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

Solenn Ferron¹, Olivier Berry¹, Damien Olivier-Jimenez¹, Isabelle Rouaud¹, Joël Boustie¹, Françoise Lohézic-Le Dévéhat¹* & Rémy Poncet²*

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 (1°51′W to 47°17′E) covering the Eastern Atlantic and Western Indian Ocean areas along an extended latitudinal range (48°49′N to 22°23′S). 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 Island 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.

Key words: Roccella applanata, Roccella belangeriana, Roccella fuciformis, Roccella montagnei, Roccella phycopsis, chemical profile

Introduction

Roccella is a diverse genus of fruticose lichens numbering approximately 30 species [24 according to Tehler et al. (2010); 31 according to Aptrood and 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, b, 2010) brought significant advances for assessing the diversity and distribution of the genus, and together with the world-key provided by Aptrood and 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 1967; Huneck & Follmann 1967; Aberhart 1969), as well as their sub-units, the monooaromatic phenols, e.g., beta-orcinol, ethyl orsellinate, and montagnetol (Huneck & Follmann 1968; Aberhart 1969; Parrot et al. 2014; Duong et al. 2017; Duong & Bui 2018; Mallavadhani & Sudhakar 2018). Aliphatic acids like roccellic acid (Huneck & Follmann 1964) and the chormone 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 
& Geyer 1986). They have also been reported from the following *Roccellaceae* genera: *Dendrographa*, *Enterographa*, *Lecanactis*, and *Opegrapha*. Dibenzo furans are also uncommon; the only one known is schizopeltic acid, reported from *R. capensis*, as well as the genus *Schizopeltia* (*Roccellaceae*) (Follman & Geyer 1986; Elix et al. 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*, *Roccella belangeriana*, *Roccella fuciformis*, *Roccella montagnei* and *Roccella phycopsis*. 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 (Fig. 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 1](image)

**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 Tomé and Príncipe (Sample ID (field): Poncet, RPO050219_201); D – *Roccella belangeriana*, general view and close-up showing apothecia, Europa Island (Sample ID (field): Poncet, Europa20190411_13); E–F – *Roccella applanata*, Europa Island (‘Sample ID (field)’: Poncet, Europa20190411_4 and Europa20190411_8).
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 Principe 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 for 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 weighed 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.
### 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 study; collectors : AHP = Anne-Hélène Paradis (MBG), CF = Christian Fontaine (CBNM), EB = Eloïse Bidault (MBG), FP = Frederic Picot (CBNM), JB = Joël Boulitre (Univ. Rennes, UMR 2006), JH = Jean Hivert (CBNM), MG = Martin Grube (Karls-Franzens-Universität Graz), RP = Renny Ponzet (UMS 2006 PâtrinNat (OFB – CNRS – MNHN)); the trees/shrubs species indicated in ‘Support’ have been identified by JH).

| Species                | Location                          | Latitude       | Longitude       | Elevation (m) | Year | Support                          | Sample ID (field) | Sample ID (chem) | Collect         | Rennes lab Herbarian |
|------------------------|-----------------------------------|----------------|-----------------|---------------|------|----------------------------------|-------------------|------------------|------------------|---------------------|
| R. planatana           | Europa Island*                    | 24°22′37.64″S  | 40°22′22.44″E   | 2019          | 0    | Guettarda speciosa (tree)        | Europa20190410_6 | ApEGs100         | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Guettarda speciosa (tree)        | Europa20190410_6 | ApEGs312A        | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Coriops segal (mangrove tree)    | Europa20190411_13| ApEC334          | RP, CF, JH, EB   | –                   |
| R. belangeriana        | Europa Island*                    | 24°22′33.13″S  | 40°21′51.53″E   | 2019          | 0    | Hiscopha mircruana (mangrove tree)| Europa20190409_12| BeEAn305         | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Guettarda speciosa (tree)        | Europa20190410_6 | BeEGs12B         | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Euphorbia stenocladula (tree)    | Europa20190410_4 | BEEEs310B        | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Rhizochna mircruana (mangrove tree)| Europa20190409_15| BeErM306         | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Coriops segal (mangrove tree)    | Europa20190411_13| BeEC339          | RP, CF, JH, EB   | –                   |
|                        |                                   |                |                 |               |      | Coriops segal (mangrove tree)    | Europa20190411_14| BeEC340          | RP, CF, JH, EB   | –                   |
| R. fuciformis          | France mainland (Ploumanac’h)     | 48°49′N        | 3°29′W          | 2005          | 0    | Rock (granite)                   | JB/05/57          | FuBs57           | JB, MG           | REN 000 157        |
|                        | France mainland (Saint-Malo)      | 48°38′N        | 2°1′W           | 2005          | –    | Rock (granite)                   | JB/05/71          | FuBs71           | JB               | REN 000 624        |
|                        | France mainland (Cancale)         | 48°40′N        | 1°51′W          | 2013          | 0    | Rock (granite)                   | JB/13/6375        | FuBs375          | JB               | REN 015 121        |
| R. montagni            | Europa Island*                    | 24°20′39.35″S  | 40°20′21.95″E   | 2019          | 0    | Saurina martisa (shrub)          | Europa20190411_19| MoEsm324A        | RP, JH, EB        | –                   |
|                        |                                   |                |                 |               |      | Saurina martisa (shrub)          | Europa20190411_18| MoEsm334        | RP, JH, EB        | –                   |
|                        |                                   |                |                 |               |      | Saurina martisa (shrub)          | Europa20190411_18| MoEsm344        | RP, JH, EB        | –                   |
|                        |                                   |                |                 |               |      | Saurina martisa (shrub)          | Europa20190411_18| MoEsm354        | RP, JH, EB        | –                   |
|                        |                                   |                |                 |               |      | Saurina martisa (shrub)          | Europa20190411_18| MoEsm364        | RP, JH, EB        | –                   |
|                        |                                   |                |                 |               |      | Saurina martisa (shrub)          | Europa20190411_18| MoEsm374        | RP, JH, EB        | –                   |
| Juan de Nova*          |                                   | 17°02′57.11″S  | 42°43′16.75″E   | 2019          | 0    | Saurina martisa (shrub)          | Juandenova20190415_23| MoJNsm418       | RP, JH, EB        | –                   |
|                        |                                   | 17°02′57.24″S  | 42°43′16.99″E   | 2019          | 0    | Saurina martisa (shrub)          | Juandenova20190415_24| MoJNsm419       | RP, JH, EB        | –                   |
|                        |                                   | 17°02′56.44″S  | 42°43′17.78″E   | 2019          | 0    | Saurina martisa (shrub)          | Juandenova20190415_25| MoJNsm420       | RP, JH, EB        | –                   |
| Grande Glorieuse*      |                                   | 11°34′50.86″S  | 47°17′26.28″E   | 2019          | 0    | Casuarina equisetifolia (tree)   | Gglorieuse20190420_15| MoGGc450A      | RP, JH, EB        | –                   |
|                        |                                   | 11°34′18.07″S  | 47°17′57.54″E   | 2019          | 0    | Casuarina equisetifolia (tree)   | Gglorieuse20190421_15| MoGGc450B      | RP, JH, EB        | –                   |
|                        |                                   | 11°34′50.86″S  | 47°17′25.27″E   | 2019          | 0    | Casuarina equisetifolia (tree)   | Gglorieuse20190421_15| MoGGc450B      | RP, JH, EB        | –                   |
|                        |                                   | 11°34′18.07″S  | 47°17′57.54″E   | 2019          | 0    | Casuarina equisetifolia (tree)   | Gglorieuse20190421_15| MoGGc486B      | RP, JH, EB        | –                   |
| São Tomé and Principe* |                                   | 0°19′33.13″N   | 6°30′29.16″E    | 2002          | 0    | unidentified tree                | RPP050219_201     | MoStu140        | RP, AHP           | –                   |
|                        |                                   | 0°19′33.13″N   | 6°30′29.16″E    | 2005          | 0    | unidentified tree                | RPP050219_200     | MoStu141A       | RP, AHP           | –                   |
|                        |                                   | 0°19′33.13″N   | 6°30′29.16″E    | 2001          | 0    | unidentified tree                | RPP050219_201     | MoStu141B       | RP, AHP           | –                   |
| R. phycopsis           | France mainland (Saint-Malo)      | 48°38′N        | 2°0′W           | 2002          | 0    | Rock (granite)                   | JB/05/72          | PhB372           | JB               | REN 000 625        |
|                        |                                    |                |                 | 2005          | 0    | Rock (granite)                   | JB/02/64          | PhB64            | JB               | REN 000 818        |
|                        | France mainland (Cancale)         | 48°40′N        | 1°51′W          | 2013          | 0    | Rock (granite)                   | JB/13/6376        | PhB376           | JB               | REN 015 122        |
Multivariate analysis

All MZmine files (MZattributes.csv) were uploaded to MetaboAnalyst 4.0 software (https://www.metaboanalyst.ca/MetaboAnalyst) (Chong et al. 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 et al. 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 (Fig. 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-C6H7O3]-, base peak)); the aliphatic roccellic acid (RT = 29.7 min; m/z = 299); and the chromone lepraric acid (RT = 19.4 min; m/z = 361 ([M-H]−, molecular peak) and 317 ([M-CHO]−, base peak)). The depside lecanoric acid and the chromone lepraric acid have the same RT, and present a similar m/z (317), but they are distinguishable by the analysis of complete mass spectrum (Fig. 4). *R. fuciformis* contains only leprac 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 lepraric acid replaced it. No roccellacic acid was detected in *R. fuciformis* and *R. applanata*, and the ethyl ether of lepraric acid was only detected in *R. fuciformis*. All of 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 analyzed 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 showed a separation between species up to 52.5% of the total variance by the first three principal components (Fig. 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 (Fig. 5A). *R. fuciformis* specimens constitute a separated outgroup due to the presence of lepraric 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 (Fig. 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 also notice that *R. applanata* and *R. fuciformis*, the only two species where no roccellic acid was found (Table 2), only segregate according to PC2.

**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.
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 \textit{R. phycopsis}; erythrin, roccellic acid and montagnetol/roccellatol derivative were important for distinguishing \textit{R. montagnei}; and lecanoric acid and lepraric acid were important metabolites for segregating \textit{R. fuciformis}. By contrast, \textit{R. belangeriana} was distinguished by the presence of lecanoric acid, and \textit{R. applanata} by the presence of erythrin together with roccellic acid.

Focus on the \textit{R. montagnei-belangeriana} complex

The phylogenetically close species \textit{R. montagnei} and \textit{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 (Fig. 6A). The PLS-DA was then applied, highlighting significant differences between \textit{R. montagnei} and \textit{R. belangeriana} (Fig. 6B). The variable importance in projection (VIP scores) indicated roccellic acid and erythrin as discriminant metabolites for \textit{R. montagnei}, while lecanoric acid and erythrin were discriminant metabolites for \textit{R. belangeriana} (Fig. 6C) (VIP > 1). All of these metabolites were detected in thalli (Table 2) of both species, but with different intensities.

Metabolite variation in \textit{R. montagnei} at different latitudes

Metabolites data restricted to \textit{Roccella} species of this study comprising four tropical territories (Europa Island, 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 \textit{Roccella} metabolome. The mass data processing was based on the ionization of each metabolite and, in
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 (Fig. 7). Among the 7 other metabolites identified, two were present in only one location: angardianic acid (Europa Island) and roccellaric acid (Grande Glorieuse), three were present in two locations: montagnetol or roccellatol derivative and lecanoric acid (Europa Island and Grande Glorieuse), orsellinylmontagnetol D (Europa Island and São Tome and Principe), and two were present in three locations. In addition, R. montagnei specimens growing in Europa Island 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 (Fig. 8A), followed by a supervised PLS-DA (Fig. 8B). Although the R2 and Q2 values for PLS-DA were quite low, it appears that specimens found in Europa Island 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

### 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 dereplicated 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.

| Identified or putative compound | Classification | [M-H] | Main ions detected (m/z) | RT (min) | R. app | R. bel | R. fuc | R. montagnei | 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 | + | + | + | – | + |
| lepracic 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 | – | – | – | + | – |
| lepracic acid ethylether       | 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 | – | – | – | + | – |
| angardanic acid                | aliphatic acids | 327.2 | 327.2 | 33.2 | – | – | – | + | + |
| 8-methoxysterethylone methylether or bis-2,4-dihydroxy-6-n-propylphenyl)-methane or pannaric acid | nd | 315.0 | 315.0 | 20.4 | – | – | + | – | – |
| 6,8-di-O-methylhidurufin or pyremulic acid G | nd | 411.0 | 411.0 | 24.0 | + | – | – | + | – |
| isidiophorin or siphulin       | nd              | 425.2 | 425.2 | 25.5 | + | – | – | – | + |
| montagnetol or roccellatol derivative | monophenolic compounds | 271.0 | 271.0 | 26.2 | – | – | + | – | – |
| orcinylecanorate               | 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 | – | – | – | – | + |

Abbreviations: R. app: R. applanata; R. bel: R belangeriana; R. fuc: R fuciformis, R mon: R. montagnei; R. phy: R phycopsis
S. Ferron et al.: Chemical diversity of five coastal Roccella

(VIP > 1) to discrimination were montagnetol/roccellatol derivative, erythrin and roccellaric acid for thalli collected in Europa Island; erythrin for R. montagnei collected in Juan de Nova; and montagnetol and orsellinyl montagnetol for specimens collected in Sao Tomé (Fig. 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); orcinyl lecanorate (R. phycopsis; French mainland); roccellaric acid (R. montagnei; Grande Glorieuse); cholestata-3,5-dien-7-one (R. belangeriana; Europa Island); and oleic acid (R. belangeriana; Europa Island). Moreover, the chemistry of R. applanata (Europa Island) 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 Island) 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

Figure 6. A – APCA 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.
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), orcinyl 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 have been 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 Island.

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) (Huneck 1967; Huneck & Follmann 1967, 1968; Strack et al. 1979; Thadhani et al. 2012; Parrot et al. 2014; Duong et al. 2017; Sweidan et al. 2017; Brakni et al. 2018). 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 & Follmann 1968). The chromone lepracic acid was only found in *R. fuciformis*; it appears to be diagnostic for this species (Aberhart 1969). Surprisingly, acetylportentol, previously described as a major compound in *R. fuciformis* (Parrot et al. 2014; Sweidan et al. 2017), was not found here. However this aliphatic compound is not expected to be detected under UV, and appears to be hardly ionized. Therefore, we analyzed 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 lepracic acids. Moreover, given the high intensities of the peaks for the main compounds, it was difficult to detect picrorocellin (Marcuccio & Elix 1983) and 6-hydroxy-methyleugenitin in *R. fuciformis* (Huneck 1972).

Three acids were detected or suspected: roccellaric acid was detected in *R. belangeriana* (two specimens out of 6: BeEEs310B and BeECt339), *R. montagnei* (all specimens) and *R. phycopsis* (all specimens); andrographic 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, MoES310, MoESm334); and roccellaric acid was suspected in one specimen of *R. montagnei* (MoGGCe450A). As seen in Figure S3, roccellaric acid differs from andrographic 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 cyclization of the andrographic acid. Thus, it is necessary for the lichen

**Figure 7.** Ratio of the metabolites (in % of ion intensity) calculated in the samples of *R. montagnei* collected in Europa Island, 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).
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 produces angardianic and roccellaric acids.

**R. montagnei-belangeriana complex**

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/roccelatol derivative), as well as by their aliphatic acids (roccellaric and angardianic acids). Nevertheless, the statistical analyses also suggest that roccelic acid, erythrin and lecanoric acid are the only reliable discriminant metabolites, even though these three compounds are present in both species (Fig. 6C). This can be explained by the difference in 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 analyzed 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 Island, 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 colonize as efficiently in these rather close territories (Fig. 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 Island, Juan de Nova, Grande Glorieuse, São Tomé and Príncipe display metabolic differences, both qualitatively and quantitatively (Fig. 8A–C). However, we can note that PCA did not show clear segregation between locations, as clearly shown in the PLS-DA scatter plot (Fig. 8B). Erythrin was the only diagnostic compound for the 5 *montagnei* samples collected in Juan de Nova, while monophenolic compounds (montagnetol, orsellinyl-montagnetol) differentiated thalli growing in São Tomé and Príncipe. Thalli collected in Europa Island contain roccelic acid in addition to montagnetol/roccellatol derivatives and erythrin; however, six of the 16 *R. montagnei* thalli were collected in Europa Island, 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 Island 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 equisetifola* 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 an area of an unidentified tree, and thus protected from the sun.

The results showed that the only *Roccella* species common in Europa Island, Juan de Nova, Grande Glorieuse, São Tomé and Príncipe display metabolic differences, both qualitatively and quantitatively (Fig. 8A–C). However, we can note that PCA did not show clear segregation between locations, as clearly shown in the PLS-DA scatter plot (Fig. 8B). Erythrin was the only diagnostic compound for the 5 *montagnei* samples collected in Juan de Nova, while monophenolic compounds (montagnetol, orsellinyl-montagnetol) differentiated thalli growing in São Tomé and Príncipe. Thalli collected in Europa Island contain roccelic acid in addition to montagnetol/roccellatol derivatives and erythrin; however, six of the 16 *R. montagnei* thalli were collected in Europa Island, 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 Island 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 equisetifola* 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 an area of an unidentified tree, and thus protected from the sun.

Finally, friendly thanks go also to Jean Hivert (CBNM) for his help in designing the field sampling and identification of trees/shrubs in the Scattered Islands; and to Christian Fontaine (UMS 2006 PatriNat) and Laurent Poncet (UMS 2006 PatriNat) and Serge Muller (ISYEB – MNHN) for their trust and mostly for their invaluable help in this project, and without whom none of this would have been possible. Sophie Marinesque (TAAF), Jonathan Grand (TAAF) and Cedric Marteau (TAAF) who coordinated the consortium and the field campaigns, and invested countless energy and time for improving the Scattered Islands’ natural heritage knowledge and conservation, are also warmly thanked.
Some species studied in this paper were collected in the frame of the ‘Scattered Island’ inter-agency research consortium (2017–2021) coordinated by the French Southern and Antarctic Lands (TAAF) in partnership with: Centre National de la Recherche Scientifique, Institut de Recherche pour le Développement, Ifremer, Agence Française pour la Biodiversité, Université de La Réunion & Université de Mayotte. Fieldwork was funded by the inter-agency research consortium and was done jointly by the Conservatoire Botanique National de Mascarin, the Missouri Botanical Garden and the Museum National d’Histoire Naturelle (UMS 2006 PatrinNat OFB – CNRS – MNHN) in the frame of the RECOFFIE (Renforcement des Connaissances sur la Flore et la Fonge des Îles Éparses) project, implemented as part of the ‘Scattered Island’ inter-agency research consortium (2017–2021).

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 district head of the Scattered Island. The RECOFFIE (CBN-CPIE Mascarin, MBC, UMS 2006 PatrinNat (OFB – CNRS – MNHN)) project was authorized by order no 2019-003 of April 1, 2019. Collecting of the lichen species was authorized in Saint 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. Aromatic constituents of the lichen Roccella fuciformis DC. A revised structure for lepraric acid. Journal of the Chemical Society 5: 704–707.

Aptroot, A. & Schummm, 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 fchumen[online], The Netherlands.

Brakni, R., Ahmed, M. A., Burger, P., Schwing, A., Michel, G., Pomares, C., Hasseine, L., Boyer, I., Fernandez, X., Landreau, A. & Michel, T. 2018. ‘UHPLC-HRMS/MSE 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. University of North Carolina Press, Chapel Hill.

de Lamarck, J. B. & de Candolle, A. P. 1805. Flore Francaise, or 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, H. T. H., 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. Phytom-chemistry 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: 63–67.

Elix, J., Naidu, R. & Launond, J. R. 1992. Synthesis of the lichen dibenzofuran panaric acid 2-methyl ester and its isomer 5-O-methylpanaric acid. Australian Journal of Chemistry 45: 785–779.

Follmann, G. & Geyer, M. 1986. Preliminary Studies towards a monograph of the lichen family Roccellaceae Chev. V II. Secondary products and relationships of the genera Combrea de Not. and Schizoplete T. M. Fries. Zeitschrift für Naturforschung 41c: 1117–1118.

Gadea, A., Le Lamer, A. C., Le Gall, S., Jonard, C., Feron, S., Catheline, D., Ertz, D., Le Pogam, P., Boustie, J., Lohezic-Le Devehat, F. & Charrier, M. 2018. Intrathalline Metabolite Profiles in the Lichen Argopisia friesiania Shape Gastropod Grazing Patterns. Journal of Chemical Ecology 44: 471–482.

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

Huneck, S. 1967. Über die Inhaltsstoffe von Combrea molluca (Ach.) De Not., Roccella vicentina (Wain.) Wain., Roccella gayana Mont. und Roccella fucoides (Neck.) Wain. Zeitschrift für Naturforschung 22b: 1369–1370.

Huneck, S. 1972. 6-hydroxyhexylmethylugenitin, ein neues chromon aus Roccella fuciformis. Phytochemistry 11: 1489–1490.

Huneck, S. & Follmann, G. 1964. Das Vorkommen von Psoromsäure in Chidecton stictalactinum Nyld. und Roccellasäure in Dirina lutosa Zahlbr. Zeitschrift für Naturforschung 19b: 658–659.

Huneck, S. & Follmann, G. 1967. Zur Chemie chilenischer Flechten. XVI. Über die Inhaltsstoffe einiger Roccellaceen. Zeitschrift für Naturforschung 22: 362–366.

Huneck, S. & Follmann, G. 1968. Mitteilungen über Flechteninhaltsstoffe LV. Zur Phytochemie und Chemotaxonomie einiger Chiodectonaceae und Roccellaceae. Berichte der Deutschen Botanischen Gesellschaft. 81, H.3(45): 125–134.

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: 268–274.

Marcuccio, S. & Elix, J. A. 1983. A structural revision of picrocerocellin. 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: 8696–8703.

Nylander, W. 1889. Lichenes insularum guineensium. Paris.

Parrot, D., T. Peresse, Hitti, E., Carrie, D., Grube, M. & Tomasi, S. 2014. Qualitative and spatial metabolite profiling of lichens by UHPLC-HRMS/MS based profiling of Algae and Detecting Chromatographic Peaks. Natural Product Research 26: 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

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

Funding

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.
montagnei and Roccella belangeriana using DNA sequence data of nuclear ribosomal internal transcribed spacer region. *Journal of Plant Biochemistry and Biotechnology* 17: 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* 34c: 695–698.

Sweidan, A., Chollet-Krugler, 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: 432–428.

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

Tehler, A., Irestedt, M., Wedin, M. & Ertz, D. 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: 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: 223–246.

Tenenhaus M. 1998. *La régression PLS: Théorie et pratique*, Editions Technip, Paris.

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. V. & Prokopenko, V. V. 2005. Virtual computational chemistry laboratory — design and description. *Journal of Computer-Aided Molecular Design* 19: 453–463. http://www.vcclab.org

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

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