 599. 1810.

Type: Peru, *Lagasta* (H-ACH!—holotype; MEL! —isotype).

MB#403795

Fig. 1C.

Description: Thallus shrubby, corticolous, up to 7 cm long, whitish green in field, stramineous in herbaria, growing from a common or delimited holdfast, concolorous with the thallus, up to 4.0 mm in diameter; branching irregularly to dichotomously anisotomic; solid branches, irregularly flattened in main branches becoming irregularly to terete in distal branches, 0.3–1.0 mm in wide, surface smooth to irregular, usually striate; soralia marginal and laminal, rarely orbicular, usually ellipsoid to rarely elongate, soredia abundant, 200–500(–700) μm in diameter, presence of cylindrical fibrils on soralia is common, up to 0.8 mm tall; pseudocyphellae laminal and marginal, at surface level, ellipsoid to short linear, $0.05\text{--}0.1 \times 0.2\text{--}1.5\text{--}(2.5)$ μm . Cortex hyaline, 5–7 μm , chondroid tissue not cracked, irregular, mainly continuous, 40–100 μm thick, algal layer discontinuous, 20–40 μm thick, medulla white, loose to compact. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+ yellowish. Medulla K- or K+ rose evanescent, C-, KC-. TLC: Usnic acid, homosekikaic acid, and sekikaic acid.

Distribution: Pantropical (e.g., Kashiwadani & Nash 2004; Stevens 1987; Krog & Swinscow 1976).

Ramalina peruviana is characterized by the shrubby thallus with irregular branches, laminal and marginal soralia, the presence of a continuous and not cracked chondroid tissue, and by the production of homosekikaic and sekikaic acids.

This species could be confused with *R. dendriscoides* Nyl., however, the later has a distinctly cracked chondroid tissue, and has medullar salazinic acid (Kashiwadani & Kalb 1993).

Ramalina solediosa have shrubby thallus with not cracked chondroid tissue, and laminal soralia. However, *R. solediosa* differs from *R. peruviana* mainly by the terete branches, and by the production of medullar salazinic acid (Kashiwadani & Kalb 1993).

Specimens examined: BRAZIL. State of Sergipe, Municipality of Nossa Senhora da Glória, locality of Povoado Retiro 1, Assentamento Nossa Senhora Aparecida, 175 m alt., Caatinga, corticolous, 10°06'13.5"S, 37°25'12.9"W, 19.IX.2013, *E. Gumboski* 4857, 4862, 4863, 4871, 4882, 4887, 4890 (ICN). State of Pernambuco, Municipality of São Vicente Férrer, Mata do Estado, sobre córtex de árvore na margem do açude, corticolous, 16.XI.1998, *W.B. Sanders* 98n16.15D (UFP), Lagoa dos Gatos, limite com Jaqueira, Pedra do Cruzeiro, corticolous, 08°43'23"S, 35°50'19"W, ca. 700 m alt., 24.V.1999, *J.A. Siqueira-Filho & S.B. Neves* 923 (UFP); Municipality of São Vicente Férrer, Mata do Estado, margem de trilha, corticolous, 22.II.1999, *W.B. Sanders* 99222.6 (UFP), Municipality of Caruaru, Estação Experimental da Empresa Pernambucana de Pesquisa Agropecuária, Caatinga, on branches, corticolous, 08.XII.1999, *W.B. Sanders* 99D08.2, 99D08.3 (UFP).

Ramalina solediosa (de Lesd.) Landrón, *The Lichenologist* 25 (1): 25. 1993.

Basionym: *Ramalina dasypoga* var. *solediosa* B. de Lesd., *Rev. Bryol. Lichen.* 7: 59. 1934.

Type: Cuba, Boqueron, Estacion Naval, *B. Hiorami* (FH—lectotype).

MB#358582

Fig. 1D.

Description: Thallus shrubby to subpendulous, corticolous, up to 10 cm long, whitish in field, weakly stramineous in herbaria, growing from a common holdfast, concolorous with the thallus, 3.0 mm in diameter, branching dichotomously anisotomic to irregularly branched, solid branches, mainly terete with irregular thickness in main branches, slightly flattened on main branches near the base, 0.2–0.6 mm in wide, surface smooth; soralia laminal and terminar, mainly orbicular to rarely irregular ellipsoid, 0.1–0.3 mm in diameter, soredia 200–300 µm in diameter, cylindrical branchlets on soralia are common, usually with soredia in apical part; pseudocyphellae laminal, punctiform to short ellipsoid, at surface level, 0.03–0.08 mm wide. Cortex absent, chondroid tissue not cracked, continuous, 40–75 µm thick, algal layer discontinuous, 20–35(–50) µm, medulla white, loose. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+ yellow, UV-. Medulla K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in America (Kashiwadani & Kalb 1993).

Ramalina solediosa is characterized by the shrubby to subpendulous thallus, terete branches, laminal soralia, continuous and not cracked chondroid tissue, and by the production of salazinic acid.

This species could be confused with *R. dendriscooides* by the sorediate branches, and by the production of salazinic acid in medulla. However, *R. dendriscooides* has a distinctly cracked chondroid tissue, added has irregular flat to irregular cylindrical branches (Kashiwadani & Kalb 1993).

The species is similar to *R. peruviana* (see comments under that species).

Specimens examined: BRAZIL. State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, *E. Gumboski* 4759, 4763, 4774, 4780, 4785, 4789 (ICN), 195 m alt., Cerrado, corticolous, 10°45'40.5"S, 37°20'25.2"W, 18.IX.2013, *E. Gumboski* 4796, 4839 (JOI), *E. Gumboski* 4847, 4849 (ICN). State of Pernambuco, Municipality of Poção, Brejo de altitude, pendulous on branches of *Caesalpinia pyramidalis* Tul. (catingueira), corticolous, 27.VI.1998, *S. Ribeiro & E. Pereira s.n.* (UFP 28293); Municipality of São Vicente Férrer, Mata do Estado, sobre córtex de árvore na margem do açude, corticolous, 16.XI.1998, *W.B. Sanders* 98n16.15C, 98n16.6 (UFP).

Ramalina subpollinaria Nyl., Bulletin de la Société Linnéenne de Normandie 4: 125. 1870.

Type: Peru, Callao, *Gaudichaud* (H-NYL 37050—lectotype, Photo!).

MB#403848

Fig. 1E.

Description: Thallus caespitose, corticolous, up to 5 cm long, whitish green in field, stramineous in herbaria, growing from a common holdfast, concolorous with the thallus, 4.0 mm in diameter, branching dichotomously anisotomic to irregularly branched, solid branches, flat to canaliculate, 0.3–1.3(–2.0) mm in wide, surface smooth to slightly foveolate; soralia subterminal to terminar, orbicular to somewhat labriform, 0.3–1.5 mm in diameter, 250–500 µm in diameter; pseudocyphellae marginal, discrete, orbicular, at surface level, 0.05–0.1 mm in diameter. Cortex distinct, hyaline to stramineous, 15–18 µm thick, chondroid tissue cracked, discontinuous, in irregular bundles, 13–50 µm

thick, algal cells dispersed in medulla, medulla white to weakly beige, loose. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+ yellow, UV-. Medulla K+ yellow→red or K+ rose evanescent, C-, KC-, UV-. TLC: (Race 1). Usnic acid and salazinic acid; (Race 2) usnic acid and sekikaic acid.

Distribution: Tropical in America (Nylander 1890; Kashiwadani & Kalb 1993).

Ramalina subpollinaria is characterized by the shrubby thallus with flat to canaliculated branches, cracked chondroid tissue, by the presence of subterminal to terminal soralia, and by the production of salazinic acid or sekikaic acid.

The species is similar to *R. dendriscoides* in have terminal soralia, by the presence of a cracked chondroid tissue, and by the production of salazinic acid. However, *R. subpollinaria* have clearly flat branches, while *R. dendriscoides* have the main branches irregularly flat to irregularly cylindrical, and the secondary branches are mainly terete (Kashiwadani 1987; Kashiwadani & Kalb 1993).

Specimens examined: BRAZIL. State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, E. Gumboski, 4782 (ICN), 220 m alt., 10°44'50.8"S, 37°20'29.6"W, 18.IX.2013, E. Gumboski, 4844, 4850 (ICN); Municipality of Nossa Senhora da Glória, locality of Povoado Retiro 1, Assentamento Nossa Senhora Aparecida, 175 m alt., Caatinga, corticolous, 10°06'13.5"S, 37°25'12.9"W, 19.IX.2013, E. Gumboski, 4853, 4866, 4867, 4873, 4877, 4881, 4889 (ICN). State of Pernambuco, Municipality of São Vicente Férrer, Mata do Estado, sobre córtex de árvore na margem do açude, corticolous, 16.XI.1998, W.B. Sanders, 98n16.15E (UFP), Municipality of Caruaru, Estação Experimental da Empresa Pernambucana de Pesquisa Agropecuária, Caatinga, em ramos, corticolous, 08.XII.1999, W.B. Sanders, 99D08.4B, 99D08.4A (UFP).

***Ramalina usnea* (L.) R. Howe**, *The Bryologist* 17: 81, pl. 12, figs 1-2. 1914.

Basionym: *Lichen usnea* L., *Mantissa* 1: 131-132 (1767).

Type: Martinica (LINN 1273-278—lectotype, Photo!).

MB#403866

Fig. 1F.

Description: Thallus pendulous, corticolous, up to 20 cm long, stramineous in herbaria, growing from a narrow holdfast, concolours with the thallus, up to 3.0 mm in diameter; branching irregularly to dichotomously (anisotomic), solid branches, branches mainly flattened, but often with secondary branches irregularly cylindrical to subterete, often contorted, 0.1–2.0 mm wide, surface mainly smooth to weakly striate; symbiotic propagules absent; pseudocyphellae linear, marginal and laminal, flat to weakly depressed, rarely sharply depressed, 0.05–0.1 mm wide. Cortex distinct, hyaline to dirty yellow, 08–12 µm thick, chondroid tissue distinctly cracked, continuous, 50–150 µm thick, algal layer discontinuous, 20–30 µm thick, medulla white, loose. Apothecia common, rounded, marginal or laminal, up to 1.2 mm diameter, flat, becoming convex with age, anfithecium smooth, without pseudocyphellae, margin entire to rarely cropped, concolorous with the thallus, disc beige weakly pruinous; epihymenium darkened, 8–12 µm thick, hymenium hyaline, 40–55 µm thick, subhymenium hyaline, 20–25 µm thick, hypothecium hyaline, continuous, 20–55 µm thick, with agglomerates of algae dispersed in medulla under the hypothecium; ascospores 1-septate, fusiform, 18–22(–25) × 4–7 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-. Medulla: K+ pinkish, C-, KC-. TLC: usnic acid, homosekikaic and sekikaic acid.

Distribution: Tropical in America (Kashiwadani & Nash 2004).

Ramalina usnea is characterized by the pendulous thallus with flat and usually contorted branches, by the cracked chondroid tissue, absence of symbiotic propagules, and by the production of homosekikaic and sekikaic acids.

Comparing with the Brazilian *Ramalina* species, *R. usnea* could be confused with *R. anceps* Nyl. by the morphological and anatomically similar thallus. However, *R. anceps* present marginal pseudocyphellae, rarely contorted branches, and produces salazinic acids. While *R. usnea* present laminal pseudocyphellae, usually contorted branches, and produces divaricatic and/or homosekikaic and sekikaic acids (Kashiwadani & Kalb 1993; Gumboski *et al.* A).

Ramalina sprengelii Krog & Swinscow and *R. kashiwadanii* Gumboski, Eliasaro & R. M. Silveira also have pendulous thallus without soredia, however, both species have a not cracked chondroid tissue and don't produces medullar acids (Gumboski *et al.*, A).

Specimens examined. BRAZIL. State of Alagoas, Municipality of Água Branca, Calderão do Zamba, Serra do Carcuman, 14.IX.1948, *H.C.Monteiro Neto s.n.* (RB 220162). State of Pernambuco, Alagoinha, Caatinga, I.1998, *E. Pereira s.n.* (UFP 36.432).

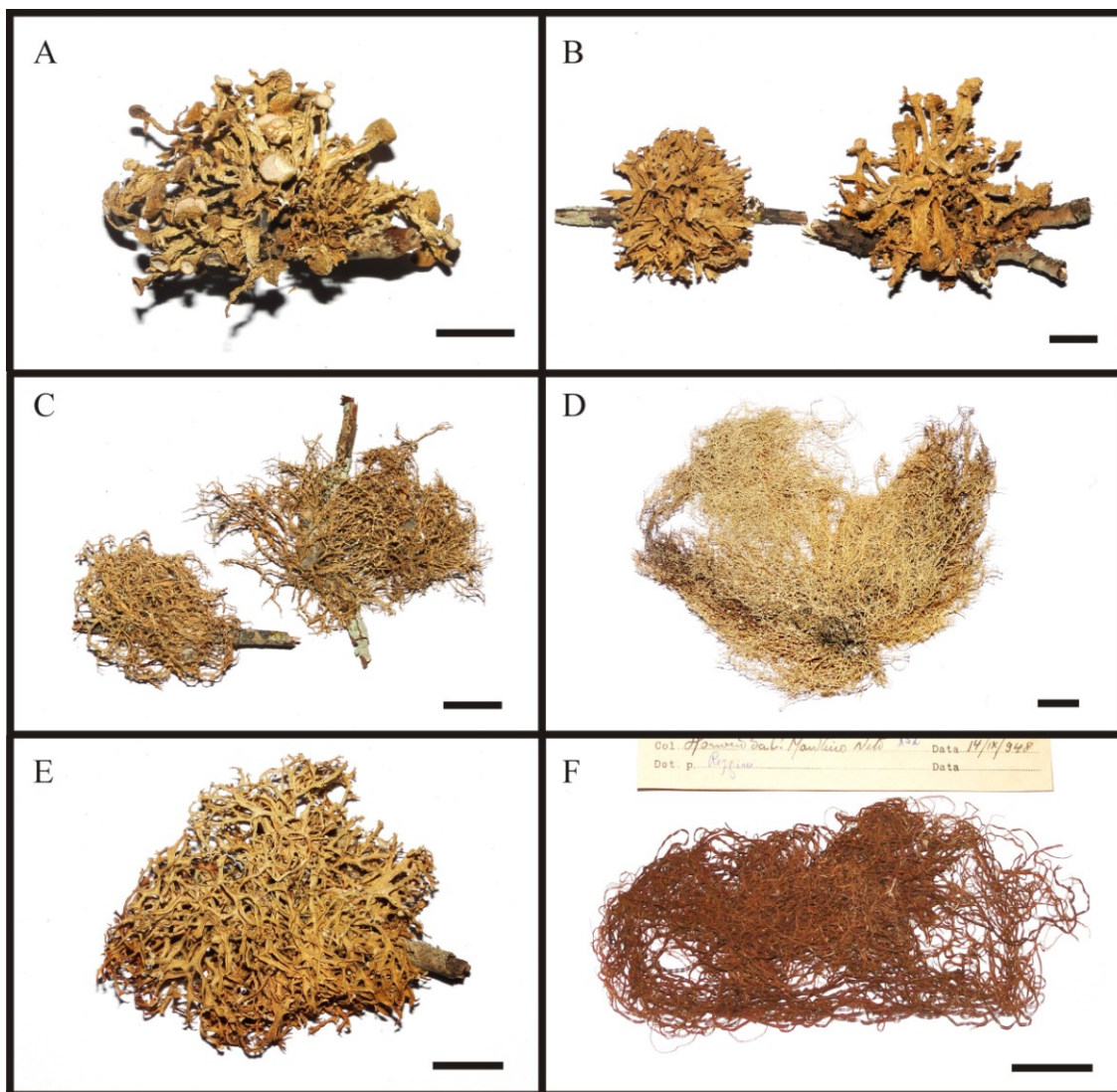


Fig. 1. A–F. *Ramalina* species from Northeastern Brazil. A – *R. aspera* (E. Gumboski 4854); B – *R. complanata* (E. Gumboski 4865); C – *R. peruviana* (E. Gumboski 4878); D – *R. soresdiosa* (E. Gumboski 4759); E – *R. subpollinaria* (E. Gumboski 4853); F – *R. usnea* (RB 220162).

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4.5. Manuscrito V

Gumboski, E.L.; Eliasaro, S. & Borges da Silveira, R.M. New records of species of *Ramalina* Ach. (Ramalinaceae, lichenized Ascomycota) from Southern Brazil.

Mycotaxon: a ser submetido.

New records of species of *Ramalina* Ach. (Ramalinaceae, lichenized Ascomycota) from Southern Brazil

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Abstract. The knowledge about mycobiota occurring in the Southern Brazil is quite dispersed, and many environments remain with a lack of information about the species that compose it. The aim of this study is to contribute to the knowledge of the species of *Ramalina* present in Southern Brazil. The specimens were collected in different environments from the three states of Southern Brazil: Paraná, Santa Catarina, and Rio Grande do Sul. Added were analyzed collections from six herbaria. Thirteen species of *Ramalina* were found, of which ten are new records to State of Santa Catarina, five to State of Rio Grande do Sul, and three to State of Paraná. *Ramalina camptospora* is the only new record to the three states. Seven species were found only in plateau regions, and five species were found only in coastal environments. Descriptions, comments and pictures of each species are given.

Keywords. Fungi, geographic distribution, lichen, South Region, taxonomy.

Introduction

The lichen mycobiota in Brazil is highly diverse, but unfortunately the information about the distribution of many groups is dispersed and many environments remain with a great lack of information about the species that compose it (Marcelli 1996, 1998; Marcelli *et al.* 1998).

The knowledge about the lichenized mycobiota occurring in the Southern Brazil began to be organized mainly with the checklist produced by Spielmann (2006) to the State of Rio Grande do Sul, and later by Gumboski & Eliasaro (2011) to the State of Santa Catarina. The checklist of State of Paraná is being produced (Eliasaro & Gumboski, in prep.), but it is known that the Rio Grande do Sul has almost three times more species of lichenized fungi [912 species according to Spielmann (2006)] than the states of Paraná and Santa Catarina.

The information about the species of *Ramalina* occurring in Southern Brazil also has some gaps in the knowledge. The region has recorded 19 species of the genus, 15 of which are known to the State of Rio Grande do Sul (Spielmann 2006; Gumboski *et al.* 2014, Gumboski *et al.* A, Gumboski *et al.* B), 13 to State of Paraná (Eliasaro & Gumboski, in prep.), and nine to State of Santa Catarina (Gumboski & Eliasaro 2011; Gumboski *et al.* B).

The aim of this study is to contribute to the knowledge of the species of *Ramalina* present in Southern Brazil.

Material and Methods

Specimens were collected in different environments from the three states of Southern Brazil: Paraná, Santa Catarina, and Rio Grande do Sul; an area of 576 774.31 km². The Southern Brazil is mainly dominated by the Atlantic Forest, and has environments such as grasslands, 'restinga' vegetation, mangroves, high altitude fields, Araucaria forest, deciduous forest and savanna (IBGE 1992). Collections from the herbaria DUKE, G, FLOR, H, HAS, ICN, JOI, MPUC, RB and W were analyzed.

The specimens examined were collected using a knife and dried at room temperature. Specimens were examined using standard techniques with stereoscopic microscope (20–40×) and light microscope (400–1000×). Freehand sections of the thalli and apothecia were mounted in water. The chemical analysis: spot tests, observation under UV light, and thin layer chromatography (TLC) using solvent C; were conducted according to Huneck & Yoshimura (1996) and Orange *et al.* (2001).

Results and Discussion

Thirteen species of *Ramalina* were found, of which ten are new records to State of Santa Catarina [*R. anceps* Nyl., *R. asahinae* W.L. Culb. & C.F. Culb., *R. aspera*

Räsänen, *R. camptospora* Nyl., *R. dendroides* (Nyl.) Nyl., *R. exiguella* Stirt., *R. puiggarii* Müll. Arg., *R. pusiola* Müll. Arg., *R. sprengelii* Krog & Swinscow, and *R. subpollinaria* Nyl.], five to State of Rio Grande do Sul [*R. anceps*, *R. camptospora*, *R. cochlearis* Zahlbr., *R. dendriscooides* Nyl., and *R. pusiola*], and three to State of Paraná [*R. camptospora*, *R. canalicularis* (Nyl.) Kashiw., and *R. dendroides*]. *Ramalina camptospora* is the only new record to the three states, and was found mainly in altitudes between 620 to 1260 m near the ‘Serra do Mar’ mountain range.

Ramalina asahinae, *R. camptospora*, *R. cochlearis*, *R. dendriscooides*, *R. puiggarii*, *R. pusiola*, and *R. sprengelii* were found only in plateau regions, all growing on trunks and/or twigs. Whiles *R. aspera*, *R. canalicularis*, *R. dendroides*, *R. exiguella*, and *R. subpollinaria* were found only in coastal environments, growing on trunks, twigs or on rocks.

Four species [*R. asahinae*, *R. canalicularis*, *R. cochlearis*, and *R. puiggarii*] have a rare occurrence in Southern Brazil. Only few specimens of each species were found, what turns difficult to interpret the species preferences of habitat or environment.

Ramalina anceps Nyl., Syn. meth. lich. 1(2): 290. 1860.

Type: Guadeloupe, *L'Herminier et al.* (PC–holotype; H-NYL 37018!—syntype).

MB#357046

Fig. 1A

Description: Thallus pendulous, corticolous or saxicolous, up to 10 cm long, greenish in field, dark stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching dichotomously, almost isotomic; solid branches, subterete to almost flat, 0.2–0.4 mm wide, sometimes weakly contorted, surface smooth; symbiotic propagules absent; pseudocyphellae mainly marginal, rarely laminal, linear, flat, 0.04–0.07(–0.15) wide. Cortex distinct, hyaline to yellowish, 12–20 µm thick, chondroid tissue distinctly cracked, continuous, 50–150 µm thick, algal cells not forming a distinct layer, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry: Cortex: K-, C-, KC-, UV-. Medulla: K+ yellow→red, C-, KC-, UV-. TLC: usnic acid and norstictic acid.

Distribution: Tropical in Central and South America (Nylander 1890; Kashiwadani & Kalb 1993; Aptroot & Bungartz 2007).

Ramalina anceps is characterized by the pendulous thallus with flat branches, the pseudocyphellae are marginal and linear, have a cracked chondroid tissue, and produces salazinic acid.

This species is similar to *R. usnea* (L.) R. Howe by the pendulous thallus with flat branches, distinctly cracked chondroid tissue, and anatomical characteristics of apothecia. However, *R. anceps* have mainly marginal pseudocyphellae and produces norstictic acid, while *R. usnea* have mainly laminal pseudocyphellae and don't produce norstictic acid (Kashiwadani & Kalb 1993; Gumboski *et al.* A).

Ramalina anceps differ from *R. kashiwadanii* Gumboski *et al.* and *R. sprengelii* by the distinctly cracked chondroid tissue, and by the presence of norstictic acid in the medulla (Kashiwadani & Kalb 1993; Gumboski *et al.* B).

Specimens examined: BRAZIL. State of Rio Grande do Sul: Municipality of Camará do Sul, Itaimbezinho, near the hotel, corticolous, 20.04.1982, *Osorio & M. Fleig T34* (ICN); Municipality of São Francisco de Paula, Loteamento Colina de São Francisco, on *Araucaria angustifolia*, 19.IV.1982, *Osorio & M. Fleig SF2* (ICN). State of Santa Catarina, Municipality of São Francisco do Sul, Morro da Enseada, rocky shore, saxicolous, 13.V.2008, *E. Gumboski 364* (JOI).

Ramalina asahinae W.L. Culb. & C.F. Culb., *Journal of Japanese Botany* 51 (12): 374. 1976.

Type: Mexico, Chiapas: 11 km west of San Cristobal las Casas, on *Quercus* sp., alt. 2154 m (DUKE!—holotype).

MB#343493

Fig. 1B

Description: Thallus shrubby, corticolous, up to 5 cm long, whitish green in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 2.0 mm in diameter; branching dichotomously anisotomic, rarely irregular, branches solid, flat to irregular flat, usually canaliculate in old branches, 0.3–1.3 mm wide, surface smooth, somewhat striate due the presence of ellipsoid pseudocyphellae; soralia mainly marginal, infrequent laminal, ellipsoid to labriform, 0.2–1.5 × 0.1–0.5 mm, soredia 30–100 µm in diameter; pseudocyphellae mainly laminal, ellipsoid to short linear, at surface level to depressed, 0.1–0.3 × 0.05–0.10 mm.

Cortex absent, chondroid tissue not cracked, continuous, 10–50 µm thick, algal cells dispersed on medulla, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry: Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic and boninic acids.

Distribution: Tropical America (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Ramalina asahinae is recognized by the shrubby thallus with mainly flat branches, mainly marginal soralia, not cracked chondroid tissue, and by the production of boninic acid.

This rare species in Southern Brazil is similar to *R. peruviana* Ach. in shrubby thallus, soredia presence, and not cracked chondroid tissue. However, *R. peruviana* have irregular flat to irregular terete branches, usually with striate surface, laminal soralia, and produces homosekikaic and sekikaic acids (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Specimens examined: BRAZIL. State of Santa Catarina, Municipality of Mafra, margins on road BR-280, corticolous, 23.IX.2012, *E. Gumboski 4048* (JOI).

Ramalina aspera Räsänen, Ann. Bot. Soc. Zool. Bot. Fenn. Vanamo 20: 5. 1994.

Type: Paraguay, Chaco, Rio Verde, Villa Hayes, *W. G. Herter 3* (H!—holotype).

MB#369881

Fig. 1C

Description: Thallus caespitose, corticolous, up to 5 cm long, stramineous whitish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 5.0 mm in diameter; branching irregularly, rarely almost palmate, solid branches, mainly flat, dorsiventral, 0.5–3.5 mm wide, surface rugose, reticulately to irregularly ridged; symbiotic propagules absent; pseudocyphellae laminal and marginal, raised, tuberculate, 0.1–0.3 mm wide, 0.05–0.15 mm tall. Cortex distinct, hyaline to yellowish, 10–20 µm thick, chondroid tissue not cracked, discontinuous, almost in irregular bundles, 50–100 µm thick, algal cells dispersed in medulla, medulla white, loose. Apothecia rounded, subterminal to terminal, up to 4.0 mm in diameter, concave to flat, amphithecia with tuberculate pseudocyphellae, margin usually indented,

concolorous with the thallus, disc whitish to beige, weakly pruinous; epihymenium greenish, 15–20 µm thick, hymenium hyaline to grayish, 20–25 µm thick, hipohymenium yellowish, 20–35 µm thick, with chondroid tissue under the hypothecium, 10–70 µm thick, algal layer continuous, 40–70 µm thick; ascospores 1-septate, straight to slightly curved, 11–15 × 3–5 µm. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC-, UV-. Medulla K-, C-, KC-, UV-. TLC: Usnic acid and divaricatic acid.

Distribution: Tropical in America and Africa (Krog & Swinscow 1976; Kashiwadani & Nash 2004).

Ramalina aspera is characterized mainly by the caespitose thallus with flat branches, the tuberculate pseudocyphellae, the not cracked chondroid tissue, and by the production of divaricatic acid.

In Brazil, this species could be confused with *R. complanata* (Sw.) Ach. by the morphologically similar thallus and habitat preferences. However, *R. complanata* have a flat to almost canaliculate branches with a cracked chondroid tissue, and produces salazinic acid and/or divaricatic acid (Kashiwadani & Kalb 1993).

Selected specimens examined: BRAZIL. State of Santa Catarina, Municipality of Florianópolis, Praia dos Ingleses, 21.IV.1983, *I.C. Pereira s.n.* (MPUC 3899 B), na borda do mangue do Itacorubi na Ilha de SC, corticolous, 08.IV.1989, *Jeane I. Dal Bó 30* (FLOR), Ilha do Francês, corticolous, 08.V.1993, *D. Farias s.n.* (MPUC 7110), Praia dos Ingleses, 21.IV.1983, *I.C. Pereira s.n.* (MPUC 3899 B), Praia do Forte de São José da Ponta Grossa, on trunk of mango, corticolous, 22.IV.1983, *I.C. Pereira s.n.* (MPUC 3909); Municipality of Imbituba, on dunes, corticolous, 10. XI.2011, *E. Gumboski; S. Eliasaro & F. Beilke 3029* (JOI); Municipality of São Francisco do Sul, praia de Capri, urban area, corticolous, 01.VIII.2008, *E. Gumboski 1145* (JOI).

Ramalina camptospora Nyl., Bulletin de la Société Linnéenne de Normandie 4: 120. 1870.

Type: Cuba, *C. Wright* (H-NYL 37248!—holotype)

MB#403670

Fig. 1D

Description: Thallus shrubby, corticolous, up to 7 cm long, greenish to weakly stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 4.0 mm in diameter; branching dichotomously to irregular, usually with tiny branchlets on margins, up to 2.0 mm tall, c. 0.1 mm wide, possibly generating new branches, branches solid, flat, usually weakly canaliculate in main branches, 0.3–1.2 mm wide, secondary branches 0.1–0.4 mm wide, surface smooth to rarely rugose; soralia marginal, ellipsoid to linear, scattered, depressed, often very scarcely formed, $0.5\text{--}1.0\text{--}(2.0) \times 0.1\text{--}0.2$ mm, soredia 20–50 μm in diameter; pseudocyphellae marginal, linear, depressed. Cortex discrete, hyaline, 6–10 μm , chondroid tissue not cracked, continuous, 100–330 μm thick, algal cells dispersed on medulla, medulla white, compact. Apothecia rounded, marginal, subterminal, up to 5.0 mm in diameter, flat, amphithecia smooth, without pseudocyphellae, margin entire, concolorous with the thallus, disc whitish to beige, weakly pruinous; epihymenium blackish, c. 10 μm thick, hymenium hyaline, 35–45 μm thick, hipohymenium yellowish, 20–35 μm thick, with chondroid tissue under de hypothecium, 25–50 μm thick, algal layer continuous, 20–50 μm thick, same algal cells dispersed in medulla; ascospores 1-septate, curved to sigmoid, $11\text{--}14 \times 4\text{--}7$ μm . Pycnidia not found.

Chemistry: Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic acid.

Distribution: Tropical in America (Kashiwadani & Kalb 1993).

Ramalina camptospora is characterized by the shrubby thallus with flat branches, marginal soralia, not cracked chondroid tissue, ascospores curved to sigmoid, and absence of medullar acids. This combination of features makes *R. camptospora* a distinct Brazilian species, once the other sorediate species have different patterns of soralia production and usually produces some medullar acids, as *R. asahinae* [boninic acid], *R. peruviana* [homosekikaic and sekikaic acids], and *R. subpollinaria* Nyl. [salazinic acid] (Kashiwadani & Kalb 1993).

Selected specimens examined: BRAZIL. State of Paraná: Municipality of Piraquara, Morro do Canal, Mata Atlântica Montana, corticolous, 09.VI.2009, *E. Gumboski 1537* (UPCB); Municipality of Ponta Grossa, Passo dos Pupos, corticolous, 3.VII.2003, *C. G. Donha 779* (UPCB); Municipality of Campina Grande do Sul, Rio Pardinho, FOD montana (620 m.s.n.m.), corticolous, 29.XI.2003, *C. G. Donha 1585* (UPCB); Municipality of Morretes, Parque Estadual Marumbi, Morro Facãozinho, aprox. 1100

m.s.n.m., 17.I.2000, *M. Borgo & S. DalaRosa s.n.* (UPCB); Municipality of Piraquara, Morro do Canal, c. 1200 m alt., 8.V.2002, *S. Eliasaro 2210* (UPCB), 29.V.2002, *S. Eliasaro 2246, 2337* (UPCB); Municipality of Guaratuba, Morro dos Perdidos, c. 1260 m alt., corticolous, 31.VIII.2002, *S. Eliasaro 2443, 2458* (UPCB). State of Rio Grande do Sul: Municipality of Cambará do Sul, Fortaleza dos Aparados, corticolous, 29.X.1983, *M. Fleig 2122, 2198* (ICN); Municipality of São Francisco de Paula, Colinas de São Francisco, open forest, corticolous, 22.VII.1981, *M. Fleig 1371, 1372* (ICN), Reserva Pro-Mata, corticolous, 18.IV.2012, *E. Gumboski 3543, 3544, 3545* (ICN). State of Santa Catarina: Municipality of Campo Alegre, nearly 7 Km south of central falls, 11.IX.2011, *E. Gumboski 2577, 2578* (JOI), Campos do Quiriri, Campos de Altitude, corticolous, 17.XI.2012, *E. Gumboski 4134, 4135, 4137, 4176, 4202* (JOI); Municipality of Garuva, Monte Crista, 15.V.2010, *E. Gumboski & F. Beilke 1941, 1988* (JOD); Municipality of Santo Amaro da Imperatriz, Pico Tabuleiro, 13.VIII.2011, *E. Gumboski et al. 2370, 2371* (JOI).

Ramalina canalicularis (Nyl.) Kashiw., Lichen Flora of the Greater Sonoran Desert Region 2: 444. 2004.

Basionym: *Ramalina denticulata* var. *canalicularis* Nyl., Bull. Soc. linn. Normandie, sér. 2 4(2): 126. 1870.

Type: Mexico, Orizaba, *F. Müller* (H-NYL 37063! —holotype).

MB#365899

Fig. 1E

Description: Thallus caespitose, corticolous, up to 7(–11) cm long, whitish green in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching mainly dichotomously anisotomic, rarely irregular, solid branches, flat, canaliculate, 0.5–1.5 mm wide, surface smooth, infrequent ridged; symbiotic propagules absent; pseudocyphellae mainly marginal to laminal, raised, tuberculate, 0.05–0.1 mm wide, 0.05–0.1 mm tall. Cortex distinct, hyaline, 18–22 µm thick, chondroid tissue cracked, discontinuous, 50–200 µm thick, algal cells dispersed in medulla, medulla white, compact. Apothecia rounded, marginal, subterminal to terminal, up to 3.0 mm in diameter, concave to mainly flat, amphithecia with tuberculate pseudocyphellae, margin entire, concolorous with the thallus, disc whitish to beige, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline,

50–55 µm thick, hypohyemium yellowish, 20–25 µm thick, with discontinuous chondroid tissue under de hypothecium, 10–25 µm thick, algal dispersed on medulla; ascospores 1-septate, straight to slightly curved, 13–20 × 3–5 µm. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+yellow, UV-. Medulla K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in America (Kashiwadani & Nash 2004).

Ramalina canalicularis is characterized by the caespitose thallus with distinct canaliculated branches, the presence of tuberculate pseudocyphellae, cracked chondroid tissue, and by the production of salazinic acid.

Ramalina complanata is similar with *R. canalicularis* in anatomical and chemistry features. However, *R. complanata* usually have a flat to irregular flat branches, almost but not distinct canaliculated branches, while *R. canalicularis* have a distinct canaliculated branches since the base (Kashiwadani & Kalb 1993).

Specimens examined: State of Paraná, Municipality of Morretes, Reserva IAPAR, corticolous, III.2014, *D. Imig s.n.* (JOI).

Ramalina cochlearis Zahlbr., Bull. Herb. Boissier, 2 sér. 5: 542. 1905.

Type: Brazil, ad ramos arborum in sylvis ad Cachoeira do Campo, *L. Damazio 1449* (W!—holotype; RB! —isotype).

MB#403680

Fig. 1F

Description: Thallus caespitose, corticolous, up to 4.0 cm long, whitish green in field, stramineous in herbaria, growing from a narrow holdfast, up to 4.0 mm in diameter; branching irregular, solid branches, flat to weakly canaliculated, canaliculated mainly near the base, 0.5–3.0 mm wide, surface smooth; soralia subterminal to terminal, helmet-shaped, 0.35–2.50 mm wide, soredia 270–500 µm in diameter; pseudocyphellae rare, ellipsoid, at level surface, 0.05–0.20 × 0.05–0.10 mm. Cortex hyaline, 10–13 µm thick, chondroid tissue not cracked, discontinuous, 20–60 µm thick, algal cells dispersed in medulla, medulla white, loose. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+yellow, UV-. Medulla K-, C-, KC-, UV-. TLC: Usnic acid and boninic acid.

Distribution: Tropical in America (Kashiwadani & Nash 2004).

Among the Brazilian species, *Ramalina cochlearis* is the unique that produces soredia originating from a helmet-shaped soralia. Other species with subterminal to terminal soralia, as *R. dendriscoides* Nyl. and *R. subpollinaria*, never produce soralia with helmet-shaped format (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Specimens examined: BRAZIL. State of Rio Grande do Sul: Municipality of Camará do Sul, Parque Nacional Aparados da Serra, 00.10.1970, *J. Goergen s.n.* (ICN 73540); Municipality of Maquiné, *N. M. Koch 326* (HAS 49138).

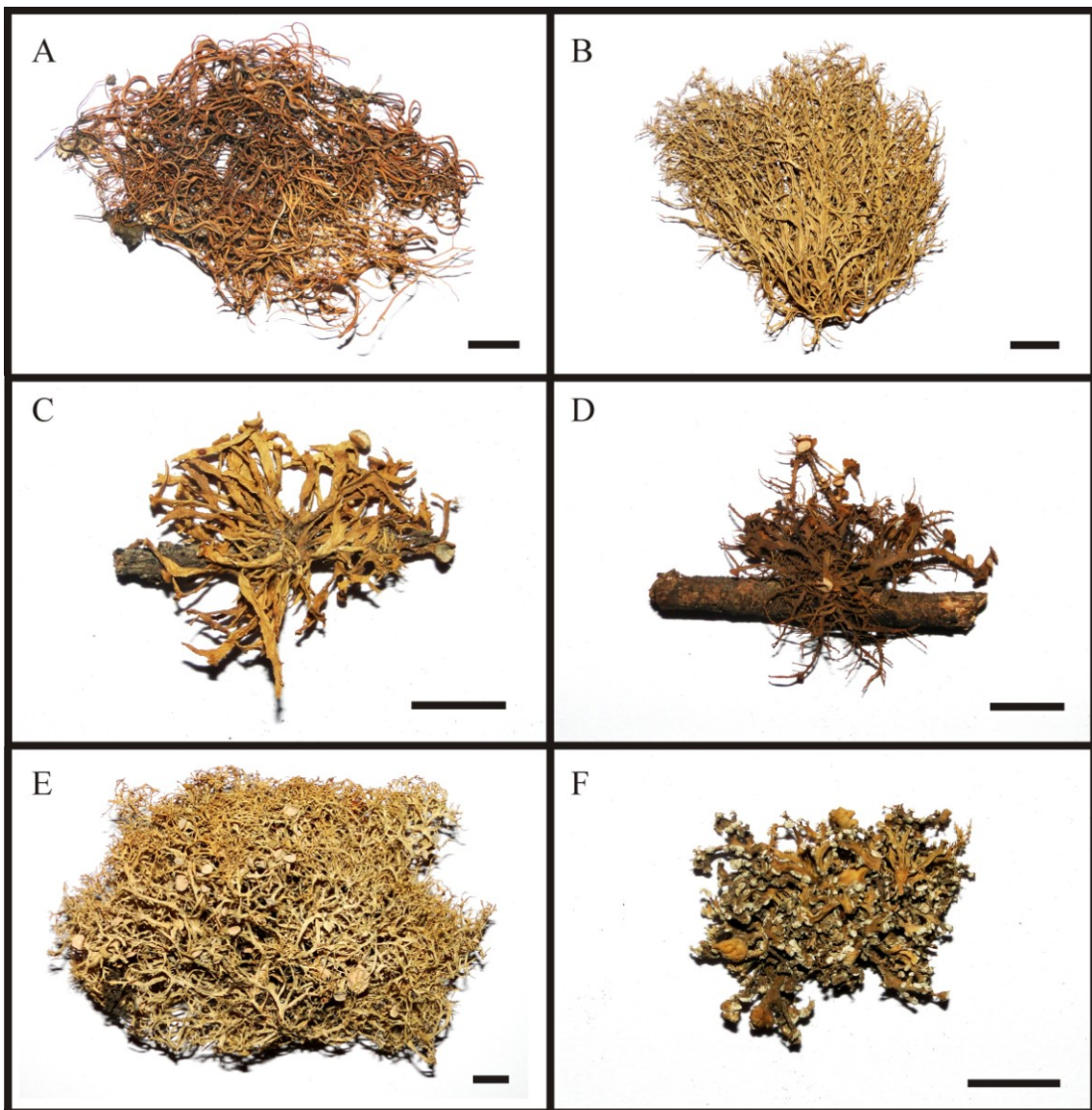


Fig. 1. A–F. A – *R. anceps* (*E. Gumboski 364*); B – *R. asahinae* (*E. Gumboski 4048*); C – *R. aspera* (*E. Gumboski et al. 3029*); D – *R. camptospora* (*E. Gumboski 4202*); E – *R. canalicularis* (*D. Imig s.n.*); F – *R. cochlearis* (HAS 49138).

Ramalina dendriscoides Nyl., Flora (Regensburg) 59: 412. 1876.

Type: Cuba, *C. Wright*, Lich. Cub. Ser. 2, 738 (H-NYL 37025— lectotype; FH, G!— isotypes).

MB#403700

Description: Thallus shrubby, corticolous, up to 7 cm high, greenish to whitish in field, stramineous in herbaria, growing from a restricted or common holdfast, concolorous with the thallus to blackish, up to 5.0 mm in diameter; branching dichotomously anisotomic to irregularly, solid branches, main branches irregularly flat to irregularly cylindrical, 0.5–1.0 mm wide, secondary branches mainly terete, 0.2–0.5 mm wide, surface smooth, slightly striate, rarely with depressions remembering some canaliculated branches; soralia mainly terminal in secondary branches, soredia 20–50 μm in diameter; pseudocyphellae orbicular to ellipsoid, at level surface, 0.1–0.5 \times 0.05–0.1 mm, scattered. Cortex hyaline, 15–20 μm thick, chondroid tissue cracked, discontinuous, 40–80 μm , algal cells dispersed in medulla, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC-, UV-. Medulla K+ yellow \rightarrow red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in America and Africa (Kashiwadani & Kalb 1993; Swinscow & Krog 1988).

Ramalina dendriscoides is characterized by the shrubby thallus with mainly terminal soralia in secondary branches, by the cracked chondroid tissue, and by the production of medullar salazinic acid.

The species is similar to *R. peruviana* and *R. solediosa* (B. de Lesd.) Landrón by the shrubby thallus and sorediate branches. However, both *R. peruviana* as *R. solediosa* have not cracked chondroid tissue and have laminal soralia. Added, *R. peruviana* produces homosekikaic and sekikaic acids, and *R. solediosa* have mainly terete branches (e.g., Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004; Stevens 1987).

Specimens examined: BRAZIL. State of Rio Grande do Sul: Municipality of Barracão, Espigão Alto, open forest, wetland, corticolous, 30.10.1988, *M. Fleig* 3719 (ICN), near a tributary of Uruguay river, open forest, corticolous, 09.10.1992, *M. Fleig* 4629, 4599, 4607 (ICN).

Ramalina dendroides (Nyl.) Nyl., Flora, (Regensburg) 26: 412. 1876.

Basionym: *Ramalina rigida* f. *dendroides* Nyl., Bull. Soc. Linn. Normandie 4: 112. 1870.

Type: Martinique Island, *Husnot 460* (H-NYL 37028!—holotype).

MB#403701

Fig. 2A

Description: Thallus pendulous, corticolous or saxicolous, up to 10 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 4.0 mm in diameter; branching dichotomously, solid branches, flat, 0.3–2.0 mm wide, surface smooth; soralia mainly marginal, orbicular to elongate, erumpent, 0.3–1.0(–2.0) × 0.15–0.30 mm, soredia 300–500 µm in diameter, in old branches the soralia can developed on lamina, usually near the basal parts; pseudocyphellae marginal and laminal, continuous and linear on margins, depressed, orbicular to ellipsoid on lamina, at level surface, mainly in the basal part of the main branches, 0.1–0.6 × 0.1–0.2 mm. Cortex hyaline, 10–16 µm thick, chondroid tissue cracked, continuous, 40–140 µm thick, algal layer continuous, 20–50 µm thick, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC-, UV-. Medulla K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in Central and South America (Kashiwadani & Kalb 1993).

Ramalina dendroides is characterized by the pendulous with flat branches, by the mainly marginal soralia, distinct cracked chondroid tissue, and by the production of salazinic acid.

This species differ from the other pendulous *Ramalina* [*R. anceps*, *R. kashiwadanii*, *R. sprengelii*, and *R. usnea*] mainly by the presence of soralia on margins (Kashiwadani & Kalb 1993; Gumboski *et al.* B).

Specimens examined: BRAZIL. State of Santa Catarina: Municipality of São Francisco do Sul, Morro da Enseada, rocky shore, saxicolous, 05/05/08, *E. Gumboski 243* (JOI), *ibid.*, 07/05/08, *E. Gumboski 346* (JOI), *ibid.*, 19/06/08, *E. Gumboski 516, 527, 637* (JOI), *ibid.*, Morro da Enseada, east, 22/09/11, *E. Gumboski 2641, 2647, 2722* (JOI). State of Paraná, Municipality of Guaraqueçaba, Reserva Natural de Itaqui - manguezal, 17.II.2004, *R. Reis 274, 298a* (UPCB), Parque Nacional do Superagui, 9.IV.2003, S.

Eliasaro & C. G. Donha 2634 (UPCB), Municipality of Paranaguá, Ilha do Mel, corticolous, 27.VIII.2009, *S. Eliasaro & E. Gumboski 3169* (UPCB).

Ramalina exiguella Stirt., Transactions and Proceedings of the Royal Society of Victoria 17: 68. 1881.

Type: Queensland, Brisbane, *I. M. Bailey 91* (GLAM— holotype).

MB#403713

Fig. 2B

Description: Thallus shrubby to subpendulous, corticolous, up to 2.0 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 2.0 mm in diameter; branching dichotomously anisotomic, solid branches, subterete to angular, 0.15–0.50 mm wide, surface smooth to weakly furrowed; symbiotic propagules absent; pseudocyphellae laminal, linear, at level surface, 0.5–1.5 × 0.05–0.10 mm. Cortex absent, chondroid tissue not cracked, continuous, 30–100 µm thick, algal layer continuous, 30–50(–70) µm thick, medulla beige, compact. Apothecia rounded, laminal, up to 1.5 mm in diameter, geniculate branches, concave to mainly flat, amphithecia smooth, margin entire, orange to stramineous, disc whitish to beige, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline, 40–50 µm thick, hipohymenium yellowish, 20–25 µm thick, with continuous chondroid tissue under de hypothecium, 13–25 µm thick, algal layer discontinuous, 30–80 µm thick; ascospores 1-septate, straight, 13–15 × 7–8 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution: Tropical in Central and South America, Africa, and Australia (Kashiwadani & Kalb 1993; Krog & Swinscow 1976; Stevens 1987).

Ramalina exiguella is characterized by the shrubby thallus with subterete to angular branches, absence of soralia, not cracked chondroid tissue, and without production of medullar acids.

This coastal species is morphologically similar with *R. gracilis* (Pers.) Nyl. and both produces usnic acid only. However, *R. gracilis* have a distinct cracked chondroid tissue, medulla whitish and loose, while *R. exiguella* have a not cracked chondroid tissue, and have medulla beige and compact (Kashiwadani & Kalb 1993; Krog & Swinscow 1976; Stevens 1987).

Specimens examined: BRAZIL. State of Santa Catarina, Municipality of Joinville, Bom Retiro, Univille, urban area, corticolous, on palm tree (*Archontophoenix cunninghamii*), 26°15'05", 48°51'22", 07.XII.2011, *E. Gumboski, J. C. S. Silva & K. Z. Ferreira 3333* (JOI); Municipality of Balneário Gaivota, near the beach, coastal, corticolous, on palm tree, 30.IX.2012, *E. Gumboski 4081* (JOI), on wood, 30.IX.2012, *E. Gumboski 4084, 4085* (JOI).

Ramalina puiggarii Müll. Arg., *Flora*, (Regensburg) 64: 83. 1881.

Type: Brazil, Crescit prope Apiahi, March 1880, *J.-J. Puiggari* (G!— holotype).

MB#403808

Fig. 2C

Description: Thallus shrubby, corticolous, up to 4.0 cm long, greenish to whitish in field, weakly stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 2.0 mm in diameter; branching dichotomously anisotomic, rarely irregular, solid branches, flat, canaliculate, 0.4–1.0 mm wide, surface smooth to weakly furrowed; symbiotic propagules absent; pseudocyphellae marginal, discrete, linear, at level surface, up to 0.05 mm wide. Cortex absent, chondroid tissue not cracked, continuous, 30–90 µm thick, algal cells dispersed in medulla, medulla white, loose. Apothecia rounded, submarginal, up to 1.7 mm in diameter, geniculate branches, concave to mainly flat, amphithecia smooth, slightly furrowed, margin entire, orange to stramineous, disc beige, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline, 50–65 µm thick, hipohymenium yellowish, 50–60 µm thick, with continuous chondroid tissue under de hypothecium, 20–50 µm thick, algal cells randomly intermixed with chondroid tissue and medulla; ascospores 1-septate, fusiform, straight, 18–25 × 4–6 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution: Tropical in Central and South America (Kashiwadani & Kalb 1993).

Ramalina puiggarii is characterized by the shrubby thallus with canaliculated branches, absence of symbiotic propagules, not cracked chondroid tissue, and by the long-fusiform ascospores with 18–25 µm long.

Ramalina celastri resemble *R. puiggarii* mainly by the branches without soredia, not cracked chondroid tissue, and absence of medullary acids. However, *R. celastri* have

abundant pseudocyphellae on lamina and shorter ascospores with 12–16 µm long, while *R. puiggarii* have discrete marginal pseudocyphellae and longer ascospores with 18–25 µm long (Kashiwadani & Kalb 1993).

Specimens examined: BRAZIL. State of Santa Catarina, Municipality of Campo Alegre, Campos do Quiriri, Campos de Altitude, corticolous, 17.XI.2012, *E. Gumboski 4136, 4175* (JOI).

Ramalina pusiola Müll. Arg., *Flora, (Regensburg) 73: 388. 1890.*

Type: Tanzania, suden von Usambara, Tumakanya, *Dr. H. Meyer (Stein no. 12)* (WRS�—lectotype)

MB#403812

Fig. 2D

Description: Thallus caespitose, corticolous, up to 4.0 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a broad base, concolorous with the thallus, up to 1.5 cm wide; branching irregularly, hollow branches, irregularly flattened, 0.5–3.0 mm thick, surface smooth; symbiotic propagules absent; pseudocyphellae absent; perforations present mainly in under surface, orbicular to irregularly orbicular, entire, 0.3–0.8 mm in diameter; medulla white, loose, somewhat arachnoid, continuous. Cortex absent, chondroid tissue not cracked, continuous, 20–90 µm thick, algal layer almost continuous, 10–20 µm thick. Apothecia rounded, subterminal to terminal, up to 3.0 mm in diameter, mainly concave, amphithecium smooth, without pseudocyphellae, margin entire, without spurs, concolorous with the thallus to almost orange, disc whitish, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline, 25–30 µm, hypohymenium yellowish, 10–12 µm thick, chondroid tissue under the hypothecium, continuous, 10–15 µm thick, algal cells dispersed in medulla; ascospores 1-septate, short-fusiform, straight to weakly curved, 10–16 × 4–5 µm. Pycnidia not found.

Chemistry: Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic acid, homosekikaic acid, and sekikaic acid.

Distribution: Tropical in Central and South America (Kashiwadani & Kalb 1993).

Ramalina pusiola is characterized by the hollow branches with orbicular to irregularly orbicular perforations presents mainly in the under surface, and by the production of homosekikaic and sekikaic acids.

This species is very similar morphological and anatomically with *R. calcarata* Krog & Swinscow. The mainly characteristics that distinguish that two species are: *R. pusiola* have orbicular to irregularly orbicular perforations mainly in the under surface, and produces homosekikaic and sekikaic acids. Whiles *R. calcarata* has perforations usually distributed sequentially through the branches, almost orbicular to mainly irregular, and produces divaricatic acid with (\pm) salazinic acid (Krog & Swinscow 1974; Kashiwadani & Kalb 1993).

Specimens examined: BRAZIL. State of Rio Grande do Sul: Municipality of Canela, FLONA, 25.IV.1997, *C. Mansan s.n.* (HAS 33846); Municipality of Esmeralda, Estação Ecológica Aracuri, corticolous, on *Araucaria angustifolia*, 17.IX.1983, *M. Fleig 2072* (ICN). State of Santa Catarina, Municipality of Rio Negrinho, Fazenda Velha, rural area, corticolous, 11.IV.2009, *E. Gumboski 951* (JOI), *ibid.*, 01.XI.2008, *E. Gumboski 1182* (JOI), *ibid.*, 31.I.2010, *E. Gumboski 1788, 1789* (JOI).

Ramalina sprengelii Krog & Swinscow, Norwegian Journal of Botany 23 (3): 172. 1976.

MB#343520

Typus: South Africa, Uitenhagen, *Ecklon 212* (Holotype – S: Photo!).

Fig. 2E

Description: Thallus pendulous, corticolous, up to 20 cm long, greenish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching main dichotomously to irregularly, solid branches, branches mainly flat and usually cylindrical to terete in distal parts, 0.2–0.7(–1.0) mm wide, not contorted, surface smooth in cylindrical to terete branches and striated in flat branches; symbiotic propagules absent; linear pseudocyphellae, marginal and laminal, flat, 0.05–0.2 mm wide. Cortex distinct, hyaline to yellowish, 12–20 μ m thick, chondroid tissue not cracked, mainly discontinuous in cylindrical bundles, and discontinuous but not in bundles in branches wider than 0.5 mm; medulla white, loose. Apothecia marginal or lateral, up to 1.2 mm in diameter, flat to convex, amphithecia

smooth without pseudocyphellae, margin entire, concolorous with the thallus, disc beige; epihymenium hyaline, 2–4 µm thick, hymenium hyaline, 40–60 µm thick, subhymenium hyaline, 20–30 µm thick, hypothecium hyaline to yellowish, 25–40 µm thick, without algal layer under the hypothecium and at margins of apothecia; ascospores 1-septate, fusiform, 12–15 × 3–5 µm. Pycnidia not found.

Chemistry: Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K-, C-, KC-. TLC: usnic acid.

Distribution: Tropical in Africa and South America (Krog & Swinscow 1976; Kashiwadani & Kalb 1993).

Ramalina sprengelii is characterized by the pendulous thallus with flat to cylindrical branches, absence of symbiotic propagules, not cracked chondroid tissue usually disposed in bundles, and absence of medullar metabolites.

This species is similar with *R. kashiwadanii* in morphology of branches and absence of medullar metabolites. However, *R. sprengelii* have mainly laminal pseudocyphellae and the chondroid tissue discontinuous is usually disposed in bundles, while *R. kashiwadanii* have mainly marginal pseudocyphellae and a continuous chondroid tissue, never disposed in bundles (Gumboski *et al.* B).

Specimens examined: BRAZIL. State of Santa Catarina: Municipality of Rio Negrinho, Estrada da Volta Grande, rural area, 01.XI.2008, *E. Gumboski* 975 (JOI), Fazenda Velha, rural area, 06.X.2007, *E. Gumboski* 1010, 1026 (JOI), urban area, Bairro Bela Vista, *E. Gumboski* 1035 (JOI), Rio Dos Bugres, 06.I.2009, *E. Gumboski* 1218 (JOI), on *Araucaria angustifolia*, 20.VII.2012, *E. Gumboski* 3707 (JOI); Municipality of São Bento do Sul, Estrada Dona Francisca, rural area, on *A. angustifolia*, 07.VII.2012, *E. Gumboski* 3629, 3632 (JOI); Municipality of Monte Castelo, road BR 116, corticolous, 23.IX.2012, *E. Gumboski* 4009 (JOI); Municipality of Mafra, side road near the BR 280, rural area, corticolous, 23.IX.2012, *E. Gumboski* 4050, 4054 (JOI).

Ramalina subpollinaria Nyl., Bulletin de la Société Linnéenne de Normandie 4: 125. 1870.

Type: Peru, Callao, *Gaudichaud* (H-NYL 37050—lectotype, Photo!).

MB#403848

Fig. 2F

Description: Thallus caespitose, corticolous, up to 5 cm long, whitish green in field, stramineous in herbaria, growing from a common holdfast, concolorous with the thallus, 4.0 mm in diameter, branching dichotomously anisotomic to irregularly branched, solid branches, flat to canaliculate, 0.3–1.3(–2.0) mm in wide, surface smooth to slightly foveolate; soralia subterminal to terminal, orbicular to somewhat labriform, 0.3–1.5 mm in diameter, 250–500 µm in diameter; pseudocyphellae marginal, discrete, orbicular, at surface level, 0.05–0.1 mm in diameter. Cortex distinct, hyaline to stramineous, 15–18 µm thick, chondroid tissue cracked, discontinuous, in irregular bundles, 13–50 µm thick, algal cells dispersed in medulla, medulla white to weakly beige, loose. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+ yellow, UV-. Medulla K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in America (Nylander 1890; Kashiwadani & Kalb 1993).

Ramalina subpollinaria is characterized by the shrubby thallus with flat to canaliculated branches, subterminal to terminal soralia, cracked chondroid tissue, and by the production of salazinic acid.

This species is similar to *R. dendriscoides* by the presence of terminal soralia, cracked chondroid tissue, and by the production of salazinic acid. However, *R. subpollinaria* have flat to canaliculated branches, while *R. dendriscoides* have the main branches irregularly flat to irregularly cylindrical, and the secondary branches are mainly terete (Kashiwadani 1987; Kashiwadani & Kalb 1993).

Specimens examined. BRAZIL. State of Santa Catarina, Municipality of São Francisco do Sul, Morro da Enseada, rocky shore, saxicolous, 26°13'11,8"S, 48°29'40,4"W, 28.IV.2008, *E. Gumboski* 146 (JOI), 19.VI.2008, *E. Gumboski* 492, 568 (JOI), corticolous, 19/06/08, *E. Gumboski* 438, 439, 440, 441, 570 (JOI), saxicolous, 26°13'33,5"S, 48°29'52,3"W, 07.V.2008, *E. Gumboski* 293, 295 (JOI), Praia Grande, saxicolous, 26.VIII.2011, *E. Gumboski* & *F. Beilke* 2458, 2467 (JOI); Municipality of Penha, west rocky shore from Praia do Quilombo, rocky shore, saxicolous, 26°46'03"S, 48°38'27"W, 25.X.2011, *E. Gumboski* & *F. Beilke* 2890 (JOI).

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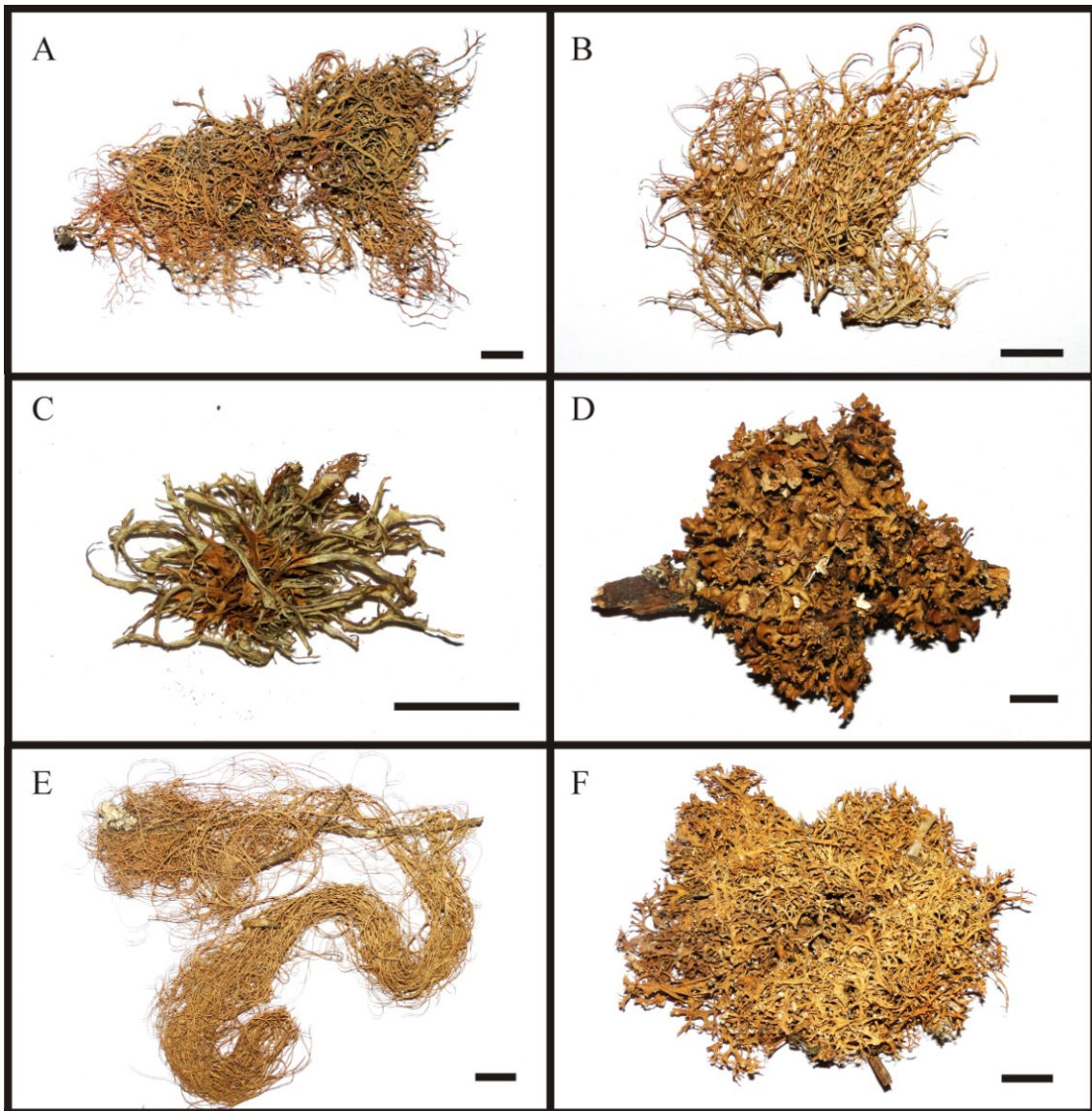


Fig. 2. A–F. A – *R. dendroides* (E. Gumboski 2722); B – *R. exiguella* (E. Gumboski 3814); C – *R. puiggarii* (E. Gumboski 4136); D – *R. pusiola* (E. Gumboski 951); E – *R. sprengelii* (E. Gumboski 1010); F – *R. subpollinaria* (E. Gumboski & F. Beilke 2890).

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4.6. Manuscrito VI

Gumboski, E.L.; Eliasaro, S. & Borges da Silveira, R.M. The Brazilian species of *Ramalina* (Ramalinaceae; Ascomycota). **Phytotaxa**: a ser submetido.

The Brazilian species of *Ramalina* (Ramalinaceae; lichenized Ascomycota)

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Abstract. Species of *Ramalina* has been recorded from Brazil since 1833, and currently has 39 registered species. However, possibly due to the fact that Brazil is a country with continental dimensions, there are many gaps concerning the distribution of *Ramalina* species in various environments. The aim of this work is increase knowledge about the species of *Ramalina* from Brazil. 566 specimens of *Ramalina* were collected in 43 municipalities (seven states) and 618 specimens from 20 herbaria were analyzed. Twenty-seven species were found, of which ten are new records from three States. Seven are new records from State of Espírito Santo, two are new records from State of Mato Grosso do Sul, and one is a new record from State of São Paulo. The species *R. subfraxinea* was excluded from the mycobiota of the State of Rio Grande do Sul. Descriptions, comments, figures and a key for identification of the species are given.

Keywords: Biodiversity, geographic distribution, lichen, inventory, taxonomy.

Introduction

Ramalina Ach. is a cosmopolitan genus of lichenized fungi that inhabit from coastal to mountainous regions (Stevens 1987; Marcano & Méndez 1994; Kashiwadani & Nash 2004). The genus is characterized by the fruticose thallus with pseudocyphellae on branches, presence of a prosoplectenchymatous supporting tissue (chondroid tissue), photobiont of *Trebouxia* Puymaly, production of hyaline ascospores, 1(–3)-septate (Kashiwadani & Kalb 1993; Brodo *et al.* 2001), and by the production of usnic acid (Krog & James, 1977; Krog & Østhagen 1980).

The genus is relatively well represented in most lichenized mycobiota of different environments, both in number of species as biomass (e.g. Krog & Swinscow 1976; Brodo *et al.* 2001; Aptroot & Bungartz 2007). Currently, it is recognized around 150 to 200 species of *Ramalina* (Kashiwadani & Nash 2004; Sérusiaux *et al.* 2010.). Some species can colonize different types of substrate, such as *R. farinacea* (L.) Ach. that inhabiting trunks, twigs, cladodes, and even rocks (Brodo *et al.* 2001; Krog & Østhagen 1980.). Whiles others occur in specific substrates, such as: *R. laevigata* Fr. which only occurs on rocks (Malme 1934), and *R. puiggarii* Müll. Arg. that only occurs on trunks (Kashiwadani & Kalb 1993).

The information on Brazilian *Ramalina* species are scarce and dispersed, and the only complete study was performed by Kashiwadani & Kalb (1993) who made a historical summary regarding the references to this genus in the country. In this work the authors described 22 species, seven taxa were reduced to synonymy, and five species were recorded for the first time to the country. The work relies primarily on specimens collected by the lichenologist Dr. Klaus Kalb mainly from the Southeast Brazil.

Almost 15 years later, Kashiwadani *et al.* (2007) described the species *R. gallowayi* Kashiw. *et al.* from Brazil, based on specimens from State of Minas Gerais. Recently, Gumboski *et al.* (2014) has reported the occurrence of *R. lacera* (With.) J.R. Laundon for the country. Gumboski *et al.* (A) described *R. kashiwadanii* Gumboski *et al.* from Southern Brazil, and Gumboski *et al.* (D) described *R. fleigiae* Gumboski *et al.* from State of Rio Grande do Sul.

Currently, Brazil has 39 registered species of *Ramalina*, of which: 24 to the South Region (Gumboski *et al.* B), 30 to Southeast, nine for the Northeast (Gumboski *et al.* C), four for the Midwest and only two species to the North Region. The states of Rio de Janeiro, São Paulo, and Minas Gerais have the highest number of recorded species, with 21, 18, and 16 species, respectively (e.g., Eschweiler 1833; Meyen & Flotow 1843; Nylander 1870; Müller Argoviensis 1881; Vainio 1890; Zahlbruckner 1902, 1905; Malme 1934; Fleig 1988; Kashiwadani & Kalb 1993; Kashiwadani *et al.* 2007; Gumboski *et al.* A, D). Fourteen states have no *Ramalina* recorded, which makes clear the lack of the studies in the country.

The aim of this work is contribute substantially with the knowledge about the species of *Ramalina* occurring in Brazil, as well as to give support for that new studies could be conducted mainly in poorly studied regions.

Material and methods

The collections were made in 43 municipalities from seven Brazilian States, of which: Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, São Paulo, and Sergipe. Higher collection effort was directed to State of Santa Catarina and State of Paraná due the lack of *Ramalina* specimens in the herbaria, and by the ease of access. The State of Rio Grande do Sul has a vast collection stored in the ICN herbarium, essentially specimens collected by the lichenologist Dr. Mariana Fleig. Added, were analyzed collections from the herbaria BHCB, CESJ, CTES, CGMS, DUKE, G, FLOR, H, HAS, ICN, JOI, MEL, MPUC, O, RB, SP, UFP, UPCB, UPS e W.

The specimens examined were collected using a knife and dried at room temperature. Specimens were examined using standard techniques with stereoscopic microscope (20–40×) and light microscope (400–1000×). Freehand sections of the thalli and apothecia were mounted in water. The chemical analysis: spot tests, observation under UV light, and thin layer chromatography (TLC) using solvent C; were conducted according to Huneck & Yoshimura (1996) and Orange *et al.* (2001).

Since some species recorded by other studies were not found in the present study (*R. grumosa* Kashiw., *R. rigida* Ach., and *R. gallowayi* Kashiw. *et al.*), the key for the Brazilian species of *Ramalina* was made based on data from Kashiwadani & Kalb (1993), Kashiwadani *et al.* (2007), and this present work. Nine species were not considered in the key for being doubtful occurrence in the country, as follows: *R. dasypoga* Tuck. (Nylander 1870), *R. denticulata* (Eschw.) Nyl. (Eschweiler 1833), *R. digitata* Meyen & Flot. (Meyen & Flotow 1843), *R. farinacea* and *R. farinacea* var. *multifida* Ach. (Zahlbruckner 1902), *R. geniculata* Hook. f. & Taylor (Vainio 1890), *R. hypodectodes* Nyl. (Nylander 1870), *R. inflata* (Hook. f. & Taylor) Hook. f. & Taylor (Vainio 1890), *R. pumila* Mont. (Nylander 1870), and *R. yemensis* var. *minima* Zahlbr. (Zahlbruckner 1905).

Results and Discussion

A total of 566 specimens of *Ramalina* were collected, and 618 specimens from the 20 herbaria were analyzed. Twenty-seven species were found, of which ten are new records from three States. Seven are new records from State of Espírito Santo: *R. calcarata* Krog & Swinscow, *R. camptospora* Nyl., *R. celastri* (Spreng.) Krog & Swinscow, *R. cochlearis* Zahlbr., *R. complanata* (Sw.) Ach., *R. gracilis* (Pers.) Nyl., and *R. usnea* (L.) R. Howe. Two are new records from State of Mato Grosso do Sul: *R. aspera* Räsänen and *R. continentalis* Malme. One is new record from State of São Paulo: *R. subpollinaria* Nyl.

Ramalina subfraxinea Nyl., a species recorded to the State of Rio Grande do Sul by Fleig (1988) is excluded from the mycobiota of Brazil. The specimens stored in ICN herbarium were analyzed and all specimens represent samples of *R. celastri* (Spreng.) Krog & Swinscow.

Key to species of *Ramalina* from Brazil

1. Thallus fistulose, hollow2
Thallus solid, not hollow (including species with loose medulla)3
2. Perforations distributed sequentially through the branches, almost orbicular to mainly irregular orbicular. Divaricatic acid present..... **R. calcarata**
Perforations irregularly distributed through the branches, orbicular to irregular orbicular. Homosekikaic and sekikaic acid present **R. pusiola**
3. Thallus pendulous, usually larger than 7 cm long.....4
Thallus caespitose to shrubby, usually shorter than 7 cm long9
4. Soredia present **R. dendroides**
Soredia absent.....5
5. Chondroid tissue not cracked6
Chondroid tissue cracked7
6. Chondroid tissue continuous; pseudocyphellae mainly marginal **R. kashiwadanii**
Chondroid tissue discontinuous, mainly disposed in bundles; pseudocyphellae mainly laminal..... **R. sprengelii**
7. Medulla K- or K+ pinkish (divaricatic or sekikaic acids) **R. usnea**
Medulla K+ yellow → red.....8
8. Branches rectangular in cross section; salazinic acid present **R. rectangularis**
Branches not rectangular in cross section; norstictic acid present **R. anceps**

9. Symbiotic propagules present.....	10
Symbiotic propagules absent.....	19
10. Isidia present, terminal	R. grumosa
Soredia present, laminal to terminal.....	11
11. Branches distinctly flat, canaliculate or not	12
Branches terete, subterete or irregularly flat	16
12. Branches broad, up to 1.3 cm wide, surface usually scrobiculate.....	R. lacera
Branches slender, up to 2.5 mm wide, surface smooth to irregular rugose.....	13
13. Soredia originating from a helmet-shaped soralia, soralia subterminal to terminal.....	
.....	R. cochlearis
Soralia flat, orbicular to labriform, laminal to terminal	14
14. Medulla K+ yellow→red or K+ pinkish (salazinic or sekikaic acids); soralia subterminal to terminal.....	R. subpollinaria
Medulla K-; soralia laminal or marginal	15
15. Pseudocyphellae linear, mainly marginal; no medullar acids	R. camptospora
Pseudocyphellae ellipsoid to linear, mainly laminal; boninic acid present.....	
.....	R. asahinae
16. Chondroid tissue cracked	17
Chondroid tissue not cracked	18
17. Branches irregular flat to irregular terete; pseudocyphellae orbicular to ellipsoid	
.....	R. dendriscoides
Branches terete to subterete; pseudocyphellae orbicular.....	R. rigida
18. Branches irregular flat to irregular terete; medulla K+ pinkish	R. peruviana
Branches terete to subterete; medulla K+ yellow→red.....	R. sorediosa
19. Branches distinctly flat, irregularly broad, canaliculate, or lanceolate	20
Branches irregularly flat to subterete, terete, or angular	23
20. Branches clearly terete; protocetraric acid presente	R. gallowayi
Branches subterete, angular, or irregular flat	21
21. Branches irregular flat to suterete; pseudocyphellae abundant, ellipsoid to linear; occurring in plateau region.....	R. fleigiae
Branches subterete to angular; pseudocyphellae linear; occurring in coastal plain	22
22. Chondroid tissue not cracked	R. exiguella
Chondroid tissue cracked	R. gracilis

23. Branches flat irregular in width to canaliculate; pseudocyphellae tuberculate orbicular.....24
 Branches mainly lanceolate; pseudocyphellae orbicular, ellipsoid, or linear, but not tuberculate26
24. Branches distinctly canaliculate **R. canalicularis**
 Branches flat but not distinctly canaliculate, usually irregular in width25
25. Chondroid tissue not cracked; K- or K+ pinkish (divaricatic or cryptochloropheic acids) **R. aspera**
 Chondroid tissue cracked; K- or K+ yellow→red (divaricatic or salazinic acids).....
 **R. complanata**
26. Pseudocyphellae mainly orbicular to ellipsoid, mainly laminal..... **R. prolifera**
 Pseudocyphellae ellipsoid to linear, laminal or marginal.....27
27. Saxicolous habit28
 Corticolous habit29
28. Branches distinctly canaliculate, slender, mainly 0.5–2.0 mm wide..... **R. laevigata**
 Branches not canaliculate, broad, 1.0–15.0 mm wide..... **R. continentalis**
29. Branches distinctly canaliculate; pseudocyphellae discrete, linear, marginal; ascospores 18–25 µm long **R. puiggarii**
 Branches not distinctly canaliculate; pseudocyphellae abundant, ellipsoid to linear, laminal; ascospores 12–16 µm long **R. celastri**

Ramalina anceps Nyl., Syn. meth. lich. 1(2): 290. 1860.

MB#357046

Typus: Guadalupe, *L'Herminier et al.* (PC–holotype; H-NYL 370128!–syntype).

Fig. 1A.

Thallus pendulous, corticolous or saxicolous, up to 20 cm long, greenish in field, dark stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching dichotomously, almost isotomic; solid branches, subterete to almost flat, 0.2–0.5 mm wide, sometimes weakly contorted, surface smooth; symbiotic propagules absent; pseudocyphellae mainly marginal, rarely laminal, linear, flat, 0.04–0.07(–0.20) wide. Cortex distinct, hyaline to yellowish, 12–20 µm thick, chondroid tissue distinctly cracked, continuous, 50–170 µm thick, algal cells not forming a distinct layer, medulla white, compact. Apothecia rare, marginal, shortly

stipitate, up to 0.7 mm in diameter; disc flat to slightly convex, anfithecia smooth, without pseudocyphellae, margin entire, concolorous with the thallus, disc beige not to weakly pruinous; epihymenium darkened, 4–6 µm thick, hymenium hyaline, 40–55 µm thick, subhymenium hypothecium yellowish, 55–70 µm thick, without algal layer under the hypothecium and at margins of apothecia; ascospores 1-septate, short-fusiform, 11–16 × 5–8 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ bright yellow. Medulla: K+ yellow → red, C-, KC-. TLC: usnic acid, norstictic acid and (±) connorstictic acid.

Distribution. Tropical in Central and South America, from sea level up to 800 m alt. (Nylander 1890; Kashiwadani & Kalb 1993; Aptroot & Bungartz 2007).

Ramalina anceps is characterized by the pendulous thallus with flat branches, marginal and linear pseudocyphellae, distinctly cracked chondroid tissue, and by the production of salazinic acid.

This species differs from *R. usnea* by the mainly marginal pseudocyphellae and by the production of norstictic acid. *Ramalina anceps* differ from *R. kashiwadani* and *R. sprengelii* by the distinctly cracked chondroid tissue and by presence of norstictic acid in the medulla (Kashiwadani & Kalb 1993; Gumboski *et al.* A).

Selected specimens examined: BRAZIL. State of Rio Grande do Sul, Municipality of São Francisco de Paula, Loteamento Colina de São Francisco, 19.IV.1982, *Osorio & M. Fleig SF2* (ICN 54772); Municipality of Cambará do Sul, Itaimbezinho, próx. Hotel, 20.IV.1982, *Osorio & M. Fleig T34* (ICN 54842). State of São Paulo, Municipality of Cananéia, on the Atlantic Coast S of São Paulo, Ilha Comprida, near road to Cananéia ferry, c. 10 m tall shrubby forest on sand, near the beach, 16.VII.1979, *H.J.M. Sipman 14148* (SP). State of Santa Catarina, Municipality of São Francisco do Sul, Morro da Enseada, rocky shore, saxicolous, 13.V.2008, *E. Gumboski 364* (JOI).

Ramalina asahinae W.L. Culb. & C.F. Culb., *Journal of Japanese Botany* 51 (12): 374. 1976.

Type: Mexico, Chiapas: 11 km west of San Cristobal las Casas, on *Quercus* sp., alt. 2154 m (DUKE!—holotype).

MB#343493

Fig. 1B.

Thallus shrubby, corticolous, up to 7 cm long, whitish green in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 2.0 mm in diameter; branching dichotomously anisotomic, rarely irregular, branches solid, flat to irregular flat, usually canaliculate in old branches, 0.3–1.5 mm wide, surface smooth, somewhat striate due the presence of ellipsoid pseudocyphellae; soralia mainly marginal, infrequent laminal, ellipsoid to labriform, 0.2–1.5 × 0.1–0.5 mm, soredia 30–100 µm in diameter; pseudocyphellae mainly laminal, ellipsoid to short linear, at surface level to depressed, 0.1–0.3 × 0.05–0.10 mm. Cortex absent, chondroid tissue not cracked, continuous, 10–50 µm thick, algal cells dispersed on medulla, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic and boninic acids.

Distribution. Tropical in America, in plateau region up to c. 2200 m alt. (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Ramalina asahinae is characterized by the shrubby thallus with mainly flat branches, mainly marginal soralia, not cracked chondroid tissue, and by the production of boninic acid.

This rare species in Southern Brazil is similar to *R. peruviana* Ach. in shrubby thallus, soredia presence, and not cracked chondroid tissue. However, *R. peruviana* has irregular flat to irregular terete branches, usually with striate surface, laminal soralia, and produces homosekikaic and sekikaic acids (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Specimens examined: BRAZIL. State of São Paulo, Municipality of Campos do Jordão, Ad corticem araucariae bras. Prope hotel Toriba, 1700 m, 1977, *H. Schindler s.n.* (SP 129028), Hotel Toriba, 06.XI.1977, *H. Schindler 7186* (SP 128999, 128973). State of Santa Catarina, Municipality of Mafra, margins on road BR-280, corticolous, 23.IX.2012, *E. Gumboski 4048* (JOI).

Ramalina aspera Räsänen, Ann. Bot. Soc. Zool. Bot. Fenn. Vanamo 20: 5. 1944.

Type: Paraguay, Chaco, Rio Verde, Villa Hayes, *W. G. Herter 3* (H!—holotype).

MB#369881

Fig. 1C.

Thallus caespitose, corticolous, up to 5 cm long, stramineous whitish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 5.0 mm in diameter; branching irregularly, rarely almost palmate, solid branches, mainly flat, dorsiventral, 0.5–4.0 mm wide, surface rugose, reticulately to irregularly ridged; symbiotic propagules absent; pseudocyphellae laminal and marginal, raised, tuberculate, 0.1–0.3 mm wide, 0.05–0.2 mm tall. Cortex distinct, hyaline to yellowish, 10–22 μm thick, chondroid tissue not cracked, discontinuous, almost in irregular bundles, 50–110 μm thick, algal cells dispersed in medulla, medulla white, loose. Apothecia rounded, subterminal to terminal, up to 4.5 mm in diameter, concave to flat, anfithecium with tuberculate pseudocyphellae, margin usually indented, concolorous with the thallus, disc whitish to beige, weakly pruinous; epihymenium greenish, 15–20 μm thick, hymenium hyaline to grayish, 20–25 μm thick, hipohymenium yellowish, 20–35 μm thick, with chondroid tissue under de hypothecium, 10–70 μm thick, algal layer continuous, 40–70 μm thick; ascospores 1-septate, straight to slightly curved, 12–15 \times 3–5 μm . Pycnidia not found.

Chemistry. Spot tests: Cortex K-, C-, KC-, UV-. Medulla: K- or K+rose, C-, KC-, UV-. TLC: (Race 1) Usnic acid and divaricatic acid. (Race 2) Usnic acid and cryptochlorophaeic acid.

Distribution: Tropical in America and Africa, from sea level up to 700 m alt. (Krog & Swinscow 1976; Kashiwadani & Nash 2004).

Ramalina aspera is characterized by the caespitose thallus with flat branches, tuberculate pseudocyphellae, not cracked chondroid tissue, and by the production of divaricatic acid or cryptochlorophaeic acid.

In Brazil, this species could be confused with *R. complanata* by the morphologically similar thallus and habitat preferences. However, *R. complanata* has a flat to almost canaliculate branches with a cracked chondroid tissue, and produces salazinic acid and/or divaricatic acid (Kashiwadani & Kalb 1993).

Selected specimens examined: BRAZIL. State of Mato Grosso do Sul, Municipality of Porto Murтинho, Fazenda Santa Verginia, 15 Km da sede, final do Retiro 13., on trunk of *Prosopis* sp., 22°06'42"S, 57°50'01"N, 15.XII.2010, L. S. Canêz; A. A. Spielmann; A. L. B. Sartori; T. E. Lima 3668 (CGMS). State of Minas Gerais, Municipality of Congonhas, 23.XI.1977, H. Schindler 7370 (SP). State of Pernambuco, Municipality of

Alagoinha, locality of Sítio Lagoa Seca, on twigs of 'algaroba', in Caatinga, corticolous, 14.XII.1998, W.B. Sanders, 98d14.19, 98d14.6 (UFP). State of Rio Grande do Sul, Municipality of Porto Alegre, Jardim Botânico, 18.03.2009, F. Lucheta s.n. (HAS 48158), Glória, 04.05.1994, S. Mazzitelli & M. Käffer s.n. (HAS 33555); Municipality of Triunfo, Estação Santa Maria, 30.09.2005, M. Käffer 375 (HAS 46721); Municipality of Barra do Quaraí, Parque Estadual do Espinilho, P4, 15.VIII.2002, I. Mota s.n. (MPUC 15586). State of São Paulo, Municipality of Itanhaém, 26.VII.1962, D. Altimari s.n. (SP 100214). State of Santa Catarina, Municipality of Florianópolis, Praia dos Ingleses, 21.IV.1983, I.C. Pereira s.n. (MPUC 3899B), on Itacorubi mangrove, corticolous, 08.IV.1989, Jeane I. Dal Bó 30 (FLOR). State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, E. Gumboski, 4757, 4765, 4781 (ICN); Municipality of Nossa Senhora da Glória, locality of Povoado Retiro1, Assentamento Nossa Senhora Aparecida, 175 m alt., Caatinga, corticolous, 10°06'13.5"S, 37°25'12.9"W, 19.IX.2013, E. Gumboski, 4854, 4858 (ICN).

Ramalina calcarata Krog & Swinscow, Norwegian Journal of Botany 21: 115. 1974.

Type: Kenya, Rift Valley Prov., Uasin Gishu District, 5 km NW of Timboroa Summit, 0-04 N, 35-32 E, YR-82-109, in moist brush by lake, 2650 m, H. Krog & T. D. V. Swinscow no. 2K19/127 (O!—holotype; BM—isotype)

MB#343495

Fig. 1D.

Thallus caespitose, corticolous, up to 3.5 cm long, greenish to whitish green in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 3.0 mm wide; branching dichotomously anisotomic to irregularly, hollow branches, irregularly inflated, 0.8–2.0 mm thick, surface smooth; symbiotic propagules absent; pseudocyphellae absent; perforations present, usually distributed sequentially through the branches, almost orbicular to mainly irregular, entire, 0.2–1.0 × 0.1–0.5 mm, rarely the perforations unite fully exposing the medulla, giving a foliose aspect to the branches; medulla white, loose, arachnoid, mainly continuous. Cortex absent, chondroid tissue not cracked, continuous, 50–120 µm thick, algal layer almost continuous, 20–70 µm thick. Apothecia rounded, subterminal to terminal, up to 4.0(–7.0) mm in diameter, concave to flat, rarely convex, amphithecia smooth to weakly

foveolate, without pseudocyphellae, margin entire, without spurs, concolorous with the thallus to almost orange, disc beige, pruinous; epihymenium blackish, 7–10 µm thick, hymenium hyaline, 25–30 µm thick, hipohymenium yellowish, 12–15 µm thick, chondroid tissue under the hypothecium, continuous, 12–32 µm thick, algal layer mainly continuous, 15–40 µm thick; ascospores 1-septate, straight to weakly curved, 12–16 × 4–5 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic acid, divaricatic acid, and (±)salazinic acid.

Distribution. Tropical in Africa and South America, from 600 m up to 2600 m alt. (Krog & Swinscow 1974, 1976; Kashiwadani & Kalb 1993).

Ramalina calcarata is characterized by the hollow branches with perforations distributed sequentially through the branches, almost orbicular to mainly irregular orbicular, and by the production of divaricatic acid.

This species is very similar morphological and anatomically with *R. pusiola*. However, *R. pusiola* has orbicular to irregularly orbicular perforations mainly in the under surface, and produces homosekikaic and sekikaic acids (Krog & Swinscow 1974; Kashiwadani & Kalb 1993).

Although the Brazilian specimens agrees with the morphology, anatomy and chemistry with the holotype of *R. calcarata*, this morphological group of fistulose thallus (e.g., *R. calcarata*, *R. pusiola*, and *R. inflata* (Hook. f. & Taylor) Hook. f. & Taylor) needs an special attention on new researches using phylogenetic analyzes including molecular data from African specimens in order to elucidate the systematic of it.

Specimens examined: BRAZIL. State of Espírito Santo, Municipality of Rio Preto, Parque Nacional de Caparaó, corticolous, 06.II.1999, *W.B. Sanders* 99206.3 (UFP). State of Minas Gerais, Municipality of Passa Quatro, III.1921, *J.F. Zikán* s.n. (SP 5604); Municipality of Catas Altas, Serra do Caraça, Parque Natural do Caraça, on trunks of ‘candeia’, 08.IX.1997, *M.P. Marcelli & C.H. Ribeiro* 31919 (SP). State of São Paulo, s. col. (SP 34242), Municipality of São Paulo, Ipiranga, V.1921, s.col. s.n. (SP 34077); Municipality of Ibiúna, near 3 Km from SP-250 road, Km 63, on trunk, forest fragment, 14.V.1998, *O. Yano & I. Cordeiro* 25343 (SP).

Ramalina camptospora Nyl., Bulletin de la Société Linnéenne de Normandie 4: 120. 1870.

Type: Cuba, *C. Wright* (H-NYL 37248!—holotype)

MB#403670

Fig. 1E.

Thallus shrubby, corticolous, up to 8 cm long, greenish to weakly stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 4.0 mm in diameter; branching dichotomously to irregular, usually with tiny branchlets on margins, up to 2.0 mm tall, c. 0.1 mm wide, possibly generating new branches, branches solid, flat, usually weakly canaliculate in main branches, 0.3–1.2 mm wide, secondary branches 0.1–0.4 mm wide, surface smooth to rarely rugose; soralia marginal, ellipsoid to linear, scattered, depressed, often very scarcely formed, 0.5–1.0(–2.0) × 0.1–0.2 mm, soredia 20–50 µm in diameter; pseudocyphellae marginal, linear, depressed. Cortex discrete, hyaline, 6–10 µm, chondroid tissue not cracked, continuous, 100–350 µm thick, algal cells dispersed on medulla, medulla white, compact. Apothecia rounded, marginal, subterminal, up to 5.0 mm in diameter, flat, amphithecia smooth, without pseudocyphellae, margin entire, concolorous with the thallus, disc whitish to beige, weakly pruinous; epihymenium blackish, c. 10 µm thick, hymenium hyaline, 35–45 µm thick, hypohymenium yellowish, 20–35 µm thick, with chondroid tissue under de hypothecium, 25–50 µm thick, algal layer continuous, 20–50 µm thick, same algal cells dispersed in medulla; ascospores 1-septate, curved to sigmoid, 11–15 × 4–7 µm.. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic acid.

Distribution. Tropical in America, in plateau region (Kashiwadani & Kalb 1993).

Ramalina camptospora is characterized by the shrubby thallus with flat branches, marginal soralia, not cracked chondroid tissue, ascospores curved to sigmoid, and absence of medullar acids.

Similar species could be distinguished by the different patterns of soralia and by the production of medullar acids. *Ramalina asahinae* has almost labriorm soralia and produces boninic acid. While *R. peruviana* has laminal soralia and produces homosekikaic and sekikaic acids (Kashiwadani & Kalb 1993).

Selected specimens examined: BRAZIL. State of Espírito Santo, Municipality of Rio Preto, Parque Nacional de Caparaó, on trunk, margins of the road, 06.II.1999, *W.B. Sanders 99206.18* (UFP). State of Minas Gerais, Municipality of Monte Verde, saxicolous, 00.03.1999, *H. L. Wagner 6225* (ICN); Municipality of Catas Altas, Serra do Caraça, Parque Natural do Caraça, 12.IX.1997, *M.P. Marcelli & C.H. Ribeiro 32143* (SP); Municipality of Ouro Preto, Parque Estadual de Itacolomi, on trunk, 03.II.1999, *W.B. Sanders 99203.11* (UFP). State of Paraná, Municipality of Piraquara, Morro do Canal, Mata Atlântica Montana, corticolous, 09.VI.2009, *E. Gumboski 1537* (UPCB). Municipality of Ponta Grossa, Passo dos Pupos, corticolous, 3.VII.2003, *C. G. Donha 779* (UPCB). State of Rio Grande do Sul, Municipality of Cambará do Sul, Fortaleza dos Aparados, corticolous, 29.X.1983, *M. Fleig 2122, 2198* (ICN). State of Santa Catarina: Municipality of Campo Alegre, near 7km south of central falls, 11.IX.2011, *E. Gumboski 2577, 2578* (JOI); Municipality of Garuva, Monte Crista, 15.V.2010, *E. Gumboski & F. Beilke 1941, 1988* (JOI). State of São Paulo, Municipality of Pindamonhangaba, ‘Matas de Neblina’ near São José dos Alpes belvedere, 24.IX.1997, *M.P. Marcelli, A. Aptroot, I. Yoshimura, H. Sipman, S. Stenroos & J. Hyvonen 32332* (SP).

Ramalina canalicularis (Nyl.) Kashiw., Lichen Flora of the Greater Sonoran Desert Region 2: 444. 2004.

Basionym: *Ramalina denticulata* var. *canalicularis* Nyl., Bull. Soc. linn. Normandie, sér. 2 4(2): 126. 1870.

Type: Mexico, Orizaba, *F. Müller* (H-NYL 37063! —holotype).

MB#365899

Fig. see Gumboski *et al.* (B).

Thallus caespitose, corticolous, up to 7(–11) cm long, whitish green in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching mainly dichotomously anisotomic, rarely irregular, solid branches, flat, canaliculate, 0.5–1.5 mm wide, surface smooth, infrequent ridged; symbiotic propagules absent; pseudocyphellae mainly marginal to laminal, raised, tuberculate, 0.05–0.1 mm wide, 0.05–0.1 mm tall. Cortex distinct, hyaline, 18–22 µm thick, chondroid tissue cracked, discontinuous, 50–200 µm thick, algal cells dispersed in medulla, medulla white, compact. Apothecia rounded, marginal, subterminal to

terminal, up to 3.0 mm in diameter, concave to mainly flat, amphithecia with tuberculate pseudocyphellae, margin entire, concolorous with the thallus, disc whitish to beige, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline, 50–55 µm thick, hipohymenium yellowish, 20–25 µm thick, with discontinuous chondroid tissue under the hypothecium, 10–25 µm thick, algal dispersed on medulla; ascospores 1-septate, straight to slightly curved, 13–20 × 3–5 µm. Pycnidia not found.

Chemistry. Spot tests: Cortex K-, C-, KC- or KC+ yellow, UV-. Medulla: K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in America, from sea level up to plateau regions (Vainio 1890, as *R. denticulata* var. *canalicularis*; Kashiwadani & Nash 2004).

Ramalina canalicularis is characterized by the caespitose thallus with distinct canaliculated branches, the presence of tuberculate pseudocyphellae, cracked chondroid tissue, and by the production of salazinic acid.

Ramalina complanata is similar with *R. canalicularis* by the caespitose thallus with distinctly cracked chondroid tissue, and by the production of salazinic acid. However, *R. complanata* usually has a flat to irregular flat branches, almost but not distinct canaliculated branches, while *R. canalicularis* has a distinct canaliculated branches since the base (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Specimens examined: BRAZIL. State of Paraná, Municipality of Morretes, Reserva IAPAR, corticolous, III.2014, *D. Imig s.n.* (JOI).

Ramalina celastri (Spreng.) Krog & Swinscow, Norwegian Journal of Botany 23 (3): 159. 1976.

Basionym: *Parmelia celastri* Spreng., Syst. Veg. 4: 328. 1827.

Type: South Africa, Cap., *C. F. Ecklon* (S—holotype, Photo!)

MB#343497

Fig. 1F.

Thallus caespitose, corticolous, up to 12.0 cm long, greenish to whitish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 2.0 mm in diameter; branching dichotomously anisotomic to irregular, solid branches, flat, lanceolate, rarely weakly canaliculate, 0.1–5.0 mm wide, surface smooth to furrowed, sometimes almost foveolate or with an intricate network due the presence

of chondroid tissue; symbiotic propagules absent; pseudocyphellae mainly laminal to marginal, linear, abundant, at level surface to depressed, $0.3\text{--}1.5 \times 0.03\text{--}0.06$ mm. Cortex almost indistinct, $3\text{--}5$ μm thick, chondroid tissue not cracked, mainly continuous, $30\text{--}90$ μm thick, algal cells dispersed in medulla, medulla whitish, loose. Apothecia rounded, laminal, up to 1.5 mm in diameter, mainly flat, amphithecia smooth, margin entire, orange to stramineous, disc beige, pruinous; epihymenium blackish, $5\text{--}8$ μm thick, hymenium hyaline, $45\text{--}55$ μm thick, hypohymenium yellowish, $50\text{--}60$ μm thick, with continuous and irregular chondroid tissue under de hypothecium, $10\text{--}120$ μm thick, algal cells randomly intermixed with chondroid tissue and medulla; ascospores 1-septate, short-fusiform, straight to slightly curved, $12\text{--}16 \times 4\text{--}6$ μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution. Pantropical, with many records around the world, except in Antarctica and Arctic, from sea level up to 1000 m alt. (*e.g.*, Krog & Swinscow 1976; Stevens 1987; Kashiwadani & Kalb 1993, Kashiwadani & Nash 2004).

Ramalina celastri is characterized by the caespitose thallus with flat and lanceolate branches, corticolous habit, absence of symbiotic propagules, laminal ellipsoid to linear pseudocyphellae, ascospores with $12\text{--}16$ μm long, and absence of medullar acids.

Ramalina puggarii is quite similar in has caespitose thallus with flat branches, corticolous habit, absence of symbiotic propagules, and absence of medullar acids. However, *R. puiggarii* has a canaliculated branches, discrete and marginal pseudocyphellae, and ascospores with $18\text{--}25$ μm long (Kashiwadani & Kalb 1993; Gumboski *et al.* B).

The species *R. prolifera* differs from *R. celastri* in having wider branches and by the presence of mainly orbicular to short ellipsoid pseudocyphellae (Kashiwadani & Kalb 1993).

Ramalina subfraxinea resembles *R. celastri* but, although both species have caespitose thallus with flat and lanceolate branches, *R. celastri* has linear pseudocyphellae, and no production of medullar acids. While *R. subfraxinea* has orbicular pseudocyphellae, and produces homosekikaic, sekikaic, and/or divaricatic acids (Stevens 1987).

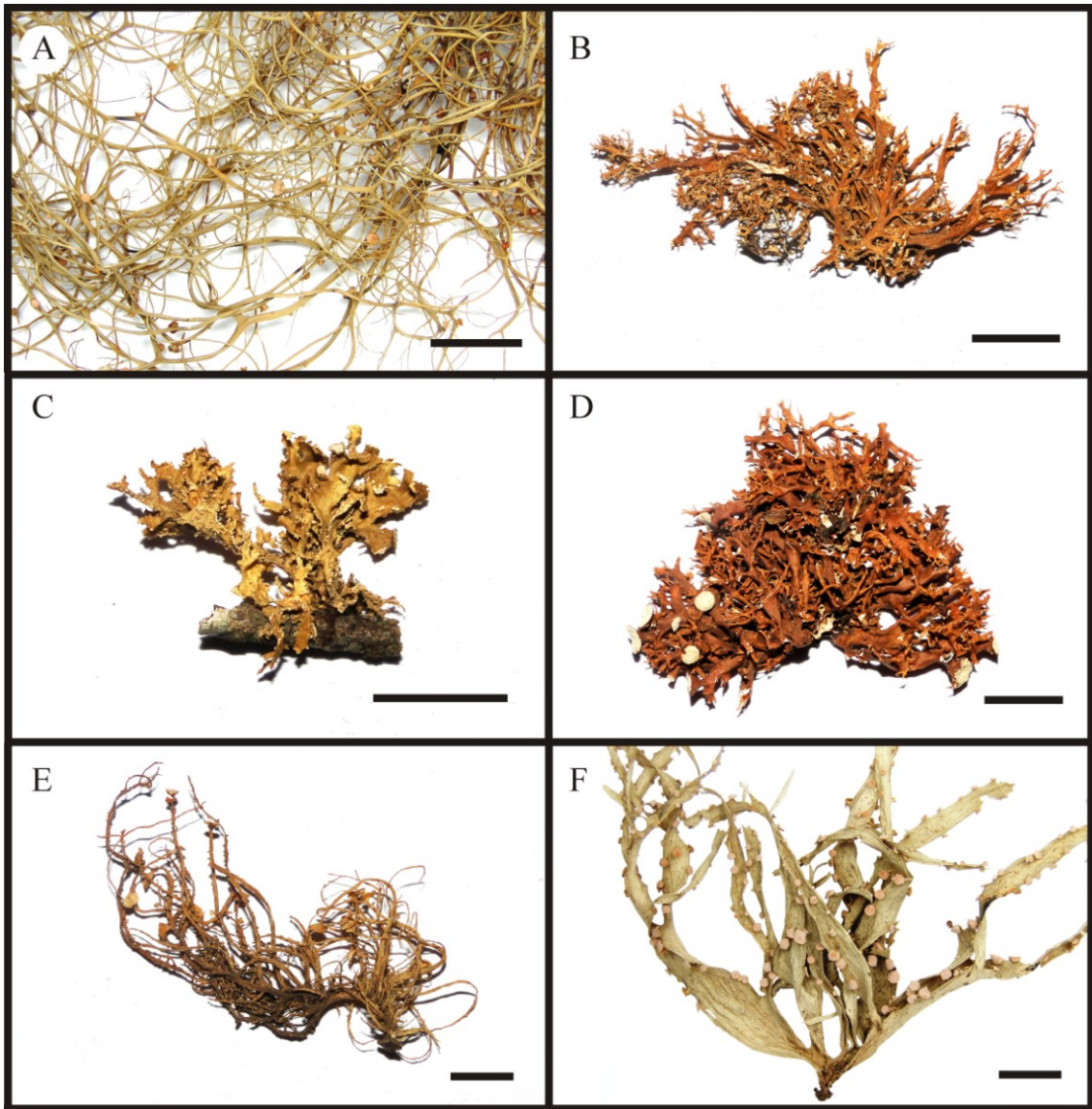


Fig. 1. A–F. A – *R. anceps* (Osorio & M. Fleig T34); B – *R. asahinae* (SP 129028); C – *R. aspera* (E. Gumboski 3216); D – *R. calcarata* (SP 34077); E – *R. camptospora* (E. Gumboski et al. 2127); F – *R. celastri* (C. G. Donha 888).

Selected specimens examined: BRAZIL. State of Espírito Santo, Municipality of Rio Preto, Parque Nacional de Caparaó; on trunk, 06.II.1999, *W.B. Sanders* 99206.5, 99206.1 (UFP). State of Minas Gerais, Municipality of Catas Altas, Caraça, 19.XI.1977, *H. Schindler* 7283 (SP); Municipality of Lambari, Parque das Águas, c. 10 km from the city, 03.V.2002, *O. Yano & S.S. Yano* 26818 (SP). State of Paraná, Municipality of Iguacu, near Hotel Cataratas, 23.VIII.1973, *H. Schindler s.n.* (SP 100906); Municipality of Campo Magro, on Atlantic Forest, 24.V.2003, *C. G. Donha* 888 (UPCB); Municipality of Curitiba, Conjunto Solar, corticolous, 19.II.2002, *C. G. Donha* 516

(UPCB); Municipality of Ponta Grossa, Buraco do Padre, corticolous, 10.VII.1999, C. G. Donha, Kretzl, Takemori, Vieira 137 (UPCB). State of Rio de Janeiro, Municipality of Petrópolis, 24.XI.1977, H. Schindler 7375 (SP), Parque Nacional do Itatiaia, 21.III.1962, D. Altimari s.n. (SP 100190); Municipality of Agulhas Negras, 2000 m alt., 25.XI.1977, H. Schindler 7402 (SP). State of Rio Grande do Sul, Municipality of Porto Alegre, Jardim Botânico, 01.10.2008, F. Lucheta 25 (HAS); Municipality of Triunfo, Copesul, Parque Proteção, s.d., S. Mazzitelli & M. Käffer s.n. (HAS 46723); Municipality of Alegrete, vicinal road near BR 290, margins of Caverá River, riparian forest, 06.V.1993, M. Fleig 5424 (HAS); Municipality of Santana do Livramento, Km 49 of BR 158 road, 22.I.1983, O. Yano & J.R. Pirani 5784 (SP). State of Santa Catarina, Municipality of Concórdia, Presidente Kennedy, 25.05.1986, C. Grabauska 417 (HAS). State of São Paulo, Municipality of Limeira, 20.VI.1942, F.C. Hoehne s.n. (SP 46994); Municipality of Campos do Jordão, Hotel Toriba, 06.XI.1977, H. Schindler 7190 (SP).

Ramalina cochlearis Zahlbr., Bull. Herb. Boissier, 2 sér. 5: 542. 1905.

Type: Brazil, ad ramos arborum in sylvis ad Cachoeira do Campo, L. Damazio 1449 (W!—holotype; RB! —isotype).

MB#403680

Fig. 2A.

Thallus caespitose, corticolous, up to 4.0 cm long, whitish green in field, stramineous in herbaria, growing from a narrow holdfast, up to 4.0 mm in diameter; branching irregular, solid branches, flat to weakly canaliculated, canaliculated mainly near the base, 0.5–3.0 mm wide, surface smooth; soralia subterminal to terminal, helmet-shaped, 0.35–2.50 mm wide, soredia 270–500 µm in diameter; pseudocyphellae rare, ellipsoid, at level surface, 0.05–0.20 × 0.05–0.10 mm. Cortex hyaline, 10–13 µm thick, chondroid tissue not cracked, discontinuous, 20–60 µm thick, algal cells dispersed in medulla, medulla white, loose. Apothecia not found. Pycnidia not found.

Chemistry. Spot tests. Cortex: K-, C-, KC- or KC+yellow, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic acid and boninic acid.

Distribution. Tropical in America, in plateau region (Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004).

Ramalina cochlearis is the unique Brazilian *Ramalina* species that produces soredia originating from a helmet-shaped soralia (Kashiwadani & Kalb 1993).

Specimens examined: BRAZIL. State of Espírito Santo, Municipality of Rio Preto, Parque Nacional de Caparaó, on trunks, margins of the road, 06.II.1999, *W.B. Sanders 99206.9A* (UFP). State of Minas Gerais, Municipality of Ouro Preto, Parque Estadual de Itacolomi, on trunk, 03.II.1999, *W.B. Sanders 99203.16B, 99202.3A* (UFP); Municipality of Caeté, Serra da Piedade, 17.XI.1986, *S. Eliasaro 157, 231, 240* (BHCB), 15.VII.1987, *S. Eliasaro 345* (BHCB). State of Rio Grande do Sul: Municipality of Cambará do Sul, Parque Nacional Aparados da Serra, 00.10.1970, *J. Goergen s.n.* (ICN 73540); Municipality of Maquiné, *N. M. Koch 326* (HAS 49138).

Ramalina complanata (Sw.) Ach., *Lichenographia Universalis*: 599. 1810.

Basionym: *Lichen complanatus* Sw. in Ach., *K. Vet. Nya. Handl.* 18: 290. 1797.

Type: India occidentalis, Jamaica, *Swartz* (S—holotype; H-ACH 1821!—isotype).

MB#403682

Fig. 2B.

Thallus caespitose, corticolous, up to 7 cm long, stramineous whitish in field, dark stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 4.0 mm in diameter; branching irregularly, almost densely branched, solid branches, mainly flat, dorsiventral, almost but not clearly canaliculate, 1.0–3.0 mm wide, surface smooth to rugose, sometimes sulcate, almost scrobiculate; symbiotic propagules absent; pseudocyphellae laminal and marginal, raised, weakly to clearly tuberculate, 0.05–0.2 mm wide, 0.05–0.2 mm tall. Cortex distinct, hyaline, 10–18 μm thick, chondroid tissue weakly to distinctly cracked, discontinuous, (50–)100–220 μm thick, algal cells dispersed in medulla, medulla whitish, loose. Apothecia rounded, subterminal to terminal, up to 4.0 mm in diameter, mainly concave, anfithecia usually with tuberculate pseudocyphellae, margin usually entire, concolorous with the thallus, disc beige, weakly pruinous; epihymenium stramineous to brownish, 10–14 μm thick, hymenium hyaline, 25–30 μm thick, hipohymenium yellowish, 30–50 μm thick, with chondroid tissue under de hypothecium, 25–35 μm thick, algal layer discontinuous, 20–50 μm thick; ascospores 1-septate, straight to slightly curved, 12–16 \times 4–5 μm . Pycnidia not found.

Chemistry. Spot tests: Cortex: K-, C-, KC-, UV-. Medulla: K- or K+ yellow→red, C-, KC-, UV-. TLC: (Race 1) Usnic acid and salazinic acid. (Race 2) Usnic acid and divaricatic acid.

Distribution: Tropical in America, from sea level up to 800 m alt. (e.g., Kashiwadani & Kalb 1993).

Ramalina complanata is characterized by the flat to subcanaliculate branches with tuberculate pseudocyphellae, by the cracked chondroid tissue, and by the production of divaricatic acid and/or salazinic acid.

Similar with *R. aspera* and *R. canalicularis* (see comments under those species).

Selected specimens examined: BRAZIL. State of Bahia, Municipality of Euclides da Cunha, along road from Canudos to Euclides da Cunha, about 45 km south of Canudos, dense moist Caatinga shrubs 1-4m tall, 10°16-17'S, 39°4'W, 18.IV.1962, *G. Eiten & L.T. Eiten*, 5010 (SP). State of Espírito Santo, Municipality of Piuma, 15.IV.1965, *D.M. Vital* 398 (SP). State of Paraná, Municipality of Guaraqueçaba, Parque Nacional do Superagui, 10.IV.2003, *S. Eliasaro & C. G. Donha* 2694 (UPCB), Ilha das Peças, 20.III.2010, *E. Gumboski & F. Beilke* 1821 (JOI); Municipality of Paranaguá, Ilha do Mel, corticolous, 27.VIII.2009, *S. Eliasaro & E. Gumboski* 3134 (UPCB); Municipality of Matinhos, Praia de Caiobá, 03.II.2011, *E. Gumboski; A. C. L. Gerlach & F. Beilke* 2270 (JOI). State of Pernambuco, Municipality of Caruaru, Estação Experimental da Empresa Pernambucana de Pesquisa Agropecuária; Caatinga; on twigs, with *Tillandsia* sp., corticolous, 08.XII.1999, *W.B. Sanders*, 99D08.1, 99D08.4B, 99D08.4B (UFP); Municipality of Poção, Mata de altitude, VIII.1994, *E. Pereira s.n.*, (UFP 28299). State of Rio Grande do Sul, Municipality of Porto Alegre, Jardim Botânico, 21.09.2008, *F. Lucheta* 18 (HAS); Municipality of Itapuã, praia das Pombas, 16.09.2004, *S. M. Mazzitelli & A. Lemos* 113 (HAS); Municipality of Itaquí, Arroio do Salso, margins of road, riparian forest, 08.09.1994, *M. Fleig* 6461 (HAS); Municipality of Caçapava do Sul, grassland, 06.12.1993, *M. Fleig* 6029 (HAS); Municipality of Viamão, Lombas, Capão da Porteira, 26.06.1983, *M. Fleig* 1989 (HAS). State of Santa Catarina, Municipality of São Francisco do Sul, Baía da Babitonga, Ilha dos Papagaios, 16.XI.2009, *F. Woitexem s.n.* (UPCB), Praia de Capri, 01.VIII.2008, *E. Gumboski* 1141 (JOI); Municipality of Florianópolis, Praia do Campeche, corticolous, c. 50m from the sea, 4.XI.2000, *L. M. C. Cordeiro s.n.* (UPCB), Lagoa do Peri, 01.IX.2008, *E.*

Gumboski 1028 (JOI). State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, *E. Gumboski 4784* (ICN).

Ramalina continentalis Malme, Ark. Bot. 26A(12): 7a. 1935.

Type: Paraguay, Paraguari, Cerro Negro, in rupibus praeruptis, apricis, 1/8 1893, *G. O. A. Malme 1479* (S-L3535—holotype, Photo!; UPS!—isotype).

MB#411740

Fig. 2C.

Thallus caespitose, saxicolous, up to 9.0 cm long, yellowish green in field, dark stramineous in herbaria, growing from a broad holdfast, concolorous with the thallus, up to 5.0 mm in diameter; branching irregularly to dichotomously anisotomic, solid branches, flat, lanceolate, rarely weakly canaliculate, 1.0–15.0 mm wide, surface smooth to furrowed, sometimes almost foveolate in broad branches; symbiotic propagules absent; pseudocyphellae laminal to marginal, linear, abundant, at level surface to slightly depressed, laminal pseudocyphellae 0.8–1.5 × 0.05–0.07 mm, marginal pseudocyphellae long linear along the branches, up to 0.1 mm wide. Cortex almost indistinct, up to 3.0 µm thick, chondroid tissue not cracked, mainly continuous, 50–120 µm thick, algal cells dispersed in medulla, medulla whitish, loose. Apothecia rounded, mainly marginal to laminal, up to 2.0 mm in diameter, flat to convex, amphithecia smooth, margin entire, orange to stramineous, disc whitish to beige, pruinous; epihymenium blackish, 5–8 µm thick, hymenium hyaline, 45–55 µm thick, hipohymenium yellowish, 25–35 µm thick, with continuous chondroid tissue under de hypothecium, 40–90 µm thick, algal layer continuous, 40–60 µm thick; ascospores 1-septate, short-fusiform, mainly straight to rarely slightly curved, 12–15 × 4–5 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution. Known only to Paraguay and Midwestern Brazil, in plateau region (Malme 1934).

Ramalina continentalis is characterized by the caespitose thallus with flat and lanceolate branches, broad branches, saxicolous habit, abundant laminal/marginal linear pseudocyphellae, ascospores with 12–15 µm long, and absence of medullar acids.

The saxicolous habit together with lanceolate and broad [up to 15 mm wide] branches, added and occurrence in plateau regions, distinguish this species from all other American *Ramalina* species.

Specimens examined: BRAZIL. State of Mato Grosso, Municipality of Rondonópolis, Fazenda Verde, Parque Ecológico João Passo, saxicolous, VII.2000, *G. Ceccantini s.n.* (UPCB); Municipality Santo Antonio pr. Cuyabá, in rupibus praeruptis satis apricis, 25.IV.1894, *G.O. Malme s.n.* (UPS L-46976). State of Mato Grosso do Sul, Municipality of Aquidauana, encosta da Serra de Maracaju, near Acampamento Batista in Piraputanga, 216 m alt., 20°27'23.6''S, 55°29'16.3''W, saxicolous, 26.XI.2014, *A.A.Spielmann 11852* (CGMS, JOI).

Ramalina dendriscoides Nyl., *Flora* (Regensburg) 59: 412. 1876.

Type: Cuba, *C. Wright*, *Lich. Cub. Ser. 2*, 738 (H-NYL 37025— lectotype; FH, G!— isotypes).

MB#403700

Fig. 2D.

Thallus shrubby, corticolous, up to 9 cm high, greenish to whitish in field, stramineous in herbaria, growing from a restricted or common holdfast, concolorous with the thallus to blackish, up to 5.0 mm in diameter; branching dichotomously anisotomic to irregularly, solid branches, main branches irregularly flat to irregularly cylindrical, 0.5–1.0 mm wide, secondary branches mainly terete, 0.2–0.5 mm wide, surface smooth, slightly striate, rarely with depressions remembering some canaliculated branches; soralia mainly terminal in secondary branches, soredia 0.02–0.05 mm in diameter; pseudocyphellae orbicular to ellipsoid, 0.1–0.5 × 0.05–0.1 mm, scattered, not depressed. Cortex hyaline, 15–20 µm thick, chondroid tissue cracked, discontinuous, 40–80 µm, algal cells dispersed in medulla, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry. Spot tests: Cortex K-, C-, KC-, UV-. Medulla: K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution. Tropical in America and Africa, from sea level up to 1000 m alt. (Kashiwadani & Kalb 1993; Swinscow & Krog 1988).

Ramalina dendriscoides is characterized by the shrubby thallus, mainly branching anisotomic dichotomously, mainly terminal soralia in secondary branches, distinct cracked chondroid tissue, and by the production of medullar salazinic acid.

This species could be confused with *R. peruviana* and *R. soresdiosa* by the similar thallus and sorediate branches. However, both *R. peruviana* as *R. soresdiosa* have not cracked chondroid tissue and have laminal soralia (e.g., Stevens 1987; Kashiwadani & Kalb 1993; Gumboski *et al.* B).

Specimens examined: BRAZIL. State unknown, 02.XI.1977, *H. Schindler* 7082 (SP 128980). State of Paraná, Municipality of Rio Branco do Sul, on *Araucaria angustifolia*, 15.V.2001, *L. M. C. Cordeiro s.n.* (UPCB 46289); Municipality of Pontal do Paraná, Pontal do Sul, restinga, 5.IX.2007, *M. Dal-Forno* 175b (UPCB); Municipality of Guaraqueçaba, Reserva Natural de Itaquí, mangrove, 17.II.2004, *R. Reis* 275 (UPCB). State of Rio Grande do Sul: Municipality of Barracão, Espigão Alto, mata aberta, zona úmida, corticolous, 30.10.1988, *M. Fleig* 3719 (ICN). State of Santa Catarina, Municipality of São Francisco do Sul, Parque Estadual do Acaraí, Restinga arbustiva, saxicolous, 29.III.2009, *E. Gumboski & S. Eliasaro* 1421 (JOI), 23.IX.2011, *E. Gumboski* 2743 (JOI).

Ramalina dendroides (Nyl.) Nyl., *Flora*, (Regensburg) 26: 412. 1876.

Basionym: *Ramalina rigida* f. *dendroides* Nyl., *Bull. Soc. Linn. Normandie* 4: 112. 1870.

Type: Martinique Island, *Husnot* 460 (H-NYL 37028!—holotype).

MB#403701

Fig. 2E.

Thallus pendulous, corticolous or saxicolous, up to 10 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 4.0 mm in diameter; branching dichotomously, solid branches, flat, 0.3–2.0 mm wide, surface smooth; soralia mainly marginal, orbicular to elongate, erumpent, 0.3–1.0(–2.0) × 0.15–0.30 mm, soredia 300–500 µm in diameter, in old branches the soralia can developed on lamina, usually near the basal parts; pseudocyphellae marginal and laminal, continuous and linear on margins, depressed, orbicular to ellipsoid on lamina, at level surface, mainly in the basal part of the main

branches, 0.1–0.6 × 0.1–0.2 mm. Cortex hyaline, 10–16 µm thick, chondroid tissue cracked, continuous, 40–140 µm thick, algal layer continuous, 20–50 µm thick, medulla white, compact. Apothecia not found. Pycnidia not found.

Chemistry. Spot tests: Cortex K-, C-, KC-, UV-. Medulla: K+ yellow→red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution. Tropical in Central and South America, from sea level up to 800 m alt. (Kashiwadani & Kalb 1993).

Ramalina dendroides is characterized by the pendulous with flat branches, by the mainly marginal soralia, distinct cracked chondroid tissue, and by the production of salazinic acid.

This species differ from the other pendulous Brazilian *Ramalina* [*R. anceps*, *R. kashiwadanii*, *R. sprengelii*, and *R. usnea*] mainly by the presence of soralia on margins (Kashiwadani & Kalb 1993; Gumboski *et al.* A).

Specimens examined: BRAZIL. State of Minas Gerais, Municipality of Itamonte, Parque Nacional das Agulhas Negras, Estrada das Prateleiras, 03.X.1997, *M.P. Marcelli & O. Vitikainen 32415* (SP). State of Paraná, Municipality of Guaraqueçaba, Reserva Natural de Itaqui, mangrove, 17.II.2004, *R. Reis 274, 298a* (UPCB), Municipality of Paranaguá, Ilha do Mel, corticolous, 27.VIII.2009, *S. Eliasaro & E. Gumboski 3169* (UPCB). State of Santa Catarina: Municipality of São Francisco do Sul, Morro da Enseada, on rocky shore, saxicolous, 05.V.2008, *E. Gumboski 243* (JOI), Morro da Enseada, east, 22.IX.2011, *E. Gumboski 2641, 2647, 2722* (JOI).

Ramalina exiguella Stirt., Transactions and Proceedings of the Royal Society of Victoria 17: 68. 1881.

Type: Queensland, Brisbane, *I. M. Bailey 91* (GLAM— holotype).

MB#403713

Fig. 2F.

Thallus shrubby to subpendulous, corticolous, up to 2.0 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 2.0 mm in diameter; branching dichotomously anisotomic, solid branches, subterete to angular, 0.15–0.50 mm wide, surface smooth to weakly furrowed; symbiotic propagules absent; pseudocyphellae laminal, linear, at level

surface, 0.5–1.5 × 0.05–0.10 mm. Cortex absent, chondroid tissue not cracked, continuous, 30–100 µm thick, algal layer continuous, 30–50(–70) µm thick, medulla beige, compact. Apothecia rounded, laminal, up to 1.5 mm in diameter, geniculate branches, concave to mainly flat, amphithecia smooth, margin entire, orange to stramineous, disc whitish to beige, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline, 40–50 µm thick, hipohymenium yellowish, 20–25 µm thick, with continuous chondroid tissue under de hypothecium, 13–25 µm thick, algal layer discontinuous, 30–80 µm thick; ascospores 1-septate, straight, 13–15 × 7–8 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution. Tropical in Central and South America, Africa, and Australia, in coastal plain (Kashiwadani & Kalb 1993; Krog & Swinscow 1976; Stevens 1987).

Ramalina exiguella is characterized by the shrubby thallus with subterete to angular branches, absence of soralia, not cracked chondroid tissue, and absence of medullar acids.

This coastal species is morphologically similar with *R. gracilis* and both only produce usnic acid. However, *R. gracilis* has a distinct cracked chondroid tissue, and whitish and loose medulla, while *R. exiguella* has a not cracked chondroid tissue, and has beige and compact medulla (Kashiwadani & Kalb 1993; Krog & Swinscow 1976; Stevens 1987).

Selected specimens examined: BRAZIL. State of Rio Grande do Sul, Municipality of Porto Alegre, Jardim Botânico, 21.X.2008, *F. Lucheta s.n.* (HAS 48159); Municipality of Mostardas, Lagoa do Peixe, 15.04.1991, *S. M. Mazzitelli s.n.* (HAS 33494); Municipality of Rio Grande, Estação Ecológica Taim, 13.XI.1980, *L. Cestaro s.n.* (HAS 54200); Municipality of Torres, on dunes, near the vegetation, 13.XI.1980, *M. Fleig 1107* (HAS), at margins of Cemetery road, coastal, corticolous, on wood, 30.IX.2012, *E. Gumboski 4061, 4068* (JOD). State of Santa Catarina, Municipality of Laguna, Morro Nossa Senhora da Glória, 22.I.1984, *Krapovickas y Cristóbal 39373* (CTES); Municipality of Florianópolis, Praia dos Ingleses, 21.IV.1983, *I.C. Pereira s.n.* (MPUC 4322); Municipality of Bombinhas, 02.II.1984, *O. Ra, s.n.* (MPUC 4325); Municipality of Balneário Gaivota, coastal, corticolous, on palm tree, 30.IX.2012, *E. Gumboski 4081* (JOD); Municipality of Joinville, Bom Retiro, Univille, urban area, corticolous, Palmeira

real (*Archontophoenix cunninghamii*), 07.XII.2011, E. Gumboski, J. C. S. Silva & K. Z. Ferreira 3333 (JOI).

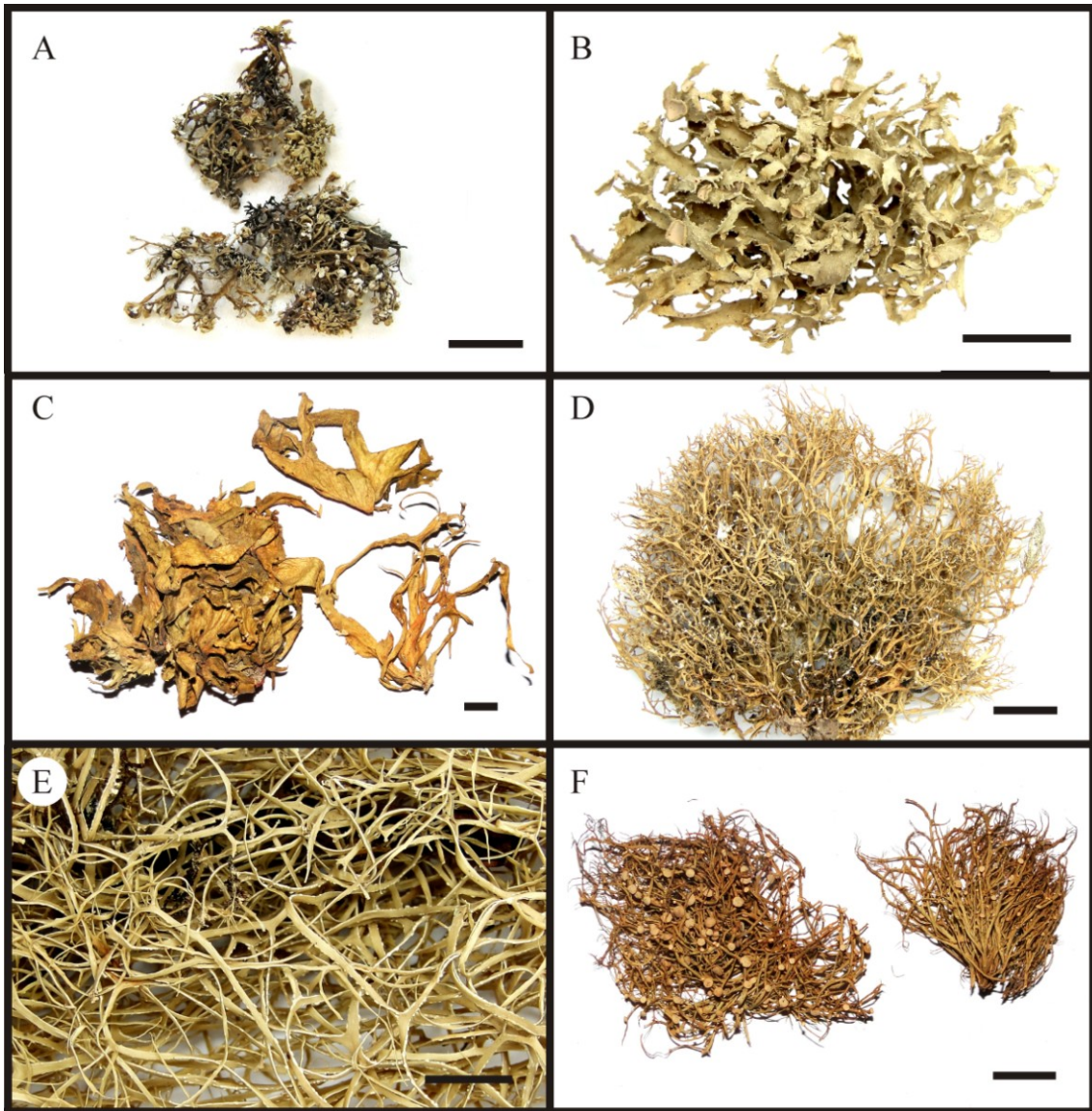


Fig. 2. A–F. A – *R. cochlearis* (S. Eliasaro 157); B – *R. complanata* (S. Eliasaro & C. G. Donha 2694); C – *R. continentalis* (A.A. Spielmann 11852); D – *R. dendriscooides* (UPCB 46289); E – *R. dendroides* (R. Reis 274); F – *R. exiguella* (E. Gumboski 4085).

Ramalina fleigiae Gumboski, Eliasaro & R. M. Silveira, *The Lichenologist* XX: XX. 2016.

Mycobank No.: MB XXXXX

Barcoding GenBank No.: XXXXXX

Typus: BRAZIL. State of Rio Grande do Sul, Municipality of São José dos Ausentes, locality of ‘Cachoeirão dos Rodrigues’, on rock in the middle of Silveira River, ca. 1150

m alt., 28°35'59.85"S, 49°59'19.89"W, 20.I.2015, *E. Gumboski 5050* (ICN!—holotype; UPCB!, SP!, H!, F!—isotypes).

Fig. see Gumboski *et al.* (D).

Thallus saxicolous, shrubby, up to 5.0 cm tall and 6.0 cm wide, whitish green in the field, becoming stramineous in the herbarium; densely branched in their upper half in best developed thalli; branches originating from a single holdfast, consisting of dense groups of 2–7 branches rooting in a common, necrotic base of up to 0.7 cm high; branching dichotomously anisotomic; main branches ramify mainly from the upper half; branches solid, rigid, irregularly flat to subterete, somewhat inflated, but not hollow, distinctly flattened mainly at their tips; main branches 0.4–1.5 × 0.2–1.1 mm broad; secondary branches 0.15–0.6 × 0.1–0.5 mm wide; apex somewhat truncated to slightly acute, sometimes curved as a hook-shape; surface opaque, irregular, ±striate due the presence of abundant pseudocyphellae resembling maculae; symbiotic propagules absent; pseudocyphellae numerous on lamina and margins, present across both the main and secondary branches, depressed to on level surface, irregular ellipsoid to shortly linear, 0.15–1.0 × 0.05–0.15 mm, rarely orbicular, 0.1–0.2 mm diameter; cortex distinct, 10–35 µm thick; chondroid tissue not cracked, discontinuous, in irregular bundles intermixed with medulla (but sometimes the majority portion of the branches); main branches 80–620(–700) µm thick; secondary branches usually with one ±circular to irregular bundle, 30–210 µm thick; medulla compact; algal layer below the cortex and around the chondroid tissue, 15–70 µm thick. Ascomata and conidiomata not found.

Chemistry. Cortex and medulla: K-, C-, KC-, PD-, UV-. TLC: usnic acid.

Distribution. Endemic from few localities from high altitudes grassland in Southern Brazil (Gumboski *et al.* D).

Ramalina fleigiae is characterized by the saxicolous habitat, branches originating from the same base and the densely branched upper half, irregularly flat to subterete branches, abundant laminal and irregular ellipsoid to short linear pseudocyphellae, not cracked chondroid tissue, and absence of medullar acids.

This species is similar with *R. exiguella* and *R. gracilis*, but both have angular in cross section branches, long linear pseudocyphellae, and absence of a cortex. Added these two species are strictly coastal (Fleig 1988; Kashiwadani & Kalb 1993; Gumboski *et al.* D).

Selected specimens examined: BRAZIL. State of Rio Grande do Sul, Municipality of Cambará do Sul, Cachoeira dos Venâncios, on rock, 08.I.2000, *W.B. Sanders 00108.3B* (UFP 29592); Municipality of Jaquirana, locality of Cachoeira dos Venâncios, on rock, c. 850 m alt., 29°01'03.72"S, 50°15'31.55"W, 20.I.2015, *E. Gumboski 5054, 5055* (ICN), *E. Gumboski 5056, 5057* (SP).

Ramalina gracilis (Pers.) Nyl., *Synopsis Methodica Lichenum* 1: 296. pl. 8, fig. 30. 1860.

Basionym: *Physcia gracilis* Pers. in Gaudich, *Voyage Uranie, Bot.*: 209. 1826.

Type: Brazil, Rio de Janeiro (L?; PC?).

Synonym: *Ramalina bicolor* Müll. Arg., *Flora, Regensburg* 60: 476. 1877. (G!—holotype). *Ramalina costata* Meyen & Flot., *Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur., Suppl.* 1 19: 212. 1843. Type: unknown.

MB#403733

Fig. 3A.

Thallus shrubby to subpendulous, corticolous, up to 10 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching dichotomously anisotomic, solid branches, subterete to angular, 0.25–1.50 mm wide, apices usually blackish, surface smooth to furrowed; symbiotic propagules absent; pseudocyphellae laminal, linear, at level surface to depressed, 0.5–2.0 × 0.03–0.06 mm. Cortex absent, chondroid tissue distinctly cracked, mainly continuous, 20–100 µm thick, algal layer discontinuous, 30–50 µm thick, medulla whitish, loose. Apothecia rounded, laminal, up to 1.5 mm in diameter, geniculate branches, flat to mainly convex, amphithecia smooth, margin entire, concolorous with the thallus to stramineous, disc whitish to beige, pruinous; epihymenium blackish, 8–10 µm thick, hymenium hyaline, 45–55 µm thick, hipohymenium yellowish, 20–30 µm thick, with continuous chondroid tissue under de hypothecium, 50–90 µm thick, algal layer mainly continuous, 40–50 µm thick; ascospores 1-septate, broad ellipsoid, straight, 14–20 × 7–8 µm. Pycnidia laminal, globose, black, up to 200 µm wide, pale ostiole, conidia rod-shaped, 4–6 × 1 µm.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution. Tropical in Central and South America, in coastal plain (Kashiwadani & Kalb 1993). The specimens cited by Nylander (1890) from Madagascar and Australia were identified by Stevens (1987) as *R. exiguella*.

Ramalina gracilis is characterized by the shrubby thallus with subterete to angular branches, absence of symbiotic propagules, distinct cracked chondroid tissue, and absence of medullar acids.

This species is similar to *R. exiguella* (see comments under that species).

The holotype of *Ramalina bicolor* agree in morphology, anatomy, chemistry and distribution with *R. gracilis* as already point out by Kashiwadani & Kalb (1993). The name *R. costata* was considered synonym under *R. gracilis* due the description, image and distribution published in Meyen & Flotow (1843) which agree with the features of *R. gracilis*, and already point out by Nylander (1890). However, the name *R. costata* var. *compressa* Meyen & Flot. remain on doubt because the description remember the morphology of *R. aspera* or *R. complanata*.

Selected specimens examined: BRAZIL. State of Espírito Santo, Ilha do Francês [?], 17.IV.1965, *D.M. Vital 399* (SP). State of Paraná, Municipality of Matinhos, Caiobá corticolous, 22.V.1999, *Donha, Kretzel, Takemori & Vieira 123* (UPCB); Municipality of Pontal do Paraná, Balneário Jardim Albatroz, corticolous, 23.IV.2000, *F. L. Brock 17* (UPCB). State of Rio de Janeiro, Municipality of Rio de Janeiro, 00.X.1977, *G. Hilt s.n.* (MPUC 3892). State of Santa Catarina, Municipality of São Francisco do Sul, Baía da Babitonga, Ilha dos Papagaios, 16.XI.2009, *F. Woitexem s.n.* (UPCB). State of São Paulo, Municipality of Itanhaém, Suarão, costal vegetation, 28.VIII.1962, *D. Altimari s.n.* (SP 100210), 22.VII.1962, *D. Altimari s.n.* (SP 100100).

Ramalina kashiwadani Gumboski, Eliasaro & R. M. Silveira, Fungal Diversity XX. 2016.

MycoBank No.: MB XXXXX

Barcoding GenBank No.: XXXXXX

Typus: BRAZIL. State of Santa Catarina, Municipality of Rio Negrinho, locality of Rio dos Bugres, Araucaria Forest, corticolous, 07.IX.2012, *E. Gumboski 3946* (ICN!—holotype; JOI!, SP!, G!, H!—isotype).

Fig. see Gumboski *et al.* (A).

Thallus pendulous, corticolous, up to 35 cm long, green whitish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus to blackish, up to 4.0 mm in diameter; branching dichotomously, anisotomic, solid branches, main branches flattened to irregularly flat at the basal part, rarely weakly canaliculated, 0.5–1.0 mm wide, secondary branches flattened to irregularly cylindrical, 0.1–0.3 mm wide, not contorted, surface weak to clearly striated transversely; symbiotic propagules absent; in flat branches pseudocyphellae marginal and linear, flat to weakly depressed, 0.05–0.15 mm wide, rarely in irregular cylindrical branches pseudocyphellae are ellipsoid to short linear, 0.2–0.3 mm long and 0.05–0.10 mm wide. Cortex weakly distinguish, hyaline, 3–5 μm thick, chondroid tissue not cracked, continuous, 50–80 μm , algal layer discontinuous, up to 25 μm thick, medulla white, loose. Apothecia rounded, marginal, up to 1.5 mm in diameter, at first concave, flat when mature, then convex with age, branches weakly geniculate, anfithecia smooth, without pseudocyphellae, margin entire, concolorous with the thallus, disc beige weakly pruinous; epihymenium hyaline, 4–6 μm thick, hymenium hyaline, 40–53 μm thick, hypohymenium yellowish, 50–70 μm , without algal layer under the hypohymenium and at margins of apothecia; ascospores 1-septate, fusiform, straight, 13–16 \times 3–5 μm . Pycnidia not found.

Chemistry. Spot test: Thallus K-, C-, KC- or KC+ dirty yellow and UV-. Medulla: K-, C-, KC- and UV-. TLC: usnic acid.

Distribution. Known to Southern Brazil, from sea level up to 900 m alt. in Araucaria forest (Gumboski *et al.* A).

Ramalina kashiwadani is characterized by the pendulous thallus with flat branches, absence of symbiotic propagules, mainly marginal pseudocyphellae, continuous and not cracked chondroid tissue, and absence of medullar acids.

This species is similar with *R. sprengelii* in chemistry and thallus morphology. However, *R. sprengelii* has branches with continuous and linear pseudocyphellae on lamina and margins, and the chondroid tissue form almost cylindrical bundles of hyphae (Kashiwadani & Kalb 1993; Gumboski *et al.* A)

Ramalina anceps and *R. usnea* have branches with distinctly cracked chondroid tissue. Addicted, *R. anceps* produces norstictic acid and *R. usnea* produces homosekikaic and sekikaic or divaricatic acids (Rundel 1978; Kashiwadani & Kalb 1993; Gumboski *et al.* A).

Specimens examined: BRAZIL. State of Paraná, old Graciosa road, margins of the road, near the bridge, corticolous, 03.VIII.1999, *W.B. Sanders 99803.19A, 99803.18* (UFP). State of Rio Grande do Sul, Municipality of Rio Grande, Estação Ecológica do Taim, 3 Km near the Albardão forest, corticolous, 10 m alt., 11.XII.1985, *M. Fleig 2720* (ICN); Municipality of Amaral Ferrador, margin at Camaquã river, corticolous, 30 m alt., 12.VIII.1993, *M. Fleig 5874* (ICN). State of Santa Catarina, Municipality of Rio Negrinho, Fazenda Velha, Araucaria forest, corticolous, 21.VIII.2010, *E. Gumboski 2199* (JOI), locality of Volta Grande, Araucaria Forest, corticolous, 20.I.2012, *E. Gumboski 3410* (ICN).

Ramalina lacera (With.) J.R. Laundon, *The Lichenologist* 16 (3): 221. 1984.

Basionym: *Lichen lacerus* With., *Bot. Arr. Veg. Gr. Brit.*: 716. 1776.

Type: Dillenius (1742: 163): tab. 21, fig. 57B (OXF—lectotype, Photo!).

MB#106434

Fig. see Gumboski *et al.* (2014).

Thallus shrubby, saxicolous, up to 6.0 cm tall, pale stramineous in herbaria, growing from a common holdfast, concolorous with the thallus, up to 4.0 mm in diameter; irregularly palmate branched, dichotomous in narrow branches, solid branches, flat, bifacial, main branches up to 1.3 cm wide, terminal branches up to 1.0 mm wide, 200–500 µm thick, surface weakly to clearly scrobiculate; soralia at first marginal then becoming laminal, mainly in upper parts of thallus, laminal soralia growing mainly on top of the wrinkle, soredia (300–) 400–600 µm in diameter; pseudocyphellae very rare, orbicular, at level surface, up to 0.1 mm in diameter. Cortex dirty yellow, 25–45 µm thick, chondroid tissue absent, algal layer almost continuous, 50–60 µm thick, medulla white, compact. Apothecia rounded, concave to mainly flat, up to 5.0 mm in diam., with marginal to rarely submarginal soralia, up to 0.6 mm long, disc beige without pruinose; epihymenium brownish, 10–15 µm thick, hymenium hyaline, 35–40 µm thick, subhymenium hyaline to weakly grayish, 25–35 µm thick, algal layer under the hymenium present, continuous, 50–55 µm thick; ascospores hyaline, 1-septate, weakly curved to straight, 10–14 × 4–5 µm. Pycnidia not found.

Chemistry. Spot test: Thallus K-, C-, KC+ yellow, UV-. Medulla: K-, C-, KC- and UV-. TLC: usnic acid and bourgeanic acid.

Distribution. Pantropical, in plateau region (e.g., Rundel *et al.* 1972; Krog & James 1977; Rundel 1978; Krog & Østhaugen 1980; Sipman 2002; Kashiwadani & Nash 2004; Gumboski *et al.* 2014).

Ramalina lacera is the unique Brazilian species that don't has chondroid tissue and produces bourgeanic acid. Added the saxicolous habit with flat branches, scrobiculate surface, and laminal soralia; this species is easily distinguish from all other species recorded from Brazil.

Ramalina mollis Krog is very similar to *R. lacera* in their chemistry, morphology and ecological features. Krog and Østhaugen (1980) distinguished *R. lacera* from *R. mollis* by the presence of soralia and rare presence of apothecia in the former, and by the absence of soralia and to usual presence of apothecia in the latter species (Gumboski *et al.* 2014).

Specimens examined: BRAZIL. State of Rio Grande do Sul: Municipality of Bagé, Casa de Pedra, 14 Km in parallel road to highway BR 153, 210 m alt., 03.XI.1989, *M. Fleig* 3907 (ICN), 04.XI.1989, *M. Fleig* 4001 (ICN), 350 m alt., 15.XII.1989, *M. Fleig* 4144 (ICN); Municipality of Caçapava do Sul, Pedra do Segredo, undisturbed place, 27.XI.1993, *M. Fleig* 5948 (ICN), on rock protected by shrubs, 300 m alt., 15.IX.1998, *M. Fleig* 7247 (ICN).

Ramalina laevigata Fr., Syst. Orb. Veg. (Lundae) 1: 283. 1825.

Type: Insula Malovina (UPS?).

MB#403756

Fig. 3B.

Thallus caespitose, saxicolous, up to 10.0 cm long, whitish green to stramineous in field, stramineous in herbaria, growing from a narrow to broad holdfast, concolorous with the thallus, up to 5.0 mm in diameter; branching dichotomously anisotomic, very little branched, solid branches, flat, lanceolate, weakly to distinctly canaliculate, 0.5–2.0(–4.0) mm wide, surface smooth to furrowed; symbiotic propagules absent; pseudocyphellae mainly laminal, ellipsoid to short linear, abundant, at level surface, 0.2–0.5 × 0.05–0.10 mm. Cortex almost indistinct, c. 3 µm thick, chondroid tissue not cracked, discontinuous, 30–90 µm thick, algal layer almost continuous, 20–30 µm thick, medulla white, compact. Apothecia rounded, mainly marginal to occasionally laminal,

up to 2.0 mm in diameter, flat to mainly convex, amphithecia smooth, margin entire, orange to stramineous, disc concolorous with margin, without pruine; epihyemenium hyaline to stramineous, 5–8 μm thick, hyemenium hyaline, 40–50 μm thick, hipohyemenium yellowish, 10–20 μm thick, with continuous chondroid tissue under de hypothecium, 45–55 μm thick, algal cells randomly intermixed with chondroid tissue and medulla; ascospores 1-septate, short-fusiform, straight to curved, 12–15 \times 3–5 μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution. Known only to South America, in plateau region (Malme 1934; Kashiwadani *et al.* 2007).

Ramalina laevigata is characterized by the caespitose thallus with flat, lanceolate and canaliculated branches, saxicolous habit, absence of symbiotic propagules, laminal ellipsoid to short-linear pseudocyphellae, ascospores with 12–15 μm long, and absence of medullar acids.

This species is very similar in morphology, anatomy and chemistry with *R. celastri*, however, *R. laevigata* has a saxicolous habit usually growing near of rivers, the canaliculated branches grow from a shared holdfast, therefore, several branches originating from the same point, little branched. While *R. celastri* has a corticolous habit, the branches grow from a narrow holdfast, usually with one or few branches from the same point. The branches are flat and rarely somewhat canaliculated.

Specimens examined: BRAZIL. State of Rio Grande do Sul, Municipality of Bom Jesus, Rocinha, on river, 30.10.1983, *M. Fleig* 2228 (HAS); Municipality of Guaíba, 24.08.1985, *C. Grabauska* 224 (HAS); Municipality of São José dos Ausentes, Rio do Marco, margins of the river, 09.12.1994, *M. Fleig* 6657b (HAS), in metal handrails, High altitude fields, saxicolous, 28°40'16.97"S, 49°57'57.12"W, 19.I.2015, *E. Gumboski*, 5037, 5038, 5039, 5045, 5046 (JOI); Municipality of Jaquirana, Cachoeira dos Venâncios, 29°01'03.72"S, 50°15'31.55"W, 20.I.2015, *E. Gumboski* 5059 (JOI); Municipality of Esmeralda, Estação Ecológica de Aracuri, 11.12.1982, *M. Fleig* 1776 (HAS).

Ramalina peruviana Ach., Lichenographia Universalis: 599. 1810.

Type: Peru, *Lagasta* (H-ACH!—holotype; MEL! —isotype).

MB#403795

Fig. 3C.

Thallus shrubby, corticolous, up to 8 cm long, whitish green in field, stramineous in herbaria, growing from a common or delimited holdfast, concolorous with the thallus, up to 4.0 mm in diameter; branching irregularly to dichotomously anisotomic; solid branches, irregularly flattened in main branches becoming irregularly to terete in distal branches, 0.3–1.0 mm in wide, surface smooth to irregular, usually striate; soralia marginal and laminal, rarely orbicular, usually ellipsoid to rarely elongate, soredia abundant, 200–500(–700) μm in diameter, presence of cylindrical fibrils on soralia is common, up to 0.8 mm tall; pseudocyphellae laminal and marginal, at surface level, ellipsoid to short linear, $0.05\text{--}0.1 \times 0.2\text{--}1.5\text{(--}2.5)$ μm . Cortex hyaline, 5–7 μm , chondroid tissue not cracked, irregular, mainly continuous, 40–100 μm thick, algal layer discontinuous, 20–40 μm thick, medulla white, loose to compact. Apothecia not found. Pycnidia not found.

Chemistry. Spot tests: Cortex K-, C-, KC- or KC+ yellowish. Medulla: K- or K+ rose evanescent, C-, KC-. TLC: Usnic acid, homosekikaic acid, and sekikaic acid.

Distribution. Pantropical, from sea level up to 2000 m alt., but mainly in plateau region (e.g., Krog & Swinscow 1976; Stevens 1987; Kashiwadani & Nash 2004).

Ramalina peruviana is characterized by the shrubby thallus with irregular flat to irregular terete branches, laminal and marginal soralia, not cracked chondroid tissue, and by the production of homosekikaic and sekikaic acids.

This species could be confused with *R. dendriscoides* (see comments under that species).

Similar with *Ramalina solediosa* due the shrubby thallus with not cracked chondroid tissue, and laminal soralia. However, *R. solediosa* has mainly terete branches, and produces salazinic acid (Kashiwadani & Kalb 1993).

Selected specimens examined: BRAZIL. State of Paraná, Municipality of São José dos Pinhais, at margins of Iguaçu river, 23.01.1985, *M. Fleig* 2660 (HAS); Municipality of Curitiba, Parque ADEA, at margins of Iguaçu river, 23.01.1985, *M. Fleig* 2640 (HAS). State of Pernambuco, Municipality of São Vicente Férrer, Mata do Estado, on trunk

near the river, corticolous, 16.XI.1998, *W.B. Sanders 98n16.15D* (UFP). State of Rio de Janeiro, Municipality of Macaé, Serra de Macaé, V.1910, *s.col. s.n.* (SP 34332). State of Rio Grande do Sul, Municipality of Porto Alegre, Jardim Botânico, 21.10.2008, *F. Lucheta 23* (HAS); Municipality of Montenegro, 08.11.1979, *L. W. Aguiar 441* (HAS); Municipality of Torres, morro do Farol Sul, 26.04.1983, *Osorio & M. Fleig 2T/27* (HAS); Municipality of Viamão, Lombas, Capão da Porteira, 26.06.1983, *M. Fleig 1985* (HAS). State of Santa Catarina, Municipality of Rio Negrinho, Rio dos Bugres, corticolous, 20.VII.2012, *E. Gumboski 3706* (JOI); Municipality of Itaiópolis, at margins of the BR 116 road, corticolous, 23.IX.2012, *E. Gumboski 3989* (JOI); Municipality of Major Vieira, parallel road of BR 116, corticolous, 23.IX.2012, *E. Gumboski 4036b* (JOI); Municipality of Mafra, near BR 280 road, corticolous, 23.IX.2012, *E. Gumboski 4047* (JOI). State of São Paulo, Municipality of São Paulo, Distrito de Parelheiros, Itaim road, Rodoanel trecho sul 4, 25.IX.2007, *O. Yano & L.S. Inácio 30015* (SP). State of Sergipe, Municipality of Nossa Senhora da Glória, locality of Povoado Retiro 1, Assentamento Nossa Senhora Aparecida, 175 m alt., Caatinga, corticolous, 10°06'13.5"S, 37°25'12.9"W, 19.IX.2013, *E. Gumboski, 4857, 4862, 4863* (ICN).

Ramalina prolifera Taylor, London Journal of Botany 6: 189. 1847.

Type: Uruguay River, *James Baird* (FH—holotype; BM, Photo!, G—isotypes)

MB#403805

Fig. 3D.

Thallus caespitose to subpendulous, corticolous, up to 15.0 cm long, bluish green in field, weakly stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 2.0 mm in diameter; branching dichotomously anisotomic to irregular, solid branches, flat, lanceolate, 0.3–20.0 mm wide, surface smooth to furrowed; symbiotic propagules absent; pseudocyphellae laminal, orbicular to irregularly orbicular (almost elliptic), abundant, at level surface to weakly depressed, 0.05–0.20 mm in diameter. Cortex absent, chondroid tissue not cracked, irregular, mainly continuous, 20–60 µm thick, algal layer mainly continuous, 20–40 µm thick, medulla white, loose. Apothecia rounded, laminal, up to 1.0 mm in diameter, concave to mainly flat, amphithecia smooth, margin entire, yellowish, disc beige, slightly pruinous; epihymenium hyaline, 8–12 µm thick, hymenium hyaline, 50–60 µm thick,

hipohyemium weakly brownish, 10–25 µm thick, with continuous chondroid tissue under de hypothecium, 50–80 µm thick, algal layer discontinuous, 10–40 µm thick; ascospores 1-septate, short-fusiform, straight to slightly curved, 14–18 × 4–6 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution: Known only to Argentina, Brazil and Uruguay (Malme 1934; Kashiwadani & Kalb 1993).

Ramalina prolifera is characterized by the caespitose to subpendulous thallus with flat and lanceolate branches, absence of symbiotic propagules, orbicular to irregularly orbicular laminal pseudocyphellae, not cracked chondroid tissue, and absence of medullar acids.

Similar with *R. celastri* (see comments under that species).

Selected specimens examined: BRAZIL. State of Rio Grande do Sul, Municipality of Arroio do Tigre, Barragem de Itaúba, April.1978, *Arno Lise s.n.* (CTES 581229); Municipality of Canela, Usina dos Bugres, 28.X.1997, *C. Mansan s.n.* (HAS 36127); Municipality of São Francisco de Paula, Passo do S, 30.I.2006, *S. A. Martins s.n.* (HAS 46120); Municipality of Viamão, Fazenda Refúgio, 27.VIII.1992, *S. M. Mazzitelli s.n.* (HAS 33507); Municipality of Amaral Ferrador, margem afluyente rio Camaquã, riparian forest, ramos de arbustos, 12.VIII.1993, *M. Fleig 5871* (HAS); Municipality of Taquara, margem rio dos Sinos, 14.I.1991, *M. Fleig & S. Eliasaro 2* (HAS); Municipality of Itaqui, 2º Distrito, Mariano Pinto, Fazenda Bola de Ouro, 08.IX.1994, *M. Fleig 6467* (HAS); Municipality of Lavras do Sul, divisa com Caçapava do Sul, Arroio do Hilário, margem, sobre ramos de sarandi, zona inundável, 07.XII.1993, *M. Fleig 6065* (HAS); Municipality of Caçapava do Sul, fazenda dos irmãos Dotto, ao sul da cidade, parte baixa, campo de pastagem, 06.XII.1993, *M. Fleig 6036* (HAS). State of Santa Catarina, Capinzal, Lageado Barra Grande, Arroio Santa Cruz, 08.IX.1988, *C.S.A. Martins s.n.* (MPUC 12591).

Ramalina puiggarii Müll. Arg., Flora, (Regensburg) 64: 83. 1881.

Type: Brazil, Crescit prope Apiahi, March 1880, *J.-J. Puiggari* (G!— holotype).

MB#403808

Fig. 3E.

Thallus shrubby, corticolous, up to 6.0 cm long, greenish to whitish in field, weakly stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 2.5 mm in diameter; branching dichotomously anisotomic, rarely irregular, solid branches, flat, canaliculate, 0.4–1.5 mm wide, surface smooth to weakly furrowed; symbiotic propagules absent; pseudocyphellae marginal, discrete, linear, at level surface, up to 0.05 mm wide. Cortex absent, chondroid tissue not cracked, continuous, 30–90 μm thick, algal cells dispersed in medulla, medulla white, loose. Apothecia rounded, submarginal, up to 1.7 mm in diameter, geniculate branches, concave to mainly flat, amphithecium smooth, slightly furrowed, margin entire, orange to stramineous, disc beige, pruinous; epihymenium blackish, 8–10 μm thick, hymenium hyaline, 50–65 μm thick, hipohymenium yellowish, 50–60 μm thick, with continuous chondroid tissue under de hypothecium, 20–50 μm thick, algal cells randomly intermixed with chondroid tissue and medulla; ascospores 1-septate, straight, 18–25 \times 4–6 μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: usnic acid.

Distribution. Tropical in Central and South America, in plateau region (Kashiwadani & Kalb 1993).

Ramalina puiggarii is characterized by the shrubby thallus with canaliculated branches, absence of symbiotic propagules, not cracked chondroid tissue, and by the long-fusiform ascospores with 18–25 μm long.

This species is similar with *Ramalina celastri* (see comments under that species).

Specimens examined: BRAZIL. State of Minas Gerais, Municipality of Caeté, Serra da Piedade, 17.XI.1986, *S. Eliasaro* 226 (BHCB), 15.VII.1987, *S. Eliasaro* 372 (BHCB). State of Santa Catarina, Municipality of Campo Alegre, Campos do Quiriri, High altitude fields, corticolous, 17.XI.2012, *E. Gumboski* 4136, 4175 (JOI).

Ramalina pusiola Müll. Arg., Flora, (Regensburg) 73: 388. 1890.

Type: Tanzania, suden von Usambara, Tumakanya, *Dr. H. Meyer* (*Stein no. 12*)
(WRS�—lectotype)

MB#403812

Fig. 3F.

Thallus caespitose, corticolous, up to 6.0 cm long, greenish to stramineous in field, stramineous in herbaria, growing from a broad base, concolorous with the thallus, up to 1.5 cm wide; branching irregularly, hollow branches, irregularly flattened, 0.5–3.0 mm thick, surface smooth; symbiotic propagules absent; pseudocyphellae absent; perforations present mainly in under surface, orbicular to irregularly orbicular, entire, 0.3–0.8 mm in diameter; medulla white, loose, somewhat arachnoid, continuous. Cortex absent, chondroid tissue not cracked, continuous, 20–90 μm thick, algal layer almost continuous, 10–20 μm thick. Apothecia rounded, subterminal to terminal, up to 3.0 mm in diameter, mainly concave, amphithecia smooth, without pseudocyphellae, margin entire, without spurs, concolorous with the thallus to almost orange, disc whitish, pruinous; epihymenium blackish, 8–10 μm thick, hymenium hyaline, 25–30 μm , hipohymenium yellowish, 10–12 μm thick, chondroid tissue under the hypothecium, continuous, 10–15 μm thick, algal cells dispersed in medulla; ascospores 1-septate, short-fusiform, straight to weakly curved, 10–16 \times 4–5 μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC-, UV-. Medulla: K-, C-, KC-, UV-. TLC: Usnic acid, homosekikaic acid, and sekikaic acid.

Distribution. Tropical in Central and South America, in plateau region (Kashiwadani & Kalb 1993).

Ramalina pusiola is characterized by the hollow branches with orbicular to irregularly orbicular perforations, and by the production of homosekikaic and sekikaic acids.

This species is very similar morphological and anatomically with *R. calcarata* (see discussion under that species).

Specimens examined: BRAZIL. State of Paraná, Municipality of São José dos Pinhais, at margins of Iguaçu river, 23.I.1985, *M. Fleig s.n.* (ICN 173733); Municipality of Curitiba, Conjunto Solar, corticolous, 5.VII.2003, *C. G. Donha 790* (UPCB); Municipality of Campo Magro, on Atlantic Forest, 24.V.2003, *C. G. Donha 884*

(UPCB); Municipality of Ponta Grossa, Buraco do Padre, corticolous, 10.VII.1999, C. G. Donha, Kretzl, Takemori, Vieira 161 (UPCB). State of Rio Grande do Sul, Municipality of Esmeralda, Estação Ecológica Aracuri, 17.IX.1983, M. Fleig 2072 (ICN). State of Santa Catarina, Municipality of Rio Negrinho, Fazenda Velha, rural area, corticolous, 11.IV.2009, E. Gumboski 951, 01.XI.2008, E. Gumboski 1182 (JOI), 31.I.2010, E. Gumboski 1788, 1789 (JOI).



Fig. 3. A–F. A – *R. gracilis* (Donha et al. 123); B – *R. laevigata* (E. Gumboski 5035); C – *R. peruviana* (E. Gumboski 3706); D – *R. prolifera* (E. Gumboski 5043); E – *R. puiggarii* (E. Gumboski et al. 2064); F – *R. pusiola* (C. G. Donha 884).

Ramalina rectangularis Nyl., Bull. Soc. linn. Normandie, sér. 2 4(2): 121. 1870.

MB#403814

Typus: Brazil, Bahia, *Blanchet* (G!, H-NYL 37509!, PC, UPS, W!—syntypes).

Fig. 4A–B.

Thallus pendulous, stramineous to almost weakly brown in herbaria, branching dichotomic anisotomic, solid branches, flat, distinctly rectangular in cross-section, 1.4–3.0 mm wide, not contorted, surface smooth to rarely weakly rough; symbiotic propagules absent; pseudocyphellae linear, continuous, marginal, depressed to weakly elevated, (0.4–)0.6–5.0(–10.0) × 0.2–0.7 mm, the protuberances at margins are verruciformis, sometimes reminding an stalked apothecia, 0.2–1.3 mm tall × 0.5–1.8 mm wide, it seems to have originated in pseudocyphellae, mainly marginal, rarely submarginal. Cortex distinct, yellowish, 15–20 µm thick, chondroid tissue distinctly cracked, continuous, 200–550 µm thick, algae dispersed throughout the medulla, medulla whitish, loose. Apothecia not found. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K+ yellow→red, C-, KC-. TLC: usnic acid, salazinic acid (*vide* Kashiwadani & Kalb 1993).

Distribution. Known only from the type collection from State of Bahiate, Northeastern Brazil (Kashiwadani & Kalb 1993).

Ramalina rectangularis is characterized by the distinctly rectangular branches in cross-section, distinctly cracked chondroid tissue, and by the production of salazinic acid in medulla. These characteristics combined turn the morphology of *R. rectangularis* unique when comparing with all recorded Brazilian species of *Ramalina*.

Specimen examined: syntypes (see above).

Ramalina solediosa (de Lesd.) Landrón, The Lichenologist 25 (1): 25. 1993.

Basionym: *Ramalina dasygoga* var. *solediosa* B. de Lesd., Rev. Bryol. Lichen. 7: 59. 1934.

Type: Cuba, Boqueron, Estacion Naval, *B. Hiorami* (FH—lectotype).

MB#358582

Fig. 4C.

Thallus shrubby to subpendulous, corticolous, up to 10 cm long, whitish in field, weakly stramineous in herbaria, growing from a common holdfast, concolorous with the thallus,

3.0 mm in diameter, branching dichotomously anisotomic to irregularly branched, solid branches, mainly terete with irregular thickness in main branches, slightly flattened on main branches near the base, 0.2–0.6 mm in wide, surface smooth; soralia laminal and terminar, mainly orbicular to rarely irregular ellipsoid, 0.1–0.3 mm in diameter, soredia 200–300 µm in diameter, cylindrical branchlets on soralia are common, usually with soredia in apical part; pseudocyphellae laminal, punctiform to short ellipsoid, at surface level, 0.03–0.08 mm wide. Cortex absent, chondroid tissue not cracked, continuous, 40–75 µm thick, algal layer discontinuous, 20–35(–50) µm, medulla white, loose. Apothecia not found. Pycnidia not found.

Chemistry: Spot tests. Cortex K-, C-, KC- or KC+ yellow, UV-. Medulla K+ yellow → red, C-, KC-, UV-. TLC: Usnic acid and salazinic acid.

Distribution: Tropical in America, from sea level up to 800 m alt. (Kashiwadani & Kalb 1993).

Ramalina solediosa is characterized by the shrubby to subpendulous thallus, mainly terete branches, laminal soralia, continuous and not cracked chondroid tissue, and by the production of salazinic acid.

The species is similar to *R. dendriscoides* and *R. peruviana* (see comments under those species).

Specimens examined: BRAZIL. State of Paraná, Estrada antiga da Graciosa, at margins of the road, near the bridge, river, corticolous, 03.VIII.1999, *W.B. Sanders 99803.13* (UFP); Municipality of Guaraqueçaba, near Pousada, corticolous, 7.VIII.2003, *C. G. Donha et al. 1038* (UPCB); Municipality of Curitiba, Parque Iguaçu, 17.VIII.1994, *D. Ramirez s.n.* (UPCB 35322); Municipality of Pontal do Paraná, Pontal do sul, restinga, 21.III.2006, *S. Eliasaro s.n.* (UPCB); Municipality of Paranaguá, Ilha do Mel, rocky shore, saxicolous, 27.VIII.2009, *E. Gumboski & S. Eliasaro 1629* (JOI), Restinga, corticolous, *E. Gumboski & S. Eliasaro IM56* (JOI). State of Pernambuco, Municipality of Poção, Brejo de altitude, pendent on twigs of *Caesalpinia pyramidalis* Tul. (catingueira), corticolous, 27.VI.1998, *S. Ribeiro & E. Pereira s.n.* (UFP 28293). State of Santa Catarina, Municipality of Rio Negrinho, Rio Dos Bugres, Araucaria forest, corticolous, 01.XII.2008, *E. Gumboski 1049, 1073* (JOI); Municipality of Três Barras, margins of road SC-303, Araucaria forest, corticolous, 24.IX.2008, *E. Gumboski 1160* (JOD); Municipality of São Francisco do Sul, Parque Estadual do Acaraí, Restinga

arbuscula, corticolous, 29.III.2009, *E. Gumboski & S. Eliasaro 1404, 1405* (JOI). State of São Paulo, Municipality of São Paulo, Parque Estadual das Fontes do Ipiranga, Instituto de Botânica, 17.VII.2004, *M.P. Marcelli & S.S. Bocalini 10610* (SP), 22.V.1989, *M.P. Marcelli 6289* (SP). State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, *E. Gumboski, 4759, 4763* (ICN), 195 m alt., Cerrado, corticolous, 10°45'40.5"S, 37°20'25.2"W, 18.IX.2013, *E. Gumboski, 4796, 4839* (ICN).

Ramalina sprengelii Krog & Swinscow, *Norwegian Journal of Botany* 23 (3): 172. 1976.

MB#343520

Typus: South Africa, Uitenhagen, *Ecklon 212* (Holotype – S: Photo!).

Fig. 4D.

Thallus pendulous, corticolous, up to 25 cm long, greenish in field, stramineous in herbaria, growing from a narrow holdfast, concolorous with the thallus, up to 3.0 mm in diameter; branching main dichotomously to irregularly, solid branches, branches mainly flat and usually cylindrical to terete in distal parts, 0.2–0.7(–1.0) mm wide, not contorted, surface smooth in cylindrical to terete branches and striated in flat branches; symbiotic propagules absent; linear pseudocyphellae, marginal and laminal, flat, 0.05–0.2 mm wide. Cortex distinct, hyaline to yellowish, 12–20 µm thick, chondroid tissue not cracked, mainly discontinuous in cylindrical bundles, and discontinuous but not in bundles in branches wider than 0.5 mm; medulla white, loose. Apothecia marginal or lateral, up to 1.2 mm in diameter, flat to convex, amphithecia smooth without pseudocyphellae, margin entire, concolorous with the thallus, disc beige; epihymenium hyaline, 2–4 µm thick, hymenium hyaline, 40–60 µm thick, subhymenium hyaline, 20–30 µm thick, hypothecium hyaline to yellowish, 25–40 µm thick, without algal layer under the hypothecium and at margins of apothecia; ascospores 1-septate, fusiform, 12–15 × 3–5 µm. Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K-, C-, KC-. TLC: usnic acid.

Distribution. Tropical in Africa and South America, from sea level up to 900 m alt. (Krog & Swinscow 1976; Kashiwadani & Kalb 1993).

Ramalina sprengelii is characterized by the pendulous thallus with flat to cylindrical branches, absence of symbiotic propagules, mainly laminal and linear pseudocyphellae, not cracked chondroid tissue usually disposed in bundles, and by the absence of medullar acids.

Similar in morphology, chemistry and habitat with *R. kashiwadanii* (see discussion under that species).

Specimens examined: BRAZIL. State of Paraná, Municipality of Curitiba, ADEA reserve, at margins of Iguaçu river, 23.I.1985, *M. Fleig 2633* (ICN). State of Rio Grande do Sul, Municipality of Cambará do Sul, Itaimbezinho, 20.IV.1982, *Osorio & M. Fleig T18* (ICN); Municipality of Santa Maria, Represa da Corsan, nascente rio Ibicui, beira riacho, 13.V.1989, *Osorio & M. Fleig 89/70* (ICN); Municipality of Júlio de Castilhos, Passo do Felício, nascente rio Saturno, 7Km a SE BR 392, edge of the forest, 14.X.1989, *Osorio & M. Fleig 89/179* (ICN); Municipality of Caçapava do Sul, arroio Seival, riparian forest near the grassland, edge of the forest, 22.V.1993, *M. Fleig 5651* (ICN); Municipality of Bagé, BR 153 road, Casa de Pedra, margins of the river, 14.XII.1989, *M. Fleig 4077* (ICN). State of São Paulo, Municipality of Pirituba, 28.X.1893, *A. Loefgren s.n.* (SP 34267). State of Santa Catarina, Municipality of São Bento do Sul, Dona Francisca road, rural area, on trunk of *A. angustifolia*, 07.VII.2012, *E. Gumboski 3629, 3632* (JOI); Municipality of Monte Castelo, road BR 116, corticolous, 23.IX.2012, *E. Gumboski 4009* (JOI); Municipality of Mafra, side road near the BR 280, rural area, corticolous, 23.IX.2012, *E. Gumboski 4050, 4054* (JOI).

Ramalina subpollinaria Nyl., Bulletin de la Société Linnéenne de Normandie 4: 125. 1870.

Type: Peru, Callao, *Gaudichaud* (H-NYL 37050—lectotype, Photo!).

MB#403848

Fig. see Gumboski *et al.* (B, C).

Thallus caespitose, corticolous, up to 8 cm long, whitish green in field, stramineous in herbaria, growing from a common holdfast, concolorous with the thallus, 4.0 mm in diameter, branching dichotomously anisotomic to irregularly branched, solid branches, flat to canaliculate, 0.3–1.4(–2.0) mm in wide, surface smooth to slightly foveolate; soralia subterminal to terminar, orbicular to somewhat labriform, 0.3–1.5 mm in

diameter, 250–500 µm in diameter; pseudocyphellae marginal, discrete, orbicular, at surface level, 0.05–0.1 mm in diameter. Cortex distinct, hyaline to stramineous, 15–18 µm thick, chondroid tissue cracked, discontinuous, in irregular bundles, 13–50 µm thick, algal cells dispersed in medulla, medulla white to weakly beige, loose. Apothecia not found. Pycnidia not found.

Chemistry. Spot tests: Cortex K-, C-, KC- or KC+ yellow, UV-. Medulla: K+ yellow → red or K+ rose evanescent, C-, KC-, UV-. TLC: (Race 1). Usnic acid and salazinic acid; (Race 2) usnic acid and sekikaic acid.

Distribution. Tropical in America, from sea level up to 1500 m alt. (Nylander 1890; Kashiwadani & Kalb 1993).

Ramalina subpollinaria is characterized by the shrubby thallus with flat and canaliculate branches, distinctly cracked chondroid tissue, subterminal to terminal soralia, and by the production of salazinic acid or sekikaic acid.

This species is similar to *R. dendriscooides* in morphology, anatomy and chemistry. However, *R. subpollinaria* has clearly flat branches, while *R. dendriscooides* has the main branches irregularly flat to irregularly cylindrical, and the secondary branches are mainly terete (Kashiwadani 1987; Kashiwadani & Kalb 1993; Gumboski *et al.* C).

Specimens examined: BRAZIL. State of Pernambuco, Municipality of São Vicente Férrer, Mata do Estado, on trunk near the lake, corticolous, 16.XI.1998, *W.B. Sanders 98n16.15E* (UFP). State of Santa Catarina, Municipality of São Francisco do Sul, Morro da Enseada, rocky shore, saxicolous, 26°13'11,8"S, 48°29'40,4"W, 28.IV.2008, *E. Gumboski, 146* (JOI), 19.VI.2008, *E. Gumboski 492, 568* (JOI). State of São Paulo, Municipality of Campos do Jordão, Fazenda Toriba, 04.XI.1977, *H. Schindler 7125, 7180, 7186* (SP). State of Sergipe, Municipality of Areia Branca, Parque Nacional Serra de Itabaiana, 450 m alt., Cerrado, corticolous, 10°45'01.1"S, 37°21'54.9"W, 17.IX.2013, *E. Gumboski 4782* (ICN), 220 m alt., 10°44'50.8"S, 37°20'29.6"W, 18.IX.2013, *E. Gumboski 4844, 4850* (ICN).

Ramalina usnea (L.) R. Howe, *The Bryologist* 17: 81, pl. 12, figs 1–2. 1914.

Basionym: *Lichen usnea* L., *Mantissa* 1: 131–132. 1767.

Typus: (Lectotype – LINN 1273-278: Photo!).

MB#403866

Fig. 4E–F.

Thallus pendulous, corticolous, up to 50 cm long, greenish in field, stramineous in herbaria, growing from a narrow holdfast, concolours with the thallus to blackish, up to 3.0 mm in diameter; branching irregularly to dichotomously (anisotomic), solid branches, branches mainly flattened, but often with secondary branches irregularly cylindrical to subterete, often contorted, 0.1–2.0(–2.5) mm wide, surface mainly smooth to weakly striate; symbiotic propagules absent; pseudocyphellae linear, marginal and laminal, flat to weakly depressed, rarely sharply depressed, 0.05–0.1 mm wide. Cortex distinct, hyaline to dirty yellow, 8–12 μm thick, chondroid tissue distinctly cracked, continuous, 50–150 μm thick, algal layer discontinuous, 20–30 μm thick, medulla white, loose. Apothecia common, rounded, marginal or laminal, up to 1.3 mm diameter, flat, becoming convex with age, anfruticium smooth, without pseudocyphellae, margin entire to rarely cropped, concolorous with the thallus, disc beige weakly pruinous; epihymenium darkened, 8–12 μm thick, hymenium hyaline, 40–55 μm thick, subhymenium hyaline, 20–25 μm thick, hypothecium hyaline, continuous, 20–55 μm thick, with agglomerates of algae dispersed in medulla under the hypothecium; ascospores 1-septate, fusiform, 18–22(–25) \times 4–7 μm . Pycnidia not found.

Chemistry. Cortex: K-, C-, KC- or KC+ dirty yellow. Medulla: K+ pinkish or K-, C-, KC-. TLC: Race 1. usnic acid, homosekikaic and sekikaic acid. Race 2. Usnic acid, divaricatic acid.

Distribution. Tropical in America, from sea level up to 1000 m alt. (*e.g.*, Rundel 1978; Kashiwadani & Kalb 1993; Kashiwadani & Nash 2004; Aptroot & Bungartz 2007; Gumboski *et al.* A).

Ramalina usnea is characterized by the pendulous thallus with mainly flat branches, usually contorted branches, absence of symbiotic propagules, mainly laminal and linear pseudocyphellae, distinctly cracked chondroid tissue, and by the production of homosekikaic, sekikaic acid or divaricatic acid.

Similar in morphology, anatomy, and ecology with *R. anceps* (see discussion under that species).

Selected specimens examined: BRAZIL. State of Espírito Santo, Municipality of Piuma, 15.IV.1965, *D.M. Vital* 398 (SP). State of Paraná, Municipality of Guaraqueçaba, Reserva Natural de Itaquí, magrove, 06.XI.2003, *C. G. Donha* 1552 (UPCB), near the sea, corticolous, 07.VIII.2003, *C. G. Donha et al.* 1001 (UPCB), Reserva Natural de Itaquí, mangrove, 17.II.2004, *R. Reis* 298b (UPCB), Parque Nacional do Superagui, 09.IV.2003, *S. Eliasaro & C. G. Donha* 2602 (UPCB), 25°27'49"S, 48°14'20"W, 09.IV.2003, *S. Eliasaro & C. G. Donha* 2686 (UPCB). State of Pernambuco, Municipality of Alagoinha, Caatinga, I.1998, *E. Pereira s.n.* (UFP 36.432). State of Rio de Janeiro, Municipality of Rio das Ostras, restinga, corticolous, 20.VII.1975, *M. Brugger* 13732 (CESJ). State of Rio Grande do Sul, Municipality of Porto Alegre, Jardim Botânico, 28.XI.2008, *F. Lucheta s.n.* (HAS 48160); Municipality of Viamão, on *Luehea divaricata*, 30.VIII.2002, *S. M. Mazzitelli & M. Käffer s.n.* (HAS 41835); Municipality of Viamão, Itapoan, on dunes near Lagoa dos Patos, 20.X.1984, *M. Fleig s.n.* (ICN 55901); Municipality of Camaquã, Arroio Velhaco, edge of the forest, near BR 116 road, 20.II.1985, *C. Grabauska* 12 (ICN). State of Santa Catarina, Municipality of São Francisco do Sul, Morro da Enseada, saxicolous, on rocky shore, 26°13'11,8"S, 48°29'40,4"W, 19.VI.2008, *E. Gumboski* 479, 597 (JOI); Municipality of Rio Negrinho, Fazenda Velha, Araucaria Forest, corticolous, 11.IV.2009, *E. Gumboski* 957 (JOI), 01.XII.2013, *E. Gumboski* 5009 (JOI); Municipality of Fraiburgo, margins of road SC 453 (302), corticolous on *Syagrus sp.*, Araucaria Forest, 18.VII.2013, *E. Gumboski* 4743 (JOI).

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Fig. 4. A–F. A – *R. rectangularis* (W–syntype); B – *R. rectangularis* in details, showing the marginal protuberances (W–syntype); C – *R. solediosa* (C. G. Donha et al. 1038); D – *R. sprengelii* (E. Gumboski 3629); E – *R. usnea* (Brugger 13732); F – *R. usnea* in details, showing the contorted branches (S. Eliasaro & C. G. Donha 2602).

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5. CONSIDERAÇÕES FINAIS

As espécies de *Ramalina* distribuem-se por todo o território nacional, embora em muitos Estados ainda seja desconhecido o real número de espécies presentes. A Região Norte merece especial atenção devido ao alto nível de desconhecimento a respeito da micota liquenizada.

Ficou evidente a preferência de *Ramalina* por ambientes com umidade atmosférica mais elevada, principalmente em regiões onde a ocorrência de neblina é mais frequente. A associação entre um número expressivo de talos e de espécies com a presença de grandes corpos hídricos pode ser notada principalmente em matas ciliares e até mesmo na borda de represas artificiais mais antigas.

As espécies podem potencialmente colonizar diversos substratos naturais (i.e., galhos, troncos de árvores e rochas) e de origem antrópica (i.e., mourões de madeira ou concreto, estruturas de metal, fios de condução de energia elétrica). Entretanto, boa parte das mesmas pode apresentar preferência por substratos exclusivos, tais como *R. laevigata* e *R. fleigiae* que só ocorrem sobre rochas ou *R. sprengelii* que só ocorre sobre troncos ou galhos de árvores. Portanto, é muito importante que conste nas etiquetas das exsicatas o substrato sobre o qual os espécimes foram coletados.

Algumas espécies podem ter distribuição bastante restrita, como parece ser o caso de *R. fleigiae* que foi encontrada apenas em algumas localidades dos Campos de Cima da Serra no Rio Grande do Sul. Outras espécies, embora restritas a alguns ambientes, possuem distribuição um pouco mais ampla. Como é o caso de *R. exiguella* e *R. gracilis* que ocorrem apenas em regiões litorâneas, adentrando pouco na planície em direção a serra, e ocorrendo em maior quantidade em áreas próximas ao mar. Entretanto, há aquelas com ampla distribuição, como *R. celastri* que possui registro em diversas partes do mundo, desde regiões litorâneas até regiões de planalto. Vale ressaltar a necessidade de revisão de muitos registros históricos!

A importância de ter boas descrições da morfologia e da anatomia foi comprovada através dos estudos morfo-anatômicos com respaldo das análises moleculares. Características anatômicas outrora negligenciadas precisam estar bem detalhadas, inclusive para facilitar a comparação entre espécimes. A revisão de espécimes tipos se faz indiscutivelmente necessária, principalmente porque há ainda muitos nomes que depois de publicados não foram novamente estudados. Destes,

muitos possuem apenas descrições muito curtas que acabam por ser subjetivas frente a grande diversidade presente no gênero.

As análises moleculares deram um bom panorama sobre os problemas que cercam as espécies do gênero *Ramalina*. Dentro de alguns grupos a distinção entre as espécies se tornou clara. Ao passo que para outros grupos, análises mais complexas deverão ser feitas, possivelmente com um número maior de espécimes e de mais marcadores moleculares para tentar resolver as relações filogenéticas entre as espécies.

Paralelamente à revisão de espécimes tipos que seguirá para aprimoramento da taxonomia clássica, também serão desenvolvidos novos estudos buscando resolver questões a respeito de espécies crípticas, tais como as relativas aos grupos de *R. aspera* e *R. celastri*. Indícios de polifiletismo em alguns nomes já haviam sido detectados nos poucos trabalhos publicados até 2015. Contudo, para algumas espécies brasileiras com alta ocorrência nos ambientes brasileiros, tais como *R. solediosa* e *R. peruviana*, não era esperado tamanho grau de diversidade gênica.

Felizmente, o presente estudo gerou um grande conhecimento a cerca dos problemas taxonômicos e sistemáticos das espécies de *Ramalina*, não apenas em nível nacional, mas mundial com a utilização das sequências depositadas no GenBank. O estudo forneceu uma base sólida sobre a qual diversos trabalhos (alguns já em andamento) serão realizados em parceria com colegas brasileiros, bem como com novas parcerias firmadas com liquenólogos estrangeiros.

Com os resultados apresentados, sobe para 38 o número de espécies de *Ramalina* conhecidas para o Brasil. A Região Sul teve substancial ganho no conhecimento a respeito das espécies presentes, bem como parte da Região Sudeste e Nordeste. As Regiões Centro-Oeste e Norte ainda carecem de coleções suficientes mesmo em herbários nacionais.

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