



**HAL**  
open science

## Chemical diversity of five coastal *Roccella* species from mainland France, the Scattered Islands, and São Tomé and Príncipe

Solenn Ferron, Olivier Berry, Damien Olivier-Jimenez, Isabelle Rouaud, Joel Boustie, Françoise Le Lohézic-Devehat, Remy Poncet

### ► To cite this version:

Solenn Ferron, Olivier Berry, Damien Olivier-Jimenez, Isabelle Rouaud, Joel Boustie, et al.. Chemical diversity of five coastal *Roccella* species from mainland France, the Scattered Islands, and São Tomé and Príncipe. *Plant and Fungal Systematics*, 2020, 65 (2), pp.247-260. 10.35535/pfsyst-2020-0021 . hal-03581819

**HAL Id: hal-03581819**

**<https://hal.science/hal-03581819v1>**

Submitted on 6 Mar 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**HAL**  
open science

## Chemical diversity of five coastal *Roccella* species from mainland France, the Scattered Islands, and São Tomé and Príncipe

Solenn Ferron, Olivier Berry, Damien Olivier-Jimenez, Isabelle Rouaud, Joel Boustie, Françoise Le Lohézic-Devehat, Remy Poncet

### ► To cite this version:

Solenn Ferron, Olivier Berry, Damien Olivier-Jimenez, Isabelle Rouaud, Joel Boustie, et al.. Chemical diversity of five coastal *Roccella* species from mainland France, the Scattered Islands, and São Tomé and Príncipe. *Plant and Fungal Systematics*, W. Szafer Institute of Botany, Polish Academy of Science, 2020, 65 (2), pp.247-260. 10.35535/pfsyst-2020-0021 . hal-03581819

**HAL Id: hal-03581819**

**<https://hal.archives-ouvertes.fr/hal-03581819>**

Submitted on 6 Mar 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Main text**

[Download source file \(57.36 kB\)](#)



**1 Chemical diversity of five coastal *Roccella* species from mainland  
2 France, the Scattered Islands, and São Tomé and Príncipe**

**3 Authors**

4 Solenn Ferron<sup>1</sup>, Olivier Berry<sup>1</sup>, Damien Olivier-Jimenez<sup>1</sup>, Isabelle Rouaud<sup>1</sup>, Joël Boustie<sup>1</sup>,  
5 Françoise Lohézic – Le Dévéhat<sup>1\*</sup>, Rémy Poncet<sup>2\*</sup>

6 Françoise Lohezic - Le Dévéhat and Remy Poncet contributed equally to this work

7 Corresponding authors : [francoise.le-devehat@univ-rennes1.fr](mailto:francoise.le-devehat@univ-rennes1.fr) and [remy.poncet@mnhn.fr](mailto:remy.poncet@mnhn.fr)

8 <sup>1</sup>Univ Rennes, CNRS, ISCR (Institut des Sciences Chimiques de Rennes) - UMR 6226, F-  
9 35000 Rennes, France

10 <sup>2</sup>UMS 2006 PatriNat (OFB - CNRS - MNHN), Muséum national d'Histoire naturelle,  
11 Maison Buffon, CP41, 36 rue Geoffroy Saint-Hilaire 75005 Paris

**12 Keywords**

13 *Roccella applanata*, *Roccella belangeriana*, *Roccella fuciformis*, *Roccella montagnei*,  
14 *Roccella phycopsis*, Chemical profile

**15 Abstract**

**Main text**[Download source file \(57.36 kB\)](#)

17 *Roccella* species constitute interesting models to address questions regarding lichen metabolite  
18 diversity across taxonomic, ecological and geographic gradients. Indeed, owing to their wide  
19 distribution, their taxonomic diversity and the narrow ecological niche they occupy, *Roccella*  
20 species are good candidates to study the drivers of lichen chemistry. This study focuses on the  
21 chemical profiling of five species: *R. applanata*, *R. belangeriana*, *R. fuciformis*, *R. montagnei* and  
22 *R. phycopsis*. These five species were sampled in a rather narrow longitudinal range (W1°51' to  
23 E47°17') covering the Eastern Atlantic and Western Indian Ocean areas along an extended  
24 latitudinal range (N48°49' to S22°23'). High Pressure Liquid Chromatography (HPLC) analysis  
25 followed by mass spectrometry of 31 *Roccella* thalli revealed a number of interesting patterns  
26 through a multivariate (PCA) analysis, including the first detailed chemical profiles for two  
27 species from the Scattered Islands: *R. applanata* and *R. belangeriana*. Metabolite segregation  
28 amongst all studied *Roccella* species, including *R. montagnei* and *R. belangeriana*, gave some  
29 insight into the taxonomy of the latter two species, which we interpret as separate species. An  
30 additional analysis focusing on *R. montagnei* samples revealed chemical differences along both  
31 a latitudinal and ecological gradient (from Europa to São Tomé and Príncipe). Three mass  
32 spectra databases were built to dereplicate the ions, which gave an overview of the factors that  
33 could drive quantitative and qualitative metabolite composition in lichens. Additionally, several  
34 new *Roccella* species records are reported for the Scattered Islands as well as São Tomé and  
35 Príncipe.

**Introduction**

36  
37 *Roccella* DC. is a diverse genus of fruticose lichens numbering approximately 30 species (24  
38 according to Tehler *et al.* (2010); 31 according to Aptroot & Schumm (2011)). This genus, which  
39 is mainly coastal and restricted to tropical, subtropical, mediterranean and hyper-oceanic

**Main text**[Download source file \(57.36 kB\)](#)

41 localities of temperate areas, has long drawn naturalists' interests as most of its species have  
42 large, fruticose thalli (sometimes in abundant populations) and are of economic interest for their  
43 tinctorial properties. For these reasons, *Roccella* was among the first lichen genera to be  
44 described (de Lamarck & de Candolle 1805) and it has received continuous attention since then.  
45 Multiple studies by Tehler *et al.* (2004, 2007, 2009a, 2009b, 2010) brought significant advances  
46 for assessing the diversity and distribution of the genus, and together with the world-key  
47 provided by Aptroot & Schumm (2011) they provide a rather good framework for studies dealing  
48 with *Roccella*. About 54 secondary metabolites have been reported for this genus, belonging to  
49 several classes of compounds. One can find depsides such as erythrin, lecanoric acid, lepranic  
50 acid (Huneck 1967a; Huneck 1967b; Aberhart 1969) as well as their sub-units, the  
51 monoaromatic phenols, e.g. beta-orcinol, ethyl orsellinate, and montagnetol (Aberhart 1969;  
52 Duong 2017 and 2018; Huneck 1968; Mallavadhani 2018; Parrot 2015). Aliphatic acids like  
53 roccellic acid (Huneck 1964) and the chromone 6-hydroxymethyleugenitin (Huneck 1972) have  
54 also been reported. Depsidones are not as common in the genus *Roccella*; so far they have only  
55 been isolated from *R. hereroensis* and *R. mossamedana* (Follman 1986). They have also been  
56 reported from the following Roccellaceae genera: *Dendrographa*, *Enterographa*, *Lecanactis*,  
57 and *Opegrapha*. Dibenzofurans are also uncommon; the only one known is schizopeltic acid,  
58 reported from *R. capensis* as well as the genus *Schizopelte* (Roccellaceae) (Follman 1986; Elix  
59 1992).

60 Despite the wealth of knowledge amassed for the genus *Roccella*, gaps in our knowledge  
61 remain regarding chemistry, ecology and distribution of the species. This paper aims to fill some  
62 of these gaps by providing new records for several species from two tropical, equatorial  
63 localities: the Scattered Islands (Indian Ocean, French Southern and Antarctic Lands (TAAF))  
64 and São Tomé and Príncipe (Atlantic Ocean). In addition, complete chemical profiles are  
65 provided for five coastal species: *Roccella applanata* M. Choisy, *Roccella belangeriana* D.D.

**Main text**[Download source file \(57.36 kB\)](#)

67 Awasthi, *Roccella fuciformis* (L.) DC., *Roccella montagnei* Bél. and *Roccella phycopsis* Ach.  
68 Special attention is paid here to assessing chemical diversity (both quantitative and qualitative)  
69 of these species along the following gradients: species, individuals, territories and ecology.

**Materials and methods***Lichen samples for chemical analysis*

72 The five *Roccella* species studied (figure 1) were selected among available material in the  
73 authors' collection ("JB" for Univ. Rennes and "RP" for UMS PatriNat) sampled in different  
74 locations between 2002 and 2019. A total of 31 specimens were studied.

75 Figure 2 presents locations where the studied material was collected and Table 1 gives  
76 supporting information for all the samples studied in this paper, along with territories where the  
77 species were reported for the first time. The French mainland specimens (JB collection) were  
78 three samples of *R. fuciformis* collected on granite rocks in Ploumanac'h (22), Saint Malo (Pointe  
79 des Chevrets, 35) and Cancale (Pointe du Grouin, 35); and three samples of *R. phycopsis*  
80 collected from granite rocks in Saint Malo (Pointe des Chevrets, 35) and in Cancale (Pointe du  
81 Grouin, 35). The specimens collected in the Scattered Islands (RP collection) were all collected  
82 in 2019 from the bark or wood of several porophytes following a protocol dedicated to assessing  
83 the corticolous and lignicolous lichen diversity of these territories (Hivert 2019). The Scattered  
84 Islands specimens were three samples of *R. applanata* and six samples of *R. belangeriana*, all  
85 from Europa Island; and 16 samples of *R. montagnei*, all from Europa island, Juan de Nova and  
86 Grande Glorieuse. The São Tome and Príncipe specimens (RP collection) were three samples  
87 of *R. montagnei* collected on the bark of an unidentified tree species. Altogether, samples from  
88 the French mainland represent two of the three *Roccella* species known from France (Roux *et*  
89 *al.* 2017). The third species known from France, *R. tinctoria*, is mainly present in Corsica (a  
90 mediterranean climate); its presence in Brittany (a hyper-oceanic climate) remains uncertain. We

**Main text**[Download source file \(57.36 kB\)](#)

92 did not include any samples of *R. tinctoria* in our analyses. Samples from the Scattered Islands  
93 represent all *Roccella* species present on each island (Poncet *pers. obs.*) and samples from São  
94 Tome and Príncipe represent one of the two known species from this territory where *R.*  
95 *fuciformis* had been recorded by (Nylander 1889); however, we consider this data uncertain  
96 owing to its currently known distribution.

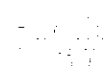
**Extraction**

97  
98 Several whole thalli of air-dried lichens (30-100 mg) were cleaned and ground under nitrogen to  
99 ensure sufficient pulverization and homogenization which allowed impregnation by the solvents  
100 during the extraction process. They were extracted using an extractor device (Heidolph  
101 Synthesis), under agitation (1000 rpm), at 35°C during 45 minutes with acetone (2 mL) four  
102 times. All filtrates were mixed and evaporated using a Speed Vac Concentrator SPD121P  
103 (Thermo Savant) to obtain extracts which were weighted to prepare the sample solutions.  
104 Triplicates (or more) were used for each species (table 1).

**High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS) Analysis**

105  
106 All extracts were dissolved in tetrahydrofuran (THF) at a concentration of 1 mg.mL<sup>-1</sup> and filtered  
107 (0.45 µm) before HPLC injection.

108 HPLC analysis was carried out using a Prominence Shimadzu LC-20AD system (Marne La  
109 Vallée, France). The samples were eluted through a C18-column (2.6 µm, 100 × 4.6 mm,  
110 Phenomenex). The mobile phase used 0.1% formic acid in HPLC-grade water (solvent A) and  
111 0.1% formic acid in acetonitrile (solvent B). The flow rate was 0.5 ml/min. Gradient elution was  
112 employed, commencing at 20% B, held for 5 min, increased to 80% B over 25 min, then  
113 increased 100% B over 5 min, held for 7 min before decreasing back to 20% B held for 3 min. A



**Main text**

[Download source file \(57.36 kB\)](#)

115 solvent blank containing solvent only was run for the purpose of subtracting background spectra  
116 (Gadea 2018).

117 Mass spectrometry analysis was carried out using an ADVION expression CMS mass  
118 spectrometer. We used an electrospray ionization source over a mass range of 99.85-999.8  $m/z$   
119 in negative mode. The ion spray voltage was set at 3.5kV, the capillary voltage at 180V, the  
120 source voltage offset at 20 V, the source voltage span at 20 V, the source gas temperature at 50  
121 °C, and capillary temperature at 250°C. LC-MS data was acquired using Advion data express  
122 software.

*Mass Spectral Data processing*

124 Each MS1 .datx file was exported as \*.cdf and mass spectral data was processed using the  
125 MZmine 2.52 freeware (<http://mzmine.sourceforge.net/>) (Pluskal 2010). The mass detection was  
126 performed with a noise level set at 2E6. ADAP chromatogram builder (Myers 2017) was run  
127 using as parameters a minimal number of scans of 4; group intensity threshold and min highest  
128 intensity of 2E6 and  $m/z$  tolerance of 0.8  $m/z$ . Then deconvolution was applied using the ADAP  
129 Wavelets algorithm. Dereplication was realized through three personal databases built in the lab.  
130 The first one, called HLDB, uses standards injected according to the same chromatographic  
131 protocol; identification was done by comparison of their retention time and MS1 data. The  
132 second one, called RoccellaDB, was realized through an extensive bibliographic study of the  
133 metabolites found in Roccellaceae family coupled with their molecular weight obtained from  
134 Huneck Data's (Huneck 1996) as well as prediction of their retention time (predicted RT). These  
135 predicted RT (text S1) were determined by a linear regression based on their lipophilicity using  
136 the online AlogPs software (Tetko 2005). The third one, called LDB-MS1, was a database built  
137 by Damien Olivier-Jimenez (*in prep.*) ; it contains a large array of lichen metabolites together



**Main text**

[Download source file \(57.36 kB\)](#)

139 with their structures, along with their calculated  $m/z$  (here for  $[M-H]^-$ ) and their predicted RT. All  
140 data were exported as .csv files for further multivariate analysis.

**Multivariate analysis**

142 All MZmine files (MZattributes.csv) were uploaded to MetaboAnalyst 4.0 software  
143 (<https://www.metaboanalyst.ca/MetaboAnalyst>)(Chong 2018) for multivariate statistical data  
144 analysis. The file comprised a list of features ( $m/z$ , retention times and intensities). Integrity was  
145 checked, missing values were replaced by very low values (half of the lowest), and data were  
146 filtered using the interquartile range (IQR) to remove variables close to the baseline. All data  
147 were normalized using log transform and scaled by the Pareto method (mean-centered and  
148 divided by the square root of standard deviation of each variable (van den Berg 2006).

**Results****Metabolic profiles of the five Roccella species**

151 The LC-MS profiles of the five species were acquired and the mass chromatograms are  
152 provided in Figure 3. Four major compounds were identified according to their retention time and  
153 their  $m/z$  using the HLDB (figure 3): the two depsides erythrin (RT=15.8 min;  $m/z=421$ ) and  
154 lecanoric acid (RT=19.4 min;  $m/z=317$  ( $[M-H]^-$ , molecular peak) and 167 ( $[M-C_8H_7O_3]^-$ , base  
155 peak)); the aliphatic roccellic acid (RT=29.7 min;  $m/z=299$ ); and the chromone lepralic acid  
156 (RT=19.4 min;  $m/z=361$  ( $[M-H]^-$ , molecular peak) and 317 ( $[M-CHO_2]^-$ , base peak)). The depside  
157 lecanoric acid and the chromone lepralic acid have the same RT, and present a similar  $m/z$   
158 (317) but they are distinguishable par the analysis of complete mass spectrum (figure 4). *R.*  
159 *fuciformis* contains only lepralic acid ( $[M-H]^- = 361.1$ ; main  $m/z$  detected: 317.0); whereas *R.*  
160 *phycopsis*, *R. applanata*, *R. belangeriana* and *R. montagnei* only contain lecanoric acid ( $[M-H]^-$   
161 =317; main  $m/z$  detected: 167.0).

**Main text**[Download source file \(57.36 kB\)](#)

163 The untargeted and automated processing of these LC-MS data sets, including alignment and  
164 clustering of all mass signals into so-called reconstructed chromatograms, resulted in a data  
165 matrix of 101 features for all samples analyzed. Finally, in addition to the four main metabolites,  
166 seventeen additional compounds were detected. Among them, ten metabolites were annotated  
167 with bibliographic support; and for the remaining seven, we found either candidates or a  
168 substructure. The depside erythrin was found in all five *Roccella* species, and lecanoric acid was  
169 detected in four species. When lecanoric acid was absent (*R. fuciformis*), the chromone lepranic  
170 acid replaced it. No roccelic acid was detected in *R. fuciformis* and *R. applanata*, and the ethyl  
171 ether of lepranic acid was only detected in *R. fuciformis*. All the putatively annotated compounds  
172 are essentially from the monophenolic compounds group. Finally, the possible presence of the  
173 butyrolactone roccellaric acid in *R. montagnei*, and the aliphatic acid angardianic acid in *R.*  
174 *montagnei* and *R. phycopsis*, can be noted.

*Assessment of metabolic segregation between the five Roccella species*

176 The data matrix obtained after MS data processing was analysed using statistical analysis. In a  
177 first step, an unsupervised multivariate analysis approach (PCA: Principal Component Analysis)  
178 was applied to determine differences between samples based on the metabolite presence and  
179 the peak intensities. The PCA scores scatter plots of MS1 data and showed a separation  
180 between species up to 52.5 % of the total variance by the first three principal components (figure  
181 5a).

182 Next, the first three components (PC1, PC2 and PC3) were extracted from the m/z, retention  
183 time and intensities of ions under chromatographic conditions. It was demonstrated that there is  
184 a clear discrimination between species along the three first components PC1 to PC3 (figure 5a).  
185 *R. fuciformis* specimens constitute a separated outgroup due to the presence of lepranic acid

## Main text

[Download source file \(57.36 kB\)](#)



187 (table 2), which is clearly visible on PC2. *Roccella phycopsis*, which is the second species (with  
188 *R. fuciformis*) to grow on rocks in mainland France, forms a compact group located peripherally  
189 to an ensemble formed by the three corticolous species *R. applanata*, *R. belangeriana* and *R.*  
190 *montagnei*, which are themselves also rather well-segregated from each other. All of the latter  
191 three species are distributed only along a two-dimensional gradient, on the PC1 and PC2 axis.

192 Then, we used PLS-DA (Supervised Partial Least Squares Discriminant Analysis)(Tenenhaus  
193 1998) to compare the metabolite profiles between samples. The multivariate analyses show  
194 clear partitioning in the metabolomic profiles among the five species (figure 5b). *R. applanata*, *R.*  
195 *belangeriana*, *R. montagnei* are mainly located along PC1 while PC2 discriminates *R. phycopsis*  
196 from *R. fuciformis* and PC3 distinguishes *R. montagnei* from *R. phycopsis*. We can also notice  
197 that *R. applanata* and *R. fuciformis*, the only two species where no roccellic acid was found  
198 (table 2), only segregates according to PC2.

199 The main discriminant metabolites (*m/z*) are reported on figure 5c using the measure of the  
200 variable's importance in PLS-DA model (VIP score), the highest VIP scores, the most  
201 contributive variable is in class discrimination. Roccellic acid was the most discriminant  
202 metabolite for *R. phycopsis*; erythrin, roccellic acid and montagnetol/roccellatol derivative were  
203 important for distinguishing *R. montagnei*; and lecanoric acid and lepranic acid were important  
204 metabolites for segregating *R. fuciformis*. By contrast, *R. belangeriana* was distinguished by the  
205 presence of lecanoric acid, and *R. applanata* by the presence of erythrin together with roccellic  
206 acid.

### 207 *Focus on the R. montagnei-belangeriana complex*

208 The phylogenetically close species *R. montagnei* and *R. belangeriana* (Tehler 2007; Prashanth  
209 2008) were further studied. Using the HPLC - MS data of both species, a PCA analysis was

**Main text**[Download source file \(57.36 kB\)](#)

211 performed for dimension reduction of multivariate data whilst preserving most of the variance for  
212 both species. It revealed two clusters representing 55.1 % of the total variance (figure 6a). The  
213 PLS-DA was then applied, highlighting significant differences between *R. montagnei* and *R.*  
214 *belangeriana* (figure 6b). The variable importance in projection (VIP scores) indicated roccellic  
215 acid and erythrin as discriminant metabolites for *R. montagnei*, while lecanoric acid and erythrin  
216 were discriminant metabolites for *R. belangeriana* (figure 6c) (VIP > 1). All these metabolites  
217 were detected in thalli (table 2) of both species, but with different intensities.

*Metabolite variation in R. montagnei at different latitudes*

218  
219 Metabolites data restricted to *Roccella* species of this study comprising four tropical territories  
220 (Europa, Juan de Nova, Grande Glorieuse and São Tome and Príncipe), under comparable  
221 ecological conditions (i.e., corticolous at sea level), was used to investigate if the geographical  
222 location (or latitude gradient) has an influence on the *Roccella* metabolome. The mass data  
223 processing was based on the ionisation of each metabolite and in a first step, for each ion, we  
224 calculated the ratio of each area intensity to build figure 7.

225 Among the compounds identified from HLDB and from the RoccellaDB, the three metabolites  
226 erythrin, roccellic acid and orsellinylmontagneton A (or O. B, or O. C) are present in rather similar  
227 proportions in the lichen thalli from the four locations (figure 7). Among the 7 other metabolites  
228 identified, two were present in only one location: angardianic acid (Europa) and roccellaric acid  
229 (Grande Glorieuse), three were present in two locations: montagneton or roccellatol derivative  
230 and lecanoric acid (Europa and Grande Glorieuse), orsellinylmontagneton D (Europa and São  
231 Tome and Príncipe), and two were present in three locations. In addition, *R. montagnei*  
232 specimens growing in Europa contain the largest diversity of metabolites (8/10), followed by

**Main text**[Download source file \(57.36 kB\)](#)

234 those from Grande Glorieuse (7/10), from São Tome and Príncipe (6/10) and from Juan de Nova  
235 (5/10).

236 In a second step, we performed a multivariate PCA analysis (figure 8a), followed by a  
237 supervised PLS-DA (figure 8b). Although the R<sup>2</sup> and Q<sup>2</sup> values for PLS-DA were quite low, it  
238 appears that specimens found in Europa differed from those from São Tome and Príncipe along  
239 the PC1 axis (22.7%). PC3 axis allows discrimination between the specimens collected in  
240 Grande Glorieuse and the others. The compounds that contributed the most (VIP > 1) to  
241 discrimination were montagnetol/roccellatol derivative, erythrin and roccellic acid for thalli  
242 collected in Europa; erythrin for *R. montagnei* collected in Juan de Nova; and montagnetol and  
243 orsellinyl montagnetol for specimens collected in Sao Tomé (figure 8c).

**Discussion***Improved knowledge of Roccella chemistry*

246 The establishment of metabolic profiles of the five *Roccella* species included in this study (table  
247 2) improves our knowledge of these species' chemistry and according to the data contained in  
248 RoccellaDB (our bibliographic database focused on metabolites found in Roccellaceae), five  
249 compounds were detected for the first time in this genus: 2,4-dihydroxyphthalide (*R. phycopsis*;  
250 French mainland); orcinyl lecanorate (*R. phycopsis*; French mainland); roccellaric acid (*R.*  
251 *montagnei*; Grande Glorieuse); cholesta-3,5-dien-7-one (*R. belangeriana*; Europa); and oleic  
252 acid (*R. belangeriana*; Europa). Moreover, the chemistry of *R. applanata* (Europa) was profiled  
253 for the first time, resulting in the identification of three compounds (all monophenolic compounds  
254 or depsides), plus four requiring further investigation (all probably polyphenolic compounds). *R.*  
255 *belangeriana* (Europa) was also profiled for the first time, resulting in the dereplication of seven

**Main text**[Download source file \(57.36 kB\)](#)

257 compounds (all monophenolic compounds, depsides, aliphatic acids, polyphenolic compounds  
258 or terpenes), plus two polyphenols requiring further investigation and two apolar putative  
259 compounds (oleic acid and cholesta-3,5-dien-7-one). Additionally, two compounds were  
260 dereplicated for the first time in *R. montagnei*: roccellaric acid (butyrolactone) and angardianic  
261 acid (aliphatic acid), plus two requiring further investigation, and three compounds in *R.*  
262 *phycopsis*: acetylerythritol (polyol), 2,4-dihydroxyphthalide (monophenolic compound), orcynyl  
263 lecanorate (depside) plus one requiring further investigation. In total, these analyses performed  
264 under the same conditions with all specimens allowed us to assess metabolite variation between  
265 species, with a maximum diversity of compounds reached by *R. belangeriana* ( $n = 11$ ), followed  
266 by *R. montagnei* ( $n = 10$ ), *R. phycopsis* ( $n = 9$ ), *R. applanata* ( $n = 8$ ) and *R. fuciformis* ( $n = 4$ ). It  
267 is possible that if our sample sizes were increased for each species, further chemical diversity  
268 would be discovered. Interestingly, our analyses—performed on just a fraction of the total known  
269 *Roccella* species—seems to indicate that metabolite richness is higher in corticolous species  
270 than in saxicolous ones; however, these results are tentative, and further investigation is  
271 necessary to make more robust conclusions. Another interesting result is that there seems to be  
272 no correlation between the extension of species distribution area and compound richness.  
273 Indeed, *R. montagnei*, *R. phycopsis* and *R. fuciformis* are widespread species, whereas *R.*  
274 *applanata* is an endemic species from Madagascar and Europa.

275 The depside erythrin was found in the five *Roccella* species, and this aromatic compound was  
276 reported in 18 taxa of *Roccella* out the 54 (infra-specific taxa included)(Brakni 2018; Duong  
277 2017; Huneck 1967a, 1967b, 1968; Parrot 2015; Strack 1979; Swedan 2017; Thadhani 2012). It  
278 appears that either this compound is present in *Roccella* species; or, another depside, lecanoric  
279 acid, is present. Only four species have been reported possessing both depsides: *R. phycopsis*,  
280 *R. linearis* (var. *guineensis* and var. *hypochromatica*) and *R. montagnei* from Asia and Africa  
281 (Huneck 1968). The chromone lepranic acid was only found in *R. fuciformis*; it appears to be

**Main text**[Download source file \(57.36 kB\)](#)

283 diagnostic for this species (Aberhart 1969). Surprisingly, acetylportentol was not found in *R.*  
284 *fuciformis* by Swedan (2017); however, this aliphatic compound is not expected to be detected  
285 under UV, and appears to be hardly ionized. Therefore, we analysed the extracts of *R. fuciformis*  
286 with HPLC-MS using an universal Evaporation Light Scattering Detector (ELSD) and confirmed  
287 the presence of a peak at RT =23.30 min corresponding to acetylportentol (data not shown).  
288 Nevertheless, *R. fuciformis* can be easily identified by its other metabolites, such as lecanoric  
289 and lepranic acids. Moreover, given the high intensities of the peaks for the main compounds, it  
290 was difficult to detect picrorocellin (Marcuccio 1983) and 6-hydroxy-methyleugenitin in *R.*  
291 *fuciformis* (Huneck 1972).

292 Three acids were detected or suspected: roccelic acid was detected in *R. belangeriana* (two  
293 specimens out of 6: BeEEs310B and BeECT339), *R. montagnei* (all specimens) and *R.*  
294 *phycopsis* (all specimens); angardianic acid was suggested for *R. phycopsis* (two out of three  
295 specimens: PhBs64, PhBs72) and three specimens out of 16 of *R. montagnei* (three specimens  
296 out of 16: MoESm324B, MoEEs310, MoESm334); and roccellaric acid was suspected in one  
297 specimen of *R. montagnei* (MoGGCe450A). As seen in figure S3, roccelic acid differs from  
298 angardianic acid by the length of the side chain with 12 vs. 14 carbons, respectively. Roccellaric  
299 acid has a side chain with thirteen carbons, but results from the cyclisation of the angardianic  
300 acid. Thus, it is necessary for the lichen to have sufficient energy to produce longer chains, and  
301 also to activate enzymes like deshydratases to form the paraconic acid, roccellaric acid. This  
302 could explain why roccelic acid seems more prevalent in the *Roccella* species (e.g. *R.*  
303 *montagnei*) that also produce angardianic and roccellaric acids.

304 *R. montagnei-belangeriana* complex

**Main text**[Download source file \(57.36 kB\)](#)

306 The studied dataset showed that a clear segregation occurs in metabolite composition between  
307 specimens considered in this publication as *R. montagnei* (thallus C+ red, generally sterile, with  
308 soralia, rarely with apothecia) and *R. belangeriana* (thallus C+ red, fertile, without soralia). These  
309 two taxa are considered as synonyms by Tehler *et al.* (2010), although they could not integrate  
310 the holotype into their phylogenetic analysis of *Roccella* species from the Paleotropics (because  
311 they were unable to receive the holotype on loan). Prashanth *et al.* (2008) also asserted that *R.*  
312 *montagnei* and *R. belangeriana* are the same species, basing their conclusion on the analysis of  
313 ITS sequences and indicating that these two taxa are similar in both morphology and secondary  
314 chemistry. The results obtained here tend to reinforce the existence of at least two taxa in the *R.*  
315 *montagnei-belangeriana* complex. First, Table 2 and figures 6a and 6b show that the species *R.*  
316 *belangeriana* and *R. montagnei* differed statistically by their monophenolic compounds (orsellinic  
317 acid roccellatol, orsellinylmontagnetol, montagnetol/roccelatol derivative) as well as by their  
318 aliphatic acids (roccellaric and angardianic acids). Nevertheless, the statistical analyses also  
319 suggest that only roccellic acid, erythrin and lecanoric acid are the only reliable discriminant  
320 metabolites, even though these three compounds are present in both species (figure 6c). This  
321 can be explained by the difference of intensities of these major compounds: when a metabolite  
322 is concentrated, some charged dimers and monophenolic compounds are generated in the mass  
323 spectrometer. Because of this, all these ions are analysed and are thus taken into account for  
324 the multivariate analysis. Moreover, as we can see in figure S2 (supporting info), all the  
325 monophenols are units of erythrin, which is itself an ester of erythritol with lecanoric acid. In  
326 addition, their uneven distribution across the Scattered Islands (*R. montagnei* and *R.*  
327 *belangeriana* are both present in Europa, but *R. montagnei* is the only *Roccella* found in Juan de  
328 Nova and Grande Glorieuse) suggests that their dispersal strategy (or perhaps some difference  
329 in their ecological niches) do not allow them to colonise as efficiently these rather close  
330 territories (figure 2). This difference in their distribution suggests the existence of at least two  
331 ecotypes, or two species, among which one has the ability to abundantly produce apothecia,



**Main text**[Download source file \(57.36 kB\)](#)

333 when the other only uses an asexual dispersion strategy (apothecia can rarely be found, but  
334 even so, the thalli remain sorediate). Finally, based on our limited sample sizes for both species,  
335 it appears that the two have rather distinct ecological niches (*R. belangeriana* occurs more often  
336 on mangrove trees), even if they sometimes were found together on the same tree with  
337 significantly bigger thalli of *R. belangeriana* (wider branches) compared to *R. montagnei*. So,  
338 combined with these observations, the distinct metabolite profiles of these two taxa is an  
339 incentive for further phylogenetic analysis based on several loci and on additional material from  
340 the *Roccella montagnei-belangeriana* complex.

*Metabolites profiling across location of R. montagnei*

342 The results showed that the only *Roccella* species common in Europa, Juan de Nova, Grande  
343 Glorieuse, São Tomé and Príncipe display metabolic differences, both qualitatively and  
344 quantitatively (figure 8a, 8b, 8c). However, we can note that PCA did not show clear segregation  
345 between locations, as clearly shown in the PLS-DA scatter plot (figure 8b). Erythrin was the only  
346 diagnostic compound for the *S. montagnei* samples collected in Juan de Nova, while  
347 monophenolic compounds (montagnetol, orsellinylmontagnetol) differentiated thalli growing in  
348 São Tomé and Príncipe. Thalli collected in Europa contain roccellic acid in addition to  
349 montagnetol/roccellatol derivatives and erythrin; however, six of the 16 *R. montagnei* thalli were  
350 collected in Europa, and this higher chemical diversity could be a result of greater sample size.  
351 As stated previously, erythrin is the final step of the proposed biosynthetic pathway resulting  
352 from two esterification reactions: the first, between monophenolic units; and the second, the  
353 joining of the depside lecanoric acid with the polyol erythritol (Culberson 1969)(Fig. S3). A  
354 possible explanation of variations of this metabolite between sampling locations could be  
355 variation in exposure of the thalli to solar radiation (UV). Indeed, thalli growing in Europa and  
356 Juan de Nova were often collected in less sheltered conditions, on the shrub *Suriana maritima*,  
357 while those from Grande Glorieuse were collected on the tree *Casuarina equisetifolia* where they

**Main text**[Download source file \(57.36 kB\)](#)

359 probably benefited from the shade of its canopy. The same can be said for those collected in  
360 São Tomé and Príncipe, which were growing on the trunks and branches of an unidentified tree,  
361 and thus protected from the sun. In other words, an interesting gradient is seen between  
362 specimens growing in a tropical climate. Specifically, material collected from Europa and Juan  
363 de Nova with supposed UV-exposed conditions produce large quantities of erythrin, while  
364 specimens collected from Grande Glorieuse in rather sheltered locations produce a mixture of  
365 erythrin and monophenolic compounds. The latter are also the dominant compounds in  
366 specimens collected from São Tomé and Príncipe, which are also more sheltered from UV  
367 exposure. For the two studied species growing in the Northern hemisphere and generally found  
368 in sheltered rocks, the diagnostic compounds for *R. fuciformis* were the two depsides lecanoric  
369 and lepraric acids, while the diagnostic compound for *R. phycopsis* was the aliphatic acid  
370 roccellic acid. Future studies could incorporate complementary molecular analyses to help  
371 address whether these chemical variations among thalli are either inherited, or acquired due to  
372 environmental conditions. However, no ecological variables (e.g. amount of solar radiation) was  
373 measured as part of this study, thus these proposed explanations are conjectural and further  
374 data measurements of UV radiation are needed to address their reliability.

**Conclusion**

375  
376 The following six compounds were found in large amounts in the five *Roccella* species studied:  
377 erythrin, lecanoric acid, lepraric acid, orsellinyl montagnetol, montagnetol and roccellic acid. Of  
378 these five species, the chemistry of *R. applanata* and *R. belangeriana* were studied in detail for  
379 the first time. Distinct chemical profiles support segregation of all these species regardless of  
380 their ecological or geographic similarity or phylogenetic relationship. Analyses of the chemical  
381 profiles of *R. montagnei* and *R. belangeriana*—together with other, anecdotal observations related

**Main text**[Download source file \(57.36 kB\)](#)

383 to distribution and ecology—give additional support to distinguish them as separate species.  
384 Molecular phylogenetic analyses involving duplicates of the material analyzed, here along with  
385 additional material from the *R. montagnei-belangeriana* complex, would be helpful to better  
386 circumscribe species in this complex. Finally, a comparison of the profiles and relative amounts  
387 of metabolites between the sub-tropical *Roccella* species (from Europa, Juan de Nova, Grande  
388 Glorieuse, São Tomé and Príncipe) suggests an effect of sun exposure on chemical composition  
389 of these lichens. Our results indicate that species explains the majority of chemical diversity  
390 seen amongst the species studied here, with slight modifications driven by ecological factors  
391 such as substrate (corticolous species seem chemically richer than saxicolous ones) and sun-  
392 exposure (specimens from more sheltered conditions being more chemically diverse ).

**Acknowledgements**

393  
394 Aurélie Bernard is acknowledged for her technical assistance. The authors warmly thank Rémy  
395 Ferron for his help in the set-up of the retention time prediction model used for the RoccellaDB  
396 and LDB-MS1 databases. We are also grateful to Léonie Gourdon who initiated the data  
397 analysis. RP gives special thanks to Sylvie Chevalier (UMS 2006 PatriNat), Mélanie Hubert  
398 (UMS 2006 PatriNat), Laurent Poncet (UMS 2006 PatriNat), Julien Touroult (UMS 2006  
399 PatriNat) and Serge Muller (ISYEB – MNHN) for their trust and mostly for their invaluable help in  
400 this project, and without whom none of this would have been possible. Sophie Marinesque  
401 (TAAF), Jonathan Grand (TAAF) and Cedric Marteau (TAAF) who coordinated the consortium  
402 and the field campaigns, and invested countless energy and time for improving the Scattered  
403 Islands natural heritage knowledge and conservation, are also warmly thanked. Finally, friendly  
404 thanks go also to Jean Hivert (CBNM) for his help in designing the field sampling and  
405 identification of trees/shrubs in the Scattered Islands; and to Christian Fontaine (CBNM),



## Main text

[Download source file \(57.36 kB\)](#)

407 Frédéric Picot (CBNM), Ehoarn Bidault (MBG) and Anne-Hélène Paradis (MBG) for their  
408 invaluable help in the sampling and foraging of lichens species in the Scattered Islands (JH, CF,  
409 FP, EB) and in Sao Tomé (AHP). We are grateful to S. LaGreca for its useful comments which  
410 greatly improved the manuscript.

## 411 Funding

412 Some species studied in this paper were collected in the frame of the "Scattered Island" inter-  
413 agency research consortium (2017-2021) coordinated by the French Southern and Antarctic  
414 Lands (TAAF) in partnership with: Centre National de la Recherche Scientifique, Institut de  
415 Recherche pour le Développement, Ifremer, Agence Française pour la Biodiversité, Université  
416 de La Réunion & Université de Mayotte. Fieldwork was funded by the inter-agency research  
417 consortium and was done jointly by the Conservatoire Botanique National de Mascarin, the  
418 Missouri Botanical Garden and the Muséum National d'Histoire Naturelle (UMS 2006 PatriNat  
419 AFB - CNRS - MNHN) in the frame of the RECOFFIE (Renforcement des COnnaisances sur la  
420 Flore et la Fonge des Iles Éparses) project, implemented as part of the "Scattered Island" inter-  
421 agency research consortium (2017-2020).

## 422 Permits

423 Collecting of the lichen species were authorized in the Scattered Islands according to the permit  
424 delivered by C. Geoffroy, General Secretary of French Southern and Antarctic Lands and district  
425 head of the Scattered Island. The RECOFFIE (CBN-CPIE Mascarin, MBG, UMS 2006 PatriNat  
426 (OFB - CNRS - MNHN)) project was authorized by order n°2019-40 of April 1, 2019. Collecting  
427 of the lichen species were authorized in São Tomé and Príncipe according to the permit  
428 003/2019 delivered by CIAT-STP (Ministério de Agricultura, Pesca e Desenvolvimento Rural) in  
429 February 2019.

## Main text

[Download source file \(57.36 kB\)](#)



## Competing interests

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

## References

Aberhart, D.J., Overton, K.H., Huneck, S. 1969. Studies on lichen substances. Part LXII. Aromatic constituents of the lichen *Roccella fuciformis* DC. A revised structure for lepranic acid. *Journal of the Chemical Society* 5: 704-707.

Aptroot, A. & Schumm, F. 2011. Fruticose Roccellaceae: an anatomical-microscopical atlas and guide with a worldwide key and further notes on some crustose Roccellaceae or similar lichens. Published by the authors and BoD [Books on Demand, available from fschumm@online.de], The Netherlands.

Brakni, R., Ahmed, M. A., Burger, P., Schwing, A., Michel, G., Pomares, C., Hasseine, L., Boyer, L., Fernandez, X., Landreau, A., Michel, T.. 2018. 'UHPLC-HRMS/MS based profiling of Algerian lichens and their antimicrobial activities, *Chemistry and Biodiversity*, 15, e1800031.

Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., et al. 2018. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research* 46, W1: 486- 494.

Culberson, C.F. 1969. Chemical and botanical guide to lichen products. Chapel Hill (N.C.): University of North Carolina press.

## Main text

[Download source file \(57.36 kB\)](#)



451 de Lamarck, J.B. & de Candolle, A.P. 1805. Flore Française, ou descriptions succinctes de  
452 toutes les plantes qui croissent naturellement en France, 3(2). H. Agasse, Paris.

453 Duong, T. H., Huynh, B. L. C., Chavasiri, W., Chollet-Krugler, M., Nguyen, V. K., Nguyen, T. H.  
454 T., Hansen, P. E., Le Pogam, P., Thüs, H., Boustie, J., & Nguyen, K. P. P. 2017. New erythritol  
455 derivatives from the fertile form of *Roccella montagnei*. *Phytochemistry*, 137, 156–164.

456 Duong, T. H., Bui, H. 2018. Chemical constituents of the lichen *Roccella sinensis* growing in  
457 Binh Thuan province. *Science and Technology Development Journal - Natural Sciences*, 2(5),  
458 63-67.

459 Elix, J., Naidu, R., Laundon, J.R. 1992. Synthesis of the lichen dibenzofuran pannaric acid 2-  
460 methyl ester and its isomer 3-O-methylpannaric acid. *Australian Journal of Chemistry* 45 (4):  
461 785-779.

462 Follman, G., Geyer, M. 1986. Preliminary Studies towards a monograph of the lichen family  
463 Roccellaceae Chev. V ll. Secondary products and relationships of the genera *Combea* de Not.  
464 and *Schizopelte* T. M. Fries. *Zeitschrift für Naturforschung* 41c: 1117-1118.

465 Gadea, A., Le Lamer, A.C., Le Gall, S., Jonard, C., Ferron, S., Catheline, D., Ertz, D., Le  
466 Pogam, P., Boustie, J., Maryvonne, C., Lohezic - Le Devehat, F. 2018. Metabolite profiles of the  
467 lichen *Argopsis friesiana* may explain sectorial land snail damage. *Journal of Chemical Ecology*.  
468 44(5): 471-472.

469 Hivert, J., Bidault, E., Poncet, R., Fontaine, C., Picot, F., 2019, CONSORTIUM DE  
470 RECHERCHE « ILES EPARSEES 2017-2020 » - RECOFFIE : Renforcement des  
471 COnnaissances sur la Flore et la Fonge des îles Éparses - Rapport de campagne (4-30 avril  
472 2019). 100p.

**Main text**

[Download source file \(57.36 kB\)](#)

474 Huneck, S., Follmann, G. 1964. Das Vorkommen von Psoromsäure in *Chiodecton stalactinum*  
475 NYL. und Roccellsäure in *Dirina lutosa* ZAHLBR. *Zeitschrift für Naturforschung* 19 b: 658-659.

476 Huneck, S., Follmann, G. 1967a. Zur Chemie chilenischer Flechten. XVI. Über die Inhaltsstoffe  
477 einiger Roccellaceen. *Zeitschrift für Naturforschung* 22(3): 362-366.

478 Huneck, S. 1967b. Über die Inhaltsstoffe von *Combea mollusca* (ACH.) DE NOT., *Roccella*  
479 *vicentina* (WAIN.) WAIN., *Roccella gayana* MONT. und *Roccella fucoides* (NECK.) WAIN.  
480 *Zeitschrift für Naturforschung* 22b: 1369-1370.

481 Huneck, S., Follmann, G. 1968. Mitteilungen über Flechteninhaltsstoffe LV. Zur Phytochemie  
482 und Chemotaxonomie einiger Chiodectonaceen und Roccellaceen). *Berichte der Deutschen*  
483 *Botanischen Gesellschaft*. 81, H.3/4 (5): 125-134.

484 Huneck, S. 1972. 6-hydroxymethyleugenitin, ein neues chromon aus *Roccella fuciformis*.  
485 *Phytochemistry* 11: 1489-1490.

486 Huneck, S, Yoshimura I. 1996. *Identification of lichen substances*. Berlin, Heidelberg, New York:  
487 Springer Verlag.

488 Mallavadhani U. V., Sudhakar A. V. S. 2018. Roccellatol, a new  $\beta$ -orcinol based metabolite from  
489 the lichen *Roccella montagnei*, *Natural Product Research*, 32:3, 268-274.

490 Marcuccio, S., Elix, J.A. 1983. A structural revision of picroroccellin. *Tetrahedron Letters* 24 (13):  
491 1445-1448.

492 Myers O.D., Sumner S.J., Li S., Barnes S., Du X. 2017. One Step Forward for Reducing False  
493 Positive and False Negative Compound Identifications from Mass Spectrometry Metabolomics  
494 Data: New Algorithms for Constructing Extracted Ion Chromatograms and Detecting  
495 Chromatographic Peaks. *Analytical Chemistry* 89 (17): 8696-8703.

## Main text

[Download source file \(57.36 kB\)](#)



- 497 Nylander, W. 1889. Lichenes insularum guineensium. Paris.
- 498 Parrot, D., T. Peresse, Hitti, E., Carrie, D., Grube, M., Tomasi, S. 2015. Qualitative and spatial  
499 metabolite profiling of lichens by a LC–MS approach combined with optimised extraction.  
500 *Phytochemical Analysis*, 26, pp. 23-33.
- 501 Pluskal T., Castillo S., Villar-Briones A., Oresic M. 2010. MZmine 2: Modular framework for  
502 processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC*  
503 *Bioinformatics* 11, 395.
- 504 Prashanth, S.R., Bharath, P., Valarmathi, R., Balaji, P., Parida, A., Hariharan, G.N. 2008.  
505 Species status and relationship between *Roccella montagnei* and *Roccella belangeriana* using  
506 DNA sequence data of nuclear ribosomal internal transcribed spacer region. *Journal of Plant*  
507 *Biochemistry and Biotechnology* 17(1): 91-94.
- 508 Roux, C., *et al.*, 2017. Catalogue des lichens et champignons lichénicoles de France  
509 métropolitaine. 2e édition revue et augmentée (2017). Edit. Association française de lichénologie  
510 (A.F.L.), Fontainebleau.
- 511 Strack, D., Feige, G.B., Kroll, R. 1979. Screening of Aromatic Secondary Lichen Substances by  
512 High Performance Liquid Chromatography. *Zeitschrift für Naturforschung* 34 c, 695-698.
- 513 Swedan, A., Chollet-Krugler M., Sauvager, A., Van de Weghe, P., Chokr, A., Bonnaure-Mallet,  
514 M., Tomasi, S., Bousarghin, L. 2017. Antibacterial activities of natural lichen compounds against  
515 *Streptococcus gordonii* and *Porphyromonas gingivalis*. *Fitoterapia* 121: 164-169.
- 516 Tehler, A., Dahlkild, Å., Eldenäs, P. & Feige, G. B. 2004. The phylogeny and taxonomy of  
517 Macaronesian, European and Mediterranean *Roccella* (Roccellaceae, Arthoniales). *Symbolae*  
518 *Botanicae Upsalienses* 34(1): 432-428.



**Main text**

[Download source file \(57.36 kB\)](#)

520 Tehler, A., Irestedt, M. 2007. Parallel evolution of lichen growth forms in the family Roccellaceae  
521 (Arthoniales, Ascomycota). *Cladistics* 23(5): 432-454.

522 Tehler, A., Irestedt, M., Bungartz, F. & Wedin, M. 2009a. Evolution and reproduction modes in  
523 the *Roccella galapagoensis* aggregate (Roccellaceae, Arthoniales). *Taxon* 58, 438–456.

524 Tehler, A., Irestedt, M., Wedin, M. & Ertz, D. 2009b. Origin, evolution and taxonomy of American  
525 *Roccella* (Roccellaceae, Ascomycetes). *Systematics and Biodiversity* 7(3): 307-317.

526 Tehler, A., Irestedt, M., Wedin, M. & Ertz, D. 2010. The Old World *Roccella* species outside  
527 Europe and Macaronesia: taxonomy, evolution and phylogeny. *Systematics and Biodiversity*  
528 8(2): 223-246.

529 Tenenhaus M. 1998. La régression PLS: Théorie et pratique / Editions Technip.

530 Tetko, I.V., Gasteiger, J.; Todeschini, R., Mauri, A., Livingstone, D., Ertl, P., Palyulin, V.A.,  
531 Radchenko, E.V., Zefirov, N.S., Makarenko, A.S., Tanchuk, V.Y., Prokopenko, V.V. 2005. Virtual  
532 computational chemistry laboratory - design and description. *Journal of Computer-Aided*  
533 *Molecular Design* 19: 453-463. <http://www.vcclab.org>, 2005.

534 Thadhani, V.M., Choudhary, M.I., Khan, S. and Karunaratne, V., 2012. Antimicrobial and  
535 toxicological activities of some depsides and depsidones. *Journal of the National Science*  
536 *Foundation of Sri Lanka*, 40(1), pp.43–48.

537 van den Berg, R.A., Hoefsloot, H.C.J, Westerhuis, J.A. and Smilde, A.K. and van der Werf, M.J.  
538 2006. Centering, scaling, and transformations: improving the biological information content of  
539 metabolomics data. *BMC Genomics* 7(1): 142



541

**Figure and Table captions**

542

**Figure 1.** Habit of the five *Roccella* species studied; **a.** *Roccella fuciformis*, France mainland, Bretagne (A.-H. Paradis); **b.** *Roccella phycopsis*, France mainland, Bretagne (A.-H. Paradis); **c.** *Roccella montagnei*, general view and close-up showing soralia, São Tome and Príncipe (Poncet, RPO050219\_201); **d.** *Roccella belangeriana*, general view and close-up showing apothecia, Europa, (Poncet, EUROPA20190411\_13); **e.** and **f.** *Roccella applanata*, Europa (Poncet, EUROPA20190411\_4 and Europa20190411\_8).

543

544

545

546

547

548

**Figure 2.** Map of sampling locations (E = Europa, G = Grande Glorieuse, J = Juan de Nova, WC = Saint-Malo and Cancale, P = Ploumanac'h, S = São Tome and Príncipe).

549

550

**Figure 3.** Base peak chromatograms from the HPLC-MS analyses without data processing showing the four major compounds: erythrin, lecanoric, lepralic and roccellic acids. The total ion current (TIC) of the samples of each species were merged in MZmine and the resulting chromatograms were exported into Excel.

551

552

553

554

**Figure 4.** ESI mass spectra (in negative mode) of lecanoric and lepralic acids with their chemical structures. Dash lines show in-source fragmentation of the compounds.

555

556

**Figure 5. a)** PCA scores scatter plot of the five species of *Roccella* (*R. applanata*, *R. belangeriana*, *R. fuciformis*, *R. montagnei*, and *R. phycopsis*) showing discrimination between

557

558

**Main text**

[Download source file \(57.36 kB\)](#)

559 species. **b)** PLS-DA graphs performed on the metabolites (*m/z*) detected in the five *Roccella*  
560 with 3-D scores plotted between selected components. **c)** Variable Importance in Projection  
561 (VIP) identified by PLS-DA. The colored boxes on the right indicate the relative presence of the  
562 corresponding metabolite in each group studied.

563 **Figure 6.** **a)** PCA scores scatter plot of *R. belangeriana* and *R. montagnei* showing  
564 discrimination between the species. **b)** 3-D scores plot of PLS-DA between selected  
565 components performed on the metabolites (*m/z*) detected in *R. montagnei* and *R. belangeriana*.  
566 **c)** Variable Importance in Projection (VIP) identified by PLS-DA. The colored boxes on the right  
567 indicate the relative presence of the corresponding metabolite in each group studied.

568 **Figure 7.** Ratio of the metabolites (in % of ion intensity) calculated in the samples of *R.*  
569 *montagnei* collected in Europa, Juan de Nova, Grande Glorieuse and Sao Tomé. The  
570 identification of the metabolites was realized using the HLDB (in bold) and RoccellaDB/LDB-  
571 MS1 databases and was based on the molecular mass in negative mode ESI (reported *m/z*) as  
572 well as their retention time (RT). The data are from HLDB (bold and black), roccellaDB (bold and grey), and  
573 LDB-MS1 (italic and grey).

574 **Figure 8.** **a)** PCA scores scatter plot of *R. montagnei* collected in Europa, Juan de Nova,  
575 Grande Glorieuse and Sao Tomé **b)**. 3D PLS-DA scores scatter plot of the different samples of  
576 *R. montagnei* collected at the four locations. **c)** Variable Importance in Projection (VIP) identified  
577 by PLS-DA. The colored boxes on the right indicate the relative concentrations of the  
578 corresponding metabolite in each group studied.



580 **Table 1.** Supporting information of all specimens included in this study (\* indicates species  
581 reported for the first time in the territory; 'Sample ID (field)' indicates the identifier given to one or  
582 several vouchers collected in the field (the same ID may be attributed to different  
583 specimens/species in the Scattered Islands, meaning that samples have been collected in the  
584 same location and on the same tree/shrub); 'Sample ID (chem)' indicates the unique identifier  
585 given to specimens in this studies; collectors : AHP = Anne-Hélène Paradis (MBG), CF =  
586 Christian Fontaine (CBNM), EB = Ehoarn Bidault (MBG), FP = Frederic Picot (CBNM), JB = Joël  
587 Boustie (Univ. Rennes, UMR 2006), JH = Jean Hivert (CBNM), MG = Martin Grube (Karl-  
588 Franzens-Universität Graz), RP = Rémy Poncet (UMS 2006 PAtriNat (OFB - CNRS - MNHN));  
589 the trees/shrubs species indicated in 'Support' have been identified by JH).

590 **Table 2.** Chemical profiling by HPLC - MS of the five *Roccella* species studied (R. app: *R.*  
591 *applanata* ; R. bel: *R. belangeriana* ; R. fuc: *R. fuciformis*, R mon: *R. montagnei* ; R. phy : *R.*  
592 *phycopsis*). Metabolites were identified by their retention time (RT), but also by their molecular  
593 mass in negative mode ESI with a major ion corresponding to [M-H]<sup>-</sup> and the fragments  
594 (reported *m/z*). The families to which each compound belongs are noted. Compounds in black  
595 and bold are dereplicatated identities against HLBD; in grey and bold are putative identities  
596 determined by dereplication against RoccellaDB; and in grey and italics against LDB-MS1. Key  
597 to symbols: -, absence of metabolites; +, presence of metabolites; nd, not determined.



Table 1

[Download source file \(19.88 kB\)](#)

Species	Location	Latitude	Longitude	Elevation (m)	Year	Support
<i>R. appianata</i>	Europa*	22°23'37.64"S	40°22'22.44"E	2	2019	<i>Guettarda speciosa</i> (tree)
		22°23'37.64"S	40°22'22.44"E	2	-	-
<i>R. belangeriana</i>	Europa*	22°20'55.47"S	40°22'20.15"E	0	-	<i>Cerriops tagal</i> (mangrove tree)
		22°22'33.13"S	40°21'51.53"E	0	-	<i>Avicennia marina</i> (mangrove tree)
		22°23'37.64"S	40°22'22.44"E	2	-	<i>Guettarda speciosa</i> (tree)
		22°21'37.69"S	40°21'06.01"E	4	-	<i>Euphorbia stenoclada</i> (tree)
		22°22'32.20"S	40°21'53.44"E	0	-	<i>Rhizophora mucronata</i> (mangrove tree)
		22°20'55.47"S	40°22'20.15"E	0	-	<i>Cerriops tagal</i> (mangrove tree)
<i>R. fuciformis</i>	France mainland (Ploumanac'h)	48°49'N	3°29'W	0	2005	Rock (granite)
	France mainland (Saint-Malo)	48°38'N	2°1'W	0	2005	-
	France mainland (Cancalle)	48°40'N	1°51'W	0	2013	-
<i>R. montagnei</i>	Europa*	22°20'39.35"S	40°20'21.95"E	2	2019	<i>Suriana maritima</i> (shrub)
		22°20'24.29"S	40°21'29.51"E	2	-	-
		22°20'39.35"S	40°20'21.95"E	2	-	-
		22°21'42.22"S	40°21'15.03"E	5	-	-
		22°21'17.53"S	40°19'57.67"E	2	-	-
		22°21'37.69"S	40°20'06.01"E	3	-	<i>Euphorbia stenoclada</i> (tree)
		17°02'57.11"S	42°43'16.75"E	3	-	<i>Suriana maritima</i> (shrub)
		17°02'57.24"S	42°43'16.99"E	3	-	-
		17°02'56.44"S	42°43'17.78"E	2	-	-
		11°34'50.86"S	47°17'26.28"E	2	-	<i>Casuarina equisetifolia</i> (tree)
Grande Glorieuse*		11°34'18.07"S	47°17'57.54"E	3	-	-
		11°34'50.86"S	47°17'25.27"E	2	-	-
		11°34'18.07"S	47°17'57.54"E	3	-	-
		0°19'33.13"N	6°30'29.16"E	3	-	unidentified tree
São Tome and Príncipe*		0°19'33.13"N	6°30'29.16"E	3	-	-
		0°19'33.13"N	6°30'29.16"E	3	-	-
		0°19'33.13"N	6°30'29.16"E	3	-	-
<i>R. phycopsis</i>	France mainland (Saint-Malo)	48°38'N	2°0'W	0	2002	Rock (granite)
	France mainland (Cancalle)	48°40'N	1°51'W	0	2005	-
				0	2013	-



Table 2

Download source file (17.75 kB)



identified or putative compound	classification	[M-H] <sup>-</sup>	main ions detected (m/z)	RT (min)	R. app.	R. bel.	R. fuc.	R. mon.	R. phy.
orsellinic acid	monophenolic compounds	167.1	167.3; 123	6.2	+	+	-	-	-
erythrin	depsides	420.9	420.9; 843.1; 571.0; 534.8; 271.0; 167.0; 149.0; 122.9; 104.9	15.8	+	+	+	+	+
lecanoric acid	depsides	317	317.0; 167.1; 149.0	19.4	+	+	-	+	+
lepraric acid	chromones	361	760.5; 723.0; 504.8; 382.9; 361.2; 339.2; 317.1; 143.0	19.5	-	-	+	-	-
roccellic acid	aliphatic acids	299.1	299.1; 255.1; 621.3	29.7	-	+	-	+	+
montagnetol	monophenolic compounds	271.1 (nd)	167.0; 149.0	3.9	+	+	+	+	+
acetylerythritol	polyols	163.1	163.1	10.9	-	-	-	-	+
roccellatol	monophenolic compounds	271.0	271.0	12.0	-	+	-	-	-
2,4-dihydroxyphthalide	monophenolic compounds	165.0	165.0	12.3	-	-	-	-	+
ethylether du lepraric acid	chromones	388.9	388.9	20.8	-	-	+	-	-
orsellinylmontagnetol A or orsellinylmontagnetol B or orsellinylmontagnetol C	polyphenolic compounds	420.9	420.9	21.4	+	+	-	+	-
orsellinylmontagnetol D	polyphenolic compounds	570.9	570.9; 420.9	21.7	-	+	-	+	-
orsellinylmontagnetol A or orsellinylmontagnetol B or orsellinylmontagnetol C	polyphenolic compounds	420.9	420.9	22.0	-	+	-	-	-
roccellaric acid	paraconic acids	325.0	325.0	31.7	-	-	-	+	-
angardianic acid	aliphatic acids	327.2	327.2	33.2	-	-	-	+	+
8-methoxytryptelone methylether or bis-(2,4-dihydroxy-6-n-propylphenyl)- methane or pannaric acid	nd	315.0	315.0	20.4	-	-	-	+	+
6,8-di-O-methylidurufin or pyrenullic acid G	nd	411.0	411.0	24.0	+	-	-	-	-
isidapharan or siphulin	nd	425.2	425.2	25.5	+	-	-	-	-
montagnetol or roccellatol derivative	monophenolic compounds	271.0	271.0	26.2	-	-	-	+	-
orcinyll Lecanorate	depsides	422.6	422.6	31.2	-	-	-	-	+
cholesta-3,5-dim-7-one	terpene	381.9	381.9	45.2	-	+	-	-	-
oleic acid	aliphatic acids	280.8	280.8	45.3	-	+	-	-	-

R. app: *R. applanata*; R. bel: *R. belangeriana*; R. fuc: *R. fuciformis*; R. mon: *R. montagnei*; R. phy: *R. phycopsis*

## Figure 1

[Download source file \(983.02 kB\)](#)

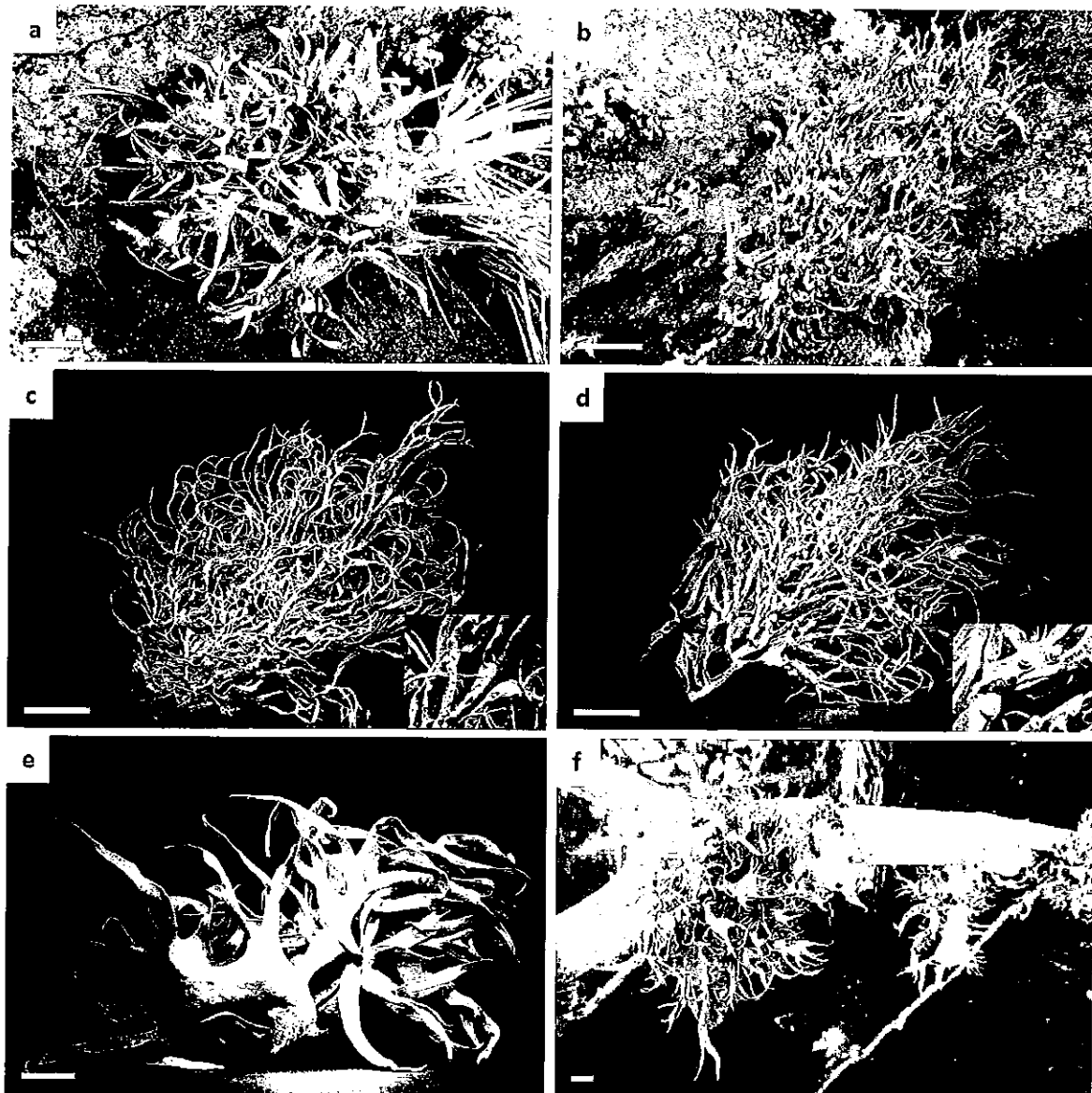


Figure 1. Habit of the five *Roccella* species studied; a. *Roccella fuciformis*, France mainland, Bretagne (A.-H. Paradis); b. *Roccella phycopsis*, France mainland, Bretagne (A.-H. Paradis); c. *Roccella montagnei*, general view and close-up showing soralia, São Tome and Príncipe (Poncet, RPO050219\_201); d. *Roccella belangeriana*, general view and close-up showing apothecia, Europa, (Poncet, EUROPA20190411\_13); e. and f. *Roccella applanata*, Europa (Poncet, EUROPA20190411\_4 and Europa20190411\_8).



**Figure 2**

[Download source file \(228.26 kB\)](#)

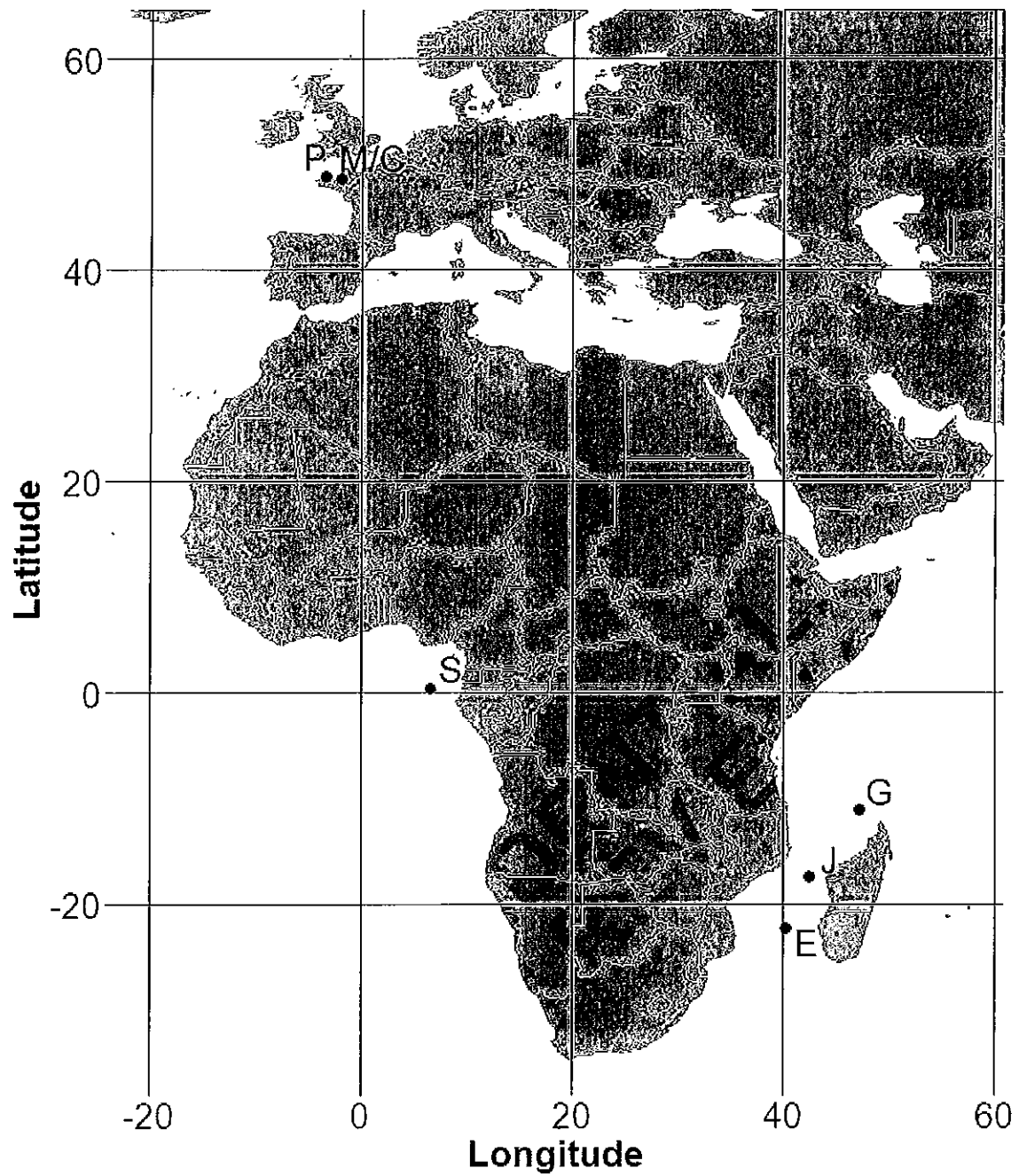


Figure 2. Map of sampling locations (E = Europa, G = Grande Glorieuse, J = Juan de Nova, M/C = Saint-Malo and Cancale, P = Ploumanac'h, S = São Tome and Príncipe).



Figure 3

[Download source file \(265.39 kB\)](#)

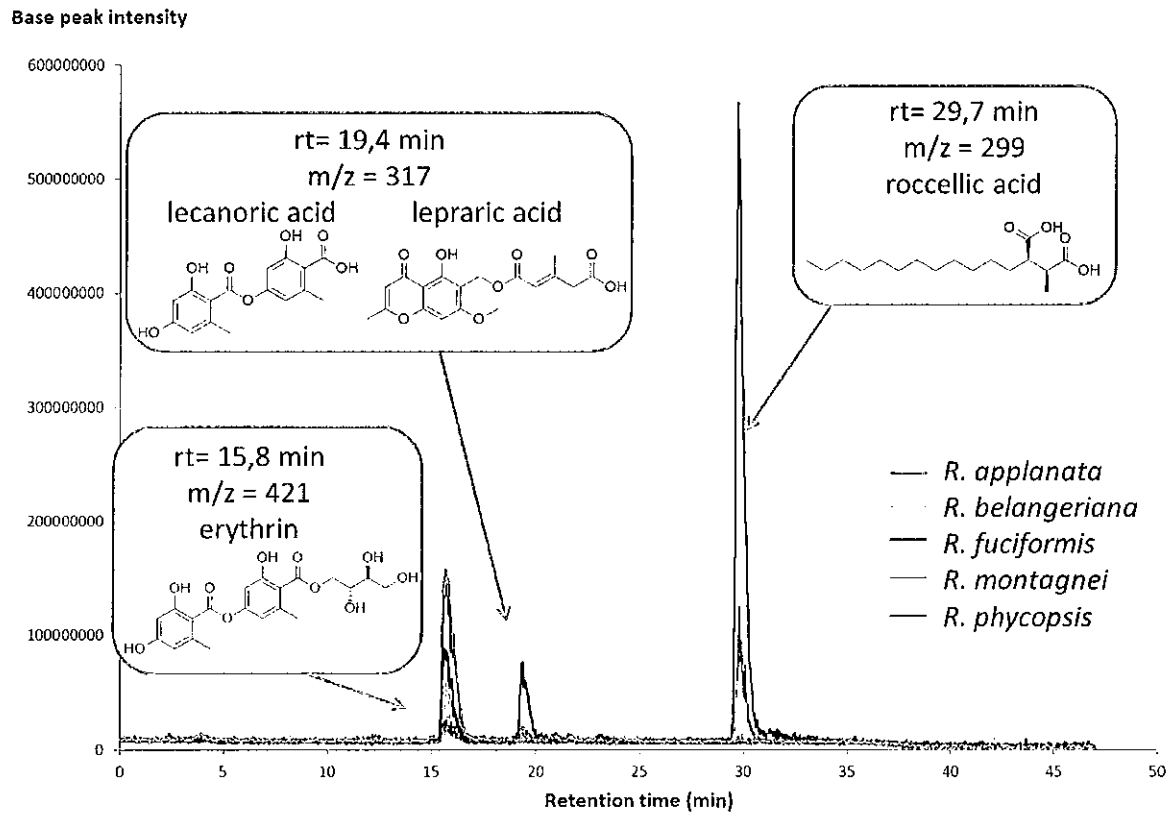


Figure 3. Base peak chromatograms from the HPLC-MS analyses without data processing showing the four major compounds: erythrin, lecanoric, lepranic and roccellic acids. The total ion current (TIC) of the samples of each species were merged in MZmine and the resulting chromatograms were exported into Excel.



Figure 4

[Download source file \(776.36 kB\)](#)

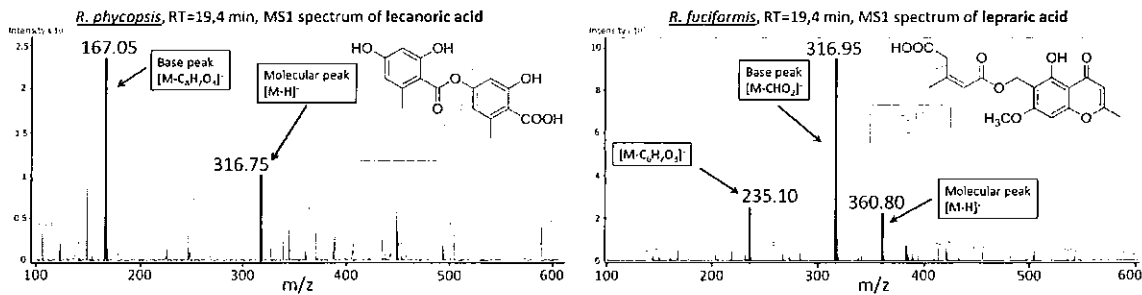


Figure 4. ESI mass spectra (in negative mode) of lecanoric and leproric acids with their chemical structures. Dash lines show in-source fragmentation of the compounds.



Figure 5

Download source file (1.29 MB)

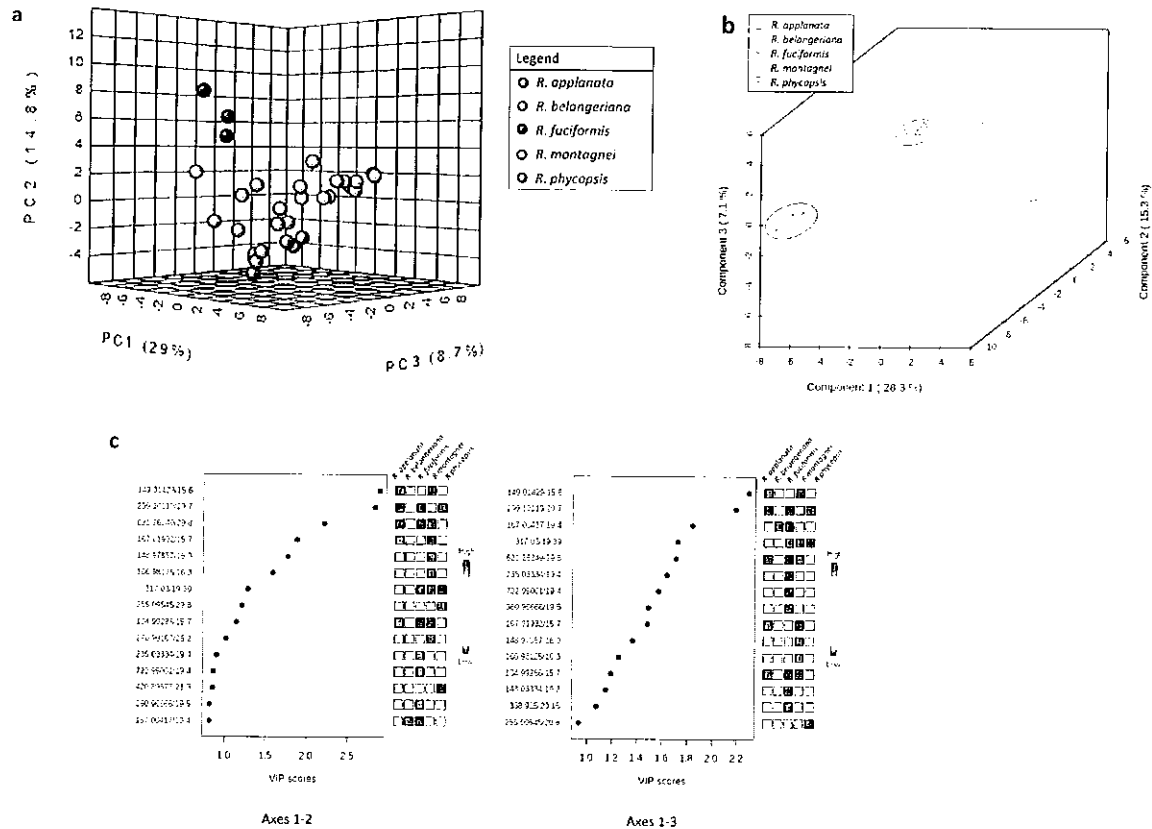


Figure 5. a) PCA scores scatter plot of the five species of Roccella (*R. appplanata*, *R. belangeriana*, *R. fuciformis*, *R. montagnei*, and *R. phycopsis*) showing discrimination between species. b) PLS-DA graphs performed on the metabolites (m/z) detected in the five Roccella with 3-D scores plotted between selected components. c) Variable Importance in Projection (VIP) identified by PLS-DA. The colored boxes on the right indicate the relative presence of the corresponding metabolite in each group studied.

**Figure 6**

[Download source file \(865.25 kB\)](#)

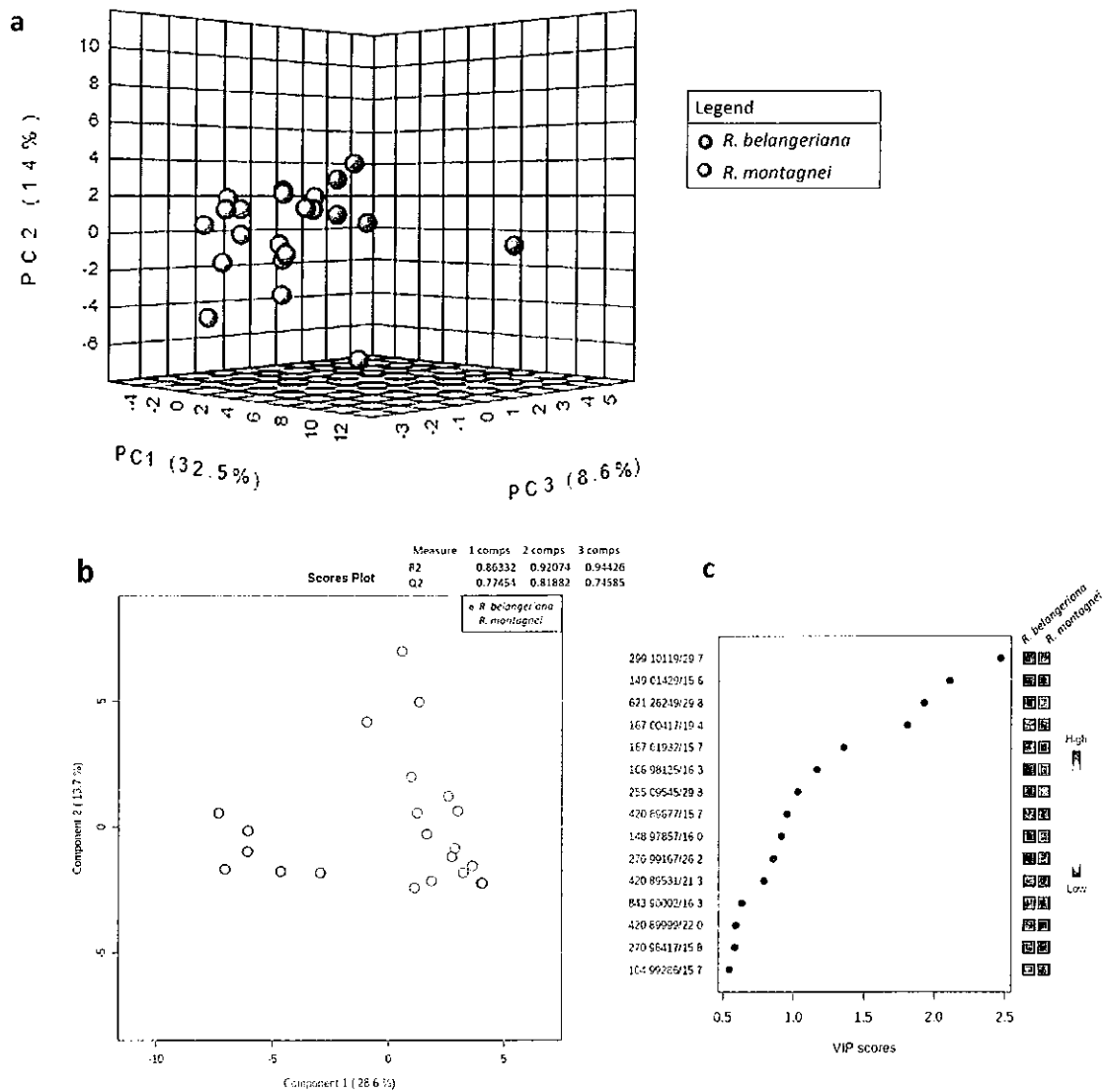


Figure 6. a) PCA scores scatter plot of *R. belangeriana* and *R. montagnei* showing discrimination between the species. b) 3-D scores plot of PLS-DA between selected components performed on the metabolites (m/z) detected in *R. montagnei* and *R. belangeriana*. c) Variable Importance in Projection (VIP) identified by PLS-DA. The colored boxes on the right indicate the relative presence of the corresponding metabolite in each group studied.

Figure 7

Download source file (165.38 kB)

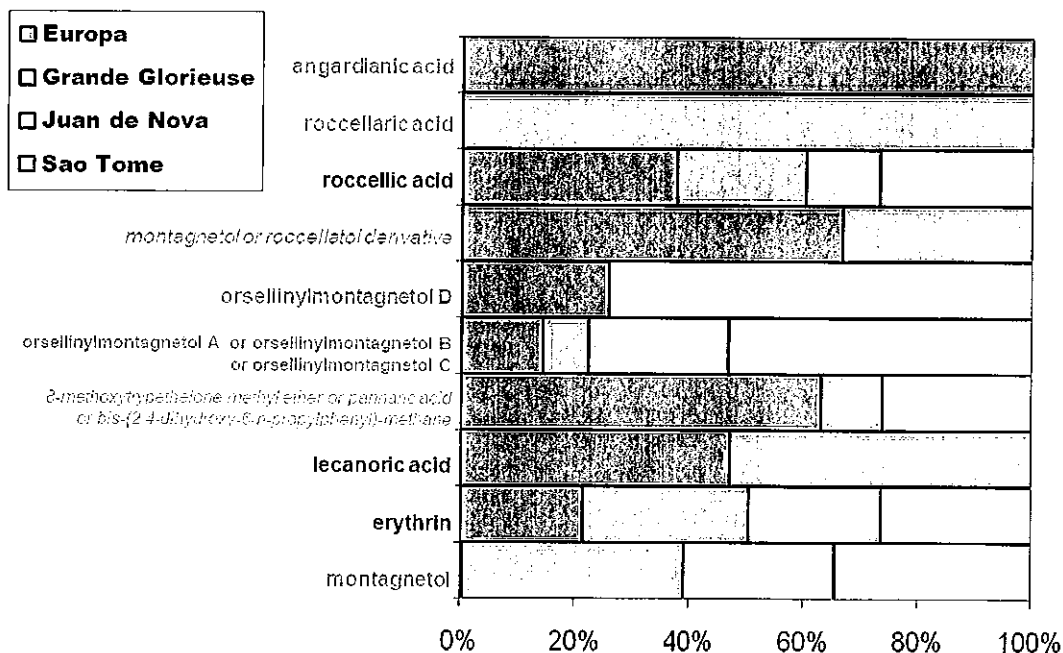


Figure 7. Ratio of the metabolites (in % of ion intensity) calculated in the samples of *R. montagnei* collected in Europa, Juan de Nova, Grande Glorieuse and Sao Tomé. The identification of the metabolites was realized using the HLDB (in bold) and RoccellaDB/LDB-MS1 databases and was based on the molecular mass in negative mode ESI (reported  $m/z$ ) as well as their retention time (RT). The data are from HLDB (bold and black), roccellaDB (bold and grey), and LDB-MS1 (italic and grey).

Figure 8

[Download source file \(342.92 kB\)](#)

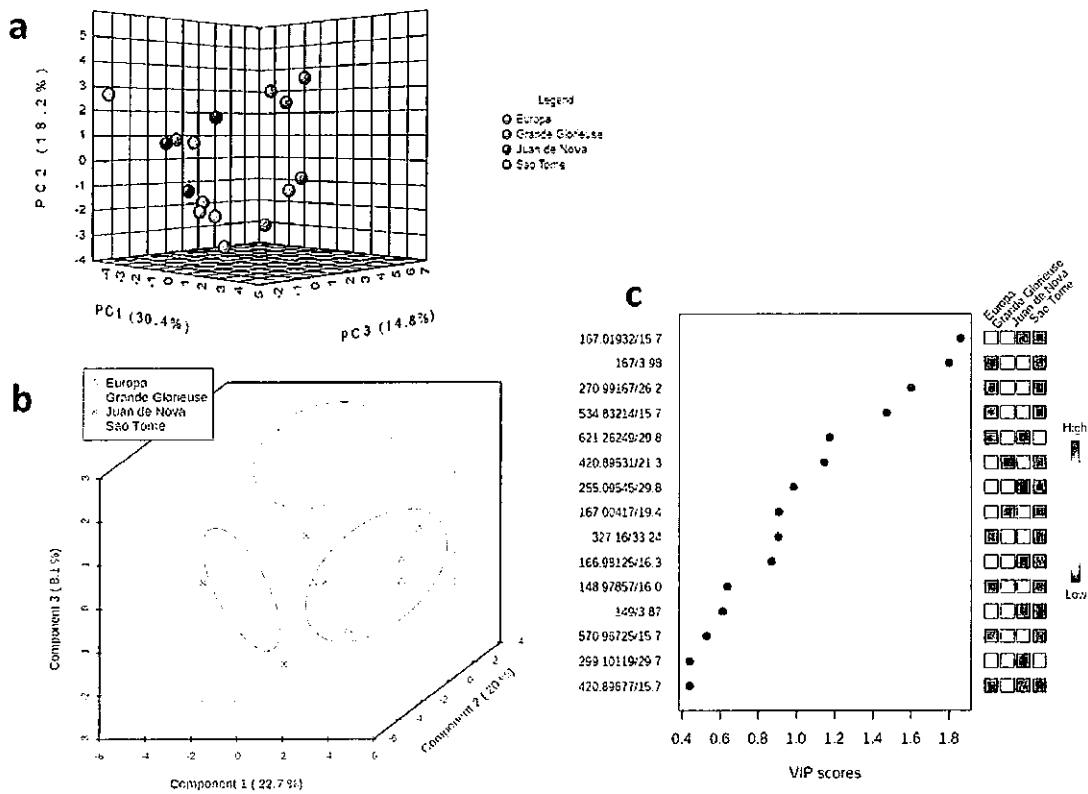


Figure 8. a) PCA scores scatter plot of *R. montagnei* collected in Europa, Juan de Nova, Grande Glorieuse and Sao Tomé b) 3D PLS-DA scores scatter plot of the different samples of *R. montagnei* collected at the four locations. c) Variable Importance in Projection (VIP) identified by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group studied.



Main text

[Download source file \(57.36 kB\)](#)

Tables

Table 1 - [Download source file \(19.88 kB\)](#)

Table 1 - Download source file (19.88 kB)

Table 2 - [Download source file \(17.75 kB\)](#)

Table 2 - Download source file (17.75 kB)

Figures

Figure 1 - [Download source file \(983.02 kB\)](#)

Figure 1 - Download source file (983.02 kB)

Figure 2 - [Download source file \(228.26 kB\)](#)

Figure 2 - Download source file (228.26 kB)

Figure 3 - [Download source file \(265.39 kB\)](#)

Figure 3 - Download source file (265.39 kB)

Figure 4 - [Download source file \(776.36 kB\)](#)

Figure 4 - Download source file (776.36 kB)

Figure 5 - [Download source file \(1.29 MB\)](#)

Figure 5 - Download source file (1.29 MB)





[Download source file \(865.25 kB\)](#)

**Figure 6 - [Download source file \(865.25 kB\)](#)**

[Download source file \(865.25 kB\)](#)

**Figure 7 - [Download source file \(165.38 kB\)](#)**

[Download source file \(165.38 kB\)](#)

**Figure 8 - [Download source file \(342.92 kB\)](#)**

[Download source file \(342.92 kB\)](#)

**Supplementary material**

**File 1 - [Download source file \(226.94 kB\)](#)**

[Download source file](#)

**File 2 - [Download source file \(72.32 kB\)](#)**

[Download source file](#)