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Short communication

Discrimination of allied species within the genus *Turbinaria* (Fucales, Phaeophyceae) using HRMAS NMR spectroscopy

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Abstract

A novel chemotaxonomical method based on 1D ^{1}H HRMAS NMR spectroscopy is being tested for taxonomical purposes. This powerful technique allowed us to discriminate between specimens belonging to two sister species of *Turbinaria*, which are difficult to tell apart using only morphological characters. Based on spectra analysis, the results allowed us to successfully group the specimens according to their species. Thus, the efficiency of HRMAS NMR spectroscopy for the discrimination of algal species and for the pre-screening of potential chemomarkers is demonstrated.

Keywords: HRMAS NMR spectroscopy; *Turbinaria* species; Phaeophyceae; Chemotaxonomy; Species discrimination

1. Introduction

Turbinaria is a widespread tropical genus within the Phaeophyceae. It is relatively species-poor; with so far only 17 species having been described and assigned to the genus [1]. However, the web site AlgaeBase lists 54 species names, of which 22 are actually flagged as current [2]. In the Indo-Pacific region, *Turbinaria ornata*, *Turbinaria conoides* and *Turbinaria decurrens* are currently recorded. While *T. ornata* is the most common and widespread species extending its distribution from the African east coast to the central Pacific Ocean including Hawaii and French Polynesia, *T. conoides* is not recorded from the French Polynesian area or Hawaiian waters, and *T. decurrens* is restricted to the western part of the tropical Pacific Ocean. Taxonomically speaking, *T. decurrens* is the most easily distinguishable from morphological features, while both *T. conoides* and *T. ornata* display great morphological variations which can generate identification ambiguities, especially when looking at the shapes of the leaf blades [3].

Despite the limited number of *Turbinaria* species, this genus is morphologically complex. Several authors recognized a wide variety of forms [1] (for review). This plasticity is correlated with habitat in response to environmental conditions [1,4,5]. Various studies on *T. ornata* have shown the effect of hydrodynamism on the thallus morphology, the buoyancy and the phenological plasticity [6–9]. Despite this high phenological variability, the morphological species concept remains the basis for algal taxonomy [10], including the genus *Turbinaria* [1] for which the shape of the leaf is one of the major taxonomical criteria to tell the species apart [1,3,11,12]. Because of the complex morphological variations, this criterion may be a source of confusion in identifications, and morphological classifications need to be improved by molecular studies [12]. However these tools depend on the nature of the biological material, i.e. buds or reproductive organs for DNA extraction, and the availability of good genetic markers; these studies can be long, difficult and expensive, especially since *T. ornata* and *T. conoides* are two closely related sister species [12]. In this context, high-resolution magic angle spinning (HRMAS) NMR spectroscopy, a recent

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non-invasive technique, has been considered as a promising analytical method to discriminate between the species concerned. It was shown, for instance, to be a reliable tool to differentiate strains of bacteria [13,14], to analyse food materials [15–20] or to diagnose the spread of prostate cancer [21]. This method is a fast, non-destructive technique, which does not require any preliminary extraction and requires only a little sample preparation. Moreover, HRMAS allows obtaining high-resolution spectra from intact samples, comparable to those achievable with liquid extracts [22]. At this time, with HRMAS, it is possible to analyse minute amounts of intact plant material using 1D and 2D spectra of *in vivo* low molecular mass compounds, as well as spectra of structural polymers [23]. The HRMAS technique is also well suited to the monitoring of the accumulation or disappearance of various classes of metabolites. Some studies indicated that this method could discriminate food material samples according to their geographical origin [17–20].

In this study, the usefulness of HRMAS as an analytical tool to discriminate closely related macroalgal species of the genus *Turbinaria* is demonstrated for the first time.

2. Materials and methods

2.1. Sampling sites and species

Specimens of *T. conoides*, *T. decurrens* and *T. ornata* were collected during the austral winter, from two different regions of the South Pacific, namely the Solomon Island (SI) and New Caledonia (NC), as shown in Table 1. Some samples were not named because morphological ambiguities could not allow us to differentiate *T. ornata* from *T. conoides* and they were then labelled as *Turbinaria* sp. (Table 1).

Field–collected samples were separated into two batches: one was freeze-dried and sent to the first author for chemotaxonomical analysis (this study) and the second was used for a molecular and taxonomical studies [12]. *T. decurrens* was considered as the outgroup of our analysis, since this species is easily distinguishable from the two closely related species, *T. conoides* and *T. ornata*.

2.2. NMR spectroscopy

The method developed in this study is based on the use of HRMAS NMR spectroscopy.

All HRMAS spectra were acquired on a Bruker DRX 500 spectrometer equipped with an indirect HRMAS $^{1}H/^{31}P$ probehead with gradient *Z* at 25 °C. A typical proton ^{1}H HRMAS spectrum consisted of 64 scans, and was performed with presaturation of the water peak. Each spectrum was phased and baseline-corrected using a polynomial function.

2.3. HRMAS sample preparation

For the HRMAS spectra, peduncles of leaves were randomly selected on the sampled thalli belonging to each region (SI and NC). About 5 mg of each peduncle was put in a vial with zirconium oxide, and set into a 4 mm MAS rotor. Approximately 30 μL of D_2O was added into the rotor with the leaf sample for ^{2}H field locking (depending on the quantity of the freeze-dried leaf sample). The sample was placed in a rotor spinning around an axis, which is oriented at the so-called magic angle of 54°7 with respect to the magnetic field $\mathbf{B}_0$. Good homogenization was obtained at a spinning rate of 5000 Hz. This results in a high-resolution NMR spectrum approaching that obtained using liquid samples, making spectra analysis possible.

In this study, three HRMAS spectra, corresponding to three independently prepared rotors, were recorded for each sample. Thus 6 spectra for *T. decurrens*, 9 for *T. ornata*, 15 for *T. conoides* and 9 for *Turbinaria* sp. were obtained.

Peduncles of leaves were chosen because: (1) leaves are useful for morphological taxonomy [12] and (2) the shape of peduncles allows a very good fit within the HRMAS rotor. In this way, spectra resolutions were improved.

Table 1
List of species, sampling data and herbarium accession numbers of *Turbinaria* species used in this present study

Species	Locality	Accession number	Date	Collector
Turbinaria decurrens	Solomon, Malaïta island, Anuta Paina	IRD—S.852-740	July 18, 2004	Payri
T. decurrens	Solomon, Malaïta island, Lau Lagoon	IRD—S.848-685	July 16, 2004	Payri
Turbinaria conoides	Solomon, Nggela Island, NW	IRD—S.842-566	July 12, 2004	Payri
T. conoides	Solomon, Malaïta island, Lau Lagoon	IRD—S.850-702	July 17, 2004	Payri
T. conoides	New Caledonia, Séche Croissant	KLL—Tc.1	April 5, 2006	Payri
T. conoides	New Caledonia, Séche Croissant	KLL—Tc.2	April 5, 2006	Payri
T. conoides	New Caledonia, Séche Croissant	KLL—Tc.3	April 5, 2006	Payri
Turbinaria ornata	Solomon, Malaïta island, Anuta Paina	IRD—S.852-741	July 18, 2004	Payri
T. ornata	New Caledonia, Séche Croissant	KLL—To.1	April 5, 2006	Payri
T. ornata	New Caledonia, Séche Croissant	KLL—To.2	April 5, 2006	Payri
T. ornata	New Caledonia, Séche Croissant	KLL—To.3	April 5, 2006	Payri
Turbinaria sp.	New Caledonia, Nouville	KLL—T.sp.1	April 4, 2006	Payri
Turbinaria sp.	New Caledonia, Nouville	KLL—T.sp.2	April 4, 2006	Payri
Turbinaria sp.	New Caledonia, Nouville	KLL—T.sp.3	April 4, 2006	Payri
Turbinaria sp.	New Caledonia, Nouville	KLL—T.sp.4	April 4, 2006	Payri
Turbinaria sp.	New Caledonia, Nouville	KLL—T.sp.5	April 4, 2006	Payri
Turbinaria sp.	New Caledonia, Nouville	KLL—T.sp.6	April 4, 2006	Payri