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French Mediterranean and Atlantic populations of the brown algal genus *Taonia* (Dictyotales) display differences in phylogeny, surface metabolomes and epibacterial communities

Benoît Paix[1,†], Christophe Vieira[2], Philippe Potin[3], Catherine Leblanc[3], Olivier De Clerck[4], Jean-François Briand[1*], Gérald Culioli[1,‡*]

[1]*Université de Toulon, Laboratoire MAPIEM, EA 4323, Toulon, France*

[2]*Kobe University Research Center for Inland Seas, Rokkodai, Kobe 657-8501, Japan*

[3]*Sorbonne Université, CNRS, Integrative Biology of Marine Models (LBI2M), UMR8227, Station Biologique de Roscoff (SBR), Roscoff, France*

[4]*Ghent University, Department of Biology, Phycology Research Group, Ghent, Belgium*

*Corresponding authors: J.-F. Briand (briand@univ-tln.fr) and G. Culioli (gerald.culioli@univ-avignon.fr)

†New affiliation: Marine Biodiversity, Naturalis Biodiversity Center, Leiden, The Netherlands
‡New affiliation: Institut Méditerranéen de Biodiversité et d'Ecologie marine et continentale (IMBE), UMR CNRS-IRD-Avignon Université-Aix-Marseille Université, Avignon, France.

Abstract

Considered as holobiont systems, marine macroalgae and their microbiota constitute functional units displaying a large diversity of interactions. Main factors driving the assembly of epiphytic microbiota, and subsequent interactions with the host, are often associated to environmental differences but also to host taxonomy displaying specific chemical properties. Here, through a large sampling effort focused on the brown algal genus *Taonia* (Dictyotales) on both the French Mediterranean and Atlantic coasts, we aimed to highlight the relative importance of the effects of environment, host taxonomy and surface metabolome on the epibacterial community of these seaweeds. Phylogenetic analyses revealed two distinct clusters, one grouping only the specimens from the French Mediterranean coasts and the second one composed of samples from Brittany. Both metabarcoding and surface metabolomics revealed clear differences between Mediterranean thalli and those collected in Brittany. Strong environmental differences associated with algae from these two different geographical areas could both be involved in this clustering. For example, oxidative stress due to higher irradiance intensities might induce a higher expression of fucoxanthin in Mediterranean samples, while higher eutrophication levels could explain the higher abundance of *Alteromonas* spp. on algal samples from Brittany coast. In a lesser extent, genetic differences observed between thalli from these two locations could also influence surface metabolomes and thus host-microbiota interactions.

Keywords: Holobiont, seaweed, phylogeny, surface metabolome, surface microbiota, multi-omics.

1. Introduction

The concept of the holobiont (i.e. the host-microbiota association as a functional entity), initially coined by [1] for model corals and their symbionts (e.g. Symbiodiniaceae and associated bacteria), was later applied to other organisms (e.g., plants [2] or human [3]] including seaweeds [4]). Knowing that the association between a host and its microbiome contributes to the functioning of the holobiont system, this holistic view has been proposed for seaweeds in the light of recent studies which have demonstrated that epibacterial communities could impact the algal physiology through beneficial (e.g. morphogenesis or defense, [5,6]) or detrimental processes (e.g. bleaching [7,8]).

While the functions of the associated microbiota are still being explored in seaweed holobionts, a parallel line of research aims to determine to which extent the microbial assembly is specific to a host and which factors influence this community structure. Depending on the host taxonomy, differences in the structure of the bacterial microbiota can also occur at the algal surface in response to environmental drivers [9,10]. However, the relative importance of both factors is often raised [10–12]. For instance, variations in the endophytic bacterial microbiota of the green algae *Caulerpa prolifera* and *C. cylindracea* (Bryopsidales, Ulvophyceae) were found to be: (i) host-specific, (ii) compartment-specific (e.g. rhizome, uprights), and (iii) influenced by the biogeography [10]. While for some *Ulva* species (Ulvales, Ulvophyceae) biogeography and host-specificity also showed an effect on the epiphytic bacterial assemblages, a large proportion of core functions were shared between *U. australis* (from Spain and Australia), *U. rigida* (Spain) and *U. ohnoi* (Australia) [13,14].

In the first instance, the host surface chemistry could explain the specificity of the microbial community assembly associated to a given algal species [9]. Some surface metabolites act as chemical defenses displaying anti-adhesion activities (e.g., halogenated furanones [15]) while others show chemo-attracting properties allowing a specific microbial gardening (e.g., DMSP [5,16]). Polysaccharides are also key biochemical components of algal surfaces and these biopolymers can be degraded by algal-specific associated bacterial taxa, such as *Zobellia galactanivorans* which has been hypothesized to form profitable and stable interactions with its algal host [17,18].

In addition, geographic differences, e.g. Mediterranean *vs* Atlantic coasts, also imply differences in physicochemical parameters, such as temperature, nitrogen concentrations, irradiance, pH, salinity and anthropogenic pollutions, and thus could also explain variations observed in microbial community assembly on macroalgae [7,19–23]. Environmental factors are notably known to shape the algal epiphytic microbiota but also the host physiological condition leading in some cases to dysbiosis [24–27]. A continent-scale study conducted on the brown algal kelp *Ecklonia radiata* collected along Australian coasts has highlighted that geographical differences of microbiota could be more strongly associated to host condition rather than to environmental variables [28].

The Dictyotales represent an ecologically important and highly diverse order of brown algae [29–31]. However, host-bacteria interactions within this diversified order are still poorly considered [32], since *Taonia* J. Agardh (Dictyotales, Phaeophyceae) and to a lesser extent *Lobophora* and *Dictyota*, are to date the only genera being investigated in association with their bacterial communities [33–37]. The present study focused on the genus *Taonia* whose representative species are commonly observed along French Mediterranean and

North Atlantic coasts. The genus *Taonia* currently comprises six taxonomically accepted species [29]: *T. abbottiana* D.S. Littler & Littler (Jamaica), *T. atomaria* (Woodw.) J. Agardh (United Kingdom), *T. australasica* J. Agardh (Australia), *T. lacheana* Cormaci, G. Furnari & Pizzuto (Italy), *T. lennebackerae* Farl. ex J. Agardh (California), and *T. pseudociliata* (J.V. Lamour.) Nizam. & Godeh (Haiti). *Taonia atomaria*, the type species of the genus, is considered to show a cosmopolitan distribution [38]. However, this is neither supported by molecular, nor by morphological data and, to our knowledge, North Atlantic or English Channel populations of this species have never been compared with Mediterranean ones using sequence data. Mediterranean populations of. *Taonia atomaria* have been already used as a holobiont model in previous studies focusing on epibacterial communities and surface metabolites [35,36,39]. Briefly, covariations between surface metabolome and epibacterial communities were observed according to the temporality but also to zonal variation along the thallus [35,36]. A control of the epibacterial community by the host metabolome was notably proposed as a main hypothesis to explain such correlated variations, involving the expression of several surface compounds such as sesquiterpenes, geranylgeranylglycerol [36,39] or DMSP [35].

Using DNA-assisted identification, flow cytometry and a multi-omics approach, coupling LC-MS-based metabolomics and 16S rRNA gene metabarcoding, the present study aimed to compare French Mediterranean populations of an unidentified species of *Taonia* (referred to hereafter as *Taonia* sp.) with *Taonia atomaria* collected in this study from the North Brittany coast. More precisely, the objectives were to: (1) determine if populations of *Taonia* collected along French Mediterranean and North Brittany coasts were conspecific;

and (2) assess the relative role of the environment, the host taxonomy and the surface metabolome on the epiphytic microbial community assembly.

2. Material and Methods

2.1. Sampling strategy

Specimens of *Taonia* were collected in May and July 2018 at 18 sites along the French Mediterranean coast and one site on the French North Brittany coast (Fig. 1, Table S1). One measure of temperature, pH, salinity, oxygen and turbidity was performed for each site using two multiparameter probes: Hydrolab® DS5X (Hatch Hydromet, USA) for Mediterranean sites and YSI™ Professional Plus (YSI, USA) for the Brittany site. Three different individuals (considered as triplicates) were collected at each site resulting in a total of 57 specimens, used for the four analyses: (1) barcoding of algal host, (2) metabolomics of algal surfaces, (3 and 4) metabarcoding and flow cytometry of epiphytic prokaryotic communities. For each of these four analyses, four adjacent fronds were respectively used from the same individual. Right after the sampling, a frond of each *Taonia* samples were dried in silica gel for algal DNA-barcoding. Surface metabolome was extracted as described in [36], by dipping a frond during 5s in 5 mL of methanol. The epiphytic microbiome was sampled by gently scraping squares of 1 cm^2 of two other fronds with a sterile scalpel as previously described in [35,36] and conserved, respectively, in a Tris-EDTA buffer for DNA extraction and in 4 mL of 1% glutaraldehyde filtered seawater solution for flow cytometry. Samples were kept on ice in a cool box and transported to the lab within one hour. Surface extracts were conserved at -20°C and epiphytic microbial samples at -80°C.

2.2. Barcoding analyses of algal specimens

Total genomic DNA was extracted from *Taonia* tissue samples using a cetyltrimethylammonium bromide (CTAB) extraction method [40]. Sequences were generated from the mitochondrial encoded cytochrome c oxidase III gene (*cox*3), the chloroplast encoded ribulose-1,5-biphosphate carboxylase (*rbc*L) and the photosystem II protein D1 (*psb*A) genes following [41]. Sequences from the three genes were concatenated and aligned using MUSCLE v.3.5 [42]. Phylogenetic trees were reconstructed based on the concatenated alignment using Bayesian (BI) and maximum likelihood (ML) methods following [41].

2.3. UHPLC-based metabolomics analyses

Metabolomics analyses were performed as described in [35] and detailed in supplementary information. Briefly, analyses were conducted by UHPLC-ESI-HRMS using a UPLC system (Dionex Ultimate 3000 rapid Separation; Thermo Fisher Scientific) equipped with an analytical core-shell reversed phase column (150 × 2.1 mm, 1.7 μm, Kinetex Phenyl-Hexyl; Phenomenex, Le Pecq, France) and coupled with a ESI-QToF Impact II mass spectrometer (Bruker Daltonics, Bremen, Germany) working in the positive ionization mode. Raw UPLC-MS data were processed for peak finding, integration and alignment using the open source XCMS package [43] in the R 3.2.3 environment. Data were filtered according to [44]. The resulting data matrix (Dataset S1) was $\log_{10}$-transformed, mean-centered, normalized using the sum of the chromatographic peak areas as described in [36] and analyzed using a hierarchical clustering dendrogram (Euclidean distance, mean method). A PERMANOVA test was performed with the "vegan" R package, using the Euclidean distance, to evaluate the overall difference of surface metabolomes between

Mediterranean and Atlantic samples. Partial Least Square – Discriminant Analysis (PLS-DA) was performed to reveal the most discriminant metabolites involved in the differences between the two geographical areas. The metabolome annotation focused on these metabolites and was performed as described in [35,36,45] and detailed in supplementary information. A db-RDA approach was performed to investigate the effect of salinity, temperature and pH (Table S1) as explanatory variables of metabolome profiles. This analysis was performed with the Euclidean distance and the *anova.cca()* function (999 permutations) using the "vegan" R package. O_2 concentration and turbidity were not considered for this analysis since missing values occurred for both parameters (Table S1).

2.4. Quantitative flow cytometry analyses

Flow cytometry analyses were used to assess densities of prokaryotic heterotrophs at the surface of the algal samples. Cells were stained using SYBR green I (Invitrogen, Carlsbad, CA, USA) and enumerated using a BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA) as described in [36]. Data were analyzed using ANOVA followed by an HSD Tukey's test.

2.5. Epibacterial community analyses

Metabarcoding approaches were performed as previously described in [36] and were detailed in supplementary information. Briefly, DNA extraction of algal biofilm samples was performed using the DNeasy PowerBiofilm kit (MoBio, Qiagen, Germantown, MD, USA). V4-V5 region of the 16S rRNA gene was amplified using the 515F-Y and 926R primers [46]. Amplicons were sent to the GeT platform (Toulouse, France) for MiSeq Illumina sequencing (2×250 bp). The OTU table (Dataset S2) was obtained after the 16S

rRNA gene reads processing using the FROGS workflow under the Galaxy environment [47]. Hierarchical clustering dendrogram was constructed using the UPGMA method with Bray-Curtis distances. PERMANOVA was performed with the "vegan" R package, using the Bray-Curtis distance, to test the overall difference of epibacterial community structure between Mediterranean and Atlantic algal samples. A SIMPER analysis was conducted with the "vegan" R package to reveal taxa which contribute the most to the overall dissimilarity between the two geographical areas [48,49]. A db-RDA approach was performed, to investigate the effect of salinity, temperature and pH (Table S1) as explanatory variables of the β-diversity, using the Bray-Curtis distance, and tested with the *anova.cca()* function (999 permutations) using the "vegan" R package. O_2 concentration and turbidity were not considered for this analysis since missing values occurred for both parameters (Table S1). In this study, the core community was defined by keeping only OTUs occurring at least in one replicate of each site.

3. Results and discussion

Taonia populations formed rather dense covers on rocky shores along the Mediterranean coast at the times of collection in May and June 2018, while specimens were observed on sandy substrates with local patches of small buried rocks on the North Brittany coast. Samples from both locations differed morphologically: the Mediterranean specimens displayed small thalli (up to 11 cm long and 0.5 cm wide) characteristically branched and twisted, while the North Brittany specimens showed larger thalli (up to 19 cm long and 1.6 cm wide) with fewer branches (Table S2; Fig. S1).

3.1. Populations of *Taonia* from the Mediterranean coast are genetically distinct from Atlantic ones

Phylogenetic results based on the concatenated alignment of the *cox*3, *psb*A and *rbc*L datasets indicated that the *Taonia* specimens collected in this study composed two distinct and well-supported genetic clusters in both analyses (ML and BI; Fig. 2). These genetic clusters reflected the two different geographic sampling locations. The first cluster (Cluster 1, Fig. 2) was composed only of specimens from the French Mediterranean (mainland) coast and the second one of specimens from North Brittany (Cluster 2, Fig. 2). Mediterranean sub-clusters were clearly not related to specific locations along the Mediterranean coast. The literature reports the occurrence of two species from western Mediterranean coasts: *T. atomaria* and *T. pseudociliata* [29]. Of these two species, only *T. atomaria* is reported in Brittany. The species collected in this study along the coast of North Brittany was morphologically closer to the description of *T. atomaria* than the species from the French Mediterranean coast. Admitting that the species from North Brittany corresponded to the genuine *T. atomaria* – originally described from the English Channel (Norfolk, UK) –, the species collected from the French Mediterranean coast could correspond to *T. pseudociliata*. However, no taxonomically confirmed sequence data are presently available from neither *T. atomaria* nor *T. pseudociliata* to check this assumption.

3.2. Atlantic and Mediterranean populations of *Taonia* support distinct epibacterial communities and surface metabolomes

For both hierarchical dendrograms constructed with metabarcoding and surface metabolome datasets, the main clustering pattern revealed clear differences between the

North Brittany samples (site LOCQ) and the Mediterranean ones, respectively (Fig. 3 and 4). For both datasets, these differences between both geographical areas were confirmed through PERMANOVA tests (p = 0.001, Table S3). Although the sampling sites number was uneven on the two coasts, Brittany samples exhibited a significant lower α-diversity compared to all Mediterranean ones (Fig. S2, ANOVA test: $p < 0.001$, Table S4). Considering Mediterranean sub-clusters of both metabolomics and metabarcoding analyses, they were not related to geographic areas. More interestingly, the clustering pattern (i.e. Brittany *vs* Mediterranean samples) appeared similar to the algal phylogenetic pattern described above. Two possible scenarios can be considered to explain such biogeographical features at the holobiont scale. Either, host phylogenetic differences and speciation processes observed between North Brittany and Mediterranean samples involve physiological and functional differences, which could notably result in various selective pressures including the differential expression of surface metabolites involved in the selection of a specific microbiota. Alternatively, environmental differences between the English Channel and Mediterranean coasts could also constitute factors explaining the clustering since Mediterranean conditions differ from those found on the English Channel coasts, notably with higher temperatures (21.9°C *vs* 18°C] and salinities (39.2 ppt *vs* 35.2 ppt) (Fig. S3, ANOVA.CCA: $p < 0.001$ for both datasets). Besides, seawater on the North Brittany coast are mainly mesotrophic to eutrophic [50] with substantial tidal regime, while those of the French Mediterranean coast are generally oligotrophic to mesotrophic, depending on the anthropic pressures [51,52], and non-tidal. Moreover, collection sites differed also in terms of irradiance and substrate types since North Brittany samples were collected on a sandy bottom with patches of small rocks in the infralittoral zone between 3 to 5m depth (varying

with the level of low tide) while the Mediterranean ones were sampled on rocky substrates at 1 to 2m depth. Local Mediterranean specific conditions of wind, sea currents, pollutants and nature of rocky substrates, like volcanic stones close to Agde, may also provide dissimilar environmental conditions for the development of *Taonia* populations. However, these parameters could probably appear as less relevant factors since no clear local geographical patterns were observed in the clustering of the epibacterial community within Mediterranean samples.

3.3. Relationships between environmental context and the epibacterial community structure

The major epibacterial taxa observed for *Taonia* samples belonged to families Hyphomonadaceae (average relative abundance in all samples: 6.7 ± 5.3%), Rhodobacteraceae (9.8 ± 3.7%), and Sphingomonadaceae (4.7 ± 3.0%) for Alpha-proteobacteria, Saprospiraceae (19.3 ± 4.9%) and Flavobacteriaceae (13.0 ± 4.8%) for Bacteroidetes, and Thiohalorhabdaceae (6.2 ± 3.8%) for Gamma-proteobacteria (Fig. 3). Compared to the total community, the mean percentage of sequences of core OTUs reached 65%, while the mean percentage of number of core OTUs reached 21.1%. Conversely, a high level of intraspecies variability was observed for the core epibacterial OTUs of *Ulva* species including Australian and Spanish samples [13,14,53], kelp species from different habitat types in Australian and North-East Pacific locations [28,54] or *Caulerpa* species [10]. The core community of *Taonia* was mainly affiliated to Hyphomonadaceae represented primarily by the genus *Litorimonas* (5.7% of sequences of all core OTUs), Thiohalorhabdaceae by the genus *Granulosicoccus* (9.5%), Saprospiraceae by the genera *Lewinella*, *Portibacter* and *Rubidimonas* (5.0, 3.8, and 3.6%), and Flavobacteriaceae by the

genera *Algitalea*, *Tenacibaculum* and *Maribacter* (2.5, 2.1, and 2.0%). In addition, this core community appeared relatively high in comparison with a previous study focused on temporal variations of "*T. atomaria"* on a single Mediterranean site (CARQ) [35]. It could indicate that temperature, that was proposed to drive temporal variations at CARQ, impacted more strongly epibacterial communities than salinity or nutrients that mainly differentiated Brittany from Mediterranean locations. However, several authors considered epibacterial recruitment as only partially deterministic and mentioned that environmental factors alone could not explain epiphytic community structures [10,13,54]. Especially, functional aspects should have to be considered [10,13,28].

Among the core taxa, the genus *Granulosicoccus* was previously described as a major core and pioneer taxon [35–37]. In addition, OTUs from the genus *Granulosicoccus* have been already encountered at the surface of many other algal species, such as the brown *Laminaria hyperborea* [55], *Fucus vesiculosus* [12], *Macrocystis pyrifera* [54], the red *Mastocarpus* spp. [56], and the green *Ulva* spp. [57]. In the case of *Mastocarpus* spp., this bacterial genus has been also found as a core taxon, common to both life history phases of the algal host (sporophytes *vs* gametophytes). For *L. hyperborea*, it has been suggested that *Granulosicoccus* spp. could be pioneer colonizers which benefit from a specific adaptation allowing a rapid attachment and development on young algal surfaces. For North-East Pacific kelp species, it has been identified as the dominant genus [54]. In accordance with these observations, we also suggested that OTUs among this genus may be especially adapted to the surface macroalgal niche in a large range of environmental conditions and host species.

When comparing all the samples, Gamma-proteobacteria showed higher percentages in North Brittany specimens of *Taonia* compared with Mediterranean ones, notably with the family Alteromonadaceae (Fig. 3). The SIMPER analysis (Table S5) revealed *Litorimonas* (Hyphomonadaceae), *Granulosicoccus* (Thiohalorhabdaceae) and *Alteromonas* (Alteromonadaceae) as major significant genera involved in the dissimilarity between samples collected from these two geographical areas (6.5, 4.9, and 6%, respectively), with higher percentages at the surface of samples from North Brittany. In contrast, the genus *Croceitalea* (Flavobacteriaceae) appeared as a major taxon of Mediterranean samples and contributed significantly to 1.7% of the dissimilarity with samples from North Brittany.

The genus *Alteromonas* was notably described in the literature by diverse strains known for their abilities to degrade algal polysaccharides [58]. Some of them are also reported as r-strategists and affiliated to *Alteromonas macleodii* which has been notably characterized by a quick growth in nutrient-enriched environments [59–62]. Consequently, the eutrophic status of the French North Brittany coast may constitute a favorable environment for the development of *Alteromonas* spp. on algae.

3.4. Relative importance of host phylogeny, the surrounding environment, and surface metabolites for epibacterial communities

The PLS-DA analyses conducted using the untargeted LC-MS-based metabolomics dataset allowed to reveal the most discriminant metabolites [those with a high Variable Importance in Projection (VIP) score] between these two geographical regions, and an annotation was proposed for some of the first fifteen ones (Table S6). Among them, different chemical classes were putatively annotated through the elucidation of their fragmentation pathway in comparison to the literature, such as the aminolipids

diacylglycerylhydroxymethyl-*N*,*N*,*N*-trimethyl-β-alanines (DGTAs or *Lyso*-DGTAs), but also other lipids, including two diacylglycerols (DG) and a monogalactosyldiacylglycerol (MGDG), and more polar compounds including an amino acid (phenylalanine) and a dipeptide (glutamineleucine), which all appeared as biomarkers of Mediterranean samples (excepted for a *Lyso*-DGTA) (Table S6, Fig. 4). Moreover, the two first discriminant metabolites were identified through the comparison of their MS data with those of purified standards [63,64]: they corresponded to fucoxanthin (VIP score = 3.16) and a bicyclic sesquiterpene (δ-cadinene, VIP score = 2.61)(Fig. 4 and S4, Table S6). According to ANOVA tests (Table S4) and pairwise comparison results (Fig. S4), fucoxanthin was observed in significant higher concentrations in all Mediterranean sites compared to the Atlantic one (LOCQ) (ANOVA: $p < 0.001$), while the opposite tendency was observed for δ-cadinene (ANOVA: $p = 0.01$), which was characterized by significant higher concentrations at LOCQ compared with 5 Mediterranean sites (AGD, MRSL, PRQN, LLND and THEO). Fucoxanthin is a common photosynthetic pigment of brown algae and differences in its concentration can be linked to irradiance variations. Indeed, fucoxanthin content is known to increase with depth for many brown seaweeds. Such a pattern is explained as a photo-adaptation of the algae to lower light intensity and quality which occurs at greater depths in order to ensure an optimal photosynthetic activity [65,66]. Herein, on the contrary, fucoxanthin was observed in lower concentrations in Brittany samples where algae were collected in rather deeper zones than Mediterranean ones. Consequently, in this case the differences of fucoxanthin production at the surface of *T. atomaria* might not result to a shading adaptation linked with the depth. In addition to the irradiance itself, other factors such as the turbidity, but also the nutrient concentrations and the salinity could constitute

important environmental parameters to consider as suggested by [67] in the case of the brown seaweed model *Fucus vesiculosus*. Among the parameters investigated, the surface fucoxanthin concentration was found to decrease with an increasing salinity along a geographical gradient from the Baltic sea to the North Sea [67]. Moreover, fucoxanthin is also involved in the protection against several oxidative stresses. In particular, its radical scavenging activity constitutes a protection which allows to reduce damages caused by reactive oxygen species produced under high irradiance conditions [68]. Through their oligo- to mesotrophic status and highly transparent waters, French Mediterranean seawaters are characterized by higher irradiance, in terms of both light intensities and duration. Consequently, high irradiance may involve a photoprotective adaptation of Mediterranean *Taonia* individuals [37]. Besides, other factors resulting in an oxidative stress can also be implied in the differences observed in the fucoxanthin production. As an example, it could be interesting to take into account in further works the oxidative stress caused by copper in contaminated areas [69,70].

Furthermore, previous studies conducted on *F. vesiculosus* and *Dictyota* sp. have shown that fucoxanthin acts as an antimicrobial compound at the algal surface by inhibiting the settlement of bacteria through bio-assays experiments [71,63]. Besides, an *in situ* approach has also confirmed that a fraction of algal extracts containing fucoxanthin deposited at the surface of an experimental device significantly decreases the epibacterial colonization [72]. However, heteroprokaryotic cell densities measured with cytometry analyses showed values ranging from 3×10^5 to 7×10^6 cells.cm^{-2} without any significant differences between Brittany and Mediterranean samples, and neither between the Mediterranean sites, according to the ANOVA test ($p < 0.001$, Table S4) and pairwise comparisons (post-hoc

Tukey's test, Fig. S2). Moreover, when comparing cells densities and fucoxanthin concentrations, these two variables did not appear significantly correlated (Pearson correlation: $r = 0.26$, $p > 0.05$), suggesting no quantitative effect of fucoxanthin on the microbiota (Fig. S2, S4). Moreover, Shannon and Chao1 indexes were significantly higher for all Mediterranean samples compared to those from Brittany according to ANOVA tests ($p < 0.001$, Table S4) followed by pairwise comparisons (Tukey's test, Fig. S2). Similarly, for both indexes, no significant correlation was observed with the fucoxanthin concentration (Pearson correlation: $r = 0.21$, $p > 0.05$ for both indexes). The fact that the epibacterial density at the surface of *Taonia* sp. was not directly related to the fucoxanthin concentration was not really surprising as it is more than likely that several other factors should be involved in the regulation of this density at the algal surface. Thus, fucoxanthin antibacterial activity could contribute to the selection of specific bacteria (as it has been previously demonstrated for several other metabolites isolated from the same algal model which had been assayed against bacterial strains isolated from its surface or from artificial substrates [39]) rather than exhibiting broad-spectrum antimicrobial properties. Nevertheless, it cannot be excluded either that other surface metabolites, but not fucoxanthin, might act as major antimicrobial compounds reducing either the cell density (e.g., CASS, LLND) or the bacterial α-diversity (e.g., TAMR) at the surface of some Mediterranean samples of *Taonia*.

The bicyclic sesquiterpene δ-cadinene was observed as a chemical biomarker of Brittany samples. Terpenoids compounds were widely described among Dictyotales, such as cyclic diterpenes mainly found within the genus *Dictyota* [73–75]. The main terpenoids isolated within the genus *Taonia* are geranylgeranylglycerol and cyclic sesquiterpenes [64,76,77].

Some of these compounds have been reported for their anti-adhesion activities against marine bacteria [39,64]. Moreover, the carbon skeletons of sesquiterpenes differ sharply between *Taonia* species: *T. lacheana* is characterized by aromadendrane sesquiterpenes [77] while sesquiterpenoids with germacrane, cadinane and spiroaxane skeletons were observed in the extracts of "*T. atomaria"* [64,76,78]. Here, the presence of a cadinane sesquiterpene as a biomarker of Brittany samples tended to confirm that these algal samples correspond to *T. atomaria* while their relative low abundance in Mediterranean samples may be consistent with phylogenetical differences. Neither in previous studies conducted at the site CARQ [35,39,64], nor in this work, aromadendrane sesquiterpenes were observed while some cadinane and germacrane sesquiterpenoids were identified in these studies. This finding suggested that the Mediterranean thalli investigated in this study may also differ in terpenoid content from previously described samples of *T. lacheana* [77] for geographic or genetic features.

Sesquiterpenes constitute a chemical class of natural products found in a large diversity of organisms [79]. These compounds often demonstrated antimicrobial activities such as bicyclic sesquiterpenes within floral organs of several plants [80,81] or halogenated sesquiterpenes from red algae of the genus *Laurencia* [82]. In the case of *T. atomaria*, the spiroaxane sesquiterpene gleenol has been found to show specific anti-adhesion activities against a panel of marine bacteria at ecological relevant concentrations [39,64]. However, anti-adhesion activities of *δ*-cadinene have not been described in these latter studies and its effect on the selection of a specific microbiota at the surface of *T. atomaria* could be interesting to consider in order to better support the relative importance of the host-phylogeny in the Atlantic *vs* Mediterranean clustering

The other discriminant metabolites showed drastically lower VIP scores (ranging from 1.12 to 1.67; Table S6) than those of fucoxanthin and δ-cadinene. Among them, several DGTAs have been identified. Such non-phosphorus-containing betaine membrane lipids have already been described at the surface of *Taonia* sp. [35,36] but apart from the fact that these lipids are differentially expressed depending the season or the part of the thallus, no ecological role has been described for DGTAs in algae. It is interesting to note that DGTAs were characteristic of metabolomes of Mediterranean samples while *Lyso*-DGTA was mainly found in Brittany samples. Another discriminant compound of Mediterranean samples was identified as glutamineleucine. This dipeptide was already described in *Taonia* sp. as a metabolite predominantly produced at the algal surface in May-June [35]. Finally, phenylalanine was also identified as a chemomarker of Mediterranean samples of *Taonia* sp. As this aromatic amino acid has been already described for its implication in the photoprotection of a wide range of organisms [83,84], its higher proportions in Mediterranean samples could be correlated with the higher irradiance conditions inherent to this geographical area. From an ecological point of view, even if we have no idea of the stereochemistry of the compound detected here, it is interesting to note that *D*-phenylalanine had been previously described for its capacity to inhibit biofilm development of a marine bacterial strain (*Pseudoalteromonas* sp.) [85] indicating that this amino acid may also have a role in the selection of specific microbial taxa.

4. Conclusion

Focusing on the brown algal genus *Taonia*, we provide a growing body of evidence that host taxonomy, environment, and surface metabolomes need to be further considered in biogeographical studies on seaweed holobionts. To our knowledge, this work constitutes the first biogeographical study conducted at a large scale, which coupled these three complementary approaches. The genetic diversity within the genus *Taonia* was scarcely studied before and we revealed through phylogenetic analyses that algal samples from the French Mediterranean coast potentially constitute a distinct species from *Taonia atomaria* samples encountered in the French North Brittany. Here, we brought evidence that environmental, and probably to a lesser extent genetic differences may both contribute to the specificity of metabolome and microbiome composition at the algal surface. More particularly, while differential expression of fucoxanthin at the surface could be more related to environmental adaptation, the case of *δ*-cadinene could be considered as a result of phylogenetic differences. Such a contrasted metabolome at the surface of the *T. atomaria* species complex may consequently result in the selection of a specific microbiome while environmental differences could also constitute a major factor directly shaping the microbiota structure, notably when considering the *Alteromonas* enrichment in surface communities from Brittany.

CRediT authorship contribution statement

All authors designed the study. BP, GC, JFB and PP performed field samplings. BP performed the flow cytometry, metabolomics and metabarcoding experiments. CV performed the barcoding of algal specimens. BP, CV, GC and JFB analyzed all the data and participated to

the writing of the original draft. All authors participated to the review and editing of the final manuscript.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

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Ethical consent

This article does not contain any studies with human participants or animals performed by any of the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

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Data accessibility

Sequences data were deposited and are publicly available in the NCBI Sequences Read Archive (SRA) under the BioProject ID PRJNA686721, accession number. Raw data for LC-ESI-(+)-MS/MS experiments were deposited and are publicly available in the MassIVE platform under the ID MSV000084472.

Author contributions

Figures

Figure 1. Map with the location of the 19 sampling sites.

From right to left: VILF: Villefranche-sur-Mer; ANTB: Cap d'Antibes; THEO: Théoule-sur-Mer; DRAM: Le Dramont; STMA: Sainte-Maxime; STCL; Saint-Clair; LLND: La Londe-les-Maures; PRQN: Porquerolles island (Northern coast); PRQS: Porquerolles island (Southern coast); CARQ: Carqueiranne; TAMR: Tamaris; BRUS: Le Brusc; BAND: Bandol; CASS: Cassis; MRSL: Marseille; AGD: Agde; BANY: Banyuls-sur-Mer; CERB: Cerbère; LOCQ: Locquirec.

Figure 2. Bayesian phylogenetic tree of *Taonia* based on the concatenated alignment of *cox*3, *psb*A and *rbc*L datasets.

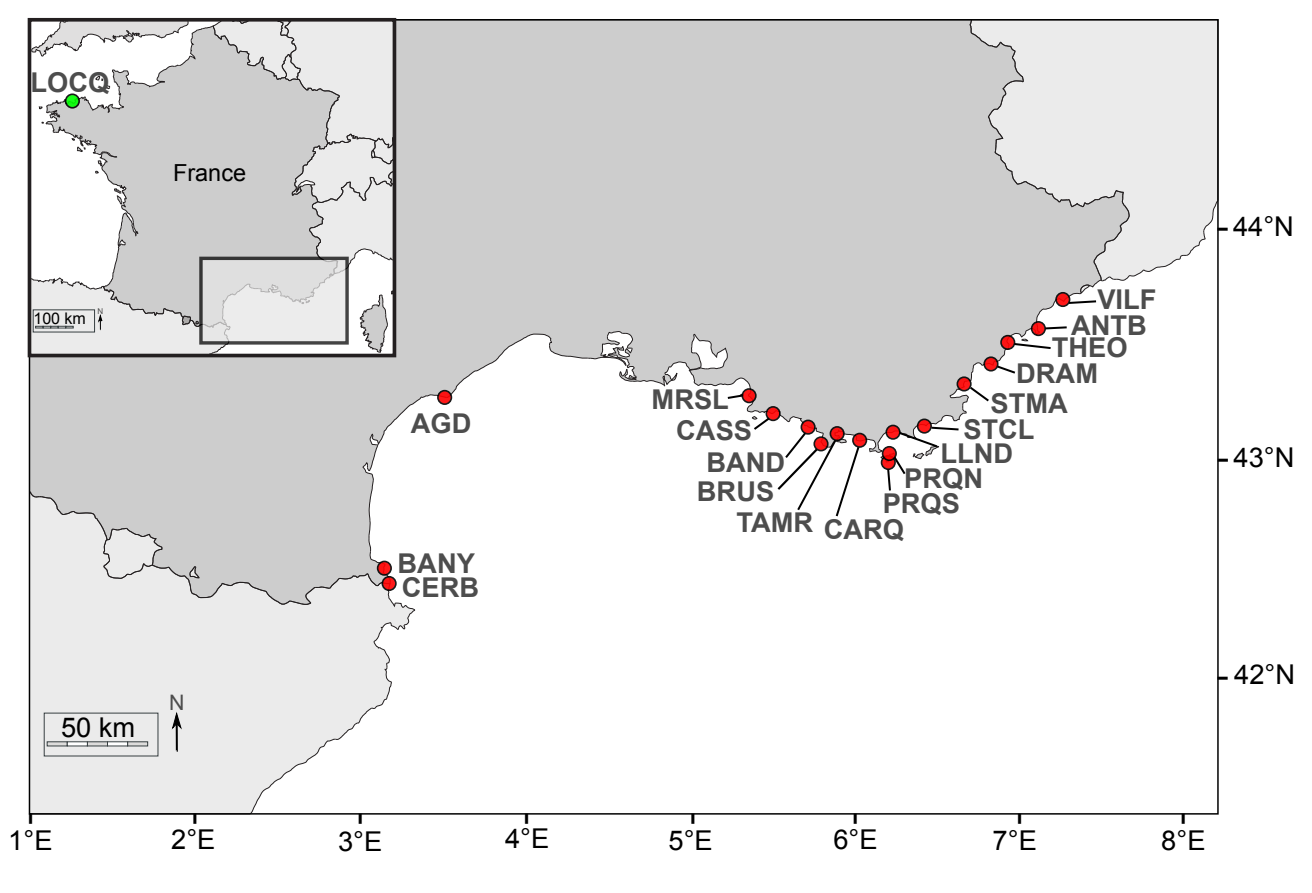
Values at the nodes represent Bayesian (left) and maximum likelihood (right) support values.

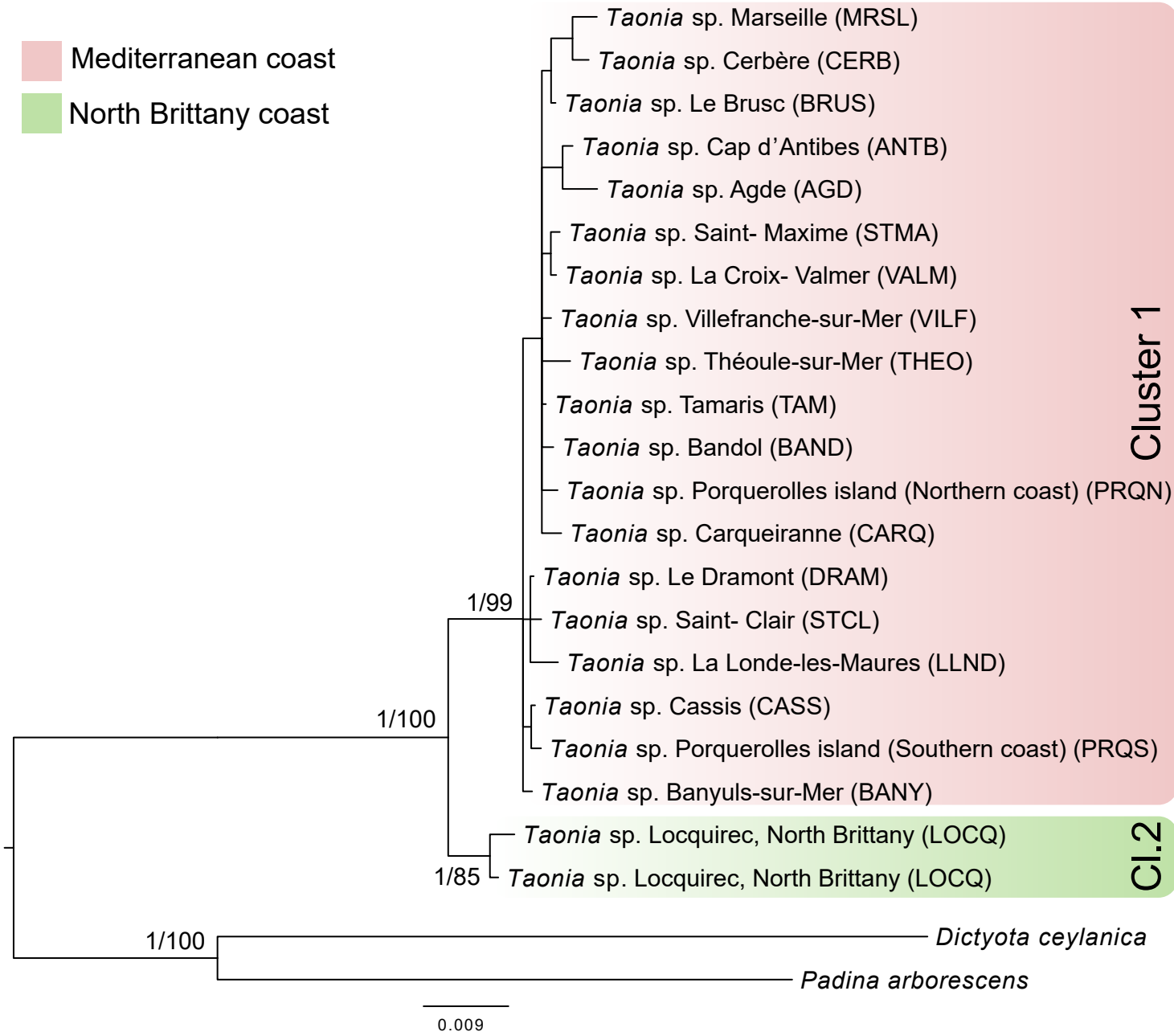
Figure 3. β-diversity and structure of prokaryotic communities at the surface of *Taonia* samples.

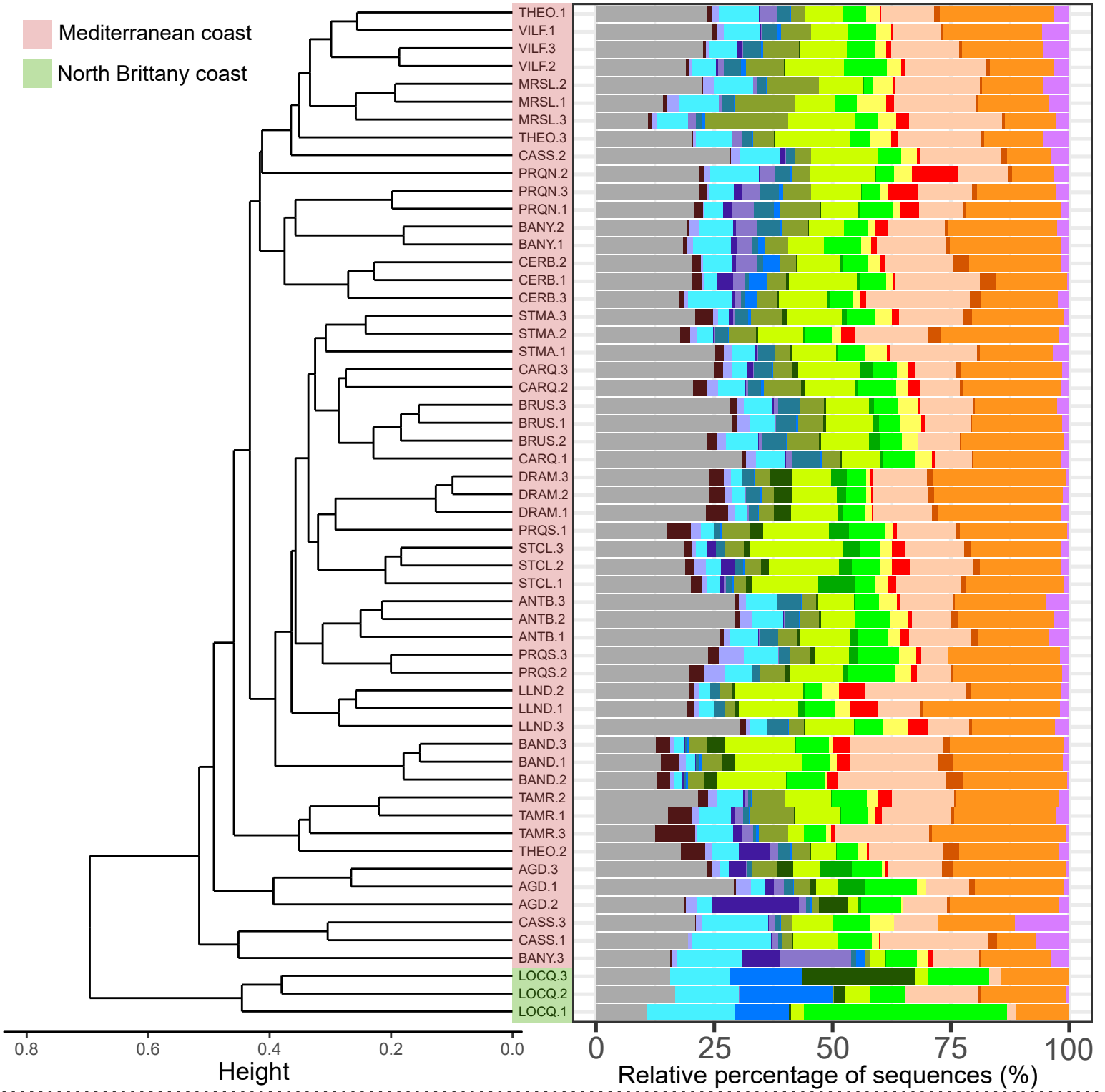
The dendrogram corresponds to a UPGMA clustering based on Bray-Curtis distance and calculated at the OTU level. Histograms represent the relative abundances of the main families. * "Other" corresponds to unaffiliated families and those below 1%.

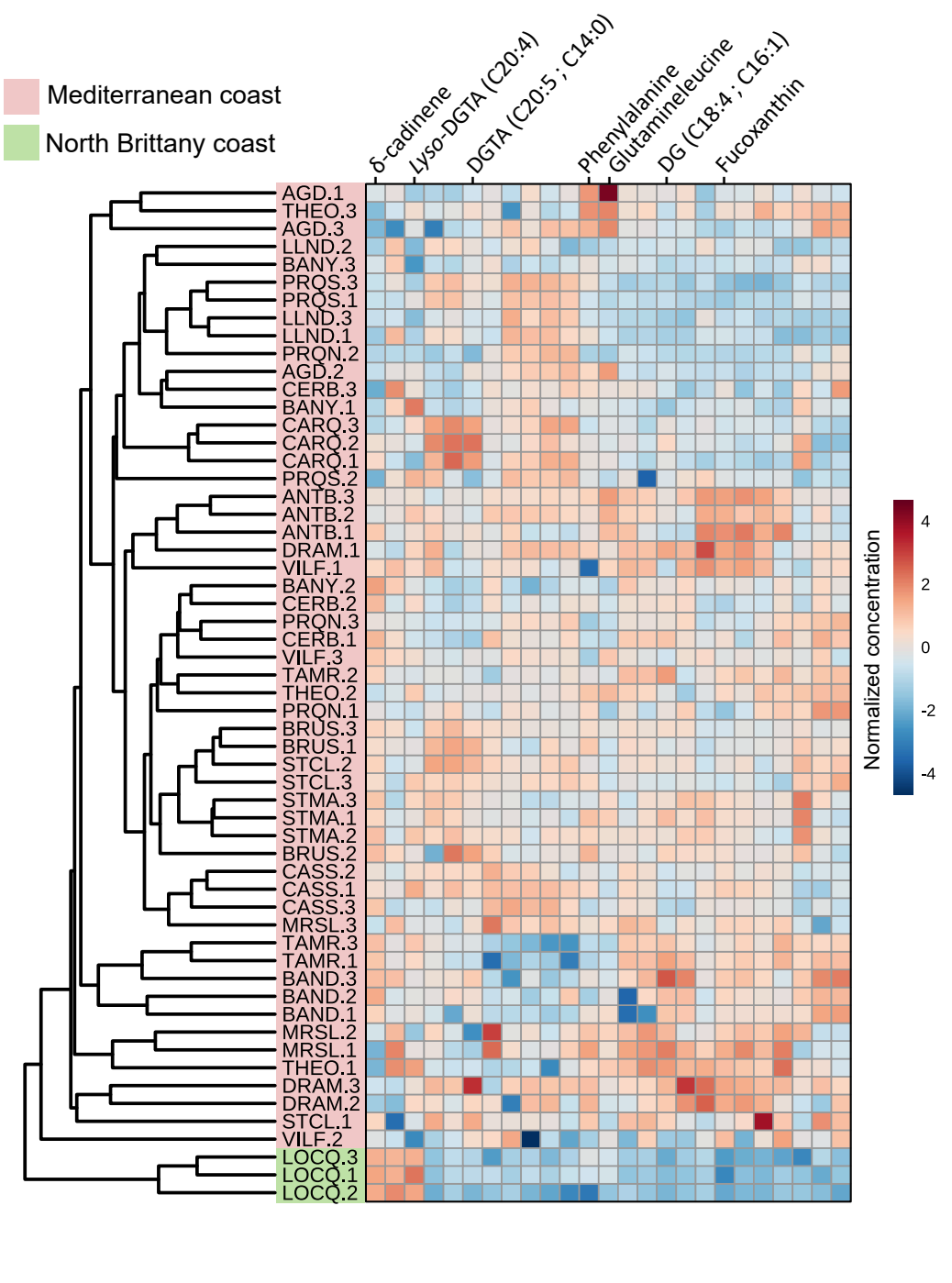
Figure 4. Metabolomics fingerprinting of surface extracts of *Taonia* samples.

The dendrogram corresponds to a Euclidean clustering based on the average method and calculated with all metabolites of the metabolomics dataset. The heatmap shows normalized concentrations of the 25 most discriminant metabolites according to both geographical areas (Mediterranean *vs* North Brittany sites). Normalized concentrations are resulting from the ion peak areas of data matrix (Dataset S1) once log-10 transformed, mean-centered and normalized with the sum of all the ion peak areas.


LOCQ
France
100 km
AGD
MRSL
CASS
BAND
BRUS
TAMR
CARQ
PRQS
PRQN
LLND
STCL
STMA
DRAM
THEO
ANTB
VILF
BANY
CERB
50 km
N
44°N
43°N
42°N
1°E
2°E
3°E
4°E
5°E
6°E
7°E
8°E


Mediterranean coast
North Brittany coast
Taonia sp. Marseille (MRSL)
Taonia sp. Cerbère (CERB)
Taonia sp. Le Brusc (BRUS)
Taonia sp. Cap d'Antibes (ANTB)
Taonia sp. Agde (AGD)
Taonia sp. Saint- Maxime (STMA)
Taonia sp. La Croix- Valmer (VALM)
Taonia sp. Villefranche-sur-Mer (VILF)
Taonia sp. Théoule-sur-Mer (THEO)
Taonia sp. Tamaris (TAM)
Taonia sp. Bandol (BAND)
Taonia sp. Porquerolles island (Northern coast) (PRQN)
Taonia sp. Carqueiranne (CARQ)
Taonia sp. Le Dramont (DRAM)
Taonia sp. Saint- Clair (STCL)
Taonia sp. La Londe-les-Maures (LLND)
Taonia sp. Cassis (CASS)
Taonia sp. Porquerolles island (Southern coast) (PRQS)
Taonia sp. Banyuls-sur-Mer (BANY)
Taonia sp. Locquirec, North Brittany (LOCQ)
Taonia sp. Locquirec, North Brittany (LOCQ)
Cluster 1
Cl.2
1/99
1/100
1/85
1/100
Dictyota ceylanica
Padina arborescens
0.009


Mediterranean coast
North Brittany coast
THEO.1
VILF.1
VILF.3
VILF.2
MRSL.2
MRSL.1
MRSL.3
THEO.3
CASS.2
PRQN.2
PRQN.3
PRQN.1
BANY.2
BANY.1
CERB.2
CERB.1
CERB.3
STMA.3
STMA.2
STMA.1
CARQ.3
CARQ.2
BRUS.3
BRUS.1
BRUS.2
CARQ.1
DRAM.3
DRAM.2
DRAM.1
PRQS.1
STCL.3
STCL.2
STCL.1
ANTB.3
ANTB.2
ANTB.1
PRQS.3
PRQS.2
LLND.2
LLND.1
LLND.3
BAND.3
BAND.1
BAND.2
TAMR.2
TAMR.1
TAMR.3
THEO.2
AGD.3
AGD.1
AGD.2
CASS.3
CASS.1
BANY.3
LOCQ.3
LOCQ.2
LOCQ.1
0.8
0.6
0.4
0.2
0.0
Height
0
25
50
75
100
Relative percentage of sequences (%)
Taxonomic affiliations
Acidimicrobiia ; Microtrichaceae
Bacteroidia ; Saprospiraceae
Bacteroidia ; Cyclobacteriaceae
Bacteroidia ; Flavobacteriaceae
Oxyphotobacteria ; Phormidesmiaceae
Planctomycetacia ; Pirellulaceae
Alphaproteobacteria ; Hyphomonadaceae
Alphaproteobacteria ; Hyphomicrobiaceae
Alphaproteobacteria ; Rhodobacteraceae
Alphaproteobacteria ; Kiloniellaceae
Alphaproteobacteria ; Sphingomonadaceae
Gammaproteobacteria ; Alteromonadaceae
Gammaproteobacteria ; Spongiibacteraceae
Gammaproteobacteria ; Ectothiorhodospiraceae
Gammaproteobacteria ; Pseudomonadaceae
Gammaproteobacteria ; Thiohalorhabdaceae
Gammaproteobacteria ; Thiotrichaceae
Verrucomicrobiae ; Rubritaleaceae
Other *


Mediterranean coast
North Brittany coast
δ-cadinene
Lyso-DGTA (C20:4)
DGTA (C20:5 ; C14:0)
Phenylalanine
Glutamineleucine
DG (C18:4 ; C16:1)
Fucoxanthin
AGD.1
THEO.3
AGD.3
LLND.2
BANY.3
PROS.3
PROS.1
LLND.3
LLND.1
PRON.2
AGD.2
CERB.3
BANY.1
CARQ.3
CARQ.2
CARQ.1
PROS.2
ANTB.3
ANTB.2
ANTB.1
DRAM.1
VILF.1
BANY.2
CERB.2
PRON.3
CERB.1
VILF.3
TAMR.2
THEO.2
PRON.1
BRUS.3
BRUS.1
STCL.2
STCL.3
STMA.3
STMA.1
STMA.2
BRUS.2
CASS.2
CASS.1
CASS.3
MRSL.3
TAMR.3
TAMR.1
BAND.3
BAND.2
BAND.1
MRSL.2
MRSL.1
THEO.1
DRAM.3
DRAM.2
STCL.1
VILF.2
LOCQ.3
LOCQ.1
LOCQ.2
Normalized concentration
4
2
0
-2
-4