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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

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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

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38 Introduction

39 The lichen genus *Ramalina* Ach. exhibits a cosmopolitan distribution and hosts an important
40 taxonomic diversity. With about 240 species currently recognized, it is the eighteenth most
41 diverse lichen genera and is thus considered as 'hyperdiverse' (Lücking et al. 2017). This
42 genus counts a large number of species with restricted geographical ranges of extension, that
43 are endemic to islands, or to ecologically particular territories (Krog & Østhagen 1980, Stevens
44 1987, Krog 1990, Blanchon et al. 1996, Aptroot & Bungartz 2007, Aptroot 2008, Aptroot &
45 Schumm 2008, Pérez-Vargas & Pérez-Ortega 2014, Sparrius et al. 2017). Regarding the
46 African realm, the genus has been extensively studied (Dodge 1971, Krog & Swinscow 1974,
47 1975, 1976, Swinscow & Krog 1988, Krog 1994, Bock et al. 2007, Fryday 2014), notably in
48 East Africa, where a large part of the *Ramalina* species recorded are restricted to the African
49 continent (Krog & Swinscow 1976). However, when considering islands of the Western Indian
50 Ocean, available data concerning *Ramalina* become scarcer, though some territories have
51 recently been targeted by field work or subjected to literature surveys: Seychelles (Seaward &
52 Aptroot 2009, Neuwirth & Stocker-Wörgötter 2017), Mauritius (Diederich & Ertz 2020), and
53 Madagascar (Aptroot 2016). Taken together, these last references list eleven accepted
54 *Ramalina* species for the region, which is notably lower than the fifteen species recorded from
55 the Galapagos only (Aptroot & Bungartz 2007), or the fifty-four species recorded from the
56 Atlantic Islands (Aptroot & Schumm 2008). This paper aims at contributing to the inventory of
57 *Ramalina* species from the Mozambique Channel and Western Indian Ocean, in particular the
58 Scattered Islands (French Southern and Antarctic Lands): Europa, Juan de Nova, Glorioso
59 Islands, and Tromelin (Fig. 1) where extensive field surveys has been performed in 2019.

60 Material and Methods

61 *Surveyed territories and sampling methodology*

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



64 64 French Southern and Antarctic Lands (TAAF). The emerged surface of these territories is less
65 65 than 40.4 km² altogether (Boullet et al. 2018), and the elevation is very low, as the higher
66 66 recorded point is 14 m asl at Glorioso Islands. The Scattered Islands constitute oceanic
67 67 sanctuaries of primitive nature and host a remarkable land and marine biological heritage,
68 68 which has been mostly preserved due to the geographical isolation, and a historically very
69 69 limited human occupation. Today, these territories are uninhabited, except for the military,
70 70 meteorologists, and scientists, and most of them benefit from protection status: Europa,
71 71 Bassas da India, and Tromelin are protected by a prefectural decree which classifies them as
72 72 a nature reserve since 1975, and Glorioso Islands are classified as national nature reserve
73 73 since 10 June 2021. Juan de Nova benefits from no regulatory conservation status. Regarding
74 74 climate, according to Beck et al. (2018) Köppen-Geiger climate classification, Europa climate
75 75 is 'BSh' (main climate: arid, precipitation: steppe, temperature: hot arid), and Juan de Nova,
76 76 Glorioso Island, and Tromelin are 'Aw' (main climate: tropical savannah, precipitation: dry winter).
77 77 *Ramalina* species were surveyed in 2019 within the frame of the RECOFFIE Project
78 78 ('*Renforcement des Connaissances sur la Flore et la Fonge des Iles Eparses*'), in four of the
79 79 five territories constituting the Scattered Islands (Fig. 1). During 3 to 4 days for each of the four
80 80 surveyed territories, all observed species of *Ramalina* were collected. Samples were stored dry
81 81 in paper envelopes, and associated with collection number, ecological information
82 82 (phorophytes, when applicable, were identified), date, and location obtained from a field GPS
83 83 device. Species were surveyed on all potential ecological substrates: bark, wood, rock, and
84 84 soil.

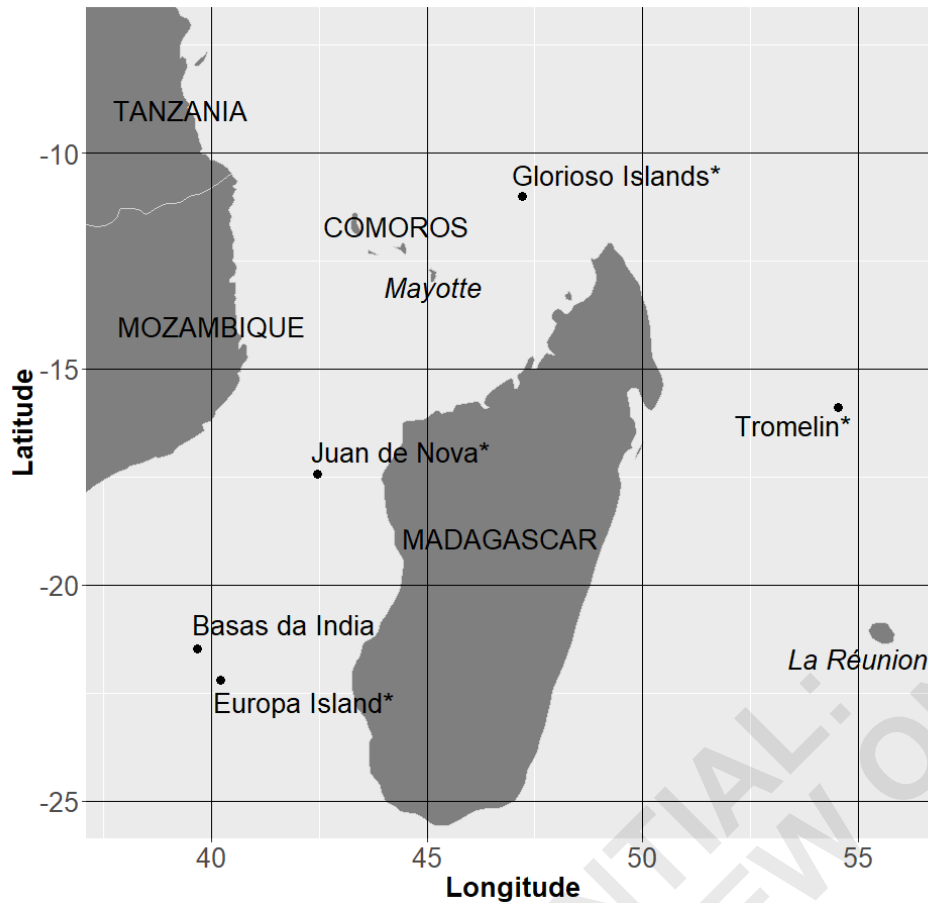


Figure 1. Map of surveyed locations. Territories in lower case not in italic correspond to the Scattered Islands, only those marked with * have been surveyed; territories in lower case in italic correspond to other French overseas territories; countries are marked in upper case.

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



98 98 dried extracts were weighted to prepare a solution at a concentration of 1 mg.mL⁻¹ in methanol.
99 99 All extracts were filtered (0.45 µm) before being subjected to high-performance liquid
100 100 chromatography coupled with diode-array detection and electrospray ionization tandem mass
101 101 spectrometry (HPLC-DAD-MS) with the method previously reported (Ferron et al. 2020). In
102 102 addition, three custom chemical databases were used: the HLDB built with authentic lichen
103 103 standards, the RamalinaDB built from an extensive bibliography on compounds identified in
104 104 *Ramalina* species coupled with their predicted retention time and their molecular weight, and
105 105 the LDB-MS1, a database built by Damien Olivier-Jimenez (Ferron et al. 2020). All databases
106 106 allowed a dereplication of the molecules suspected to be in the extracts. However, some
107 107 detected ions exhibited a m/z which may correspond to several lichen compounds of same
108 108 molecular formula. The dereplication was thus refined by the complete spectrum analysis
109 109 which allowed taking into account in-source fragments.

110 110 Mass spectrometry and UV allow to detect metabolites that wouldn't be revealed by TLC, and
111 111 gap filling step in MZmine revealed the presence at trace level of almost all detected
112 112 compounds in each sample. Atranorin is known (Le Pogam, 2017) to be hardly detected under
113 113 our ionisation conditions but is well identified by HPLC/DAD and was found to be present in
114 114 most of the samples. Moreover, some of the trace compound detected both by UV and mass
115 115 spectrometry are still unidentified.

117 117 Results

118 118 The following species and chemical compounds have been recorded from the Scattered
119 119 Islands. Five species are listed from these territories (Table 1), among which three are new to
120 120 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 marteaui</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)					
			40						
	4'-O-methylnorsekikaic acid	24.5	3	225;195	t	m	m	t	-
	4'-O-demethylsekikaic acid subpaludotic acid*	24.8	3	211;209	t	m	m	t	-
			45						
	cryptochlorophaeic acid	26.1	9	239;211	t	t	t	M	-
			43						
	4'-O-methylnorhomosekikaic acid	27.4	1	253;195	m	t	t	t	t
	2-O-methylnordivaricatic acid divaricatic acid*	28.4	7	195;177	t	t	t	t	-
	2,4'-di-O-methylnorsekikaic acid sekikaic acid*	28.9	7	225;209	M	M	M	t	-
			34						
	usnic acid	29.8	3		m	m	m	m	M
	atranorin	30.3			m	t	t	t	t
	4'-O-methylpaludotic acid homosekikaic acid ramalinic acid	31.6	5	253;209	M	m	m	t	-

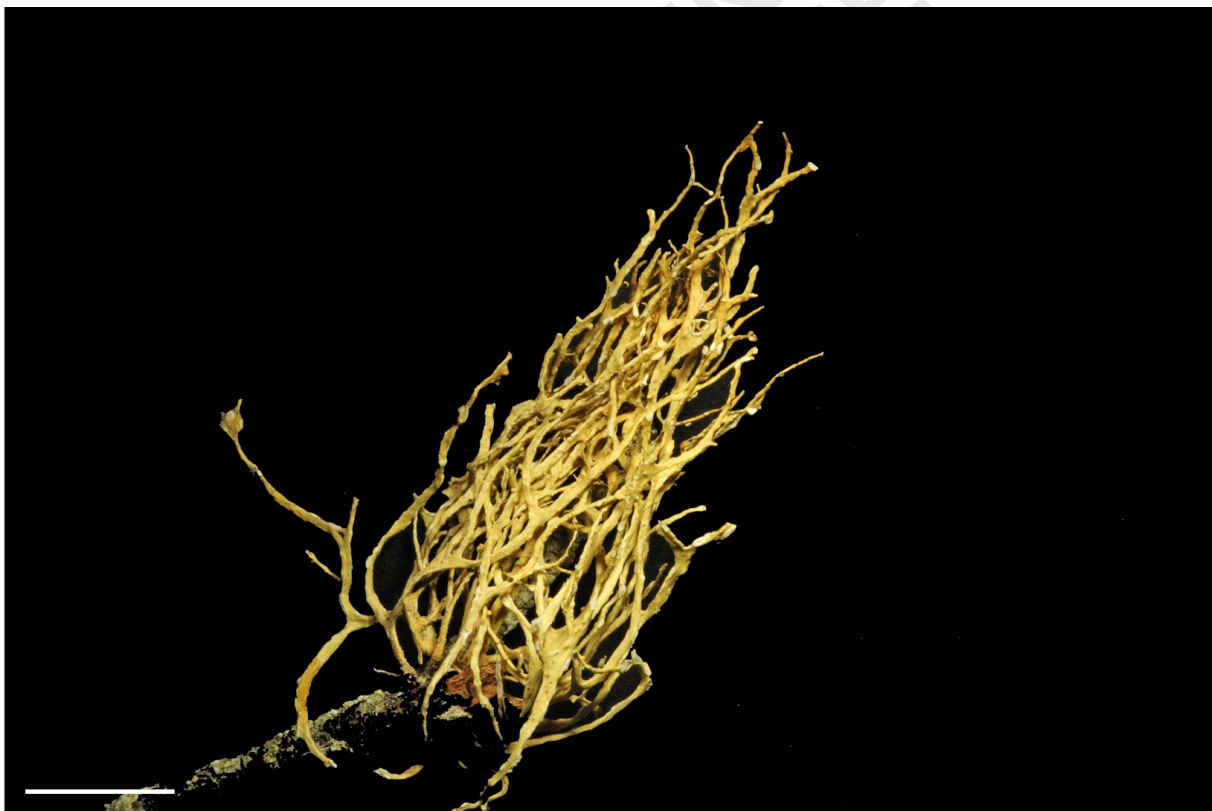
*pairs of compounds that can not be distinguished and only differ from the inversion of methoxy and hydroxy group at C2-C4 positions.



- 143 121 ***Ramalina dumeticola*** Krog & Swinscow (Fig. 2)
- 144 122 **Description.** Thallus corticolous, dull, greenish-yellowish to greyish-stramineous, fruticose, to
145 123 4-5 cm high, holdfast present, moderately branched, branching predominantly dichotomous
146 124 with angle often less than 70-80°. Branches solid, flattened (generally up to the distal part, but
147 125 sometimes subterete distally), 0.5-1.2 mm wide, apices attenuate, forked or broken, with faint
148 126 longitudinal laminal and marginal short pseudocyphellae. Soralia marginal, ellipsoid to
149 127 elongate. Apothecia rare, lateral, marginal, thalline exciple without pseudocyphellae,
150 128 sometimes slightly crenulate. Hymenium clear, 25-35 µm high, paraphyses simple, apically
151 129 furcate, 1-1.5 µm wide. Epithecium 10-12 µm high, brownish-olivaceous, with a layer of calcium
152 130 oxalate crystals (1-4 µm diam.) visible in polarized light, dissolving in K. Hypothecium hyaline,
153 131 30-40 µm high, with an algal layer below forming ovate clusters (15-20 x 20-30 µm) of rounded
154 132 photobiont cells (4-7 µm diam.). Excipulum filled with calcium oxalate crystals (2-10(12) µm
155 133 diam.) visible in polarized light, partially dissolving in K. Asci subclavate-cylindrical, 20-25 x 5-
156 134 8 µm, 8-spored. Ascospores hyaline (surface seeming micro-verrucose), 12-14(16) x 4-5 µm,
157 135 1-septate, narrowly ellipsoid to oblong with rounded ends, straight.
- 158 136 **Chemistry.** Major compounds: sekikaic acid or its positional isomer and a compound with a
159 137 molecular peak $m/z = 445$ which could be homosekikaic or 4'-O-methylpaludosic acids or
160 138 ramalinic acid B. Minor compounds: 4'-O-methylnorhomosekikaic and usnic acids (Table 1).
161 139 Spot tests: cortex P-, K-, KC-, C-, UV-; apothecial discs P-, K+ yellow turning pale wine
162 140 (sometimes fleeting, best seen on young apothecia), KC + yellow-orange, C+ yellow, UV-;
163 141 medulla P-, K+ pink, C+ pink, KC+ pink, UV-.
- 164 142 **Distribution and ecology.** Coastal corticolous species occurring in Eastern Africa, Australia,
165 143 and the Southeastern part of the Indo-Malay realm.
- 166 144 **Note.** Species belonging to the *Ramalina farinacea* complex. Similar to *Ramalina farinacea*
167 145 (L.) Ach., but differing by the yellowish thallus tinge, the presence of minute linear
168 146 pseudocyphellae, and the absence of protocetraric acid, salazinic acid, or hypoprotocetraric

169 147 acid. Also similar to *Ramalina nervulosa* (Müll. Arg.) Abbayes, but differing by the K⁺ pink
170 148 medulla, the lower size, and the absence of salazinic acid. Also similar to *Ramalina luciae*
171 149 Molho, Bodo, W.L. Culb. & C.F. Culb., but differing by the size, the branching pattern without
172 150 extreme elongation between dichotomies, and the untwisted branches. Also close to *Ramalina*
173 151 *peruviana* Ach., but differing by the ellipsoidal soralia, the not dense branching pattern, and
174 152 absence of ramalinolic acid. Also close to *Ramalina pacifica* Asahina, but differing by the
175 153 yellowish thallus tinge, the lower size, the non-splitting branches, the absence of laminal
176 154 soralia, and the absence of salazinic acid.

177 155 **Specimens examined.** JUAN DE NOVA, S 17°02'57.2400", E 42°43'16.9874", 3 m asl,
178 156 corticolous on dead wood of *Suriana maritima* L., leg. R. Poncet, C. Fontaine, J. Hivert, E.
179 157 Bidault, 15 April 2019, Poncet 151 (PC0739884); JUAN DE NOVA, S 17°02'57.1073", E
180 158 42°43'16.7523", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet, C. Fontaine, J. Hivert,
181 159 E. Bidault, 15 April 2019, Poncet 153 (PC0739883).



182 160
183 161 **Figure 2.** *Ramalina dumeticola*, PC0739884, collected in 2019 in Juan de Nova. Scale = 1 cm.



184 162

185 163 ***Ramalina gloriosensis*** R. Poncet sp. nov. (Fig. 3)

186 164 MYCOBANK MB XXXXXX

187 165 **Diagnosis.** Similar to *Ramalina dumeticola*, but differing by the greyer thallus color, the more
188 166 wavy appearance, the quite clearly canaliculated branches on well-developed thalli (which
189 167 have a more robust appearance), the wider branching angles (often close to 80-90°), the
190 168 presence of 4'-O-methylnorsekikaic acid, and another metabolite that may belong to 4'-O-
191 169 demethylsekikaic acid or subpaludodic acid, both as minor compounds. Also close to *Ramalina*
192 170 *nervulosa*, but differing by the absence of salazinic acid, and the faint longitudinal
193 171 pseudocyphellae. Also close to *Ramalina luciae*, but differing in the branching pattern without
194 172 extreme elongation between dichotomies, and the untwisted branches.

195 173 Type: GLORIOSO ISLANDS, S 11°34'13.0101", E 47°17'43.6725", 5 m asl, corticolous on
196 174 *Casuarina equisetifolia* L., leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet
197 175 155 (PC0739882 – holotype!).

198 176 **Description.** Thallus corticolous, dull, greenish-grey, fruticose, to 5 cm high but often shorter,
199 177 holdfast present, moderately branched, branching predominantly dichotomous with wide-angle
200 178 often close to 80-90°. Branches solid, flattened to canaliculated on well-developed individuals,
201 179 angular-terete in distal parts and young individuals, 0.5-1.2 mm wide, tapering apically, wavy
202 180 to slightly convoluted, with marginal pseudocyphellae (generally evolving to soralia) and faint
203 181 longitudinal laminal striae. Soralia marginal, ellipsoid or confluent and then irregularly elongate.
204 182 Apothecia not seen.

205 183 **Chemistry.** Major compound: sekikaic acid (or its positional isomer). Minor compounds: 4'-O-
206 184 methylnorsekikaic acid, usnic acid, and a compound which could be 4'-O-demethylsekikaic
207 185 acid or subpaludodic acid (Table 1). Spot tests: cortex P-, K-, KC-, C-, UV-; medulla P-, K+
208 186 pinkish-vineys (sometimes hard to see, best seen on well-developed individuals, and spot tests

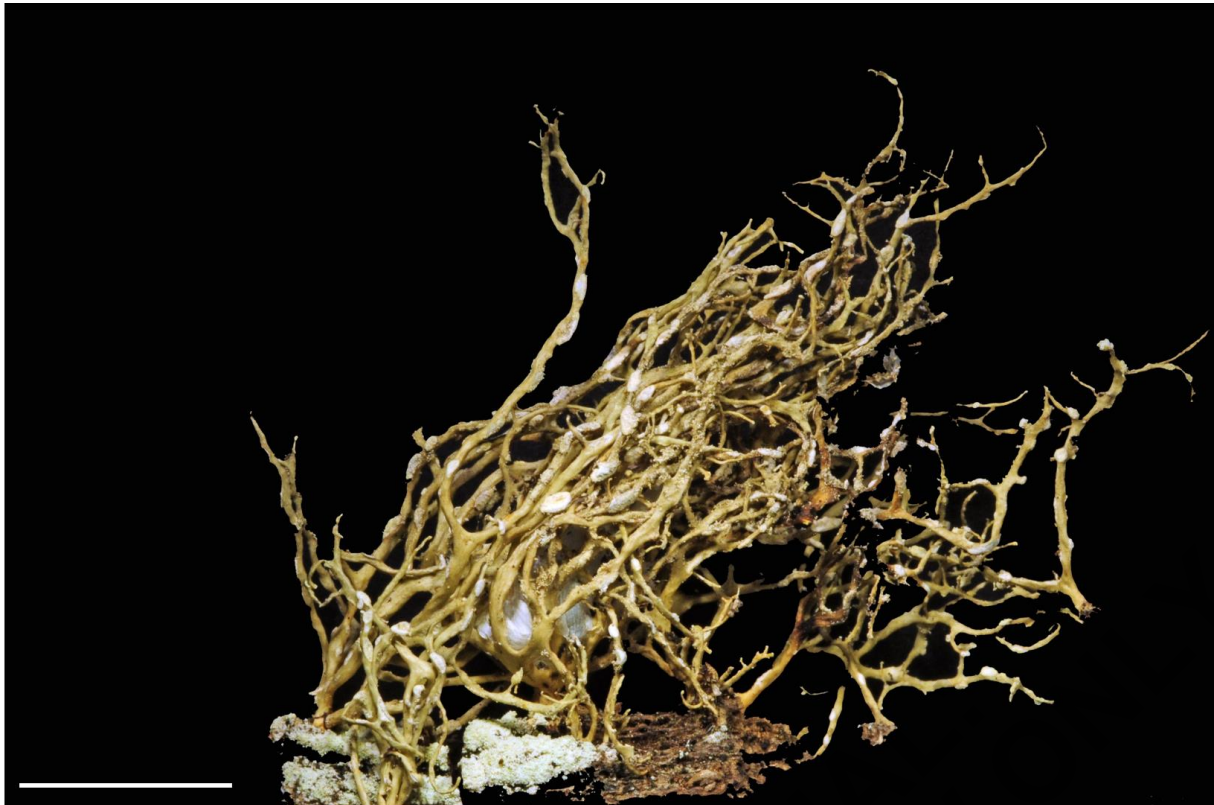


209 187 most visible after drying), C+ pink (best seen on well-developed individuals), KC+ pink (best
210 188 seen on well-developed individuals), UV-.

211 189 **Distribution and ecology.** Coastal corticolous species only known from Glorioso islands,
212 190 growing on *Casuarina equisetifolia*, *Heliotropium foertherianum* Diane & Hilger, *Guettarda*
213 191 *speciosa* L., and *Suriana maritima*.

214 192 **Etymology.** The species is named after the Glorioso Islands where it has been discovered.
215 193 The spelling of the epithet is made up of *glorios-*, derived from “Îles Glorieuses” in French, and
216 194 the Latin suffix *-ensis*, place of origin.

217 195 **Additional specimens.** GLORIOSO ISLANDS, S 11°34'17.2923", E 47°17'53.4078", 3 m asl,
218 196 corticolous on *Heliotropium foertherianum*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21
219 197 April 2019, Poncet 156 (PC0739887 – paratype!). GLORIOSO ISLANDS, S 11°34'14.2821",
220 198 E 47°17'39.1627", 3 m asl, corticolous on *Guettarda speciosa*, leg. R. Poncet, F. Picot, J.
221 199 Hivert, E. Bidault, 21 April 2019, Poncet 157 (PC0739886 – paratype!). GLORIOSO ISLANDS,
222 200 S 11°34'13.0476", E 47°17'43.0459", 3 m asl, corticolous on *Suriana maritima*, leg. R. Poncet,
223 201 F. Picot, J. Hivert, E. Bidault, 21 April 2019, Poncet 158 (PC0739885 – paratype!). GLORIOSO
224 202 ISLANDS, S 11°34'12.4942", E 47°17'30.7675", 3 m asl, corticolous on *Suriana maritima*, leg.
225 203 R. Poncet, F. Picot, J. Hivert, E. Bidault, 20 April 2019, Poncet 159 (PC0739890 – paratype!);
226 204 GLORIOSO ISLANDS, S 11°34'17.3558", E 47°17'53.6063", 3 m asl, corticolous on dead
227 205 branches of *Suriana maritima*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019,
228 206 Poncet 165 (PC0739892 – paratype!).



229 207
230 208 **Figure 3.** *Ramalina gloriosensis*, holotype, PC0739882, collected in 2019 in Glorioso Islands.

231 209 Scale = 1 cm.

232 210
233 211 ***Ramalina hivertiana*** R. Poncet, sp. nov. (Fig. 4)

234 212 MYCOBANK MB XXXXXX

235 213 **Diagnosis.** Similar to *Ramalina dumeticola*, but differing by the darker stramineous to
236 214 brownish ochraceous thallus color, the shorter, straighter and more rigid branches with
237 215 conspicuous abundant longitudinal laminal striae, the narrower branching angles (often less
238 216 than 70-80°), the absence of 4'-O-methylnorhomosekikaic as major or minor compound, the
239 217 presence of a metabolite that may belong to 4'-O-demethylsekikaic acid or subpaludodic acid
240 218 as minor compound, and a UV+ white medulla.



241 219 Type: EUROPA, S 22°20'39.1581", E 40°20'21.7748", 3 m asl, corticolous on *Suriana maritima*
242 220 L., leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 11 April 2019, Poncet 160 (PC0739891 –
243 221 holotype!).

244 222 **Description.** Thallus corticolous, dull, dark stramineous to brownish ochraceous, fruticose, to
245 223 2(3) cm high but often shorter, holdfast present, moderately branched, branching
246 224 predominantly dichotomous with angle often less than 70-80°. Branches solid, flattened,
247 225 becoming angular-terete in most distal parts, 0.3-1.1 mm wide, tapering apically, short, rather
248 226 straight and brittle when dry, with marginal pseudocyphellae (generally evolving to soralia) and
249 227 conspicuous abundant longitudinal laminal striae. Soralia marginal, ellipsoid or confluent and
250 228 then irregularly elongate. Apothecia not seen.

251 229 **Chemistry.** Major compound: sekikaic acid (or its positional isomer). Minor compounds: 4'-O-
252 230 methylnorsekikaic acid, usnic acid, a compound which could be 4'-O-methylpaludosic acid or
253 231 homosekikaic acid, or ramalinic B acid, and a last compound which could be 4'-O-
254 232 demethylsekikaic acid or subpaludosic acid (Table 1). Spot tests: cortex P-, K-, KC-, C-, UV-;
255 233 medulla P-, K+ pinkish-vineys (sometimes hard to see), C+ pink (sometimes hard to see), KC+
256 234 pink (sometimes hard to see), UV+ white.

257 235 **Distribution and ecology.** Coastal corticolous species only known from Europa, growing on
258 236 *Suriana maritima*.

259 237 **Etymology.** The species is dedicated to our friend and colleague the French botanist Jean
260 238 Hivert, who tirelessly dedicates his life to the study and conservation of the flora and natural
261 239 habitats of the Scattered Islands. Up to now, he has performed more than 470 days (32
262 240 missions) of field survey on these little uninhabited remote islands, greatly contributing to the
263 241 recent increase of knowledge on their flora and habitats. J. Hivert was also the initiator and the
264 242 coordinator of the RECOFFIE Project, within the frame of which all the recent material of
265 243 *Ramalina* was collected.

266 244 **Additional specimen.** EUROPA, S 22°20'39.1581", E 40°20'21.7748", 3 m asl, corticolous on
267 245 *Suriana maritima*, leg. R. Poncet, C. Fontaine, J. Hivert, E. Bidault, 11 April 2019, Poncet 166
268 246 (PC0739895 – isotype!).



269 247
270 248 **Figure 4.** *Ramalina hivertiana*, holotype, PC0739891, collected in 2019 in Europa. Scale = 1
271 249 cm.

272 250

273 251 ***Ramalina marteau*** R. Poncet, sp. nov. (Fig. 5)

274 252 MYCOBANK MB XXXXXX

275 253 **Diagnosis.** Similar to *Ramalina holstii* Krog & Swinscow, but differing by the inconspicuous
276 254 marginal pseudocyphellae not raised on tubercles, forming barely visible ripples, the bigger
277 255 spores and the absence of salazinic acid. Also similar to *Ramalina maritima* Krog & Swinscow,
278 256 but differing by the shorter not subpendulous thallus, generally less than 4 cm (a maximum of
279 257 5 cm was recorded among collected samples), the narrower branches (to 1-1.2 mm wide), the



280 258 smaller spores and the absence of boninic acid. Also close to *Ramalina fecunda* Krog &
281 259 Swinscow, but differing by the shorter spore size and the absence of salazinic acid, the
282 260 greenish-grey thallus (without yellow tinge), the absence of laminal pseudocyphellae, the
283 261 margin not splitting, and the smaller spores. Also close to *Ramalina sp.* A (Swinscow & Krog
284 262 1988), but with narrower branches and smaller spores.

285 263 Type: GLORIOSO ISLANDS, S 11°34'17.3558", E 47°17'53.6063", 3 m asl, corticolous on
286 264 dead branches of *Suriana maritima*, leg. R. Poncet, F. Picot, J. Hivert, E. Bidault, 21 April 2019,
287 265 Poncet 164 (PC0739894 – holotype!).

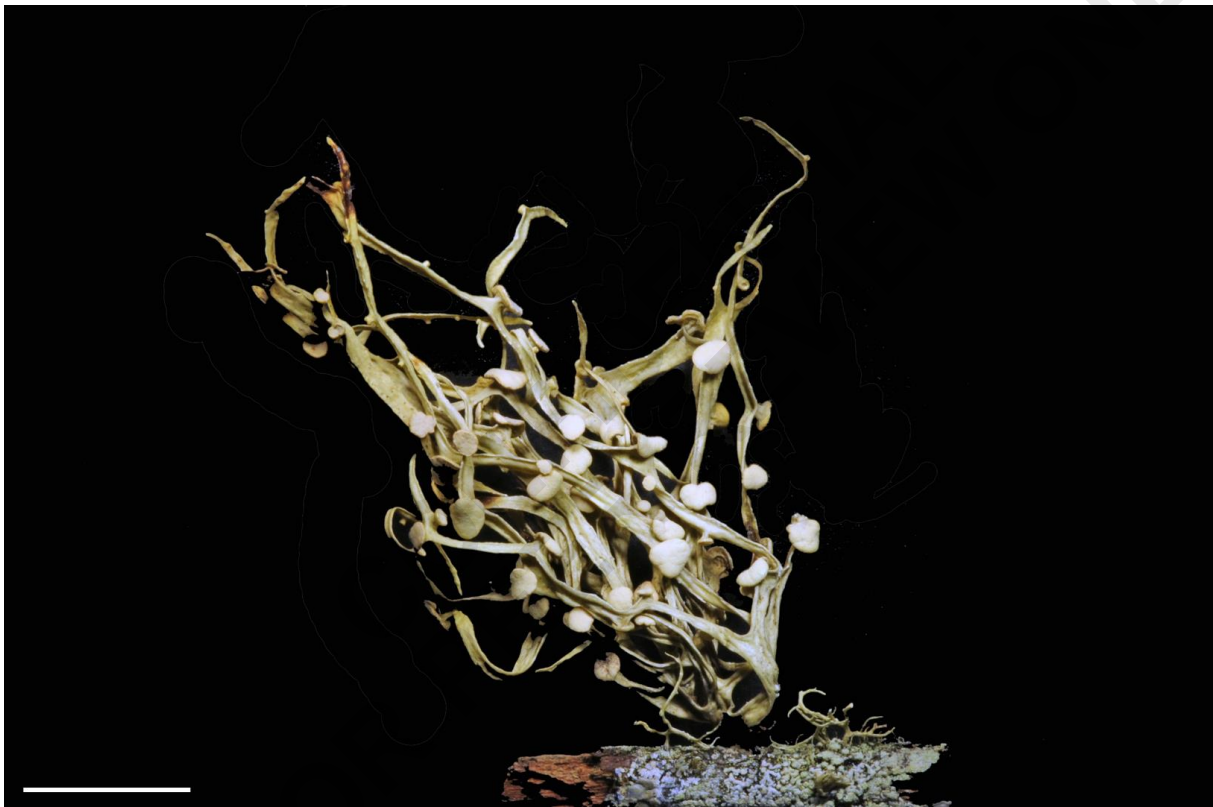
288 266 **Description.** Thallus corticolous, dull, greenish-grey, fruticose, erected to 2-4(5) cm high but
289 267 often shorter, holdfast present, moderately branched, branching predominantly dichotomous.
290 268 Branches solid, flattened, centrally canaliculated with often a distinct flattened area along
291 269 margins, 0.5-1.2 mm wide, tapering apically, short, with inconspicuous marginal
292 270 pseudocyphellae forming barely visible ripples, and no laminal pseudocyphellae. Soralia not
293 271 seen. Apothecia lateral, marginal, on (sub-)geniculate branches, thalline exciple with a few
294 272 pseudocyphellae, sometimes slightly crenulate. Hymenium clear, 50-70 µm high, paraphyses
295 273 anastomosed, apically furcate, 1-1.5 µm wide. Epithecium 7-10(12) µm high, brownish-
296 274 olivaceous, with a layer of calcium oxalate crystals (1-4 µm diam.) visible in polarized light,
297 275 dissolving in K. Hypothecium hyaline, 30-40 µm high, with an algal layer below forming ovate
298 276 clusters (20-30 x 40-50 µm) of rounded photobiont cells (4-7 µm diam.). Excipulum filled with
299 277 oxalate crystals (2-10 µm diam.) visible in polarized light, partially dissolving in K. Asci
300 278 subclavate-cylindrical, 40-45 x 10-14 µm, 8-spored. Ascospores hyaline (surface seeming
301 279 micro-verrucose), 12-16(18) x 4-5 µm, 1-septate (with sometimes 2 pseudosepta), fusiform,
302 280 straight to curved.

303 281 **Chemistry.** Major compound: cryptochlorophaeic acid. Minor compound: usnic acid (Table 1).
304 282 Spot tests: cortex P-, K-, KC-, C-, UV-; medulla P-, K+ red, C+ pink, KC+ pink, UV-; apothecial
305 283 disc: P-, K-, KC-, C-, UV+ dark reddish-brown.

306 284 **Distribution and ecology.** Coastal corticolous species only known from the type locality in
307 285 Glorioso Islands, growing on *Suriana maritima*.

308 286 **Etymology.** The species is dedicated to the French ornithologist and conservationist Cédric
309 287 Marteau, who dedicates his life to actively improve conservation, knowledge, and visibility of
310 288 the French Southern territories (Amsterdam Islands and Saint Paul, Crozet Islands, Kerguelen
311 289 Islands) and the Scattered Islands.

312 290 **Additional specimen.** GLORIOSO ISLANDS, S 11°34'17.3558", E 47°17'53.6063", 3 m asl,
313 291 corticolous on dead branches of *Suriana maritima* L., leg. R. Poncet, F. Picot, J. Hivert, E.
314 292 Bidault, 21 April 2019, Poncet 167 (PC0739896 – paratype!).



315 293
316 294 **Figure 5.** *Ramalina marteau*, holotype, PC0739894, collected in 2019 in Glorioso Islands.
317 295 Scale = 1 cm.

318 296

319 297 ***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.

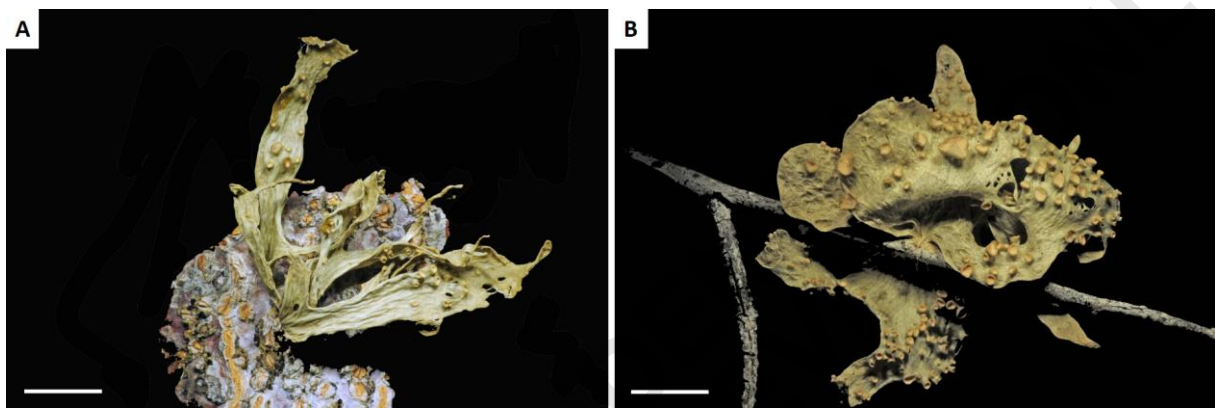
Chemistry. Major compound: usnic acid (Table 1). Spot tests: cortex P-, K-, KC+ yellow, C-, UV-; apothecial discs P-, K+ yellow turning pale wine (sometimes fleeting, best seen on young apothecia), KC + yellow-orange, C+ yellow, UV-; medulla P-, K-, KC- yellow, UV-.

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

347 325 laminal pseudocyphellae. Also close to *Ramalina sinensis* Jatta, but differing by the no
348 326 dorsiventral thallus, and by the ecology and distribution.

349 327 **Specimens examined.** EUROPA, S 22°20'39.1581", E 40°20'21.7748", 3 m asl, corticolous
350 328 on *Suriana maritima*, leg. R. Poncet, J. Hivert, C. Fontaine, E. Bidault, 11 April 2019, Poncet
351 329 161 (PC0739889); EUROPA, S 22°20'39.3518", E 40°20'21.9514", 3 m asl, corticolous on
352 330 *Suriana maritima*, leg. R. Poncet, J. Hivert, C. Fontaine, E. Bidault, 11 April 2019, Poncet 162
353 331 (PC0739888); EUROPA, S 22°20'42.8007", E 40°20'25.6197", 3 m asl, corticolous on *Delonix*
354 332 *regia* (Bojer) Raf., leg. R. Poncet, J. Hivert, C. Fontaine, E. Bidault, 10 April 2019, Poncet 163
355 333 (PC0739893).



356 334
357 335 **Figure 6.** A-B – *Ramalina ovalis*, PC0739889, thalli showing morphological variability between
358 336 individuals from the same location, collected in 2019 in Europa. Scales: A-B = 1 cm.

359 337
360 338 **Identification key to the *Ramalina* species of the Scattered Island and morphologically**
361 339 **similar species**

362 340 All species listed in this identification key are corticolous, have a solid thallus, shrubby or
363 341 subpendulous at most, with branches flat to canaliculated (at most slightly subterete or terete
364 342 apically, or when young). Identification of specimens from paleotropics remains a rather difficult
365 343 task, and the use of additional keys like the ones provided by Swinscow & Krog (1988) and
366 344 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 lacinate-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 lacinate, 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.....*R. holstii*
- 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.....*R. maritima*
- 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).....*R. marteau*
- 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.....*R. fecunda* or *R. sp. A.* [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.....*R. peruviana*
- 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>	



- 444 422 **13'** Thallus subpendulous to erect, 3-6 cm long, without extreme elongation between
 445 423 dichotomies.....14
- 446 424 **14** Thallus with a conspicuous yellowish tinge; branches not intricated, branching angle
 447 425 narrow, often less than 70-80°; presence of sekikaic acid (or its positional isomer) and
 448 426 another compound which could be 4'-O-methylpaludosic acid or homosekikaic acid or
 449 427 ramalinic acid as major compound, and presence of usnic acid and 4'-O-
 450 428 methylnorhomosekikaic acid as minor compound (in the material examined from
 451 429 Scattered islands); paleotropical-australasian species.....***R. dumeticola***
- 452 430 **14'** Thallus conspicuously greyer (yellow tinge present, but far less intense), branches
 453 431 more or less intricated, wavy, sinuous, branching angle wider, often close to 80-90°;
 454 432 presence of sekikaic acid (or its positional isomer) as major compound, and presence
 455 433 of 4'-O-methylnorsekikaic acid, usnic acid and another compound that could be 4'-O-
 456 434 demethylsekikaic acid or subpaludosic acid as minor compound; known from Glorioso
 457 435 Islands (Mozambique Channel, Comoros area).....***R. gloriosensis***

458 436 Discussion

459 437 The survey of *Ramalina* species from the Scattered Islands allowed to record five species,
 460 438 among which three are new to science. All species are corticolous and were found growing on
 461 439 widespread phorophytes. *Suriana maritima*, a pantropical coastal shrub, constitutes a suitable
 462 440 host, as all five *Ramalina* species were recorded growing on it. The other phorophytes were
 463 441 *Delonix regia* (with *R. ovalis*), *Casuarina equisetifolia* (with *R. gloriosensis*), *Heliotropium*
 464 442 *foertherianum* (with *R. gloriosensis*), and *Guettarda speciosa* (with *R. gloriosensis*). Among
 465 443 the recorded *Ramalina* species, *R. gloriosensis* was growing on four different phorophytes, *R.*
 466 444 *ovalis* on two, and the other three species only on *S. maritima*. Despite our intensive fieldwork
 467 445 efforts, no *Ramalina* was recorded from Tromelin (Table 1), which can be considered as the
 468 446 most remote island among the surveyed territories, notably because it is located outside the
 469 447 Mozambique Channel to the East of Madagascar (Fig. 1). This absence of *Ramalina* species
 470 448 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,



499 477 Seychelles), *Ramalina consanguinea* Müll. Arg. (Madagascar), *Ramalina dendriscoides* Nyl.
500 478 (Madagascar, Seychelles) *Ramalina dumeticola* (Juan de Nova), *Ramalina fecunda*
501 479 (Seychelles), *Ramalina fimbriata* Krog & Swinscow (Madagascar), *Ramalina gloriosensis*
502 480 (Glorioso Islands), *Ramalina hivertiana* (Europa), *Ramalina leiodea* (Mauritius), *Ramalina*
503 481 *litorea* G.N. Stevens (Mauritius), *Ramalina marteaui* (Glorioso Islands), *Ramalina nervulosa*
504 482 (Mauritius, Seychelles), *Ramalina ovalis* (Europa), *Ramalina praetermissa* Abbayes
505 483 (Madagascar), and *Ramalina sprengelii* Krog & Swinscow (Mauritius, Seychelles).

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536 514 **Competing interests**

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600 578

601 579 **Legends of figures and tables**

602 580 **Figures**

603 581 **Figure 1.** Map of surveyed locations. Territories in lower case not in italic correspond to the
604 582 Scattered Islands, only those marked with * have been surveyed; territories in lower case in
605 583 italic correspond to other French overseas territories; countries are marked in upper case.

606 584 **Figure 2.** *Ramalina dumeticola*, PC0739884, collected in 2019 in Juan de Nova. Scale = 1 cm.

607 585 **Figure 3.** *Ramalina gloriosensis*, holotype, PC0739882, collected in 2019 in Glorioso Islands.
608 586 Scale = 1 cm.

609 587 **Figure 4.** *Ramalina hivertiana*, holotype, PC0739891, collected in 2019 in Europa. Scale = 1
610 588 cm.

611 589 **Figure 5.** *Ramalina marteaui*, holotype, PC0739894, collected in 2019 in Glorioso Islands.
612 590 Scale = 1 cm.

613 591 **Figure 6.** A-B – *Ramalina ovalis*, PC0739889, thalli showing morphological variability between
614 592 individuals from the same location, collected in 2019 in Europa. Scales: A-B = 1 cm.

615 593 **Table**

616 594 **Table 1.** *Ramalina* species reported from the Scattered Islands and results of chemical profiling
617 595 (MS = mass spectrometry; UV = ultraviolet; M = major compound; m = minor compound; t =
618 596 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 subpaludodic acid*
	cryptochlorophaeic acid
	4'-O-methylnorhomosekikaic acid
	2-O-methylnordivarcatic acid divarcatic acid*
	2,4'-di-O-methylnorsekikaic acid sekikaic acid*
	usnic acid
	atranorin
	4'-O-methylpaludodic acid homosekikaic acid ramalinic acid

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

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

			<i>Ramalina dum</i>	<i>Ramalina glori</i>	<i>Ramalina hiv</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

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

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Table

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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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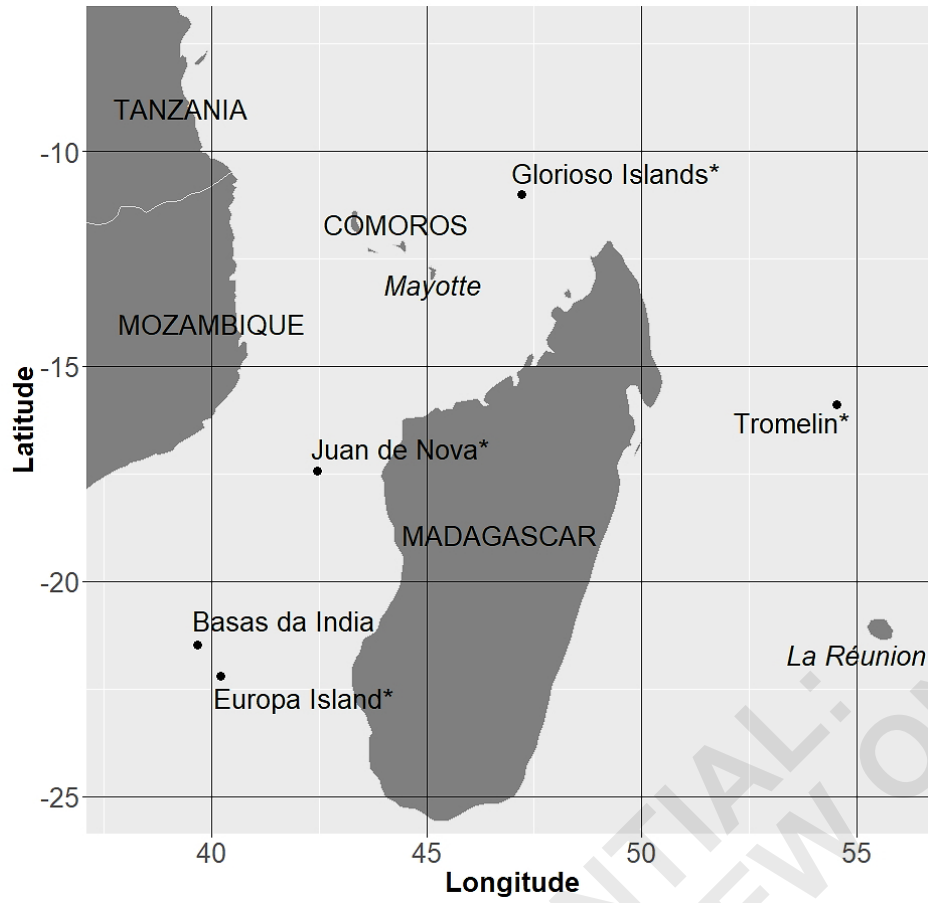


Fig. 1



Fig. 2

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Fig. 4

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Fig. 5

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Figure 6

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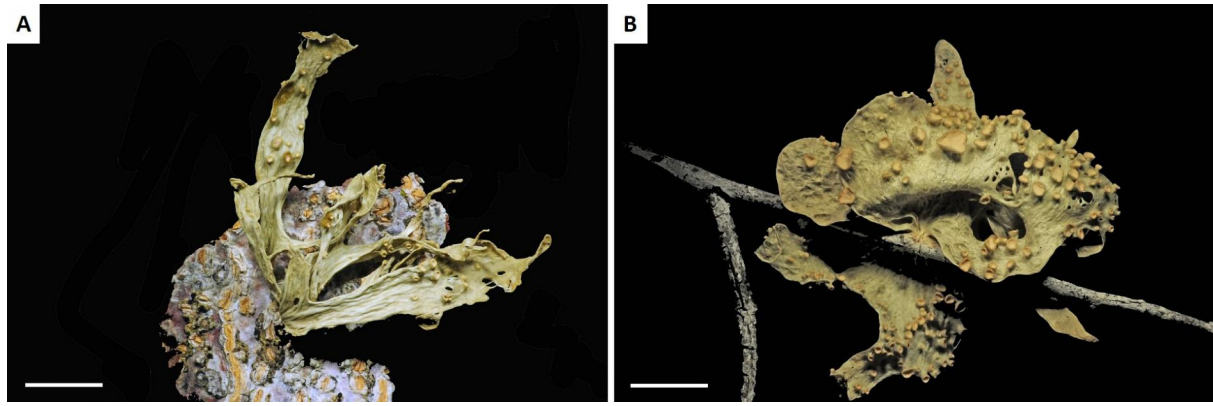


Fig. 6

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