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## Dinoflagellate diversity among nudibranchs and sponges from French Polynesia: Insights into associations and transfer

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La diversité des dinoflagellés chez les nudibranches et les éponges de Polynésie française : associations et transfert

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#### ABSTRACT

Symbioses with the dinoflagellate *Symbiodinium* are widespread among marine invertebrates and protists, especially in nutritionally demanding habitats, such as tropical coral reefs, where they play a major role in ecosystem survival. Moreover, apart from corals and sea anemones, many of the *Symbiodinium* species and clades involved in these partnerships remain to be characterized. This study provides new insights into nudibranch and sponge associations with *Symbiodinium* by sequencing regions of the *Symbiodinium* 28S rDNA and the host mitochondrial *COI* oxidase. Specimens were sampled between 2011 and 2013 from locations around the islands of Moorea and Tahiti, French Polynesia. Our results revealed that some of the sponges and nudibranchs harbored typical *Symbiodinium* from clade B or C while others harbored new, undescribed *Symbiodinium* like dinoflagellates. A detailed analysis of the different life stages of the nudibranch *Phestilla lugubris* and of its specific coral prey, *Porites rus*, suggests a prey-predator horizontal transfer of the symbiont and its vertical inheritance from the parent to the eggs. © 2015 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.

#### RÉSUMÉ

Les symbioses à zooxanthelles, dinoflagellés du genre *Symbiodinium*, sont largement répandues chez les invertébrés marins et les protozoaires, notamment dans les eaux oligotrophes, où ils jouent un rôle important dans la survie des récifs coralliens. De plus, excepté chez les coraux et les anémones, les différentes espèces et clades de *Symbiodinium* impliqués dans ces associations restent à être caractérisés. Notre étude, grâce à l'utilisation des marqueurs génétiques 28S ADNr des *Symbiodinium* et la sous-unité 1 de la cytochrome oxydase mitochondriale (*COI*) de l'hôte, procure de nouvelles données quant aux associations des éponges et des nudibranches avec les *Symbiodinium*. L'échantillonnage a

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eu lieu autour de Moorea et de Tahiti (Polynésie française), entre 2011 et 2013. Nos résultats révèlent que certaines espèces d'éponges et de nudibranches possèdent les clades B et C communément retrouvés, chez les coraux notamment, alors que d'autres présentent de nouveaux dinoflagellés « *Symbiodinium*-like ». Une analyses détaillée des *Symbiodinium* à différents stades du cycle de vie du nudibranche corallivore *Phestilla lugubris* et de son corail hôte *Porites rus* suggèrent l'existence d'un transfert horizontal de *Symbiodinium* proie–prédateur et un transfert vertical du parent *P. lugubris* à ses œufs.

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#### 1. Introduction

The high number of marine invertebrate hosts that have evolved towards establishing symbiotic relationships with photosynthetic dinoflagellates of the genus Symbiodinium [1] suggests the highly valuable competitive metabolic advantage provided by such associations [2]. This is especially true in the shallow and nutrient-poor tropical waters. Indeed, the symbiotic dinoflagellates can provide up to 90% of the host's energetic requirements, in the form of photosynthetic products [3]. Symbiodinium were initially considered as belonging to one single species, Symbiodinium microadriaticum [4]. To date up to nine different clades (from A to I) of Symbiodinium have been identified, with each clade being itself composed of numerous sub-clades [5,6]. Clades A, C and D have been reported as present in hard corals from Moorea [7]. Despite our developed knowledge on coral-Symbiodinium associations, very little is known about associations involving other invertebrate groups, such as sponges and nudibranchs.

Marine sponges (Porifera) represent the second largest biomass on tropical reefs after corals [8]. They play an important role in removing detritus and organic nutrients from water [9]. Although marine sponges are known to contain a range of microbial symbionts, e.g., ammoniumoxidizing archaea, sulfate-reducing bacteria, and cyanobacteria with a photosynthetic function [10,11 and references within], only a few of them have been reported to harbor Symbiodinium. The sponge Haliclona sp. is characterized by the presence of dinoflagellates [9], but whether these are from the genus Symbiodinium or not still needs to be addressed from a molecular point of view. To our knowledge, Symbiodinium clades have only been characterized in excavating sponges from the genus Cliona [12]. Those sponges acquire their symbionts when digesting the coral substratum they live in. Other than these two types of sponges, barely anything is known about sponge-Symbiodinium associations.

*Nudibranchs* (Mollusca) are among the most ecologically and morphologically diverse of all gastropod taxa [13]. Some of them have been identified as having *Symbiodinium* in their tissues. These are mostly carnivorous nudibranchs feeding on sponges, anemones [14], hydroids, or corals [15], which themselves contain *Symbiodinium*. These nudibranchs are referred to as "solar powered" and are assumed to obtain their *Symbiodinium* directly from their prey [16]. The dinoflagellates they obtain are stored alive within the epithelial cells of the finely branched digestive system, which leads into the skin layers of their intermittently flattened cerata. This system enables the host to adjust the symbionts' exposure to sunlight [15]. This mutualistic relationship with symbiotic algae, and its subsequent host adaptation, have been studied only on the genera *Phyllodesmium* [17] and *Pteraeolidia* [18].

The focus of this study was to identify sponge- and nudibranch-*Symbiodinium* associations, to assess their specificity, and to find out more about their acquisition by transfer as nudibranchs and sponges are crucial in the sense that they could represent important *Symbiodinium* reservoirs and vectors.

#### 2. Materials and methods

#### 2.1. Specimen collection and storage

Nudibranch and sponge samples were collected using a combination of SCUBA from vessels, shore-based SCUBA, and snorkel from locations around the islands of Moorea (17.5333°S, 149.8333°W) and Tahiti (17.6667°S, 149.4167°W), French Polynesia, between 2011 and 2013 (Table 1). In addition, a fragment of a *Porites rus* coral colony – harboring eggs and adults of the nudibranch *Phestilla lugubris* – was collected from the field and kept for one month in an aquarium (open water flow). Eggs, juveniles and adult nudibranchs were separated from their coral substratum. All specimens were preserved in 85% ETOH at 4 °C.

#### 2.2. DNA extraction, Amplification and sequencing

Whole genomic DNA was extracted from the ethanolpreserved tissue. A CTAB protocol, modified after Mieog et al. [19], was used. Specific genomic regions typically used as a reference for barcoding were amplified by PCR.

To confirm the taxonomic identification of the collected species, "universal" primers [20] were used to amplify by PCR a 658 bp DNA region of the cytochrome *c* oxidase subunit 1 gene (*COI*) from either the nudibranch or sponge mitochondrial genome. To detect symbionts, a close to 580 bp region of the *Symbiodinium* large ribosomal subunit RNA (28S rDNA) was amplified with Richter's universal primers [21]. Amplifications by PCR were performed following the manufacturer's protocol (Promega Corporation, Madison, WI). Amplicons were purified and sent for sequencing of both strands to Macrogen Download English Version:

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