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

Authors

Solenn Ferron¹, Olivier Berry¹, Damien Olivier-Jimenez¹, Isabelle Rouaud¹, Joël Boustie¹,
Françoise Lohézic – Le Dévéhat^{1*}, Rémy Poncet^{2*}

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

Corresponding authors : francoise.le-devehat@univ-rennes1.fr and remy.poncet@mnhn.fr

¹Univ Rennes, CNRS, ISCR (Institut des Sciences Chimiques de Rennes) - UMR 6226, F-35000 Rennes, France

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

Keywords

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

Abstract



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

Introduction

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



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

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



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

Materials and methods

Lichen samples for chemical analysis

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

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



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

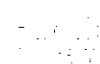
Extraction

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

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

All extracts were dissolved in tetrahydrofuran (THF) at a concentration of 1 mg.mL⁻¹ and filtered (0.45 µm) before HPLC injection.

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





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

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

Mass Spectral Data processing

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

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

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

Multivariate analysis

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

Results*Metabolic profiles of the five Roccella species*

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



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

Assessment of metabolic segregation between the five Roccella species

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

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



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

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

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

Focus on the R. montagnei-belangeriana complex

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



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

Metabolite variation in R. montagnei at different latitudes

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

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



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

In a second step, we performed a multivariate PCA analysis (figure 8a), followed by a supervised PLS-DA (figure 8b). Although the R² and Q² values for PLS-DA were quite low, it appears that specimens found in Europa differed from those from São Tome and Príncipe along the PC1 axis (22.7%). PC3 axis allows discrimination between the specimens collected in Grande Glorieuse and the others. The compounds that contributed the most (VIP > 1) to discrimination were montagnetol/roccellatol derivative, erythrin and roccellic acid for thalli collected in Europa; erythrin for *R. montagnei* collected in Juan de Nova; and montagnetol and orsellinyl montagnetol for specimens collected in Sao Tomé (figure 8c).

Discussion

Improved knowledge of Roccella chemistry

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



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

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



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

Three acids were detected or suspected: roccelic acid was detected in *R. belangeriana* (two specimens out of 6: BeEEs310B and BeECt339), *R. montagnei* (all specimens) and *R. phycopsis* (all specimens); angardianic acid was suggested for *R. phycopsis* (two out of three specimens: PhBs64, PhBs72) and three specimens out of 16 of *R. montagnei* (three specimens out of 16: MoESm324B, MoEEs310, MoESm334); and roccellaric acid was suspected in one specimen of *R. montagnei* (MoGGCe450A). As seen in figure S3, roccelic acid differs from angardianic acid by the length of the side chain with 12 vs. 14 carbons, respectively. Roccellaric acid has a side chain with thirteen carbons, but results from the cyclisation of the angardianic acid. Thus, it is necessary for the lichen to have sufficient energy to produce longer chains, and also to activate enzymes like deshydratases to form the paraconic acid, roccellaric acid. This could explain why roccelic acid seems more prevalent in the *Roccella* species (e.g. *R. montagnei*) that also produce angardianic and roccellaric acids.

R. montagnei-belangeriana complex

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



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

Metabolites profiling across location of R. montagnei

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



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

Conclusion

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



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

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Permits

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



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 LXI.I. Aromatic constituents of the lichen *Roccella fuciformis* DC. A revised structure for lepralic 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.



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

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

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

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

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

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

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

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- Huneck, S., Follmann, G. 1964. Das Vorkommen von Psoromsäure in *Chiodecton stalactinum* NYL. und Roccellsäure in *Dirina lutosa* ZAHLBR. *Zeitschrift für Naturforschung* 19 b: 658-659.
- Huneck, S., Follmann, G. 1967a. Zur Chemie chilenischer Flechten. XVI. Über die Inhaltsstoffe einiger Roccellaceen. *Zeitschrift für Naturforschung* 22(3): 362-366.
- Huneck, S. 1967b. Über die Inhaltsstoffe von *Combea mollusca* (ACH.) DE NOT., *Roccella vicentina* (WAIN.) WAIN., *Roccella gayana* MONT. und *Roccella fucoides* (NECK.) WAIN. *Zeitschrift für Naturforschung* 22b: 1369-1370.
- Huneck, S., Follmann, G. 1968. Mitteilungen über Flechteninhaltsstoffe LV. Zur Phytochemie und Chemotaxonomie einiger Chiodectonaceen und Roccellaceen). *Berichte der Deutschen Botanischen Gesellschaft*. 81, H.3/4 (5): 125-134.
- Huneck, S. 1972. 6-hydroxymethyleugenitin, ein neues chromon aus *Roccella fuciformis*. *Phytochemistry* 11: 1489-1490.
- Huneck, S, Yoshimura I. 1996. *Identification of lichen substances*. Berlin, Heidelberg, New York: Springer Verlag.
- Mallavadhani U. V., Sudhakar A. V. S. 2018. Roccellatol, a new β -orcinol based metabolite from the lichen *Roccella montagnei*, *Natural Product Research*, 32:3, 268-274.
- Marcuccio, S., Elix, J.A. 1983. A structural revision of picroroccellin. *Tetrahedron Letters* 24 (13): 1445-1448.
- Myers O.D., Sumner S.J., Li S., Barnes S., Du X. 2017. One Step Forward for Reducing False Positive and False Negative Compound Identifications from Mass Spectrometry Metabolomics Data: New Algorithms for Constructing Extracted Ion Chromatograms and Detecting Chromatographic Peaks. *Analytical Chemistry* 89 (17): 8696-8703.



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

Main text

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

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

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

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

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

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

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

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

**Figure and Table captions**

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. 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).

Figure 3. Base peak chromatograms from the HPLC-MS analyses without data processing showing the four major compounds: erythrin, lecanoric, lepraric 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. ESI mass spectra (in negative mode) of lecanoric and lepraric acids with their chemical structures. Dash lines show in-source fragmentation of the compounds.

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



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. **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. 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. **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.



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

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



Table 1

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Species	Location	Latitude	Longitude	Elevation (m)	Year	Support
<i>R. applanata</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	-	-
		22°20'55.47"S	40°22'20.15"E	0	-	<i>Cerriops tagal</i> (mangrove tree)
<i>R. belangeriana</i>	Europa*	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)
		22°20'55.66"S	40°22'19.84"E	0	-	-
		48°49'N	3°29'W	0	2005	Rock (granite)
<i>R. fuciformis</i>	France mainland (Ploumanac'h)	48°38'N	2°1'W	0	2005	-
<i>R. montagnei</i>	France mainland (Saint-Malo)	48°40'N	1°51'W	0	2013	-
		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	-	-
	Europa*	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	-	-
	Juan de Nova*	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	-	-
	Grande Glorieuse*	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)
		11°34'18.07"S	47°17'57.54"E	3	-	-
<i>R. phycopsis</i>	São Tome and Príncipe*	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
	France mainland (Saint-Malo)	0°19'33.13"N	6°30'29.16"E	3	-	-
		0°19'33.13"N	6°30'29.16"E	3	-	-
		48°38'N	2°0'W	0	2002	Rock (granite)
	France mainland (Cancale)	-	-	0	2005	-
		48°40'N	1°51'W	0	2013	-
		-	-	-	-	-
		-	-	-	-	-



Table 1

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Sample ID (field)	Sample ID (chem)	Collect
Europa20190410_6	ApEGs100	RP, CF, JH, EB
Europa20190410_6	ApEGs312A	RP, CF, JH, EB
Europa20190411_13	ApECt334	RP, CF, JH, EB
Europa20190409_12	BeEAn305	RP, CF, JH, EB
Europa20190410_6	BeEGs312B	RP, CF, JH, EB
Europa20190410_4	BEEEs310B	RP, CF, JH, EB
Europa20190409_15	BeERm306	RP, CF, JH, EB
Europa20190411_13	BeECt339	RP, CF, JH, EB
Europa20190411_14	BeECt340	RP, CF, JH, EB
JB/05/57	FuBs57	JB, MG REN 000 157
JB/05/71	FuBs71	JB REN 000 624
JB/13/é375	FuBs375	JB REN 015 121
Europa20190411_19	MoESm324A	RP, CF, JH, EB
Europa20190411_8	MoES334	RP, CF, JH, EB
Europa20190411_6	MoESm324B	RP, CF, JH, EB
Europa20190409_9	MoESm300	RP, CF, JH, EB
Europa20190410_3	MoESm309	RP, CF, JH, EB
Europa20190410_4	MoEEs310	RP, CF, JH, EB
Juandenova20190415_23	MoJNSm418	RP, CF, JH, EB
Juandenova20190415_24	MoJNSm419	RP, CF, JH, EB
Juandenova20190415_25	MoJNSm420	RP, CF, JH, EB
Gglorieuse20190420_15	MoGGCe450A	RP, FP, JH, EB
Gglorieuse20190421_27	MoGGCe486A	RP, FP, JH, EB
Gglorieuse20190420_15	MoGGCe450B	RP, FP, JH, EB
Gglorieuse20190421_27	MOGGCe486B	RP, FP, JH, EB
RPO050219_201	MoSTu140	RP, AHP
RPO050219_200	MoSTu141A	RP, AHP
RPO050219_201	MoSTu141B	RP, AHP
JB/05/72	PhBs72	JB REN 000 625
JB/02/é64	PhBs64	JB REN 000 818
JB/13/é376	PhBs376	JB REN 015 122

Table 2

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identified or putative compound	classification	[M-H] ⁺	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-methylnidurufin or pyrenullic acid G	nd	411.0	411.0	24.0	+	-	-	-	-
isidiopharin or siphulin	nd	425.2	425.2	25.5	+	-	-	-	-
montagnetol or roccellatol derivative	monophenolic compounds	271.0	271.0	26.2	-	-	-	+	-
orcinylllecanorate	depsides	422.6	422.6	31.2	-	-	-	-	+
cholesta-3,5-dien-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*

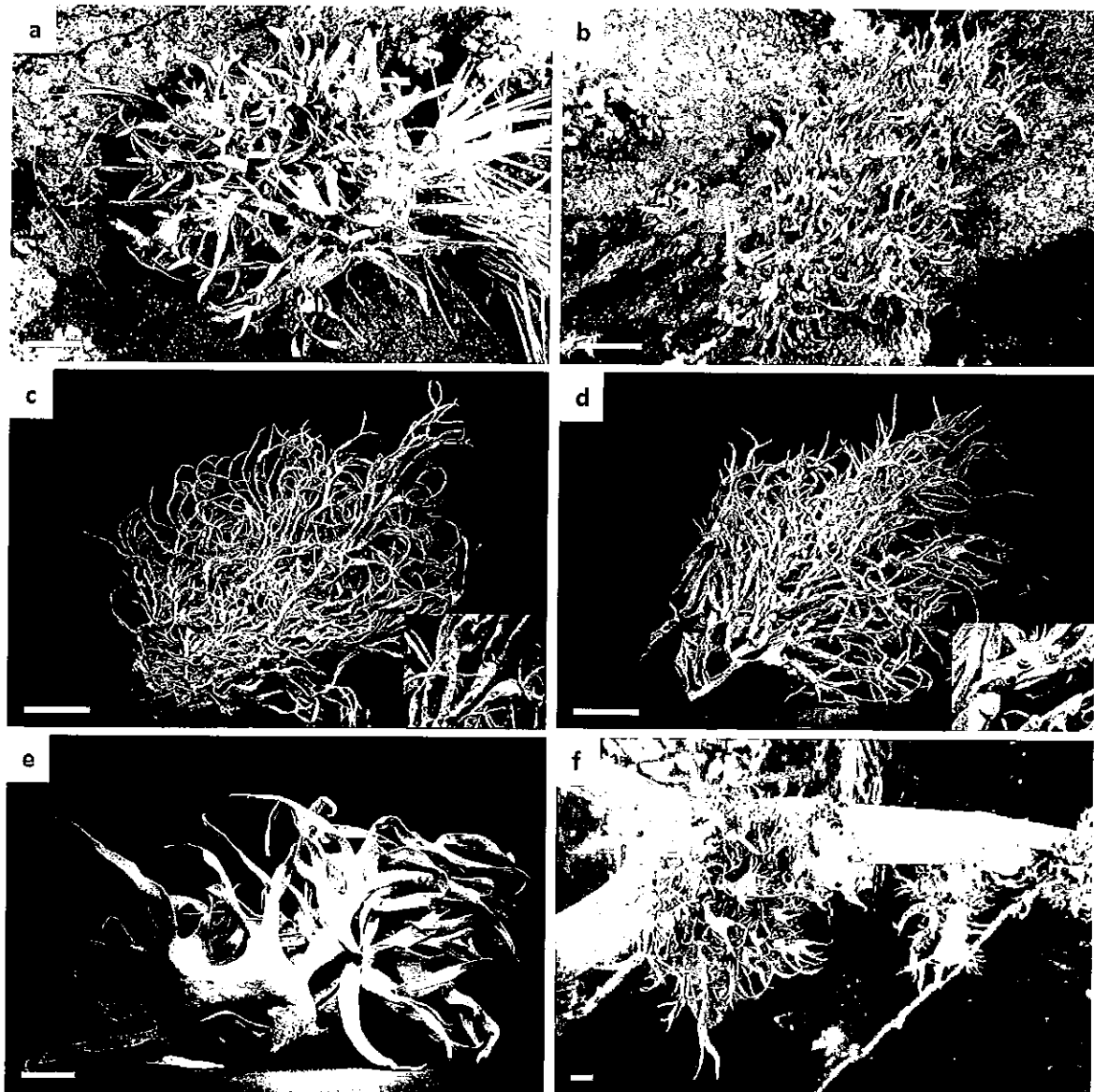


Figure 1. Habit of the five *Roccella* species studied; a. *Roccella fuciformis*, France mainland, Bretagne (A.-H. Paradis); b. *Roccella phycopsis*, France mainland, Bretagne (A.-H. Paradis); c. *Roccella montagnei*, general view and close-up showing soralia, São Tome and Príncipe (Poncet, RPO050219_201); 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

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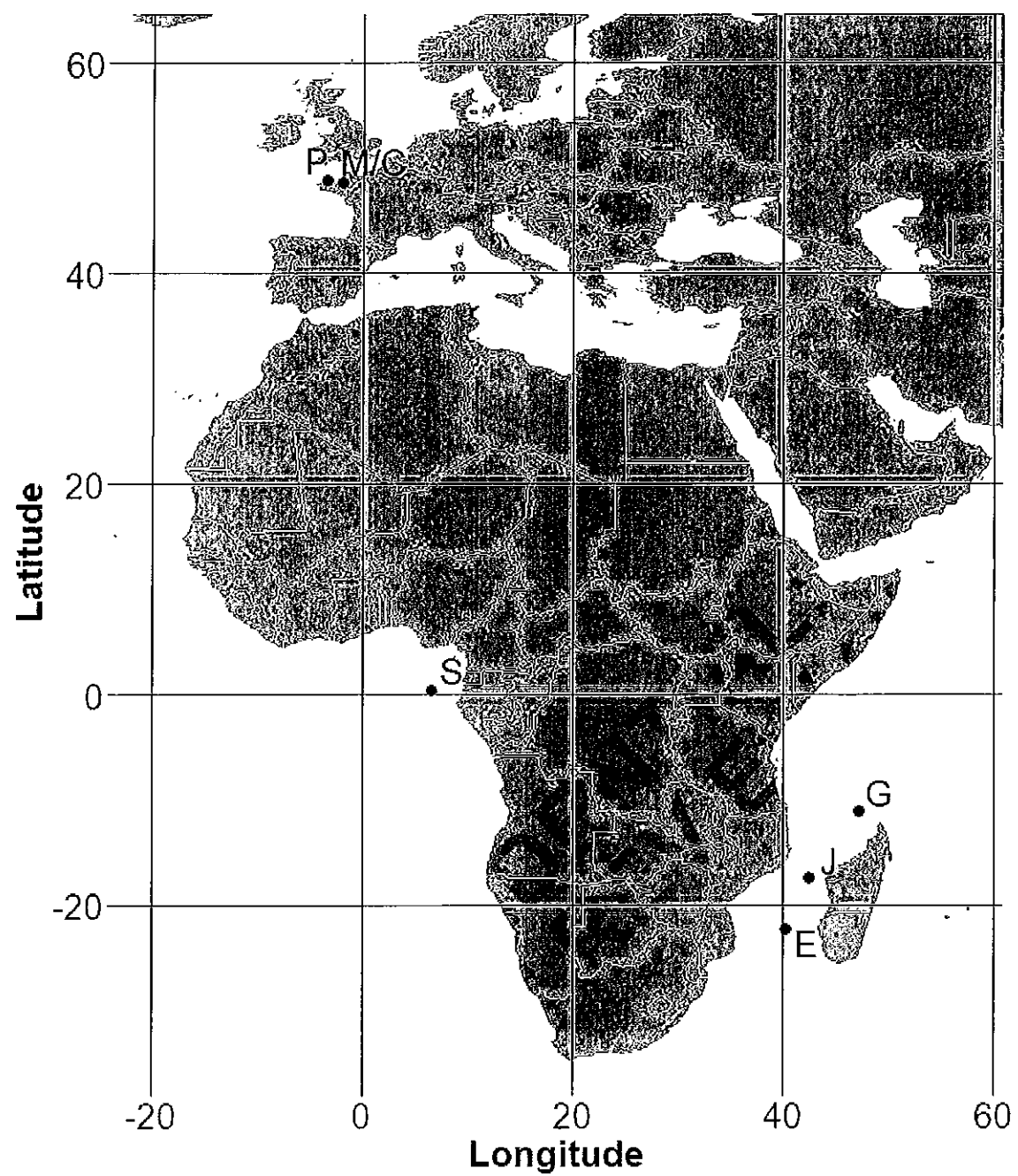


Figure 2. Map of sampling locations (E = Europa, G = Grande Glorieuse, J = Juan de Nova, M/C = Saint-Malo and Cancale, P = Ploumanac'h, S = São Tome and Príncipe).

Figure 3

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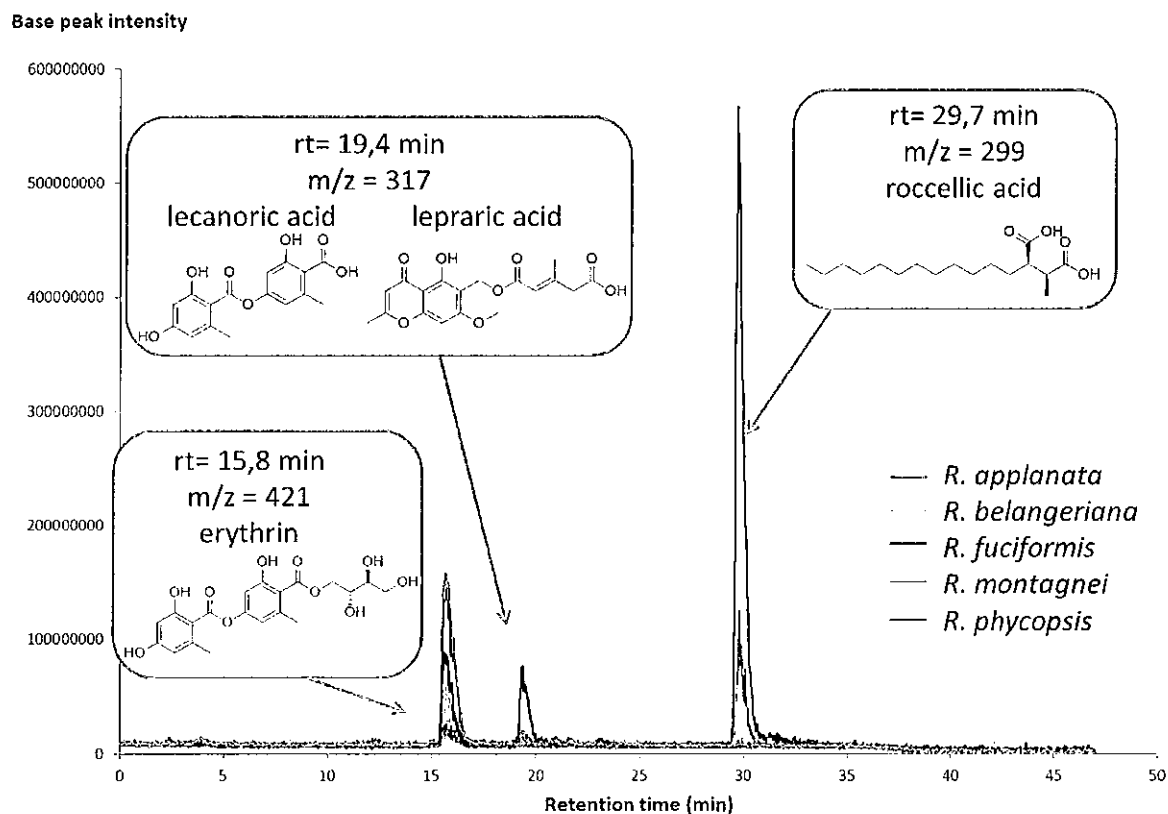


Figure 3. Base peak chromatograms from the HPLC-MS analyses without data processing showing the four major compounds: erythrin, lecanoric, lepraric and roccellic acids. The total ion current (TIC) of the samples of each species were merged in MZmine and the resulting chromatograms were exported into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

Figure 4

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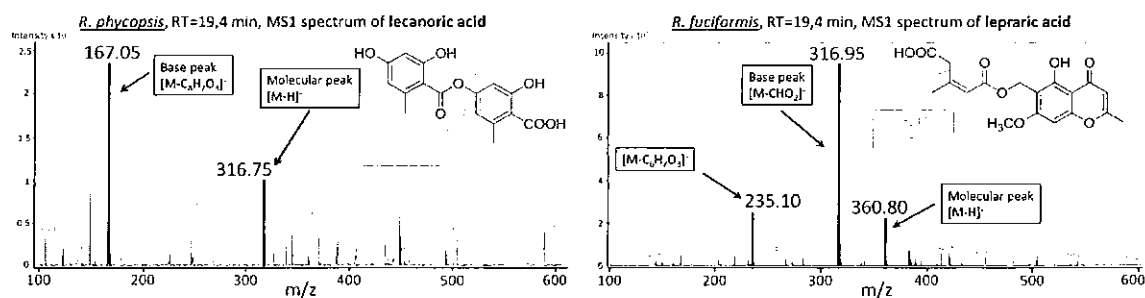


Figure 4. ESI mass spectra (in negative mode) of lecanoric and lepralic acids with their chemical structures. Dash lines show in-source fragmentation of the compounds.

Figure 5

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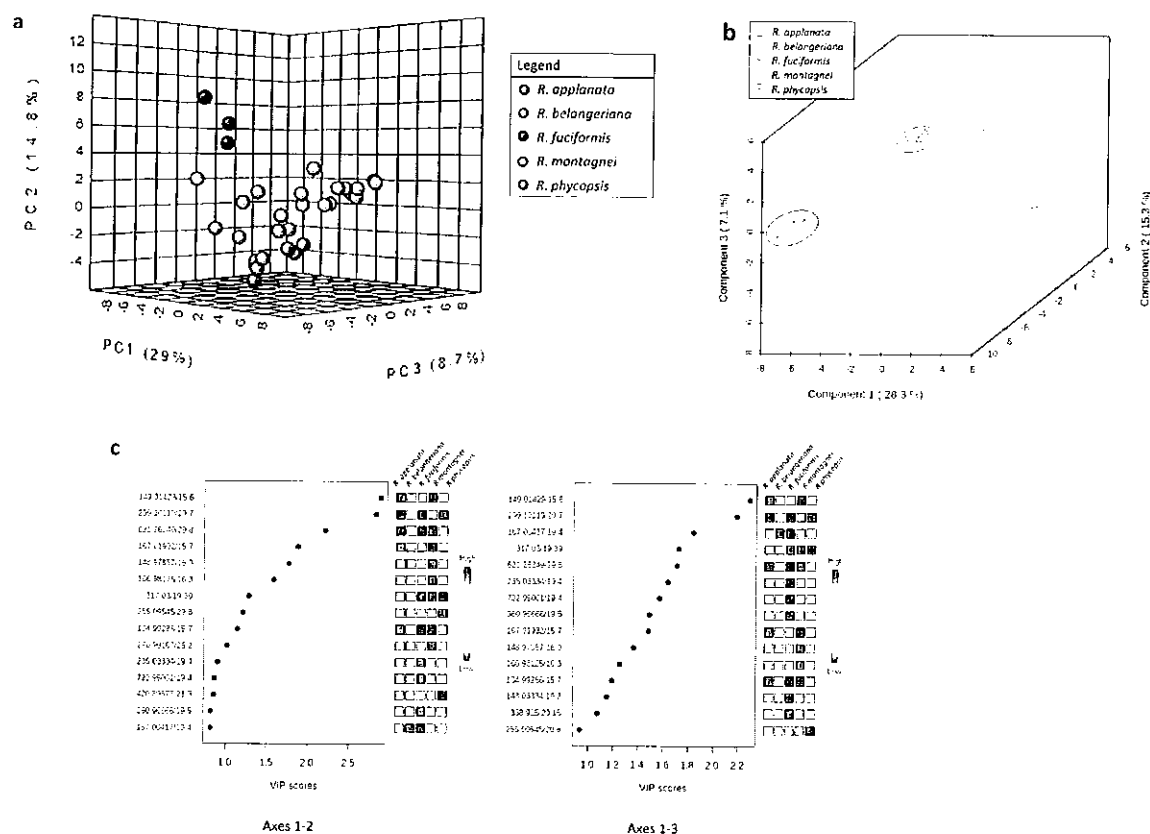


Figure 5. a) PCA scores scatter plot of the five species of *Roccella* (*R. applanata*, *R. belangeriana*, *R. fuciformis*, *R. montagnei*, and *R. phycopsis*) showing discrimination between species. b) PLS-DA graphs performed on the metabolites (m/z) detected in the five *Roccella* with 3-D scores plotted 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

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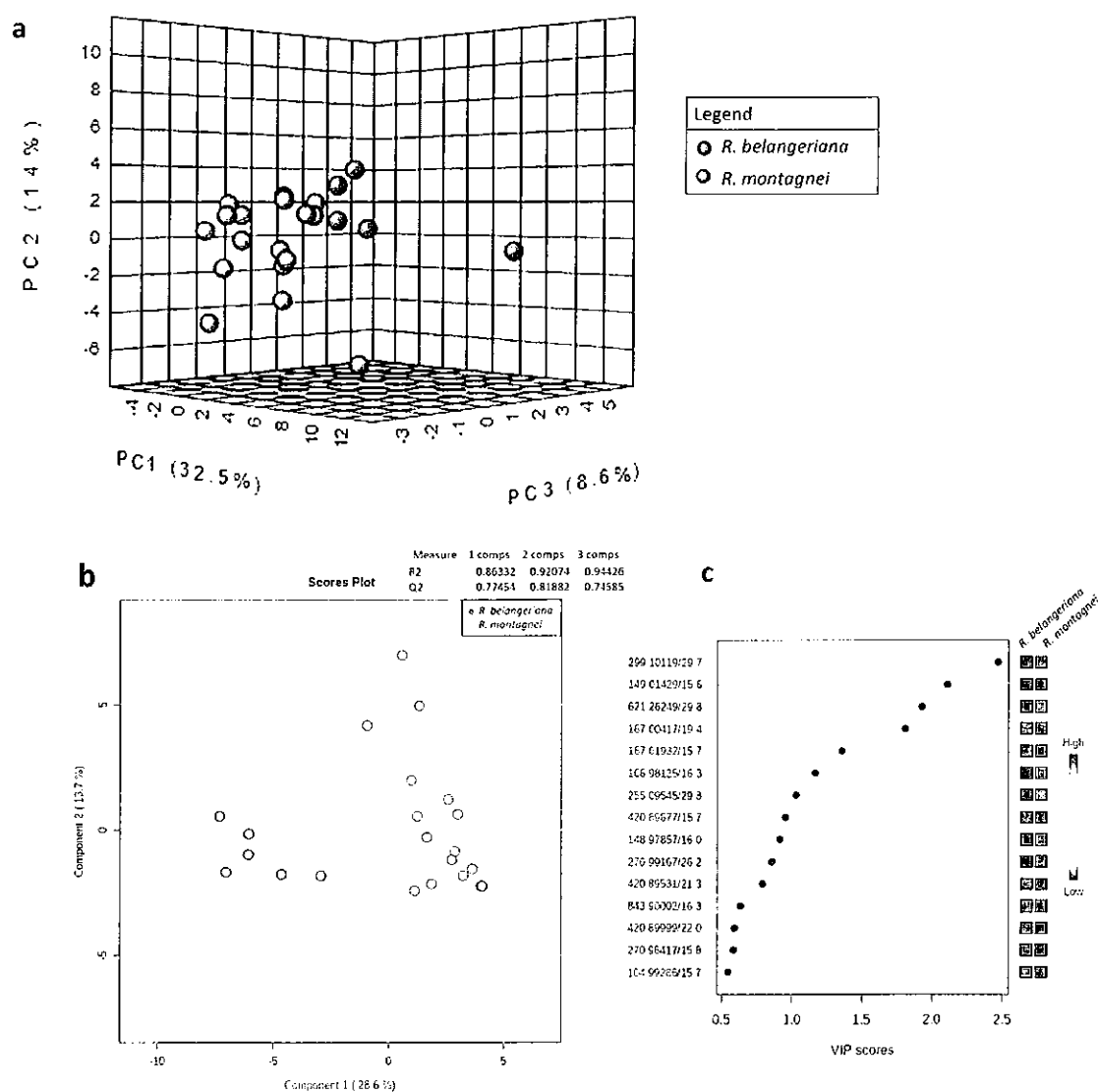


Figure 6. a) PCA scores scatter plot of *R. belangeriana* and *R. montagnei* showing discrimination between the species. b) 3-D scores plot of PLS-DA between selected components performed on the metabolites (m/z) detected in *R. montagnei* and *R. belangeriana*. c) Variable Importance in Projection (VIP) 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 \(165.38 kB\)](#)

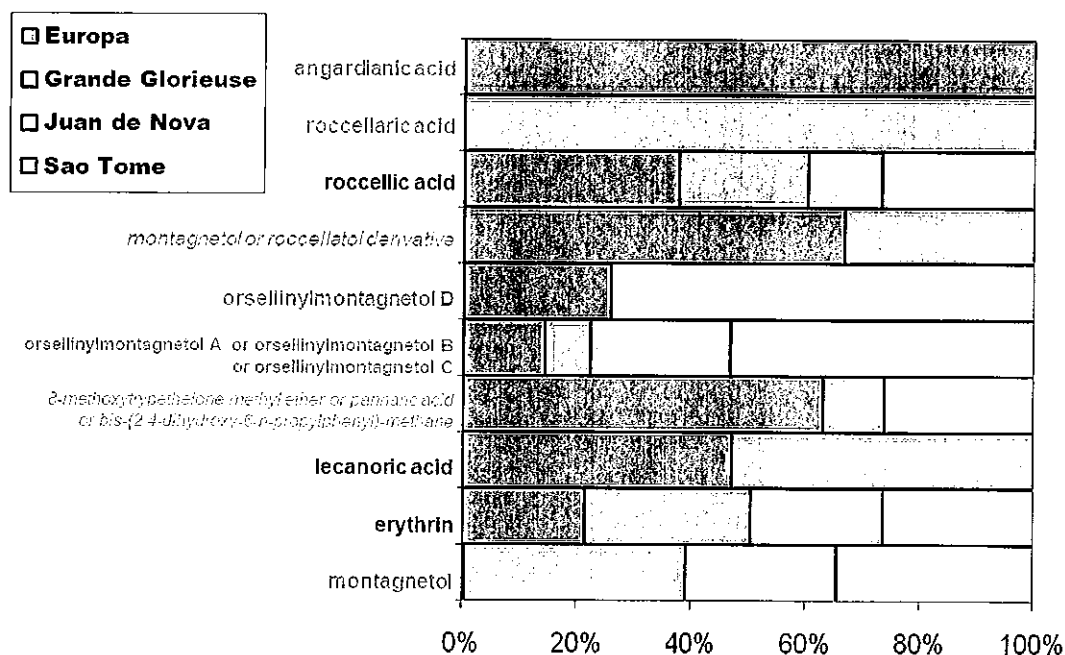


Figure 7. Ratio of the metabolites (in % of ion intensity) calculated in the samples of *R. montagnei* collected in Europa, Juan de Nova, Grande Glorieuse and Sao Tom . The identification of the metabolites was realized using the HLDB (in bold) and RoccellaDB/LDB-MS1 databases and was based on the molecular 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

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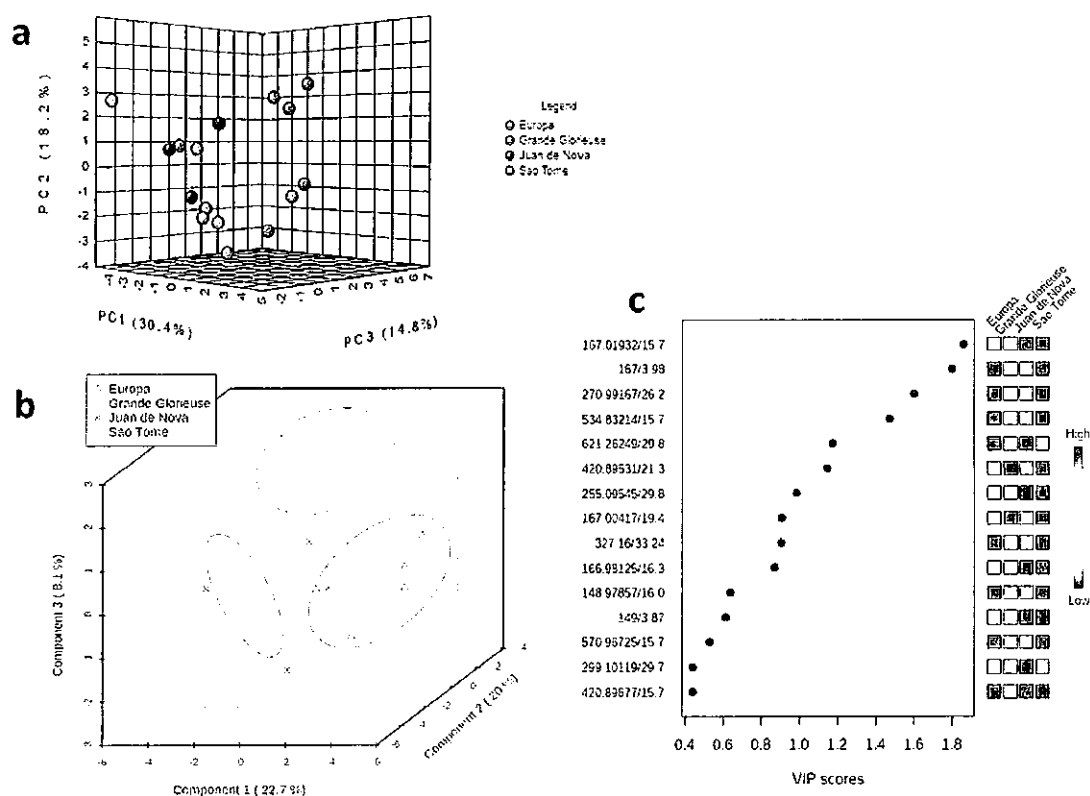


Figure 8. a) PCA scores scatter plot of *R. montagnei* collected in Europa, Juan de Nova, Grande Glorieuse and Sao Tomé b). 3D PLS-DA scores scatter plot of the different samples of *R. montagnei* collected at the four locations. c) Variable Importance in Projection (VIP) identified by PLS-DA. The colored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

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains. The *Agrobacterium* strains were grown in YEA medium for 24 h at 28 °C. The cell concentration was adjusted to 10⁸ cells/ml. The cells were then mixed with the plant tissue and incubated for 24 h at 28 °C. The plant tissue was then cultured on the selective medium. The transformation efficiency was calculated as the number of transformants per 100 µg of plant tissue. The data are the mean ± SD of three independent experiments.



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

Figure 6 shows the results of the phylogenetic analysis of the ITS region of the *Aspergillus* species. The tree is rooted with *Aspergillus nidulans* as the outgroup. The scale bar represents 0.01 substitutions per site. The bootstrap values are shown at the nodes. The species names are listed at the tips of the branches. The results of the analysis are shown in the table below.

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

Figure 7 shows the results of the phylogenetic analysis of the ITS region of the *Aspergillus* species. The tree is rooted with *Aspergillus nidulans* as the outgroup. The scale bar represents 0.01 substitutions per site. The bootstrap values are shown at the nodes. The species names are listed at the tips of the branches. The results of the analysis are shown in the table below.

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

Figure 8 shows the results of the phylogenetic analysis of the ITS region of the *Aspergillus* species. The tree is rooted with *Aspergillus nidulans* as the outgroup. The scale bar represents 0.01 substitutions per site. The bootstrap values are shown at the nodes. The species names are listed at the tips of the branches. The results of the analysis are shown in the table below.

Supplementary material

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

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

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