



The genus *Ramalina* Ach. (Ascomycota, Lecanoromycetes, Ramalinaceae) from the Scattered Islands (French Southern and Antarctic Lands), with description of three new species

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Type

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Keywords

Scattered Islands, Mozambique Channel, Ramalinaceae, *Ramalina*, Corticolous

Abstract

A systematic survey of lichens was performed in 2019 in four of the five territories constituting the French Scattered Islands (Europa, Juan de Nova, Glorioso Islands, and Tromelin), with focus on the genus *Ramalina*. Species were identified and described using morphological (macroscopic and microscopic) features and accurate chemical profiling method based on high-performance liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-MS). Five species are listed from these territories, among which two already described: *Ramalina dumeticola* and *Ramalina ovalis*, and three novelties described here: *Ramalina gloriosensis*, *Ramalina hivertiana*, and *Ramalina marteau*. An identification key to a selection of corticolous similar species (with a solid thallus, shrubby or subpendulous at most, and branches flat to canaliculated) is provided.

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TITLE PAGE

The genus *Ramalina* Ach. (Ascomycota, Lecanoromycetes, Ramalinaceae) from the Scattered Islands (French Southern and Antarctic Lands), with description of three new species

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ABSTRACT

Abstract

A systematic survey of lichens was performed in 2019 in four of the five territories constituting the French Scattered Islands (Europa, Juan de Nova, Glorioso Islands, and Tromelin), with focus on the genus *Ramalina*. Species were identified and described using morphological (macroscopic and microscopic) features and accurate chemical profiling method based on high-performance liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-MS). Five species are listed from these territories, among which two already described: *Ramalina dumeticola* and *Ramalina ovalis*, and three novelties described here: *Ramalina gloriosensis*, *Ramalina hivertiana*, and *Ramalina marteau*. An identification key to a selection of corticolous similar species (with a solid thallus, shrubby or subpendulous at most, and branches flat to canaliculated) is provided.

Keywords

Scattered Islands, Mozambique Channel, Ramalinaceae, *Ramalina*, Corticolous



Introduction

The lichen genus *Ramalina* Ach. exhibits a cosmopolitan distribution and hosts an important taxonomic diversity. With about 240 species currently recognized, it is the eighteenth most diverse lichen genera and is thus considered as 'hyperdiverse' (Lücking et al. 2017). This genus counts a large number of species with restricted geographical ranges of extension, that are endemic to islands, or to ecologically particular territories (Krog & Østhagen 1980, Stevens 1987, Krog 1990, Blanchon et al. 1996, Aptroot & Bungartz 2007, Aptroot 2008, Aptroot & Schumm 2008, Pérez-Vargas & Pérez-Ortega 2014, Sparrius et al. 2017). Regarding the African realm, the genus has been extensively studied (Dodge 1971, Krog & Swinscow 1974, 1975, 1976, Swinscow & Krog 1988, Krog 1994, Bock et al. 2007, Fryday 2014), notably in East Africa, where a large part of the *Ramalina* species recorded are restricted to the African continent (Krog & Swinscow 1976). However, when considering islands of the Western Indian Ocean, available data concerning *Ramalina* become scarcer, though some territories have recently been targeted by field work or subjected to literature surveys: Seychelles (Seaward & Aptroot 2009, Neuwirth & Stocker-Wörgötter 2017), Mauritius (Diederich & Ertz 2020), and Madagascar (Aptroot 2016). Taken together, these last references list eleven accepted *Ramalina* species for the region, which is notably lower than the fifteen species recorded from the Galapagos only (Aptroot & Bungartz 2007), or the fifty-four species recorded from the Atlantic Islands (Aptroot & Schumm 2008). This paper aims at contributing to the inventory of *Ramalina* species from the Mozambique Channel and Western Indian Ocean, in particular the Scattered Islands (French Southern and Antarctic Lands): Europa, Juan de Nova, Glorioso Islands, and Tromelin (Fig. 1) where extensive field surveys has been performed in 2019.

Material and Methods

Surveyed territories and sampling methodology

Europa, Juan de Nova, Glorioso Islands, and Tromelin, constitute, along with Bassas da India (an atoll of only 0.2 km², which is merely totally submerged at high tide), the fifth district of the



French Southern and Antarctic Lands (TAAF). The emerged surface of these territories is less than 40.4 km² altogether (Boullet et al. 2018), and the elevation is very low, as the higher recorded point is 14 m asl at Glorioso Islands. The Scattered Islands constitute oceanic sanctuaries of primitive nature and host a remarkable land and marine biological heritage, which has been mostly preserved due to the geographical isolation, and a historically very limited human occupation. Today, these territories are uninhabited, except for the military, meteorologists, and scientists, and most of them benefit from protection status: Europa, Bassas da India, and Tromelin are protected by a prefectural decree which classifies them as a nature reserve since 1975, and Glorioso Islands are classified as national nature reserve since 10 June 2021. Juan de Nova benefits from no regulatory conservation status. Regarding climate, according to Beck et al. (2018) Köppen-Geiger climate classification, Europa climate is 'BSh' (main climate: arid, precipitation: steppe, temperature: hot arid), and Juan de Nova, Glorioso Island, and Tromelin are 'Aw' (main climate: tropical savannah, precipitation: dry winter). *Ramalina* species were surveyed in 2019 within the frame of the RECOFFIE Project ('Renforcement des Connaissances sur la Flore et la Fonge des Iles Eparses'), in four of the five territories constituting the Scattered Islands (Fig. 1). During 3 to 4 days for each of the four surveyed territories, all observed species of *Ramalina* were collected. Samples were stored dry in paper envelopes, and associated with collection number, ecological information (phorophytes, when applicable, were identified), date, and location obtained from a field GPS device. Species were surveyed on all potential ecological substrates: bark, wood, rock, and soil.

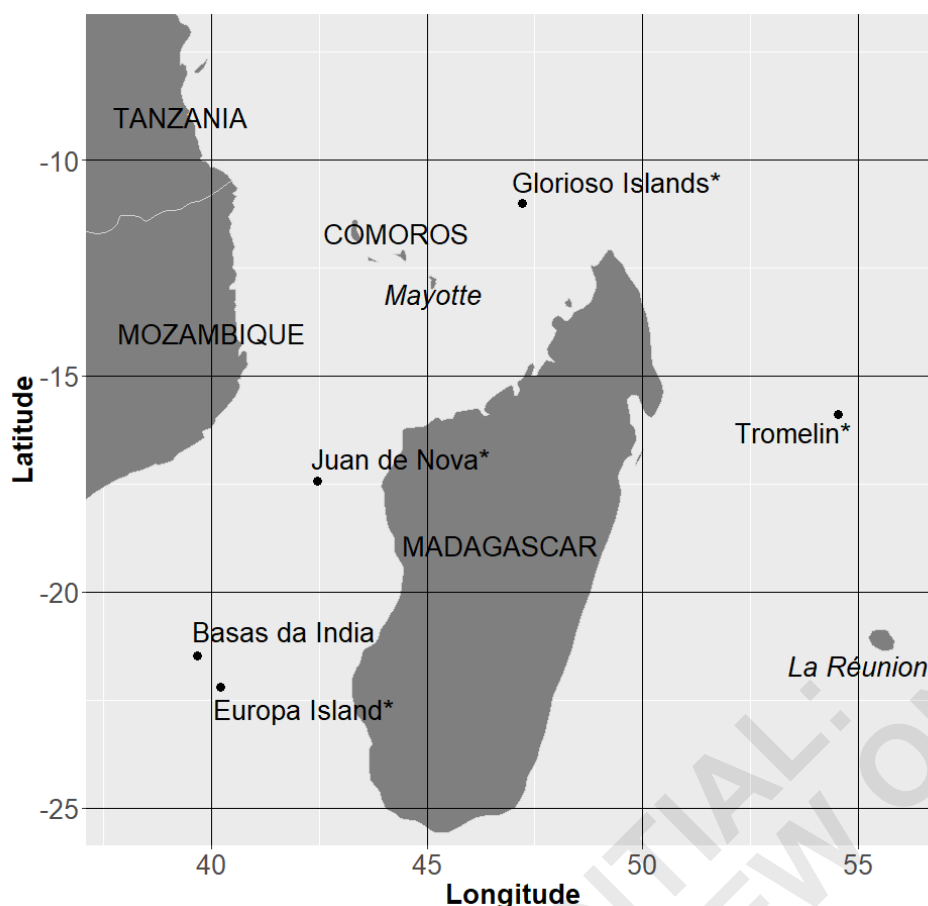


Figure 1. Map of surveyed locations. Territories in lower case not in italic correspond to the Scattered Islands, only those marked with * have been surveyed; territories in lower case in italic correspond to other French overseas territories; countries are marked in upper case.

Species identification and description

Identification and descriptive work were performed using a Zeiss Stemi SV8 stereomicroscope and a Leitz Orthoplan compound microscope with phase contrast, connected to a Sony E3CMOS camera sensor. Sections were mounted in tap water, from which all measurements were taken.

Specimens chemical profiling

About twenty milligrams (8 – 30 mg) of each lichen sample were grounded and extracted with acetone (1 mL) using an ultrasound bath at 30 – 40°C for 45 minutes. These extractions were repeated three times and all three extracts were mixed. After evaporation of the solvents, all



dried extracts were weighted to prepare a solution at a concentration of 1 mg.mL⁻¹ in methanol. All extracts were filtered (0.45 µm) before being subjected to high-performance liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-MS) with the method previously reported (Ferron et al. 2020). In addition, three custom chemical databases were used: the HLDB built with authentic lichen standards, the RamalinaDB built from an extensive bibliography on compounds identified in *Ramalina* species coupled with their predicted retention time and their molecular weight, and the LDB-MS1, a database built by Damien Olivier-Jimenez (Ferron et al. 2020). All databases allowed a dereplication of the molecules suspected to be in the extracts. However, some detected ions exhibited a m/z which may correspond to several lichen compounds of same molecular formula. The dereplication was thus refined by the complete spectrum analysis which allowed taking into account in-source fragments.

Mass spectrometry and UV allow to detect metabolites that wouldn't be revealed by TLC, and gap filling step in MZmine revealed the presence at trace level of almost all detected compounds in each sample. Atranorin is known (Le Pogam, 2017) to be hardly detected under our ionisation conditions but is well identified by HPLC/DAD and was found to be present in most of the samples. Moreover, some of the trace compound detected both by UV and mass spectrometry are still unidentified.

Results

The following species and chemical compounds have been recorded from the Scattered Islands. Five species are listed from these territories (Table 1), among which three are new to science.

Table 1. Ramalina species reported from the Scattered Islands and results of chemical profiling (MS = mass spectrometry; UV = ultraviolet; **M** = major compound; **m** = minor compound; t = trace compound)

					<i>Ramalina dumeticola</i>	<i>Ramalina gloriosensis</i>	<i>Ramalina hivertiana</i>	<i>Ramalina marteau</i>	<i>Ramalina ovalis</i>
Territories surveyed									
Europa					-	-	X	-	X
Juan de Nova					X	-	-	-	-
Glorioso Islands					-	X	-	X	-
Tromelin					-	-	-	-	-
Chemical compounds									
Identity	RT (min)	m/z	([M-H] ⁻ , in-source fragments)						
4'-O-methylnorsekikaic acid	24.5	40	3	225;195	t	m	m	t	-
4'-O-demethylsekikaic acid subpaludotic acid*	24.8	40	3	211;209	t	m	m	t	-
cryptochlorophaeic acid	26.1	45	9	239;211	t	t	t	M	-
4'-O-methylnorhomosekikaic acid	27.4	43	1	253;195	m	t	t	t	t
2-O-methylnordivaricatic acid divaricatic acid*	28.4	38	7	195;177	t	t	t	t	-
2,4'-di-O-methylnorsekikaic acid sekikaic acid*	28.9	41	7	225;209	M	M	M	t	-
usnic acid	29.8	34	3		m	m	m	m	M
atranorin	30.3				m	t	t	t	t
4'-O-methylpaludotic acid homosekikaic acid ramalinic acid	31.6	44	5	253;209	M	m	m	t	-

*pairs of compounds that can not be distinguished and only differ from the inversion of methoxy and hydroxy group at C2-C4 positions.



Ramalina dumeticola Krog & Swinscow (Fig. 2)

Description. Thallus corticolous, dull, greenish-yellowish to greyish-stramineous, fruticose, to 4-5 cm high, holdfast present, moderately branched, branching predominantly dichotomous with angle often less than 70-80°. Branches solid, flattened (generally up to the distal part, but sometimes subterete distally), 0.5-1.2 mm wide, apices attenuate, forked or broken, with faint longitudinal laminal and marginal short pseudocyphellae. Soralia marginal, ellipsoid to elongate. Apothecia rare, lateral, marginal, thalline exciple without pseudocyphellae, sometimes slightly crenulate. Hymenium clear, 25-35 µm high, paraphyses simple, apically furcate, 1-1.5 µm wide. Epithecium 10-12 µm high, brownish-olivaceous, with a layer of calcium oxalate crystals (1-4 µm diam.) visible in polarized light, dissolving in K. Hypothecium hyaline, 30-40 µm high, with an algal layer below forming ovate clusters (15-20 x 20-30 µm) of rounded photobiont cells (4-7 µm diam.). Excipulum filled with calcium oxalate crystals (2-10(12) µm diam.) visible in polarized light, partially dissolving in K. Asci subclavate-cylindrical, 20-25 x 5-8 µm, 8-spored. Ascospores hyaline (surface seeming micro-verrucose), 12-14(16) x 4-5 µm, 1-septate, narrowly ellipsoid to oblong with rounded ends, straight.

Chemistry. Major compounds: sekikaic acid or its positional isomer and a compound with a molecular peak $m/z = 445$ which could be homosekikaic or 4'-O-methylpaludosic acids or ramalinic acid B. Minor compounds: 4'-O-methylnorhomosekikaic and usnic acids (Table 1). Spot tests: cortex P-, K-, KC-, C-, UV-; apothecial discs P-, K+ yellow turning pale wine (sometimes fleeting, best seen on young apothecia), KC + yellow-orange, C+ yellow, UV-; medulla P-, K+ pink, C+ pink, KC+ pink, UV-.

Distribution and ecology. Coastal corticolous species occurring in Eastern Africa, Australia, and the Southeastern part of the Indo-Malay realm.

Note. Species belonging to the *Ramalina farinacea* complex. Similar to *Ramalina farinacea* (L.) Ach., but differing by the yellowish thallus tinge, the presence of minute linear pseudocyphellae, and the absence of protocetraric acid, salazinic acid, or hypoprotocetraric

acid. Also similar to *Ramalina nervulosa* (Müll. Arg.) Abbayes, but differing by the K⁺ pink medulla, the lower size, and the absence of salazinic acid. Also similar to *Ramalina luciae* Molho, Bodo, W.L. Culb. & C.F. Culb., but differing by the size, the branching pattern without extreme elongation between dichotomies, and the untwisted branches. Also close to *Ramalina peruviana* Ach., but differing by the ellipsoidal soralia, the not dense branching pattern, and absence of ramalinolic acid. Also close to *Ramalina pacifica* Asahina, but differing by the yellowish thallus tinge, the lower size, the non-splitting branches, the absence of laminal soralia, and the absence of salazinic acid.

Specimens examined. JUAN DE NOVA, S 17°02'57.2400", E 42°43'16.9874", 3 m asl, corticolous on dead wood of *Suriana maritima* L., leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 15 April 2019, Poncet 151 (PC0739884); JUAN DE NOVA, S 17°02'57.1073", E 42°43'16.7523", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 15 April 2019, Poncet 153 (PC0739883).

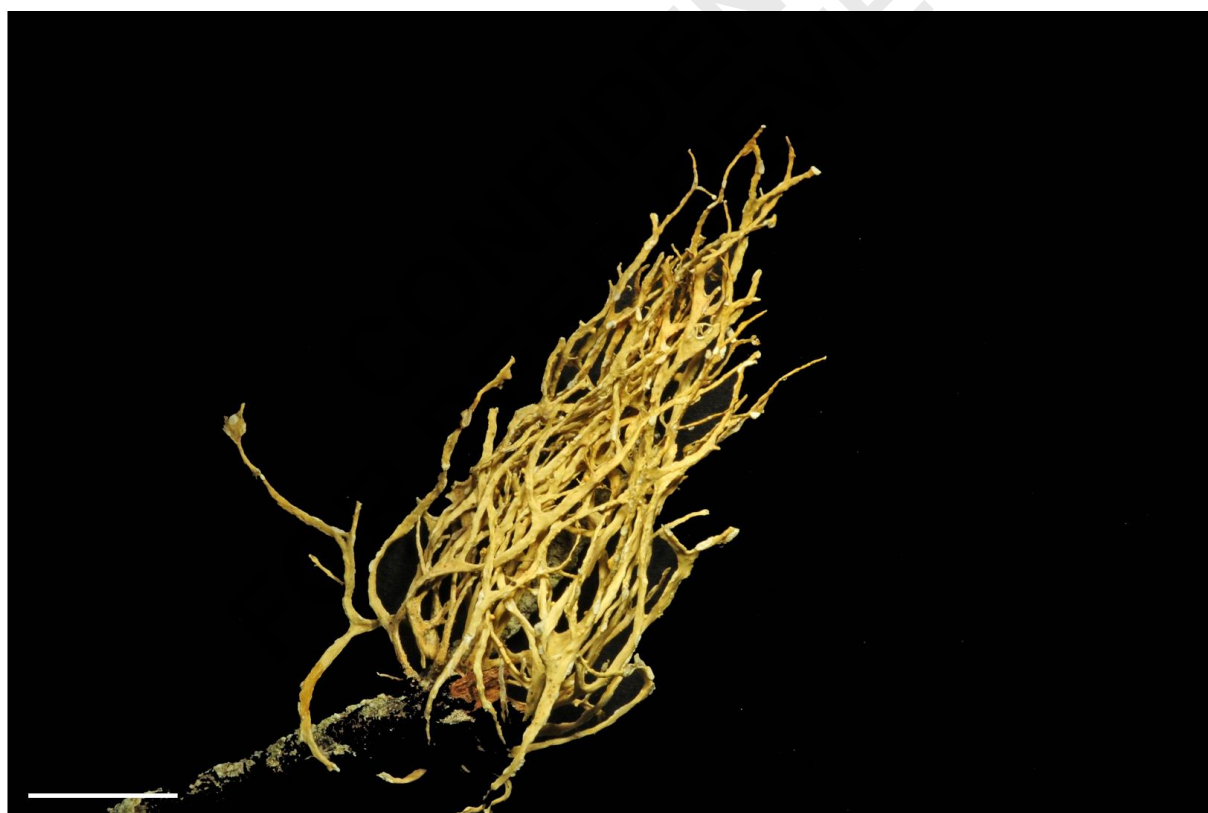


Figure 2. *Ramalina dumeticola*, PC0739884, collected in 2019 in Juan de Nova. Scale = 1 cm.



Ramalina gloriosensis R. Poncet sp. nov. (Fig. 3)

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Diagnosis. Similar to *Ramalina dumeticola*, but differing by the greyer thallus color, the more wavy appearance, the quite clearly canaliculated branches on well-developed thalli (which have a more robust appearance), the wider branching angles (often close to 80-90°), the presence of 4'-O-methylnorsekikaic acid, and another metabolite that may belong to 4'-O-demethylsekikaic acid or subpaludodic acid, both as minor compounds. Also close to *Ramalina nervulosa*, but differing by the absence of salazinic acid, and the faint longitudinal pseudocyphellae. Also close to *Ramalina luciae*, but differing in the branching pattern without extreme elongation between dichotomies, and the untwisted branches.

Type: GLORIOSO ISLANDS, S 11°34'13.0101", E 47°17'43.6725", 5 m asl, corticolous on *Casuarina equisetifolia* L., leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 155 (PC0739882 – holotype!).

Description. Thallus corticolous, dull, greenish-grey, fruticose, to 5 cm high but often shorter, holdfast present, moderately branched, branching predominantly dichotomous with wide-angle often close to 80-90°. Branches solid, flattened to canaliculated on well-developed individuals, angular-terete in distal parts and young individuals, 0.5-1.2 mm wide, tapering apically, wavy to slightly convoluted, with marginal pseudocyphellae (generally evolving to soralia) and faint longitudinal laminal striae. Soralia marginal, ellipsoid or confluent and then irregularly elongate. Apothecia not seen.

Chemistry. Major compound: sekikaic acid (or its positional isomer). Minor compounds: 4'-O-methylnorsekikaic acid, usnic acid, and a compound which could be 4'-O-demethylsekikaic acid or subpaludodic acid (Table 1). Spot tests: cortex P-, K-, KC-, C-, UV-; medulla P-, K+ pinkish-vineys (sometimes hard to see, best seen on well-developed individuals, and spot tests



most visible after drying), C+ pink (best seen on well-developed individuals), KC+ pink (best seen on well-developed individuals), UV-.

Distribution and ecology. Coastal corticolous species only known from Glorioso islands, growing on *Casuarina equisetifolia*, *Heliotropium foertherianum* Diane & Hilger, *Guettarda speciosa* L., and *Suriana maritima*.

Etymology. The species is named after the Glorioso Islands where it has been discovered. The spelling of the epithet is made up of *glorios-*, derived from “Îles Glorieuses” in French, and the Latin suffix *-ensis*, place of origin.

Additional specimens. GLORIOSO ISLANDS, S 11°34'17.2923", E 47°17'53.4078", 3 m asl, corticolous on *Heliotropium foertherianum*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 156 (PC0739887 – paratype!). GLORIOSO ISLANDS, S 11°34'14.2821", E 47°17'39.1627", 3 m asl, corticolous on *Guettarda speciosa*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 157 (PC0739886 – paratype!). GLORIOSO ISLANDS, S 11°34'13.0476", E 47°17'43.0459", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 158 (PC0739885 – paratype!). GLORIOSO ISLANDS, S 11°34'12.4942", E 47°17'30.7675", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 20 April 2019, Poncet 159 (PC0739890 – paratype!); GLORIOSO ISLANDS, S 11°34'17.3558", E 47°17'53.6063", 3 m asl, corticolous on dead branches of *Suriana maritima*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 165 (PC0739892 – paratype!).



Figure 3. *Ramalina gloriosensis*, holotype, PC0739882, collected in 2019 in Glorioso Islands.

Scale = 1 cm.

Ramalina hivertiana R. Poncet, sp. nov. (Fig. 4)

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Diagnosis. Similar to *Ramalina dumeticola*, but differing by the darker stramineous to brownish ochraceous thallus color, the shorter, straighter and more rigid branches with conspicuous abundant longitudinal laminal striae, the narrower branching angles (often less than 70-80°), the absence of 4'-O-methylnorhomosekikaic as major or minor compound, the presence of a metabolite that may belong to 4'-O-demethylsekikaic acid or subpaludonic acid as minor compound, and a UV+ white medulla.



Type: EUROPA, S 22°20'39.1581", E 40°20'21.7748", 3 m asl, corticolous on *Suriana maritima* L., leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 11 April 2019, Poncet 160 (PC0739891 – holotype!).

Description. Thallus corticolous, dull, dark stramineous to brownish ochraceous, fruticose, to 2(3) cm high but often shorter, holdfast present, moderately branched, branching predominantly dichotomous with angle often less than 70-80°. Branches solid, flattened, becoming angular-terete in most distal parts, 0.3-1.1 mm wide, tapering apically, short, rather straight and brittle when dry, with marginal pseudocyphellae (generally evolving to soralia) and conspicuous abundant longitudinal laminal striae. Soralia marginal, ellipsoid or confluent and then irregularly elongate. Apothecia not seen.

Chemistry. Major compound: sekikaic acid (or its positional isomer). Minor compounds: 4'-O-methylnorsekikaic acid, usnic acid, a compound which could be 4'-O-methylpaludosic acid or homosekikaic acid, or ramalinic B acid, and a last compound which could be 4'-O-demethylsekikaic acid or subpaludosic acid (Table 1). Spot tests: cortex P-, K-, KC-, C-, UV-; medulla P-, K+ pinkish-vineys (sometimes hard to see), C+ pink (sometimes hard to see), KC+ pink (sometimes hard to see), UV+ white.

Distribution and ecology. Coastal corticolous species only known from Europa, growing on *Suriana maritima*.

Etymology. The species is dedicated to our friend and colleague the French botanist Jean Hivert, who tirelessly dedicates his life to the study and conservation of the flora and natural habitats of the Scattered Islands. Up to now, he has performed more than 470 days (32 missions) of field survey on these little uninhabited remote islands, greatly contributing to the recent increase of knowledge on their flora and habitats. J. Hivert was also the initiator and the coordinator of the RECOFFIE Project, within the frame of which all the recent material of *Ramalina* was collected.

Additional specimen. EUROPA, S 22°20'39.1581", E 40°20'21.7748", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 11 April 2019, Poncet 166 (PC0739895 – isotype!).



Figure 4. *Ramalina hivertiana*, holotype, PC0739891, collected in 2019 in Europa. Scale = 1 cm.

Ramalina marteau R. Poncet, sp. nov. (Fig. 5)

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Diagnosis. Similar to *Ramalina holstii* Krog & Swinscow, but differing by the inconspicuous marginal pseudocyphellae not raised on tubercles, forming barely visible ripples, the bigger spores and the absence of salazinic acid. Also similar to *Ramalina maritima* Krog & Swinscow, but differing by the shorter not subpendulous thallus, generally less than 4 cm (a maximum of 5 cm was recorded among collected samples), the narrower branches (to 1-1.2 mm wide), the



smaller spores and the absence of boninic acid. Also close to *Ramalina fecunda* Krog & Swinscow, but differing by the shorter spore size and the absence of salazinic acid, the greenish-grey thallus (without yellow tinge), the absence of laminal pseudocyphellae, the margin not splitting, and the smaller spores. Also close to *Ramalina* sp. A (Swinscow & Krog 1988), but with narrower branches and smaller spores.

Type: GLORIOSO ISLANDS, S 11°34'17.3558", E 47°17'53.6063", 3 m asl, corticolous on dead branches of *Suriana maritima*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 164 (PC0739894 – holotype!).

Description. Thallus corticolous, dull, greenish-grey, fruticose, erected to 2-4(5) cm high but often shorter, holdfast present, moderately branched, branching predominantly dichotomous. Branches solid, flattened, centrally canaliculated with often a distinct flattened area along margins, 0.5-1.2 mm wide, tapering apically, short, with inconspicuous marginal pseudocyphellae forming barely visible ripples, and no laminal pseudocyphellae. Soralia not seen. Apothecia lateral, marginal, on (sub-)geniculate branches, thalline exciple with a few pseudocyphellae, sometimes slightly crenulate. Hymenium clear, 50-70 µm high, paraphyses anastomosed, apically furcate, 1-1.5 µm wide. Epithecium 7-10(12) µm high, brownish-olivaceous, with a layer of calcium oxalate crystals (1-4 µm diam.) visible in polarized light, dissolving in K. Hypothecium hyaline, 30-40 µm high, with an algal layer below forming ovate clusters (20-30 x 40-50 µm) of rounded photobiont cells (4-7 µm diam.). Excipulum filled with oxalate crystals (2-10 µm diam.) visible in polarized light, partially dissolving in K. Asci subclavate-cylindrical, 40-45 x 10-14 µm, 8-spored. Ascospores hyaline (surface seeming micro-verrucose), 12-16(18) x 4-5 µm, 1-septate (with sometimes 2 pseudosepta), fusiform, straight to curved.

Chemistry. Major compound: cryptochlorophaeic acid. Minor compound: usnic acid (Table 1). Spot tests: cortex P-, K-, KC-, C-, UV-; medulla P-, K+ red, C+ pink, KC+ pink, UV-; apothecial disc: P-, K-, KC-, C-, UV+ dark reddish-brown.

Distribution and ecology. Coastal corticolous species only known from the type locality in Glorioso Islands, growing on *Suriana maritima*.

Etymology. The species is dedicated to the French ornithologist and conservationist Cédric Marteau, who dedicates his life to actively improve conservation, knowledge, and visibility of the French Southern territories (Amsterdam Islands and Saint Paul, Crozet Islands, Kerguelen Islands) and the Scattered Islands.

Additional specimen. GLORIOSO ISLANDS, S 11°34'17.3558", E 47°17'53.6063", 3 m asl, corticolous on dead branches of *Suriana maritima* L., leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 167 (PC0739896 – paratype!).

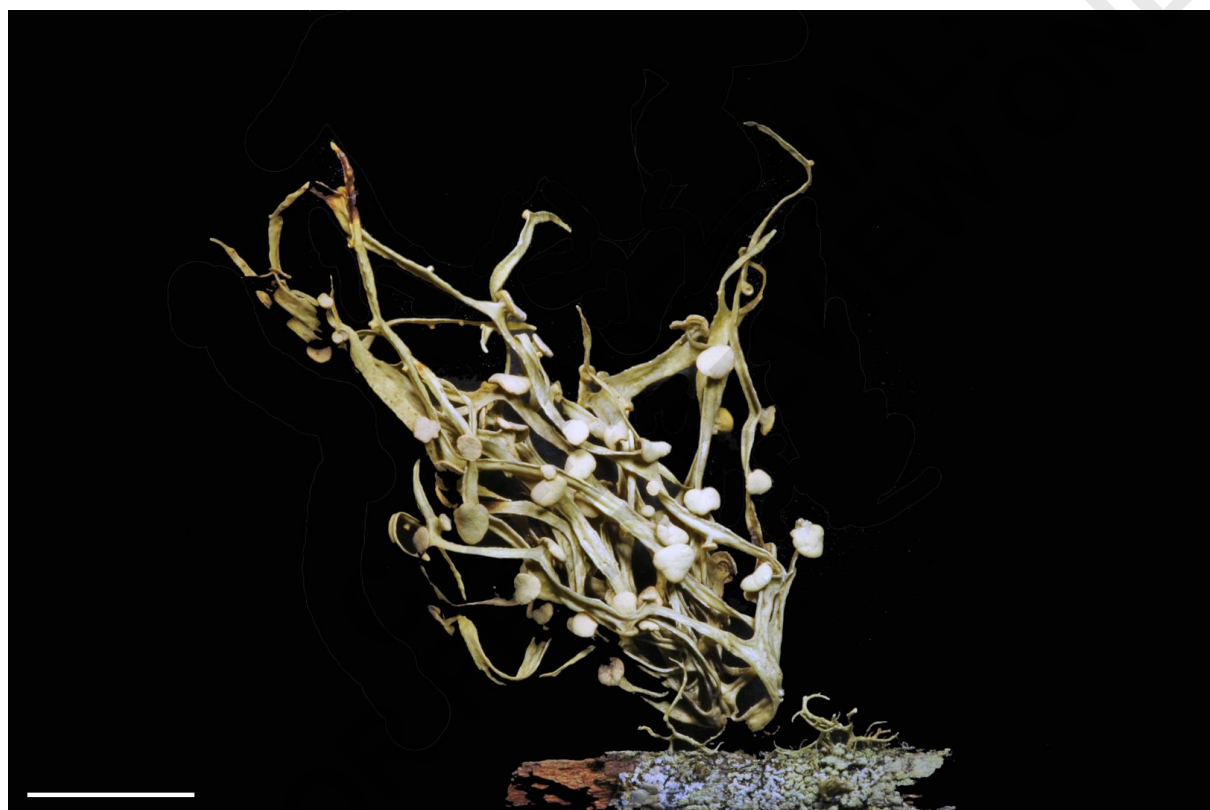


Figure 5. *Ramalina marteau*, holotype, PC0739894, collected in 2019 in Glorioso Islands. Scale = 1 cm.

Ramalina ovalis Hook. f. & Taylor (Fig. 6)



Description. Thallus corticolous, dull, greenish-grey to pale stramineous (when dry), erected, 1.5-4(5) cm high and 1-4(5) cm wide, holdfast present, fan-shaped to palmate, or sparingly branched. Branches flattened, with rounded to blunt apices, solid and rigid, almost brittle when dry, with surface longitudinally ridged from strands of cartilaginous tissue (medulla often almost inexistent), pseudocyphellae present, subpunctiform to shortly linear. Soralia not seen. Apothecia mostly laminal, sometimes marginal, stipited, numerous, discs 1-2(3) mm diam, mainly concave, thalline excipulum entire, sometimes slightly crenate. Hymenium clear, 60-80 µm high, paraphyses anastomosed, apically furcate, 1-1.5 µm wide. Epithecium 8-12 µm high, pale brownish-olivaceous, with a layer of calcium oxalate crystals (1-4 µm diam.) visible in polarized light, dissolving in K. Hypothecium hyaline, 30-40 µm high, with an algal layer below forming ovate clusters (25-35 x 40-50 µm) of rounded photobiont cells (5-8 µm diam.). Excipulum filled with oxalate crystals (4-10 µm diam.) visible in polarized light, partially dissolving in K. Asci subclavate-cylindrical, 50-75 x 11-15 µm, 8-spored. Ascospores hyaline (surface seeming micro-verrucose), (10-)12-16 x 4-5 µm, 1-septate, ellipsoid or slightly fusiform, straight to curved.

Chemistry. Major compound: usnic acid (Table 1). Spot tests: cortex P-, K-, KC+ yellow, C-, UV-; apothecial discs P-, K+ yellow turning pale wine (sometimes fleeting, best seen on young apothecia), KC + yellow-orange, C+ yellow, UV-; medulla P-, K-, KC- yellow, UV-.

Distribution and ecology. *Ramalina ovalis* is known from Australia and New Zealand where it mostly grows under warm temperate climates with warm summers. Stevens (1987) also indicates that it is present among collections of *R. celastri* from high elevation locations in Tanzania (1,440 m asl) and Uganda (1,950-2,100 m asl). Only recorded from Europa in the present study, growing at sea level on *Suriana maritima* and *Delonix regia*.

Note. Close to *Ramalina celastri* (Spreng.) Krog & Swinscow, but differing by the broader and fewer branches, often fan-shaped to palmate, with rounded to blunt apices. Also close to *Ramalina leiodea* (Nyl.) Nyl., but differing by the wider branches, which are not canaliculated and not tapering distally, by the mostly laminal apothecia, and by the less abundant and shorter

laminal pseudocyphellae. Also close to *Ramalina sinensis* Jatta, but differing by the no dorsiventral thallus, and by the ecology and distribution.

Specimens examined. EUROPA, S 22°20'39.1581", E 40°20'21.7748", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, J. Hivert, C. Fontaine, E. Bidault, 11 April 2019, Poncet 161 (PC0739889); EUROPA, S 22°20'39.3518", E 40°20'21.9514", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, J. Hivert, C. Fontaine, E. Bidault, 11 April 2019, Poncet 162 (PC0739888); EUROPA, S 22°20'42.8007", E 40°20'25.6197", 3 m asl, corticolous on *Delonix regia* (Bojer) Raf., leg. R. Poncet, J. Hivert, C. Fontaine, E. Bidault, 10 April 2019, Poncet 163 (PC0739893).

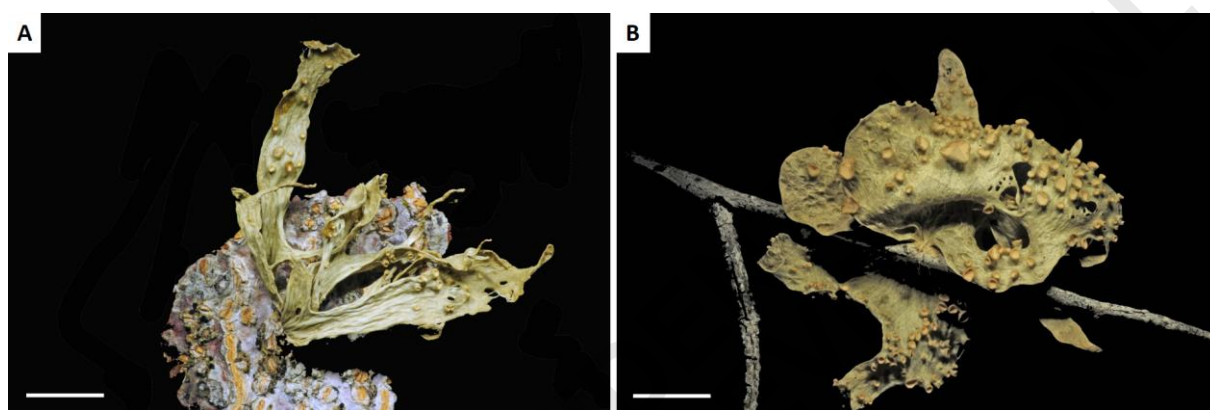


Figure 6. A-B – *Ramalina ovalis*, PC0739889, thalli showing morphological variability between individuals from the same location, collected in 2019 in Europa. Scales: A-B = 1 cm.

Identification key to the *Ramalina* species of the Scattered Island and morphologically similar species

All species listed in this identification key are corticolous, have a solid thallus, shrubby or subpendulous at most, with branches flat to canaliculated (at most slightly subterete or terete apically, or when young). Identification of specimens from paleotropics remains a rather difficult task, and the use of additional keys like the ones provided by Swinscow & Krog (1988) and Stevens (1987) will be of great help. Unfortunately, the lack of knowledge regarding species



367	345		distribution in the Southern hemisphere and morphological variability makes the use of	
368	346		chemical criteria still necessary for many species.	
369	347	1	Soralia absent, apothecia common.....	2
370	348	1'	Soralia present, apothecia rare.....	9
371	349	2	Thallus unbranched to moderately branched, from fan-shaped to palmate; branches	
372	350		strap-shaped, flattened to canaliculate, generally rather wide, from 0.5 to 2-3(4) cm.....	3
373	351	2'	Thallus moderately branched; branches flattened to subterete or canaliculated, thin,	
374	352		from 0.5 to 2-3(4) mm wide.....	6
375	353	3	Apothecia mostly on or close to the tips of the branches; branches often fan-shaped to	
376	354		more or less laciniate-fenestrate (particularly in older thallus), generally with a slightly	
377	355		dorsiventral aspect (lower surface paler than the upper one); circumboreal	
378	356		species.....	<i>R. sinensis</i>
379	357	3'	Apothecia mostly on the branches surface and/or along margin; branches if fan-shaped	
380	358		no laciniate, or strap-shaped, canaliculated or not; no dorsiventral aspect; pantropical	
381	359		to austral species.....	4
382	360	4	Apothecia mostly marginal (subterminal on small individuals), rarely laminal; branches	
383	361		flat, sometimes canaliculated when narrow, usually (0.5-)2-4(-10) mm wide tapering	
384	362		distally; australasian species.....	<i>R. leiodea</i>
385	363	4'	Apothecia mostly laminal (and sometimes marginal); branches flat to canaliculate, fan-	
386	364		shaped to strap-shaped.....	5
387	365	5	Branches lanceolate, with apices mostly acute (sometimes blunt); pantropical species;	
388	366		pantropical species.....	<i>R. celastri</i>
389	367	5'	Branches with variable shape, from fan-shaped to strap-shaped, with apices rounded	
390	368		to blunt; paleotropical species.....	<i>R. ovalis</i>



391	369	6	Pseudocyphellae punctiform, raised on conspicuous tubercles, mainly marginal;
392	370		salazinic acid and usually also cryptochlorophaeic acid present (P+ orange, K+ red);
393	371		East African species..... <i>R. holstii</i>
394	372	6'	Pseudocyphellae linear, or irregular, not raised on conspicuous tubercles, or absent...7
395	373	7	Thallus erect or subpendulous, usually more than 4 cm long; with boninic acid; East
396	374		African species..... <i>R. maritima</i>
397	375	7'	Thallus tufted, erected, rarely more than 4 cm long; with salazinic acid, or
398	376		cryptochlorophaeic acid, or an undetermined substance.....8
399	377	8	Laminal pseudocyphellae absent (but marginal often present, inconspicuously raised);
400	378		branches margin not splitting, branches centrally canaliculated with often a distinct
401	379		flattened area along margins; ascospores 12-16(18) x 4-5 µm; presence of
402	380		cryptochlorophaeic acid as major compound and usnic acid as minor compound; known
403	381		from Glorioso Islands (Mozambique channel)..... <i>R. marteau</i>
404	382	8'	Laminal pseudocyphellae present; branches margin splitting or not, branches not
405	383		canaliculated, on only weakly; ascospores comprised between 16-26 x 5-6 µm;
406	384		presence of salazinic acid or another undetermined substance; East African
407	385		species..... <i>R. fecunda</i> or <i>R. sp. A.</i> [see Swinscow & Krog (1988)]
408	386	9	Soralia punctiform (sometimes ellipsoid), often producing small fibrils, marginal and
409	387		apical; thallus delicate and fragile, densely branched; branches flat, compressed
410	388		becoming angularly subterete or terete distally; presence of acids in the sekikaic
411	389		aggregate; pantropical species..... <i>R. peruviana</i>
412	390	9'	Soralia ellipsoidal or round, no producing fibrils, mostly marginal; thallus no delicate
413	391		and constituted of fragile branchlets; branches mostly flat.....10
414	392	10	Branches without pseudocyphellae; thallus extremely variable, usually with a greyish-
415	393		greenish, or greyish-bluish, or bluish-greenish tinge (on West European material at
416	394		least; contrary to what is frequently stated, we find that this species lacks a significant



417	395		yellow tinge), generally sub-pendant to pendant; branches broad to narrow, 0.5-3(-4)
418	396		mm wide, 3-7 cm long, usually flattened, but sometimes almost terete (mostly distally);
419	397		soralia mostly marginal, but sometimes laminal also; presence of usnic acid, and other
420	398		metabolites depending on chemotype (protocetraric acid, salazinic acid,
421	399		hypoprotocetraric acid), or no other metabolite; circumboreal species..... <i>R. farinacea</i>
422	400	10'	Branches with pseudocyphellae (sometimes inconspicuous); thallus variable, but
423	401		usually with at least a conspicuous yellowish tinge; tropical to austral
424	402		species.....11
425	403	11	Thallus dark stramineous to brownish ochraceous, short, to 2(3) cm high, moderately
426	404		branched to unbranched; branches flattened, solid, rigid, brittle (or almost) when dry;
427	405		pseudocyphellae conspicuous, laminal and marginal; without divaric acid, with sekikaic
428	406		acid (or its positional isomer) as major compound, and 4'-O-methylnorsekikaic acid,
429	407		usnic acid, and two other unidentified acids as minor compounds (belonging to
430	408		paludotic, sekikaic or ramalinic aggregates); known from Europa (Mozambique
431	409		Channel)..... <i>R. hivertiana</i>
432	410	11'	Thallus paler, greenish-yellowish to greyish-stramineous, generally longer and more
433	411		branched; branches not brittle when dry; pseudocyphellae more or less visible; with or
434	412		without divaric acid; paleotropical-australasian species.....12
435	413	12	Medulla K-; thallus subpendulous to pendulous, 4-10 cm long (to 30 cm); cortex marked
436	414		with longitudinal pseudocyphellae; presence of divaricated and salazinic acids;
437	415		paleotropical-australasian species..... <i>R. nervulosa</i>
438	416	12'	Medulla K+ pinkish-vineys.....13
439	417	13	Thallus subpendulous to pendulous, up to 7 cm long (exceptionally to 18 cm); branches
440	418		sparsely dichotomous, producing extreme elongation between dichotomies, slightly
441	419		twisted along the whole length, the fusion between branches common; presence of
442	420		acids in the sekikaic aggregate, and more or less faint homosekikaic acid; australasian
443	421		species..... <i>R. luciae</i>



- Thallus subpendulous to erect, 3-6 cm long, without extreme elongation between dichotomies.....14
- Thallus with a conspicuous yellowish tinge; branches not intricated, branching angle narrow, often less than 70-80°; presence of sekikaic acid (or its positional isomer) and another compound which could be 4'-O-methylpaludodic acid or homosekikaic acid or ramalinic acid as major compound, and presence of usnic acid and 4'-O-methylnorhomosekikaic acid as minor compound (in the material examined from Scattered islands); paleotropical-australasian species.....***R. dumeticola***
- Thallus conspicuously greyer (yellow tinge present, but far less intense), branches more or less intricated, wavy, sinuous, branching angle wider, often close to 80-90°; presence of sekikaic acid (or its positional isomer) as major compound, and presence of 4'-O-methylnorsekikaic acid, usnic acid and another compound that could be 4'-O-demethylsekikaic acid or subpaludodic acid as minor compound; known from Glorioso Islands (Mozambique Channel, Comoros area).....***R. gloriosensis***

Discussion

The survey of *Ramalina* species from the Scattered Islands allowed to record five species, among which three are new to science. All species are corticolous and were found growing on widespread phorophytes. *Suriana maritima*, a pantropical coastal shrub, constitutes a suitable host, as all five *Ramalina* species were recorded growing on it. The other phorophytes were *Delonix regia* (with *R. ovalis*), *Casuarina equisetifolia* (with *R. gloriosensis*), *Heliotropium foertherianum* (with *R. gloriosensis*), and *Guettarda speciosa* (with *R. gloriosensis*). Among the recorded *Ramalina* species, *R. gloriosensis* was growing on four different phorophytes, *R. ovalis* on two, and the other three species only on *S. maritima*. Despite our intensive fieldwork efforts, no *Ramalina* was recorded from Tromelin (Table 1), which can be considered as the most remote island among the surveyed territories, notably because it is located outside the Mozambique Channel to the East of Madagascar (Fig. 1). This absence of *Ramalina* species could partly be explained by the absence of the most suitable phorophyte species, *S. maritima*



(Boullet et al. 2018). However *H. foertherianum* constitutes rather vast shrublands in this territory, which was shown from Glorioso Islands to be a suitable phorophyte for *Ramalina* species. Another notable result is that despite the relative ecological and climatic similarities among the surveyed territories, none of the *Ramalina* species were recorded in several islands (Table 1). Two species were recorded from Europa, two others from Glorioso Islands and only one from Juan de Nova. Compared with the only other lichen genus producing fruticose corticolous species recorded from the Scattered Islands: *Roccella* DC. (Ascomycota, Arthoniomycetes, Roccellaceae). This result contributes to shedding light on the dispersal abilities of *Ramalina* species. Indeed, among *Roccella* species previously reported from the Scattered Islands, two species with sexual dispersal means were only reported from Europa: *Roccella applanata* M. Choisy and *Roccella belangeriana* D.D. Awasthi, while one species with asexual dispersal means (soralia) was reported from three of the four surveyed territories: *Roccella montagnei* Bél. from Europa, Juan de Nova, and Glorioso Islands (Ferron et al. 2020). So, when considering *Roccella* genus from the Scattered Islands, the species with asexual dispersal means seem to have better dispersal abilities, but this cannot be generalized to the *Ramalina* genus, for which species with sexual or asexual dispersal strategy seems to have rather low dispersal abilities. These hypothesized low dispersal abilities of species belonging to the *Ramalina* genus could explain that none of them was recorded from several territories from the Scattered Islands, and is in line with the high endemism rate observed within this genus. Indeed, Aptroot & Bungartz (2007) and Aptroot & Schumm (2008) reported many endemic *Ramalina* species from insular territories: Azores (4), Canary Islands (11), Galapagos (4), Madeira (7), and Saint Helena (5). It is still unclear if the three species described here are really endemic to their respective islands, but owing to the general trend inside the *Ramalina* genus and available data from the Western Indian Ocean and Eastern Africa, we can infer that these three new species may have rather restricted extension areas. Finally, combined with already available recent records from the Western Indian Ocean and the Mozambique Channel, including Madagascar, sixteen species are now reported from this area: *Ramalina arabum* (Dill. ex Ach.) Meyen & Flot. (Madagascar, Mauritius), *Ramalina celastri* (Madagascar,



Seychelles), *Ramalina consanguinea* Müll. Arg. (Madagascar), *Ramalina dendriscoides* Nyl. (Madagascar, Seychelles) *Ramalina dumeticola* (Juan de Nova), *Ramalina fecunda* (Seychelles), *Ramalina fimbriata* Krog & Swinscow (Madagascar), *Ramalina gloriosensis* (Glorioso Islands), *Ramalina hivertiana* (Europa), *Ramalina leiodea* (Mauritius), *Ramalina litorea* G.N. Stevens (Mauritius), *Ramalina marteau* (Glorioso Islands), *Ramalina nervulosa* (Mauritius, Seychelles), *Ramalina ovalis* (Europa), *Ramalina praetermissa* Abbayes (Madagascar), and *Ramalina sprengelii* Krog & Swinscow (Mauritius, Seychelles).

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Permits

Collecting of the lichen species were authorized in the Scattered Islands according to the permit delivered by C. Geoffroy, General Secretary of French Southern and Antarctic Lands and district head of the Scattered Island. The RECOFFIE (CBN-CPIE Mascarin, MBG, UMS 2006 PatriNat (OFB – CNRS – MNHN)) project was authorized by order n°2019-40 of April 1, 2019.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

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Legends of figures and tables

Figures

Figure 1. Map of surveyed locations. Territories in lower case not in italic correspond to the Scattered Islands, only those marked with * have been surveyed; territories in lower case in italic correspond to other French overseas territories; countries are marked in upper case.

Figure 2. *Ramalina dumeticola*, PC0739884, collected in 2019 in Juan de Nova. Scale = 1 cm.

Figure 3. *Ramalina gloriosensis*, holotype, PC0739882, collected in 2019 in Glorioso Islands. Scale = 1 cm.

Figure 4. *Ramalina hivertiana*, holotype, PC0739891, collected in 2019 in Europa. Scale = 1 cm.

Figure 5. *Ramalina marteau*, holotype, PC0739894, collected in 2019 in Glorioso Islands. Scale = 1 cm.

Figure 6. A-B – *Ramalina ovalis*, PC0739889, thalli showing morphological variability between individuals from the same location, collected in 2019 in Europa. Scales: A-B = 1 cm.

Table

Table 1. *Ramalina* species reported from the Scattered Islands and results of chemical profiling (MS = mass spectrometry; UV = ultraviolet; M = major compound; m = minor compound; t = trace compound)

**Table 1.** Ramalina species reported from the Scattered Islands and results (compound)

Territories surveyed	
Chemical compounds	
Identity	
	4'-O-methylnorsekikaic acid
	4'-O-demethylsekikaic acid subpaludonic acid*
	cryptochlorophaeic acid
	4'-O-methylnorhomosekikaic acid
	2-O-methylnordivaricatic acid divaricatic acid*
	2,4'-di-O-methylnorsekikaic acid sekikaic acid*
	usnic acid
	atranorin
	4'-O-methylpaludonic acid homosekikaic acid ramalinic acid

*pairs of compounds that can not be distinguished and only differ from the

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s of chemical profiling (MS = mass spectrometry; UV = ultraviolet; **M** = major compound; **m** = minor compound)

			<i>Ramalina dum</i>	<i>Ramalina glori</i>	<i>Ramalina hivi</i>
Europa			-	-	X
Juan de Nova			X	-	-
Glorioso Islands			-	X	-
Tromelin			-	-	-
RT (min)	m/z	[[M-H] ⁻ , in-source fragments)			
24.5	403	225;195	t	m	m
24.8	403	211;209	t	m	m
26.1	459	239;211	t	t	t
27.4	431	253;195	m	t	t
28.4	387	195;177	t	t	t
28.9	417	225;209	M	M	M
29.8	343		m	m	m
30.3			m	t	t
31.6	445	253;209	M	m	m

the inversion of methoxy and hydroxy group at C2-C4 positions.



und; t = trace

Ramalina m ***Ramalina ovalis***

-	X
-	-
X	-
-	-

t	-
t	-
M	-
t	t
t	-
t	-
m	M
t	t
t	-

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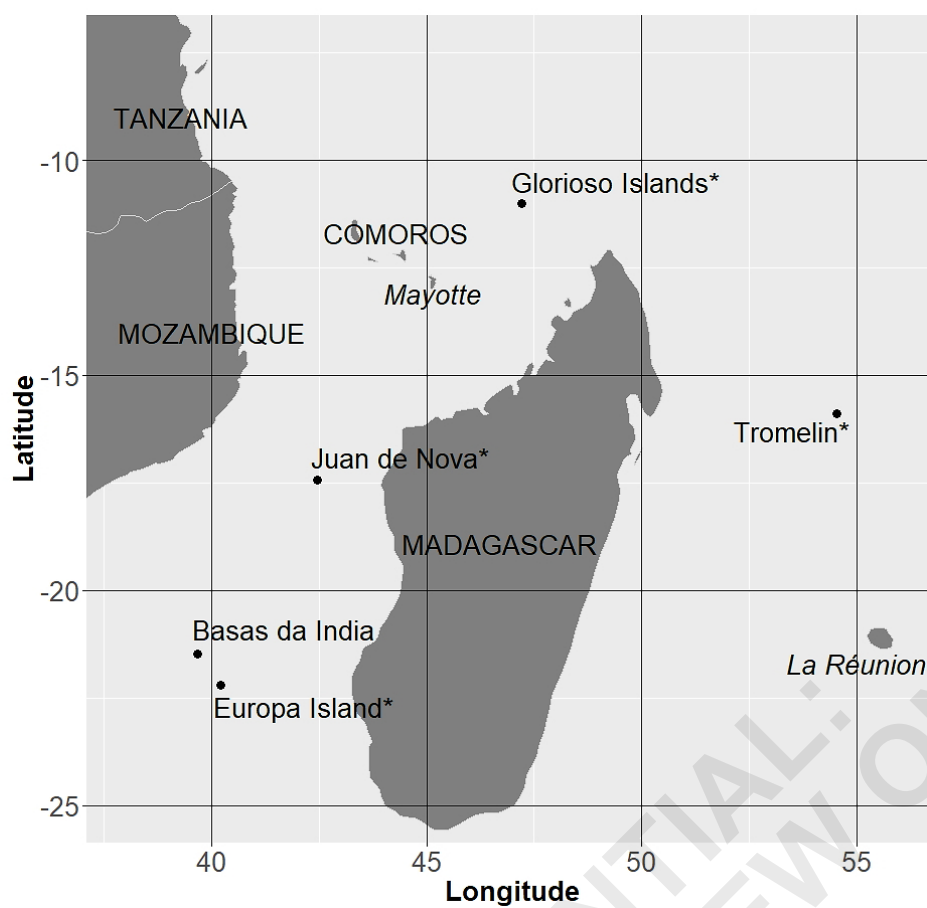


Fig. 1



Fig. 2

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Fig. 3

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Fig. 4

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Fig. 5

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Figure 6

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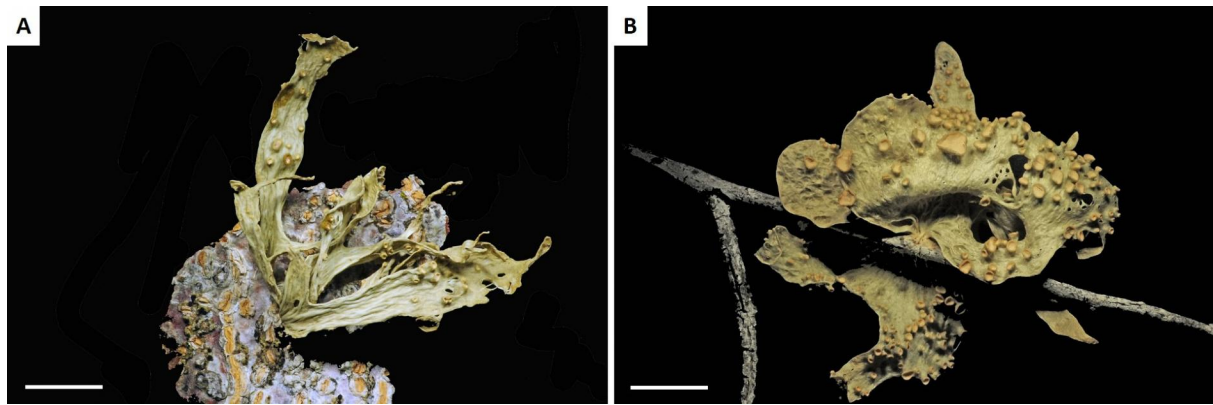


Fig. 6

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**Main text**

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Fig. 6

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