Lobophora: biotic interactions and diversification

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PhD in Marine Biology

Supervised by Claude PAYRI and Olivier DE CLERCK

Presented and publicly defended on the 3rd of July 2015

Before a Jury composed of:

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Interactions biologiques et diversification du genre *Lobophora*

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Dirigée par Claude PAYRI et Olivier DE CLERCK

Présentée et soutenue publiquement le 3 Juillet 2015

Devant un jury composé de :

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This thesis is dedicated to my beloved wife, Sumi and to my loving family

Acknowledgements

To my scientific parents

I will begin these acknowledgements by thanking my supervisors Claude Payri and Olivier De Clerck without whom this Doctoral journey would never have happened. I feel greatly indebted to both of them not only for giving me this tremendous opportunity to realize this PhD, but also and especially for the wonderful scientific mentors they have been to me. As I often heard stories of PhD students that have been abandoned to themselves, I feel I have been really fortunate. I truly consider them as my scientific parents!

To the technical and administrative staff, to my scientific peers & collaborators

A number of people have made valuable contributions. Also I greatly acknowledge the technical staff from IRD and UGent for their precious help: the IRD divers: Christophe Peignon, Gerard Mou-Tham, John Butscher; the IRD boat crew: Miguel Clarque, Philippe Naudin and Samuel Tereua; but also the Jack of all trades Joseph Baly; and a special thanks to Sofie D'hondt and Tine Verstraete from UGent who thought me the bases of molecular techniques. I would also like to thank IRD and UGent researchers for the fruitful discussions we had and for their valuable advices: Mehdi Adjeroud, Serge Andrefouet, Linda Dombrowsky, Cécile Fauvelot, Véronique Guillemard, Fanny Houlbreque, Frederik Leliart, Ricardo Rodolfo-Metalpa, Heroen Verbruggen and Laurent Vigliola. I would also like to thank the administrative staff of IRD and in particular Catherine Hartmann, Juliette Lelong, Emanuelle Raffy, Marie-Noëlle Segura, Christian Turmolle and Catherine Verleye; and of UGent, Anja Hondekyn, for their kindness and their assistance with all the maddening administrative work. I deeply acknowledge the numerous people with whom I have collaborated: Robert J. Anderson, John J. Bolton, Olga Camacho, Gérald Culioli, Suzanne Fredericq, Lydiane Mattio, Thibaud Moleana, Marta Sansón, Michael J. Wynne, Sun Zhongmin and Maya Zubia. I am grateful to Aqualagon's owner, Frank Le Garrec, for letting me perform the grazing experiments in their fish farm, and to its crew, Renaud Condoya and Marie-Claude Qalue, for assisting us in the deployment of the experiments at the fish farm. I am very grateful to Olivier Thomas not only for letting me perform a major part of the chemical work

at his instituion but especially for our invaluable collaboration. I am also thankful to Marie-Aude Tribalat for her kindness and great help with the chemical work.

To my friends & colleagues

I thank my friends & fellow PhD & post-doc comrades and from IRD Nouméa, Elvan Ampou, Laure Barrabé, Laurent Bordez, Christophe Cléguer, Paul Coulerie, Marion Cuif, Julien Debreuil, Laury Dijoux, Simon Gensous, Daphné Grulois, Adrien Jacotot, Mohsen Kayal, Audrey Léopold, Sofia Motuhi, Christelle Paillon, Héloïse Rouze, Josina tiavouane, Sébastien Trape, Simon Van Wynsberge and from UGent, Kenny Bogaert, Samuel Bosch, Andrea Del Cortona, Agnieszka Lipinska, Van Tu Nguyen, Frédérique Steen, Lennert Tyberghein, for the long and inspiring talks.

To name a few I would like also to acknowledge Xavier Girard, J. Michael Hicks, Fabian Carriconde, Swen & Géraldine Bidau, Laurent & Mylène Millet, for your friendship and for the great moments we had together during these past four years. A very special thank goes to the friends from New Caledonia whom have been like family to my wife & I: Brice & Coralie, Joel & Yorita Lauvray, Laurent & Nadia Guy, Christophe & Christine Peignon and Yves & Evelyne.

To my family

My before final acknowledgments go to my family, in particular my parents, brothers and sister Edgar, Kévin and Bélinda, and my in laws Patricia, Hyo Shim and David, for their unwavering love, supports and encouragements throughout these years.

To my wife

Last, but certainly not least, I would like to extend my heartfelt gratitude to the most precious person in my life, my wife, Sumi. Thanks to her love, patience, support and unwavering belief in me, I have been able to complete this long doctoral journey.

Thank you all!

Christophe VIEIRA was granted by UPMC (ED 129) as part of the MARES programme. MARES is a joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016). Check www.mares-eu.org for extra information. During his PhD, CV has shared his time between IRD Nouméa and Ghent University with part of his time at the Institut de Chime de Nice. CV has also contributed to the NetBiome Program SEAPROLIF, notably in the settings of the *in situ* bioassays in Noumea, New Caledonia.

Contents

Thesis Outline

This thesis aims at investigating the role of biotic interactions in the diversification of coral reefs algae. Our goal is to explore how biotic interaction can lead to diversification in marine benthic algae and how this results in ecological preferences.
The species-rich brown algal genus *Dictyota* (Dictyotales, Phaeophyceae) was originally considered as a model to conduct this study. However, preliminary field observations led us to another alga, *Lobophora*, which was chiefly observed associated with corals. In an interesting turn of events, this PhD thesis took a taxonomical detour to reassess the species diversity of this genus, which then, in 2011, contained only a handful of accepted species. This taxonomical work, which consumed half of the time of this PhD, was essential to investigate the role of biotic interactions in reef algal speciation. The benefit of this detour justified its cost as it led us to unveil an unexpected species richness. Most importantly, these taxonomical results allowed putting names on entities that are clearly ecologically distinct, and thereto to proceed in studying biotic interactions to answer the primary objectives of this PhD. More precisely, these studies focused on the interactions between *Lobophora*, scleractinian corals and herbivores. They are multifaceted and integrate ecology, taxonomy, phylogeny, present and historical biogeography, microbial ecology and chemical ecology.
Chapter 1 discusses the nature of macroalgal – coral interactions and reviews the literature dealing with *Lobophora* – corals interactions.
Chapter 2 explores biotic interactions in coral reefs. The first part of this chapter is dedicated to documentating macroalgal-coral interactions in the southwest lagoon of New Caledonia. The prime objective of this descriptive study was to define what is the natural situation of macroalgal-coral interactions in healthy coral reefs. Furthermore, this first chapter allowed identifying the genus *Lobophora* as a model organism for the rest of the study. A box is dedicated to macroalgal-coral interaction in seagrass beds. The second part of this chapter documents a unique case of a negative interaction between a *Lobophora* species and a scleractinian coral. The third part of this chapter reviews all the work done on *Lobophora* susceptibility to herbivory and investigates the susceptibility to herbivory of several *Lobophora* species genetically more or less distant. A second box is dedicated to exploring the role of microbial mediation in *Lobophora*-coral interaction.

Chapter 3 is dedicated to re-examining the diversity of the genus *Lobophora* using a DNA-based taxonomic approach. The first part focuses on the diversity in New Caledonia. The second part of this chapter is dedicated to the re-examination of old type specimens with the objective to link them to the clades unveiled in the molecular analyses. The third part addresses the global *Lobophora* diversity. This part also explores patterns of diversity at multiple spatial scales as well as the historical biogeography of the genus.

In **chapter 4**, we begin by reviewing all the natural compounds of *Lobophora* and their associated bioactivities. The second part aims at testing if *Lobophora* species naturally associated or not with corals present negative allelopathy against the latter.

Chapter 5 summarizes the main findings of this research work, and discusses the role of ecological speciation in *Lobophora* diversification in coral reefs.

Notes to reader:

Chapters 1 – 4, composed of subparts, are presented as manuscripts with CV as first author. Specific contributions are mentioned at the end of each chapter.

All chapters are either accepted, submitted, or are in preparation. Therefore some overlap in the content of the chapters does occur.

Chapter 1: General introduction and thesis outline

Part 1. A fresh look at macroalgal-coral interactions: are macroalgae a threat to corals? [1]

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Abstract

Corals and reef algae fulfill important ecological functions in tropical reef ecosystems. In an environment where space is a limiting factor, competition between both players is critical in defining the structure of coral reef communities. Dramatic shifts from coral- to macroalgal-dominated reefs have put the spotlights on competitive interactions between macroalgae and corals. But have those studies not overrated the former has on the latter? Defining the nature of the interaction between corals and reef algae, however, has been challenging. Although it is commonly accepted that macroalgae may outcompete corals under conditions of reduced herbivory or enhanced nutrient levels, there is also evidence that algae may have a negligible or even a positive effect on corals in healthy reefs. Interactions between macroalgae and corals date back to the Paleocene, when 'modern' coralgal reefs became established. Macroalgae and corals share a long evolutionary history. A combination of abiotic and biotic interactions shaped coral reef ecosystems as we presently know them, reaching stable ecological dynamics. However, natural and anthropogenic disturbances may rupture this equilibrium resulting in unbalanced population dynamics. Intensified competition between macroalgae and corals is therefore symptomatic of damaged reefs, and usually results from decrease in herbivory as well as coral morbidity and mortality.

1. Introduction

The idea that every single living organism interacts in one way or another with other organisms, led Elton (1968) to the famous 'boutade' that 'no organism is an island'. The interactions of organisms with one another and with the environment resulted in complex adaptive ecosystem over evolutionary time-scales (Levin, 2005). These complex adaptive systems operate as a whole, with their specific structure and functioning, and with each organism having their own functional position in the community, referred to as its ecological niche (Whittaker *et al.*, 1973; but see the Neutral Theory Hubbell, 2001). Ecosystems have usually an equilibrium state in which the communities have achieved relative stability in structure, function, biomass, energy flow, species-diversity and species-interactions (Hawley, 1950). While ecosystem ecologists have been mostly interested in energy flow and biogeochemical cycling, community ecologists have been concerned with the interactions between individuals. The fundamental challenge for community ecologists in defining the nature of the interactions between species has been to assess whether the net effects

are deleterious or beneficial for the partakers (Wootton & Emmerson, 2005). In addition to the difficulty measuring and defining interactions between species, interactions may depend on the scale, the evolutionary context and environmental conditions in which they occur (Smith *et al.*, 1995).

Macroalgae in coral reef ecosystems perfectly illustrate the difficulty in defining the exact nature of their interactions with other benthic organisms such as corals. Dramatic shifts from coral- to macroalgal-dominated habitats (e.g. Hughes, 1994) have put the spotlights on the competitive nature of the interaction between macroalgae and corals and may have overrated macroalgal threat (Bruno *et al.*, 2009; Vroom, 2010; Vroom *et al.*, 2010). However, the significance researchers have accorded to macroalgal-coral competition represents an inherent bias based on research interest, and may not be proportional to the ecological importance/relevance in healthy coral reefs. In fact, macroalgae play a variety of significant roles in healthy reef ecosystems and the nature of their interaction with corals is not strictly competitive but can also be mutualistic (Morse, 1992).

2. Reef algae: a vital reef component

The term 'reef algae' is presently defined as benthic algae naturally growing in coral reef ecosystems *sensu lato* encompassing scleractinian reefs themselves but also adjacent ecosystems, e.g. seagrass beds, macroalgal beds, etc. Benthic algae have been categorized into three major functional groups, namely crustose coralline algae (CCA), macroalgae and turf algae. Reef algae act as key coral reefs engineers significantly contributing to the structure of coral reefs (Fong & Paul, 2011) in four main ways as (1) primary producers, (2) reef builders, (3) sediment producers, and (4) autogenic engineers. Jointly with symbiotic *Symbiodinium* (zooxanthellae), reef algae are the major contributors to coral reefs primary production, and stand as basal actors in the food-web sustaining a wide diversity of herbivores (mostly algal turf, which supports high grazing pressure). Like corals, calcareous reef algae, and particularly CCA, make an important contribution to calcification and play an major role in cementing reef frameworks (Adey, 1998). Calcareous algae (e.g. CCA, *Halimeda*) also largely contribute to sediment production due to bioerosion and other biological, physical, and chemical erosion processes (Neumann & Land, 1975; Drew, 1983). Finally and although much less documented compared to temperate regions, reef algae also act as habitats for organisms, that benefit from the refuges from predators and harsh physical conditions. Some CCA also may act as settlement platforms for benthic organism such as corals (Morse, 1992), and certain reef algae

may stimulate early life history processes (e.g. swimming, recruitment, settlement) of some benthic reef organisms (Birrell *et al.*, 2008a). While the benefits of macroalgae to coral reef ecosystems as a whole have been relatively well documented, catastrophic regime shift to macroalgae notably in the Caribbean reefs (Hughes, 1994) but also in the Pacific (Diaz-Pulido *et al.*, 2009) have conveyed a negative image of reef macroalgae and pictured them as the 'sworn enemies' of corals. Some authors went as far as dichotomizing reef algae, based on the functional groups, into 'good' versus 'bad' for coral reefs (Barott & Rohwer, 2012).

3. Macroalgae and corals: convicted to each other since ...

Associations between macroalgae and corals are natural in healthy reefs (Haas *et al.*, 2010; Vroom, 2010; Vroom & Braun, 2010; Vroom *et al.*, 2010; Barott *et al.*, 2012). The relative abundance of macroalgae and hard corals is generally related to coral reefs status and geography. Large macroalgal cover is generally commonly observed in subtropical regions located in high latitudes such as Northern Hawaii archipelago (Vroom, 2010; Vroom & Braun, 2010; Vroom *et al.*, 2010), Southern Polynesia archipelago, Pines Isle in New Caledonia (personal observations).

The term 'coralgal' used by geologists will subsequently be used not to confuse with the coral-algal symbiosis. It was postulated that the Paleozoic Era was the "Age of Algae" in reefs, with large stands of algae dominating shallow benthic communities (Steneck, 1983), although absence of fossil records does not allow confirmation of this hypothesis. Interactions between coralline algae and scleractinian corals probably date back from the Jurassic when both taxa started radiating (Steneck, 1983; Veron, 2009). The Mesozoic Marine Revolution, involved in the origin and diversification of bioturbators, herbivores, predators and bioerodors, played an important role in regulating modern reef community structure (Vermeij, 1977), and is largely responsible for the radiation of corals and reef algae. Fossil records revealed that interactions between macroalgae (e.g. *Halimeda, Clypeina, Sporolithon, Peyssonneliaceae*) and corals clearly occurred from at least the Paleocene (Danian to Late Thanetian), when 'modern' coralgal reefs became first established (Moussavian & Vecsei, 1995; Scheibner & Speijer, 2008). Coralgal communities became prominent somewhere between the Paleocene (Scheibner & Speijer, 2008) and the mid-Cenozoic (Wood, 1998). During this long and chaotic period starting from the Paleocene, reef macroalgae and corals have evolved and adapted to their local environment to occupy the specific ecological niches that we know of today. Although fundamentally macroalgae are competing for space and light with other benthic organisms, species

interactions between reef macroalgae and benthic organisms including herbivores have evolved to reach stable dynamics, also termed a stable state (Knowlton, 1992), on healthy coral reefs. Over this long evolutionary history, herbivory, benthic organisms defenses and to a lesser extent oligotrophy have kept macroalgae within certain limits (Hay, 1981b; Lewis, 1986). In particular, herbivorous fishes, echinoids and other invertebrates are maintaining a low macroalgal cover on coral dominated habitats. Dense stand of several macroalgal species are thriving in areas where herbivory is low (Hay, 1981b, a). Coralgal interactions may occur in coral-dominated, algal-dominated habitats and even in habitats where the presence of the two is limited (e.g. seagrass beds). On coral dominated habitats, low macroalgal cover is limiting the competition between macroalgae and other benthic organisms (Steneck, 1988).

4. Macroalgal-coral coexistence

Macroalgae and corals are thus coexisting in healthy coral reefs in a state of what can be termed competitive equilibrium or co-existence, which may be largely mediated by herbivores (Steneck, 1988; Jompa & McCook, 2002b; Mumby & Steneck, 2008). Nevertheless, Carassou *et al.* (2013) showed that macroalgal cover was not related to the biomass, density or diversity of macroalgae feeders, which also stresses the importance of corals defense in preventing macroalgal cover expansion. While herbivory may primarily prevent significant interaction between corals and reef algae, coralgal competition is an active process, highly variable depending on macroalgal groups, coral species, life history stages of corals (recruits vs. adults) (McCook *et al.*, 2001; Nugues *et al.*, 2004a; Nugues & Bak, 2006; Birrell *et al.*, 2008b). A community in competitive equilibrium is one that is not undergoing compositional or structural change due to competition. Under this stable ecological setting, in which competition between macroalgae and benthic organisms is being largely hampered by a third party – the herbivores –, the evolution of complex strategies/interactions including taking advantage of the other have been favored.
For instance, the constant release of a plethora of organic compounds (Morse *et al.*, 1996), such as allelopathic compounds or necrotic tissue, offered ample opportunities for other species such as corals to evolve chemosensitivity to those compounds, and to use them as inductors in important life processes such as recruitment stages including metamorphosis and settlement. This idea is perfectly illustrated by the positive role played by crustose coralline algae (Harrington *et al.*, 2004) and fleshy macroalgae such as *Lobophora* in facilitating coral establishment (Morse, 1992; Morse

et al., 1996; Birrell *et al.*, 2008a). On the other side, some reef algae have developed compounds inhibiting coral settlement (Birrell *et al.*, 2008a; Diaz-Pulido *et al.*, 2010).

Although anecdotal, macroalgae have also been reported playing protective roles towards corals, such as decreasing coral bleaching (Jompa & McCook, 1998), reducing corallivory (Bulleri *et al.*, 2013). But overall, positive interactions, such as mutualism and facilitation between corals and macroalgae, have been largely underappreciated.

5. Healthy reefs immune to serious algal threat

Herbivory is nevertheless not uniform across coral reefs, thus leaving heterogeneous patches of macroalgae to develop, leading to competition with other benthic organisms. Unquestionably, some macroalgae can have a major influence on the demography, growth, fecundity and recruitment of scleractinian corals (Sammarco, 1982; Tanner, 1995; Lirman, 2001; Mumby & Steneck, 2008), but these unfavorable effects are clearly not sufficient to allow a takeover of macroalgae on healthy coral reefs, owing to corals defense mechanisms in addition to herbivory (Nugues & Bak, 2006). In fact, most macroalgae in physical contact with corals are not overgrowing the latter (Tanner, 1995). Several studies have demonstrated that corals are not only able to prevent algal-overgrowth, attachment or survival of algal recruits (Diaz-Pulido & McCook, 2004), but are also capable of overgrowing colonizing algae (Bak *et al.*, 1977; Meesters & Bak, 1994; Diaz-Pulido *et al.*, 2009) and inhibiting algal growth (De Ruyter van Steveninck *et al.*, 1988b). And while macroalgae have been anecdotally identified as vectors of coral pathogens (Nugues *et al.*, 2004b), it was also demonstrated that some macroalgae do not aggravate corals affected with the Caribbean yellow band disease (Vu *et al.*, 2009), although it should be noted that the algae were placed next to the coral colonies infected with CYBD, but not in direct contact.

6. Native versus introduced algae effects on coral reefs

Introductions of non-indigenous species to new ecosystems represent major threats to biodiversity and ecosystems functions and macroalgal invaders are no exception to this rule (Schaffelke *et al.*, 2007; Williams & Smith, 2007). When introduced to a new ecosystem, invasive plants have a competitive advantage over native ones because they are freed from the normal ecological influences that control their

growth (Vila & Weiner, 2004). Note, however, that only a small percentage of introduced species turn out to be invasive, which largely depends on the algal families (Williams & Smith, 2007). Native species can also become invasive following ecosystems disequilibrium (e.g. hurricanes, herbivores die-off, etc.). But while bloom in coral-dominated reefs by native reef macroalgae has been little documented in healthy coral reefs (e.g. Martinez *et al.*, 2007; Vroom *et al.*, 2009), several exotic species that have been introduced in coral reefs were capable of spreading aggressively over coral reef communities causing negative effects or changes to the native biota (Fernández & Cortés, 2005; Williams & Smith, 2007; Kružić *et al.*, 2008). Herbivores that co-evolved with native species generally prefer native to introduced algae (Williams & Smith, 2007). This contrast in the ecological dynamics between native vs. alien species is strongly corroborating how native algal species have evolved and adapted to their ecosystems over evolutionary time-scales. Numerous examples can be cited here (see Smith *et al.*, 2002 for review of invasive algae in Hawaii), also we will only cite couple cases to illustrate our point: *Caulerpa cylindracea* and *C. sertularioides*. The south-western Australian green algae, *C. cylindracea* is, in its native range, a common and opportunistic species that grows from the intertidal down to only 6 m depth on reef flats and in intertidal pools (Womersley, 1984; Carruthers *et al.*, 1993). In contrast, in the Mediterranean Sea, it thrives under a large array of environmental conditions and is found on all kinds of soft and hard substrata (Klein & Verlaque, 2008). *Caulerpa sertularioides* is a remarkable illustration of an alien species which is severely impacting native algal flora and overgrowing corals in coral reefs, diminishing significantly the local biodiversity in Costa Rica (Fernández & Cortés, 2005).

7. Competition between corals and algae in damaged reefs

Regime shifts from coral- to macroalgal-dominated reefs stressed out the competitive nature of macroalgal-coral interaction. Onsets of macroalgal takeover on coral reefs have always been attributed to anthropogenic or natural disturbances. The change from coral to macroalgal dominance has been attributed to (1) coral mortality, (2) a reduction of herbivorous fish and sea urchins, and (3) an increase in nutrients (e.g. Lapointe, 1997; Jompa & McCook, 2002b, a; Nugues & Bak, 2006). Nonetheless, it is still debated if macroalgal overrun results from (1) a bottom-up process, i.e. the competitive overgrowth of corals by algae released from herbivory pressure, or (2) a top-down process, i.e. to coral mortality freeing space for algal colonization. Nevertheless, evidence tend to agree that generally, coral morbidity and mortality

are necessary conditions for regime shifts to occur (Nugues *et al.*, 2004a; Vieira *et al.*, in prep.-a). Subsequent studies targeted at elucidating the competitive mechanisms, allowing macroalgal takeover, have been conducted in experimental conditions generally admitting disturbance (e.g. herbivore exclusion, increased nutrient load), or employing experimental approaches forcing contact between algae and corals (Jompa & McCook, 2002b, a; Rasher & Hay, 2010). Those studies clearly showed that algae possess the potential to alter the structure and communities of tropical reef ecosystems. Evidently, following disturbance, macroalgae exhibit competitive interactions with corals that exacerbate the negative effect of environmental change. One should, however, bear in mind that while biotic interactions have evolved over a long evolutionary period, they can evolve rapidly under changed ecological conditions (Smith *et al.*, 1995). Macroalgal-coral interactions are a perfect illustration of this in the marine realm. For in stance, in damaged reefs, dense stand of macroalgae may (1) interfere with coral recruitment, (2) suppress coral growth and fecundity, and (3) cause localized coral mortality to certain species (Birrell *et al.*, 2008b; Mumby & Steneck, 2008). Habitat degradation, be it physical, chemical or biological, may therefore have severe consequences on species-interactions.

8. An exclusive fight club in damaged reefs

McCook *et al.* (2001) pointed out that interactions between a limited set of corals (e.g. *Montastrea* spp., e.g. *M. annularis*, *Agaricia agaricites*, *Acropora tenuifolia*, *Acropora palmata* and *Porites astreoides*) and algae (e.g. *Dictyota*, *Lobophora, Sargassum, Turbinaria, Dictyosphaeria* and *Halimeda*) may account for most of the significant interactions in terms of shifts in reef status on Caribbean reefs. Shifts from coral- to macroalgal-dominated assemblage, usually involves recurrent species: *Dictyota, Lobophora, Halimeda, Dictyosphaeria, Codium, Turbinaria, Sargassum,* crustose coralline algae. Generally, only a few algal taxa appear able to actually overgrow healthy corals by direct contact. In the literature, records of overgrowth predominantly involve *Lobophora*, *Dictyota*, *Halimeda*, *Dictyosphaeria* and crustose coralline algae. On the coral side, *Acropora* species also appear more susceptible than several other common corals to diseases (Page & Willis, 2006; Haapkylä *et al.*, 2007), and it is possible that competition with macroalgae exacerbates this susceptibility (Nugues *et al.*, 2004b; Smith *et al.*, 2006).

9. Putting macroalgal threat on the global scale in perspective

Coral reefs worldwide are undeniably facing major threats, and significant declines began several decades ago (Gardner *et al.*, 2003; Bruno & Selig, 2007; Wilkinson, 2008), with an estimated loss of the original area of coral reefs of 19% (Wilkinson, 2008). But while it is still largely accepted that damaged corals reefs are turning and being locked into a macroalgal-dominated state, it was shown that this assumption is being overly exaggerated (Bruno *et al.*, 2009) based on the widely cited, striking 1980s Jamaican anomaly (Hughes, 1994). On a global scale, Roff and Mumby (2012) showed that Caribbean reefs are far less resilient that Indo-Pacific reefs, and that heavy degradation is necessary to results in coral-macroalgal phase shift. Furthermore, as pointed out by Mumby (2009) the term "macroalgal dominated" is potentially misleading because the coral-depauperate state can be associated with various levels of macroalgal cover. Consequently, the term "coral depauperate" is preferable to "macroalgal-dominated" when describing alternate stable states of Caribbean reefs. And while phase shifts on coral reefs are often associated with shift from coral- to macroalgal-dominated communities, there exist alternative states, such as reefs dominated by corallimorpharia, soft corals, sponges and sea urchins (Norström *et al.*, 2009).

10. Conclusion

In healthy coral reefs, macroalgae and corals have no, negligible or positive effects on each other (Tanner, 1995; Jompa & McCook, 1998; McCook *et al.*, 2001). Sudden macroalgal dominance is symptomatic of an equilibrium lost often as a result of decreased grazing pressure and or coral morbidity and mortality. Even then, a limited number of macroalgal species represent a threat to corals, and the latters have shown remarkable resilience in some cases (Idjadi *et al.*, 2006; Diaz-Pulido *et al.*, 2009).

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Author contributions

CV wrote the manuscript. ODC and CP commented on the manuscript.

Part 2. Don't blame it on the algae! A review of *Lobophora* effects on corals[2]

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Abstract

The genus *Lobophora* is a highly successful, species-rich phaeophycean lineage, which is present in all ocean basins from tropical to warm-temperate waters. The species can be found in almost all habitats with hard substrata down to 140 m, adopting a variety of morphologies. *Lobophora* is a major algal component in tropical coral reefs and is a representative species in algal-coral competition. Cited in no less than 50 studies, in the Caribbean and the Pacific, it is generally considered to be a potent competitor to scleractinian corals, especially in damaged reefs. It is often one of the chief algae in reefs that have turned into an algal-dominated assemblage. However, while some studies agree that it is an aggressive alga, others concluded that it had no, a negligible or even a positive effect on corals. These contrasting results primarily indicate species-specific responses of corals, but also possibly *Lobophora* species-specific effects, which have been completely ignored until now. Unaware of the species-richness of this genus, nearly all publications refer to the Caribbean species assigned as *Lobophora variegata*. However, *Lobophora* is a species-rich genus comprising 21 described species, and close to 80 more operational taxonomic units yet to be described. Recent studies focused on species-specificity in algal-coral interaction outcomes. Consequently, ecological studies with incorrectly identified species are inconclusive or irrelevant, and taxonomic consultation is therefore essential to ensure correct interpretation of ecological patterns. The present review demonstrates that beyond species-specificity, effects on coral may vary across different coral-life stages, and environmental conditions (depth, reef types, etc.), thus highlighting the complexity of algal-coral interactions. As a result, studies focusing on algal-coral competitive interactions should not be extrapolated, but considered in a case-per-case basis.

1. A successful genus

The phaeophycean genus *Lobophora* J.Agardh (1894) belongs to the species-rich and widespread order of the Dictyotales, and to the Zonarieae tribe in the Dictyotaceae family. Its species richness has only recently begun to be recognized. The taxonomical history of *Lobophora* can be divided into two eras: the pre-molecular era (1809-2012) and the molecular era (2012-present). The two centuries composing the pre-molecular era resulted in the description of only five species (Vieira *et al.*, 2014a). During this time frame, many more species were described, but were eventually reduced to synonymies. With the description of 14 new species, from the

Pacific, in a time-span of only two years (Sun *et al.*, 2012; Vieira *et al.*, 2014a), the use of molecular tools unvealed a substantial hitherto unknown biodiversity. Today, the genus comprises 21 taxonomically accepted species (Guiry & Guiry, 2015), but phylogenies point toward at least another 80 (Vieira *et al.*, in prep.-c) in need of description, many of which may be cryptic or pseudo-cryptic species.

Lobophora has is present in all ocean basins from tropical to warm-temperate waters (Vieira *et al.*, in prep.-c). *Lobophora* is a polymorphic genus, ranging from crustose species tightly attached to the substrata to stipitate and erect species, with thalli up to 20 cm in diameter (Vieira *et al.*, 2014a). *Lobophora* is found from the surface down to 140 m (Markager & Sand-Jensen, 1992); inhabiting almost any habitat with hard substrata, from inter-tidal pools to subtidal areas, including shallow waters in sheltered coasts or exposed reef face, offshore coral reefs and rocky outcrops surrounded by sand. *Lobophora* grows on a variety of substrata encompassing mangrove prop roots, sunken logs, dead, unhealthy or live corals, the bases of branching and massive corals, epilithic, epiphytic to other algae (e.g; crustose coralline algae, other *Lobophora* species, large fleshy algae); in habitats ranging from seagrass and macroalgal beds to coral fields (Littler & Littler, 2000; Payri *et al.*, 2000; Abbott & Huisman, 2004; De Clerck *et al.*, 2005a; Coppejans *et al.*, 2009; Kraft, 2009; Sun *et al.*, 2012; Vieira *et al.*, 2014a). Numerous *Lobophora* species are associated with corals, growing under branching corals canopy or niched between coral branches (Vieira *et al.*, 2014b; Vieira *et al.*, in revision), certainly finding refuge from herbivory. Overall, *Lobophora* is a remarkable phaeophycean genus that significantly diversified and successfully colonized a wide variety of habitats and substrates. Most importantly, we will presently keep in mind that *Lobophora* is a fully-fledged member of coral reef ecosystems, significantly associated with corals.

2. *Lobophora* and regime shifts: an opportunistic player?

It is essential to begin this section by saying that *Lobophora* is a native component of the marine flora associated to coral reefs, that co-evolved with coral reef organisms probably since its origin in Upper Cretaceous (Vieira *et al.*, in prep.-c). In healthy coral reefs, numerous *Lobophora* species are found associated with corals, and occupy only a small percentage of the benthic cover (Vieira *et al.*, in revision).

Following anthropogenic or natural disturbances, some coral reefs have been reported to shift from an environment that favors coral dominance to one that favors other benthic organisms, such as macroalgae, corallimorpharians, soft corals, sponges and sea urchins (Hughes, 1994; Norström *et al.*, 2009). While anthropogenic and natural

disturbance could individually cause a shift, it is often the synergistic effect of multiple disturbances that ultimately lead to a phase shift (Folke *et al.*, 2004). Importantly, an increase in algal abundance does not necessarily imply coral overgrowth or a decrease in coral cover. Increase in algal cover may be at the expense of benthic organisms other than corals (e.g. coralline algae, clionid sponges) (De Ruyter Van Steveninck & Bak, 1986). In the Caribbean, reports of coral reefs dominated by macroalgae have been increasing since the 1980s (Hughes, 1994; Wilkinson, 2008). *Lobophora,* which is a major benthic macroalga in the Caribbean and in the Indo-Pacific, has been reported in several of these events, suggesting a susceptibility of coral reefs to *Lobophora* phase shifts from coral to macroalgal dominance (Cheal *et al.*, 2010).

The presence of *Lobophora* in reefs that have undergone a shift from coral- to algal-dominated assemblage was principally reported in the Caribbean in at least six different countries (Antigua, Bahamas, Belize, Curaçao, Jamaica, Navassa) (Table 1.2.1). However, there are also reports from the Pacific (Great Barrier Reef, Australia) and in the Indian Ocean (Andavadoaka, Madagascar) (Table 1.2.1). Shifts to algal-dominated assemblage were documented in different reef types and at varying depths (Table 1.2.1). In all the phase-shifts where *Lobophora* has been reported, seemingly clear events (natural and/or anthropogenic disturbances) marked the onset of increase in *Lobophora* abundance (Table 1.2.1). The change from coral to macroalgal dominance has been attributed to (1) coral mortality, (2) a reduction of herbivorous fish and sea urchins, and (3) an increase in nutrients (e.g. Lapointe, 1997; Jompa & McCook, 2002b, a; Nugues & Bak, 2006). Nonetheless, it is still debated if macroalgal overrun results from (1) a top-down process, i.e. the competitive overgrowth of corals by algae released from herbivory pressure, or (2) a to coral mortality freeing space for algal colonization. In virtually all the regime shifts involving *Lobophora*, the decrease in coral cover was chiefly attributable to storm damage, coral diseases, thermal stress and mass bleaching (Table 1.2.1), and the subsequent algal bloom appears to be opportunistic. Following a *Diadema antillarum* die-off in Curaçao, *Lobophora* cover increased at 27 m at the expense of crustose coralline algae, while decrease in coral cover was not significant (De Ruyter Van Steveninck & Bak, 1986). The shift to algal dominated communities in the mesophotic coral reefs of the Bahamas represent the only exception, where the lionfish invasion appears to be the prime cause (Lesser & Slattery, 2011). We should, however, point out that the coral cover is quite low in the mesophotic zone (Lesser & Slattery, 2011), and processes leading to a regime shift are certainly different from

shallow coral-dominated reefs. Decline in herbivory (e.g. sea urchin die-off, overfishing) was on the other hand only reported in four out of the ten regime shifts (reported in the literature), and increase in nutrients in only one case. We can deduce from these events that coral mortality, creating available open surface for colonization, appears to be a chief process allowing the proliferation of *Lobophora*. But as illustrated by the regime shift in the mesophotic zone in the Bahamas, herbivory may also contribute to *Lobophora* increase leading to coral overgrowth. Artificial and natural manipulation of herbivores, in addition to herbivory exclusion in damselfishes territories, showed that *Lobophora* abundance may significantly increase following a reduction in herbivory (Brawley & Adey, 1977; Sammarco, 1982; De Ruyter Van Steveninck & Bak, 1986). However, it would be a far stretch to conclude from these large scale events that decline in herbivory by itself may allow overgrowth of corals by *Lobophora* leading to a regime-shift. These events only allow concluding that a decline in herbivory may promote algal blooms, and discernibly herbivory does not prevent algal colonization over dead coral. While nutrient increase may also boost *Lobophora* growth rate, none of those regime shifts permit considering it as a fundamental factor. *Lobophora* was moreover not necessarily the first colonizer of dead corals as illustrated in Pandora reefs where following a bleaching event and a cyclone, dead coral colonies were colonized by algal turfs, that later became replaced by frondose macroalgae including *Lobophora* (Done *et al.*, 2007). Overall, evidences of the phase shifts suggest that an increase in abundance of *Lobophora* requires the death of corals (i.e. cascading effects). Decline in herbivory and increase in nutrients appear to facilitate *Lobophora* inception in damaged reefs. It is worth pointing out that *Lobophora* increase usually co-occurs with other recurrent macroalgae, i.e. *Dictyota, Halimeda, Turbinaria, Padina* and *Sargassum*. A monospecific bloom of *Lobophora*, following a mass bleaching event, was documented only onece and was followed by a quick – less than a year – coral recovery (Keppel Island, GBR; Diaz-Pulido *et al.*, 2009).

These reports are also indicative for what appears to be a depth-dependent proneness (depth-dependent dynamics of coral reefs) to *Lobophora* increase. Most of those shifts involving *Lobophora* are reported to occur at very specific depths, except for the reefs in Jamaica following the disastrous hurricane (Hughes, 1994). In five of these events, shifts occur at shallow depths, between 4 to 15 m (Table 1.2.1), which is not surprising since storm events have the potential to cause significant damage to shallow coral reefs (Hughes *et al.*, 2003). In Curaçao, *Lobophora* cover increased at 20, 27 and 30 m but not at 3, 10, 15 and 40 m, despite a significant decline in coral

cover at 10 m (De Ruyter Van Steveninck & Bak, 1986; Nugues & Bak, 2008). In the Bahamas, *Lobophora* cover increase was restricted within the mesophotic zone at 46 and 61 m (Lesser & Slattery, 2011). Reports from Belize, indicated that changes in coral-macroalgal abundance may vary across different adjacent reefs (barrier reef, fore reef, reef crests habitats) (McClanahan *et al.*, 1999). These observations suggest that the change to *Lobophora* is dependent on the habitat interacting with other, yet unknown, environmental factors.

Those regime shifts triggered research interest into the competition between corals and algae, and *Lobophora* has particularly been a model organism, given its recurrent presence in those shifts, notably in the Caribbean. We are subsequently reviewing those studies taking *Lobophora* for model and summarizing their contrasting effects on corals.

3. Contrasting effects on corals

3.1. The *Lobophora*-syndrome

Overgrowth of corals by *Lobophora* was first reported by Glynn (1973) in the Caribbean, and later observed by several other authors (De Ruyter van Steveninck *et al.*, 1988b; Antonius & Ballesteros, 1998; Nugues & Bak, 2006) in this region, but also in the Pacific (Jompa & McCook, 2002b; Vieira *et al.*, 2015). The term "*Lobophora*-syndrome" (*LOB*) was specifically coined to define this very phenomenon whereby *Lobophora* overgrowth leads to the coral mortality (Antonius & Ballesteros, 1998). *LOB* was only observed on a limited number of coral species (Table 1.2.2) and it is worth pointing out that in the Caribbean *LOB* observations were made in rather very unhealthy coral reefs. For instance the 1998 mass bleaching events and Hurricane Mitch caused widespread coral mortality in the Belizean reefs, which are today damaged by a combination of punctuated disturbance events and chronic stressors, leading to decline in coral cover. Epizoism syndromes, not observed 25 years prior to Antonius and Ballesteros (1998) study, are piling-up on the top of so many other coral-killing syndromes in Carrie Bow Cay reefs (Antonius & Ballesteros, 1998). On the other hand, observations of coral overgrowth by *Lobophora* are anecdotal in healthy coral reefs, where the common algal association with corals is interpreted as a refuge from herbivores (Bennett *et al.*, 2010). It would thus appear that *LOB* is tributary to reduced coral health in combination with other environmental factors, e.g. herbivory decline and nutrients increase.

Observations of coral overgrowth by *Lobophora* in addition to the presence of *Lobophora* in reefs that have turned to the "green side", led to a series of studies aimed at deciphering processes by which the alga may overgrow corals.

Table 1.2.1. *Lobophora* occurrence in reefs that have turned to algal-dominated assemblage following disturbance.

Location	Year	Form	Depth	Cause of phase shift	Associated algae	Reference
Caribbean						
Curaçao	1983	N.M.	27	*Diadema antillarum die-off*	*Dictyota*	(De Ruyter Van Steveninck & Bak, 1986)
Belize (lagoonal patch reef)	1983-1998	N.M.	N.M.	Coral disease, sea urchin die-off, overfishing, not sure	*Dictyota, Turbinaria, Sargassum*	(McClanahan *et al.*, 1998)
*Belize (barrier reef)	mid 1980s	N.M.	12-15	White Band Disease	N.M.	(McClanahan *et al.*, 1999)
Belize (Glovers Reef Atoll)	1970-1997	N.M.	N.M.	Thermal stress, nutrient increase, overfishing	*Dictyota*	(McClanahan *et al.*, 1999)
**Belize (Glovers Reef Atoll)	1998-2001	Foliose	8-12	Hurricane	*Dictyota*	(Mumby *et al.*, 2005)
Jamaica	1983-1994	N.M.	All depths up to 40	Overfishing, Hurricane damage, sea urchin die off,	*Sargassum, Dictyota, Halimeda*	(Hughes, 1994)
**Curaçao	1979-2006	N.M. Foliose?	20, 30	Bleaching, disease, storm related mortality	*Sargassum, Dictyota, Halimeda*	(Nugues & Bak, 2008)
Bahamas (mesophotic reefs)	2003-2009	Foliose	30-61	Lionfish invasion	*Halimeda copiosa, Peyssonnelia* sp.	(Lesser & Slattery, 2011)
Navassa	2002-2006	N.M.	N.M.	N.M.	*Halimeda, Dictyota*	(Wilkinson, 2008)
**Lesser Antilles	N.M.-2008	N.M.	N.M.	Thermal stress, nutrient increase, Hurrican damage	*Dictyota*	(Wilkinson, 2008)
**Antigua	2006-2007	N.M.	Shallow waters	Coral diseases, sedimentation/nutrients	*Dictyota, Halimeda, Caulerpa*	(Wilkinson, 2008)
Indian Ocean						
Andavadoaka, Madagascar	1998-2005	N.M.	N.M.	Bleaching events	*Dictyota, Turbinaria*	(Wilkinson, 2008)
Pacific						
Keppel Islands, Great Barrier Reef (reef slope)	2006	Foliose	4-7	Mass bleaching	Single species	(Diaz-Pulido *et al.*, 2009)
Havannah Is., Great Barrier Reef (reef slope)	1997-2007	Foliose	6-9	Thermal stress, storm damage	*Sargassum, Padina, Turbinaria*	(Cheal *et al.*, 2010)
Pandora Reef, Great Barrier Reef (fore reef, back reef)	1981-2005		4, 10	Thermal stress	*Dictyota, Boodlea*	(Done *et al.*, 2007)

*** *Lobophora* is not mentioned in the text, but it is obvious on the picture. ** Not a regime shift, but abundance increase. In the Lesser Antilles, it is only mentioned "in sheltered areas".N.M.: not mentioned**

Table 1.2.2. Review of the papers studying *Lobophora* impact on corals.

Coral	Effect	Mechanism	Context	Location	Reference
Negative effects on corals					
Porites cylindrica	Tissue mortality	Overgrowth	Nutrients & Herbivory	Great Barrier Reef, Pacific	(Jompa & McCook, 2002a)
Porites cylindrica	Tissue mortality	Overgrowth	Herbivory	Great Barrier Reef, Pacific	(Jompa & McCook, 2002b)
Agaricia agaricites	Tissue mortality				(Nugues & Bak, 2006)
Agaricia spp.	Growth decrease and mortality of juvenile	Shading and abrasion	Herbivory	Roatan Island, Caribbean	(Box & Mumby, 2007)
Acropora			Acidification & temperature increase, decrease in herbivory	Simulation model	(Anthony *et al.*, 2011)
Acropora digitifera *Acropora florida* *Acropora formosa* *Acropora gemmifera* *Acropora hyacinthus* *Acropora nasuta* *Acropora* sp1, sp4, sp6 *Acropora tenuis* *Cyphastrea* sp. *Favia favus* *Goniastrea capitata*	*Lobophora* promoted in some instances first stage elongation of the larvae, but further development rarely if ever occurred.				(Morse *et al.*, 1996)
Acropora intermedia (**L. papenfussii*)	Mortality	Allelopathy	Acidification increase	Southern Great Barrier Reef, Pacific	(Diaz-Pulido *et al.*, 2011)
Stylophora pistillata *Acropora palifera* (**L.* sp)	Metamorphosis inhibition	Waterborne			(Baird & Morse, 2004)
Montastraea annularis	Fecundity	Contact			(Foster *et al.*, 2008)
Millepora complanata *Millepora alcicornis* *Porites porites* *Porites astreoides*	Tissue mortality	Overgrowth (tightly attached skin on the coral surface	Other coral-killing syndromes	Belize, Caribbean	(Antonius & Ballesteros, 1998)
Porites sp. *Favia stelligera*	Tissue mortality	Overgrowth	Partially very sick coral populations	Mauritius, Atlantic	(cited by Antonius & Ballesteros, 1998) (Antonius, 1991, 1995)
			Macroalgal-dynamics	Simulation model	(Mumby, 2009)
Porites astreoides	Recruitment inhibition	Allelopathy		Laboratory, Florida	(Kuffner *et al.*, 2006)
Porites porites *Porites cylindrica*	Bleaching	Allelopathy		Panama, Caribbean Fiji, Pacific	(Rasher & Hay, 2010)
Montastrea cavernosa	Bleaching	Allelopathy		Bahamas, Caribbean	(Slattery & Lesser, 2014)
Montastrea faveolata	Tissue mortality	Allelopathy and/or microbial activity		Curaçao, Caribbean	(Wolf *et al.*, 2012b)
Platygyra daedalea	Settlement, swimming			Great Barrier Reef, Pacific	(Diaz-Pulido *et al.*, 2010)
Porites lutea	Colonized injured area & overgrowth	Overgrowth	In low light & injured	Okinawa, Pacific	(Titlyanov *et al.*, 2009)
Montastrea faveolata *Porites astroides*	Coral-associated bacterial assemblage shift to a entirely new state.	Aqueous extract		Florida & Belize, Caribbean	(Morrow *et al.*, 2012)
Hydnophora *Platygyra* *Favia* *Goniastrea* *Pavona*	Tissue mortality	Overgrowth	Synergetic interaction with potential disease	Majuro Atoll, Marshall Islands	(Jacobson)

(**Lobophora*-like)				
Agaricia tenuifolia *Porites astreoides*	Bleaching		Belize, Caribbean	(Longo & Hay, 2014)
No effects on corals				
	Coral recovery			(Bender *et al.*, 2012)
Positive effects on corals				
Acropora millepora	Pre-settlement & settlement enhancer	Waterborne effect	Aquarium, Pacific	(Birrell *et al.*, 2008a)
Small juvenile corals (* *L.* sp.)	Settlement substrate	Chemical cues	Palmyra Atoll, Pacific	(Roth & Knowlton, 2009)
Porites astreoides	Protection of juvenile from parrotfish		Colombia, Caribbean	(Venera-Ponton *et al.*, 2011)
Detrimental effect of coral on *Lobophora*				
*Agaricia agaricites** *Agaricia lamarcki** *Meandrina meandrites*** *Mycetophyllia aliciae*** *Stephanocoenia michelinii**	Growth inhibition, reduction of growth rate of *Lobophora*	Mesenterial filaments or sweeper tentacles injure *Lobophora blades upon contact*;* Small grazers living near coral margins damage *Lobophora* blades. Allelopathy**	Curaçao, Caribbean	(De Ruyter van Steveninck *et al.*, 1988b)
Montastrea cavernosa *Colpophyllia natans*	Growth inhibition	Mesenterial filaments extrusion: Notches and frayed margins	Curaçao, Caribbean	(Nugues *et al.*, 2004a)
*Agaricia lamarcki** *Meandrina meandrites* *Mycetophyllia aliciae* *Montastrea franski* *Porites astreoides*	Growth inhibition of alga		Curaçao, Caribbean	(Nugues & Bak, 2006)
Porites australiensis *Favites russelli* *Galaxea astreata* *Cyphastrea chalcidicum* *Goniastrea retiformis* *Astreopora listeria*	Effect of live coral on algal recruitment	Propagules cannot directly settle on and colonize healthy coral tissue.		(Diaz-Pulido & McCook, 2004)
Positive effect of coral on *Lobophora*				
Acropora	Refuge		Great Barrier Reef, Pacific	(Bennett *et al.*, 2010)

3.2. Competitive experiments

Lobophora aggressiveness towards corals was initially attributed to its creeping growth form and opaque, thick foliose thallus (Jompa & McCook 2002a,b). The first experiments on competition between *Lobophora* and scleractinian corals were conducted by De Ruyter van Steveninck *et al.* (1988b), who showed that the four corals tested (Table 1.2.2) were capable of preventing *Lobophora* overgrowth on live tissue. Jompa and McCook (2002b) similarly showed that (untreated or not damaged) *Porites cylindrica* (branches) inhibited the overgrowth by *Lobophora*, although the alga was markedly a superior competitor. Nugues and Bak (2006) showed that coral species have different competitive abilities, and all the tested

corals (Table 1.2.2), but *Agaricia agaricites*, inhibited *Lobophora* growth. Overall, these studies showed that most of the tested corals demonstrated the capacity to inhibit *Lobophora* overgrowth either by (1) mechanical damage by mesanterial filaments or sweeper tentacles, (2) allelopathy, and (3) the involvement of grazers defending coral margins. Furthermore, Diaz-Pulido and McCook (2004) showed that *Lobophora* cannot settle on living corals.

3.3. Negative allelopathic experiments

Lobophora allelopathy against corals was later experimentally tested by Rasher and Hay (2010) who showed that hydrophilic extract had bleaching effects against *Porites porites and P. cylindrica*. Slattery and Lesser (2014) showed similar effects on the coral *Montastrea cavernosa.* Those latter authors isolated an allelochemicals from *Lobophora*. While Morrow *et al.* (2012) showed that *Lobophora* extracts may trigger a shift in the bacterial community associated to corals, Antonius and Ballesteros (1998) observed that *Lobophora* overgrowing *Porites porites* triggered White Band Disease.

3.4. Effects on coral recruitment

Starting from the late 80s, researchers brought to light the role of chemosensory mechanisms in the early life stages of corals (i.e. recruitment), and showed that waterborne compounds from crustose coralline algae and other calcareous red algae acted as chemical signals inducing coral settlement and metamorphosis (Morse *et al.*, 1988; Morse, 1991; Morse & Morse, 1991; Morse *et al.*, 1994; Heyward & Negri, 1999; Raimondi & Morse, 2000; Negri *et al.*, 2001). While the role of chemical inducers in coral recruitment from crustose coralline algae has been well studied (and presented variable effects), very few studies have evaluated the roles of other algal taxa (Birrell *et al.*, 2008b; Diaz-Pulido *et al.*, 2010; Sin *et al.*, 2012).

A small number of studies testing the effect of *Lobophora* on coral recruitment yielded contrasting results. *Lobophora* has been found to enhance coral larval settlement of *Acropora millepora* larvae on *Hydrolithon reinboldii* (Birrell *et al.*, 2008a), and to induce the first stage elongation of larvae from seven acroporid corals (Morse *et al.*, 1996). However, in the latter study, further development severely compromised (Morse *et al.*, 1996). Baird and Morse (2004) reported apparent avoidance behavior of *Acropora palifera* and *Stylophora pistillata* coral larvae in response to fragmented portions of *Lobophora*. Also while *Lobophora* was chosen as

substratum for coral settlement in Roth and Knowlton (2009), it was not the case in the study of Morse *et al.* (1996), where the alga served as a control to investigate coral settlement on crustose coralline algae. In this latter case, it is possible that while *Lobophora* could have very well served as a substratum, coral larvae showed a clear "preference" for the crustose coralline algae (attraction to crustose coralline algae was stronger), as shown between different species of CCA (Harrington *et al.*, 2004). In New Caledonia, we observed the regular presence of Acroporidae coral juvenile on the fronds of *Lobophora rosacea,* which is niched between *Acropora* spp. corals branches (personal observations). Finally, while some members of *Lobophora* epiphytic algae community are deleterious to some corals as mentioned earlier, others such as *Hydrolithon* spp. serve as substratum, and induce coral larvae settlement and metamorphosis (Morse *et al.*, 1994; Morse *et al.*, 1996; Heyward & Negri, 1999). However, it was argued that settlement on algal fronds is likely to lead to dislodgement and mortality of the coral recruit, as shown on the green alga *Halimeda* (Nugues & Szmant, 2006). Also, future studies should investigate the fate of corals that have settled on *Lobophora* fronds, to conclude if it is a fatal attraction or not. Diaz-Pulido *et al.* (2010) showed that *Lobophora* did not negatively affect the swimming activity of the 2-day-old larvae of the coral *Platygyra daedalea*, but that the larval settlement onto discs of *Porolithon onkodes* was six times lower in *Lobophora* treatment (5% settlement) than in the treatment with no algae added (*P. onkodes* only; 30% settlement).

These conflicting results suggest divergence in the effect of *Lobophora* between different corals species or stages of the recruitment, or the role of different species-specific biofilms.

4. *Lobophora* biology and ecology

When studying the effects of macroalgae on corals, it is important to take into account algal life-history traits and ecological information. For instance, algal patch dynamism is of great importance when considering processes of coral recruitment and coral-algal competition (Mumby *et al.*, 2005).

It is very likely that different *Lobophora* species have different life-history strategies. In most studies on *Lobophora*-coral interactions, however, species-level differences of the alga were not considered. Since no inter-specific comparisons have yet been made, the following life-history traits remarks will be considered at the genus-level.

Lobophora displays a high dynamism in the colonization and extinction of patches, which increases the frequency of coral-algal interactions but reduces the average

duration of coral-algal interactions durations (Mumby *et al.*, 2005). Patch dynamism is not only species-specific but varies across habitats with contrasting levels of wave exposures. In Glovers Reef (Belize), *Lobophora* exhibited lower temporal and spatial variations in its patch dynamics in comparison to *Dictyota pulchella* (Mumby *et al.*, 2005).

Furthermore, *Lobophora* abundance has different seasonal variation depending on the latitude. While in tropical regions, *Lobophora* appears to be perennial without seasonal cover fluctuation (De Ruyter van Steveninck & Breeman, 1987b), in sub-tropical to temperate regions, it may undergo large seasonal changes in abundance (Bernatowicz, 1950; Tsuda, 1974; Peckol & Searles, 1984) and may even not be present during a part of the year (Tsuda & Kami, 1973; Mathieson & Dawes, 1975). Seasonal die-back, appeared to be a major process allowing coral recovery in Keppel Islands (GBR, Australia) (Diaz-Pulido *et al.*, 2009). Such differences may very well be species-specific.

Lobophora has a very high blade turnover, which may vary in time and space, with a half-life of blades being on average 20 days (De Ruyter van Steveninck & Breeman, 1987b). High turnover rates of *L. variegata* blades (a result of intense herbivory), together with defense mechanisms of the corals, generally prevent *L. variegata* from overgrowing coral colonies (De Ruyter van Steveninck & Breeman, 1987b).

Taking into account *Lobophora* the high blade turnover rates, high patch dynamics and seasonal fluctuation, the net outcome on corals will *in fine* depend on the susceptibility of corals to relatively short term algal contacts.

5. *Lobophora* epiphytes: spectators or players?

Most studies on algal-coral interaction, have considered the algae as single entities, except for algal-turf, which usually are an assemblage of species. However, most macroalgae harbor a community of epibionts including microorganisms and algae. Such is the case of *Lobophora,* whose blades act as an important living substratum, harboring a community of up to 70 species of epiphytic algae (Fricke *et al.*, 2011). In photographs from studies on *Lobophora*-coral competition, the presence of dense filamentous turf-like epiphytes is clearly visible (Jompa & McCook, 2002a; Diaz-Pulido *et al.*, 2009; Vieira *et al.*, 2015). Yet, none of those studies discriminated the effects of *Lobophora* from those of its associated epiphytes on the tested corals. As justly commented by Fricke *et al.* (2011), some members of *Lobophora* epiphytic community have shown detrimental effects on corals (Table 1.2.3). Particularly, the red alga *Anotrichium tenue,* commonly present on *Lobophora* blades, is able to

overgrow some corals (Jompa & McCook, 2003b). Furthermore, the lower surface of the alga, which is the most susceptible side to enter in contact with corals, generally harbors a denser and more diverse epiphytic community than the upper-side (Fricke *et al.*, 2011). While, Fricke *et al.* (2011) clearly showed that the epiphytic community associated to *Lobophora* varied with depth and sites, future studies should investigate if these differences could represent a species-specificity. In New Caledonia, for instance, different species of *Lobophora* were more or less epiphytized, and for those heavily epiphytized, they appeared to visually present different epiphytes (personal observation). It remains to be determined if these visual observations can be interpreted as (1) species-dependent substrata or host properties or (2) the biotic and abiotic factors specific to the habitats of each species (Belegratis *et al.*, 1999; Fricke *et al.*, 2011) and (3) assess the negative effects of epiphytes on corals.

Table 1.2.3. Epiphytic macroalgal species and cyanobacteria found on *Lobophora* and their effects on corals.

Species	**Effect on corals**	**References**
Anotrichum tenue	Overgrowth	(Jompa & McCook, 2003b)
Jania spp.	Settlement substratum*	(Harrington *et al.*, 2004)
Hydrolithon spp.		
Phormidium	Reduce survival and settlement and growth of adult	(Kuffner & Paul, 2004)
Lyngbya	corals or act as a pathogen for coral diseases	(Titlyanov *et al.*, 2007)
		(Richardson & Kuta, 2003)

***Unstable substrate not suitable for future coral growth (Nugues & Szmant, 2006)**.

6. Role of herbivory in preventing *Lobophora* proliferation

Lobophora is highly susceptible to grazing by sea urchins and herbivorous fishes, although grazing intensity may vary across habitats and depths (Lewis, 1985; De Ruyter van Steveninck & Breeman, 1987a; Bennett *et al.*, 2010). Also, in healthy coral reefs, *Lobophora* is consumed in considerable quantities by herbivores (Lewis, 1985; De Ruyter van Steveninck & Breeman, 1987a, b). During some of the regime shifts reviewed earlier, reduction in herbivory occurred (e.g. sea urchin die-off, overfishing), raising the question whether grazing plays a critical role in preventing macroalgal overgrowth on corals or not. De Ruyter van Steveninck *et al.* (1988b) suggested that aggressive and defensive mechanisms by corals were not by themselves sufficient and that intense herbivory is the most important factor preventing overgrowth of corals by *Lobophora*. Several studies have subsequently experimentally tested the effects of herbivory exclusion on *Lobophora*-corals

competition (Table 1.2.2). Those limited number of studies concluded that herbivory exclusion resulted in faster algal growth and consequent overgrowth and mortality of coral tissue, demonstrating the critical importance of herbivory on the outcome of the competitive interaction (Jompa & McCook, 2002b). Artificial and natural manipulation of herbivores, in addition to herbivory exclusion in damselfishes territories, showed that *Lobophora* abundance may significantly increase following a reduction in herbivory (Brawley & Adey, 1977; Sammarco, 1982; De Ruyter Van Steveninck & Bak, 1986). It confirms the hypothesis by van den Hoek et al. (1978), that grazing can restrict *Lobophora*.

Lobophora is commonly being farmed by different species of damselfishes (*Segastes apicalis, S. adustus, Eupomacentrus planifrons, E. fuscus*), by defending their territories against intruders of different species. A rapid reduction in the biomass of *Lobophora* was noted when damselfish were permanently removed from their territories (Brawley & Adey, 1977). *Lobophora* had been heavily grazed within on day (Brawley & Adey, 1977).

But we should keep in mind that herbivore exclusion procedure may produce artifacts detrimental to corals (e.g. shading, reduction in flow; not all experiments included procedural controls) (McCook *et al.*, 2001). Declines in corals could also result from the incidental exclusion of predators that would otherwise restrict corallivores (e.g. gastropods) (McCook *et al.*, 2001).

7. The need for dead coral surface for settlement

McCook *et al.* (2001) commented that leathery algae can rarely colonize healthy coral tissue, and that such observations of overgrowth often result from prior coral injury or death. Indeed, evidence stemming from regime shifts reviewed earlier strongly suggests that coral mortality is a primary condition for *Lobophora* increase. *Lobophora* obviously benefits from the increase in substratum generated by the diverse causes killing areas of coral tissue (Mumby *et al.*, 2005). Several studies, testing whether or not coral tissue mortality could be caused by algal overgrowth, yielded contrasting results. The experimental studies from Jompa and McCook (2002b); (2002a) clearly showed that *Lobophora* was capable of overgrowing *Porites cylindrica* and thus directly causing tissue mortality. Although competitive inhibition by these two species was mutual, *Lobophora* was competitively superior to *P. cylindrica*. We should nevertheless point out that caging artifacts and stress caused by the section and transplantation of corals branches may bias experimental results. Furthermore, Jompa and McCook (2002b); (2002a) worked on colonies that were

already covered with *Lobophora*. It can therefore not be ruled out that the initial presence of *Lobophora* also resultos from tissue injury, caused for example by the 1998 mass bleaching event. This compromises the evidence that *Lobophora* is capable of settling on healthy live tissue of *P. cylindrica*. Nugues and Bak (2006), who tested six different species of corals, demonstrated that except for one species, *Agaricia agaricites*, prior death of corals was a requirement for *Lobophora* to become established. Diaz-Pulido and McCook (2004) also showed that *Lobophora* propagules were not able to settle on healthy tissue of six different coral species but only on dead tissue areas besides healthy tissue. These studies show that generally *Lobophora* appears unable to directly settle, overgrow and kill the living tissue of healthy corals, and therefore prior coral death is necessary for coral overgrowth. Also, it reveals that some corals, such as *P. cylindrica* and *A. agaricites*, may be more vulnerable to *Lobophora* overgrowth. In the light of those experimental studies, it is more likely that the increase in *Lobophora* in reefs that have shifted to macroalgal-dominance, was primarily a consequence of coral mortality rather than a cause (but see Vieira *et al.*, 2015).

8. Post-shift situation

While in healthy coral reefs *Lobophora may* not represent a threat to corals, this may not be the case in damaged reefs. The factorial combination effects of (1) broad-scale coral mortality, (2) reduction in grazing pressure and (3) increase in the alga reproductive capacity may result in rapid increase of *Lobophora* cover. Areas of high macroalgal density may in turn no longer be efficiently grazed (McClanahan *et al.*, 1999; Hoey *et al.*, 2011) and eventually avoided by herbivores (Nugues & Bak, 2008), resulting in the formation of denser and uniform beds of though mature thalli gorged in chemical deterrents, thus farther less susceptibly grazed by herbivores (Paul & Hay, 1986; Cheal *et al.*, 2010). A set of direct and indirect, physical, chemical and biological mechanisms will subsequently prevent the recovery and recruitment of corals (McCook *et al.*, 2001; Birrell *et al.*, 2008b; Barott & Rohwer, 2012).

9. Contrasting effects between *Lobophora* species

Contrasting effects of *Lobophora* on corals are clearly indicative of differential competitive abilities between coral species. However, the reverse is also possible, that is different species of *Lobophora* have contrasting effects on corals, which has been completely ignored until now. Unaware of the species-richness of the genus, nearly all

of these publications are referring to the Caribbean species assigned as *Lobophora variegata*. However, *Lobophora* is a species-rich genus comprising 21 described species, and close to 80 more species yet to be described (Vieira *et al.*, in prep.-c). In New Caledonia, several species of *Lobophora* are naturally associated with corals. Among those species, only one has been observed showing apparent signs of negative effect on one coral species, *Lobophora hederacea* on *Seriatopora caliendrum* (Vieira *et al.*, 2015). In New Caledonia, however, *L. hederacea,* which grows at the basal parts of *P. cylindrica* branches, represents no apparent threat to the latter (Vieira *et al.*, 2015). The Caribbean also comprises numerous different species with contrasting morphologies (Vieira *et al.*, in prep.-c), the foliose form was the most documented in the studies on *Lobophora*-coral competition, suggesting that a limited number of species could chiefly be implicated in those regime shifts.

10. Conclusion

The brown alga *Lobophora* has become since the late 80s a model organism in the studies on algal-coral competition. Based on dramatic regime-shifts in the Caribbean and on few experimental studies, it has been generally accepted that *Lobophora* is a potent competitor against corals. However, the evidences reviewed here showed that *Lobophora* yielded controversial effects on corals ranging from positive to negative, apparently reflecting differences amongst coral species or life-stages. Also, although unaccounted for until today, these differences could also be attributed to the differential effects of different *Lobophora* species. The majority of the evidence is in favor of *Lobophora* having no or negligible effects on corals. These divergent results question the negative effects of *Lobophora* on corals, and the alga could even be playing an important role in coral recruitment on some reefs, which is furthermore supported by the frequent association of several *Lobophora* species to corals in healthy reefs. Yet, further studies would be necessary to investigate the positive interaction between *Lobophora* and corals. Reviews of *Lobophora* involved in reefs that have turned to algal-dominated assemblage clearly occurred following disturbances that have damaged corals, and experimental studies tend to agree that prior death of corals may be generally required for *Lobophora* to become established (i.e. necessity of having the substratum liberated). Although, herbivory may play a role in maintaining the population within limits, presently reviewed evidences do not allow concluding that by itself it may be a sufficient process enabling a regime-shift. While the presence of *Lobophora* in reefs that have shifted to algal-dominance is frequent in the Caribbean, reports in other places are rather anecdotal and generally

constrained to small spatial scales (specific reefs and depths). Occurrences of *Lobophora* succeeding coral decline at very specific depths may be interpreted in terms of (1) depth-specific coral decline, (2) *Lobophora* species habitat preferences, (3) specific coral-species susceptibility to *Lobophora* overgrowth, (4) presence of specific herbivores. Persistence in reefs that have turned to *Lobophora*, in association with other algae or forming monospecific mats, is dependent on the alga ecological and biological traits, such as seasonal diebacks, patch dynamics, blade growth rates. Taking into account that (1) coral susceptibility to *Lobophora* overgrowth of adverse effects is species-specific, and (2) most coral species tested were capable of preventing *Lobophora* overgrowth, it is hardly conceivable that *Lobophora* may cause regime-shift from coral-dominated to algal-dominated reefs by coral overgrowth. Otherwise, it would imply that *Lobophora* is capable of overgrowing a multitude of corals species unlike it was experimentally shown.

In conclusion, *Lobophora* is a perfect illustration of the complexity and species-specific nature of coral-algal interactions, and algal-coral competitive studies should (1) ensure correct taxonomical identification, (2) take into account a multitude of factors before concluding that an alga has adverse effects on a coral and (3) one should be careful extrapolating from field or aquarium experiments. Rather experiments should be considered on a case-per-case basis.

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Author contributions

CV wrote the manuscript. ODC and CP commented on the manuscript.

Chapter 2: *Lobophora* biotic interactions in coral reefs

Part 1. Macroalgal-coral interactions in healthy coral reefs[3]

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Abstract

Although the competitive nature of macroalgal-coral interactions draws a lot of attention since a couple of decades, these biotic interactions are complex and should not be simply labeled as negative. While many studies have focused on elucidating the competitive mechanisms between these two major benthic marine protagonists, there is surprisingly little knowledge on interaction between macroalgae and corals in healthy reefs, compared to in damaged reefs. The present study aims at documenting macroalgal-coral interactions (MCI) in coral-dominated habitats across multiple healthy reefs in New Caledonia. We document the MCI typologies, diversity, abundance, and distribution. Over 40 MCI were recorded with a limited number of dominant/frequent MCI, indicating the "preference" of association of some macroalgae for some corals and the proneness of some coral to algal association. Multivariate analyses indicate that while some coral genera are clearly more associated with algae and that some macroalgae genera are more associated to corals, MCI are predominantly habitat specific. Interactions between corals and macroalgae are not uncommon on healthy reefs and display what appears to be a neutral interaction or a competitive equilibrium.

1. Introduction

Corals and macroalgae represent two major groups of benthic reef organisms standing at the basis of the incredible diversity occurring in coral reefs (Done et al., 1996). Increasingly witnessed shifts from coral-dominated to fleshy macroalgal-dominated habitats (e.g. Hughes, 1994) put the spotlights on the competitive interactions between macroalgae and corals. However, such interactions are not necessarily exclusively negative. Biological interactions in coral reef ecosystems are complex, making it difficult to label them simply into categories such as predation, herbivory, and competition. Interactions are frequently dependent on the scale and ecological conditions (Harrison & Cornell 2008, Ricklefs 2008, Brooker et al 2009). Furthermore, assemblages of organisms within an ecological community are the results of a long evolutionary history (Brown & Maurer, 1987), making interactions dependent on the evolutionary context as well (Smith *et al.*, 1995).

In healthy coral reefs, a set of ecological factors resulted in spatial segregation of coral assemblages and fleshy macroalgal communities (Hay, 1981b), limiting interactions between the two. Corals outcompete macroalgae for space in largely

owing to herbivory (Hay, 1981b) but also thanks to a set of defense mechanisms (McCook *et al.*, 2001). Following natural or anthropogenic disturbance, affecting corals and herbivores, this ecological state may be overturned, with macroalgae or other reef organisms taking advantage and shifting the ecosystem for example into a macroalgal-dominated reef (Folke *et al.*, 2004). Many studies have investigated the mechanisms by which macroalgae are able to outcompete corals and the threat they represent in reducing corals resilience (e.g. McCook *et al.*, 2001; Rasher & Hay, 2010). From these studies emerged that corals are differentially susceptible to macroalgae (Nugues & Bak, 2006), that in damaged reefs macroalgae can preclude coral resilience (Birrell *et al.*, 2008b; Rasher & Hay, 2010), but also that corals can be remarkably resilient (Diaz-Pulido *et al.*, 2009; Gilmour *et al.*, 2013).

Under natural conditions, macroalgae are present in coral reefs without necessarily representing a threat to the corals. From a competitive perspective, it appears a competitive equilibrium exists between macroalgae and corals, i.e. a state of co-existence. Those interactions, largely undocumented may actually suggest more than just competition, but possibly include beneficial interactions (e.g. Steinberg & De Nys, 2002; Bennett *et al.*, 2010). Few studies have, however, investigated MCI diversity, typologies of associations and spatial distribution in healthy reefs (e.g. Haas *et al.*, 2010; Barott *et al.*, 2012), which will give ground for future studies aiming to elucidate the complex nature of MCI.

The present study aims at documenting natural interactions between macroalgae and scleractinian corals, to identify (1) the major MCI incidences, (2) the different types of interactions, and (3) to estimate the nature of their interaction. This will form a baseline for future studies, which may explicitly investigate the nature of these interactions. In healthy to mildly disturbed reefs in the southwest lagoon of New Caledonia, we (1) documented quantitatively and qualitatively natural occurrence of MCI, involving the most conspicuous macroalgae and corals; and (2) investigated the spatial distribution of MCI in coral-dominated habitats.

2. Material and methods

2.1. Survey area

The present research was conducted in the southwest lagoon of New Caledonia in April 2012 (Fig. 2.1.1). An interaction is defined as a direct contact, constant in time or not, between a coral colony and a neighboring macroalga.

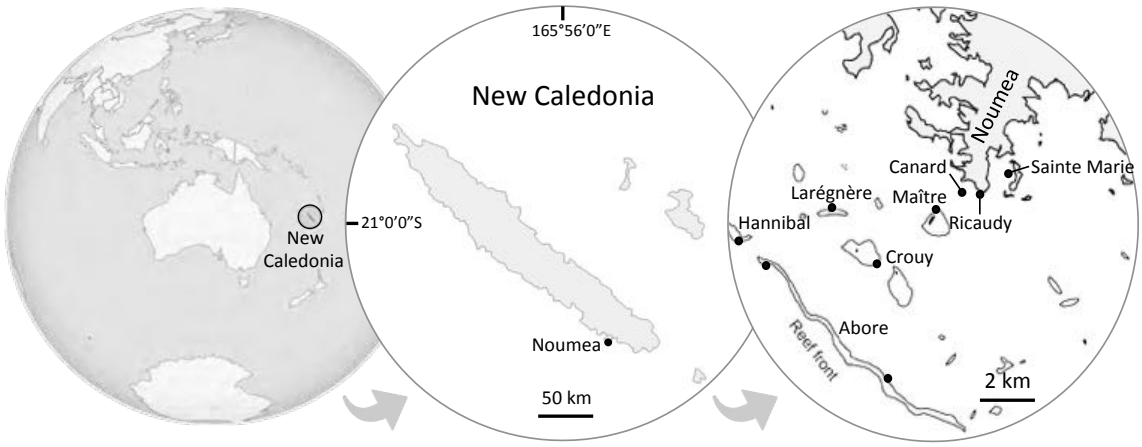

Figure 2.1.1. Map indicating survey sites in the southwest lagoon of New Caledonia.

MCI were surveyed on eight reefs, along a gradient from the shore to the fore reef (Fig. 2.1.1, Table 2.1.1). Reef types included fringing platform reef, islet reef, patch reef, back reef and fore reef (Fig. S2.1.1) with contrasting anthropogenic influences (Table 2.1.1).

Table 2.1.1. Survey sites in the southwest lagoon of New Caledonia

Site name	**Reef type**	**Distance to shore (km)**	**Human influence**
Bovis	Fringing reef	0.1	Natural reserve
Ricaudy	Fringing reef	0.1	No restrictions
Canard	Islet reef	1	Natural reserve
Crouy	Patch reef	10	No restrictions
Larégnère	Islet reef	12	Natural reserve
Abore	Back reef	18	Integral marine reserve
Abore	Fore reef	19	Integral marine reserve
Mbere	Fore reef	21	No restrictions

Although MCI occur in different coral reefs habitats (e.g. macroalgal beds, seagrass beds, sandy bottom, coral fields), coral-dominated habitats were specifically targeted in the present study. Habitats were defined based on reef geomorphology and coral benthic cover (Table 2.1.2). The reef geomorphology was decomposed into two levels, the reef type and the reef zonation (Fig. S2.1.1, S2.1.2). In other words, different reef types could present similar zonation and benthic covers.

Table 2.1.2. Habitat levels.

Reef type	**Reef zonation**	**Benthic cover**
Fringing	Flat	Branching coral field
Islet	Slope	Sparse corals and bedrock
Patch	Wall	Coral heads
Back reef	Bottom	Coral patches
Fore reef	Slope	Coral patches

A total of twenty-six different habitats were selected based on benthic cover and coral structure and represent most of the reef habitat diversity in the southwest lagoon of New Caledonia (Table 2.1.3).

Table 2.1.3. Habitats selected to quantify MCI in the southwest lagoon of New Caledonia.

Habitat	Location	Reef type	Zonation	Benthos	Depth (m)	Note
ABO_0001	Abore	Patch reef	Slope	BCF	2-5	Patch reef
ABO_0002	Abore	Patch reef	Flat	SCBR	0-2	Patch reef
ABO_0003	Abore	Back reef	Wall	SCBR	2-5	Spur & groove
ABO_0004	Abore	Back reef	Flat	SCBR	0-2	Spur
ABO_0005	Abore	Back reef	Bottom	BCF	5-10	Groove
ABO_0006	Abore	Back reef	Bottom	CH	5-10	Groove
ABOOUT	Abore	Fore reef	Slope	SCBR	10-15	-
BOV_0001	Bovis	Fringing reef	Bottom	CH	5-10	-
BOV_0002	Bovis	Fringing reef	Slope	BCF	2-5	-
BOV_0003	Bovis	Fringing reef	Flat	BCF	0-2	-
BOV_0004	Bovis	Fringing reef	Flat	SBC	0-2	-
CAN_0001	Canard	Islet reef	Bottom	BCF	5-10	Windward
CAN_0003	Canard	Islet reef	Slope	BCF	2-5	Windward
CAN_0004	Canard	Islet reef	Flat	SCBR	0-2	Windward
CAN_0009	Canard	Islet reef	Slope	BCF	2-5	Leeward
CAN_0014	Canard	Islet reef	Flat	SPCB	0-2	Leeward
CRO_0003	Crouy	Patch reef	Flat	BCF	0-2	Leeward
CRO_0004	Crouy	Patch reef	Flat	BCF	0-2	Leeward
CRO_0010	Crouy	Patch reef	Flat	BCF	0-2	Windward
RIC_0002	Ricaudy	Fringing reef	Slope	BCF	0-2	Windward
LAR_0001	Laregnere	Islet reef	Bottom	CH	5-10	Leeward
LAR_0002	Laregnere	Islet reef	Slope	BCF	2-5	Leeward
LAR_0003	Laregnere	Islet reef	Flat	BCF	0-2	Outside islet lagoon
LAR_0007	Laregnere	Islet reef	Flat	BCF	0-2	Inside islet lagoon
LAR_0010	Laregnere	Islet reef	Flat	SCBR	0-2	-
MBEOUT	Mbere	Fore reef	Slope	SCBR	10-15	-

BCF: Branching Coral Field; SCBR: Sparse Corals and Bedrock; CH: Coral Heads; CP: Coral Patches

2.2. Data collection

2.2.1. Preliminary qualitative survey

A preliminary survey was conducted (1) to qualitatively assess MCI in the study area, and (2) to select habitats presenting the most conspicuous MCI for the succeeding quantitative survey. Survey sites were chosen using raw imagery satellite pictures from Google Earth version 7.1.2.2041 (Landsat satellite images; http://www.earth.google.com [April 26, 2012]) in order to target the most representative sites (Fig. 2.1.2A). During this preliminary survey, Linear Point Intercept (LPI) transects, as described in English *et al.* (1994), were implemented along a cross-shore section (i.e. vertical transects) from the sandy bottom up to the reef (Fig. 2.1.2B, S2.1.2). MCI were assessed every 50 cm along the LPI transects,

which could reach up to 300 m. For the islets and patch reefs, LPI transect were made in the four main cardinal directions of the reefs. For the fringing, back and fore reefs, four transects with contrasting wind exposition (e.g. leeward and windward) were done. A total of 36 LPI were realized in the studied area. During this preliminary survey, close-up pictures of each MCI were taken. Identifications of corals and macroaglae were carried out up to genus-level.

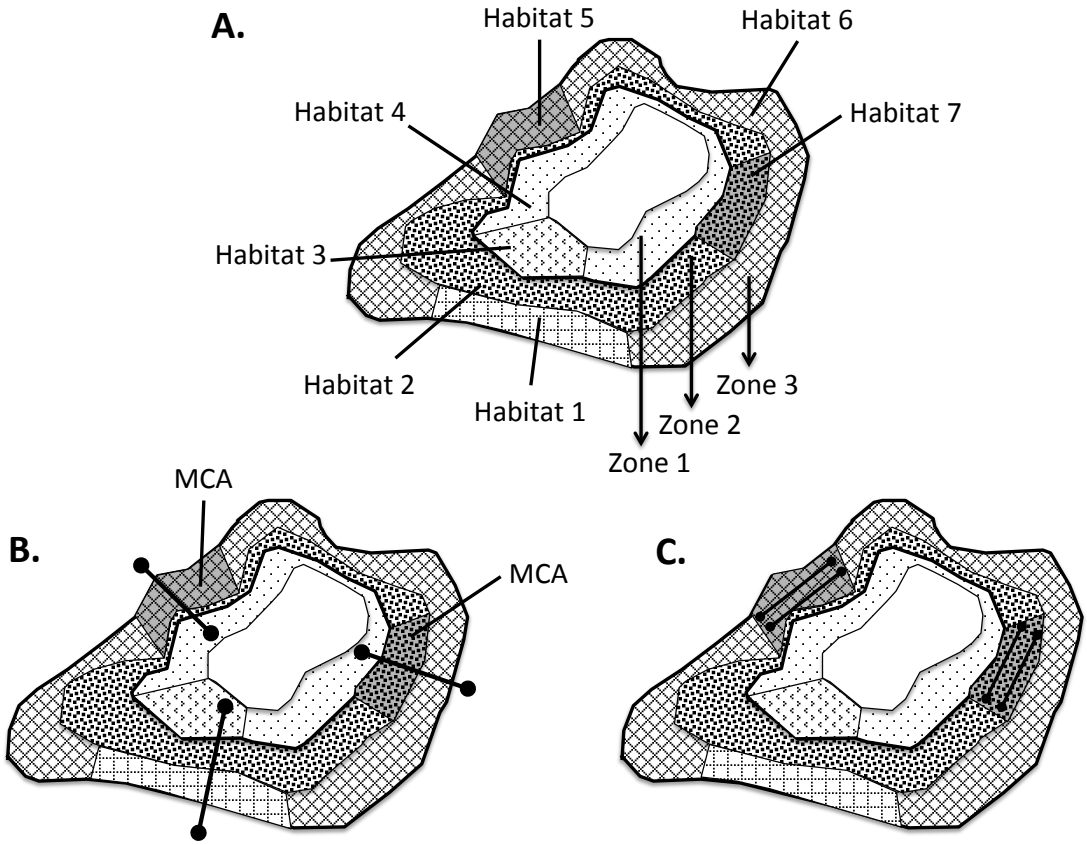

Figure 2.1.2. Schematic representation of the sites selection in an islet reef by satellite imagery (A), the linear point transects during the preliminary survey (B) and the belt transects in the selected habitats during the quantitative survey (C). MCA: macroalgal-coral associations.

2.2.2. Quantitative survey

Benthic cover and MCI quantitative assessments were done using a 10 m belt transects as described in English *et al.* (1994) in selected habitats with conspicuous MCI. Per habitat transects were deployed in triplicate parallel to the isobaths (i.e. horizontal transects), 10 m apart from each other. This resulted in a total of 78 transects. Within a transect, 50 x 50 cm quadrats were positioned 20 times

consecutively left and right along a defined line. Photographs were taken directly above each quadrat using a Lumix Panasonic digital camera (12 megapixels), set on a photoquadrat framer (i.e. tetrapod). In addition, close-up pictures from the different MCI within the transect were taken.

2.3. Typologies of associations

Based on visual observations during both surveys, we attempted to categorize and define typologies of associations between macroalgae and corals.

2.4. MCI inventory and abundance

Relative benthic cover (i.e. corals, macroalgae, other benthic organisms) and MCI quantification, assessed with the pictures taken in the horizontal transect, was determined using a stratified random point count method (CPCe; Kohler & Gill, 2006). Individual pictures were subdivided into 16 equal squares, and each cell was populated with one random point lying within the cell borders. The circle with crosshairs with a diameter of 150 pixels was chosen as data point object shape. The feature lying under the circle-crosshairs was recorded, according to the code identifier available in the Supplementary Information. If the crosshairs fell on a coral or macroalgae, the occurrence of macroalgae or corals in direct contact within the circle were recorded. Abundance of specific interactions was subsequently calculated.

2.5. MCI richness estimation

To estimate the MCI richness in the southwest lagoon of New Caledonia, we used three sample-based richness estimators, namely the incidence-based coverage estimator (ICE; Chao & Lee, 1992), the Chao 2 richness estimators (Chao 2; Chao, 1987), and the Jackknife 1 first-order Jackknife richness estimator (Jack 1; Burnham & Overton, 1979). Species richness estimators are based on statistical procedure and are conventionally used to assess species richness from a sub-sample of individuals selected at random from a larger sample. ICE distinguishes between frequent and infrequent species in analysis. Jack 1 does not differentiate the species frequency and relies on the number of MCI only found once. Chao 2 relies on the number of unique units and duplicates. The MCI sample-based data was used for the calculation of three MCI richness estimators with EstimateS (v9.1.0; Colwell, 2013).

2.6. MCI spatial patterns

To determine spatial patterns of MCI across multiple habitats in the southwest lagoon of New Caledonia we carried out a multiple correspondence analysis (MCA) (Greenacre & Blasius, 2006), which is an extension of correspondence analyses when multiple variables are being considered for categorical data. MCA allows analyzing the pattern of relationships of several categorical dependent variables. Here, we consider two biological variables, namely the occurrence of macroalgae and corals, and three environmental variables, namely the reef type, the reef zonation and the benthic cover. For the MCA analysis, only the six most common macroalgae and corals were selected. The MCA was carried out using FactoMineR (Husson *et al.*, 2007; Lê *et al.*, 2008) in R (R Development Core Team, 2013).

3. Results

3.1. Typologies of interactions

Six types of association were defined based on observations, namely (1) niched among (e.g. Lobophora, Halimeda, Dictyota, Hypnea) (Fig. 2.1.3A, 2.1.4A), (2) adjacent to (e.g. Asparagopsis) (Fig. 2.1.3B, 2.1.4B), (3) growing at the base (e.g. Lobophora, CCA) (Fig. 2.1.3C, 2.1.4C), (4) overgrowing live tissue (e.g. Lobophora) (Fig. 2.1.3D, 2.1.4D), (5) growing in (dead) interstices (e.g. Turbinaria) (Fig. 2.1.3E, 2.1.4E) and (6) on dead surfaces (e.g. Padina) (Fig. 2.1.3F, 2.1.4F) of corals.

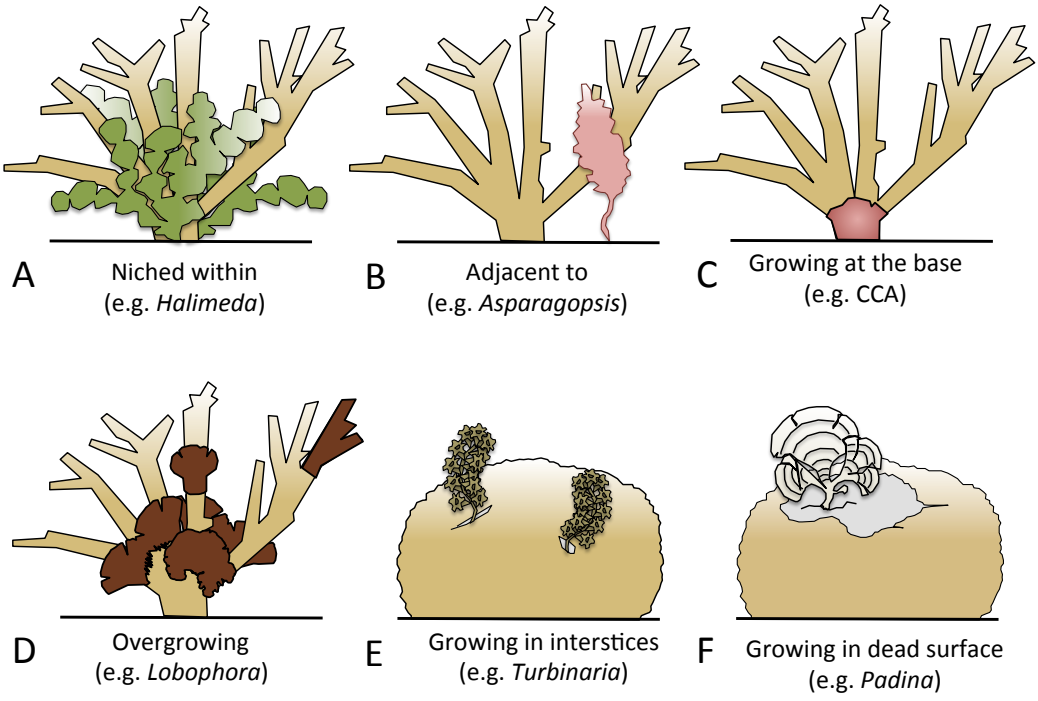

Figure 2.1.3. Schematic representation of the six typologies of interactions identified.

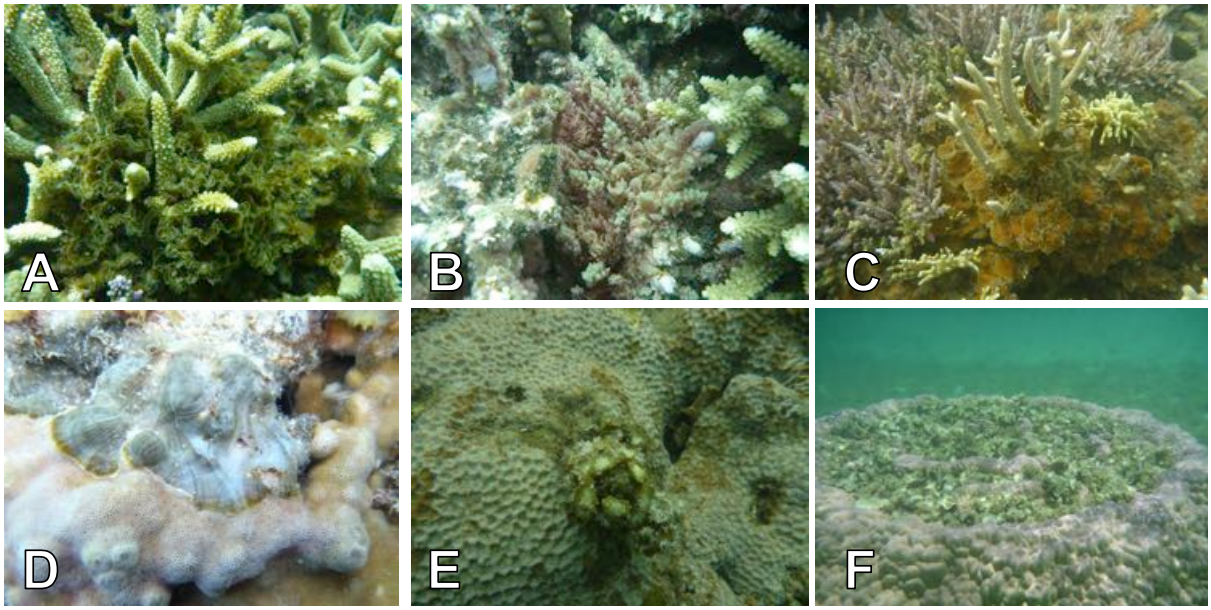

Figure 2.1.4. Illustration of the six typologies of interactions identified. A. *Lobophora rosacea* niched within *Acropora* sp. branches. B. *Asparagopsis taxiformis* adjacent to *Acropora* sp.. C. *Lobophora monticola* growing at the base of *Acropora muricata*. D. *Lobophora crassa* overgrowing *Montipora* sp.. E. *Turbinaria ornata* growing in interstice of *Porites* sp. F. *Padina* sp. growing on dead surface of *Porites* sp.. Photo credit: C. Vieira.

3.2. MCI diversity

During the preliminary survey a total of 43 interactions (Table S2.1.1) were visually recorded involving 10 coral genera (*Acropora, Galaxea, Montipora, Pavona, Pocillopora, Porites, Seriatopora, Stylophora, Turbinaria*) as well as the Hydrozoa *Millepora*, and 16 macroalgal genera (*Asparagopsis, Amphiroa, Caulerpa, Ceratodictyon, Chaetomorpha, Chlorodesmis, Colpomenia, Dictyota, Galaxaura, Halimeda, Hydroclathrus, Hypnea, Liagora, Lobophora, Padina, Sargassum*) in addition to crustose coralline (CCA) and turf algae. But many of these interactions were only rarely encountered and observed only during the prospection period (Fig. S2.1.3). Furthermore, despite our efforts, some macroalgae growing underneath branching corals, attached at the coral's base, may have been overlooked.

Species richness estimators were applied to obtain an estimate of the number of MCI to be expected in the southwest lagoon of New Caledonia. The three species richness estimators (ICE, Jack 1 and Chao 2) converged on similar values (Fig. 2.1.5) ranging between 21 (Chao 2) to 23 (Jack 1) MCI, which were slightly higher than the observed diversity, i.e. 20 MCI (Sobs; Fig. 2.1.5) based on the quantitative survey data.

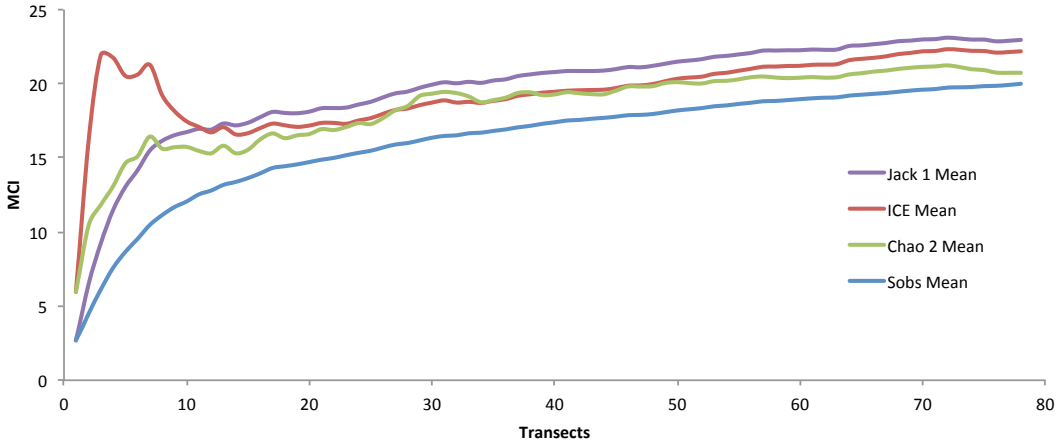

Figure 2.1.5. Observed and estimator-based MCI accumulation curves obtained with incidence-based coverage (ICE), Chao 2 and first-order Jackknife (Jack 1) richness estimators.

3.3. MCI abundance and variability

Based on the preliminary survey, we estimated the frequency of MCI on a reef scale to represent an average of 16.4 % of the benthic cover in coral-dominated habitats. Within the selected habitat where MCI were the most conspicuous, the percentage of interaction between benthic reef macroalgae and scleractinian corals reached up to 70% with an average of 30% within the surveyed belt transects. Note that these numbers cannot be extrapolated to estimate macroalgal presence in coral-dominated habitats within New Caledonian reefs, since we specifically targeted sites where MCI were the most abundant. *Lobophora* was the most frequent macroalgal representative, being involved in 47% of all MCI, followed by *Halimeda* (20%), and *Hypnea* (9%) (Fig. 2.1.6A). *Acropora* was the most abundant scleractinian coral genus observed in direct contact with macroalgae (Fig. 2.1.6B) and accounted for 61% of all the MCI, followed by *Montipora* (19%), *Seriatopora* (13%) and *Porites* (5%). Macroalgae were preferentially found on branching, columnar and digitate corals, but some genera like *Lobophora* and crustose coralline algae were also found growing at the basal part or on dead surfaces (e.g. *Padina*, *Chlorodesmis*) of large, massive and encrusting corals (e.g. *Porites, Montipora*).

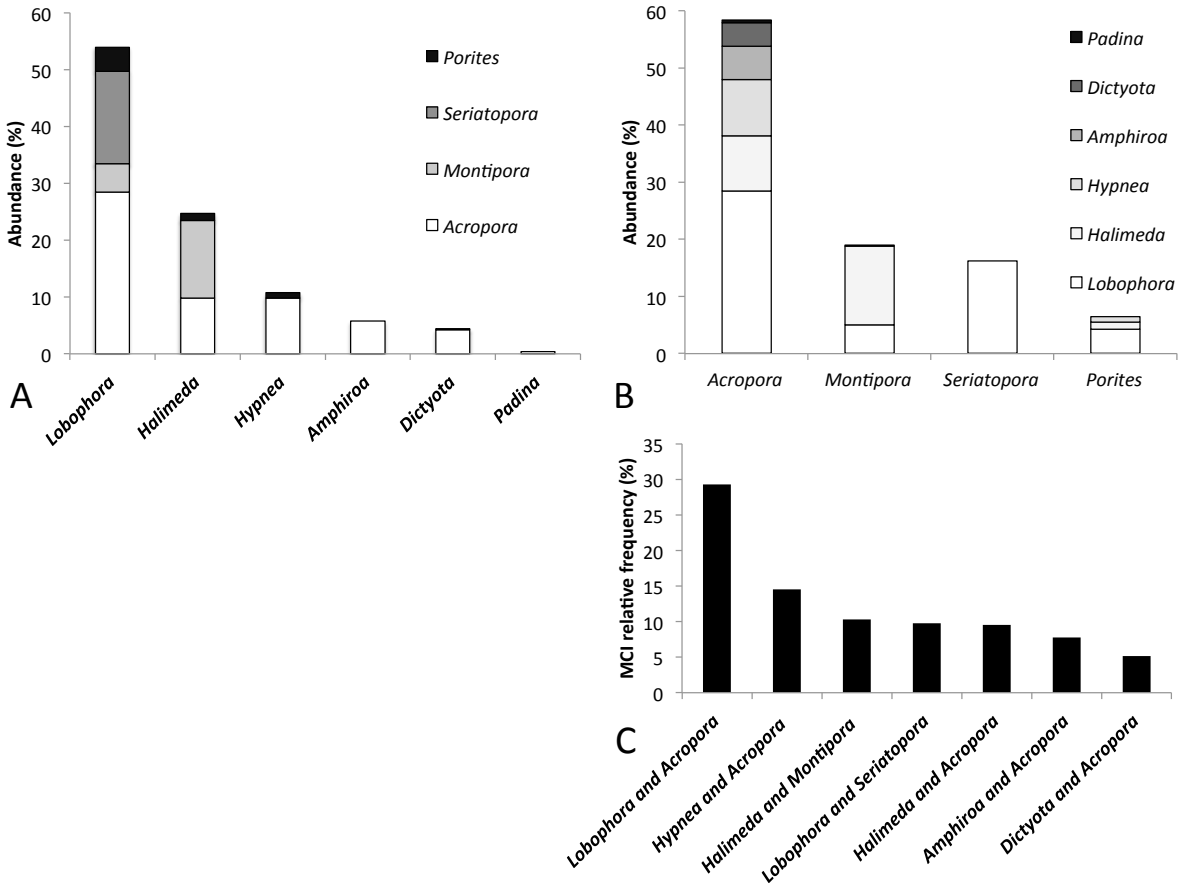

Figure 2.1.6. Macroalgal-coral interactions abundance in the southwest lagoon of New Caledonia.

Macroalgal-coral associations which accounted for more than 5% of all the associations scored across transects are represented in Fig. 2.1.6C. *Lobophora-Acropora* represented the most common/abundant and conspicuous MCI (29%), followed by *Hypnea-Acropora* (15%), and by *Halimeda-Montipora*, *Lobophora-Seriatopora* and *Halimeda-Acropora*, which accounted for ca. 10%. Note that *Lobophora-Seriatopora*, which represent for a non-negligible percentage of all the MCI, was only observed in the barrier reef, where *Seriatopora* preferentially grows.

3.4. MCI spatial patterns

A multiple correspondence analysis (MCA) was conducted to test possible links between MCI and habitat variables (i.e. reef type, reef zonation and benthic cover). The MCA showed that some MCI are closely related to some habitats. The first dimension of the MCA (45%) separates barrier from the other reef types (Fig. 2.1.7). The second dimension of the MCA (16%) mostly separates the fringing from the islet reefs (Fig. 2.1.7). *Lobophora-Seriatopora*, *Lobophora-Turbinaria*, *Lobophora-Porites* and turf-*Acropora* were mainly observed in the inner barrier on sparse coral in

bedrock, on walls. *Halimeda-Acropora*, *Halimeda-Montipora* and *Lobophora-Montipora* were mostly found in flat fringing reef. MCI occurred independently of anthropogenic disturbance.

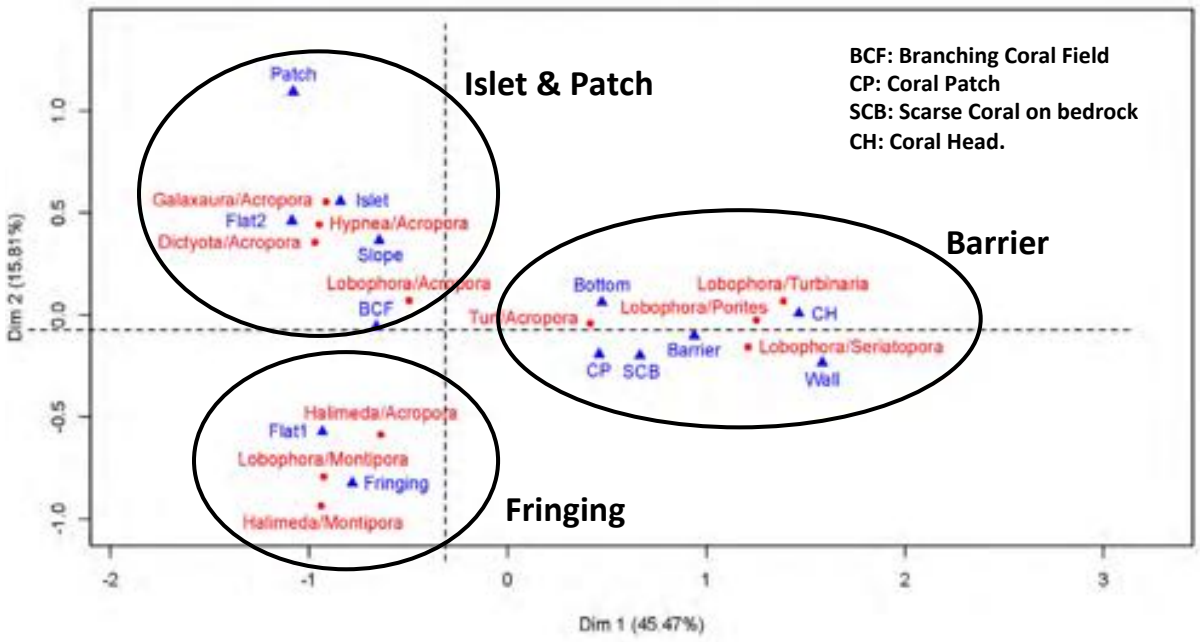

Figure 2.1.7. Multiple correspondence analysis map of MCI and habitat variables (reef type, reef zonation and benthic cover).

4. Discussion

Documenting MCI in coral-dominated habitats across multiple reefs, from fringing to fore reefs, across the southwest lagoon of New Caledonia, we showed that (1) MCI are relatively rare but not uncommon in coral coral-dominated habitats – with ca. 16% of the benthic cover – in the southwest lagoon of New Caledonia, and that (2) MCI are not randomly occurring but that on the contrary there obviously exists non-arm's-length relationships between certain macroalgae and corals. Finally, our data reveal spatial distribution patterns in macroalgal-coral occurrence related to the reef types.

4.1. Diversity and abundance of MCI

In New Caledonia MCI occur on undisturbed to mildly disturbed (i.e. anthropogenized) reefs and represent a relatively small percentage of the benthic cover. While in some habitats MCI are homogeneously distributed, in others they are clumped or randomly distributed. This study showed that while a significant

numbers of MCI were documented, only a few are common and relatively abundant such as *Lobophora*-, *Halimeda*- and *Hypnea-Acropora*. The three most common macroalgae found interacting with corals belong to the three major algal divisions, by order of importance, *Lobophora* (Phaeophyceae), *Halimeda* (Chlorophyceae) and *Hypnea* (Rhodophycea). It is noteworthy, however, to point out that in one specific site *Dictyota* was found growing abundantly niched within *Acropora* branches, which is suggesting that this macroalga is also potentially an important interactant with corals as shown in previous studies (e.g. Lirman, 2001; Box & Mumby, 2007; Titlyanov *et al.*, 2007). *Lobophora* and *Acropora* were by far the most abundant macroalgal and coral genera associated to each other. And while macroalgae with diverse morphotypes (e.g. crustose, articulated calcareous, leathery, filamentous, etc.) are involved in MCI, corals with complex morphologies (e.g. branching, columnar, etc.) are more significantly subjected to interactions with macroalgae.

Since this study was conducted in coral-dominated habitats, we reason in terms of macroalgal 'preference' for coral hosts and reciprocally in terms of coral 'receptiveness' to macroalgal association. The present results suggest that some macroalgae are preferentially associated to corals and some corals are more susceptibly associated with macroalgae. For example *Acropora* hosted the most diverse, in number, macroalgae (e.g. *Lobophora, Hypnea, Halimeda, Padina, Amphiroa, Dictyota*). On the other hand *Seriatopora* was mostly targeted by *Lobophora*. This reflects a combination of corals susceptibility to occupancy and algal successful occupation.

Preference and susceptibility are closely related to the macroalgal settlement and survival success. Given the complex morphologies of branching corals providing refuge to macroalgae from larger herbivores (e.g. sea urchins, large fishes) (Bennett *et al.*, 2010), it only makes sense that they are the most targeted hosts. And from a competitive perspective, it was shown that some coral taxa are more susceptible than others to macroalgal aggressiveness (Nugues & Bak, 2006). But regardless of the exact nature of the interaction, e.g., commensal or competitive, the commonness of some specific interactions indicates that some macroalgae are more successfully interacting with some corals. This study showed that the three genera *Lobophora*, *Halimeda* and *Hypnea* are the most commonly found macroalgae in association with corals in the southwest lagoon of New Caledonia. The leathery brown macroalgae *Lobophora* has been documented as one of the major competitor with corals and has been reported to be involved in regime shift from coral- to macroalgal-dominated ecosystems following disturbances in coral reefs in the Caribbean (e.g. De Ruyter van

Steveninck & Breeman, 1987b). *Lobophora* has increased on many degraded reefs (Mumby *et al.*, 2005), such as in Belize in the Caribbean (De Ruyter van Steveninck & Breeman, 1987b) or in even in the Great Barrier Reef (Diaz-Pulido *et al.*, 2009), and poses a threat to coral populations by overgrowing adult colonies, reducing growth rates and inhibiting recruitment (Nugues & Bak, 2006). In Curaçao, in 10 years a significant increase in cover has been documented (Nugues & Bak, 2008). But the question remains if *Lobophora* really is a serious threat to corals or simply an opportunistic player. A, recent review leaned towards the latter (Vieira *et al.*, in prep.-a). Recently, it has been shown that *Lobophora* is a species rich genus with species occupying a variety of different ecological niches (Vieira *et al.*, 2014b). And while some species of *Lobophora* have been documented associated to corals (e.g. *L. rosacea, L. monticola, L. hederacea, L. undulata*) others were found having different substrata preferences (e.g. *L. nigrescens, L. crassa*). Furthermore, *Lobophora* species associated with corals presented different impacts on the latter (Vieira *et al.*, 2015). This highlights the importance of interpreting MCI on species level. The calcareous articulated green macroalgal *Halimeda opuntia* is found growing niched within coral branches. *Halimeda* has been already documented in the literature as a space competitors with potential detrimental effects on corals like reducing growth rates of *Porites cylindrica* (Lirman, 2001). *Halimeda* was also documented as a vector of the white plague type II, triggered by physical contact and causing widespread mortality in most Caribbean coral species (Nugues *et al.*, 2004b). In New Caledonia, however, we did not observe any severe visual bleaching on the underlying coral host tissues. The corticated red macroalgae *Hypnea pannosa*, forming mats within coral branches, was not observed to have a major impact on underlying coral tissue and was neither documented in the literature as a threat, apparently because its relatively translucent and porous thallus structure does not strongly inhibit coral tissue functions (Jompa & McCook, 2003a).

4.2. Typologies of interactions and coralgal biotic interaction compass

Macroalgal association with corals occurred in different ways which we attempted to categorize into six different typologies of interactions (TI), namely **(1)** "niched within", **(2)** "adjacent to", **(3)** "growing at the base of", **(4)** "overgrowing", **(5)** "growing in interstices", and **(6)** "growing on dead surfaces" of corals. Some types of interactions may intergrade such as **(3)**, **(5)** and **(6)** since in these cases the algae grow on dead surfaces, but we deliberately distinguished them as distinct types since

they involve different corals and macroalgae and thus modes of settlement and interaction nature. Interaction between macroalgae and corals can be of four natures: competitive, amensal, commensal or mutualistic. We represented the possible biotic interaction between macroaglae and corals into what we coined coralgal biotic interaction compass (CBIC; Fig. 2.1.8, Table S2.1.2). Among the five types of interaction, overgrowth of live coral tissue is manifestly considered to be a negative interaction, without necessarily resulting in coral death. Overgrowth is certainly kept within limits by herbivory in addition to the coral's defense mechanisms (Jompa & McCook, 2002b; Nugues & Bak, 2006). An illustration of overgrowth in New Caledonia would be the interaction between the alga *Lobophora hederacea* and the coral *Seriatopora caliendrum* (Vieira *et al.*, 2015). The remaining four interaction types on the other hand are not necessarily negative associations. Algae niched within or growing at the base of corals could either be neutral, commensal, of mutual interactions. For instance, corals may provide macroalgae with substratum and refuge from herbivores (Bennett *et al.*, 2010), and macroalgae may harbor free-living *Symbiodinium* communities (see below). However, if macroalgae produce allelopathic compounds adverse to corals or stimulate the growth of coral pathogens, direct contact may be detrimental to corals on the area where it is restricted (Nugues *et al.*, 2004b; Rasher & Hay, 2010), which may turn the biotic interaction into amensalism. Examples of seaweeds niched among corals branches are *Hypnea-, Halimeda-* and *Lobophora-Acropora*. Some macroalgae such as *Hypnea* never caused visual bleaching on coral hosts. Algae growing adjacent to corals would appear to be neutral since no traces of bleaching were observed on the corals, and the alga is exposed to herbivory. Corals may nevertheless act as environmental facilitator/enabler (e.g. reducing hydrodynamic and drag forces) allowing persistence of macroalgae in habitats characterized by strong water flow regimes. All of the types of interaction may play a beneficial role for corals, such as harboring populations of free-living *Symbiodinium* as shown with *Halimeda*, *Lobophora*, *Amphiroa*, *Caulerpa* and *Dictyota* (Porto *et al.*, 2008), necessary for hosts that must acquire their symbionts anew each generation and for the possible reestablishment of endosymbiosis in bleached adults (Takabayashi *et al.*, 2012). Algae growing in coral interstices appear to be clearly commensalism with the illustration given by *Turbinaria*. Growing in dead coral interstices may provide young *Turbinaria* recruits protection from (1) intense hydrodynamism, particularly in shallow wave-washed habitats, and (2) from grazers. Growth on dead surfaces, such as *Padina* sp. frequent presence on the top of massive corals on already dead surface, is a commensal

interaction, with the coral providing substratum for the macroalgae and protection from herbivores at low tides. Coral may also inflict damage to macroalgae by a combination of mechanisms, e.g. overgrowth, shading, abrasion, stinging, allelopathy, mucus secretion or space pre-emption (McCook *et al.*, 2001). The effects of corals on macroalgae have nevertheless received far less attention than the reverse (McCook *et al.*, 2001).

Generally, no alarming situation whereby macroalgae would represent a significant threat to coral was observed during this study, except in one case, i.e. *Lobophora hederacea* – *Seriatopora caliendrum* (Vieira *et al.*, 2015).

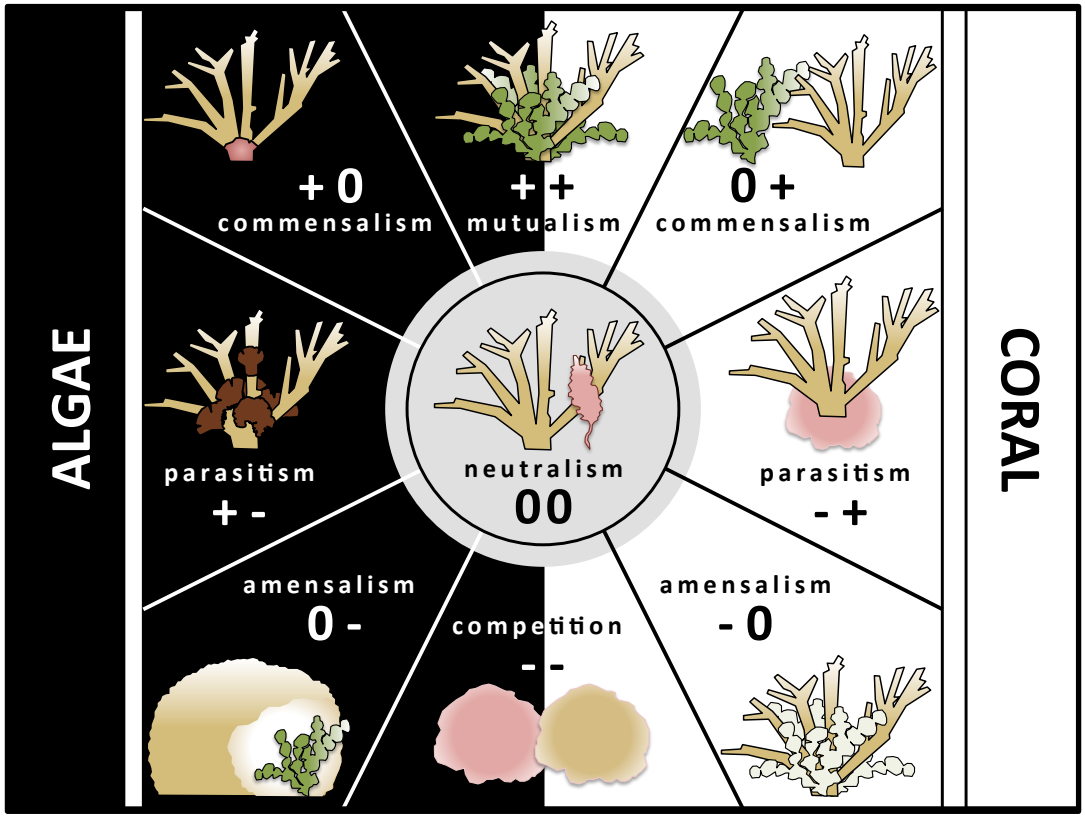

Figure 2.1.8. Coralgal biotic interaction compass. Interactions with beneficial or neutral effects for the algae on the left side (black background), and for the coral on the right side (white background). +: positive effect, 0: neutral effect, -: negative effect.

4.3. Anthropogenization influence

Results of this study demonstrated that MCI are not restricted to reefs with human influence but are naturally occurring in healthy reefs. Although MCI abundance may increase in human impacted areas, mainly due to increased nutrient load and decrease herbivory levels, it does not lead to a regime shift in New Caledonia reefs. Overgrowth by macroalgae of corals is more likely caused by coral's deteriorating

health rather than the algal competitive success (personal observations). And while abundance of macroalgae may increase in areas with strong anthropogenic influence, the type of MCI remains the same (i.e. is not qualitatively different).

4.4. MCI spatial patterns: a window to evolution of species assemblages

In the southwest lagoon of New Caledonia a limited number of recurrent MCI occur encompassing a limited number of macroalgae and corals. We investigated whether MCI are spatially structured in the southwest lagoon of New Caledonia. We broke down the habitat into three levels to see which level matters the most to MCI distribution. In other words, we wanted to see if MCI are related to the reef type, the reef zonation or the benthic cover. It appeared that reef types are the most important. The analyses first segregated the barrier reef from the islet and fringing reef and subsequently the islet from the fringing reef. In other words, MCI generally do not occur randomly, but specifically occur in specific habitats. Naturally, we would expect associations between macroalgae and corals to occur in common grounds of both organisms, where environmental conditions are suited for both organisms. For example, the *Lobophora-Seriatopora* association involves a unique species of *Lobophora*, *Lobophora hederacea,* which is mostly found growing in the barrier reef, and no other *Lobophora* species were found in the barrier reef. Similarly *Seriatopora caliendrum,* which was the most targeted species of the genus *Seriatopora,* is predominantly occurring in the barrier reef. Clearly, this association is happening where both protagonists are present. Now if we look at *Halimeda-Acropora* interaction, the latter was mainly observed occurring in fringing reefs, although *Acropora* fields are also present in all the other reef types such as in the barrier reef. This seems to reflect a combination of the coral and algal environmental preferences. *Halimeda* association to corals occurs in habitats suited to the alga. Finally, MCI spatial distribution may be reflecting (1) habitats where macroalgae and corals have common environmental preferences, or (2) corals habitats where algae would normally not be present but are there owing to the presence of corals providing substrata and shelter from herbivory and strong hydrodynamics. Knowing which corals are preferentially targeted and where they naturally occur can predict the occurrence of MCI.

4.5. Macroalgal-coral *status quo*

Undeniably, corals and macroalgae are major space competitors in coral reefs. They are comparable to two fierce armies having developed and perfectionized over time physical and chemical warfare. However, when looking at "healthy" and undisturbed reefs, we observe a competitive equilibrium between corals and algae. Although the exact mechanisms keeping the dynamic between these two competitors into a state of equilibrium is not fully understood and is a source of disagreement (e.g. bottom-up vs. top-down controls), the fact remains that they have reached some sort of "*status quo*". Degradation by human activities and occasional natural disturbances, directly affecting corals and herbivores, are breaking this equilibrium resulting in an increasing prevalence of macroalgae. But then, only corals with weaker 'fighting skills' have shown to suffer from algal overgrowth. Furthermore, corals have demonstrated remarkable resilience (Diaz-Pulido *et al.*, 2009; Roff & Mumby, 2012; Gilmour *et al.*, 2013).

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Acknowledgments

We acknowledge the IRD divers, Christophe Peignon, John Butscher, Gérard Mou-Tham who assisted CV during during the surveys, as well as the IRD boat pilots, Miguel Clarque, Samuel Tereua and Philippe Naudin.

Author contributions

CV, ODC and CP conceived and designed the study. CV carried out all the analyses and wrote the manuscript. ODC and CP commented on the manuscript.

Box 1 Macroalgal-coral interactions in seagrass beds

Interactions between seaweeds and corals are often addressed in coral dominated habitats, especially reefs that have witnessed phase-shifts from coral to macroalgal dominance. Interactions between both players, however, also occur in macroalgal-dominated habitats and more surprisingly in habitats where the presence of both seaweeds and corals is limited due to the limited availability of hard substrate. In seagrass beds, interactions between macroalgae and corals are possible thanks to successful settlement of corals on sandy substrate and subsequent macroalgal settlement on corals.

Benthic macroalgae are encountered with variable diversity and abundance in all habitats associated with tropical coral reef ecosystems. Soft bottom substrates, e.g. sandy lagoons, harbor less macroalgal species. Unlike seagrasses, which root into the sand, the vast majority of macroalgae requires hard substrate for attachment. The paucity of macroalgal abundance on sandy habitats is therefore linked to the scarcity of hard substrata. Although hard substrata may be patchy and rather insignificant, they support high percentages of the biomass and individuals in sandy habitats (Hay, 1981a). Further expansion of macroalgae is limited over a high percentage of the seagrass beds surface area by the lack of adequate attachment sites. Macroalgae associated with seagrass beds have been studied in detail by Heijs (1985b) who recorded more than 100 species in Papua New Guinea, exhibiting spatio-temporal patterns which could be related to the availability of suitable substrata (Heijs, 1985a, c, b). Suitable attachment sites are uncommon on the sand plain but may be provided by coral rubble, shells or other solid substrate. Occasional patches of live coral dot the seagrass beds, and offer fitting substrate for macroalgal settlement. Therefore interactions between some benthic reef macroalgae and corals in seagrass beds are unavoidable. Seagrass beds, in some fringing and islet reefs in the southwestern lagoon of New Caledonia, revealed the presence of locally abundant macroalgae. Besides (1) rhizophytic- and lithophytic-macroalgae (e.g. *Halimeda cylindracea*, *Caulerpa racemosa*) among the seagrasses, (2) epiphytic algae on seagrass leaves, seagrass stems and macroalgae, and (3) loose-lying or drift algae (e.g. *Sargassum spp.*); some macroalgal species (e.g. *Ceratodictyon spongiosum, Lobophora rosacea, Hydroclathrus clathratus, Halimeda opuntia, Dictyota* spp., *Hypnea* sp.; Fig. 2.Box1.1-4) were exclusively observed attached to live coral colonies with a patchy distribution. Since available substrate is very limited, competition for space on live coral between macroalgal species is a direct consequence. Representing

90% of the macroalgae associated with corals in seagrass beds, *Ceratodictyon spongiosum* Zanardini is by far the most commonly found macroalga on live corals in seagrass beds. It is intertwined between coral colonies branches (Fig. 2.Box1.2). And the coral genus *Montipora (M. hirsuta or stellata and digitata)*, representing ca. 80% of the corals associated with macroalgae in the seagrass beds, is the most common coral growing in seagrass beds. The coral 'host' do not seem to suffer from macroalgal occupation since no bleaching was observed right below the algae. Studies on competition showed that *Montipora* was less susceptible to algal threat (Rasher *et al.*, 2011), which is in agreement with our observations. These observations in seagrass beds demonstrate that macroalgae and corals may be interacting in habitats other than coral-dominated ones and that they may be positively associated, unlike in damaged reefs where macroalgae appears to be preventing coral resilience.

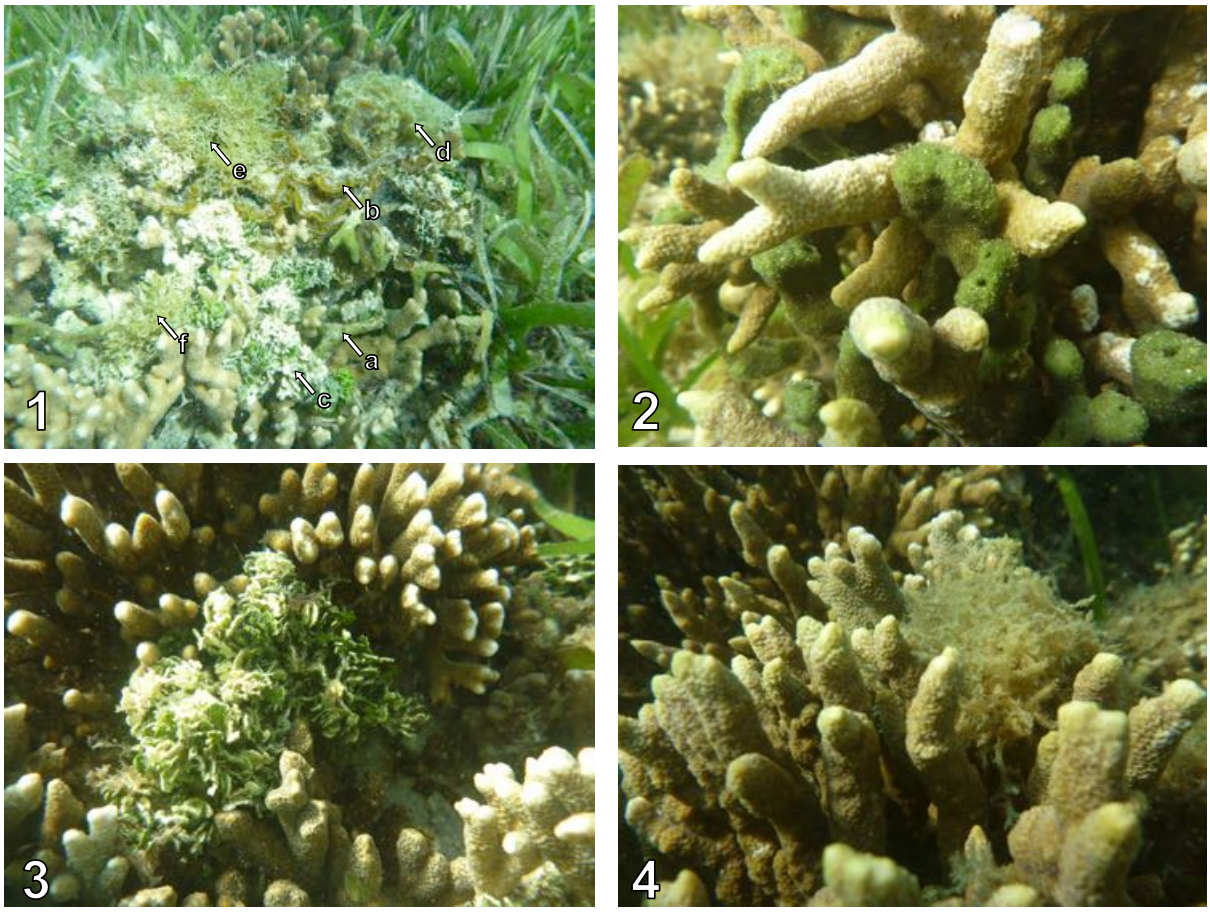

Figure 2.Box1.1-4. Macroalgal-coral interactions in seagrass beds. 1. *Montipora digitata* coral colony in seagrass bed covered with (a) *Ceratodyction spongiosum*, (b) *Lobophora rosacea*, (c) *Halimeda opuntia*, (d) *Hydoclathrus clathratus*, (e,f) *Dictyota* spp. (2 species). Maitre Islet reef, southwest lagoon of New Caledonia. 2. *Ceratodyction spongiosum* on *Montipora digitata*. 3. *Halimeda opuntia* on *Montipora stellata*. 4. *Hypnea pannosa* on *Montipora stellata*.

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Part 2. Overgrowth and killing of corals by the brown algal *Lobophora hederacea* (Dictyotales, Phaeophyceae) on healthy reefs in New Caledonia: a new case of the epizoism syndrome[4]

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Abstract

Coral reef degradation is often associated with regime shifts from coral- to macroalgal-dominated reefs. These shifts demonstrate that under certain environmental conditions (*e.g.*, decrease in herbivory and/or increased nutrients supply) some macroalgae may overgrow corals. The outcome of the competition is dependent on algal aggressiveness and the coral susceptibility. In undisturbed reefs, herbivore grazing is regulating macroalgal cover, thus preventing the latter from overgrowing corals. However, some macroalgae have evolved strategies not only to outcompete corals but also to escape herbivory to some extent, allowing overgrowth of some coral species in undisturbed reefs. Epizoism represents one of those successful strategies, and has been previously documented with red algae, cyanobacteria and *Lobophora variegata* (Dictyotales, Phaeophyceae). Here we report a new case of epizoism leading to coral mortality, involving a recently described species of *Lobophora*, *L. hederacea*, overgrowing the coral *Seriatopora caliendrum* (Pocilloporidae) in undisturbed reefs in New Caledonia.

Research note

Cases of coral overgrowth have been reported for several algae, such as *Pneophyllum conicum* (E.Y.Dawson) Keats, Y.M.Chamberlain & Baba, 1997*, Ramicrusta* textilis Pueschel & G.W.Saunders, 2009*, Anotrichium tenue* (C.Agardh) Nägeli, 1862 (Antonius & Afonso-Carillo, 2001; Jompa & McCook, 2003b; Pueschel & Saunders, 2009), including the brown algal genus *Lobophora* J.Agardh, 1894. In the Caribbean, a significant increase of *Lobophora* outcompeting *Agaracia* corals was observed following mass mortality of *Diadema* populations (De Ruyter Van Steveninck & Bak, 1986). Antonius and Ballesteros (1998) associated *Lobophora* with white banding disease in the Florida Keys. Later, several studies experimentally investigated the mechanisms by which *Lobophora* may outcompete corals (Jompa & McCook, 2002b, a; Nugues & Bak, 2006; Rasher & Hay, 2010; Rasher *et al.*, 2011; Slattery & Lesser, 2014) and demonstrated that direct contact by *Lobophora* could lead to coral bleaching or mortality. It was argued that declines of herbivores from coral reefs will lead to seaweeds becoming more abundant and further decline of reef corals.

While reviewing the diversity of the genus *Lobophora* using molecular markers it has been shown that some but not all species are specifically associated to live corals (Vieira *et al.*, 2014a). We presently document a case of epizoism involving a recently described species of *Lobophora, L. hederacea* C.W.Vieira, De Clerck and C.E.Payri

(Vieira *et al.*, 2014a), in undisturbed coral reefs in the southwestern barrier reef of New Caledonia, situated within an integral marine reserve.

Although, there is no shortage of space in the barrier reef, with one-third of the bedrock left vacant, *L. hederacea* seemed to have evolved a very specific substrate preference, as it was virtually only observed occurring on corals. The alga has been mainly observed associated with members of Pocilloporids and Acroporids. 42% of the time, the alga was found growing on two species of the genus *Seriatopora* (Pocilloporidae), *S. caliendrum* (34%; Fig. 2.2.1) and *S. hystrix* (8%). And remarkably, 100% of the colonies of *S. caliendrum* were epiphytized by *L. hederacea*. Less spectacularly, *L. hederacea* was also found growing at the base of other coral species, *e.g.*, *Pocillopora damicornis* and *Stylophora pistillata* (Pocilloporidae) (6.2%), as well as *Acropora* spp. (Acroporidae) (15%), *Porites cylindrica* (Poritidae) (22% IC) and *Turbinaria* sp. (Dendrophylliidae) (15%).

While *L. hederacea* did not seem to represent a threat to the other coral species, as it was only observed restricted to the base of the corals, it is not the case with *S. caliendrum.* In fact, the colonies of the latter displayed various stages of *L. hederacea* thalli development, which in some cases reached complete overgrowth of the entire coral colonies (Fig. 2.2.2). *Lobophora hederacea* starts growing at the basal part of the coral colony (Fig. 2.2.1, 2.2.3, arrows), devoid of living tissue, and proceeds upwards by overgrowing and killing living polyps. *Lobophora* thalli are tightly attached to the coral surface without a trace of coral tissue left below the algal cover (Fig. 2.2.3, arrow). Bleaching on the edge of the algae (Fig. 2.2.4, arrow) strongly suggests allelopathic mechanisms in the overgrowth process. *Lobophora* paves the way for subsequent colonizers, such as turf ceramiacean algae, followed by larger macroalgae, *e.g.*, *Halimeda* and *Dictyota*.

The competitive advantage taken by the brown alga over *Seriatopora* is probably due to (1) allelopathic mechanism (Rasher & Hay, 2010), (2) the complex skeletal structure of the coral, which presents very delicate and thin branches with needle-like tips, providing refuge from larger herbivores (*e.g.*, sea urchins, fishes) (Bennett *et al.*, 2010) and (3) the encrusting growth-form of *Lobophora* which renders accessibility by herbivores more difficult. Furthermore, it was shown that some corals have different competitive abilities against algae (Nugues & Bak, 2006). *Seriatopora* belongs to the Pocilloporidae family, for which another member (*Pocillopora damicornis*) has been documented as more susceptible to *Lobophora* allelopathy (Rasher & Hay, 2010; Rasher *et al.*, 2011) than other coral taxa (*e.g.*, *Montipora*, *Porites*). It is possible that coral bleaching at the edge of *L. hederacea*

could be caused by the filamentous algae, which heavily epiphytize *L. hederacea* (Fig. 2.2.4, arrow), and which were documented to directly kill branched as well as massive corals (Littler & Littler, 1997; Jompa & McCook, 2003b, a).

There are 14 described species of *Lobophora* in New Caledonia and probably 15 more undescribed lineages (Vieira *et al.*, 2014a). Some, but not all species of *Lobophora* are found associated to corals. It remains to be determined whether the allelopathic activities differ between them and whether their substrate preferences, being associated with living coral or not, can be linked to allelopathy.

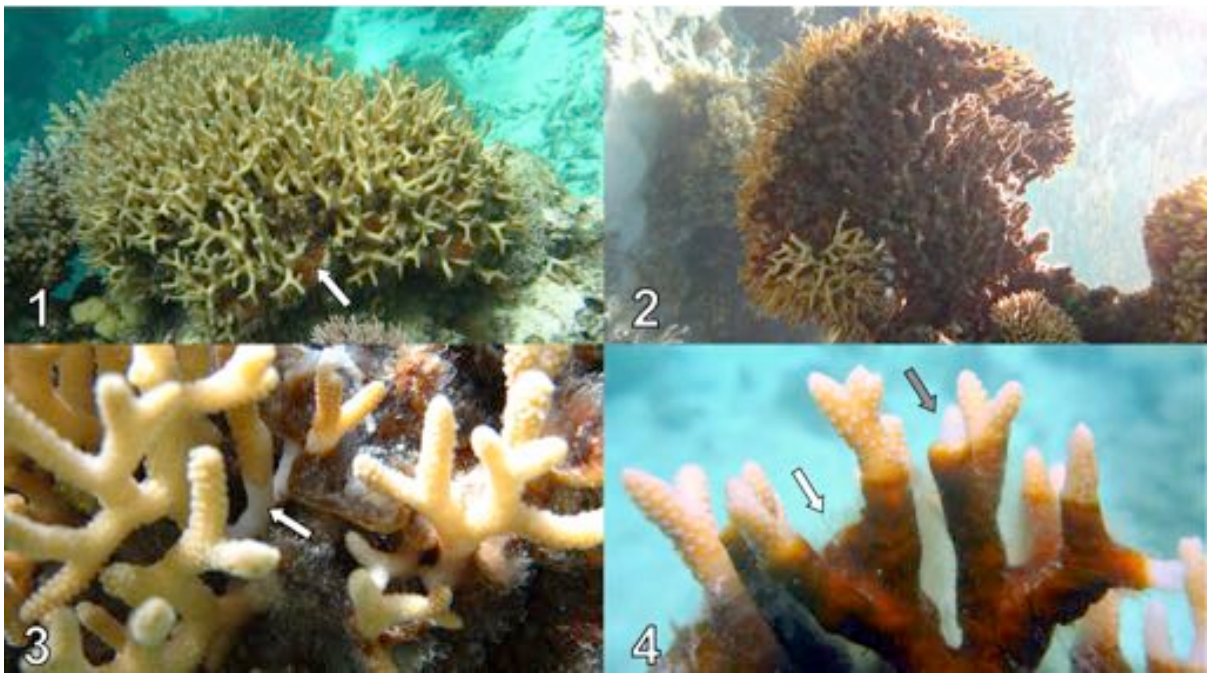

Figure 2.2.1-4. Epizoism on *Seriatopora caliendrum* by *Lobophora hederacea*. 1. Mildly impacted colony at the base. The arrow indicates *L. hederacea* growing at the base of the colony. 2. Severely impacted colony. 3. Close-up on *L. hederacea* growing at the base of *S. caliendrum*. The arrow indicates the coral surface devoid of living tissue after removal of *L. hederacea* thallus. 4 Close-up on an a severely impacted *S. caliendrum* colony by *L. hederacea*. The grey and white arrows are respectively pointing out to (1) the bleaching at the edge of *L. hederacea*, and (2) the filamentous algae epiphytizing *L. hederacea*.

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Acknowledgments

We acknowledge the IRD divers, Christophe Peignon, John Butscher, Gérard Mou-Tham who assisted CV during during the surveys, as well as the IRD boat pilots, Miguel Clarque, Samuel Tereua and Philippe Naudin.

Author contributions

CV, ODC and CP conceived and designed the study. CV carried out all the analyses and wrote the manuscript. ODC and CP commented on the manuscript.

Part 3. *Lobophora* susceptibility to herbivory[5]

Publication title: Why fight if you can run? Strategies of the brown algal genus *Lobophora* (Dictyotales, Phaeophyceae) against herbivores

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[5] In preparation for submission in JEMBE as an Original Article

Abstract

The brown alga *Lobophora* represents a major benthic component in tropical coral reefs, capable of dominating large reef areas following coral motality and herbivory declines. The alga, however, has been the object of contradictory observations in terms of susceptibility to herbivory. Unaware of the species-richness of this genus, virtually all the previous studies referred to the single Caribbean species referred to as *Lobophora variegata*, which was presumably polymorphic, with different chemical compositions and occupying diverse ecological niches. Variation in susceptibility of this single algal species to herbivory have been consequently interpreted as intraspecific variation in terms of morphology and chemical composition as well as differences in herbivore guild compositions and diet across different locations (e.g. habitat, reef, region). Recent taxonomical studies of the genus *Lobophora* disclosed a high species diversity, which could conceivably explain previous contradictory results. However, the present study, which compared the susceptibility to herbivory of eight different species of *Lobophora*, which differed in growth form as well as their fine-scale alpha-niche on coral reefs in the southern lagoon in New Caledonia, showed that they were all consumed without outstanding differences. These results suggest that *Lobophora* strategies in forms of escapes – associational or spatial – have been privileged by this brown tropical alga over defenses – chemical or morphological – against herbivores.

1. Introduction

Herbivory is a key top-down process in many ecosystems, aquatic and terrestrial, regulating abundance, dynamics, diversity and assemblages of primary producers (Huntly, 1991; Cyr & Face, 1993). On coral reefs, herbivory is one of the important processes controlling macroalgal biomass and thus regulating population dynamics of algae and other benthic organisms (Hay, 1981b, a; Carpenter, 1986; Hughes *et al.*, 1987). Herbivorous fishes, sea urchins and microherbivores are important species structuring and maintaining algal assemblages (Carpenter, 1986; Duffy & Hay, 2000), fundamental to the benthic population and community structure of coral reefs. Although the relative importance of top-down vs. bottom-up processes is still debated (e.g. Lapointe, 1997; Jompa & McCook, 2002b, a; Nugues & Bak, 2006), cases of massive herbivore die-off and experimental herbivore exclusion experiments clearly demonstrated the importance of herbivory in regulating algal abundance (De Ruyter van Steveninck & Breeman, 1987b; Hughes *et al.*, 1987).

1.1. Unpalatability against a paradox

Some experimental studies have concluded that some macroalgae are largely unpalatable. Admitting that herbivory is a critical process keeping macroalgae within limits, this would intuitively entail that unpalatable algae could easily takeover coral reefs, if not controlled by other mechanisms (e.g. chemical coral defense strategies). While some invasive algae have demonstrated this capacity, it has not been the case with native species in healthy coral reefs. Plants and herbivores have co-evolved over a long period of time. During this long evolutionary history, plants have evolved strategies against herbivores in forms of escapes and defenses possibly making some of them unpalatable at some point, but herbivores have responded by evolving counter-defenses (Hay, 1981a; Hay & Fenical, 1988; Hay, 1997). Therefore, for every alga, even the so-called unpalatable ones, an herbivore has evolved to eat them. This in turn, questions the exact meaning of palatability. First, it is important to distinguish between edibility and palatability, since both terms are often wrongly used as synonyms. Edibility is defined as "fit to be eaten" that is not poisonous, while palatability is defined as the preference a consumer has for a particular feed when offered a choice – that is the sum of factors, which operate to determine whether and to what degree food is attractive to the animal – (Tribe & Gordon, 1950). Palatability depends on factors such as the herbivore itself, growth stage and development of the alga and alternative food sources offered to the latter. Consequently, the terms "relative palatability" or "preference" are preferable when describing algal susceptibility to herbivory. Accordingly, preferences determined from grazing experiments may not represent the general palatability of the alga and thus not be ecologically relevant since (1) other herbivores than the ones tested may be the main consumers of the alga, and (2) although not highly preferred in grazing experiments, the alga may be naturally (i.e. *in situ*) significantly consumed given the absence or rarity of the more preferred algae in natural settings. Therefore, one should be careful drawing ecological conclusions based on grazing experiments with a limited number of herbivores.

1.2. A controversial alga

Lobophora J.Agardh (1894) (Dictyotales, Phaeophycea) is the perfect illustration of an alga with contrasting observations in terms of consumption. This genus represents a major benthic component in tropical coral reefs. Following natural and anthropogenic disturbances, *Lobophora* has frequently been observed blooming in

reefs that have shifted from coral- to macroalgal-dominated assemblages (e.g. De Ruyter van Steveninck & Breeman, 1987b; Diaz-Pulido *et al.*, 2009). Although the relative importance of coral death over a decrease in grazing pressure is still debated, these large-scale events are strongly suggesting that *Lobophora* is being intensely consumed by herbivores. Nonetheless, experimental studies yielded contradictory results thus questioning the consumption of *Lobophora*.

1.3. Evidences of *Lobophora* consumption

1.3.1. Herbivores mortality and exclusion

Mass mortality of *Diadema antillarum* in the Caribbean in the mid-80s (De Ruyter van Steveninck & Breeman, 1987b; Steneck, 1993) and herbivores exclusion experiments (Kennelly, 1991; Jompa & McCook, 2002b, a; Diaz-Pulido & McCook, 2003; Burkepile & Hay, 2008; Morrow *et al.*, 2011) resulted in a significant increase in *Lobophora* abundance clearly demonstrating not only the susceptibility to herbivory but the intense consumption necessary to restrict the algal cover.

1.3.2. *Lobophora* in damselfish territories

Damselfish are cultivating within their territories their favorite algae, weeding out the less desirable ones (Low, 1971; Brawley & Adey, 1977; Hata *et al.*, 2002). *Lobophora* has frequently been reported being farmed by damselfishes across the globe (Table 2.3.1). However, it is not absolutely clear if *Lobophora* is being consumed or simply provides substratum for more palatable epiphytic algae (Ceccarelli *et al.*, 2005). From studies reporting *Lobophora* presence in damselfish territories, the few that analyzed gut contents or the bite marks of the fish on the algae, demonstrated that *Lobophora* is eaten (Souza *et al.*, 2011; Feitosa *et al.*, 2012). Yet, these studies do not determine if it concerns primary or secondary consumption. Although consumption by damselfish is not evident, exclusion of damselfishes from their algal gardens on the other hand resulted in a rapid decrease of *Lobophora* cover, showing the palatability by other surrounding herbivores (Brawley & Adey, 1977).

1.3.3. Field experiments

Grazing experiments have shown that *Lobophora* is consumed by a wide variety of herbivores comprising sea urchins, herbivorous fishes (grazers and browsers), meso-

and macro-grazers (e.g. crabs, sea-snails) (Table 2.3.1). Those grazing experiments have shown low (Pillans *et al.*, 2004) to high (Lewis, 1985) preference for *Lobophora* relatively to other macroalgae. For a same family of herbivore such as the Siganidae (rabbitfish) some studies found low preference (Pillans *et al.*, 2004) while others high preference (Bennett *et al.*, 2010). Contradictory results were also reported of the herbivore species, e.g. *Diadema antillarum* (De Ruyter van Steveninck & Breeman, 1987a, b; Morrison, 1988; Solandt & Campbell, 2001; Tuya *et al.*, 2001).

1.3.4. Assessing susceptible herbivores based on functional groups?

The macroalgal functional-form groups, proposed by Littler *et al.* (1983), and the herbivore functional groups (e.g. scrapers, grazers, browsers) approaches may probably inform us on the most susceptible organisms that could be feeding on *Lobophora* (Mantyka & Bellwood, 2007b). However, given the diet range variability within a herbivore functional group, and the differential susceptibility to deterrent compounds, it is hazardous to speculate susceptibility of *Lobophora* to herbivores. Predictions of susceptibility to herbivory based on algal toughness and external morphology has been shown to be of limited value in explaining differing resistances to herbivory (Hay, 1984). More solid evidence is provided by direct observations (Fox & Bellwood, 2008).

Although strong evidence stemming from field observations, herbivores die-off or exclusion and grazing experiments clearly showed *Lobophora* susceptibility to herbivory, (Diaz-Pulido *et al.*, 2009) still concluded that *Lobophora* was an unpalatable/inedible species, a conclusion based on its low preference by a limited number of herbivores. Some studies have subsequently investigated the putative chemical and physical defense mechanisms deployed by this alga to deter herbivore grazing.

1.4. *Lobophora* a defended alga?

1.4.1. Chemical defenses

Brown algal polyphenolic secondary metabolites (pholorotannins) have been shown to deter certain marine vertebrate and invertebrate herbivores (Amsler & Fairhead, 2005). Understandably, the low preference for *Lobophora* by certain herbivores (e.g. sea urchin, fishes and sea snails; Bolser & Hay, 1996; Pillans *et al.*, 2004; Ng *et al.*, 2013) has been attributed to the production of such feeding deterrent secondary

metabolites (Targett *et al.*, 1995; Bolser & Hay, 1996; Arnold & Targett, 1998, 2000). Bolser and Hay (1996) concluded that the greater consumption of temperate (North Carolina) versus tropical (the Bahamas) *Lobophora* by the sea urchin *Arbacia punctulata* was likely due to the higher concentrations of secondary metabolites such as pholorotannins in *Lobophora* from the temperate regions than in tropical regions. Edibility would therefore be negatively correlated with the concentrations of polyphenolic secondary metabolites, which (in the case of phlorotannins) are inversely related to nitrogen availability and do increase with the C:N ratio (Targett *et al.*, 1995). However, Coen and Tanner (1989) showed that the most consumed morphotypes (i.e. ruffled and encrusting forms) of *Lobophora* possessed the highest and the lowest C:N ratios (33 and 18) and the least consumed morphotype (i.e. decumbent form) possessed an intermediate C:N ratio (23). Similarly, Vergés *et al.* (2011) who found near-significant differences in the C:N ratio (21.87 ± 03, 23.65 ± 0.81, ± SD) between two polymorphic *Lobophora* species did not observe differences in grazing intensity between the two. These latter results do not support the idea that edibility is correlated with phlorotannins concentration. Boettcher and Targett (1993) concluded that polyphenolics, as a chemical class, do not all have the same bioactivity, but differ in their activity in a size-dependent manner. Their result based on the fish *Xiphister mucosus*, concluded that phlorotannins fraction size $> 16.60 \times 10^{-21}$ g (= > 10 kDa) significantly decreased assimilation efficiency in *X. mucosus*; and those $< 8.30 \times 10^{-21}$ g (= < 5 kDA) rarely, if ever, had an effect. In their study, Boettcher and Targett (1993) also measured the total polyphenolic concentrations and percentage distribution of polyphenolics among molecular size fractions in the three *Lobophora* forms from Belize. *Lobophora* ruffled, decumbent and encrusting forms respectively had 93.69 ± 2.56, 94.50 ± 0.33 and 96.01 ± 1.68 % of polyphenolic size fractions $< 16.60 \times 10^{-21}$ g, and 10.48 ± 0.53, 12.01 ± 3.06 and 8.57 ± 0.84 total polyphenolic concentration in % of dry mass. First of all we notice that differences between the three forms in terms of total polyphenolic concentration and of polyphenolic size fractions $< 16.60 \times 10^{-21}$ g are not stupendous, and would intuitively not explain consumption differences between the three forms. Furthermore, the encrusting form, which possess the highest polyphenolic size fractions $< 16.60 \times 10^{-21}$ g of the three forms, was according to Coen and Tanner (1989) consumed at equal rates as the ruffled form, which has the lowest polyphenolic size fractions $< 16.60 \times 10^{-21}$ g of the three. In order words a clear relationship between polyphenol content and susceptibility to consumption is not evident.

Furthermore, consumers of *Lobophora*, and of any algae for that matter, may significantly vary in temperate and tropical regions. In fact, the dietary composition of a given fish species may differ across latitude (Lek *et al.*, 2011). Also, comparison of consumption by similar herbivores from different latitude may be ecologically irrelevant, and therefore does not substantiate the chemical defense by pholorotannins in *Lobophora*. Last and most importantly, toxicity of *Lobophora* extracts towards fish has only been suggested, but not rigorously tested. Only one study actually tested the ichtyotoxicity of *Lobophora* against a freshwater fish, the goldfish *Carassius auratus* (De Lara-Isassi *et al.*, 2000), which is therefore ecologically irrelevant.

In conclusion, disagreement between studies does not allow firm conclusions on the importance of phlorotannins or any other secondary metabolites in chemical defense of *Lobophora*. Contradicting results rather tend to devaluate the importance of this defense mechanism. It is plausible, however, that while some feeding deterrent compounds may deter *Lobophora* grazing by certain herbivores, this might not be the case with herbivores that have evolved counter-defenses, explaining the high consumption in the example presented earlier.

1.4.2. Morphological defenses

Algal resistance to herbivory has also been based on morphology (Littler, 1980; Steneck & Watling, 1982). *Lobophora* species display a variety of morphotypes, ranging from encrusting to stipitate. Therefore, Coen and Tanner (1989) suggested that the different morphotypes of *L. variegata*, displaying differential susceptibilities to herbivory by fish and crabs, could partly explain the conflicting results on *Lobophora* palatability. Conversely, Vergés *et al.* (2011) did not find differences in consumption between reef flat-decumbent and lagoon-ruffled morphotypes. Here again, these two studies do not allow far ranging conclusions on the importance of morphological defense in *Lobophora*, but rather tend to devaluate the importance of this defense mechanism.

Table 2.3.1. Review of the publications on *Lobophora* herbivory

Herbivore	Family	Palatability/Susceptibility	Defense process	Locality	Reference
Herbivory experiments					
Not identified		Yes. Medium relative to other algae.	Transplant	Belize	(Hay, 1984)
Herbivorous fish guild				Belize	(Lewis, 1985)
Diadema antillarum (sea urchin)	Diadematidae	Yes. High.		Curaçao	(De Ruyter van Steveninck & Breeman, 1987a)
Diadema antillarum (sea urchin)	Diadematidae	Yes. High.		Curaçao	(De Ruyter van Steveninck & Breeman, 1987b)
Mithrax sculptus *Mithrax coryphe* (crabs)	Majidae	Yes. Low-Medium relative to other algae.		Laboratory experiments	(Coen, 1988)
Mithrax sculptus *Mithrax coryphe* (crabs) Roving herbivorous fishes	Majidae	Yes. Low-High relative to other algae.		Laboratory experiments Belize	(Coen & Tanner, 1989)
Abracia punctulata *Lytechinus variegatus* (sea urchins)	Abraciidae Toxopneustidae	Yes. High relative to other algae in North Carolina, and low relative to other algae in Bahamas.	Considerable variation in palatability can also occur between local population of a single species. 27 vs. 17 m in North Carolina.	North Carolina Bahamas	(Bolser & Hay, 1996)
Sparisoma aurofrenatum *Sparisoma viridae* (parrot fishes) *Diplodus holbrooki* (sparid fish) *Lytechinus variegatus* (sea urchin)	Scaridae Scaridae Sparidae Toxopneustidae	Yes. Similar relative to other algae.	No chemical defense activation following damage	Florida	(Cetrulo & Hay, 2000)
Roving herbivorous fishes	Acanthuridae Scaridae Siganidae	Yes.		GBR, Australia	(Jompa & McCook, 2002b)
Roving herbivorous fishes	Acanthuridae Scaridae Siganidae	Yes.		GBR, Australia	(Jompa & McCook, 2002a)
Supposedly: Acanthurids Scarids Pomacentrids Siganids	Acanthuridae Scaridae Pomacentridae Siganidae	Yes.		Rib reef, GBR, Australia	(Diaz-Pulido & McCook, 2003)
Siganus fuscescens	Siganidae	Yes. Low relative to other algae.			(Pillans *et al.*, 2004)
Roving herbivorous fishes *Acanthurus* spp. *Kyphosus vaigiensis* *Scarus rivulatus* *Siganus doliatus*	Acanthuridae Kyphosidae Scaridae Siganidae	Yes.		GBR, Australia	(Bennett *et al.*, 2010)
Mithrax sculptus *Echinometra viridis*		Yes. Medium-High		Laboratory exeperiments	(Heckman, 2011)
Salmacis sphaeroides	Temnopleuridae	Yes. Low relatively to other		Singapore	(Ng *et al.*, 2013)

(sea urchin) *Trochus maculatus* (sea snail)	Trochidae	algae.			
Herbivorous fishes Omnivorous fish *Canthigaster rostrata* *Canthurus coeruleus* *Sparisoma atomarium*	Scaridae Tetraodontidae	Yes.	No chemical defense.	Bahamian reefs	(Slattery & Lesser, 2014)
Damselfish farming					
Eupomacentrus planifrons	Pomocentridae	Yes. Cultivated.		Jamaica	(Brawley & Adey, 1977)
Stegastes apicalis	Pomocentridae	Yes. Cultivated.		Gulf of Thailand	(Kamura & Choonhabandit, 1986)
Stegastes apicalis	Pomocentridae	Yes. Cultivated.		GBR, Australia	(Klumpp & Polunin, 1989)
Stegastes adustus	Pomocentridae	Yes. Cultivated.		Fiji and Tonga	(Cardona & Clayton, 1999)
Pomacentrus wardii *Pomacentrus tripunctatus* *Segastes apicalis*	Pomocentridae	Substratum for palatable epiphytic algae.		North Queensland, Australia	(Ceccarelli *et al.*, 2005)
Stegastes	Pomocentridae	Yes. Cultivated.		Tonga	(Gobler *et al.*, 2006)
Pomacentrus wardii	Pomocentridae	Yes. Cultivated.		GBR, Australia	(Ceccarelli, 2007)
Stegastes rosacensis	Pomocentridae	Yes. Low relatively to other algae.		Brazil	(Souza *et al.*, 2011)
Segastes spp.	Pomocentridae	Inside territories		Brazil	(Feitosa *et al.*, 2012)
Chemical defense induction experiments					
Amphipods *Elasmopus basiliensis*		Yes. Low relatively to other algae.	Chemical defense: inducible defenses.	Rio de Janeiro, Brazil	(Weidner *et al.*, 2004)
Browsers fish		Yes. High.		Ningaloo Reef, Western Australia	(Vergés *et al.*, 2011)
Laboratory ichtyotoxicity experiments					
Xiphoster mucosus	Stichaeidae	Yes.	Phlorotannins: reduction of assimilation efficiency		(Boettcher & Targett, 1993)
No herbivore			Precipitation of proteins by pholorotannins.	Laboratory exeperiments	(Stern *et al.*, 1996)
Freshwater fish *Carassius auratus*	Cyprinidae	Not relevant.	Ethanolic, acetonic, aqueous extracts: ichtyotoxic	Laboratory exeperiments	(De Lara-Isassi *et al.*, 2000)
Habitat					
Mesograzer*				Australia New Zealand	(Taylor & Steinberg, 2005)
Epifaunal invertebrates*				Exuma Cays, Caribbean	(Roff *et al.*, 2013)
Panulirus argus juvenile*				Mexican Caribbean coast	(Briones-Fourzán & Lozano-Álvarez, 2001)

* *Lobophora* act primarily as habitat.

1.5. Discrepancies on *Lobophora* susceptibility

Overall, *Lobophora* is clearly susceptible to herbivory in spite of its leathery, tough thalli and richness in phlorotannins. Yet, different studies yielded several

contradictory observations on the susceptibility of *L. variegata* to various grazers. These discrepancies on *Lobophora* susceptibility to herbivory were interpreted until now as the chemical or morphological intraspecific variations, which are tributary to the environments and geographic location (depth, habitat, reef type, temperate vs. tropical). But studies on the subject yielded contradictory results on the role of defense against herbivory, and certainly do not make a strong case in favor of defense mechanisms as major strategies against herbivores.

Theoretically, differences in susceptibility can be interpreted as: (1) differential susceptibility between different herbivores, (2) presence of more preferred algae, and prominently although completely ignored until now as (3) interspecific variation. In fact, unaware of the species-richness of this genus, those previous studies have virtually only referred to the Caribbean species assigned as *Lobophora variegata*. However, *Lobophora* is a species-rich genus comprising 21 described species, and close to 80 more species yet to be described. Also the following questions are being raised: (1) are *Lobophora* species differentially edible, and if so (2) are those differences attributable to chemical and/or physical defenses, and finally (3) are those defenses intrinsic to a given species or relative to a given habitat.

This study precisely aims at testing interspecific variation in susceptibility to herbivory, and to propose alternative strategies against herbivore based on field observations.

2. Material and methods

2.1. Experimental design and study organisms

Grazing experiments were performed in the southwest lagoon of New Caledonia between the 16th and 18th of April 2014. Three series of grazing experiments were conducted, namely in situ, in the fish farm Aqualagon (Baie N'go, New Caledonia) and in aquariums at the Institut de Recherche pour le Développement (IRD) in Noumea.

2.2. *Lobophora* sampling

Seven *Lobophora* species commonly found in New Caledonia were selected to (1) test the consumption of *Lobophora* by different herbivores, and (2) to compare the relative preference for species that are polymorphic, with high interspecific polychemistry and occupying diverse ecological niches (Table 2.3.2; Vieira *et al.*,

2014b). *Padina* sp. was included in the *in situ* and fish farm experiments as a positive control. *Lobophora* samples were collected on the 16th of April 2014, kept in a cooler until treatment in the lab. Samples were then kept in a freezer until use for the grazing experiments.

Table 2.3.2. Description of the *Lobophora* species tested in the grazing experiments

	Morphology	**Thickness (µm)**	**Habitat**	**Substrate**
L. rosacea	Fasciculate, Decumbent	146.5 ± 16	Branching coral fields	Dead coral basal part
L. nigrescens	Stipitate	211.2 ± 8.2	Macroalgae beds	Bedrock, rock
L. monticola	Shelf-like	152.9 ± 24.4	Branching coral fields	Dead coral basal part, live coral branches
L. hederacea	Shelf-like	188.6 ± 26.1	Branching coral fields	Dead coral basal part, live coral branches
L. undulata	Shelf-like	214 ± 52.3	Branching coral fields	Dead coral basal part
L. dimorpha	Procumbent	101.2 ± 12.8	Branching coral fields	Dead coral basal part
L. crassa	Crustose	291.6 ± 39.8	Shallow exposed reefs	Dead coral, coral rubble, bedrock, rock

2.3. *In situ* experiments

In situ experiments took place in different reefs in front of Noumea. Triplicates of 20 m lines were deployed at five different sites (Table 2.3.3). *Lobophora* thalli were alternatively inserted every 25 cm between strands of three-stranded polypropylene lines. We used ten replicates per species, resulting in 80 algal thalli per line. Lines were fixed horizontally by metal rods, at 1-m above the lagoon floor, and were left for 24 h.

Table 2.3.3. *In situ* grazing experiments sites information.

Reef name	**Reef type**	**Habitat**	**Depth (m)**	**Latitude**	**Longitude**	**Duration**
Crouy	Patch reef	Algae bed	2	22°21.114	166°21.084	48h
Larégnère	Islet reef	Sandy bottom	2	22°19.524	166°18.953	48h
Canard 1	Islet reef	Coral reef	2	22°18.840	166°26.266	48h
Canard 2	Islet reef	Coral reef	4	22°18.855	166°26.289	48h
Canard 3	Islet reef	Coral reef	7	22°18.858	166°26.317	72h
Senez	Patch reef	Coral reef	2	22°17.760	166°19.975	72h
Abore	Back reef	Coral reef	1	22°27.001	166°22.271	24h

2.4. Fish farm experiments

The grazing experiments in the fish farm were conducted in fish tanks (3 m^3) and in circular open-water fish cages (8 m in diameter x 6 m in depth) with a single species of rabbitfish, *Siganus lineatus* (Valenciennes, 1835), a common fish in New Caledonia and identified as a prominent herbivore in the GBR (Mantyka & Bellwood, 2007a). Similarly to the *in situ* experiments, *Lobophora* species were alternatively inserted in

three-stranded polypropylene lines. For the tank experiments, the lines were 1 m long and the samples were fixed 10 cm from each other. For the cage experiments, the lines were 5 m long and specimens were fixed every 15 cm. The lines were disposed vertically in the tubs and net pens. Grazing susceptibility of *Lobophora* was tested on juveniles and commercial size of *S. lineatus*, in the tanks (2 adult tanks, 1 juvenile tank) and the cage (2 juvenile cages, 1 adult cage).

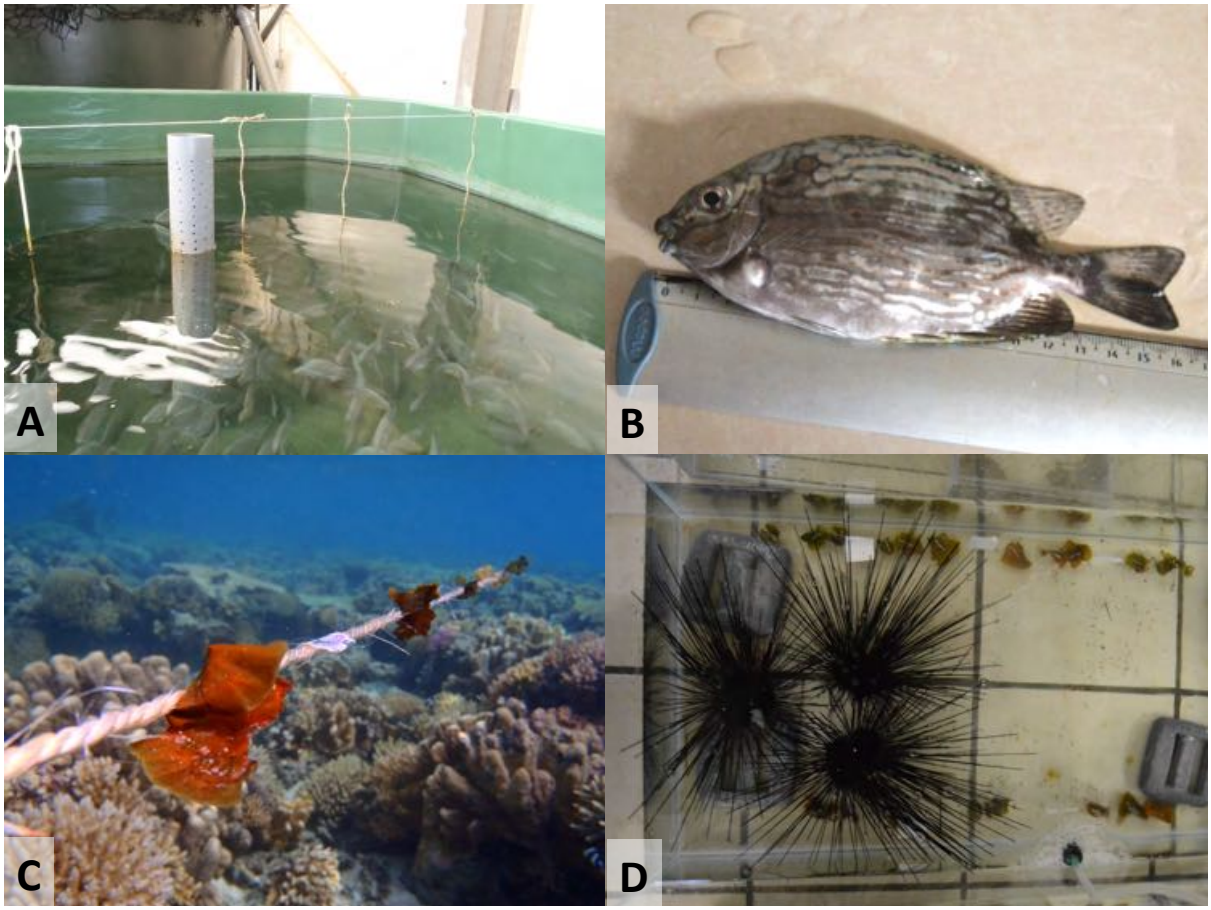

Figure 2.3.1. Pictures of the fish tank grazing experiments at Aqualagon fish farm (A), *Siganus lineatus* (B), *in situ* grazing experiment (C) and aquarium grazing experiment with *Diadema setosum* (D).

2.5. Aquarium experiments

Grazing experiments in the aquarium were conducted with the sea urchin *Diadema setosum* (Lesk, 1778), a common grazer in the Pacific tropical region. The seven *Lobophora* species were alternatively stapled along a nylon fishing line, and fixed on the aquarium walls. Nine lines (which represent nine replicates per species) were distributed in three aquariums, which represent a total of 21 *Lobophora* specimens (i.e. 7 species × 3 replicates) per aquarium. In each aquarium four individuals of sea

urchins were put together in order to limit the impact on the feeding behavior by reproducing their gregarious behavior observed in the field.

2.6. Algal consumption rates

To measure the biomass of alga consumed, we measured the algal dry-blotted weight before and after the grazing experiments to the nearest 0.001 g. Given the significant differences of thallus size and thicknesses, we also calculated the percentage of alga consumed. ANOVA's were performed on both, the consumed biomass and percentage. Results for each experiment (*in situ*, fish farm and aquarium) were pooled and averaged.

2.7. Statistical analyses

Normality of the grazing results was tested with the Shapiro-Wilk test. If the responses violated parametric assumptions, grazing results were evaluated using the Kruskal-Wallis H tests followed by Tukey honestly significant difference (HSD) post hoc comparisons test for significant Kruskall-Wallis findings. If data respected the parametric assumptions, a one-way ANOVA was performed followed by the Tukey post hoc HSD test for significant ANOVA findings. Statistical analyses were performed using the computing environment R (R Development Core Team, 2013).

3. Results

3.1. Fish farm grazing experiment

All *Lobophora* species were consumed by *S. lineatus*, with consumption ranging from 47.8 (*L. dimorpha*) to 158.3 mg (*L. monticola*) of algal material (Fig. 2.3.2A); and ranging from 38 (*L. undulata*) to 53 % (*L. rosacea*) of percentage of alga consumed (Fig. 2.3.2B). Significant differences in consumption by *S. lineatus* were observed among the seven species of *Lobophora* (Fig. 2.3.2A,B) (one-way ANOVAs, $p<$2e-16 (biomass) and $p=$9.92e-11 (percentage)). Considering the biomass consumed, no significant difference was observed between *Padina* sp. and *L. monticola,* nor between *L. crassa*, *L. hederacea*, *L. nigrescens, L. rosacea* and *L. undulata*. With an average of ca. 50 mg of biomass consumed, *L. dimorpha* stood out to be the least consumed by two orders of magnitude in comparison to the latter species, and by an order of three compared to *Padina* sp. and *L. monticola*. Although, still displaying significant differences, when considering the percentage of the alga consumed, no

outstanding differences appear between the different *Lobophora* species, with an average percentage of alga consumed close to 40 % (ranging from 37.2 to 41.2%) (Fig. 2.3.2A,B). *L. rosacea* is the only species slightly standing out with an average of 52.8%. When the lines were retrieved from the fish tanks and cages, we visually observed that practically all the entire thallus exposed to grazing was consumed for every species of *Lobophora* and *Padina*.

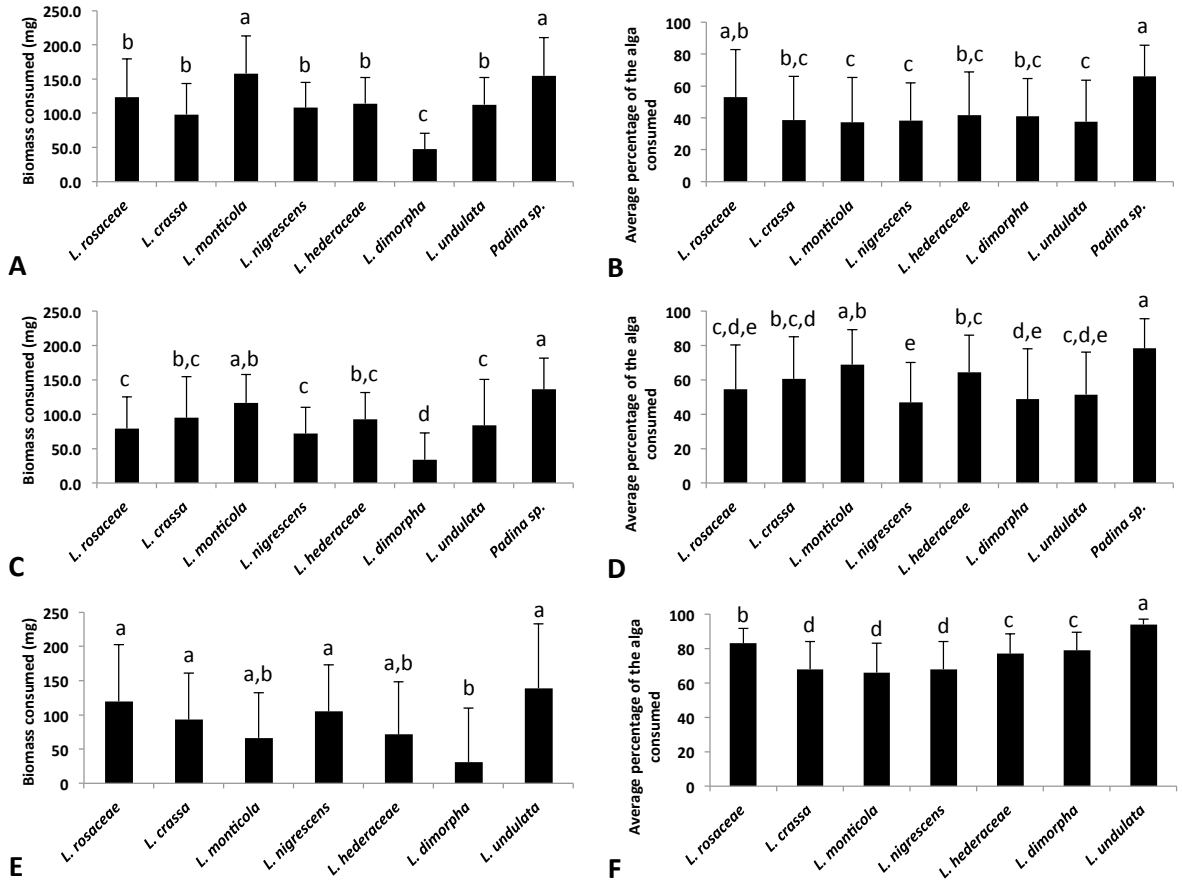

Figure 2.3.2. Grazing experiments results with seven *Lobophora* species in three grazing experiments: *in situ* (A, B), fish farm (C, D), and aquarium (E, F) experiments. Barplots represent the average biomass (A, C, E) and percentage (B, D, F) of alga consumed. Letters indicate distinct groupings based on post-hoc statistical comparison among *Lobophora* species. Error bars represent standard deviation of the mean.

3.2. *In situ* grazing experiments

All *Lobophora* species were consumed during the *in situ* experiments, with a consumption ranging from 33.7 (*L. dimorpha*) to 116.1 mg (*L. monticola*) in biomass (Fig. 2.3.2C); and ranging from 47 (*L. nigrescens*) to 69 % (*L. monticola*) in percentage of alga consumed (Fig. 2.3.2D). Significant differences in consumption were observed for *in situ* grazing experiments among the seven species of *Lobophora* (one-way ANOVAs, $p<2e\text{-}16$ (biomass consumed) and $p=5.27e\text{-}14$ (percentage

consumed)). Comparatively to the fish farm experiment, differences in the consumption between the different *Lobophora* species were not outstanding (Fig. 2.3.2D).

3.3. Aquarium grazing experiments

All *Lobophora* species were consumed by the sea urchin *D. setosum*, with a consumption ranging from 31.2 (*L. dimorpha*) to 119.7 mg (*L. rosacea*) of biomass consumed (Fig. 2.3.2E); and ranging from 66 (*L. monticola*) to 83 % (*L. rosacea*) of alga consumed (Fig. 2.3.2F).
Significant differences in consumption (Fig. 2.3.2E, F) were observed among the seven species of *Lobophora* (one-way ANOVAs, p=5.27e-14 (biomass consumed) and p=5.27e-14 (percentage consumed)).
Although statistically significant, differences in consumption between the different *Lobophora* species were not outstanding (Fig. 2.3.2F). Furthermore, in at least one experiment, every *Lobophora* species was entirely consumed as visible by the standard deviation reaching 100% for every species (Fig. 2.3.2F), suggesting that *D. setosum* consumption of *Lobophora* species was indiscriminate.

4. Discussion

4.1. New insights: no interspecific differences

The present study assessed the susceptibility to herbivory of seven different species of *Lobophora* and presenting contrasting morphologies, chemical compositions (author's unpublished data) and ecologies. We experimentally forced the contact between algae and herbivores, which naturally would not necessarily be occurring (e.g. sheltering, presence in algae beds). Results of the present grazing experiments showed that two important herbivores in New Caledonia, the rabbitfish *S. lineatus* and the sea urchin *D. setosum*, consumed all the *Lobophora* species presented to them without outstanding significant interspecific differences. As stated within the introduction, ecological conclusions in terms of consumption should be carefully drawn from grazing experiments.

4.2. Retrospective on previous studies

Previous work on *Lobophora* susceptibility to herbivory have considered studying individuals from the same species (i.e. *L. variegata*) with intraspecific variations (i.e.

contrasting morphotypes, growing at different depth or location, polychemistry (Coen & Tanner, 1989; Vergés *et al.*, 2011)). However, in the light of the recent molecular studies which revealed a high species diversity in *Lobophora* (Vieira *et al.*, 2014b), these previous studies were most likely studying different species of *Lobophora* and thus were conducting interspecific experiments. These experiments yielded contrasting results. Our results are supporting those of Vergés *et al.* (2011), who did not find significant differences in the consumption of (obviously) two difference species of *Lobophora*. Coen and Tanner (1989) also observed similitudes in the consumption of apparently two different species (ruffled and crustose), which were both more consumed than a third (decumbent) species.
As stated earlier, difference in the consumption of *Lobophora* between locations could be attributed to differences in herbivore guild composition and diet.

4.3. Escape over defenses

Our results suggest that morphological differences between *Lobophora* species do not significantly affect *S. lineatus* and *D. setosum* food choice. Food preferences and susceptibility to feeding deterrents are species-specific. Therefore, while the presence of feeding deterring compounds may be inefficient on some herbivores it might not be the case on others. Admitting that chemical composition could actually affect edibility, the question remains if chemical adversity towards certain herbivores is species-specific or depends on the environment. Previous studies lean towards intraspecific rather than interspecific variations. In fact, several studies have shown spatial and seasonal variation in the content of certain compounds, e.g. bromophenols (Chung *et al.*, 2003), polyphenols (Arnold *et al.*, 1995), phlorotannins (Targett *et al.*, 1995). While it remains to be demonstrated if previously tested individuals did not belong to different species, temporal differences would strongly support intraspecific variation. However, chemical defense has only been speculated until now, and no studies have yet explicitly demonstrated toxicity of *Lobophora* secondary metabolites against herbivores.
This leads us to dispute that while chemicals and morphological defenses have been suggested previously as strategies against herbivores, they may eventually play a limited role as a strategy against herbivory in the case of *Lobophora* species. Alternatively, and largely overlooked until now, escape would appear to be a major strategy against herbivores. We presently argue that species from the genus *Lobophora* adopt two major escape strategies, namely (1) spatial escape and (2) associational escape as chief stratagems against herbivory.

A review of the diversity of the genus in New Caledonia, showed that *Lobophora* species presented distinct habitat and substratum preferences (e.g. bedrocks, coral rubbles, live and dead corals) (Vieira *et al.*, 2014b).

4.4. Spatial escapes or refuges

Spatial escape has already been evidenced by De Ruyter van Steveninck and Breeman (1987a) who showed that *Lobophora* abundance was negatively correlated with *Diademum antiallarum* density. Therefore, in Curaçao, the erect golden-brown *Lobophora* species is finding refuge from herbivores in deep waters. In New Caledonia, *L. crassa* is mainly found in shallow wave-washed habitats consisting of bedrock, rocks, coral rubbles. *L. crassa* has thick blades and adheres strongly to the substratum, which is characteristic of intertidal populations and considered to be adaptations to increased water motion and desiccation (Norton *et al.*, 1981). In this habitat, herbivore presence is limited due the high hydrodynamism. Consequently, the presence of *L. crassa* is seen as a spatial escape from herbivores.
L. hederacea, L. monticola, L. undulata and *L. rosacea* are commonly found associated to branching corals and notably the genera *Acropora, Montipora, Porites, Stylophora, Pocillopora* and *Seriatopora* (Diaz-Pulido *et al.*, 2009; Bennett *et al.*, 2010; Vieira *et al.*, 2014b). The species *L. rosacea* has a ruffled form and is niched intermingled between coral branches. The other *Lobophora* species are usually decumbent, attached by their basal part to coral branches, or form crusts predominatly at the basal part of the coral branches, where access by large herbivores is difficult. In the Great Barrier Reef, populations of *Lobophora* growing within branching *Acropora* were less consumed than populations located in planar habitats, suggesting that branching corals act as a refuge for *Lobophora* from herbivores (Bennett *et al.*, 2010). Jompa and McCook (2002b) also concluded that the coral *Porites cylindrica* structure provides a refuge for *Lobophora* from herbivory. The refuge role played by branching corals is furthermore supported by the rare presence of *Lobophora* with other coral forms in the same habitat (personal observations).

4.5. Associational escapes

Numerous authors have suggested that palatable prey may typically be protected from consumers by living in association with less preferred prey (Poore & Hill, 2005). *L. nigrescens sensu* Sun *et al.* (2012) usually grows in sand-covered habitats,

characterized by low grazing intensity, amidst other algae usually comprising *Turbinaria* and *Sargassum*, both tough spiky and upright brown algae, which are less edible because of morphological and chemical defenses (Bittick *et al.*, 2010). *Turbinaria ornata* has been previously reported to represent a herbivory refuge for associated algae (Hay, 1986; Bittick *et al.*, 2010). This escape is not only associational but also spatial, as algal beds outside coral reefs experience a low grazing intensity. *L. rosacea* presents two distinct ecotypes. It is either associated to branching *Acropora* or as an epiphyte to another *Lobophora* species, i.e. *L. nigrescens sensu* Sun *et al.* (2012).

5. Conclusion

While it has been suggested that *Lobophora* resists herbivory by relying primarily on chemical deterrents, evidence strongly suggests that *Lobophora* primarily escapes herbivores rather than investing in chemical and physical defense mechanisms. The diversity of forms and substrate preferences demonstrate the importance of escape from herbivory as a driving mechanisms behind the genus speciation.

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Acknowledgments

We are grateful to Aqualagon's owner, Frank Le Garrec, for letting us perform the grazing experiments in their fish farm, and to its crew, Renaud Condoya and Marie-Claude Qalue, for assisting us in the deployment of the experiments at the fish farm. We also thank Julie Gaubert and the IRD boat crew, Miguel Clarque, Samuel Tereua and Philippe Naudin, for assisting us in the deployment of the experiments in situ. C. Vieira is a PhD fellow of the University of Pierre and Marie Curie and Ghent University and is part of MARES, a Joint Doctorate programme selected under Erasmus Mundus coordinated by Ghent University (FPA 2011-0016).

Author contributions

CV, ODC and CP conceived and designed the study. CV, PL, TM carried out the grazing experiments. CV and PL carried out the analyses. CV wrote the manuscript. ODC, CP and TM commented on the manuscript.

Part 4. Microbial mediation in *Lobophora* – coral interaction?[6]

Publication title: Bacteria of the brown seaweeds *Lobophora* (Dictyotales, Phaeophyceae) induce strong and rapid coral bleaching in *Acropora muricata*

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Abstract

Numerous studies have addressed the mechanisms by which macroalgae may outcompete corals and a few recent studies highlighted the putative role of bacteria at the interface between macroalgae and corals. We question if the adversity of bacteria to corals is exclusive to coral-pathogenic kinds, by means of *in situ* bioassays. When grown for 24h bacteria isolated from the surface of *Lobophora*, a brown macroalga, were placed in direct contact on the branches of the coral *Acropora muricata* by means of marine agar patches. These bioassays resulted in severe bleaching. Sequencing results confirmed the presence of ten genera, some of which related to the pathogens involved in coral diseases, but others naturally associated to corals. Results suggest that regardless of taxonomic affinities, increased in density of any bacteria can be adverse to coral. Nevertheless, the microbial community associated to macroalgal surface may not represent a threat to corals, given a specific bacterial screening exerted by the alga, which is preventing monospecific bacterial proliferation.

Research note

Contrary to disturbed reef ecosystem where macroalgae often gain dominance over scleractinian corals, in healthy reefs macroalgae and corals maintain a stable coexistence (McCook et al. 2001). In the pursuit of deciphering the mechanisms by which macroalgae may outcompete corals, the first studies focused on effects directly attributable to the alga, e.g. overgrowth, shading, abrasion, recruitment barrier and allelopathic interactions(McCook *et al.*, 2001). The concept of holobiont initially proposed for corals (Rohwer *et al.*, 2002) and more recently adopted for algae (Barott *et al.*, 2011) raised the awareness that the microbial component may play a significant ecological role in biotic interactions. A series of studies indicated (1) that macroalgae can act as reservoirs and vectors of coral pathogens (Nugues *et al.*, 2004b; Barott *et al.*, 2011; Wolf *et al.*, 2012a; Sweet *et al.*, 2013), (2) that macroalgal diffusible compounds can lead to changes in coral microbial assemblages resulting in coral vulnerability or even mortality (Smith *et al.*, 2006; Morrow *et al.*, 2011; Morrow *et al.*, 2012). Here, we question if adversity of alga-associated bacteria to coral is restricted to the coral-pathogenic species. To address this question we tested the effects of alga-associated culturable bacteria on coral's health.

We assayed the effects of the surface-associated bacteria isolated from two species of the genus *Lobophora* (Dictyotales, Phaeophyceae), *L. rosacea* and *L. monticola*, on

the Scleractinian coral *Acropora muricata*. To assess the putative role of these isolates in *Lobophora*-coral interactions, a technique was developed using monospecific bacterial inclusion culture to test microbial effects against hermatypic corals expressed in photosynthetic efficiency. Marine agar patches with 24h grown bacterial strain isolates were directly applied for 24h, *in situ*, on the coral branches of *A. muricata* colonies. Pulse Amplitude Modulated (PAM) fluorometry was used to assess the effects of bacteria on coral health (effective quantum yield). To assess the representativeness of the cultured bacterial strains, we sequenced microbial 16S rDNA extracted from the thallus surface of the two *Lobophora* species using next generation sequencing (NGS).

NGS results revealed the presence of 9809 MOTUs belonging to seven different genera from both *Lobophora* species. Sixteen strains were isolated and successfully cultured from the two species belonging to ten genera: *Bacillus*, *Erythrobacter*, *Microbulbifer*, *Muricauda*, *Paramoritella*, *Ruegeria*, *Shimia*, *Tenacibaculum*, *Thalassomonas*, and *Vibrio*. After 24h exposure, the surface area of the coral *Acropora muricata* in direct contact with each of the macroalgae-associated culturable bacterial patches, showed severe visual bleaching and an almost complete suppression of coral photosynthetic efficiency across all tested strains, with a relative average quantum yield decrease to 0.064 ± 0.051 (± S.D.), ($P < 0.001$) (Fig. 2.4.1). Nevertheless, coral tissue on which agar patches were applied was left intact.

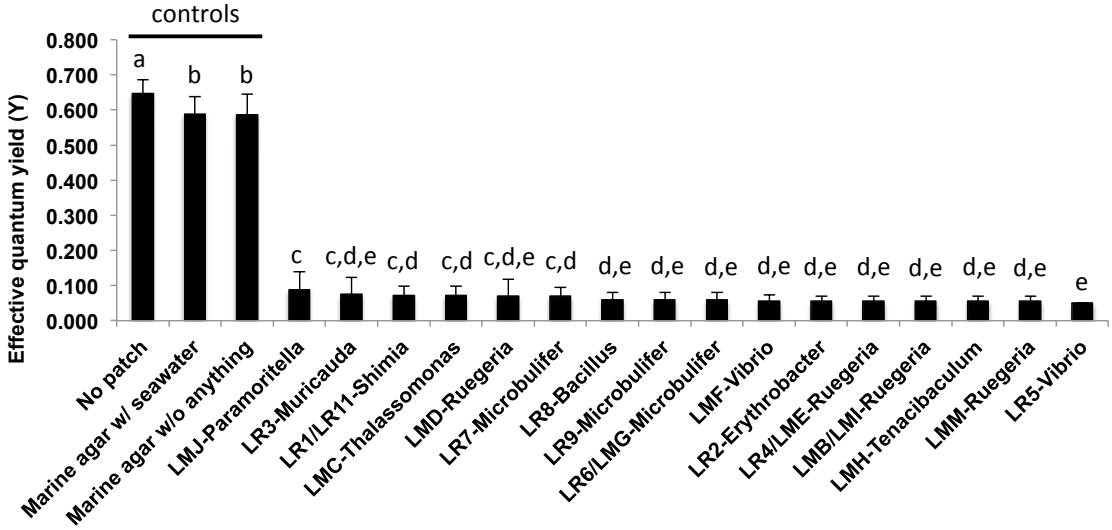

Figure 2.4.1. Barplot representation of the allelopathic bioassay results with the 16 strains isolated from *L. rosacea* and *L. monticola* on *A. muricata.* The statistical analyses, comparing the compounds-treated patchs to MeOH-treated patch and untreated controls, were performed using using one-way ANOVA and Tukey's HSD post-hoc test. Letters indicate distinct groupings based on post-hoc statistical comparison among sub-fractions. n=10 assays. Error bars represent standard deviation of the mean.

The present bioassay results evoke symptoms of "white" diseases, such as the white plague syndrome affecting massive and encrusting corals, the white band disease affecting *Acropora* spp. and the Acroporid white syndrome affecting *A. hyacinthus*. Among the bacterial genera isolated, four have been documented as coral pathogens or were found associated to coral diseases (Table 2.4.1).

Table 2.4.1. List of the strains isolated from *L. rosacea* and *L. monticola*, with the genus, family and phylum name, identified with the 16S ribosomal RNA sequences (Bacteria and Archaea) database using Megablast (optimize for highly similar sequences) in the NCBI BLAST website at http://blast.ncbi.nlm.nih.gov. * indicates the genera that have been documented as coral pathogens.

Strain voucher	**Isolated bacterial species**	**Family**	**Phylum**
LMB	*Ruegeria* sp.2*	Rhodobacteraceae	*α-proteobacteria*
LMC	*Thalassomonas* sp.*	Colwelliaceae	*γ-proteobacteria*
LMD	*Ruegeria* sp.3*	Rhodobacteraceae	*α-proteobacteria*
LME	*Ruegeria* sp.1*	Rhodobacteraceae	*α-proteobacteria*
LMF	*Vibrio* sp.2*	Vibrionaceae	*γ-proteobacteria*
LMG	*Microbulbifer* sp.1	Alteromonadaceae	*γ-proteobacteria*
LMH	*Tenacibaculum* sp.	Flavobacteriaceae	*Bacteroidetes*
LMI	*Ruegeria* sp.2*	Rhodobacteraceae	*α-proteobacteria*
LMJ	*Paramoritella* sp.	Moritellaceae	*γ-proteobacteria*
LMM	*Ruegeria* sp.4*	Rhodobacteraceae	*α-proteobacteria*
LR1	*Shimia* sp.1	Rhodobacteraceae	*α-proteobacteria*
LR11	*Shimia* sp.1	Rhodobacteraceae	*α-proteobacteria*
LR2	*Erythrobacter* sp.	Sphingonmonadaceae	*α-proteobacteria*
LR3	*Muricauda* sp.	Flavobacteriaceae	*Bacteroidetes*
LR4	*Ruegeria* sp.1	Rhodobacteraceae	*α-proteobacteria*
LR5	*Vibrio* sp.1*	Vibrionaceae	*γ-proteobacteria*
LR6	*Microbulbifer* sp.1	Alteromonadaceae	*γ-proteobacteria*
LR7	*Microbulbife*r sp.2	Alteromonadaceae	*γ-proteobacteria*
LR8	*Bacillus* sp.	Bacillaceae	*Firmicutes*
LR9	*Microbulbifer* sp.3	Alteromonadaceae	*γ-proteobacteria*

Results indicate that regardless of their taxonomic affinity, cultured macroalgal-associated bacteria are capable of bleaching corals. Smith *et al.* (2006) previously showed that macroalgal diffusible compounds enhanced the activity of coral- or seawater-associated bacteria, leading to coral mortality. These latter results support the idea that bacterial proliferation can generally be adverse to coral. Consequently, although it is true that macroalgae may harbor coral pathogens (Nugues *et al.*, 2004b; Barott *et al.*, 2011; Sweet *et al.*, 2013), bacterial adversity is not restricted to the pathogenic strains, but appears correlated to bacterial density. The natural presence of potentially pathogenic species within coral microbial communities (Barott *et al.*, 2011) supports the idea that adversity toward corals is linked to microbial

density. While any bacteria may potentially be adverse to corals, a combination of biotic (e.g. allelopathy) and abiotic (e.g. temperature) factors is regulating microbial composition and abundance on both the coral and the algae (e.g. Ritchie, 2006; Mao-Jones *et al.*, 2010; Stratil *et al.*, 2013). Comparably to corals (Ritchie, 2006), algae have the capacity to control the density of specific strains, which coexist in the algal surface biofilm (Barott *et al.*, 2011; Egan *et al.*, 2013).

Present results suggest that regulation is a key factor preventing microbial adversity towards corals. In healthy reefs, the microbial community associated to macroalgal surface may not represent a threat at the direct interface between macroalgae and corals. And although macroalgae may act as a pathogenic reservoir, it has clearly been reported as a pathogenic vector in only few cases (Nugues *et al.*, 2004b). Disruption in the coral or algal microbial community equilibrium, turning in favor of some bacterial strains, may result in a situation menacing corals. Future studies should be directed at exploring and clearly identifying factors susceptible to lead to microbial composition disruption and increase in density.

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Acknowledgments

We acknowledge Ricardo Rodolfo-Metalpa for assisting us during the experiments, as well as the IRD boat pilots, Miguel Clarque, Samuel Tereua and Philippe Naudin.

Author contributions

CV, ODC, CP and LG conceived and designed the study. LG and JG performed the bacterial isolation and culturing. CV, JG and FH carried out the bioassay experiments. AE carried out the NGS analyses. CV and JG carried out the statistical analyses. CV wrote the manuscript. LG, CP, ODC and AE commented on the manuscript.

Chapter 3: *Lobophora* diversity, distribution and diversification

Part 1. *Lobophora* diversity in New Caledonia[7]

Publication title: Towards an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia.

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[7] Published as an Original Article in Journal of Phycology

Abstract

Until the recent use of molecular markers, species diversity of *Lobophora*, an ecologically important brown algal genus with a worldwide distribution in temperate and tropical seas, has been critically underestimated. Using a DNA-based taxonomic approach, we re-examined diversity of the genus from New Caledonia in the Southwest Pacific Ocean. First, species were delineated using GMYC-based and barcoding gap approaches applied to a mitochondrial *cox*3 dataset. Results were subsequently confirmed using chloroplast *psb*A and *rbc*L datasets. Species delimitation analyses agreed well across markers and delimitation algorithms, with the barcoding gap approach being slightly more conservative. Analyses of the *cox*3 dataset resulted in 31 to 39 molecular operational taxonomic units, four of which are previously described species (*L. asiatica, L. crassa, L. nigrescens* s.l., *L. pachyventera*). Of the remaining MOTUs for which we obtained a representative number of sequences and results are corroborated across analyses and genes, we describe ten species *de novo*: *L. abaculusa, L. abscondita*, *L. densa*, *L. dimorpha*, *L. gibbera*, *L. hederacea*, *L. monticola*, *L. petila*, *L. rosacea*, and *L. undulata*. Our study presents a excellent case of how a traditional morphology-based taxonomy fails to provide accurate estimates of algal diversity. Furthermore, the level of *Lobophora* diversity unveiled from a single locality in the Pacific Ocean raises important questions with respect to the global diversity of the genus, the distributions and range sizes of the individual species, as well as the mechanisms facilitating co-existence.

1. Introduction

Contrary to substantial historical disagreement on the generic classification of the genus *Lobophora* J.Agardh (J.V.Lamouroux 1809, C.Agardh 1817, J.Agardh 1894, Papenfuss 1943, Womersley 1967), species-level taxonomy has been remarkably stable. Traditionally only three *Lobophora* species were recognized, with *L. variegata* (J.V.Lamouroux) Womersley ex E.C.Oliveira being by far the most commonly reported species. Literature data make it seem that *L. variegata* is widely distributed in temperate to tropical parts of the Atlantic (incl. Mediterranean Sea), Indian and Pacific Ocean. The other two species *L. papenfussii* (W.R.Taylor) Farghaly and *L. dichotoma* (R.H.Simons) P.C.Silva were only sporadically reported from the Indo-Pacific and South Africa respectively. From 2000 until 2012, three more species were described (*L. minima* V.Krishnamurthy and M.Baluswami (2000), *L. indica*

V.Krishnamurthy and M.Baluswami (2000) and *L. rickeri* Kraft (2009)), based on morphological criteria only.

From a molecular phylogenetic perspective *Lobophora* had not received much attention (but see Hoshina et al. 2004, Phillips et al. 2008, Bittner et al. 2008) until a recent study of Sun et al. (2012). The latter authors recognized nine major *Lobophora* clades based on chloroplast *rbc*L and mitochondrial *cox*3 gene sequences, four of which were formally described as new species (*i.e. L. asiatica* Z.Sun, Ji.Tanaka and H.Kawai*, L. crassa* Z.Sun, P.-E.Lim and H.Kawai*, L. pachyventera* Z.Sun, P.-E.Lim, Tanaka and H.Kawai*, L. australis* Z.Sun, Gurgel and H.Kawai). In total, 10 species are currently accepted taxonomically (Guiry and Guiry, 2013).

Despite the ecological importance of *Lobophora* in seaweed-coral-grazing interactions and competition (De Ruyter van Steveninck and Breeman 1987a,b, De Ruyter van Steveninck et al. 1988a,b,c, Coen and Tanner 1989, Diaz-Pulido et al. 2009, Rasher and Hay 2010, Anthony et al. 2011, Slattery and Lesser 2013), the species diversity of the genus remains largely unaddressed. Here we study the diversity of *Lobophora* in New Caledonia. New Caledonia is located just south of the coral triangle, recognized as the global center of marine biodiversity, and displays tropical to subtropical-temperate conditions. The *Lobophora* flora has been comprehensively sampled over the last decades from various regions and the large amount of material revealed a large morphological diversity associated to the ecological variation justifying the present study.

The paper of Sun et al. (2012) provided two important insights about the genus *Lobophora*, (1) the existence of a rich and yet to be discovered diversity and (2) the occurrence of cryptic diversity lacking distinctive morphological features between taxa.

Decisions on species concepts as well as the practical criteria to delimit species represent critical aspects for studies aiming to elucidate species level diversity (*e.g.* Harrison 1998, Agapow 2004). For algae it has long been recognized that diversity is often inadequately reflected in the organism's morphology. It is therefore not surprising that, coinciding with a growing ease to obtain molecular data, the latter have become the standard for delimiting algal species (see Alverson 2008; De Clerck et al. 2013; Leliaert et al. 2014). Accompanying a growing dependency on DNA sequence data in biodiversity assessment, a variety of approaches and algorithms have been proposed to detect discontinuities in genetic variation representative for species boundaries (*e.g.* Wiens and Penkrot 2002, Sites and Marshall 2004, Carstens et al. 2013). Since, species delimitation may be influenced by the gene information

content as well as the species delimitation method, we test species boundaries in *Lobophora* using three species delimitation methods, a General Mixed Yule Coalescent (GMYC) model (Pons et al. 2006, Fujisawa and Barraclough 2013), the Bayesian implementation of the GMYC model (Reid and Carstens 2012) and an Automated Barcoding Gap Discovery method (ABGD) (Puillandre et al. 2011). The combination of several molecular methods for species delimitation is becoming a reference to detect species boundaries and have been used in different taxonomical groups (Jörger et al. 2012 for sea slugs; Kekkoken and Hebert 2014 for moths; Cornils and Held 2014 for copepods; Alò et al. 2013 for fishes). To our knowledge it is the first time that such a combination is used for algae species delimitation.
Species delimitation is in the first place carried out using a mitochondrial *cox*3 dataset for which we had the most complete taxon sampling. To investigate up to which extent results were influenced by marker choice, analyses were repeated for chloroplast *rbc*L and *psb*A datasets, which contained less sequences per taxon compared to the *cox*3 dataset. Subsequently, we studied the morphology and ecology of the New Caledonian specimens to determine up to which extent the DNA-based species are morphologically and ecologically diverged.

2. Material and methods

2.1. Sampling

Lobophora specimens were collected from 41 locations in New Caledonia (Fig. 3.1.1). Most of New Caledonia was sampled, except for the remote Entrecasteaux reefs. Sampling sites included the southwest lagoon of Grande Terre (collections between 2004 and 2013), Isle of Pines (BIODIP, November 2005), the Loyalty Islands (BSM-Loyauté, March-April 2005), La Côte Oubliée (CORALCAL1, March 2007), the Chesterfield-Bellona-Bampton area (CORALCAL2, July 2008), Le Grand Lagon Nord (CORALCAL3, February 2009), and different sites along the north west and north east coasts of Grande Terre (CORALCAL4, November-December 2012). Sampling was carried out mainly by SCUBA from 3 down to 90m deep or by snorkeling and reef walking. The specimens were readily stored in a cooler and desiccated in silica gel for subsequent DNA extraction once at the laboratory. Specimens were dried and mounted on herbarium sheets and deposited at the IRD Herbarium of Nouméa (New Caledonia, IRD-NOU). For the earliest collections, dry Herbarium specimens were used as DNA source. The New Caledonia samples were complemented with a few collections from Papua New Guinea (Madang 2012) and

the Maldive Islands (2011). The origin of the specimens and accession numbers are detailed in Table S3.1.1.

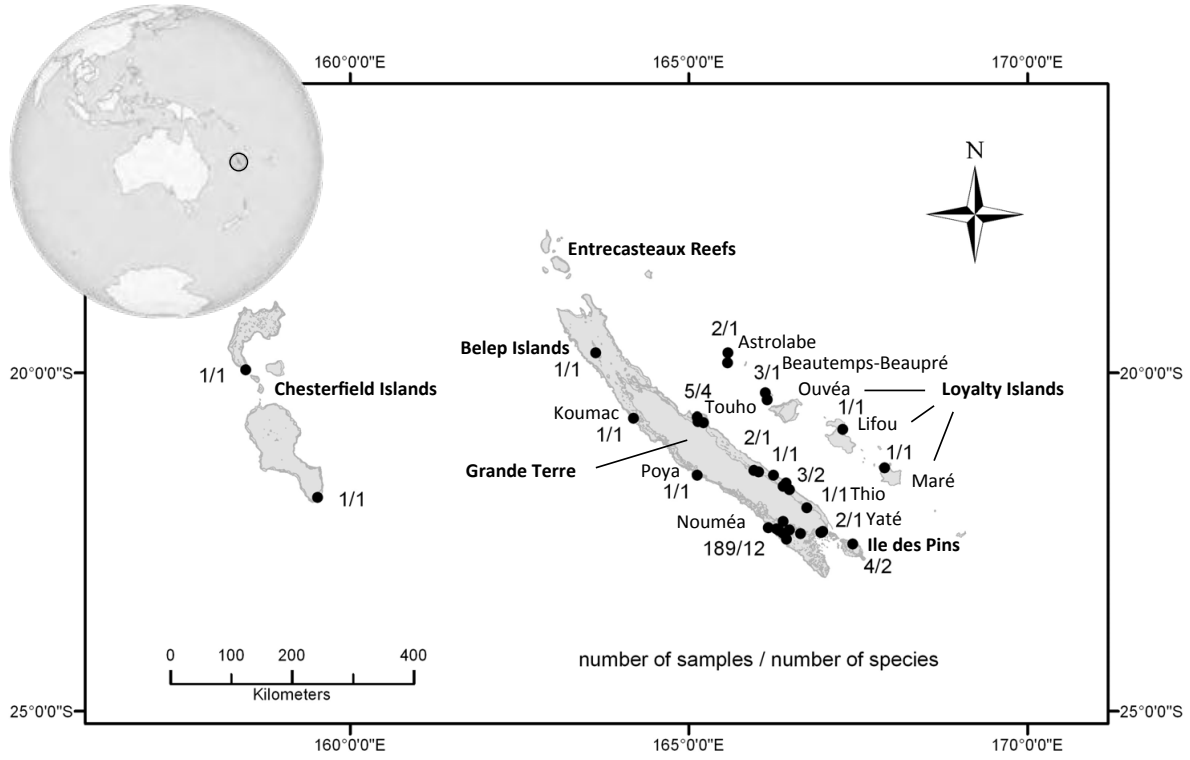


Figure 3.1.1. Map showing the sampling sites of *Lobophora* specimens around New Caledonia with indication of the sampling effort and number of species collected.

2.2. DNA extraction, amplification, sequencing and phylogenetic analyses

Total genomic DNA was extracted from 235 *Lobophora* samples, 228 from New Caledonia, 5 from Papua New Guinea and 2 from the Maldive Islands using a CTAB-extraction method (De Clerck et al. 2006). Genomic DNA was subsequently purified with a Wizard® DNA Clean-Up System (Promega Inc., Madison, WI, USA) following the manufacturer's instructions. Sequences were generated from one mitochondrial gene (*cox*3), two chloroplast genes (*psb*A, *rbc*L) and the 5'-end of the nuclear encoded large subunit rDNA (LSU, ca. 1200 bp). PCR and sequencing conditions are detailed in Table S3.1.2. LSU sequences were not tested for species delimitation because of the low number of sequences obtained, but were integrated in the concatenated alignment to generate a species tree with improved resolution. In addition to the sequences generated in the present study, 25 *cox*3, 4 *psb*A, 33 *rbc*L and 6 LSU *Lobophora* sequences from GenBank were added to the alignments (Table S3.1.1). Sequences were aligned using MUSCLE implemented in eBioX 1.5.1 (www.ebioinformatics.org). Ambiguously aligned regions in the LSU alignment were

removed by eye.

2.3. Species delimitation

Following exploratory ML and Bayesian analyses (results available upon request), ultrametric gene trees were constructed using Bayesian analyses in BEAST v1.7.5 (Drummond et al. 2012) for the *cox*3, *rbc*L and *psb*A alignments. A GTR+G substitution model was identified as the best-fitting model for each individual gene, based on the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba et al. 2012). BEAST analyses were run under a strict molecular clock in combination with a Constant Coalescent tree prior. Other priors were set to default. In order to check for convergence of the MCMC chains, we performed two independent runs for 10^7 generations each, starting from random trees and sampling every 10^4 generations. MCMC output files of the independent runs were inspected in Tracer 1.5 (Rambaut and Drummond 2009) for acceptable effective sample sizes (ESS $>$ 200). A burn-in was applied once log-likelihood values had stabilized. Maximum clade credibility trees and posterior probability for the nodes were calculated using the postburnin trees using TreeAnnotator 1.6.2 (included in the BEAST package). All tree searches were conducted on the Cipres web portal (Miller et al. 2010).

We used a Maximum Likelihood (GMYC) as well as a Bayesian Implementation (bGMYC) of the GMYC model (Pons et al. 2006; Reid and Carstens 2012). Both methods are able to discriminate between population and speciation patterns on a given ultrametric tree. GMYC analyses under a single-threshold were conducted in R (R Core Team, 2014) using the package "Splits". The bGMYC model was performed using "bGMYC" (Reid and Carstens 2012) in R using a subsample of 100 trees from the posterior distribution of BEAST as suggested by the authors. Markov chain Monte Carlo (MCMC) chains were run for each tree for 10,000 generations with a burn-in comprising the first 1,000 generations once the log-likelihood values had stabilized, and sampling every 100 generations.

Automatic Barcode Gap Discovery (ABGD, Puillandre et al. 2012) is an exploratory tool based on pairwise distances to detect automatically significant difference in intra and inter specific variation (*i.e.* barcoding gap), without an *a priori* species hypothesis. These analyses were performed on the abgd website (wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. Accessed 2013 October 12) selecting default parameters except for the relative gap width (X) which was set to 1 and the number of steps which was set to 100. The distance matrix was build under a K2P model.

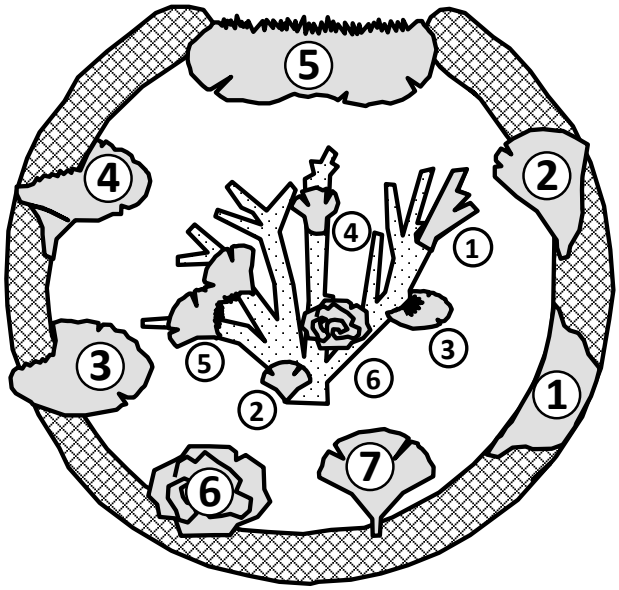
Species boundaries were subsequently defined based on the congruence of the three methods and are detailed in the discussion.

2.4. Species tree inference

Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic species trees were generated from a concatenated alignment including *cox*3 (610 bp), *psb*A (919 bp), *rbc*L (1360 bp) and LSU rDNA (1361 bp) genes, partitioned by gene and codon position. The concatenated alignment contained a single representative per Molecular Operational Taxonomic Unit (MOTU) resulting from the species delineation analyses of the *rbc*L dataset. The matrix was 70% filled at the MOTU level. A selection of *Zonaria* C.Agardh (Dictyotales, Phaeophycea), *Padina* Adanson (Dictyotales, Phaeophycea) and *Dictyota* J.V.Lamouroux (Dictyotales, Phaeophycea) species were used as outgroup taxa (cf. Table S3.1.1). ML analyses were conducted using RAxML under a GTR+CAT model (Stamatakis 2006). The robustness of the resulting phylogenies was tested using 1000 replicates of a rapid bootstrap heuristic (Stamatakis et al. 2008). BI, using MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003), initiated with a random starting tree and ran four chains of MCMC iterations simultaneously for 100 million generations. The first 100,000 (25%) trees sampled were discarded as burn-in, based on the stationarity of $\ln L$ as assessed using Tracer version 1.5 (Rambaut and Drummond 2009). A consensus topology and posterior probability values were calculated from the remaining trees.

2.5. Morphological and ecological analyses

Morphological observations of *Lobophora* species included analyses of the external and internal (anatomy) structure of the specimens. Based on our field observations we distinguished the occurrence of seven main growth forms, namely (1) stipitate, (2) fasciculate, (3) conk-like, (4) decumbent, (5) anastomosing, (6) procumbent and (7) crustose as illustrated and defined in Fig. 3.1.2.



(1) Crustose: growing firmly attached to the substratum
(2) Procumbent: growing along the substratum without setting forth rhizoids except at the basal part, loosely attached;
(3) Conk-like*: growing perpendicularly to the substratum (horizontally or vertically oriented) comparable to conks (shelf-like mushroom)
(4) Decumbent*: basal part attached to the substratum, and rest of the thallus or distal part curving upward, not in contact with the substratum.
(5) Anastomosing*: basal part attached to an elevated substratum and capacities of bridging out to another close elevated substratum and attaching to it by rhizoids, either as a single thallus or by anastomosis (attachment of one thallus to another)
(6) Fasciculate: a cluster of erect, ruffled thalli forming a rosette.
(7) Stipitate: erect thalli, attached by a clear stipe.
*** Shelf-like forms**

Figure 3.1.2 Schematic representation of the various growth forms discerned in *Lobophora*, with the circle representing the substratum. The center of the picture dipicts the various *Lobophora* growth forms on live or dead coral.

For the internal morphology, longitudinal and transverse sections were made of the basal, middle and distal portions of the thallus using a medical freezing portable microtome (Labonord®). Photographs of the sections were taken with a digital camera (Olympus Camedia C-5050 5.0 Megapixel, Tokyo, Japan) attached to a compound microscope (Olympus BH-2, Tokyo, Japan). The number and size of the cortical (dorsal and ventral) and medulla cells of the basal, middle and distal portions of the thallus were measured as shown in Fig. 3.1.3, which resulted in the measurements of 9 anatomical traits (*i.e.* number of dorsal and ventral cells; total number of cells; thallus thickness; dorsal, medullar and ventral heights; medullar width and length).

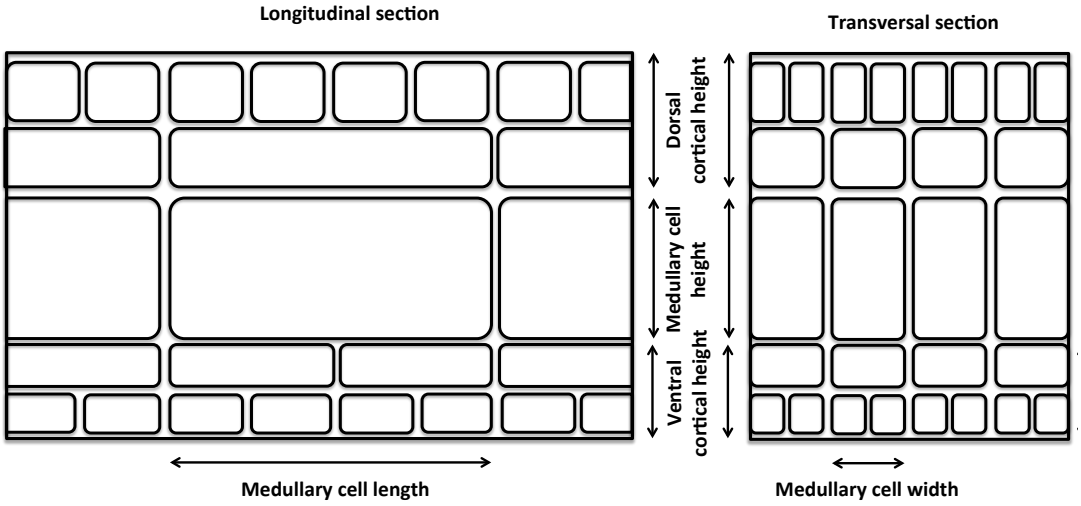


Figure 3.1.3 Schematic representation of a longitudinal and a transverse section of *Lobophora*, illustrating the anatomical characters.

The surface of the thallus with rhizoids was defined as the ventral surface. A total of 285 specimens, from one to 15 specimens per species, were examined for morphological analyses. Every specimen studied morphologically has been sequenced for at least the *cox*3 marker. A few sequences which were too short were not included in the molecular analyses. Descriptive statistics were generated for the anatomical traits and correlations between them were tested to select independent traits for subsequent univariate analyses. Mean anatomical traits were tested for equality by a one-way ANOVA and post-ANOVA Tukey Honestly Significant Difference (HSD) tests. The data were tested for normality and homogeneity of variances by means of a Shapiro-Wilk test of normality and the Bartlett test of the homogeneity of variances. The thickness data were log-transformed prior to analysis, to meet assumptions of normality and homogeneity of variance. All analyses were conducted using R. Ecologically, we identified three major substratum preferences in the field specific to some groups of species: (1) niched among or growing on live corals, (2) growing at the base of live corals, on dead corals, coral rubbles or bedrock and (3) growing niched among *Sargassum* beds.

3. Results

3.1. Species delimitation

Species delimitation based on the *cox*3 alignment (610 bp x 210 sequences) using GMYC under a single threshold resulted in an estimate of 37 for MOTUs, with a confidence interval of 36-49 (Fig. 3.1.4). The number of specimens per MOTU ranged from 1 (singletons) to 45 with an average of 6.5. bGMYC analysis of posterior probabilities of conspecificity within *cox*3 *Lobophora* clusters was high ($P > 0.9$) and resulted in a species delimitation which was marginally less conservative than GMYC, differing in 2 cases only (Fig. 3.1.4): IRD10187 was resolved as a singleton (prob. 0.59), d271 and d6625 were resolved as a separate cluster (prob. 0.648). The ABGD approach is slightly more conservative, grouping four MOTUs that were split in both GMYC analyses (Fig. 3.1.4).

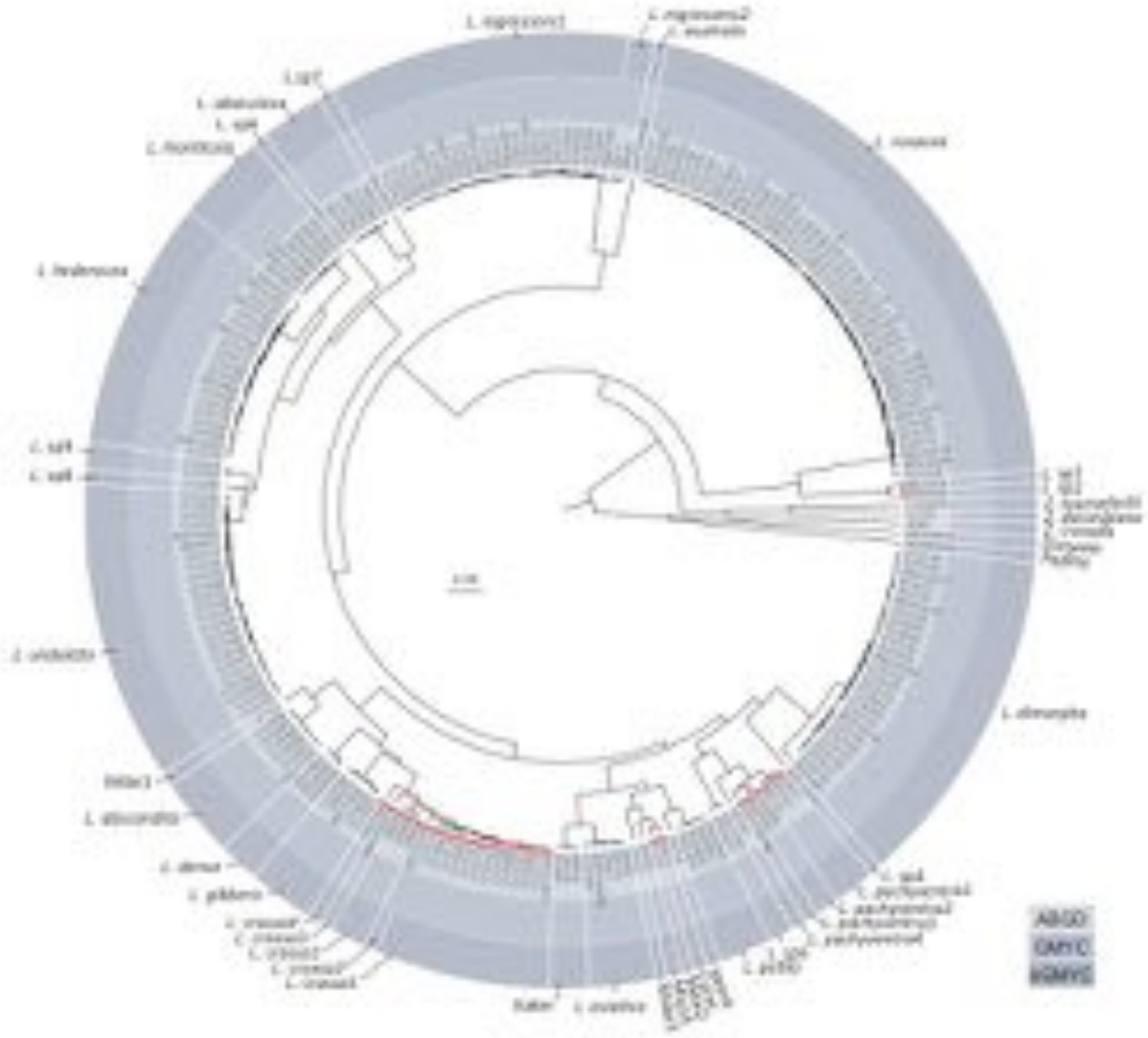


Figure 3.1.4. Results of the three species delimitation methods based on the *cox*3 data set. Species delimitation results of ABGD (inner), GMYC (middle), and bGMYC (outer) are represented by three concentric circles. The tree is the maximum clade credibility tree obtained from BEAST. Red lines and asteriks indicate conflicting results between ABGD, GMYC-based methods and both GMYC-based methods respectively.

Species delimitation analyses were repeated for *rbc*L (1345 bp x 139 sequences) and *psb*A (919 bp x 88 sequences) datasets to investigate if the *cox*3 results were stable across genes. In all analyses the likelihood of the GMYC model was significantly higher ($p < 0.001$) than that of the null model of uniform coalescent branching rates. GMYC analyses of *rbc*L data yielded (40-) 47 (-54) MOTUs while the *psb*A data resulted in (17-) 19 (-34) MOTUs (Table 3.1.1).

Table 3.1.1. Comparison of species delimitation analyses

	*cox*3			*rbc*L			*psb*A		
	ABGD	GMYC	BGMYC	ABGD	GMYC	bGMYC	ABGD	GMYC	bGMYC
Lineages	31	(36-) 37 (-49)	39	47	(40-) 47 (-54)	47	19	(17-) 19 (-34)	19
Singletons	14	18	19	27	27	27	7	7	7
number (percentage) of *cox*3 lineages absent[1]	NA	NA	NA	2	2	2	19	19	19
number of *rbc*L lineages absent	9	9	9	NA	NA	NA	25	25	25
incongruence with *cox*3 ABGD	-	-	-	6	6	6	-2	-2	-2
incongruence with *cox*3 bGMYC	-	-	-	-1	-1	-1	-2	-2	-2

[1]Lineages absent for the other markers.

Contrary to the *cox*3 dataset, no incongruence between the various delimitation methods was detected. Unequal sampling across markers complicates a detailed comparison of results from different markers, but even without a fully congruent sampling it was clear that the outcome of the analyses was stable across genes (Table S3.1.3). Six MOTUs from the *cox*3 ABGD analysis were subdivided in less inconclusive units in the *rbc*L dataset. All but one of the *cox*3 bGMYC MOTUs on the other hand were confirmed in the *rbc*L dataset. Data from the *psb*A dataset are less informative because of the high number of missing MOTUs (47%), but of the *cox*3 ABGD MOTUs present two are subdivided and one is merged with another MOTU. Similarly, two *cox*3 bGMYC MOTUs are merged. In addition, inclusion of Genbank accessions in the *rbc*L dataset yielded 9 additional MOTUs, which were not represented in either the *cox*3 or *psb*A dataset. This resulted in the *rbc*L gene alignment being the most diverse in terms of MOTUs, but with a significantly higher number of singletons than *cox*3.

3.2. Morphological and ecological characters

The morphology and ecology of the specimens from New Caledonia were studied to determine up to which extent the MOTUs are morphologically and ecologically diverged. For practical reasons we introduce names of newly described species already in the sections below. Results and interpretations of correlation analyses between the nine anatomical characters measured are given in the supplementary text (Table S3.1.4). Boxplots were used to show inter- and intra-specific variation of six anatomical traits (thallus thickness; dorsal and ventral height; medulla height, width and length) (Fig. 3.1.5).

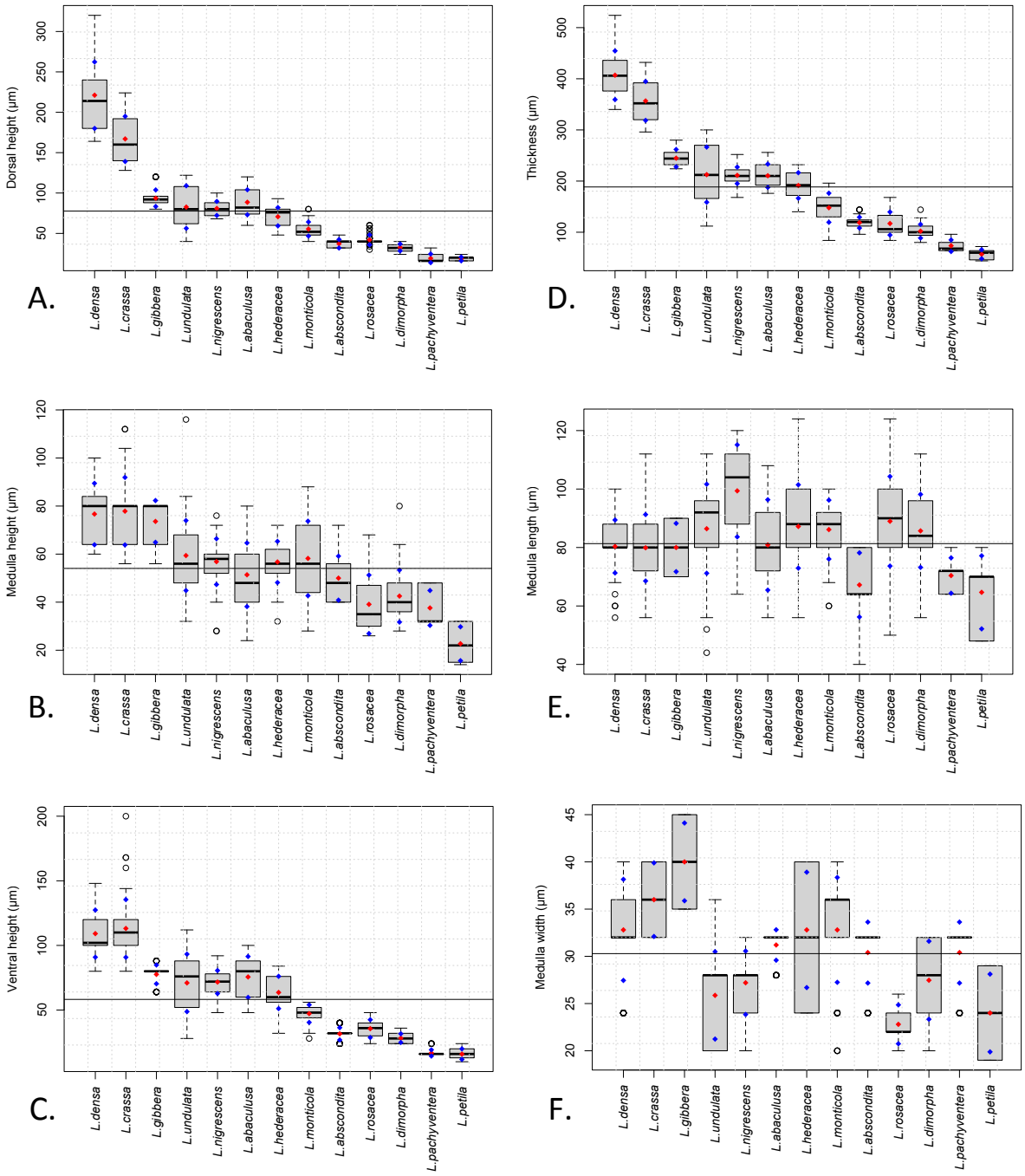


Figure 3.1.5. Boxplots of anatomical variables of New Caledonian *Lobophora* species ; rectangles and whiskers bound 25-75 percentiles, respectively, horizontal lines show the median, circles are extreme values, red and blue points show the mean standard deviation respectively.

Anatomical characters related to cell height differed significantly among species as well. On the other hand cell length and width displayed some variation but were overall less diagnostic. Among the three independent anatomical traits (*i.e.* thallus thickness, medulla width and length), the thallus thickness presented the most significant interspecific variability and was therefore retained as the only variable for the ANOVA analysis. The thallus thickness ranged from an average of 57 µm for the

thinnest species (*L. petila*) to 407 µm for the thickest species (*L. densa*). A continuous grade from these two extreme values was observed and the thickness of several species overlapped. The amount of intraspecific variation differed, with the thicker species presenting a greater variability. A one-way ANOVA analysis (Table S3.1.4) revealed statistically significant differences and subsequent post-hoc analyses (Tuckey HSD) (Table S3.1.5) confirmed significant difference between the species thallus thickness means. Seven species presented unique means and distribution (*L. densa, L. crassa, L. gibbera, L. hederacea, L. monticola, L. pachyventera* and *L. petila*) and three groups of species exhibited neighboring mean values with comparable variances (Fig. S3.1.1). Consequently, thallus thickness may serve to identify seven New Caledonian species but for some groups of species does not suffice to go down to the species level delineated with the phylogenetic approaches. However, for those 3 groups with similar thickness, external morphology and ecology allow species differentiation (see below).

3.3. Species phylogeny

ML and BI analyses of the concatenated alignment (*cox*3 + *rbc*L + *psb*A + LSU) including every MOTU discovered in the species delimitation analyses, yielded similar tree topologies except for the relationships between the MOTUs 29 to 32, and the MOTUs 45 to 47. Results are presented using the BEAST ultrametric tree topology (Fig. 3.1.6). The 4-genes analyses resulted in a fairly well-resolved phylogeny with moderate to strong support for most nodes. The phylogenetic tree revealed 6 well-supported lineages (defined as a sequence of species or MOTUs; Lineage A-F) (Fig. 3.1.6). However, the position of the MOTU 46 from Guadeloupe, for which only the *rbc*L sequence is available, is incongruent between the trees. In the BEAST and ML trees MOTU 46 is part of the Lineage A (Fig. 3.1.6 and S3.1.2), while it comes outside of the Lineage A, in the most basal position, in the Bayesian tree (Fig. S3.1.3). This inconsistency may be resolved by acquiring extra sequences for the missing markers, and for the time being we will consider it as part of the Lineage A.

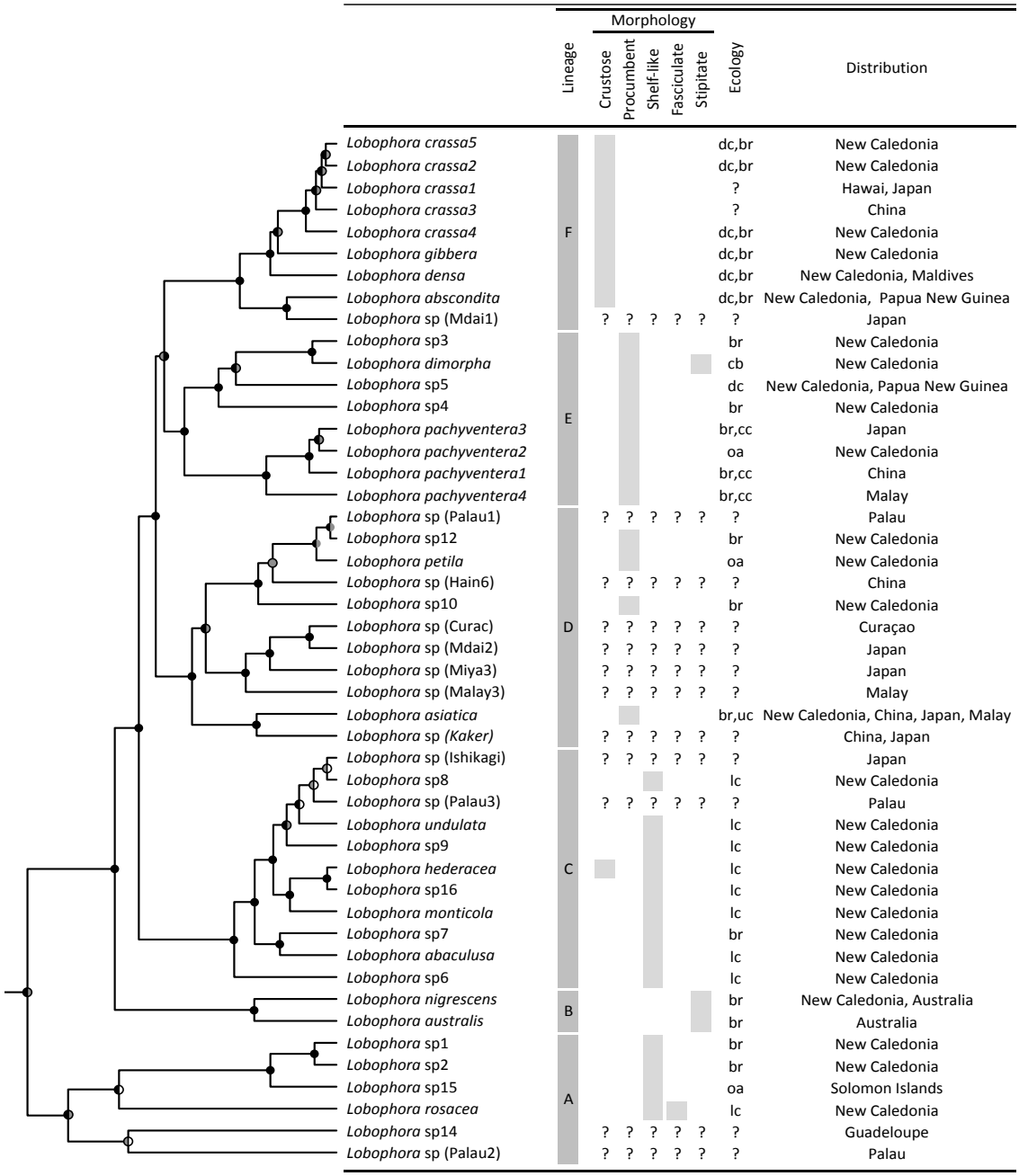


Figure 3.1.6. *Lobophora* species tree with indication of morphological and ecological characteristics as well as the distribution of the MOTUs as presently known. Species represent the MOTUs resulting from the species delimitation analyses. The tree is the maximum clade credibility tree obtained from a BEAST analysis of the concatenated alignment of four genes (*rbc*L, *cox*3, *psb*A, and LSU). The values shown at each node represent Bayesian posterior probabilities (left part of the circle) and ML boostrap values (right part of the circle) respectively. High support (posterior probabilities >0.95 and bootstrap values >0.9) is indicated in black, while low support (posterior probabilities <0.95 and bootstrap values <0.9) is indicated in gray. No color indicates configuration incongruence between the Bayesian and the maximum likelihood trees. Ecological codes: br, bedrock; cb, coral base; cc, crustose coralline algae; dc, dead coral; lc, live coral; oa, with other algae; uc, unhealthy coral.

4. Discussion

4.1. Species diversity and taxonomy

In this study we aimed to characterize the diversity of the genus *Lobophora* in New Caledonia in the South West Pacific Ocean and subsequently address the evolutionary relationships of the New Caledonian representatives. Thereto, we applied the most comprehensive sampling of the genus to date. Although expecting some levels of cryptic or pseudocryptic diversity, much to our initial astonishment *cox*3 species delimitation analyses yielded between 31, 37 and 39 MOTUs based on ABGD, GMYC and bGMYC analyses, respectively. Both GMYC-based methods were highly congruent. The bGMYC analyses segregated one specimen (IRD10187) from *Lobophora crassa*2. Likewise, d271 and d6625 were segregated from *L. nigrescens* s.l. Both results, however, were only moderately supported in the bGMYC analysis, with posterior probabilities of 0.591 and 0.648 respectively. The barcoding gap method yielded a more conservative estimate, but most discrepancies were limited to the *L. crassa* and *L. pachyventera* complexes as defined by Sun et al. (2012) and discussed below.

Subsequent analyses of *rbc*L and *psb*A dataset were highly congruent with the GMYC and bGMYC results and indicated that the ABGD estimate of the *cox*3 dataset is likely somewhat over-conservative (Table 3.1.2 and Table S3.1.6). Possibly the small sample size of some MOTUs may result in larger units as identified by the barcoding gap approach (Jorger et al. 2012, Puillandre et al. 2012). We identified one case in which the *cox*3 GMYC analyses were too conservative (SAP109520) compared to *rbc*L results, and one case in which they were too liberal (IRD10187). In both situation a single specimens was either added to or segregated from a MOTU.

Our analyses disclosed the occurrence of 29 MOTUs in New Caledonian. These results confirm findings by Sun et al. (2012) of undescribed species diversity in *Lobophora.* Species boundaries as defined by Sun et al. (2012) of *L. asiatica, L. nigrescens* sensu Sun et al. (2012) (subsequently referred to as *L. nigrescens* s.l.) and *L. australis* are mirrored by our species delimitation. However, their species delineation appeared to be more conservative for *L. crassa* and *L. pachyventera*. GMYC and bGMYC analyses split the *L. crassa* and *L. pachyventera* complexes into five and four MOTUs respectively for *cox*3 (Fig. 3.1.4). In the *L. crassa* complex the New Caledonian specimens were resolved as separate MOTUs, *L. crassa*2, *L. crassa*4 and *L. crassa*5. Likewise, in the *L. pachyventera* complex the New Caledonian specimens were resolved as a separate MOTU, *L. pachyventera*2. However, it should

be noticed that the *cox*3 ABGD results group the *L. crassa* MOTUs and *L. pachyventera1*, *L. pachyventera2* and *L. pachyventera3* in two clusters only. Four of the New Caledonian MOTUs, were assigned to existing species or species complexes (*Lobophora crassa*, *Lobophora asiatica, Lobophora pachyventera* and *L. nigrescens* s.l.). In addition, none of our samples matched the descriptions of the four *Lobophora* species for which no molecular data are available (*i.e. L. variegata, L. dichotoma*, *L. rickeri*, *L. papenfussii*). The remaining MOTUs could therefore qualify as putative species.

Decisions as to which of these putative new species should be described *de novo* are based on the availability of a representative set of specimens for a single MOTU and congruence between the various species delimitation algorithms. In this we opt for a conservative approach, describing only those species for which we had (1) at least 3 sequences (specimens) for *cox*3, (2) at least sequences for the three markers (*cox*3, *rbc*L and *psb*A), and (3) which resulted in consensual results between analyses (GMYC, bGMYC and ABGD) and genes. In other words, we opted for the least inclusive species delimitation. Based on this rationale we describe 10 species *de novo* (*L. abaculusa, L. abscondita*, *L. densa*, *L. dimorpha*, *L. gibbera*, *L. hederacea, L. monticola*, *L. petila*, *L. rosacea*, and *L. undulata*) (Table 3.1.2 and Fig. 3.1.4). Although there are strong indications that several of the remaining MOTUs could well represent new species as well, at present they are left undescribed, awaiting additional sampling.

Table 3.1.2. Description of new *Lobophora* species from New Caledonia.

***Lobophora abaculusa* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage D (figure 3.1.6); figures 3.1.8e, 3.1.9e

Thallus fan shaped, up to 3 cm wide and 2 cm tall, predominantly procumbent, green khaki-gray in color. Thallus attached to the substratum by ventral rhizoids. Margin entire. Thallus composed of single to double-cell-layered medulla, three- to five -cell-layered cortex on both dorsal and ventral sides. The thallus was 140-280 µm thick and composed of 7-11-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species in having a well-developed and symmetrical arrangement of both ventral and dorsal cortex, a cuboid medulla and the distinctive DNA sequence IRD277.

Holotype: IRD277

Distribution: Ouvea and Mare (Loyalties Islands), New Caledonia;

Ecology: growing abundantly among *Distromium* sp. from -4 to -55 m on the outer reef slope. Ecological habit must be confirmed with more field observation and samples.

Epithet: from the Latin “abaculus”, meaning small cube, in reference to the cuboid medulla.

Specimens: Beautemps-Beaupre, Ouvea, Loyalty Islands, New Caledonia, 21 March 2005,

leg. C. Payri (IRD277); (IRD7636); 3 April 2005, *leg.* C. Payri (IRD7641); Mare, Loyalty Islands, New Caledonia, 21 March 2005, *leg.* C. Payri (IRD7651).

***Lobophora abscondita* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage F (figure 3.1.6); figures 3.1.8d, 3.1.9d

Thallus reniform, up to 5 cm wide, 3 cm tall, thin, predominantly crustose, dark brown in color. Margins crenulated. Thalli, tightly to loosely attached to hard substrata (e.g rocks, dead coral) by rhizoids on the entire ventral surface. Thallus surface embossed due to the roughness of the substratum. Thallus composed of single-cell-layered medulla, four- to five- and three- to four-cell-layered cortex on the dorsal and ventral side respectively. Thallus 80-140 µm thick and composed of 4-6-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species by its thinness and by the distinctive DNA sequence IRD10198.

Holotype: IRD10198

Distribution: South-western lagoon, Ile des Pins, New Caledonia; Papua New Guinea.

Ecology: a common species, found growing abundantly on dead coral branches and bedrock down to -5 m in New Caledonia and Papua New Guinea. The specimen from Ile des Pins (IRD7919) was growing at the high level of the intertidal zone on bedrock covered by a fine layer of sand.

Epithet: from the latin "abscondita", meaning concealed, as the species is often found hidden at the bases of corals.

Specimens: Bovis, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (IRD10198); 28 January 2012, *leg.* C.W.Vieira (CV3088); 6 May 2013, *leg.* C.W.Vieira (CV3212); Brun Islet, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (CV3058); Laregnere Islet, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (CV3060); Crouy, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (CV3076); Noumea Aquarium, Noumea, South Province, New Caledonia, 29 January 2013, *leg.* C.W.Vieira (IRD11057); Kanumera, Ile des Pins, South Province, New Caledonia, 2 May 2013, *leg.* C. Payri (IRD7919).

***Lobophora crassa* Z.Sun, P.-E.Lim & H.Kawai**

Lineage F (figure 3.1.6); figures 3.1.8b, 3.1.9b

Thallus fan shaped, up to 5 cm wide and 4 cm tall, rugose surface, coarse and rigid predominantly crustose, dark brown to black in color, presenting grey iridescent lines. Thallus firmly attached to the substratum across the whole of the ventral surface by rhizoids. Thallus composed of single-cell-layered medulla, five to nine- and three to five-cell-layered cortex on the dorsal and ventral side respectively. The thallus was 184-328 µm thick and composed of 10-14-cell-layers. The species was distinguished from its related species in having grey iridescent lines, and by the distinctive DNA sequence IRD10188.

Distribution: North- and south-west of the Grande Terre, New Caledonia; China, Hawaii, Japan, Marquesas Islands (French Polynesia).

Ecology: abundant on bedrock, dead corals and coral rubble, shallow water down to -5 m, exposed reefs.

Specimens: Poya, North Province, New Caledonia, 4 March 2012, *leg.* C. Payri (IRD7884); Ricaudy, Noumea, South Province, New Caledonia, 3 April 2013, *leg.* C.W.Vieira (IRD10187), 15 March 2012 (IRD10188); Eiao, Marquesas Islands, French Polynesia, 26 November 2011, *leg.* C. Payri (IRD8918); Nuku Hiva, Marquesas Islands, French Polynesia, 27 November 2011, *leg.* C. Payri (IRD8919), (IRD8920); Tahuataa, Marquesas Islands, French Polynesia, 6 December 2011, *leg.* C. Payri (IRD8921).

***Lobophora densa* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage F (figure 3.1.6); figures 3.1.8c, 3.1.9c

Thallus fan shaped, up to 5 cm wide and 4 cm tall, rugose surface, coarse and rigid predominantly crustose, dark brown to black in color. Thallus firmly attached to the substratum by basal rhizoids on the entire ventral surface. Thallus composed of single-cell-layered medulla, eight to sixteen- and five to ten-cell-layered cortex on the dorsal and ventral side respectively. The cortex dorsal outer cell-layers (5-6 cell-layers) are smaller (10 µm thick) and strongly pigmented. The thallus was 240-524 µm thick and composed of 16-25-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species by its thickness, its unique dorsal cortex layers strongly pigmented and the distinctive DNA sequence IRD7885.

Holotype: IRD7885

Distribution: Chesterfield Islands, New Caledonia.

Ecology: found growing on dead coral on the outer slope at -50 m.

Epithet: from the latin “densa”, meaning dense, in reference to its particularly thick thallus.

Specimens: Ilots du Passage, Chesterfield Islands, New Caledonia, 20 July 2008, *leg.* C. Payri (IRD7885).

***Lobophora dimorpha* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage E (figure 3.1.6); figures 3.1.8k,l, 3.1.10c

Thallus reniform to deeply lobed with a tendency to form numerous orbicular lobes at the margin, up to 4 cm wide and 2 cm tall, predominantly procumbent and stipitate. Thallus attached to the substratum by basal rhizoids on the ventral surface or with a fibrous stipe. The thallus was 80-140 µm thick and composed of 5- to 6-cell-layers. Thallus composed of a single-cell-layered medulla, two- to three- and two-cell-layered cortex on the dorsal and ventral side respectively. Sexual reproductive organs unknown. The species was distinguished from its related species by being procumbent or stipitate and the distinctive DNA sequence IRD10218.

Holotype: IRD10218

Distribution: South-western lagoon, center-east lagoon of the Grande Terre, New Caledonia.

Ecology: common in shallow waters of the lagoon, attached on the basal part of branched corals where it grows abundantly protected from herbivore grazing.

The specific epithet from comes the latin "dimorpha", in reference to the two morphotypes of this species.

Specimens: Senez, Noumea, South Province, New Caledonia, 13 March 2012, *leg.* C.W.Vieira (IRD10218), *leg.* C.W.Vieira (IRD10220), *leg.* C.W.Vieira (IRD10216), *leg.* C.W.Vieira (IRD10219), *leg.* C.W.Vieira (IRD10217); Signal Islet, Noumea, South Province, New Caledonia, 29 April 2004, *leg.* C. Payri (IRD7614); Maître Islet, Noumea, South Province, New Caledonia, 29 April 2003, *leg.* C. Payri (IRD7654); *leg.* C. Payri (IRD7912); Bogota, Canala, North Province, New Caledonia, 22 April 2012, *leg.* C. Payri (IRD7887).

***Lobophora gibbera* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage F (figure 3.1.6); figures 3.1.8a, 3.1.9a

Thallus fan shaped, up to 3 cm wide and 2 cm tall, leather-look surface grain with a wrinkled aspect, predominantly crustose, dark brown in color. Thallus imbricated, attached to the substratum by rhizoids on the entire ventral surface. Margin entire. Thallus composed of single-cell-layered medulla, four- to five- and three- to four-cell-layered cortex on the dorsal and ventral side respectively. The thallus was 220-280 µm thick and composed of 8-10-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species by its thickness and the distinctive DNA sequence IRD275.

Holotype: IRD275

Distribution: North-east and south-west lagoon of the Grande Terre, New Caledonia.

Ecology: found on hard substratum, collected from the lagoon on a pinnacle wall at -15 m.

Epithet: from the latin "gibbera", meaning humpbacked, in reference to bumpy appearance of the thallus embracing the substrate full of hillocks.

Specimens: Les Quatre Freres, Touho, North Province, New Caledonia, 2 December 2004, *leg* C. Payri (IRD275); Mbere, Noumea, South Province, New Caledonia, 15 June 2013, *leg* C.W.Vieira (IRD11058).

***Lobophora hederacea* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage C (figure 3.1.6); figures 3.1.8h, 3.1.10a

Thallus fan shaped, up to 7 cm wide and 6 cm tall, rigid, longitudinally striated, predominantly decumbent to crustose, very smooth surface, dark orange brown in color. Thallus attached to hard substratum by basal rhizoids on the ventral side. Commonly found proliferating on the coral genus *Seriatopora*. Margin entire. Thallus composed of single-cell-layered medulla, three- to five- and two- to four-cell-layered cortex on the dorsal and ventral

side respectively. The thallus was 136-232 µm thick and composed of 6-10-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species by its specific habitat (corals) and its ivy-like growth and the distinctive DNA sequence IRD10189.

Holotype: IRD10189

Distribution: South-west and north-east lagoon of the Grande Terre, Chesterfield Islands and Belep Islands, New Caledonia.

Ecology: common on shallow coral patches (0-5 m), the inner barrier or fringing reefs, proliferating on dead (*Acropora*) or on live corals (*Turbinaria*, *Acropora*, *Porites* and predominantly *Seriatopora*).

Epithet: from the latin "hederacea", meaning ivy-like, as the species is found growing on coral branches reminds the ivy.

Specimens: Abore, Noumea, South Province, New Caledonia, 15 October 2012, *leg.* C.W.Vieira (IRD10189); (IRD10190); (IRD10191); (IRD10192); (IRD10193); (IRD10194); Loop Island, Chesterfield Islands, New Caledonia, 8 July 2008, *leg.* C. Payri (IRD7677); Art Island, Belep Islands, North Province New Caledonia, 14 March 2009, *leg.* C. Payri (IRD7621); Touho, North Province, New Caledonia, 15 April 2012, *leg.* C. Payri (IRD7880).

***Lobophora monticola* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage C (figure 3.1.6); figures 3.1.8f, 3.1.9g

Thallus fan shaped, lax, lacerated at the base, predominantly conk-like and anastomosing, rugose surface, dark orange brown in color. Thallus with a lot of epiphytes, attached to the substratum by basal rhizoids on the ventral surface. Growing attached on *Acropora* corals forming bridge connection between coral branches by connections (anastomosis) of the distal part of multiple fronds. Margin entire to crenulated. Thallus composed of single-cell-layered medulla, two- to four- and two- to three-cell-layered cortex on the dorsal and ventral side respectively. The thallus was 84-196 µm thick and composed of 5-8-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species in having big and anastomosing thalli lacerated at the base, copper (dark orange) colored thalli and the distinctive DNA sequence IRD7878.

Holotype: IRD7878

Distribution: South-west, south-east and center-east lagoon of the Grande Terre, and Loyalty Islands, New Caledonia.

Ecology: common in sheltered areas along the inner slope of reefs; growing abundantly on *Acropora* branches.

Epithet: from the latin "monticola", meaning mountain dweller, in reference to its growing habit in the apical part of branching corals.

Specimens: Baie de Canala, Canala, North Province, New Caledonia, 21 April 2012, *leg.* C. Payri (IRD7878); Port Bouquet, Ile Nemou, South Province, New Caledonia, 19 March 2007, *leg.* C. Payri (IRD7631); *leg.* C. Payri (IRD7632); *leg.* C. Payri (IRD7633); Bogota, Canala,

North Province, New Caledonia, 22 April 2012, *leg.* C. Payri (IRD7882); Astrolabe, Great Reef, Loyalty Islands Province, New Caledonia, 4 April 2005, *leg.* C. Payri (IRD7640); Sainte-Marie, Noumea, South Province, New Caledonia, 12 March 2012, *leg.* C.W.Vieira (IRD10200); Prony Bay, South Province, New Caledonia, 12 March 2012, *leg.* F. Houlbreque (IRD10199).

***Lobophora nigrescens* J.Agardh**

Lineage B (figure 3.1.6); figures 3.1.8o, 3.1.10f

Thalli in dense erect blades, medium to dark brown. Fronds composed of several lobes, stipitate, anchored by an obvious holdfast of mixed sand and slender fibers. Thallus composed of single-cell-layered medulla, four- to six- and three- to five-cell-layered cortex on the dorsal and ventral side respectively. The thallus 168-252 µm thick with 8-12-cell-layers. This species is distinguished from the other *Lobophora* lineages in having a *Zonaria*-like morphology. The New Caledonian species is ecologically distinguished from the Australian one as the former is found in shallow fringing, coastal or islets reefs mixed within Sargassum beds while the latter is found in exposed reef face.

Distribution: South-east, south-west and north-east of the Grande Terre, and south-west of Ile des Pins, New Caledonia; China, Malaysia, Japan.

Ecology: common, growing on shallow fringing, coastal or islets reefs, mixed in Sargassum beds, down to -5 m.

Specimens: Tiakan, South Province, New Caledonia, 17 July 2004, *leg.* C. Payri (IRD281). *leg.* C. Payri (IRD282); Laregnere, Noumea, South Province, New Caledonia, 14 April 2004, *leg.* C. Payri (IRD283), (IRD284), (IRD482b), (IRD7658), (IRD7659), (IRD7660), (IRD7661), (IRD7665), (IRD7674), (IRD7675); Ricaudy, Noumea, South Province, New Caledonia, 5 April 2004, *leg.* C. Payri (IRD7655); Redika Islet, South Province, New Caledonia, 4 June 2006, *leg.* C. Payri (IRD7657); Anse Vere, South Province, New Caledonia, 25 February 2004, *leg.* C. Payri (IRD7916), (IRD7664); Baie des Rouleaux, Ile des Pins, South Province, New Caledonia, 15 April 2013, *leg.* C. Payri (IRD7920); Canard Islet, Noumea, South Province, New Caledonia, 15 March 2012 *leg.* C.W.Vieira (IRD10195); Maître Islet, Noumea, South Province, New Caledonia, 15 March 2012 *leg.* C.W.Vieira (IRD10196); Senez, Noumea, South Province, New Caledonia, 15 March 2012 *leg.* C.W.Vieira (IRD10197).

***Lobophora pachyventera* Z.Sun, P.-E.Lim & H.Kawai**

Lineage E (figure 3.1.6); figures 3.1.8m, 3.1.10d

Thallus fan-shaped, up to 3 cm wide and 2 cm tall, rugose surface, predominantly crustose, dark green in color. Thalli attached to hard substratum by rhizoids on the entire ventral surface. Margin entire. Thallus composed of single-cell-layered medulla, two- and two- to three-cell-layered cortex on the dorsal and ventral side respectively. The thallus was 100-140

µm thick and composed of 5-6-cell-layers. Sexual reproductive organs are unknown. The species was distinguished from its related species in having a thicker ventral than dorsal cortex and the distinctive DNA sequence IRD7881.

Distribution: North-east of the Grande Terre, New Caledonia; China, Malaysia, Japan.

Ecology: growing among *Halimeda,* down to -10 m, on coral patches.

Specimens: Touho, North Province, New Caledonia, 15 April 2012, *leg.* C. Payri (IRD7881); Bovis, Noumea, South Province, New Caledonia, 15 January 2013, *leg.* C.W.Vieira (CV3095).

***Lobophora petila* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage D (figure 3.1.6); figures 3.1.8n, 3.1.10e

Thallus fan-shaped, up to 3 cm wide and 2 cm tall, predominantly procumbent, light to dark brown in color. Thallus attached by rhizoids on the ventral surface. Margin entire. Thallus composed of single-cell-layered medulla, one- to two- cell-layered cortex on both dorsal and ventral sides. The thallus was 40 to 70 µm thick and composed of 3-5-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species in having the thinnest thallus of all *Lobophora* species and the distinctive DNA sequence IRD7877.

Holotype: IRD7877

Distribution: Center-east lagoon of the Grande Terre, New Caledonia; Papua New Guinea, Marquesas Islands (French Polynesia).

Ecology: abundant on patch reefs in sheltered lagoon area, mixed with other Dictyotales (*Padina, Dictyota, Distromium*), -8 to -12 m.

The specific epithet from the latin "petila" referring to its very thin thallus, as this species has the thinnest thallus of all *Lobophora* species.

Specimens: Baie de Canala, Canala, North Province, New Caledonia, 21 April 2012, *leg.* C. Payri (IRD7877); Kranget Island, Madang, Papua New Guinea, 10 November 2012, *leg.* C. Payri (IRD9831); (IRD9832); (IRD9833); (IRD9834); (IRD9835); (IRD9836); (IRD9837); Duad Island, Madang, Papua New Guinea, 15 November 2012, *leg.* C. Payri (PAP399); Malamal Anchorage, Madang, Papua New Guinea, 17 November 2012, *leg.* C. Payri (IRD9838); (IRD9839); 18 November 2012 (IRD9840); Sek Island, Madang, Papua New Guinea, 20 November 2012, *leg.* C. Payri (IRD9841); (IRD9842); (IRD9843); (IRD9844); (IRD9845); (PAP900); 23 November 2012 (PAP950); *leg.* H. Verbruggen (PHV369); *leg.* H. Verbruggen (PHV385); *leg.* H. Verbruggen (PHV394); *leg.* H. Verbruggen (PHV440); *leg.* H. Verbruggen (PHV551); *leg.* H. Verbruggen (PHV773); Nuku Hiva, Marquesas Islands, French Polynesia, 27 November 2011, *leg.* C. Payri (IRD8917).

***Lobophora rosacea* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage B (figure 3.1.6); figures 3.1.10k,l, 3.1.13e

This species is composed of two morphotypes. The first morphotype had a thallus fan-shaped to circular, up to 10 cm wide and 7 cm tall, lax, predominantly erect, light orange in color. Thalli attached to the substratum by basal rhizoids on the ventral surface. Margin entire. Thallus composed of single-cell-layered medulla, two- to three- and two- to three-cell-layered cortex on the dorsal and ventral side respectively. The thallus was 80-130 µm thick and composed of 5-7-cell-layers. The second morphotype had a thallus reniform to circular, lax, ruffled, up to 9 cm wide and 8 cm tall, predominantly erect, light green in color. Thalli are spirally arranged, forming a dense rosette, attached to each other and to the substratum by a basal mound of rhizoids. Commonly found nested among branching corals, or attached to *Lobophora nigrescens.* Margin entire. The thallus was 110-170 µm thick and composed of 5-8-cell-layers. Thallus composed of single-cell-layered medulla, two- to four- and two- to four-cell-layered cortex on the dorsal and ventral side respectively. Sexual reproductive organs unknown. The species was distinguished from its related species in having a thallus medium to large in size, fan-shape and ruffled, a basal mound of hairs, and the distinctive DNA sequences IRD10213.

Holotype: IRD10213

Distribution: South-west and south-east lagoon of the Grande Terre, Chesterfield Islands, New Caledonia.

Ecology: growing in the lagoon area (-3 to -5 m), among *Sargassum spinuligerum,* attached to *Lobophora nigrescens* or nested in corals.

Epithet: from the latin “rosacea” in reference to the rose-like shape.

Specimens: Ricaudy, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (IRD10206); (IRD10213) Bovis, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (IRD10207); Canard Islet, Noumea, South Province, New Caledonia, 7 March 2012, *leg.* C.W.Vieira (IRD10205); Plum, South Province, New Caledonia, 23 April 2004, *leg.* C. Payri (IRD7662); Dumbea Bay, Dumbea, South Province, New Caledonia, 13 July 2005, *leg.* L. Mattio (IRD7673); Chesterfield Islands, New Caledonia, 4 July 2008, *leg.* C. Payri (IRD7876); Thio, South Province, New Caledonia, 23 April 2012, *leg.* C. Payri (IRD7879); Canard Islet, Noumea, South Province, New Caledonia, 13 October 2002, *leg.* C. Payri (IRD7908) Laregnere Islet, Noumea, South Province, New Caledonia, 14 April 2004, *leg.* C. Payri (IRD7913); *leg.* C. Payri (IRD7914); *leg.* C. Payri (IRD7915); *leg.* C. Payri (IRD7917).

***Lobophora undulata* sp. nov. C.W.Vieira, Payri & De Clerck**

Lineage C (figure 3.1.6); figures 3.1.8g, 3.1.9g

Thallus fan-shaped, up to 7 cm wide and 6 cm tall, undulated longitudinally, rigid, striated, predominantly decumbent, very smooth surface, dark orange brown in color. Thallus attached to the substratum by basal rhizoids on the ventral surface. Distal part, free

and tending to ascend. Margin entire to lobated. Thallus composed of single-cell-layered medulla, three- to six- and two- to five-cell-layered cortex on the dorsal and ventral side respectively. The thallus 110-300 µm thick with 6-12-cell-layers. Sexual reproductive organs unknown. The species was distinguished from its related species in being decumbent, having undulated and thick thallus and the distinctive DNA sequences IRD10202. Anatomically very similar with *L. hederaceae* but differs morphologically by its undulated shape.

Holotype: IRD10202

Distribution: Sout-west and south-east lagoon of the Grande Terre, New Caledonia.

Ecology: abundant on coral branches, especially on the base of *Acropora* colonies, protected from herbivores.

Epithet: from the latin "undulata" in reference to the undulated surface of the thallus.

Specimens: Laregnere, Noumea, South Province, New Caledonia, 12 March 2012, *leg.* C.W.Vieira (IRD10202); (IRD10201); Ilot Kouare, South Province, New Caledonia, 1 April 2013, *leg.* M. Conord (IRD11054); Kanua, Port Boise, South Province, New Caledonia, 3 October 2005, *leg.* C. Payri (IRD7669); (IRD7671).

4.2. Morphology and ecology

A combination of morphological and ecological traits allows a good differentiation of the New Caledonian species. Combinations of morphological, anatomical and ecological characters are graphically represented in Fig. 3.1.7.

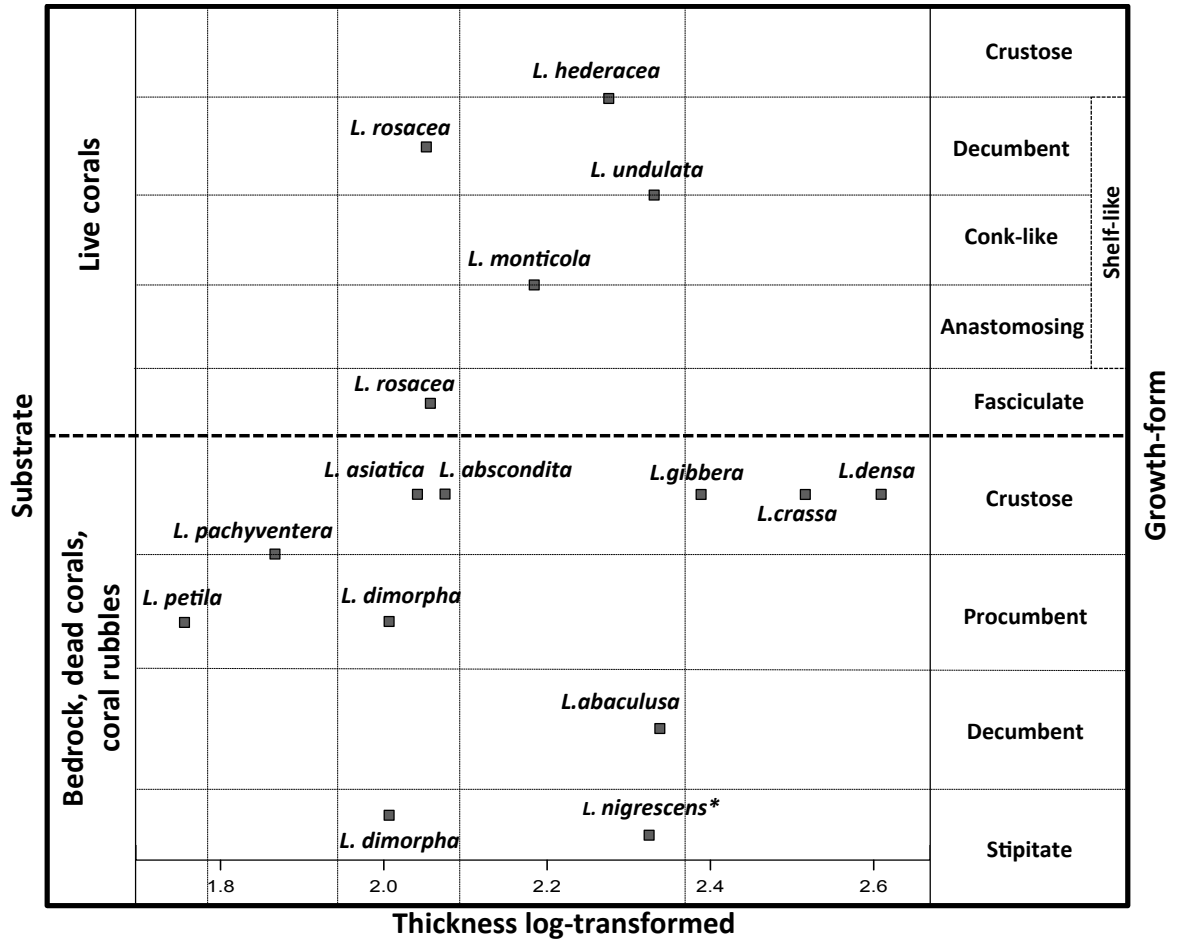


Figure 3.1.7. Schematic representation of the ecological (substrate preferences), morphological (growth forms), and anatomical (log-transformed thallus thickness) features of the New Caledonian *Lobophora* species. Horizontal dashed lines separate the substrates. **L. nigrescens* s.l. grows on hard substrates (e.g., rocks, bedrock) found in sandy bottoms.

The *Lobophora* complex provides an excellent example of the power of molecular-assisted alpha taxonomy (MAAT; Cianciola et al. 2010) in which species are delimited based on molecular data and subsequently the diagnostic value of morphological and ecological characteristics reassessed (see also Verbruggen et al. 2005; Leliaert et al. 2014). Even though the current sampling most likely fails dramatically in representing the global species diversity in the genus, several trends with regard to the evolutionary signal of morphological characters stand out. Lineage A composed of five MOTUs, including the newly described species *L. rosacea* is characterized by a decumbent or fasciculate thallus. Species of lineage B, composed of two species *L. nigrescens* s.l. and *L. australis,* is characterized by erect thalli and conspicuous basal holdfasts.

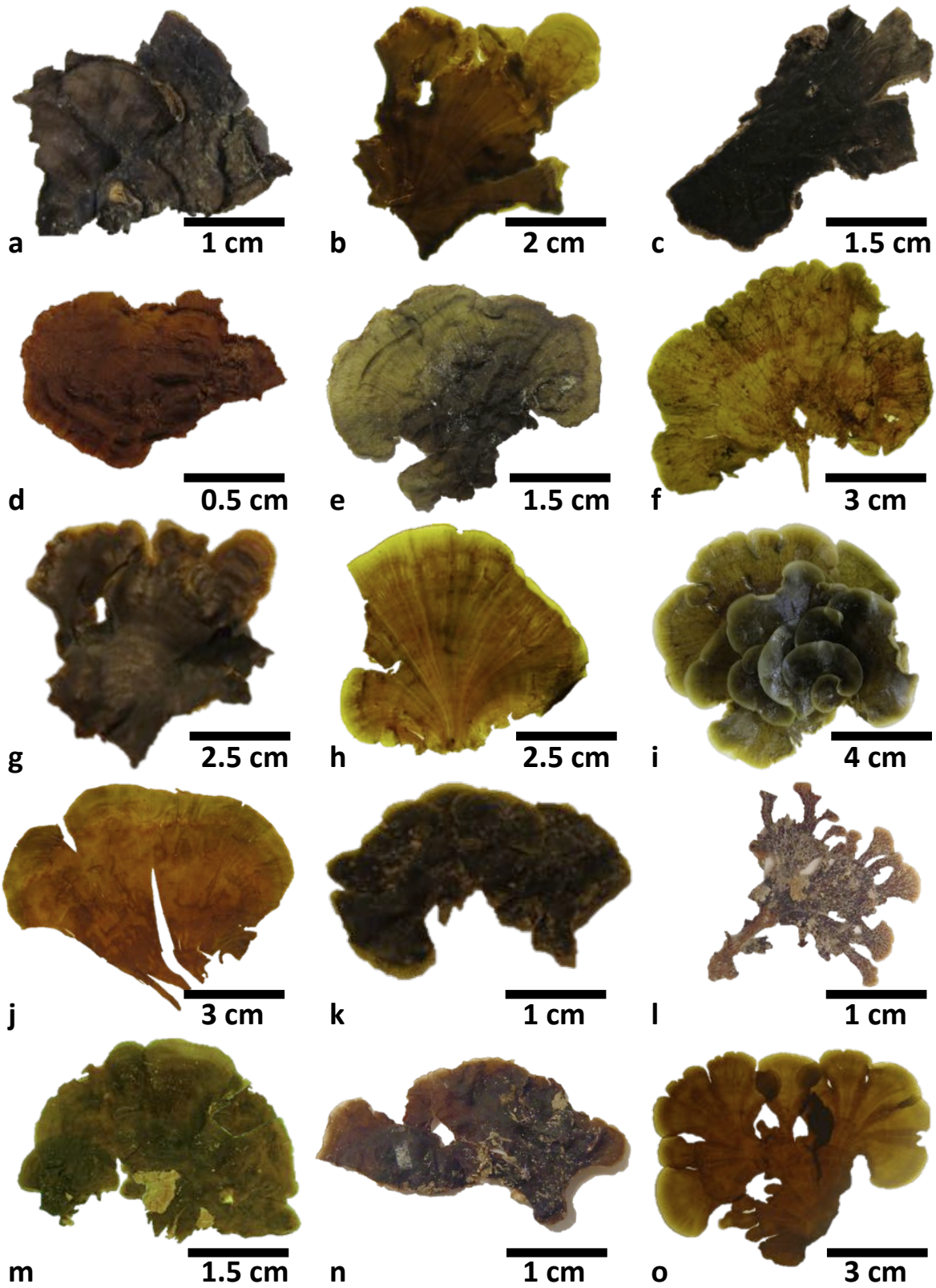


Figure 3.1.8. (a–l) External morphology of New Caledonian *Lobophora* species. For new species the picture represents the holotype. (a) *L. gibbera*; (b) *L. crassa*; (c) *L. densa*; (d) *L. abscondita*; (e) *L. abaculusa*; (f) *L. monticola*; (g) *L. undulata*; (h) *L. hederacea*; (i) *L. rosacea*; (j) *L. rosacea*; (k) *L. dimorpha*; (l) *L. dimorpha*; (m) *L. pachyventera*; (n) *L. petila*; (o) *L. nigrescens* s.l..

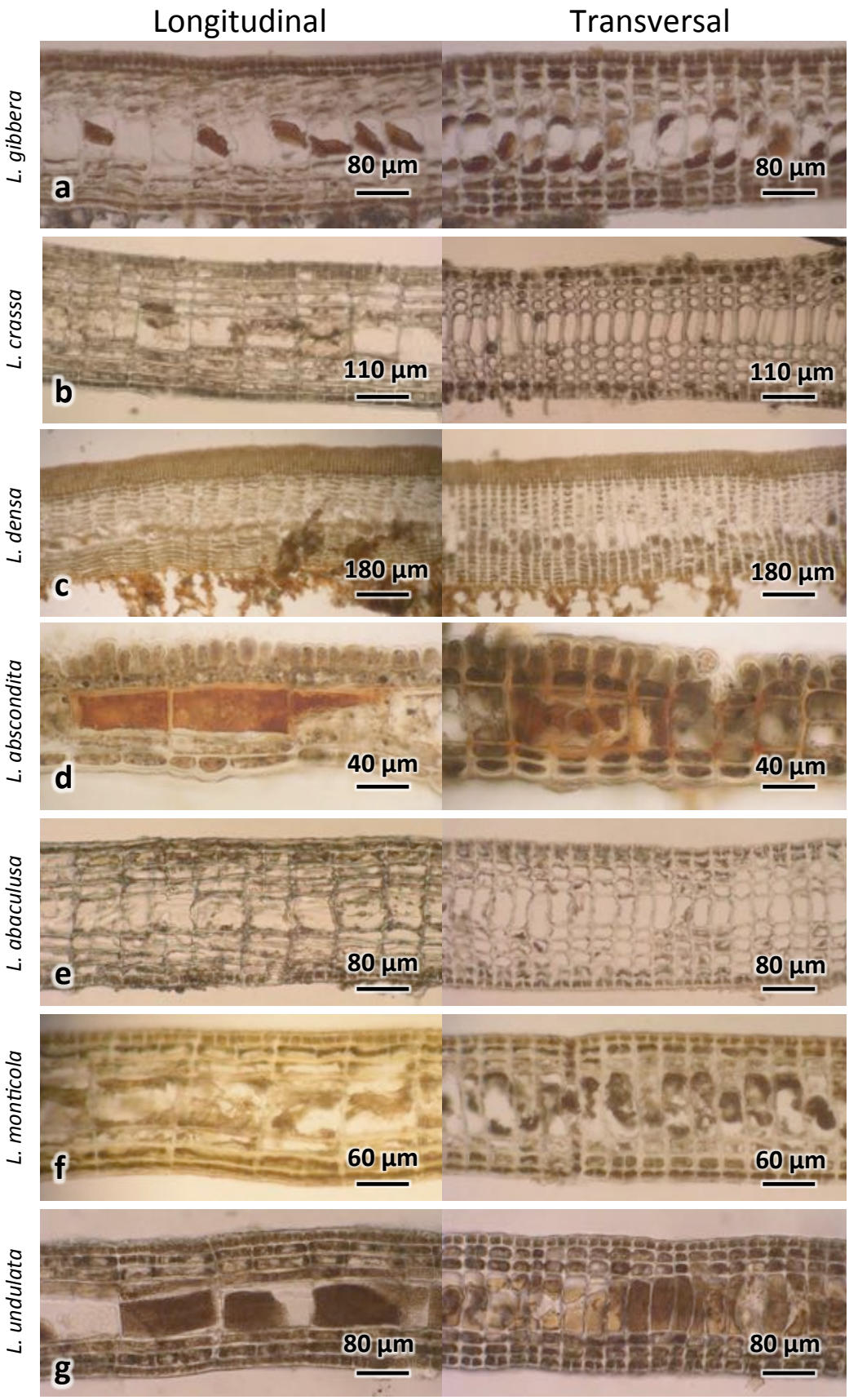


Figure 3.1.9. (a–f) Longitudinal (on the left) and transverse (on the right) sections of New Caledonian Lobophora species. (a) *L. gibbera*; (b) *L. crassa*; (c) *L. densa*; (d) *L. abscondita*; (e) *L. abaculusa*; (f) *L. monticola*; (g) *L. undulata*.

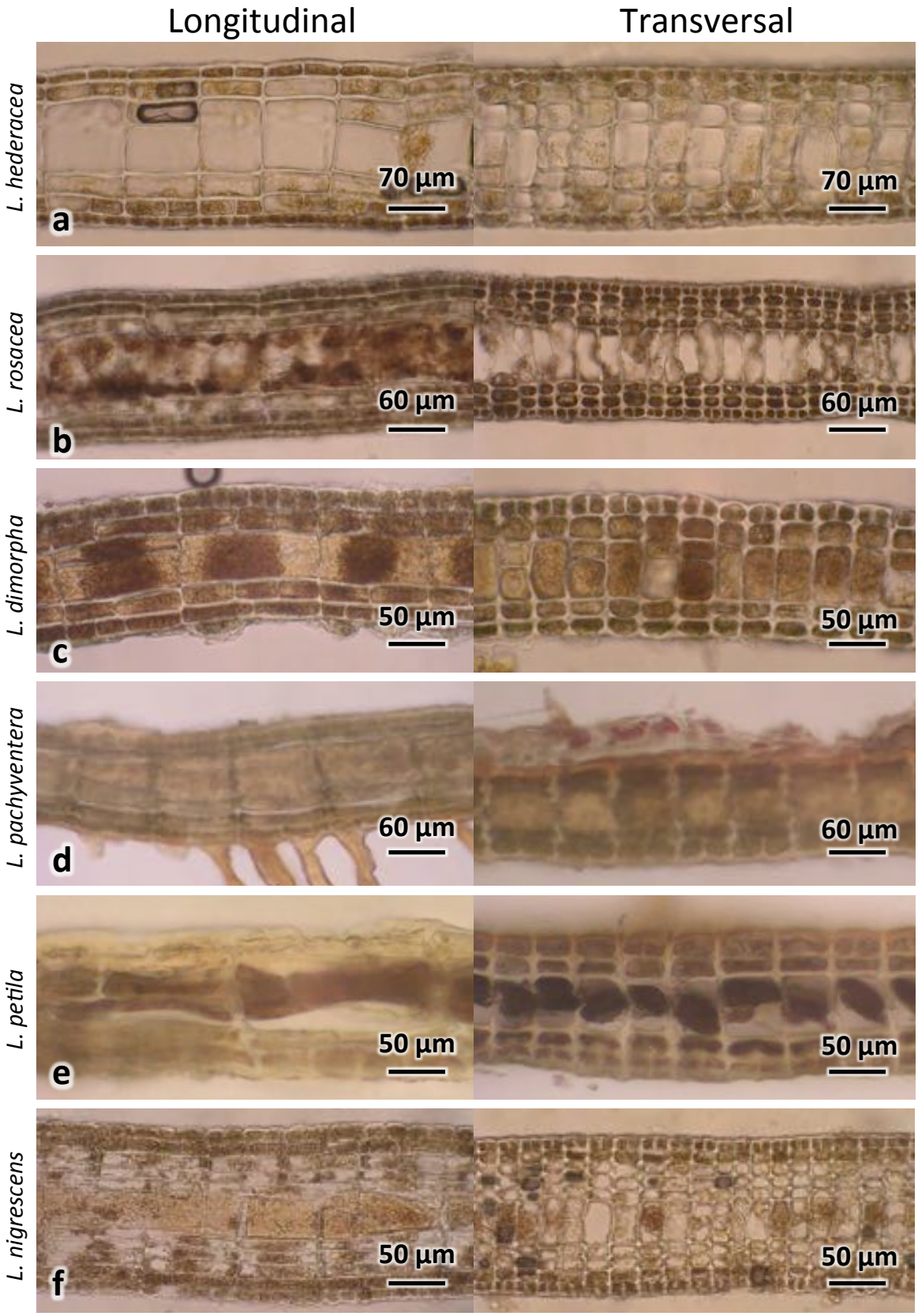


Figure 3.1.10. (a–f) Longitudinal and transverse sections of New Caledonian *Lobophora* species (continued). (a) *L. hederacea*; (b) *L. rosacea*; (c) *L. dimorpha*; (d) *L. pachyventera*; (e) *L. petila*; (f) *L. nigrescens* s.l.

Members of the lineage C, including the newly described species *L. hederacea*, *L. undulata*, *L. monticola* and *L. abaculusa*, are commonly associated with corals and present a predominantly conk-like form. *L. hederacea* may also adopt a crustose form especially when found covering specific coral genera (*e.g. Seriatopora caliendrum* Ehrengerg (1834) and *S. hystrix* Dana (1846)). The species of lineage E, including the species *L. dimorpha* and *L. pachyventera,* adopt predominantly a procumbent form. The species of the lineages D and F, including the species *L. crassa*, *L. abscondita*, *L. gibbera*, *L. densa*, *L. asiatica* and *L. petila,* are characterized by a predominant crustose form. *L. papenfussii* from Bikini Atoll (Marshall Islands) and *L. rickeri,* from Lord Howe Island (Australia), which presents a crustose form and a thick thallus, may well belong to the lineage F, whose members share the same morphological characteristics (*i.e.* a crustose form and thick thallus).

4.3. Evolutionary perspective and ecological significance of the morphology

The genus *Lobophora* illustrates the misapprehension of morphological differences for phenotypic plasticity instead of genetic diversity well. Several authors (*e.g.* De Ruyter van Steveninck et al. 1988; Littler and Littler 2000) already observed different growth forms and certainly sensed the existence of different species in relation to the different forms, but nobody ventured to look into this diversity until recently (Sun et al. 2012). The morphological diversity observed within the genus *Lobophora* was until now considered as the phenotypic plasticity (*e.g.* Coen and Tanner 1989, De Ruyter van Steveninck et al. 1988; Littler and Littler 2000) displayed by a single species, namely *Lobophora variegata*. Today, three arguments strongly stand against this misconception. First, recent studies including the present one unraveled the hotchpotch of species hidden behind the catch-all species *Lobophora variegata*. Second, comparison of phylogeny and morphological results revealed the existence of predominant growth forms in each major lineage. Lastly, in a same habitat we may find different species with different forms. However, one cannot discard phenotypic plasticity off the picture, as we can observe a certain degree of plasticity in every species, with a spectrum of shapes ranging from crustose to erect, but yet again with a predominant form per species. By comparing the morphologies shared by species of a same lineage, we were able to distinguish predominant forms in each lineage. The most basal lineages (A and B) possess

predominantly an erect form, the most recent lineages (D-F) present a procumbent to a crustose form, and the intermediate lineage (C) presents a decumbent form. Most likely the ancestral form was a *Zonaria*-like erect species with a single holdfast, which was also suggested in Sun et al. (2012). Furthermore, those forms seem to be associated with ecological features. In fact, *Lobophora* species are found to have a wide variety of habitat and substratum preferences in New Caledonia (*e.g.* bedrocks, coral rubbles, dead corals and live corals). More remarkably we noticed that this variety of substrata reflected a niche partitioning between the major lineages. For instance, species of lineage B are mostly found growing on sand bottoms, species of lineage C are strongly found in interactions with live corals. These species, present the capacity to bleach and overgrow corals, certainly by the means of secondary metabolites. Species of lineage A are also found in interactions with corals. Species of lineages D to F are mostly found on bedrocks, dead corals or coral rubbles.

5. Conclusion

The high levels of *Lobophora* diversity unveiled from a single locality in the Pacific Ocean raises important question with respect to the global diversity of the genus, the distributions and range sizes of the individual species, as well as the mechanisms facilitating co-existence. Current sampling of *Lobophora* species does not allow to draw far ranging conclusions, but it would appear that individual *Lobophora* species are restricted to one ocean basin and in this aspect it reminisces the biogeography of the genus *Padina*, for which there is no or very scanty evidence for species spanning more than one ocean basin. Our analyses included two specimens from the Caribbean Sea, the type locality of *L. variegata*. Even though the presence of genuine *L. variegata* in the Indo-Pacific Ocean seems quite unlikely, additional sampling of the Caribbean region is highly needed to precisely determine the identity of *L. variegata* and assess the species diversity in the Atlantic Ocean. In addition, at present more than half of the MOTUs are recorded only from New Caledonia, but it remains unclear which percentage of the unveiled diversity is really restricted to the study area. An extensive sampling in the Indo-Pacific region is needed to improve our understanding of *Lobophora* distribution patterns significantly.

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Acknowledgments

Some samples were collected during several sea campaigns (BIODIP; BSM-Loyaute; CORALCAL1, 2, 3, and 4; MADANG) on board of the IRD research vessel ALIS. We acknowledge the collectors who have contributed to this study: J.-L. Menou, H. Verbruggen, L. Mattio, F. Houlbreque, M. Co- nord, and C. Peignon. We also thank the boat pilots of IRD, Miguel Clarque, Samuel Tereua and Philippe Naudin, for their contribution to this work through their kind and careful assistance.

Author contributions

CV, ODC and CP conceived and designed the study. CV carried out all the surveys and morphological analyses. CV and SD carried out the molecular analyses. CV wrote the manuscript. ODC and CP commented on the manuscript.

Part 2. Shedding new lights on old algae: matching names and sequences in the brown algal genus *Lobophora* (Dictyotales, Phaeophyceae)[8]

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Abstract

Recent studies focusing on species diversity in the brown algal genus *Lobophora* (Phaeophyceae, Dictyotales) raised questions with respect to the identity and phylogenetic position of several *Lobophora* taxa described in the pre-DNA era and considered synonyms of *L. variegata*. The present study aimed at re-evaluating the identity of old names by attempting DNA extraction and amplification of historical herbarium material, and by re-examining and comparing type specimens with recently described *Lobophora* species. Seventeen names suspected to be representative of *Lobophora* species were identified. The nine types that we were able to access corresponded morphologically to *Lobophora* species, and we successfully extracted and amplified short DNA fragments from *Dictyota variegata, Pocockiella papenfussii, Zonaria isselii* and *Z. obscura*. Alternatively, new collections near type localities were made and when morphological comparisons agreed, epitypification was made. Finally, four types preserve their taxonomic identity (*D. variegata*, *P. dichotoma, P. papenfussii* and *L. rickeri*), the names of seven types are here resurrected from the synonymy of *L. variegata* (*Aglaozonia pacifica, A. canariensis, L. nigrescens*, *Ralfsia ceylanica, Z. isselii, Z. nigrescens* and *Z. obscura*), and three recently described species are reduced to synonymy (*L. crassa*, *L. densa* and *L. indica*). Epitypifications and new name combinations were made when necessary. The present study illustrates the difficulty in reassessing the identity of old types, from accessibility of type material to molecular analyses. Although certainly not ideal given cryptic diversity, new collections from type localities and morphological comparisons to type specimens offer the best alternative to reintegrate historic names into modern DNA-based taxonomy.

1. Introduction

The use of gene sequence data has profoundly altered our view of algal diversity on every taxonomic level, but perhaps most spectacularly, sequence data have unveiled the existence of massive cryptic or pseudo-cryptic diversity at the species level (De Clerck *et al.*, 2013). Although a mismatch between genetic diversity and morphology has been observed in virtually all groups of organisms (Knowlton, 1993; Bickford *et al.*, 2007; Pfenninger & Schwenk, 2007), cryptic diversity in morphologically simple and/or plastic organisms has severely reduced the utility of morphology as a criterion for species delimitation in the latter (Sáez & Lozano, 2005; Cianciola *et al.*, 2010; Leliaert *et al.*, 2014; Verbruggen, 2014). As a side effect, cryptic diversity also makes

linking DNA-based lineages to existing taxa exceedingly difficult. Both Saunders and McDevit (2012) and De Clerck *et al.* (2013) described a growing tendency in phycology to move from a formal algal taxonomy to a more informal system whereby clade-, specimen- or strain-based identifiers are used to communicate biological information. A better integration of historical collections into modern taxonomic research is therefore a pressing need (Hind *et al.*, 2014). Strategies that have been proposed to solve this problem include both generating DNA sequences from type collections and designating epitypes from which sequence data can be readily obtained (Tautz *et al.*, 2003). Although obviously DNA information from the type specimen itself would be favored, it remains to be evaluated how successful this approach is over a large selection of taxa.

A number of case studies have successfully generated DNA sequences from type material of marine macroalgae (e.g. Hughey *et al.*, 2001; Brodie *et al.*, 2007; Gabrielson, 2008b, a; Hughey & Gabrielson, 2012; Hind *et al.*, 2014; Hughey *et al.*, 2014; Sauvage *et al.*, 2014). The focus of these studies has been largely to pinpoint the identity of the types of one or a few species only, but a more encompassing study, such as establishing the identity of all types of a specific genus, has not yet been attempted. Moreover, most studies have focused on red and on green seaweeds (Hayden *et al.*, 2003), while obtaining DNA of sufficient quality from brown algae is widely regarded as more challenging (Phillips *et al.*, 2001; Varela - Álvarez *et al.*, 2006; McDevit & Saunders, 2009).

The brown algal genus *Lobophora* forms an excellent test case to investigate the feasibility of integrating sequences from type material in algal taxonomy. Recent studies have demonstrated that the genus is far more diverse than traditionally assumed (Sun *et al.*, 2012; Vieira *et al.*, 2014b; Schultz *et al.*, in press). For decades only three species were recognized, among which *Dictyota variegata* was by far the most commonly reported. Other names included *Pocockiella papenfussii* and *P. dichotoma*. From 2000 until 2012, three additional species were described based on morphological criteria only: *Lobophora indica*, *L. minima* and *L. rickeri* (Krishnamurthy & Baluswami, 2000; Kraft, 2009). The use of molecular taxonomic tools has highlighted the taxonomic deficit from which the genus *Lobophora* was suffering, i.e. Sun *et al.* (2012) recognized nine major *Lobophora* Molecular Operational Taxonomical Units, which they referred to as clades, based on chloroplast *rbc*L and mitochondrial *cox*3 gene sequences, and described four species *de novo*. Subsequently, Vieira *et al.* (2014b) described 10 species from New Caledonia using a combination of molecular delimitation methods (automatic barcode gap

discovery and general mixed Yule-coalescent models) based on chloroplast *psb*A, *rbc*L and mitochondrial *cox*3 genes. Including the latest study on *Lobophora*, there are at present 20 currently accepted species in the genus (Guiry & Guiry, 2015). These recent molecular taxonomic insights call into question the identity of several old names that have been associated with *Lobophora*, many of which are now mostly regarded as synonyms of *L. variegata*. With the continuous increase in species diversity, type specimens (currently accepted species or synonyms) described based on morphological criteria only (subsequently referred to as old types), are necessary sources of comparative material for systematic studies. In particular, with the omnipresence of cryptic species, the identity of old type specimens can only be ascertained with molecular approaches.

The present study aims to re-evaluate the identity of old type specimens by attempting DNA extraction and amplification of historical herbarium material, and by reexamining and comparing type specimens with recently described *Lobophora* species. For the sake of clarity, we refer to the basionyms for all the types.

2. Material and methods

2.1. Type material

We tried to identify all published taxa which are either currently regarded or suspected as belonging to *Lobophora* (Papenfuss, 1943; Womersley, 1967), but for which no gene sequence data are available to link them to the known *Lobophora* diversity. Type material of these species was traced in various herbaria and authorization for destructive sampling, necessary for the molecular and morphological analyses, was requested.

2.2. Taxon sampling

New specimens of *Lobophora* were collected by SCUBA at or near the type localities of five types (*A. pacifica, A. canariensis*, *D. variegata, P. dichotoma, Z. nigrescens*; Table 3.2.1, Fig. 3.2.1). Newly collected material was kept in a cooler and stored in silica gel once at the laboratory. Specimens were prepared as herbarium sheets and preserved in a 5% solution of formalehyde with seawater. New type material is housed at local herbaria.

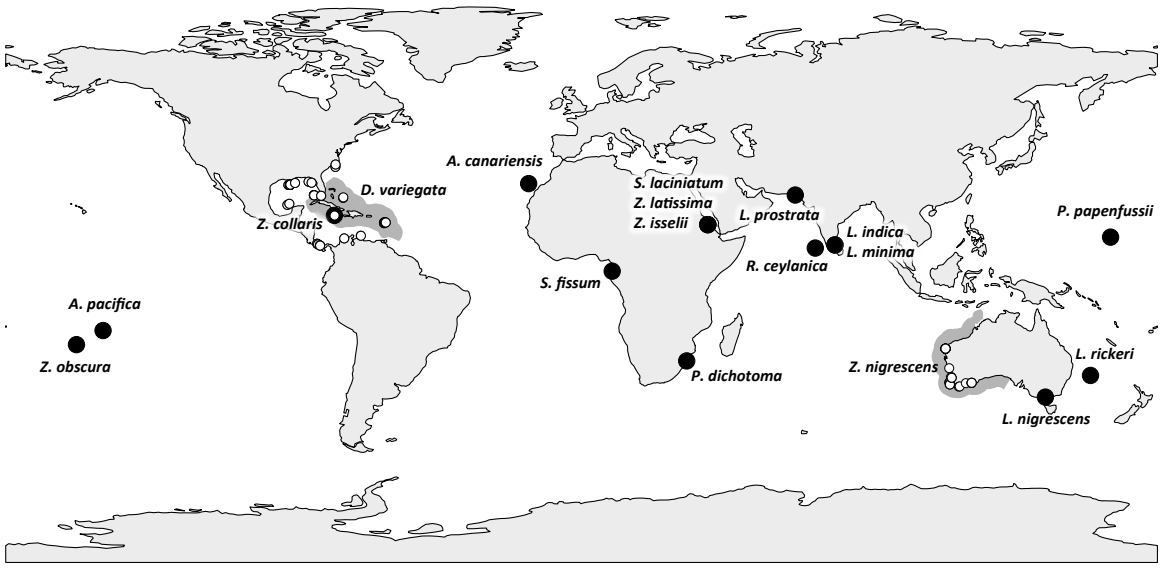

Figure 3.2.1. World map with the type localities of the 17 *Lobophora* types described prior to 2012 for which no molecular data are available. Type localities of *L. variegata* and *Z. nigrescens*, namely the Caribbean and Western Australia, respectively are shown in grey because they are only known on a regional level. Although indicated, *L. prostrata* is not a valid species.

2.3. DNA extraction, amplification, sequencing and sequence alignments

Total genomic DNA was extracted using the silica-gel membrane-based DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) for the herbarium type specimens, following the manufacturer's instructions and a standard CTAB-extraction method (De Clerck *et al.*, 2006) for recently collected material. Genomic DNA of type specimens was subsequently purified with a Wizard® DNA Clean-Up System (Promega Inc., Madison, WI, USA) following the manufacturer's instructions. Sequences were generated from one mitochondrial gene (*cox*3) and two chloroplast genes (*psb*A, *rbc*L). New primers were designed (Table S3.2.1) for the three genes to generate short fragment of c.a. 100--200 pb for the type specimens. Multiple primer combinations were tested for three genes (Table S3.2.1). PCR and sequencing conditions are detailed in Table S3.2.1. The E-Gel® Agarose Gel Electrophoresis (Life Technologies, Inc., Carlsbad, CA, USA) 2% Agarose was used to select and isolate the bands of interest. Nested PCR was then performed to further amplify the isolated fragments. *Lobophora* sequences from GenBank (Vieira *et al.*, in prep.-c) were added to the alignments (Table S3.2.2). Sequences were aligned using MUSCLE implemented in eBioX 1.6 (Lagercrantz, 2008).

2.4. Sequence similarity searches

For short sequences obtained from type specimens, sequence similarity searches were performed using Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990). BLAST searches were done against a *Lobophora* sequences database containing all available *Lobophora* sequences.

2.5. Phylogenetic analysis

Maximum likelihood (ML) and Bayesian inference (BI) gene trees were generated for *cox*3, *psb*A and *rbc*L alignments. ML analyses were conducted using RAxML (Stamatakis 2006). The robustness of the resulting phylogenies was tested by nonparametric bootstrapping (Felsenstein, 1985) using 1,000 replicates. BI analyses were conducted using MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003), initiated with a random starting tree and ran four chains of MCMC iterations simultaneously for 100 million generations. The first 100,000 (25%) trees sampled were discarded as burn-in, based on the stationarity of ln$L$ as assessed using Tracer version 1.5 (Rambaut and Drummond 2009). A consensus topology and posterior probability values were calculated from the remaining trees.

2.6. Morphological analysis

Morphological observations of *Lobophora* species comprised the analyses of the external and internal morphology of the specimens. External observations consisted in the description of the general appearance, growth form, size and color of the thallus. For the internal morphology, longitudinal and transverse sections were made of the middle portions of the thallus using a portable medical freezing microtome (Labonord®). Alternatively, hand-made cross sections were made for some type material and some Caribbean specimens. Photographs of the sections were taken with a digital camera (Olympus Camedia C-5050 5.0 Megapixel, Tokyo, Japan) attached to a compound microscope (Olympus BH-2, Tokyo, Japan). All type specimens available were examined for generic confirmation and morphologically compared to the new collections.

3. Results

We identified 17 names that are currently accepted or that have been related at some point in their taxonomic history to the genus *Lobophora* and for which no

molecular data are available (Table 3.2.1, Fig. 3.2.1). We failed to contact with the herbaria where the types of *L. indica* and *L. minima* should be housed according to the protologue in Krishnamurthy and Baluswami (2000). We were not allowed to perform destructive sampling on the types of *A. pacifica, L. nigrescens, P. dichotoma, Z. collaris* and *Z. nigrescens* because of their fragmented state. Preservation of *L. rickeri* in formaldehyde meant that is could not be subjected to molecular analyses and was therefore not requested on loan. Finally, out of the nine types on which we were able to perform molecular analyses, we were able to amplify and sequence DNA fragments from four types (*D. variegata, P. papenfussii, Z. isselii* and *Z. obscura*; Fig. 3.2.2). Morphological comparisons of newly collected material from type localities allowed the epitypification of four types (*A. canariensis, A. pacifica, P. dichotoma* and *Z. nigrescens*).

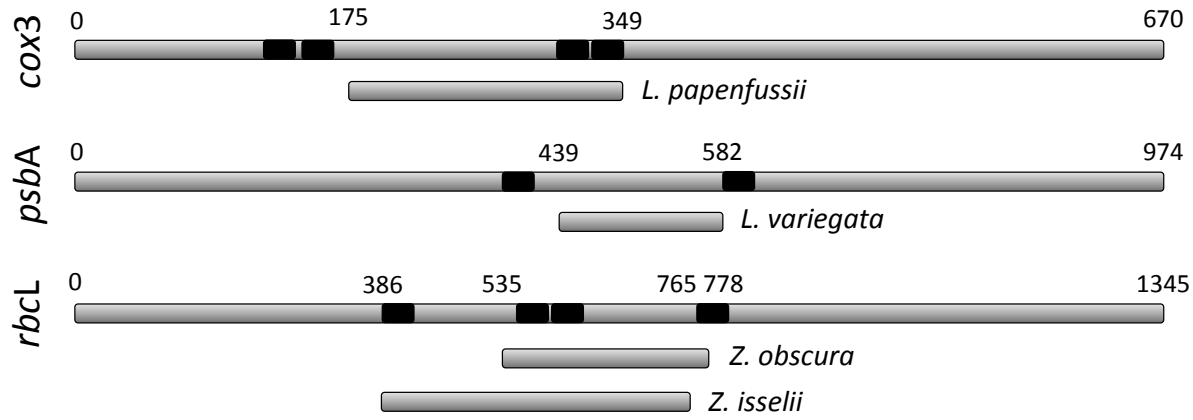

Figure 3.2.2. Graphic representation of types using short-fragment of *rbc*L, *psb*A and *cox*3 or BLAST results.

3.1. Sequence similarity and phylogenies

Results of the BLAST analyses for the four types we were able to sequence are given in Table S3.2.3. Bayesian phylogenetic inference supported the BLAST results, positioning the types next to the species with which they had the highest similarity (Figs. 3.2.11--13). Out of the four types, *D. variegata* and *Z. obscura* came out to be identical to an undescribed Caribbean lineage (*L.* sp.75) and to *L. crassa*, respectively (Table S3.2.3, Figs. 3.2.12, 3.2.13). *P. papenfussii* and *Z. isselii* were resolved as singletons, sister to *L. densa* (98% similarity) and to *L. abscondita* (97% similarity), respectively (Table S3.2.3; Figs. 3.2.11, 3.2.13). Phylogenetic results confirmed that the newly collected specimens that we have associated with *P. dichotoma* based on morphological similarities, belong to the genus *Lobophora*, and

came out as a distinct species with strong node supports (Figs. 3.2.11--13). Specimens collected near the type localities of *A. canariensis*, *A. pacifica* and *L. rickeri* also resolved as a distinct species (*L.* sp9, *L.* sp34, *L. rickeri*). *Lobophora rickeri* is sister to *L. undulata* with strong node supports (Figs. 3.2.11--13). *Aglaozonia canariensis* is sister to the clade comprising *L. undulata* and *L. rickeri* (Figs. 3.2.11--13). *Aglaozonia canariensis* is sister to *L.* sp4 with a strong node support (Figs 3.2.11--13). The phylogenetic position of *A. canariensis* and *L.* sp4 within the *Lobophora* phylogenetic tree is not congruent between the three markers, which is not surprising given the low bootstrap values in the *cox*3 (BI = 73%, Fig. 3.2.11) and *rbc*L (BI = 74%, Fig. 3.2.11) trees. In the *psb*A tree, however, *A. canariensis* and *L.* sp4 are sister species to a group of species including *L. pachyventera*, with a strong node support (BI = 100%, Fig. 3.2.12), indicative of the mostly likely correct phylogenetic position within the *Lobophora* tree.

3.2. Morphology

All the types presently examined clearly presented the generic criteria of the genus *Lobophora*, i.e., a central layer of medullary cells distinctly larger. Morphological illustrations, descriptions and measurements are given in Figs. 3.2.3--10 and Table 3.2.2.

3.3. Epitypification

For the type material for which molecular data could not be obtained, but for which we acquired new material from or near type localities that corresponded morphologically to the original description, we performed epitypification. Epitypification was made for seven species: *A. canariensis*, *A. pacifica*, *L. nigrescens, L. rickeri*, *P. dichotoma*, *R. ceylanica* and *Z. nigrescens*.

Table 3.2.1. Types associated with the genus *Lobophora*.

Type	Locality	Loan	Herbarium number	Morphology	Anatomy	New Name	Reference
Dictyota variegata (J.V.Lamour.) Womersley ex E.C.Oliveira (1809)	Antilles	•	CN C7F100	Fig.3A,B,C,D,E,F	Fig.7A,B,C,D,E,F	*Lobophora variegata*	Oliveira Filho (1977)
Zonaria collaris C.Agardh (1820)	Jamaica, Jamaica		LD 48006, 47966, 47967	Fig.6E	-	-	Agardh (1820)
Zonaria nigrescens Sonder (1845)	Western Australia, Australia		MEL 16822	Fig.5C,D	-	*Lobophora sonderii*	Sonder (1845)
Stypopodium fissum Kützing (1859)	Embouchure de la rivière de Gabon, Equatorial Guinea	•	L 937-273-354	Fig.6C	Fig.9E,F	-	Kützing (1859)
Stypopodium lacinatum Kützing (1859)	Coast of Eritrea, Eritrea	•	L 937-55-255	Fig.6D	Fig.9G,H	-	Kützing (1859)
Zonaria latissima Sonder ex Kützing (1859)	Coast of Eritrea, Eritrea	•	L 937-46-235	Fig.6F	Fig.10A,B	*Lobophora latissima*	Kützing (1859)
Zonaria obscura Dickie (1875)	Mangaia, Cook Islands	•	BM 563329	Fig.6G	-	*Lobophora obscura*	Dickie (1875)
Zonaria isselii Piccone & Grunow (1884)	Massawa, Eritrea	•	W 19388, 19389, 19390, 19393	Fig.4A	Fig.8A,B	*Lobophora isselii*	Piccone (1884)
Lobophora nigrescens J.Agardh (1894)	Dromana Bay, Victoria, Australia		LD 48307	Fig.6A,B	pub.	*Lobophora nigrescens*	Agardh (1894)
Ralfsia ceylanica Harvey ex Barton (1903)	Minicoy, Lakshadweep, India	•	BM 562896	Fig.5A	Fig.9A,B	*Lobophora ceylanica*	Barton (1903)
Aglaozonia canariensis Sauvageau (1905)	Puerto de la Cruz, Tenerife, Canary Islands, Spain		type material missing (PC)	Fig.4B	Fig.8C,D	-	Sauvageau (1905)
Aglaozonia pacifica W.A.Setchell (1922)	Papeete, Tahiti	•	UC/JEPS 5057a	Fig.4C,D	Fig.8E,F	*Lobophora pacifica*	Setchell (1926)
Pocockiella papenfussii (W.R.Taylor) Farghaly (1946)	Bikini Atoll, Marshall Islands	•	MICH WRT46-232	Fig.3G,H	Fig.7G,H	*Lobophora papenfussii*	Farghaly (1980)
Pocockiella dichotoma R.H.Simons (1966)	Kosi Bay, Kwazulu-Natal, South Africa		PRE 625	Fig.4E,F,G,H	Fig.8G,H	*Lobophora dichotoma*	Simons (1966)
Lobophora indica V.Krishnamurthy & M.Baluswami (2000)	Krusadai Island, India		KIA 1520	-	-	*Lobophora ceylanica*	V.Krishnamurthy & M.Baluswami 2000
Lobophora indica V.Krishnamurthy & M.Baluswami (2000)	Krusadai Island, India		KIA 1520	-	-	-	V.Krishnamurthy & M.Baluswami 2000
Lobophora rickeri Kraft (2009)	Wistari Reef, southern Great Barrier Reef, Queensland, Australia		MELU KA00335	pub.	pub.	*Lobophora rickeri*	Kraft (2009)

Table 3.2.2. Comparison of characters of types associated with *Lobophora*. Mean ± SD (in µm). NA : not available.

	Aglaozonia pacifica	*Aglaozonia pacifica*	*Aglaozonia canariensis*	*Pocockiella dichotoma*	*Pocockiella papenfussii*	*Pocockiella papenfussii*	*Lobophora rickeri*	*Dictyota variegata*	*Dictyota variegata*
Type	**Holotype**	**Epitype**	**Epitype**	**Epitype**	**Holotype**	**Isotype**	**Holotype**	**Isotype**	**new coll.**
Thickness									
Average	NA	185.6 ± 16.6	101.9 ± 8.0	129.7 ± 7.8	NA	347.1 ± 22.7	221 ± 17.1	125.9 ± 7.2	151.5 ± 18.5
Min-Max	NA	168 - 202	80 - 112	117 - 140	385 - 640	308 - 388	110 - 500	112 - 140	123.5 - 197
Number of cells									
Average	9	7.5 ± 1.5	5	8.9 ± 0.3	17 - 23	15.6 ± 0.9	10 ± 0.6	7	6.1 ± 0.9
Min-Max	NA	6 - 9	5	8 - 9	NA	14 - 17	8 - 9	7	5 - 7
Number of dorsal cells									
Average	4	3.5 ± 0.5	2	4.0 ± 0	8 – 11	7.5 ± 0.5	5 ± 0.4	3	2.6 ± 0.5
Min-Max	NA	3 - 4	2	4	NA	7 - 8	4 - 5	3	2 - 3
Number of ventral cells									
Average	4	3.5 ± 0.5	2	3.9 ± 0.3	8 – 11	7.1 ± 0.5	3 ± 0.3	3	2.5 ± 0.5
Min-Max	NA	3 - 4	2	3-4	NA	6 - 8	3 - 4	3	2 - 3
Medulla length									
Average	NA	70.54 ± 10.0	79.4 ± 12.2	41.2 ± 2.5	NA	35.5 ± 3.7	80.5 ± 11.8	79.9 ± 5.1	80.7 ± 6.4
Min-Max	NA	60 - 80	60 - 100	36 - 46	57 - 70	28 - 40	68 - 92	74 - 92	67.8 - 93.8
Medulla height									
Average	NA	54.4 ± 6.69	45.4 ± 5.7	24.1 ± 3.4	NA	81.7 ± 11.5	61.1.5 ± 8.8	52.5 ± 5.1	69.7 ± 11.2
Min-Max	NA	47 - 60	30 - 54	20 - 30	NA	60 - 100	52 - 70	40 - 60	50 - 93.8
Medulla width									
Average	NA	30.28 ± 6.04	33 ± 3.7	29.0 ± 2.4	NA	76.8 ± 4.6	29.7 ± 3.1	26.9 ± 2.7	33 ± 9.7
Min-Max	NA	24 - 36	30 - 40	25 - 32	NA	68 - 84	27 - 33	24 - 32	23 - 43
Dorsal height									
Average	NA	69.6 ± 13.14	29.7 ± 2.5	55.2 ± 4.1	NA	141.3 ± 10.9	81 ± 3.3	36.7 ± 3.3	16.1 ± 3.0
Min-Max	NA	57 - 83	26 - 34	50 - 62	NA	124 - 175	78 - 84	30 - 40	12.5 - 25.0
Ventral height									
Average	NA	56.8 ± 3.34	26.8 ± 2.7	50.3 ± 5.0	NA	124.1 ± 14.2	46.5 ± 3.0	36.7 ± 3.3	16.5 ± 2.0
Min-Max	NA	53 - 60	20 - 32	40 - 58	NA	100 - 152	43 - 49	30 - 40	12.5 - 21.3
Thallus									
Growth-form	crustose	crustose	Prostrate	stipitate	crustose	crustose	crustose	stipitate	
Color	dark brown	dark brown	dark green	brown	dark green to dark brown	dark green to dark brown	NA	dark orange brown	dark orange brown to dark green
Reference	Setchell (1922)	This study	This study	Simons (1966)	Taylor (1950)	This study	Kraft (2009)	This study	This study

Table 3.2.2. (suite) Comparison of characters of types associated with *Lobophora*. Mean ± SD (in µm). NA : not available.

	Lobophora indica	***Lobophora minima***	***Ralfsia ceylanica***	***Stypopodium fissum***	***Stypopodium laciniatum***	***Zonaria colaris***	***Zonaria isselii***	***Zonaria latissima***	***Zonaria nigrescens***	***Zonaria obscura***
Type	Lectotype	Lectotype	Holotype	Holotype	Holotype	Holotype	Holotype	Holotype	Holotype	Holotype
Thickness										
Average	421 ± 146	80*	>200	90 ± 10	137.5 ± 12.5	NA	132.5 ± 17.5	145 ± 20	NA	NA
Min-Max	275 - 567	80*	200 -	80 - 100	125 - 150	NA	115 - 150	125 - 165	NA	NA
Number of cells										
Average	16 ± 7	3.5 ± 0.5*	NA	6.5 ± 0.5	7	NA	6.5 ± 0.5	9	NA	NA
Min-Max	9 - 23	3 - 4*	12 -	6 - 7	7	NA	6 - 7	9	NA	NA
Number of dorsal cells										
Average	10 ± 5	1.5 ± 0.5*	NA	3	3	NA	3	4	NA	NA
Min-Max	5 - 15	1 - 2*	7 -	3	3	NA	3	4	NA	NA
Number of ventral cells										
Average	5 ± 2	1*	4.5 ± 0.5	2.5	3	NA	2.5	4	NA	NA
Min-Max	3 - 7	1*	4 - 5	2 - 3	3	NA	2 - 3	4	NA	NA
Medulla length										
Average	NA	75*	50	57.5 ± 7.5	57.5 ± 7.5	NA	57.5 ± 7.5	57.5 ± 7.5	NA	NA
Min-Max	NA	75*	50	50 - 75	50 - 75	NA	50 - 75	50 - 75	NA	NA
Medulla height										
Average	NA	30*	40	30	35	NA	42.5 ± 7.5	40	NA	NA
Min-Max	NA	30*	40	30	35	NA	35 - 50	40	NA	NA
Medulla width										
Average	NA	30*	NA	22.5 ± 2.5	22.5 ± 2.5	NA	22.5 ± 2.5	22.5 ± 2.5	NA	NA
Min-Max	NA	30*	NA	20 - 25	20 - 25	NA	20 - 25	20 - 25	NA	NA
Dorsal height										
Average	NA	35*	NA	40	50	NA	50	57.5 ± 7.5	NA	NA
Min-Max	NA	35*	NA	40	50	NA	50	50 - 75	NA	NA
Ventral height										
Average	NA	18*	NA	25 ± 5	50	NA	40 ± 10	57.5 ± 7.5	NA	NA
Min-Max	NA	18*	NA	30 - 40	50	NA	30 - 50	50 - 75	NA	NA
Thallus										
Growth-	crustose-prostrate		crustose					stipitate		
Color	dark/yellow brown		dark brown	dark brown	dark brown	green	dark brown	dark brown	dark brown	dark brown
Reference	Krishnamurthy & Baluswami (2000)		Barton (1903)	This study	This study	C.Agardh (1820)	This study	This study	Sonder (1845)	Dickie (1875)

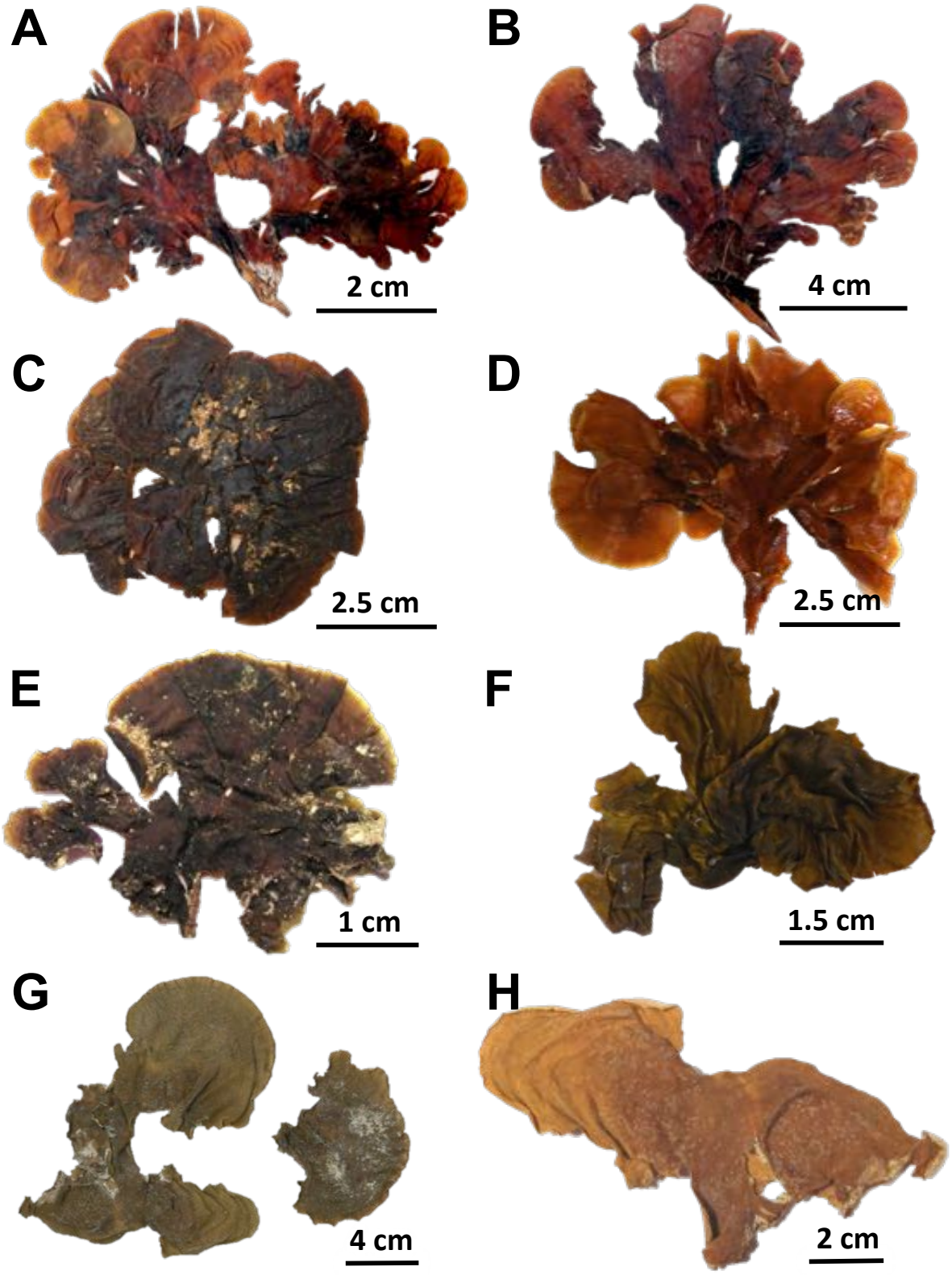

Figure 3.2.3. External morphology of *L. variegata* holotype[(1)] (A), isotype[(1)] (B), and new specimens from Bahamas[(2)] (LAF06912) (C), Florida Keys[(2)] (LAF06914) (D), Guadeloupe[(3)] (E), St Kitts and Nevis[(2)] (LAF06947) (F), *L. papenfussii* holotype[(4)] (G) and isotype[(5)] (H). Photo credits: [(1)]Courtesy of Chantal Billard of the Lamouroux Herbarium (CN, France), [(2)]O. Camacho, [(3)]C. Payri, [(4)]M. Wynne, [(5)]C. Vieira

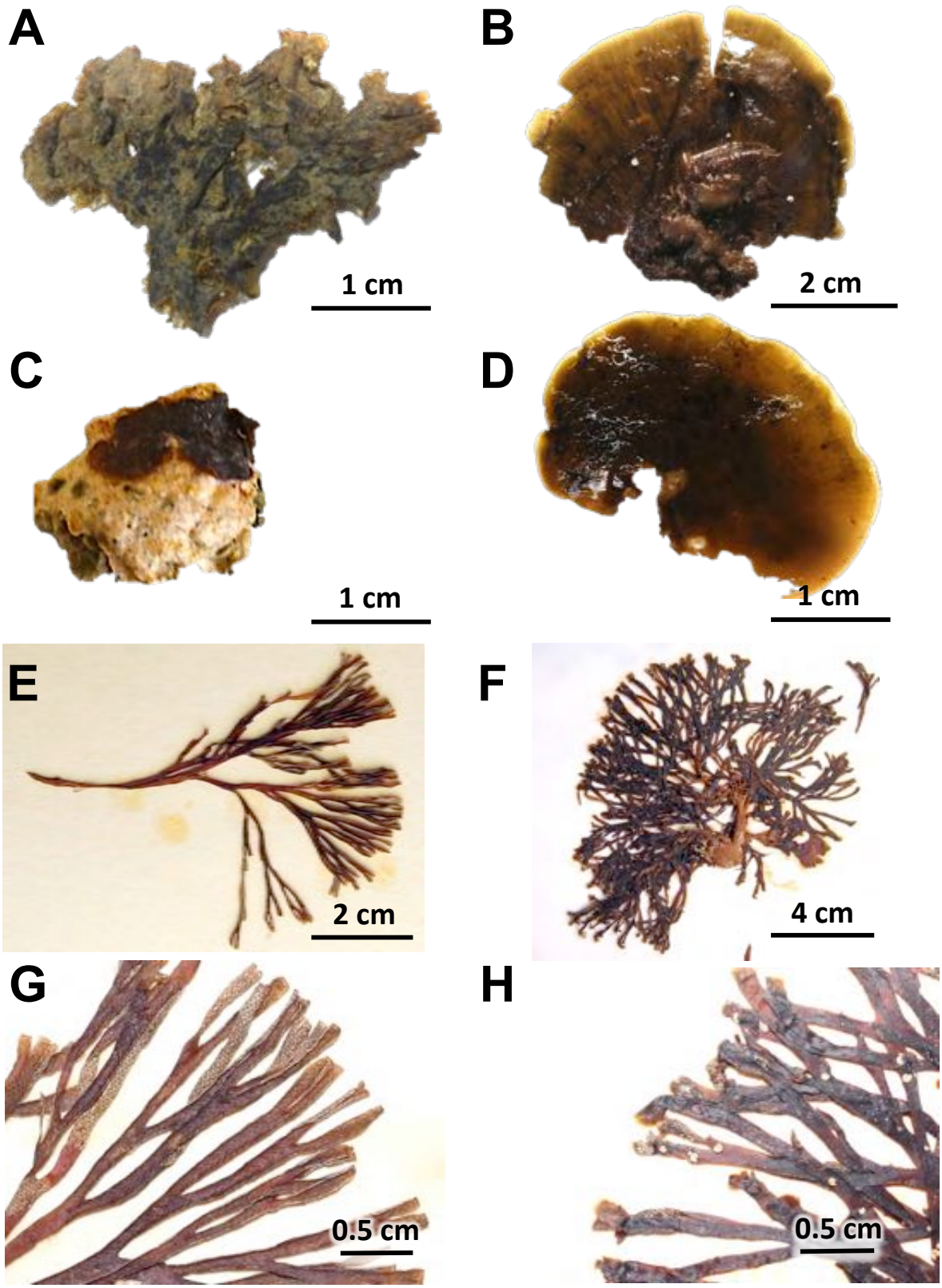

Figure 3.2.4. External morphology of *Z. issellii* type[(1)] (A), *L. canariensis* epitype (ODC2383)[(1)] (B), *A. pacifica* holotype[(1)] (C), *A. pacifica* epitype[(2)] (D), *P. dichotoma* holotype[(3)] (E), *P. dichotoma* epitype (D1006)[(4)] (F), *P. dichotoma* holotype close-up [(3)] (G), *P. dichotoma* epitype close-up (D1006) [(4)] (H). Photo credits: [(1)]C. Vieira, [(2)]M. Zubia, [(3)]R. Anderson, [(4)]L. Mattio

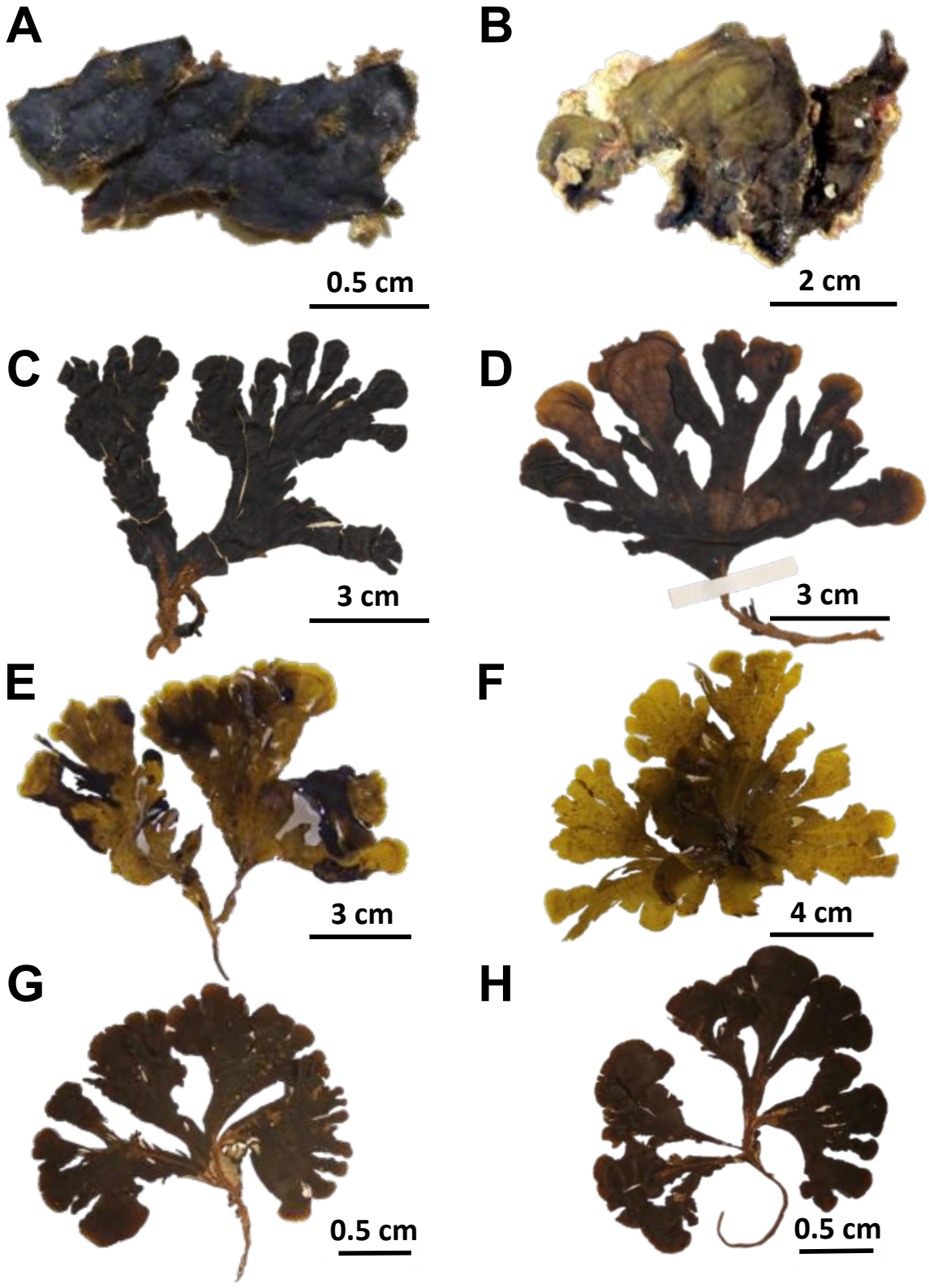

Figure 3.2.5. External morphology of *R. ceylanica* holotype[(1)] (A), *L. densa*[(1)] (B), *Z. nigrescens* holotype[(2)] (C), *Z. nigrescens* isotype[(2)] (D), *L. nigrescens* sensu Sun et al. (2012) (JFC0286)[(3)] (E), *L. nigrescens* sensu Sun et al. (2012) (JFC0215)[(3)] (F), *L. nigrescens* sensu Sun et al. (2012) (IRD7920)[(1)] (G), *L. nigrescens* sensu Sun et al. (2012) (IRD7920)[(1)] (H). Photo credits: [(1)]C. Vieira, [(2)]Courtesy of the staff of the National Herbarium of Victoria (MEL, Melbourne, Australia), [(3)]H. Verbruggen

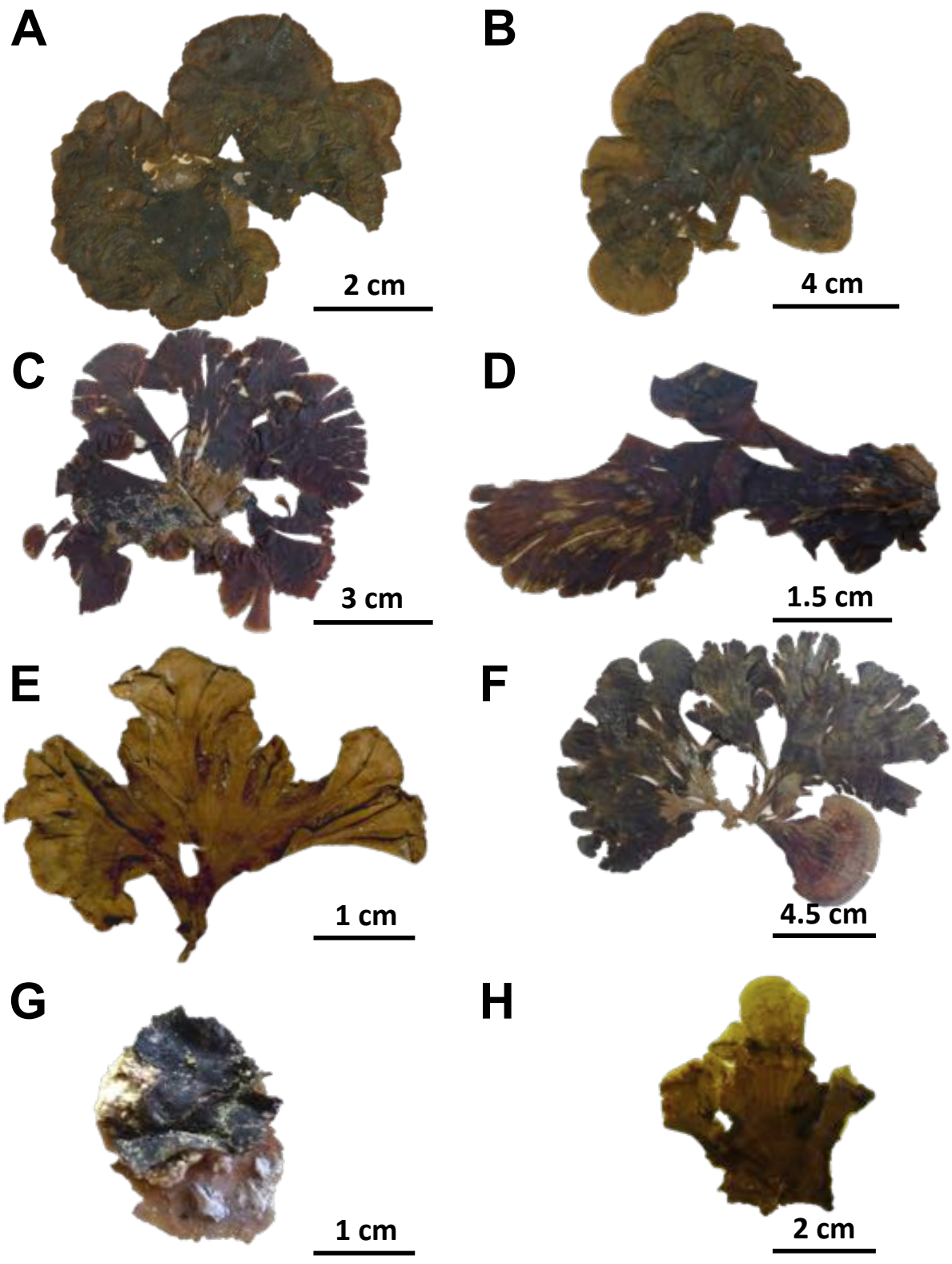

Figure 3.2.6. External morphology of *L. nigrescens* holotype[(1)] (A), *L. nigrescens* isotype[(1)] (B), *S. fissum* type[(2)] (C), *S. laciniatum* type[(2)] (D), *Z. collaris* type[(2)] (E), *Z. latissima* type[(2)] (F), *Z. obscura* type[(1)] (G), *L. densa* (CV3040)[(1)] (H). Photo credits: [(1)]Courtesy of Patrik Froden of the Botanical Museum (LD, Sweden), [(2)]C.Vieira.

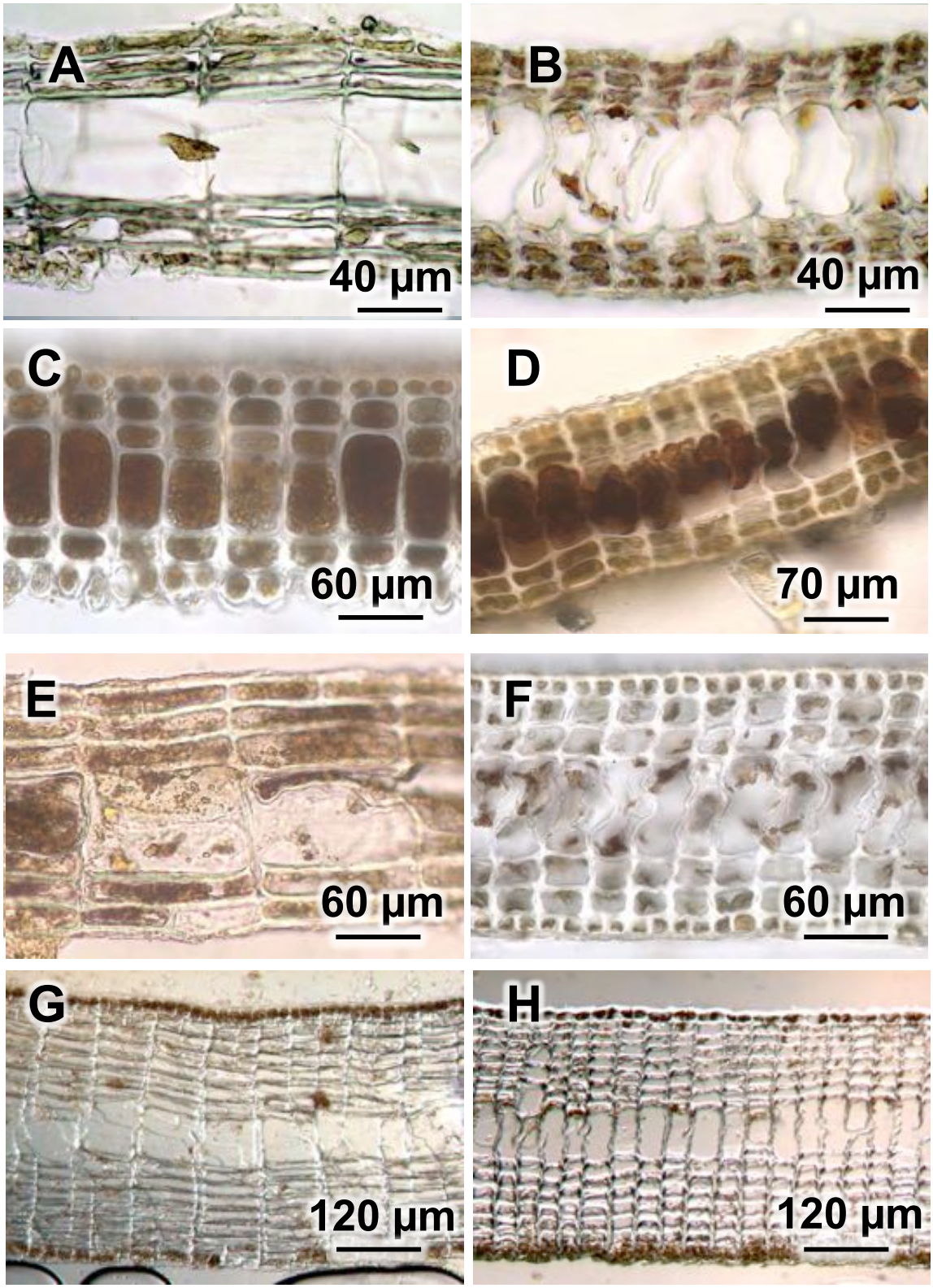

Figure 3.2.7. Anatomy of *L. variegata* isotype longitudinal section (LS)[(1)] (A) and transverse section (TS)[(1)] (B), *L. variegata* TS (Florida Keys, LAF06914)[(2)] (C), *L. variegata* TS (Bahamas, LAF06912)[(2)] (D), *L. variegata* TS (Guadeloupe, GUA009)[(3)] (E), *L. variegata* TS (St Kitts and Nevis, LAF06947)[(2)] (F), *L. papenfussii* isotype LS[(1)] (G), *L. papenfussii* TS[(1)] (H). Photo credits: [(1)]C. Vieira, [(2)]O. Camacho, [(3)]C. Payri

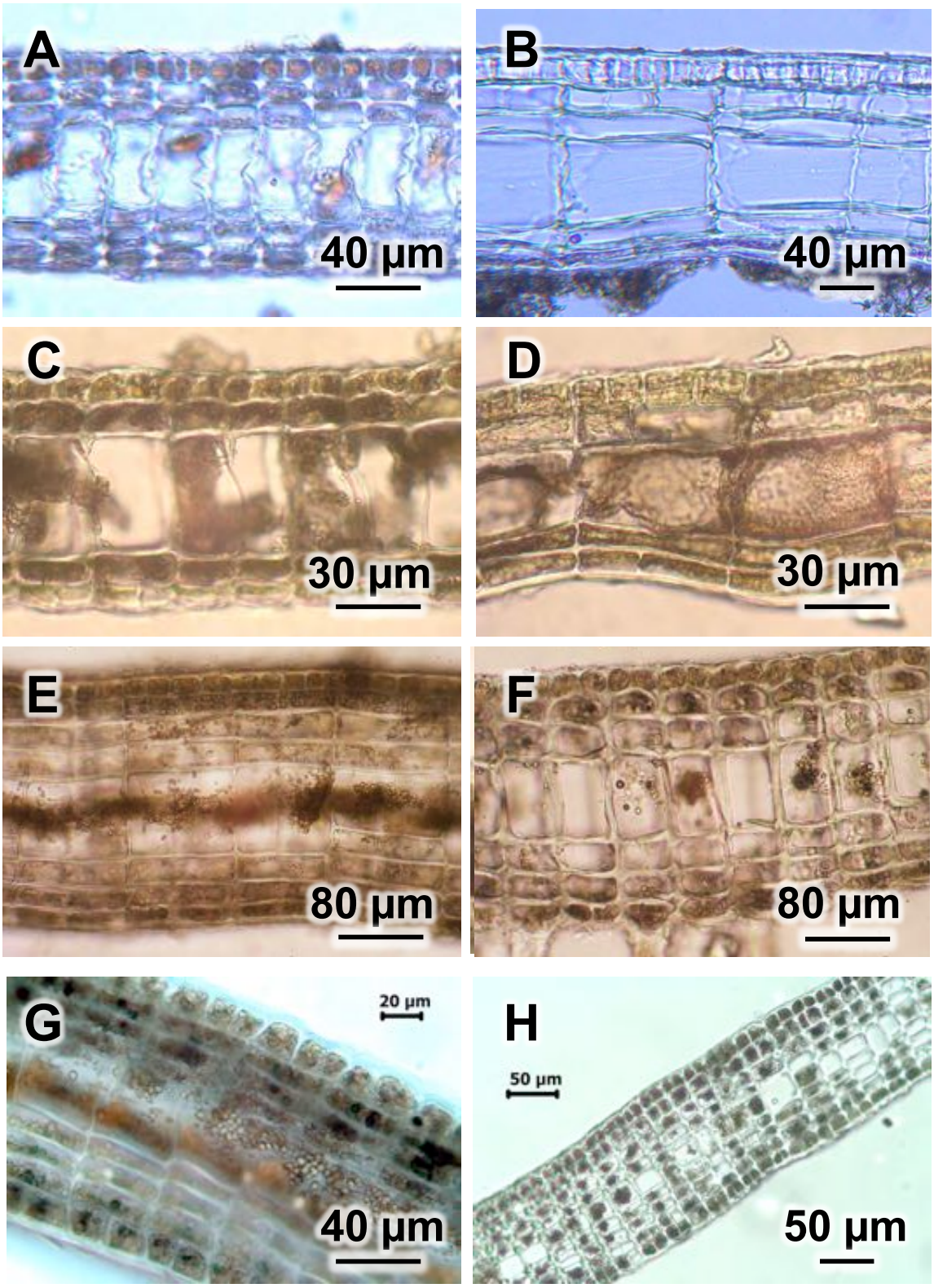

Figure 3.2.8. Anatomy of *L. isselii* LS (A) and TS (B), *L. canariensis* epitype (ODC2383) LS (C) and TS (D), *L. pacifica* epitype (UPF-026) LS (E) and TS (F), *L. dichotoma* epitype (D1006) LS (G) and TS (H). Photo credits: C. Vieira

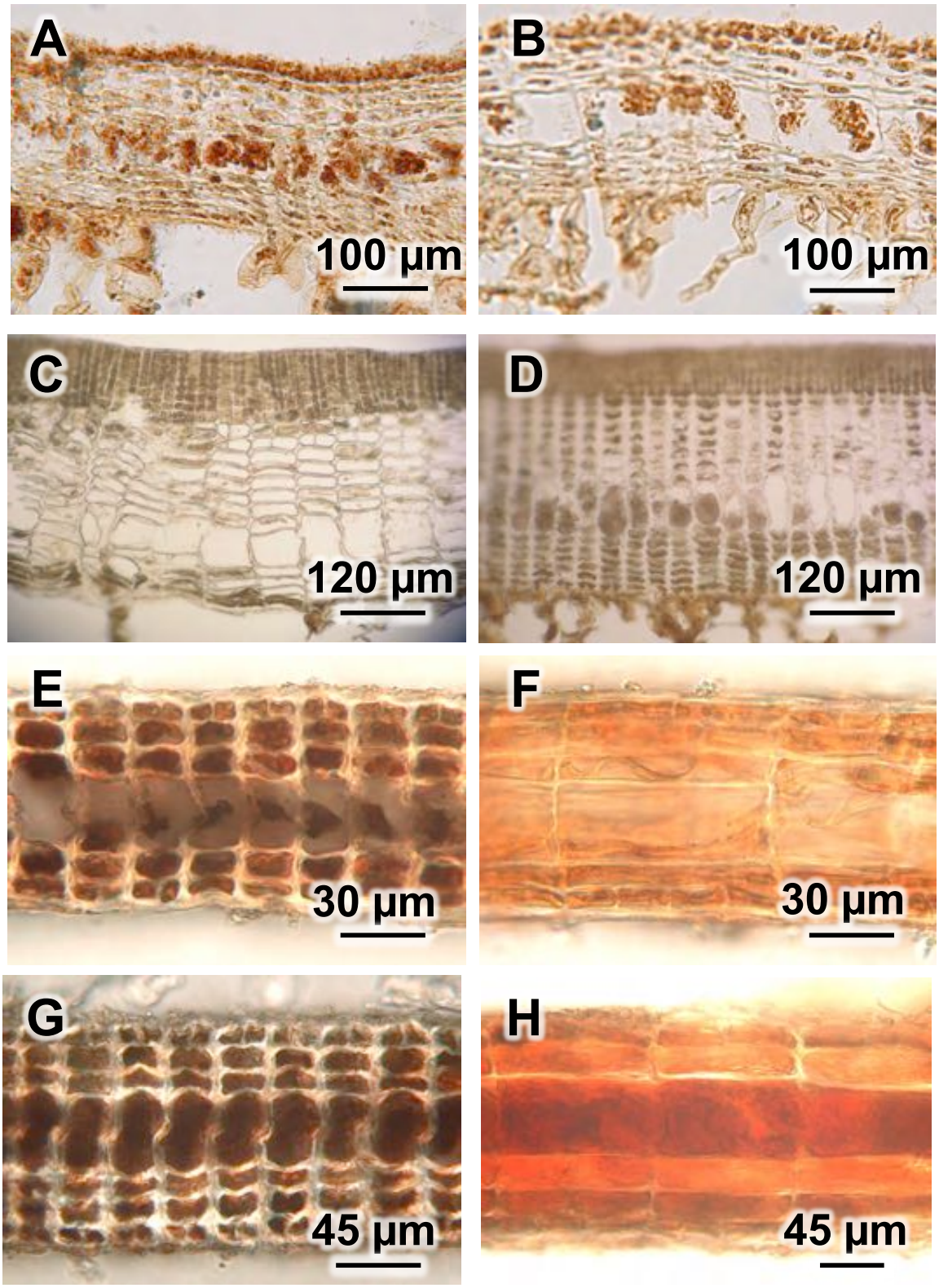

Figure 3.2.9. Anatomy of *R. ceylanica* holotype LS (A) and TS (B), *L. densa* (CV3040) LS (C) and TS (D), *S. fissum* holotype LS (E) and TS (F), *S. laciniatum* holotype LS (G) and TS (H). Photo credit: C. Vieira

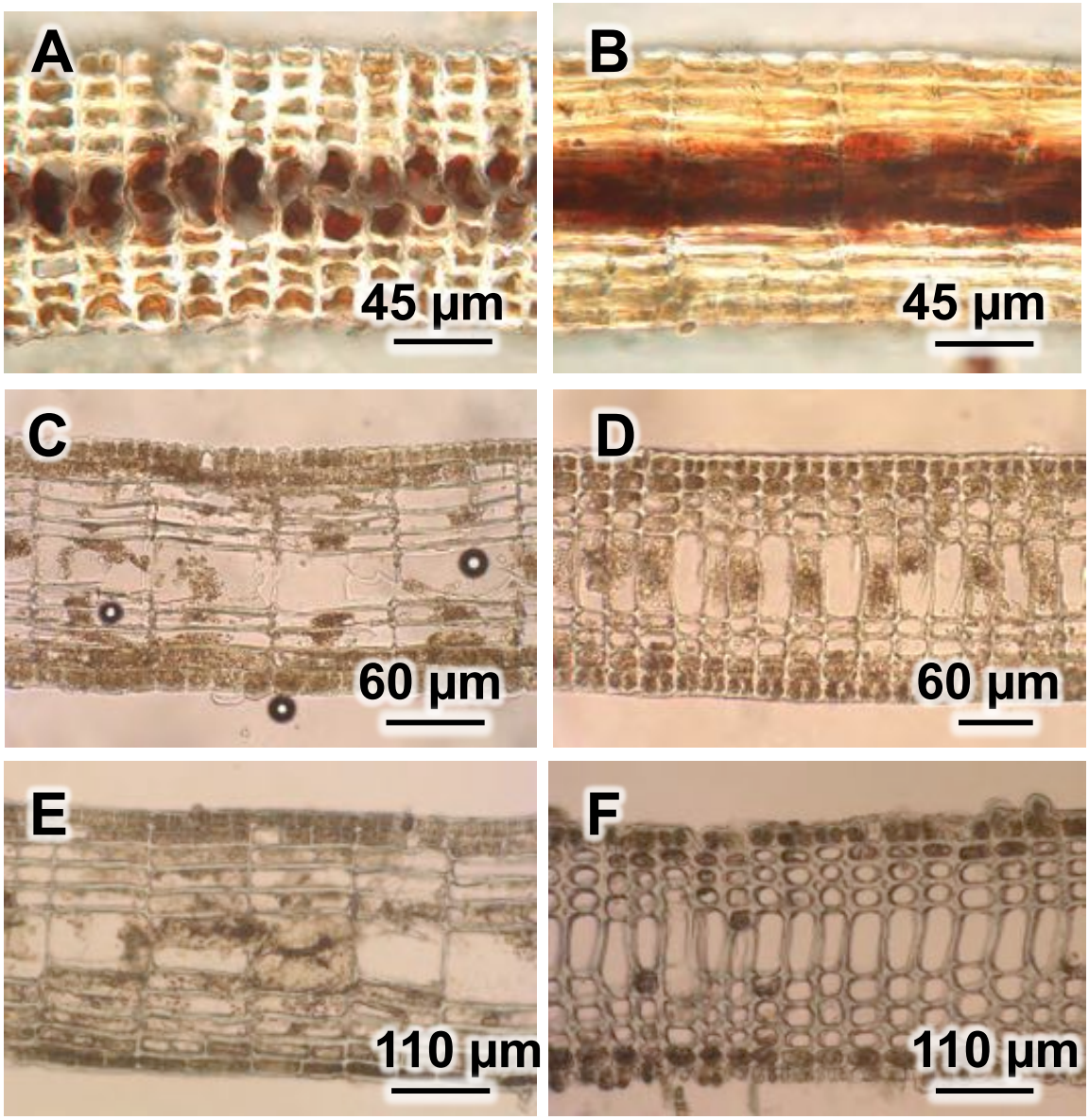

Figure 3.2.10. Anatomy of *Z. latissima* holotype LS (A) and TS (B), *L. nigrescens* sensu Sun et al. (2012) (IRD7920) LS (C) and TS (D), *L. crassa* (CV3040) LS (E) and TS (F). Photo credit: C. Vieira

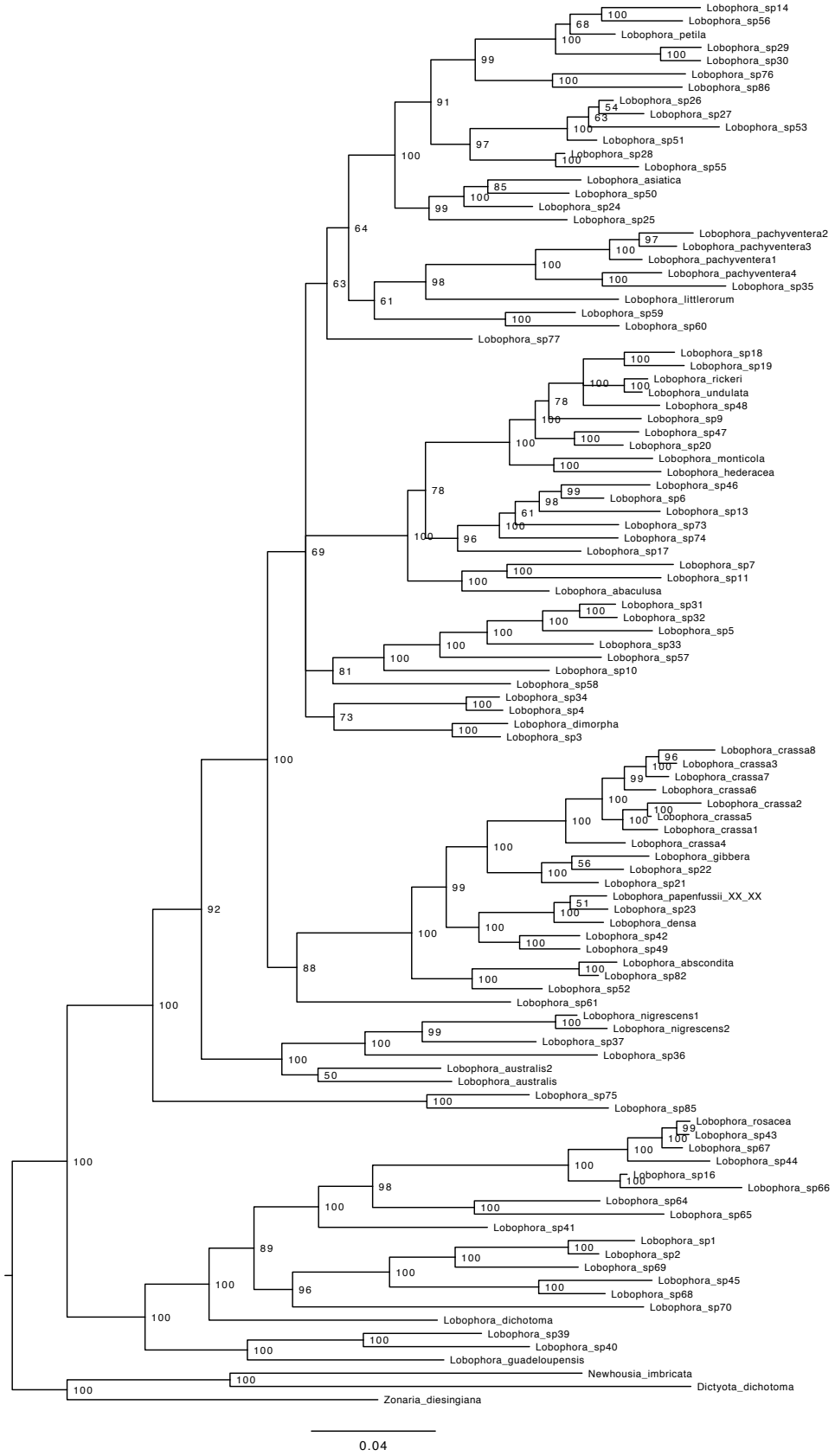

Figure 3.2.11. Bayesian phylogenetic tree based on *cox*3 sequences.

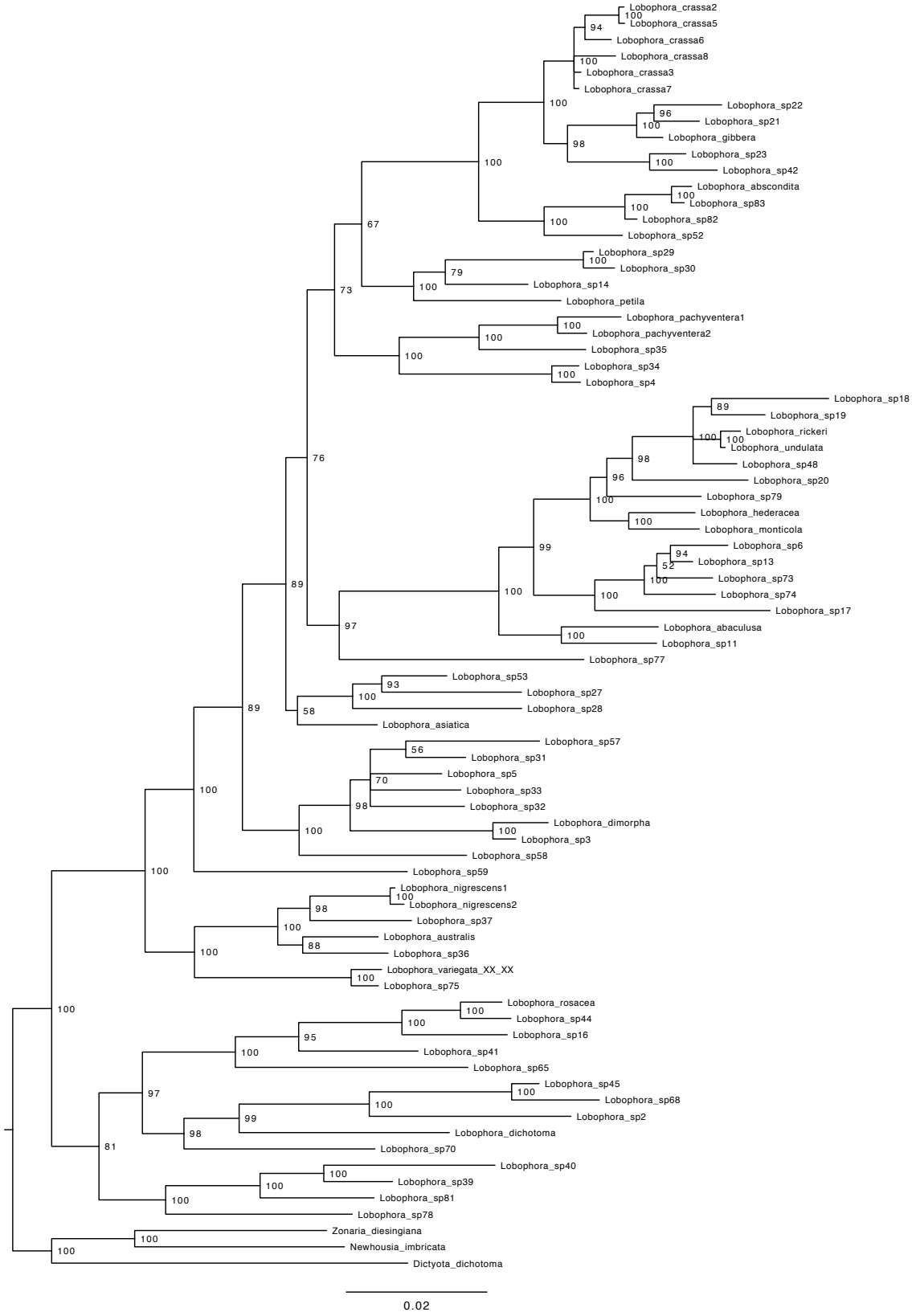

Figure 3.2.12. Bayesian phylogenetic tree based on *psb*A sequences.

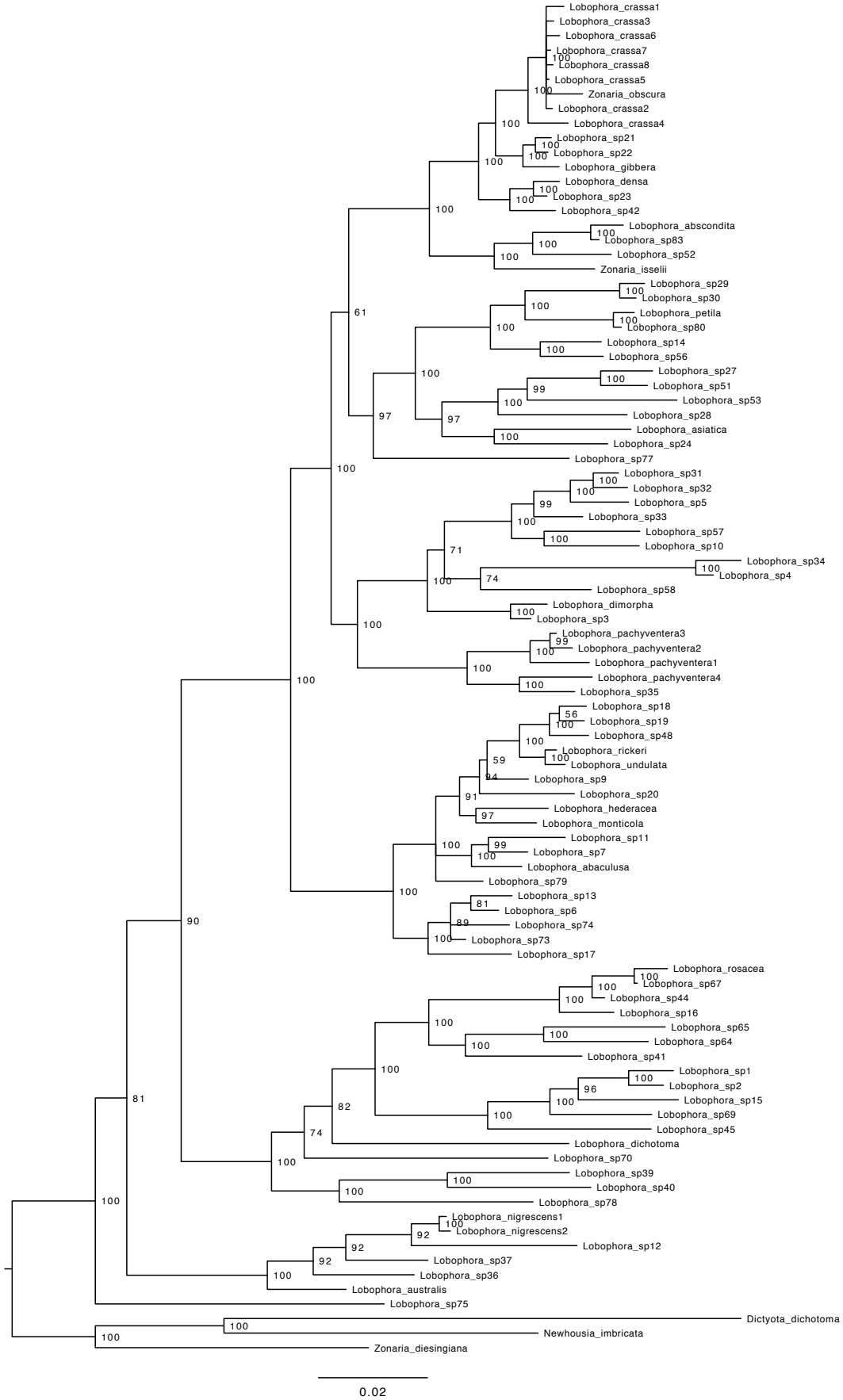

Figure 3.2.13. Bayesian phylogenetic tree based on *rbc*L sequences.

4. Discussion

Currently, 20 species of the brown algal genus *Lobophora* are accepted taxonomically (Guiry & Guiry, 2015). From the literature we identified 17 names of types associated with *Lobophora* (Table 3.2.1). We were able to perform molecular analyses on half of these types, with only four yielding useful results. Molecular analyses of the four type specimens allowed us to define three of them as distinct species (*D. variegata, P. papenfussii* and *Z. isselii*) and to match the fourth one (*Z. obscura*) to a recently described species (*L. crassa*). Based on these results, seven species are herein reinstated (*A. canariensis, A. pacifica, L. nigrescens, R. ceylanica, Z. isselii, Z. nigrescens* and *Z. obscura*), and three others are reduced to taxonomic synonyms (*L. crassa, L. densa* and *L. indica*), leading to a total of 23 taxonomically accepted *Lobophora* species (Table 3.2.3).

Table 3.2.3. Taxonomically accepted *Lobophora* species on the date of the 1st of May 2015.

Species
Lobophora abaculusa C.W.Vieira, Payri & De Clerck
Lobophora abscondita C.W.Vieira, Payri & De Clerck
Lobophora asiatica Z.Sun, Ji.Tanaka & H.Kawai
Lobophora australis Z.Sun, Gurgel & H.Kawai
Lobophora canariensis (Sauvageau) C.W.Vieira, De Clerck & Payri
Lobophora ceylanica (Harvey) C.W.Vieira, De Clerck & Payri
Lobophora dichotoma (R.H.Simons) P.C.Silva
Lobophora gibbera C.W.Vieira, Payri & De Clerck
Lobophora hederacea C.W.Vieira, Payri & De Clerck
Lobophora isselii (Piccone and Grunow) C.W. Vieira, De Clerck & Payri
Lobophora minima V.Krishnamurty & M. Baluswami
Lobophora monticola C.W.Vieira, Payri & De Clerck
Lobophora nigrescens J.Agardh
Lobophora obscura (Dickie) C.W.Vieira, De Clerck & Payri
Lobophora pachyventera Z.Sun, P.-E.Lim, Ji.Tanaka & H.Kawai
Lobophora pacifica (Setchell) C.W. Vieira, M. Zubia, De Clerck & Payri
Lobophora papenfussii (W.R.Taylor) Farghaly
Lobophora petila C.W.Vieira, Payri & De Clerck
Lobophora rickeri Kraft
Lobophora rosacea C.W.Vieira, Payri & De Clerck
Lobophora sonderii C.W.Vieira, De Clerck & Payri
Lobophora undulata C.W.Vieira, Payri & De Clerck
Lobophora variegata (J.V.Lamouroux) Womersley ex E.C.Oliveira

4.1. Taxonomic treatment

***Dictyota variegata* J.V.Lamour. (1809).** Collected by the French botanist Louis Claude Marie Richard in the Antilles, West Indies, the species was described as *Dictyota* (Lamouroux, 1809), which at the time was still broadly defined (De Clerck 2003). Although the exact type locality is unknown, it is plausible that *D. variegata* was collected in a French territory in the Lesser Antilles. Moreover, considering the geopolitical situation in 1809, *D. variegata* was most possibly collected in Guadeloupe, the only *territory* in the *Antilles* islands under *French* sovereignty during the Napoleonic wars. *D. variegata* has had a complicated taxonomic history that was well described by Sun *et al.* (2012). Lectotypification was established by Womersley (1967), and the species was validly transferred to *Lobophora* by Oliveira (1977). Type material of *D. variegata* is morphologically characterized by dark orange to dark brown thalli organized in dense, erect blades, with fronds composed of several lobes, and a rudimentary stipe (not as evident as in *Z. nigrescens*). Molecular analyses link the *D. variegata* type specimen to the species *L.* sp75 distributed in the Caribbean (Bahamas, Florida Keys, Grand Cayman, St Kitts and Nevis and Guadeloupe). Our newly sequenced specimens from this species (*L.* sp75) show internal morphological similarities with the *D. variegata* type: namely a single medullary layer and two to three upper- and lower-cortical cell layers (Fig. 3.2.7C--F). The specimen from the Florida Keys (LAF06914) is morphologically the most similar to the *D. variegata* type (Fig. 3.2.3D). Among the newly collected specimens from the Caribbean, the only ones with a rudimentary stipe (specimens from Guadeloupe) also comprise the species that matches this MOTU. The presence of a stipe was more or less evident among the different specimens in this MOTU (Fig. 3.2.3C--F). For the reasons discussed earlier, the presence of this MOTU in Guadeloupe supports the idea of this MOTU being *D. variegata*. Since morphology (external and internal) and historical geography corroborate molecular results, we are confident of having identified the genuine *L. variegata*. Unlike what was previously thought, *L. variegata* appears to be restricted to the Caribbean.

Distribution. – Caribbean: Bahamas, Florida Keys, St Kitts and Nevis, Grand Cayman, Guadeloupe.

Description. – Thallus fan-shaped, erect, more or less stipitate, up to 8 cm wide and 6 cm tall, forming clusters of ruffled dark brown to dark green blades. Blades 123--197 µm thick, composed of 5--7 cell layers, a single medullary layer and cortex of two to three cell layers on the dorsal and ventral sides. *L. variegata* occurs in shallow

waters (down to 7 m), on blocks of hard substratum or coral rubble mixed among numerous other algae including *Dictyota* J.V. Lamouroux, *Sargassum* C. Agardh, *Jania* J.V. Lamouroux and *Caulerpa* J.V. Lamouroux. In the Florida Keys, *L. variegata* LAF06914, reported as *Lobophora* sp. by Camacho *et al.* (2015), was also found growing conspicuously on the holdfast and basal branches of *Sargassum pteropleuron* Grunow and some Gorgonian corals, and also on *Thalassia testudinum* Banks *ex* König leaves.

***Pocockiella papenfussii* W.R.Taylor (1946).** *P. papenfussii* was described from Bikini Atoll, Marshall Islands, as a new species of *Pocockiella* (Taylor, 1950). Farghaly (1980) transferred *P. papenfussii* to the genus *Lobophora,* following the suggestion of Womersley (1967). Molecular analyses disclosed that *P. papenfussii* is as a distinct species closely related to *L. densa*. Although *L. papenfussii* resembles *L. densa* in morphology (Figs. 3.2.3G-H, 3.2.7G-H, Table 3.2.2), sections of the isotype do not show the distinct numerous superficial cell-layers characteristic of *L. densa*. Although molecular and anatomical evidence tends to support that *P. papenfussii* and *L. densa* are separate species, we recommend further taxonomic investigation of this species to confirm its distinctness from *L. densa*.

Description. – *Lobophora papenfussii* has a crustose thallus 308--640 µm thick, composed of 14--17 cell layers; a single layered medulla, a seven to eight layered cortex on the dorsal side and a six to eight to eight layered cortex on the ventral side.

Note. – Based on the geographical proximity, Bittner *et al.* (2008) designated the specimen (IRD1382 = *L.* sp15) collected from the Solomon Islands as *L. papenfussii*. However, their sequence did not match the *L. papenfussii* type, and the morphology of that specimen is clearly distinct from the *L. papenfussii* types (isotype and holotype).

***Zonaria isselii* Piccone and Grunow (1884).** *Z. isselii* was described from Massawa, Eritrea. It was considered a taxonomic synonym of *L. variegata* by Papenfuss (1943). Molecular and morphological results confirm that *Z. isselii* is a *Lobophora* (Figs. 4A, 8A-B) and molecular analyses concluded that it is a distinct species. Therefore, we hereby propose the reinstatement of *Z. isselii* Piccone & Grunow (1884), and the following new combination:

Lobophora isselii (Piccone and Grunow) C.W. Vieira, De Clerck & Payri ***comb. nov.*** ≡ *Zonaria isselii Piccone and Grunow* (1884: 297, pl. VII: Figs 1--4; Pl. IX: Fig. 1) in Piccone, A. Contribuzioni all'algologica Eritrea. *Nuovo Giornale*

Botanico Italiano 16: 281--332, pls VII--IX. 1884 – **Lectotype (designated here)**: ERITREA. Massawa, No. 19388 (W!).

Note. – Multiple plates of *Z. isselii* were produced by Piccone without designation of a specific specimen as the holotype. We hereby designate the specimen No. 19388 as the lectotype of *L. isselii*.

Description. – Thalli 115--150 µm thick, composed of 6--7 cell layers, a single layer of medulla, a dorsal cortex of three cell layers and ventral cortex of two cell layers. An ecological description of *L. isselii* still needs to be done.

***Zonaria obscura* Dickie (1875).** *Z. obscura* was described from Mangaia, Cook Islands, South Pacific. Dickie (1875) described the species as a procumbent, leathery, suborbicular, wavy and sparsely hairy, very dark olive-color thallus with stringy rhizoids on the ventral surface. Molecular analyses disclosed that *Z. obscura* corresponds to the species *L. crassa* Z. Sun, P.E. Lim & H. Kawai (2012), which was shown to form a complex of at least four MOTUs (Vieira *et al.*, 2014b). The original morphological description of *Z. obscura* fits the description of *L. crassa* (Sun *et al.*, 2012; Vieira *et al.*, 2014b). Additionally, the presence of *L. crassa* in different places in the Pacific (e.g., Hawaii, New Caledonia) further supports the molecular results and the morphological resemblance. We hereby propose the resurrection of *Z. obscura* Dickie and the following new combination:

Lobophora obscura (Dickie) C.W. Vieira, De Clerck & Payri ***comb. nov.*** ≡ *Zonaria obscura* Dickie 1875: 31, in Dickie, G. (1875). Notes on algae from the Island of Mangaia, South Pacific. *Journal of the Linnean Society of London, Botany* 15: 30--33. – Holotype: COOK ISLANDS: Mangaia (BM!) ≡ *L. crassa* Z.Sun, P.E. Lim & H.Kawai, in Sun, Z., Hanyuda, T., Lim, P.-E., Tanaka, J., Gurgel, C.F.D., & Kawai, H. Taxonomic revision of the genus *Lobophora* (Dictyotales, Phaeophyceae) based on morphological evidence and analyses *rbc*L and *cox*3 gene sequences. *Phycologia* 51:500--512. 2012 **syn. nov.**.

Notes. – Future molecular studies may be necessary to tease apart members of the complex *L. obscura* complex and characterize them as separate species.

***Aglaozonia canariensis* Sauvageau (1905).** *A. canariensis* was originally described from the Canary Islands, Spain. In his original description, Sauvageau (1905) already highlighted the similarity of his new species to *L. variegata* (as *Zonaria variegata*). Papenfuss (1943) suggested that *A. canariensis* is a taxonomic synonym of *L. variegata* (as *Pocockiella variegata*) based on clear morphological criteria specific to the genus *Lobophora*. In spite of the taxonomic treatment proposed by Papenfuss (1943), Abbott and Huisman (2003) proposed the

combination *Cutleria canariensis* (Sauvageau) I.A. Abbott & J.M. Huisman (2003) based on the argument that *Aglaozonia* was recognized as the sporophyte phase of *Cutleria* (Womersley, 1987). Morphological (external and internal) features of newly collected specimens (Figs. 4B, 8C-D) from Punta del Hidalgo, Tenerife, Canary Islands (Spain), located 30 km northeast of Puerto de la Cruz (type locality), match the original description of *A. canariensis* (Sauvageau, 1905) and the drawings of internal morphology by Børgesen (1926) of a plant collected at the same locality in which Sauvageau originally found it. Molecular results of newly collected specimens confirmed that the correct generic identity of *A. canariensis* is actually *Lobophora* as advocated by Papenfuss (1943). We hereby propose the following new combination:

Lobophora canariensis (Sauvageau) C.W. Vieira, De Clerck & Payri ***comb. nov.***

≡ *Aglaozonia canariensis* Sauvageau 1905: 79, in Sauvageau, C. (1905). Observations sur quelques Dictyotacées et sur un *Aglaozonia* nouveau. *Bulletin de la Station Biologique d'Arcachon* 8: 66--81 (holotype missing in PC) – **Neotype (designated here):** SPAIN. Punta del Hidalgo: Vicinity of Puerto de la Cruz, Tenerife, Canary Islands, No. ODC2383 (PC).

Note. – The herbarium material housed in the Muséum National d'Histoire Naturelle, Paris (PC) is missing.

Description. – Thalli 80--112 µm thick and composed of 5 cell layers, composed of a single cell-layered medulla, and two cell-layered cortex on the dorsal and ventral sides. It has a crustose thallus firmly attached to the substrate, following the sinuated surface of the rocks. The species is common in intertidal rock pools and subtidal down to about -20 m depth, where it can form crusts up to 20 cm in diameter. The orangey-brown color with dark brown spots is distinctive and sets the species apart from sympatric congeners.

Aglaozonia pacifica **Setchell (1926).** *A. pacifica* was described from Papeete, Tahiti (Setchell, 1926). The species was found closely appressed to a calcareous red crust (*Porolithon*), but the author did not give further details on its morphology. The original description clearly corresponds to a *Lobophora*: "a central layer of larger cells, on each side of which are four layers of flattened cells". Furthermore, the author noted the similarity to *L. variegata* (as *Z. variegata*) and *Z. latissima* (in the current paper shown to be a *Lobophora*). Among the new collections from Tahiti, we identified a specimen from Fa'aa with a shelf-like morphology and with the basal part attached to the substratum (Fig. 3.2.4D). The Fa'aa specimen shows internal morphology similar to the description by Setchell (Fig. 3.2.8E-F).

Consequently, we hereby propose the reinstatement of *Aglaozonia pacifica* Setchell and the following new combination:

Lobophora pacifica (Setchell) C.W. Vieira, M. Zubia, De Clerck & Payri ***comb. nov.*** ≡ *Aglaozonia pacifica* Setchell (1926: 90) in Setchell, W.A., Setchell, C.B.P.C., & Parks, H.E. Tahitian spermatophytes collected by WA Setchell, CB Setchell, and HE Parks. *University of California Publications in Botany* 12: 61--142, Pls 7--22. 1926 – **Epitype (designated here):** FRENCH POLYNESIA. Fa'aa: Vicinity of Papeete, Moorea, Feb. 2014, No. UPF026 (PC).

Note. – Since only a small fragment of the Holotype material remains in UC, we selected an epitype (UPF026) collected at the type locality.

Distribution. – So far *L. pacifica* is known only from French Polynesia (Moorea, Tahiti) and New Caledonia.

Description. – Thalli 168--202 µm thick, composed of 7--9 cell layers; a single layered medulla, a three to four layered cortex on the dorsal and ventral sides. The thallus is. *L. pacifica* grows on dead corals, hidden under coral assemblages, and is common on barrier reefs near the front reef, in exposed areas subject to wave action, down to -30 m depth.

Pocockiella dichotoma **Simons (1966).** *P. dichotoma* was described from Kosi Bay, Kwazulu-Natal, South Africa (Simons, 1966). Silva in Silva *et al.* (1996) made the combination *Lobophora dichotoma* by recognizing the nomenclatural priority of *Lobophora* against *Pocockiella*. *L. dichotoma* presents a very characteristic and atypical morphology, which differentiates it from any other *Lobophora* species. Although the internal morphology of *P. dichotoma* accurately fits the generic description of *Lobophora*, the external appearance does not. As shown in Fig. 3.2.4E--H, *L. dichotoma* presents dichotomizing, strap-shaped branches very similar to other Dictyotales genera (e.g. *Dictyota, Stoechospermum, Zonaria* etc.), while *Lobophora* species documented to date typically show broad and entire, flabellate thalli (Lamouroux, 1809; Agardh, 1894; Taylor, 1950; Kraft, 2009; Sun *et al.*, 2012; Vieira *et al.*, 2014b). Morphological (external and internal) comparisons of newly collected specimens from Ribbon Reef, Sodwana Bay, 70 km south of Kosi Bay (type locality), with *L. dichotoma* holotype material (PRE!) indicated that they are the same species (Figs. 4E--H, 8E--H). Molecular and anatomical data of newly collected specimens confirm that this species belongs in the genus *Lobophora*. Furthermore, molecular analyses placed *L. dichotoma* as part of the most basal lineage (Fig. 3.2.11--13). *L. dichotoma* has been reported only from Kwazulu-Natal, South Africa, and in the

southern part of Madagascar. The type species of *L. dichotoma* is epitypified by newly collected material from Sodwana Bay (D1006), Kwazulu-Natal (South Africa). *L. dichotoma* was found at 19--20 m depth attached to hard substrata (e.g. sandstone) on reefs with scattered sandy patches, and loose pebbles.

***Ralfsia ceylanica* Harvey ex Barton (1903).** *R. ceylanica* was described from Lakshadweep [formerly the Laccadive Islands], India. The original description and drawings of Barton (1903) clearly correspond to those of a *Lobophora*. The synonymy to *L. variegata* was first suggested by Papenfuss (1943). It is a crustose species with a thick and unique anatomy (Figs. 3.2.5A, 3.2.9A-B). Two species of *Lobophora, L. densa* (Figs. 3.2.5B, 3.2.9C-D) and *L. indica*, morphologically resemble *R. ceylanica*. *L. densa* was reported from the Maldives, 330 km south of Minicoy, Lakshadweep, India, and *L. indica* from the southeastern coast of India (Krishnamurthy & Baluswami, 2000). Morphological similarities and geographic proximity between these three species convince us that they are conspecific. Consequently, we hereby propose the following new combination:

Lobophora ceylanica (Harvey) C.W. Vieira, De Clerck & Payri ***comb. nov.*** ≡ *Ralfsia ceylanica* Harvey ex Barton (1903: 477, Pl. 13: Figs 1--4) in Barton, E.S. List of marine algae collected at the Maldive and Laccadive Islands by J.S. Gardiner, Esq., M.A. *Journal of the Linnean Society of London, Botany* 35: 475--482, Pl. 13. 1903 ≡ *Lobophora densa* C.W. Vieira, De Clerck, Payri, in Vieira, C., D'hondt, S., De Clerck, O., & Payri, C.E. Toward an inordinate fondness for stars, beetles and *Lobophora*? Species diversity of the genus *Lobophora* (Dictyotales, Phaeophyceae) in New Caledonia. *Journal of Phycology* 50:1101--1119. 2014 **syn. nov.** ≡ *Lobophora indica* V. Krishnamurthy & M. Baluswami in Krishnamurthy, V., & Baluswami, M. Some new species of algae from India. *Indian Hydrobiology* 3(1):45--48. 2000 **syn. nov.**.

Note. – Detailed morphological and ecological descriptions are given in Vieira *et al.* (2014b) under the epithet *L. densa*.

Lobophora nigrescens J. Agardh (1894) and ***Zonaria nigrescens*** Sonder (1845) were proposed by Womersley (1967) to be taxonomic synonyms of *L. variegata*. They were both described from Australia, the former from "Dromana Bay" (Victoria) and the latter from Western Australia (exact locality unknown). When J. Agardh established the new genus *Lobophora* to accommodate his new species *L. nigrescens* (1894), he was fully aware of the existence of *Z. nigrescens* (1845) and considered it as a separate species because he transferred it into his genus

Gymnosorus as *G. nigrescens* (Sonder) J. Agardh (1894). Prior to J. Agardh (1894), *Z. nigrescens* was transferred to *Spatoglossum* by Kützing (1849) and to *Orthosorus* by Trevisan (1849). Later, Papenfuss (1943) transferred *Gymnosorus nigrescens* (Sonder) J. Agardh to the genus *Pocockiella*. Finally, Womersley (1967) transferred *Pocockiella variegata* (Lamouroux) Papenfuss and *Pocockiella nigrescens* (Sonder) Papenfuss into *L. variegata*. During this long and complex taxonomic history the combination *Lobophora nigrescens* J. Agardh (1894) has been considered only by Womersley (1967) who treated it as a taxonomic synonym of his *L. variegata*. Conversely, Sun *et al.* (2012) assigned their newly collected material "near the type locality" of *L. nigrescens* J.Agardh to *L. nigrescens* without considering *Zonaria nigrescens* Sonder. However, the specimen they assigned to *L. nigrescens* was not collected near its type locality (i.e. Dromana, Victoria), but in Sydney, New South Wales. An error is made in their Table 3.2.2, and consequently, the type locality argument to make this assignment is not valid. In fact, two obviously distinct species (*L. nigrescens* sensu Sun & al. 2012 and *L. australis* of Sun & al. 2012) could have been associated with *L. nigrescens* J. Agardh (1894). Comparison between *L. nigrescens* J. Agardh and *Z. nigrescens* Sonder show that not only are they distinct morphological species but that *L. nigrescens sensu* Sun & al. (2012) (Fig. 3.2.4C-D) matches the description of *Z. nigrescens* Sonder and *L. nigrescens* J. Agardh (Fig. 3.2.4A-B) that of *L. australis*. To revise the taxonomic identity of *Z. nigrescens* Sonder, we compared the morphology of the type with recently collected specimens from Western Australia. Molecular analyses revealed the presence of at least eight MOTUs in Western Australia. Since among these species, only *L. nigrescens* sensu Sun & al. (2012) (Fig. 3.2.5E-H) showed a clear morphological resemblance to *Z. nigrescens*, we propose the reinstatement of *Z. nigrescens*. A new name is hereby proposed for the type *Zonaria nigrescens* Sonder:

Lobophora sonderi C.W. Vieira, De Clerck & Payri ***nomen novum*** ≡ *Zonaria nigrescens* Sonder 1845: 50 in Sonder, G. Nova algarum genera et species, quas in itinere ad oras occidentales Novae Hollandiae, collegit L. *Priess, Ph. Dr. Botanische Zeitung* 3:49--57. 1845 – **Lectotype (designated here):** AUSTRALIA. Western Australia, National Herbarium of Victoria, No.16822 (MEL!).

Note. – Detailed morphological and ecological descriptions of *L. sonderi* are given in Sun *et al.* (2012) and Vieira *et al.* (2014b) under the name *L. nigrescens*.

We propose the resurrection of the species *Lobophora nigrescens*:

***Lobophora nigrescens* J. Agardh** (1894) Analecta algologica, observationes de speciebus algarum minus cognitae earumque dispositione: Continuatio I. *Lunds Universitets Års-Skrift, Andra Afdelningen, Kongl. Fysiografiska Sällskapets i Lund Handlingar* 29:1--144. 1894 – **Holotype:** AUSTRALIA. Dromana, No. 48307 (LD!).

Note. – We suspect that the recently described species *Lobophora australis* Z. Sun, C.F.D. Gurgel & H. Kawai (2012) is a taxonomic synonym of *L. nigrescens.*

Lobophora rickeri. *L. rickeri* was described from the southern Great Barrier Reef, Queensland (Australia) and was also reported from Lord Howe Island, New South Wales (Australia). Type material has been kept in formaldehyde since 1982, including specimens pressed on the present Herbarium sheet, and consequently cannot be sequenced. New specimens were collected from different places on Lord Howe Island by G.W. Saunders for the Barcode of Life Data Systems database. According to Kraft (2009), only two species of *Lobophora* are present on Lord Howe Island: *L. rickeri* and *L. variegata*. *L. variegata*, described by Kraft (2009) from Australia and Lord Howe Island, most likely corresponds to *Z. nigrescens* based on morphological similarity (erect stipitate species). Because all specimens from Lord Howe Island matched a single species, distinct from *Z. nigrescens*, we conveniently assigned these new collections to *L. rickeri*. *L. rickeri* came out as the sister species of *L. undulata* (Figs. 3.2.11--13). The type species of *L. rickeri* is epitypified by material newly collected on Lord Howe Island (No. GWS022754):

***Lobophora rickeri* Kraft** (2009) Algae of Australia: Marine Benthic Algae of Lord Howe Island and the Southern Great Barrier Reef, 2: Brown algae. CSIRO Publishing, Melbourne. 2009 – **Epitype (designated here):** AUSTRALIA. Lord Howe Island, No. GWS022754.

Remaining types. — The five remaining types, *Zonaria collaris* C. Agardh (1820), *Stypopodium fissum* Kützing (1859), *Stypopodium laciniatum* Kützing (1859), *Zonaria latissima* Sonder ex Kützing (1859) have not been included for further taxonomic treatment because we do not at present have sufficient data,lacking DNA and type locality material. Anatomical analyses clearly demonstrated that these species that have been recognized as taxonomic synonyms of *L. variegata* (Papenfuss, 1943) belong to *Lobophora*. While *Z. collaris* described from Jamaica could possible be *L. variegata*, it is very unlikely the case for three other taxa, all collected from Eritrea. In order to reassess the identity of these remaining types, sampling near type localities and morphological comparisons with type material will be needed.

5. Conclusion

Taking the genus *Lobophora* as an example, we aimed to reassess the taxonomic identity of old types associated with a brown algal taxon, and to give to those names a molecular identity. The first obstacle in completing this task was the accession of the types and the authorization to perform destructive sampling necessary for molecular and anatomical analyses. We were able to access only half of the types requested. The second obstacle was to perform molecular analyses and get short DNA fragments from types that are, in our case study, up to 206 years old. We were able to amplify only short fragments of DNA from four types. Although we could probably have raised the yield by generating a higher numbers of primers, successful molecular results rely heavily on the preservation quality of the type material, which varies from one herbarium to another. Alternatively, we resorted to new collections from type localities and morphological comparisons to perform epitypification, and molecular identification of some types. Finally, by means of molecular analyses on old types and epitypes, we were able to assign molecular identities to 11 of the 17 types associated with *Lobophora*. Since four of the types associated with *Lobophora* remain of uncertain taxonomic identity, we raise the question whether or not to reject those names, a move not presently allowed by the ICBN code.

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Acknowledgments

We thank Chantal Billard (Curator of Lamouroux Herbarium, Caen, France), Patrik Froden (Assistant Curator of the Botanical Museum (LD), Lund University, Sweden) and the staff (in particular Josephine Milne, Catherine Gallagher and Nimal Karunajeewa) of the National Herbarium of Victoria ((MEL) Melbourne, Australia) for their assistance in providing us fragments of *L. variegata* isotype, *L. papenfussii*

isotype, *L. nigrescens* holotype and *Z. nigrescens* respectively, as well as high-resolution pictures of the types. We thank Claude Bouchon and Aschwin Engelen for collecting *Lobophora* specimens in Saint Barthélemy and Curaçao respectively.
The types' pictures of *L. variegata*, *Z. nigrescens* and *L. nigrescens* were reproduced with permission from the Lamouroux Herbarium, Caen, the National Herbarium of Victoria (MEL), Royal Botanic Gardens Melbourne, and the Botanical Museum (LD), Lund University.

Author contributions

CV, OC, ODC and CP conceived and designed the study. CV, OC, SD, LM, RA, ODC, CP carried out the analyses. CV and OC wrote the manuscript. ODC, CP, MW, LM, RA, SF, JB and MS commented on the manuscript.

Part 3. Global biogeography and diversification of the tropic-temperate brown algal genus *Lobophora* (Dictyotales, Phaeophyceae)[9]

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[9] In preparation for submission in Journal of Biogeography as an Original Article

Abstract

This study intended to reassess *Lobophora* global diversity, biogeography and historical biogeography of the brown marine algal genus *Lobophora.* A global dataset of ca. 600 DNA verified specimen records based on the mitochondrial marker *cox*3 was applied. A dated phylogeny of *Lobophora* species and distributional data were used to infer ancestral area based on a concatenated phylogeny of three markers (*cox*3, *psb*A, *rbc*L). The dated phylogeny of *Lobophora* species and distributional data were then used to infer ancestral areas. Ancestral areas were reconstructed using a maximum likelihood approach under dispersal-extinction-cladogenesis (DEC) + J model and a global model of area evolution was formulated, estimated as the best model in BioGeoBears. Species delimitation resulted in the estimation of 98 to 121 putative species (MOTUs). *Lobophora* has a worldwide distribution in tropical to warm temperate waters. Given its dispersal success, high level of diversity and ecological diversity, it represents an excellent model group for evolutionary studies of tropical marine algae. Molecular dating using a relaxed clock suggests that *Lobophora* originated in the Upper Cretaceous (-75 to -60 MY) and diversified during the Cenozoic. Extensive range overlaps between *Lobophora* sister species is evidence for sympatric speciation. In conclusion, *Lobophora* is a hyperdiverse circum-torpico-temperate genus. It's species diversity forms a bull's eye centered on the Central Indo-Pacific. *Lobophora* species are restricted to limited geographic regions. *Lobophora* probably originated from the Tethys Sea and dispersed in the Atlantic and Pacific Oceans. We present the first biogeographical hypothesis for the evolution on a global scale of the tropical marine alga *Lobophora* in space and time. Our results illustrate that global dispersal of marine algae is possible in oceans. Founder's events and sympatric speciations represented important speciation mechanisms in *Lobophora* diversification.

1. Introduction

In order to properly address biogeographical questions, a good knowledge of species diversity is essential. Several studies have recently addressed the magnitude of global eukaryotic diversity of terrestrial and marine systems (Mora *et al.*, 2011; Sweetlove, 2011; Appeltans *et al.*, 2012; Costello *et al.*, 2013). These new estimates range between 2 to 10 million species on earth. The advent of molecular taxonomy made us aware of the importance of cryptic species diversity (Adams *et al.*, 2014). Also, failure to recognize cryptic diversity may result in severe underestimation of species

diversity. These recent estimates of global biodiversity, however, did not take into account the magnitude of cryptic diversity, which are likely to be common in many organismal groups (Adams *et al.*, 2014), and could considerably inflate these recent biodiversity estimates. Algae represents a group for which the magnitude of diversity remains largely uncertain (Guiry, 2012; De Clerck *et al.*, 2013). Several regional case studies demonstrated that species diversity could be up-scaled with one or two orders of magnitude (e.g. Stiller & Waaland, 1993; Evans *et al.*, 2007; Saunders, 2008; Payo *et al.*, 2013; Leliaert *et al.*, 2014), highlighting the high level of cryptic diversity within algae. In the process of estimating global diversity of any given group, it is important to determine how diversity is geographically structured. High local diversity may not necessarily translate into high global diversity, because of possible broad geographic distribution of species and/or the paucity of species diversity in other regions. On the other hand, narrow species ranges may result in low species diversity at a local scale, but high global diversity.

High species diversity in turn raises evolutionary questions related to the causes and mechanisms of evolutionary diversification. Geographic isolation is the traditional explanation for diversification, but recent studies have shown that adaptive diversification occurring in sympatry may be an important source of diversity (Schluter, 1996, 2001; Bowen *et al.*, 2013). Estimating the relative importance of allopatric versus sympatric speciation is possible by examining historical biogeographic patterns (Avise, 2000). It has been suggested that opportunities for allopatric speciation are reduced in the ocean, since there are few physical barriers, and dispersal is extensive (Bowen *et al.*, 2013). Long-distance dispersal manifestly occurred in marine macroalgae over evolutionary time-scales allowing global colonization by different taxa above the species-level. Long-distance dispersal is, however, rare in marine macroalgae as propagules have been shown to have limited dispersal capabilities (Santelices, 1990; Norton, 1992). Furthermore, rare, if not inexistent, are cosmopolitan marine algal species. Many alleged cosmopolitan species, have eventually been shown to represent a complex of phylogenetically distinct species with more restricted distributions (Zuccarello & West, 2003; De Clerck *et al.*, 2005b; Leliaert *et al.*, 2009; Tronholm *et al.*, 2012). In conclusion, allopatric speciation may have represented a non-negligible process in macroalgal speciation. Recent work on marine fauna also showed that adaptive sympatric speciation acted as a major mode of speciation in several groups (e.g. fishes) in tropical regions (Rocha *et al.*, 2005; Bowen *et al.*, 2013).

The present study intends to assess species diversity and distributions on a global scale focusing on the brown macroalga *Lobophora* (Dictyotales, Phaeophyceae). *Lobophora* is a cosmopolitan genus that has been previously reported in tropical and temperate regions (Guiry & Guiry, 2015). The genus is distributed in the Atlantic, Indian and Pacific Oceans, from tropical to warm-temperate regions, across both hemispheres (Fig.1; this study; Guiry & Guiry, 2015). Before molecular data were available, virtually all species reported around the world had been assigned to *L. variegata* J.V.Lamouroux (Womersley) ex Oliveira, a species that is now known to be restricted to the Caribbean (Vieira *et al.*, submitted). Recent molecular studies revealed that the biodiversity of this genus has been severely underestimated by at least three-fold (Sun *et al.*, 2012; Vieira *et al.*, 2014b). In the Pacific Ocean, only three species were recognized (i.e. *L. variegata*, *L. papenfussii* and *L. rickeri*) based on morphological data. Based on a broad geographic sampling (Australia, China, Hawaii, Japan, Palau) and using genetic data, Sun *et al.* (2012) showed the existence of nine major clades, and described four new species. In New Caledonia, only *L. variegata* and *L. papenfussii* were documented prior to molecular data. Vieira *et al.* (2014b) assessed the presence of 31 – 39 species, described 10 species *de novo*, and ruled out the presence of *L. variegata* and *L. papenfussii* in New Caledonia. This exceptional diversity discovered from limited locations in the Pacific suggests the existence of a much greater diversity on a global level.

The present study aims at (1) assessing species diversity on a global scale using molecular data, (2) defining species distributional ranges, and (3) examining spatial and temporal pattern of diversification and dispersal of the genus *Lobophora*. Note that we will be using the term sympatric speciation to express speciation within the scale considered (e.g. basin, region, realm). Nevertheless, within a given region *s.l.* speciation may actually result from allopatric speciation (e.g. vicariance or founder event). Identifying actual sympatric speciation event requires working at the finest possible scale e.g. several hundred meters to several hundred kilometers.

2. Material and methods

2.1. Taxon sampling

Taxon sampling consisted of 598 *Lobophora* specimens, 307 of which were sampled in the course of this study. Sampling was carried out from intertidal down to 90 m deep by scuba diving or snorkelling. Voucher specimens were preserved in silica gel and dried as herbarium. *Lobophora* specimens were sampled in more than 40 countries,

spanning the entire range of the genus (Table S3.3.1, Fig.3.3.1). The origin of the specimens and accession numbers are detailed in Table S3.3.1.

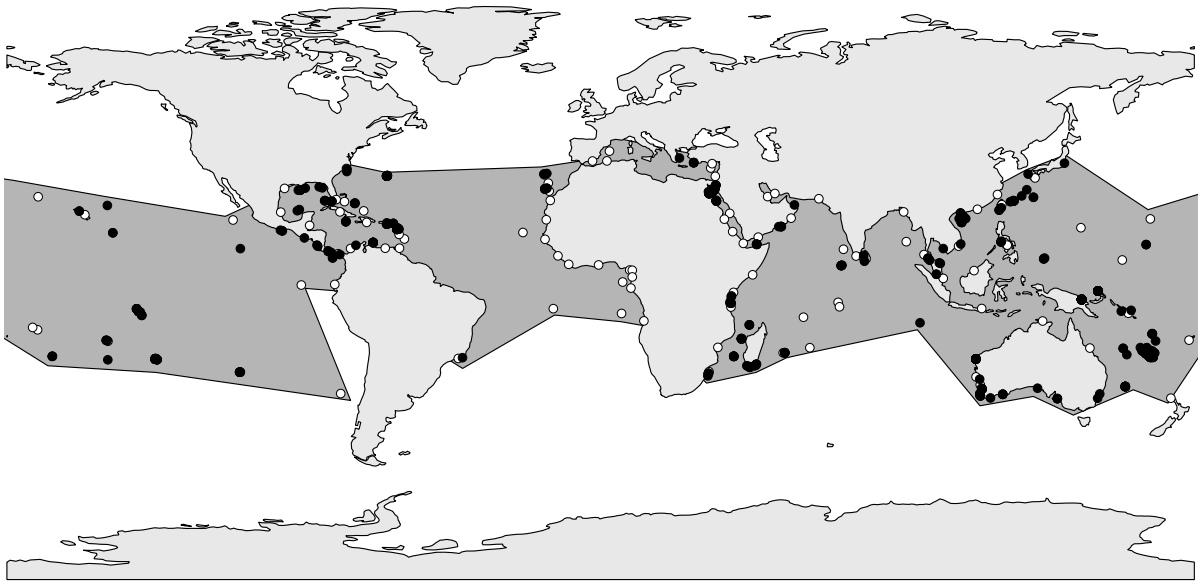

Figure 3.3.1. *Lobophora* global distribution range (dark grey area) based on DNA confirmed samples (black circles) and literature records (white circles).

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from tissue samples dried in silica gel using a cetyl-trimethyl ammonium bromide-extraction method following De Clerck *et al.* (2006). Sequences were generated from the mitochondrial encoded cytochrome c oxidase III gene (*cox*3), and the chloroplast encoded ribulose-1,5-biphosphate carboxylase (*rbc*L) and the photosystem II protein D1 (*psbA*) genes. A total of 300 *cox*3, 222 *rbc*L and 190 *psb*A sequences were generated. The datasets were complemented by 278 *cox*3, 148 *rbc*L and 90 *psb*A *Lobophora* sequences from GenBank (Table S3.3.1). Sequences were aligned using MUSCLE (Edgar, 2004) implemented in eBioX 1.6 beta (Lagercrantz, 2008; available at: http://www.ebioinformatics.org).

2.3. Species delimitation

As we are equating the terms "species" and "Molecular Operational Taxonomic Unit", we will conveniently only use the former term. Since traditional morphology-based species delimitation often yields inaccurate estimates of seaweed diversity (Leliaert *et al.*, 2014), we defined species exclusively on DNA sequence data. To do so we applied

the Maximum Likelihood implementation of the GMYC model (Pons *et al.*, 2006; Reid & Carstens, 2012) on the *cox*3 dataset. This approach has previously been applied to define *Lobophora* species from New Caledonia (Vieira *et al.*, 2014b). Application of the ML-GMYC on *cox*3 yielded highly similar results (1) with other delimitation methods such as the Bayesian implementation of the GMYC model and the Automatic Barcode Gap Discovery (Puillandre *et al.*, 2012) for the same markers *cox*3, and (2) with other markers, namely, the chloroplast markers *rbc*L and *psb*A, and the nuclear marker LSU (Vieira *et al.*, 2014b). GMYC analyses under a single-threshold were conducted in R (R Core Team, 2014) using the package "Splits". The *cox*3 ultrametric tree, used to conduct the GMYC species delineation, was constructed using Bayesian analyses in BEAST v1.8.2 (Drummond et al., 2012). A GTR + I + Γ substitution model was identified as the best-fitting model for *cox*3, based on the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba et al., 2012). BEAST analyses were run under a relaxed molecular clock in combination with a Yule tree prior. Other priors were set to default. In order to check for convergence of the MCMC chains, we performed two independent runs for 10^7 generations each, starting from random trees and sampling every 10^4 generations. MCMC output files of the independent runs were inspected in Tracer 1.5 (Rambaut & Drummond, 2007) for acceptable effective sample sizes (ESS $>$ 200). A burn-in of 25% was applied once log-likelihood values had stabilized. Maximum clade credibility trees and posterior probability for the nodes were calculated using the postburnin trees using TreeAnnotator 1.8.2 (included in the BEAST package).

2.4. Geographical scale

Different levels of geographical areas were considered to assess the patterns of diversity and historical biogeography analyses: (1) basins: Atlantic and Indo-Pacific; (2) climate zones: tropical and temperate; (3) three regions: Indo-Pacific, East Pacific and Atlantic; (4) five regions: Indo-Australian Archipelago (IAA), West Indo-Pacific, Central Pacific, East Pacific and Atlantic; and (5) 9 realms based on the Marine Ecoregions of the World from Spalding *et al.* (2007): Temperate Northern Pacific, Central Indo-Pacific, Western Indo-Pacific, Eastern Indo-Pacific, Tropical Eastern Pacific, Tropical Atlantic, Temperate Northern Atlantic, Temperate Southern Africa and Temperate Australasia. Species were assigned to one or more geographical areas.

2.5. Species richness estimation and diversity

To estimate global species diversity of the genus *Lobophora* we calculated non-parametric richness estimators and extrapolated the rarefaction curve (Shen *et al.*, 2003). Rarefaction allows the calculation of species richness for a given number of individual samples, based on the construction of rarefaction curves (Sanders, 1968; Gotelli & Colwell, 2001). We used sample-based rarefaction, rescaled to number of individuals, to interpolate species richness per individual sampled, based on the analytical formulas of Colwell *et al.* (2004). Additionaly, we computed three species richness estimators: the incidence-based coverage estimator (ICE; Chao & Lee, 1992), the Chao 2 richness estimators (Chao 2; Chao, 1987), and the first-order Jackknife richness estimator (Jack 1; Burnham & Overton, 1979). ICE distinguishes between frequent and infrequent species in analysis. Jack 1 does not differentiate the species frequency and relies on the number of MCI only found once. Chao 2 relies on the number of unique units and duplicates. Extrapolation of the rarefaction curve and species richness estimators were computed with the software EstimateS (Version 9; Colwell, 2013). We compared the observed and Chao 2 estimated species diversity between four marine regions i.e. Indo-Pacific, Atlantic, Temperate Australasia and Tropical Eastern Pacific, in order to compare the level of diversity in each of these regions. We compared the observed and Chao 2 estimated species diversity between four spatial scales i.e. local, sub-regional, regional and global. We took the most well-sampled locality (New Caledonia), sub-region (Central Indo-Pacific) and region (Indo-Pacific), in order to get the best idea of what it takes in terms of sampling to properly assess species diversity at a given spatial scale. Finally, to evaluate species range overlap between marine realms, we calculated the similarity matrix between the nine marine realms with respect to their species overlap, applying the widely used Sørensen index (Magurran, 2013).

2.6. Phylogenetic reconstruction

Based on the results of the species delimitation analyses, a concatenated alignment of the *cox*3 (610 bp) + *psb*A (919 bp) + *rbc*L (1,360 bp) dataset was made containing a single representative per MOTU. The matrix was 80% filled at the gene level. Species used as outgroup taxa are given in Table S3.3.1. Maximum Likelihood (ML) and Bayesian Inference (BI) species trees were generated from the concatenated alignment, partitioned by gene and codon position. ML analyses were conducted using RAxML under a GTR+CAT model (Stamatakis, 2006). The robustness of the

resulting phylogenies was tested using 1,000 replicates of a rapid bootstrap heuristic (Stamatakis, 2006). BI, using MrBayes v3.2.2 (Ronquist & Huelsenbeck, 2003), initiated with a random starting tree and ran four chains of MCMC iterations simultaneously for 100 million generations. The first 100,000 (25%) trees sampled were discarded as burn-in, based on the stationarity of lnL as assessed using Tracer version 1.6 (Rambaut *et al.*, 2014). A consensus topology and posterior probability values were calculated from the remaining trees.

2.7. Time calibrated phylogeny

The occurrence of Phaeophyceae as fossils is rare due to their generally soft-bodied nature (Arnold, 1947), and scientists continue to debate on the identification of some findings (Coyer *et al.*, 2001). *Padina* and *Newhousia* are the only two genera of the class Phaeophyceae to form calcium carbonate. While no fossils of *Newhousia* are documented to date, the Early Cretaceous (-145.5 to -99.6 Ma) clay shales from the Gangapur formation (Andhra Pradesh state, India) yielded a macroalgal fossil reminiscent of extant species of the genus *Padina* (Rajanikanth, 1989). *Lobophora* phylogeny was therefore calibrated with (1) a fossil of *Padina*, (2) the Dictyotales node as estimated in Silberfeld *et al.* (2010), and (3) the Phaeophyceae node as estimated in Brown and Sorhannus (2010). The age of *Padina* was constrained at -95 Ma and tailing off according to a gamma distribution with shape = 3.0 and scale = 5.5 (Silberfeld *et al.*, 2014). The split between the Dictyotales and the outgroup *Syringoderma*, i.e. the crown group Dictyotales-*Syringoderma*, was constrained between -130 and -195 Ma using a uniform prior (Silberfeld *et al.*, 2014). The age of the split between Phaeophyceae and Schizocladiophyceae lineages, i.e. the crown group Phaeophyceae-Schizocladiophyceae, was constrained in the Lower Jurassic between -125 and -253 Ma using a uniform prior (Brown & Sorhannus, 2010). The time-calibrated *Lobophora* phylogeny (i.e. chronogram) was inferred using Bayesian analyses in BEAST 1.8.2 (Drummond et al., 2012), for the concatenated (*cox*3 + *rbc*L + *psb*A) alignment partitioned by gene and codon position, using a lognormal relaxed molecular clock method, with autocorrelated rates in combination with a Yule model tree prior, and the GTR + I + Γ substitution model for the three unlinked markers. The GTR + I + Γ substitution model was identified as the best-fitting model for each gene, based on the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba et al., 2012). Other priors were set to default. In order to check for convergence of the MCMC chains, we performed two independent runs for 10^7 generations each, starting from random trees and sampling every 10^4 generations.

MCMC output files of the independent runs were inspected in Tracer 1.5 (Rambaut & Drummond, 2007) for acceptable effective sample sizes (ESS > 200). A burn-in was applied once log-likelihood values had stabilized. Maximum clade credibility trees and posterior probability for the nodes were calculated using the postburnin trees using TreeAnnotator 1.8.2 (included in the BEAST package). All tree searches were conducted on the Cipres web portal (Miller et al., 2010).

2.8. Historical biogeography

To infer the evolution of geographical ranges, we used the R package BioGeoBEARS (Matzke, 2013). This package implements the most common biogeographical history reconstruction methods in a likelihood framework: dispersal-extinction-cladogenesis model (DEC; Ree *et al.*, 2005; Ree & Smith, 2008), dispersal-vicariance analysis (DIVA; Ronquist, 1997) and the BayArea model (Landis *et al.*, 2013). Moreover, it also incorporates a model of founder-event speciation ('+J') and allows the fit of models to be compared using a model choice procedure (Matzke, 2013).

3. Results

3.1. *Lobophora* species diversity

The GMYC analysis based on the mitochondrial *cox*3 marker resulted in delimitation of 109 species (i.e. GMYC clusters), with a confidence interval of 98 – 121. Extrapolation of the rarefaction curve indicates a mean value of ~190 *Lobophora* species, with a confidence interval of 140 – 235 species (Fig. 3.3.2). The species diversity value reaches a plateau at ca. 3000 samples.

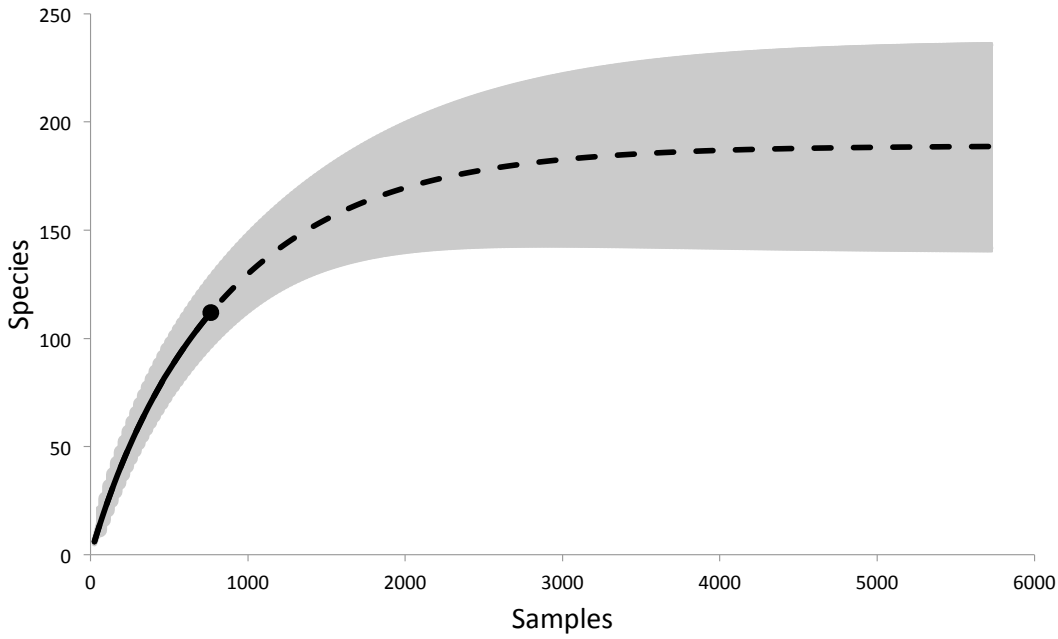

Figure 3.3.2. *Lobophora* species richness estimation by extrapolation of the rarefaction curve with 95% confidence interval. Continuous black line represents the observed species richness and the black dashed line represents the estimated diversity by extrapolation. The grayed out area represents the 95% confidence interval.

Species richness estimators projected a diversity of 179 (Jack 1) to 209 (ICE) species (Table 3.3.1). Taking the mean and the confidence interval of the GMYC results into consideration, and estimators and extrapolation values, we estimate having discovered 42 to 86 % of the *Lobophora* extant species diversity (Table 3.3.1).

Table 3.3.1. Number of estimated species and resultant percentage of species discovered. The number of species is estimated with the species-richness estimators (ICE, Chao2 and Jack 1) and with the extrapolation (mean and lower and upper 95% confidence interval). The percentage of species discovered based on the number of estimated species and the number of discovered species identified.

	Richness estimators			Extrapolation		
	ICE	Chao 2	Jack 1	Lower 95%	Mean	Upper 95%
No. of species[(1)]	209	185	179	140	188	235
Low DS (%)[(2)]	47	53	55	70	52	42
Mean DS (%)[(3)]	52	59	61	78	58	46
Upper DS (%)[(3)]	58	65	68	86	64	51

[(1)] Number of estimated species. Percentage of discovered species considering the mean and the lower ([(2)] 98) and upper ([(4)] 121) 95% confidence interval number of species identified with the GMYC model based on *cox*3. DS: described species.

3.2. Regional diversity

When comparing the level of diversity between four marine regions (i.e. Indo-Pacific, Atlantic, Temperate Australasia and Tropical Eastern Pacific), we observe a substantial difference between some of these regions. The Indo-Pacific stands out with the highest diversity with 95 species and an estimate of 150 species based on the Chao 2 species richness estimator (Fig. 3.3.3a,b). The level of diversity drops to 18 species in the Atlantic, the second most speciose marine region, with an estimate of 20 based on Chao 2 (Fig. 3.3.3a,b). The least speciose regions are the Temperate Australasia and the Tropical Eastern Pacific with six and four species, respectively, and with similar Chao 2 based-estimates (Fig. 3.3.3a,b). We also examined species diversity along a multiscale gradient from a local (i.e. New Caledonia) to a global scale (Fig. 3.3.3c,d).

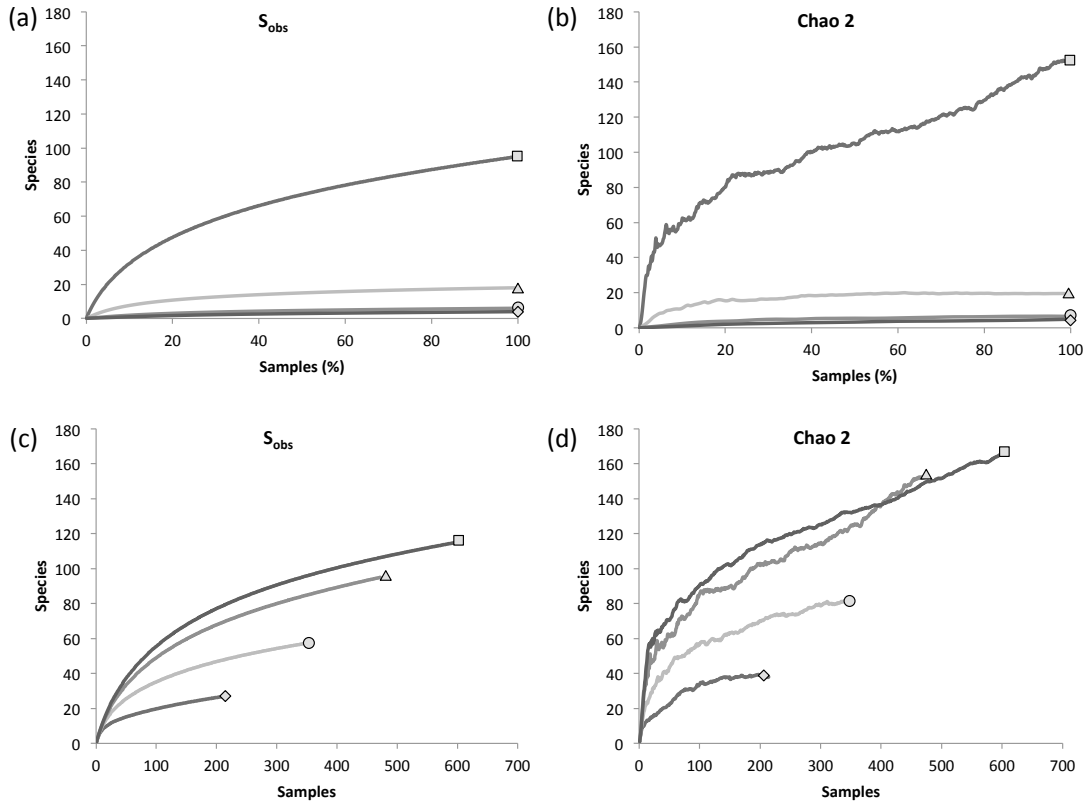

Figure 3.3.3. Observed richness (S_{obs}, a, c) and estimated richness based on the non-parametric richness estimator Chao 2 (b, d) *Lobophora* species. (a, b) Comparison between four marine regions: Indo-Pacific (square), Atlantic (triangle), Temperate Australasia (circle), Tropical Eastern Pacific (diamond). (c, d) Comparison between multiple spatial scales: local (New Caledonia, diamond), sub-regional (Central Indo-Pacific, circle), regional (Indo-Pacific, triangle), and global (square).

3.3. Inter-regional species overlap

A similarity matrix shows an overall low similarity (<0.20) between the nine marine realms in terms of species overlap (Table 3.3.2), meaning that a limited number of species are spanning more than one realm. The highest level of similarity (0.92) is observed between the Tropical Atlantic and Temperate Northern Atlantic, which have four species in common.

Table 3.3.2. Similarity matrix of *Lobophora* the diversity in 9 marine realms (Spalding *et al.*, 2007) calculated with the Sørensen index.

	CIP	WIP	EIP	Tau	TNP	TEP	TSA	TAtl	TNA
Central Indo-Pacific (CIP)	1	-	-	-	-	-	-	-	-
Western Indo-Pacific (WIP)	0.20	1	-	-	-	-	-	-	-
Eastern Indo-Pacific (EIP)	0.16	0.04	1	-	-	-	-	-	-
Temperate Australasia (TAu)	0.13	0.05	0.08	1	-	-	-	-	-
Temperate Northern Pacific (TNP)	0.03	0.00	0.10	0.00	1	-	-	-	-
Tropical Eastern Pacific (TEP)	0.00	0.00	0.00	0.00	0.33	1	-	-	-
Temperate Southern Africa (TSA)	0.00	0.06	0.00	0.00	0.00	0.00	1	-	-
Tropical Atlantic (TAtl)	0.03	0.04	0.06	0.00	0.00	0.2	0.00	1	-
Temperate Northern Atlantic (TNA)	0.03	0.05	0.08	0.00	0.00	0.18	0.25	**0.92**	1

3.4. Geographical diversity patterns

The Central Indo-Pacific is the richest realm with at least 57 species, followed by the Western Indo-Pacific with 35 species, the Eastern Indo-Pacific with 19 species and the Tropical Atlantic with 14 species. The remaining realms contain between one to 6 species (Table 3.3.3). Only three species are trans-hemispheric (*L. asiatica*, *L.* sp.18 and *L.* sp44). Ninety-nine *Lobophora* species (87%) are strictly tropical, 5 species (4%) are strictly temperate and 10 species (9%) are tropico-temperate. Nearly all *Lobophora* species are restricted to one ocean basin (Table 3.3.3), and 86 species (75%) are restricted to one marine realm, as defined by Spalding *et al.* (2007) (Table 3.3.3). Twenty-three (20%) and five (3.5%) species are spanning into two and three realms, respectively. In the Indo-Pacific, only four species are distributed across the centro-western part (*L.* sp28, *L. rosacea*, *L. gibbera*, *L. densa*) and only three in the centro-eastern part (*L.* sp9, *L. undulata, L.* sp19), but no species are found across the entire the Indo-Pacific.

Table 3.3.3. *Lobophora* species diversity per marine region. The "exclusive" column exclusively considers the species present in one given region so that the total number sums up to the actual number of species. For example species present in tropical and temperate regions will not be accounted as tropical and temperate species but exclusively as tropico-temperate species. The "inclusive" column counts every species present in a given region.

	Exclusive	Inclusive
	Species # (%)	Species # (%)
Ocean climate regions		
Tropical	99 (87)	109 (81)
Temperate	5 (4)	15 (11)
Tropical-Temperate	10 (9)	10 (7)
Ocean basins		
Pacific	98 (87)	102 (87)
Atlantic	11 (10)	15 (10)
Pacific-Atlantic	4 (4)	4 (4)
Marine regions		
Indo-Australian Archipelago	43 (38)	60 (39)
Western Indo-Pacific	24 (21)	36 (23)
Central Pacific	11 (10)	19 (12)
Eastern Pacific	3 (3)	4 (3)
Atlantic	11 (10)	15 (10)
Marine realms		
Central Indo-Pacific	37 (32)	57 (31)
Western Indo-Pacific	24 (21)	35 (19)
Eastern Indo-Pacific	11 (10)	19 (10)
Temperate Australasia	2 (2)	6 (3)
Temperate Northern Pacific	1 (1)	2 (1)
Tropical Eastern Pacific	3 (3)	4 (2)
Temperate Southern Africa	0 (0)	1 (1)
Tropical Atlantic	8 (7)	14 (8)
Temperate Northern Atlantic	0 (0)	7 (4)

3.5. Dated molecular phylogeny of *Lobophora*

Our time-calibrated phylogeny indicates that *Lobophora* originated in the Upper Cretaceous between 65 – 90 MY (Fig. 3.3.4). From the beginning of the Cenozoic onward, *Lobophora* diversification occurred rather steadily but experienced two periods of short stagnation at ca. -40 and -20 MY (Fig. 3.3.5). None of the major marine vicariance events (e.g. closure of the Tethys Sea, Benguela upwelling, Panama Isthmus closure) seem to have represented important events in *Lobophora* diversification history. On the other hand, the East Pacific barrier represents a clear dispersal barrier since the East Pacific is depleted in *Lobophora* species.

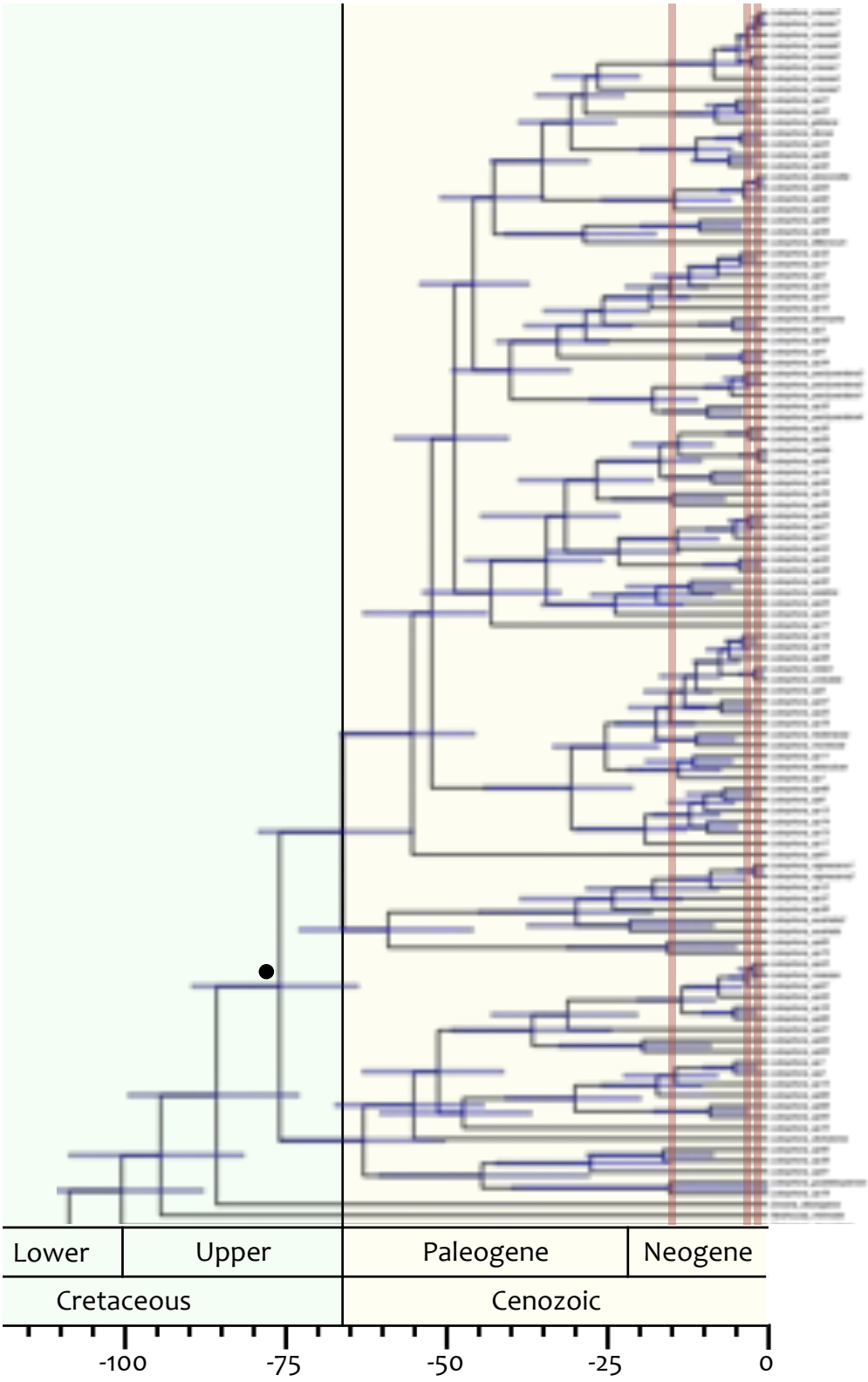

Figure 3.3.4. Chronogram resulting from the Bayesian relaxed clock analysis with BEAST 1.8.2. The purple bars display the 95% HDP (highest probability density). The black circle indicates the ancestral node of *Lobophora*. The red vertical lines display the emergence of major marine barriers: Terminal Tethian event (ca. -18 Ma), the Isthmus of Panama (ca. 3 Ma), Benguela upwelling formation (ca. 1-2 Ma). The black vertical line separate the Cretaceous from the Cenozoic.

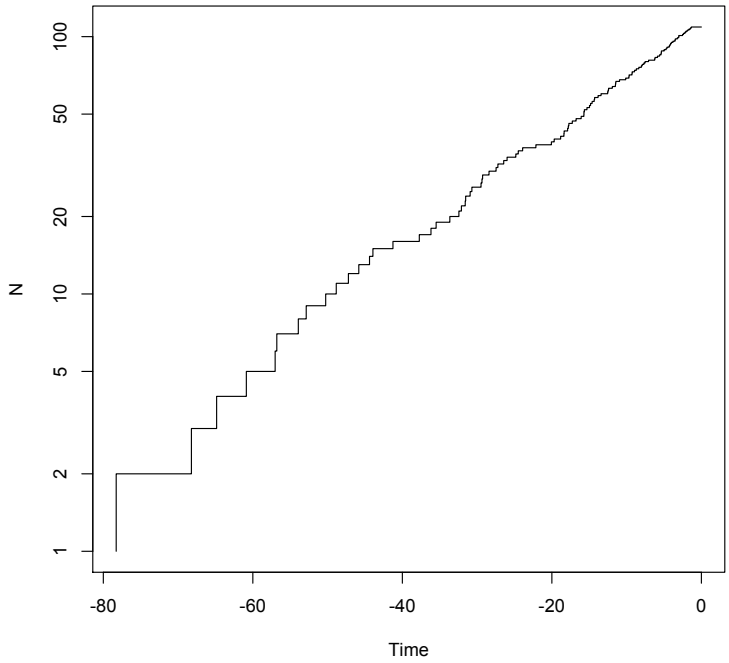

Figure 3.3.5. Lineage-through-time plot observed for the Bayesian relaxed clock analysis with BEAST 1.8.2.

3.5. Historical biogeographical inference

The Dispersal-Extinction-Cladogenesis plus the founder-event speciation model (DEC+ J) was identified as the best model in the BioGeoBEARS analyses with partitioning in nine marine realms *sensu* Spalding *et al.* (2007), and partitioning into five marine regions (Table 3.3.4). These results indicate the importance of founder-event speciation. When the number of region was reduced to three (Atlantic, Indo-Pacific and Eastern Pacific) and two (Atlantic and Indo-Pacific) regions, DIVA + J was identified as the best model. Considering the biogeographical inference based on the basins level (Atlantic and Pacific), the DEC + J model informs us that *Lobophora* ancestor originated from a region encompassing the Indo-Pacific and the Atlantic which corresponds to the Upper Cretaceous Tethys Sea (Table 3.3.4).

3.6. Relative contribution of sympatry, vicariance and founder events

The function "Biogeographical Stochastic Mapping" (BSM) implemented in BioGeoBEARS allowed quantifying speciation events. Sympatric speciation comes as the most important speciation mode (90%) at the basin level, with the remaining 10% being founder events, i.e. dispersal from one basin on to another. At a finer scale, i.e. marine realms level, sympatry remains the most important mode of

speciation (71%), followed by founder events (19%) and vicariance (9%). The relative contribution of each of these modes of speciation vary between the different realms (Fig. 3.3.6). For instance, while most of *Lobophora* diversity within the Central Indo-Pacific and the Western Indo-Pacific result from sympatric speciation, *Lobophora* diversity within the Temperate Northern Pacific and Temperate Southern Africa exclusively results from founder events (Fig. 3.3.6).

Table 3.3.4. Comparison of the fit of the dispersal-extinction-cladogenesis (DEC), dispersal-vicariance analysis (DIVA) and BayArea biogeographical reconstruction models, all with the possibility of founder-event speciation ('+J'). The log-likelihood (ln$L$) of each model is given for the analyses. Result of the best model is indicated in bold.

	10 regions	5 regions	3 regions	2 basins	Temp-Trop
DEC	-316.5	-248.0	-69.8	-50.2	**-46.3**
DEC+J	**-298.1**	**-219.9**	-63.3	-46.4	-46.3
DIVA Like	-324.8	-248.3	-64.4	-46.7	-51.1
DIVA Like + J	-309.1	-226.8	**-62.6**	**-45.9**	-51.1
BayArea Like	-339.9	-280.6	-97.9	-72.8	-53.5
BayArea Like + J	-313.8	-231.3	-66.6	-50.1	-53.3

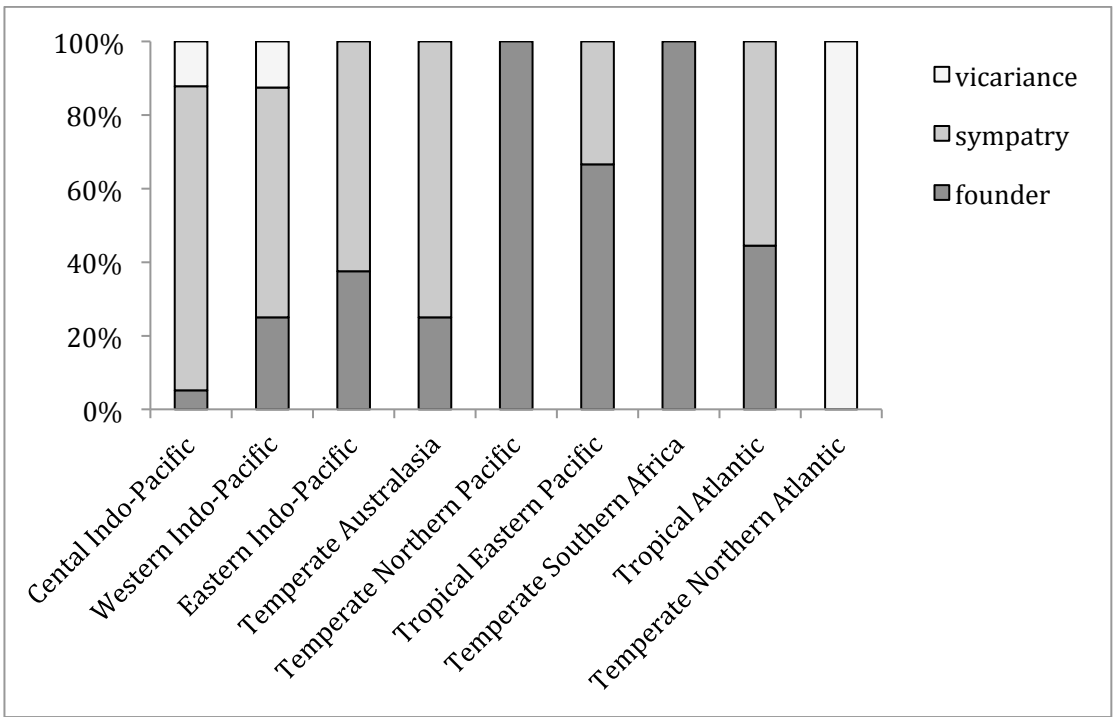

Figure 3.3.6. Relative contribution of vicariance, sympatry and founder events to *Lobophora* diversity at the marine realm (Spalding *et al.*, 2007) level.

4. Discussion

4.1. Species diversity

We assessed the species diversity of the brown algal genus *Lobophora* on a global scale. As expected, the level of *Lobophora* diversity unveiled from a limited number of localities in the Pacific Ocean (Sun *et al.*, 2012; Vieira *et al.*, 2014b) foretold a richer global biodiversity for this genus than presently recognized. DNA sequence data indicate an increase of the species diversity of the genus *Lobophora* by five to six folds, from 20 species to about 100 – 120 species, which makes of *Lobophora* a hyperdiverse genus of marine macroalgae. Our results once again show how morphology-based taxonomy dramatically failed to accurately estimates algal diversity in some groups (Packer *et al.*, 2009; De Clerck *et al.*, 2013; Leliaert *et al.*, 2014). Extrapolation beyond present sampling effort indicates 140 – 215 *Lobophora* species world-wide, denoting that we have discovered 46 – 86 % of the extant diversity.

4.2. Geographic distributions

While sister species may be geographically widely separated (Fig. 3.3.7), the distribution of single species are mostly restricted to one ocean basin and usually do not expand further than beyond a marine realm *sensu* (Spalding et al., 2007). Virtually no *Lobophora* species are pantropical.

4.3. Patterns of diversity

The majority of the species are restricted to tropical regions, and have small ranges limited to marine realms. *Lobophora* species diversity is highest in the Indo-Australian Archipelago (IAA) with declining diversity when moving away from this center, both latitudinally and longitudinally. In contrast to the general patterns of most macroalgal genera (Kerswell, 2006), the center of diversity for the genus *Lobophora* is located in the tropics. Similar patterns are observed among several other macroalgal groups such as siphonous green algae (Kerswell, 2006), but also genera belonging to the same order as *Lobophora*, i.e. *Dictyota* (Guiry & Guiry, 2015) and *Padina* (Silberfeld *et al.*, 2014). In the Atlantic Ocean, the center of diversity is located in the central Caribbean. However, diversity in the Atlantic is quite low, with only 15 species compared to 102 species in the Indo-Pacific.

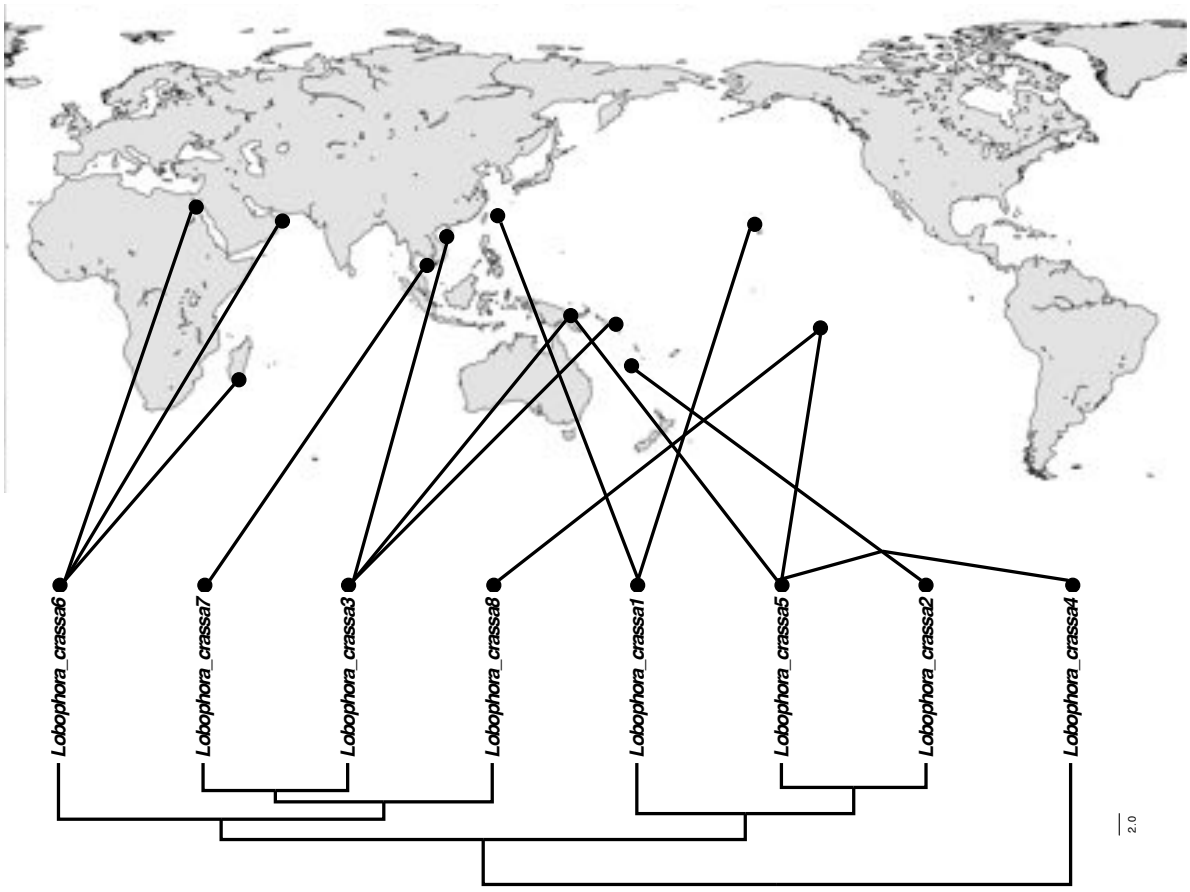

Figure 3.3.7. The geographic locations of sister species belonging to the *Lobophora crassa* complex. The phylogenetic relationships between the species are given by the phylogenetic tree resulting from the Bayesian Inference analysis.

4.4. Tethian diaspora: origin and early diversification

The time calibrated phylogeny and historical biogeographical analysis show that *Lobophora* originated in the Upper Creataceous in the remains of the Tethys Sea. Origination in the Tethys Sea is inferred from the DEC + J model reconstruction giving as ancestral area a region common to the current Atlantic and Indo-Pacific Oceans. From the Tethys Sea, *Lobophora* species dispersed to colonize other parts of the Atlantic (e.g. the Caribbean) and Indo-Pacific Oceans (e.g. IAA) where they diversified. Diversification was considerably higher in the Indo-Pacific Ocean. Given that the genus has colonized the Atlantic Ocean as early as during the Upper Cretaceous, the idea that species depletion in the Atlantic could be explained by recent colonization does not apply. Generally, colonization of a given region and subsequent speciation occurred several times throughout *Lobophora* evolutionary history, which is suggesting that occasional long-distance dispersal events played an important role in *Lobophora* diversification. High diversity within the Central Indo-

Pacific region appears to result from a combination of sympatric speciation and of regular re-colonization from adjacent regions (West Indo-Pacific and Eastern Indo-Pacific). Furthermore, 70% of the species distributed within at least two different marine realms are present in the Central Indo-Pacific. These observations are suggesting that this region acted not only as a region of origination/diversification but also of accumulation of diversity (Connolly *et al.*, 2003; Barber, 2009; Halas & Winterbottom, 2009). Caribbean species originated from different origins. Primary colonization of the Caribbean from the Tethys Sea (eastward migration), occurred during the Paleocene and resulted in regional diversification. Re-colonization of the Caribbean and subsequent speciation occurred later but this time probably from the Indo-Pacific by crossing the Eastern Pacific Barrier (westward migration). Efficiency of this barrier is illustrated by the limited number of extant Caribbean species that have probably originated from the Indo-Pacific. The presence of one species (*L.* sp44) distributed in the Western Indo-Pacific and in the Atlantic also suggests that while the Benguela upwelling may represent an efficient dispersal barrier, dispersal across it occurred at least once. Finally, colonization of temperate regions occurred at different periods of *Lobophora* evolution history. The earliest dispersal to temperate region occurred during the Paleocene (-60 Ma) in the southern hemisphere. Northern hemisphere temperate regions were colonized more recently. *Lobophora* global current taxonomic makeup shows that hard barrier formation (East Pacific Barrier, Terminal Tethian event, Isthmus of the Panama) did not act as important vicariance events for this genus. On the other hand, they constituted efficient barriers for *Lobophora* dispersal.

4.5. Cladogenic drivers

Lobophora distribution and species richness reminisce those of corals and coral reef fishes (Cowman & Bellwood, 2011). Several studies have already pointed to the central role of the coral reef association in underpinning diversification within major marine groups (Hughes *et al.*, 2002; Alfaro *et al.*, 2007; Renema *et al.*, 2008; Bellwood *et al.*, 2010; Cowman & Bellwood, 2011). Considering the major role herbivory played in macroalgal diversification (Lubchenco & Gaines, 1981; Hay, 1997), reef algae and herbivores diversification are very likely correlated through co-evolutionary arms race. The development of a complex mosaic of reef habitats also probably favored reef algal speciation by providing opportunities for new habitat colonization and ecological diversification (Alfaro *et al.*, 2007; Cowman & Bellwood, 2011). *In fine*, the biotic interaction between *Lobophora*, herbivores and corals may

have favored diversification in coral reefs. This idea that coral reefs acted as cladogenesis drivers has been already proposed for other reef organisms, such as coral reef fishes, where coral reefs would have provided the mechanisms allowing both higher rates of speciation and reduced vulnerability to extinction for associated lineages (Cowman & Bellwood, 2011).

4.6. Ecological insight

Lobophora has been considered as a potent competitor against corals, because of the proliferation it underwent following disturbances that impacted herbivores and corals and that occurred in the mid-80s (De Ruyter van Steveninck & Breeman, 1987b; Hughes, 1994). Timing of origination and patterns of distribution and diversity clearly show that *Lobophora* is a fully-fledged member of coral reefs and has evolved in these ecosystems since the rise of modern coral reefs (during the Cretaceous). Consequently, *Lobophora* should not be seen as a threat to corals, but instead as an indicator of coral reef health status. In fact, while following disturbances *Lobophora* has shown the capacity to bloom in certain reefs across the globe (De Ruyter van Steveninck & Breeman, 1987b; Diaz-Pulido *et al.*, 2009; Lesser & Slattery, 2011), corals demonstrated resilience once conditions came back to normal (Diaz-Pulido *et al.*, 2009).

5. Conclusion

This study is yet again another eye opener on our limited knowledge of algal diversity. It remains to be seen by how much our knowledge of algal diversity will increase with the help of molecular taxonomy. Will the magnitude of algal diversity reach a comparable level to other mega-diverse groups such as fungi or even beetles? It is the first study to quantify the relative importance of the different modes of geographical speciation, and it highlights the importance of within realm speciation and fonder events in the diversification of this algal taxon.

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Author contributions

CV, OC, ODC and CP conceived and designed the study. CV and OC carried out the analyses. CV wrote the manuscript. FL, ODC, CP, OC and ZS commented on the manuscript.

Box 2 A new phylometabolomical method

Abstract A new method, coined "phylometabolomics", is proposed to analyze metabolomics profiles in an evolutionary – phylogenetic context. Metabolomic chromatograms are converted into a matrix of discretized variables, which can be subsequently analyzed in a phylogenetic context. Unlike multivariate analyses, which proceed through data reduction or structural simplification, which inexorably results in information loss, with the present method, every single chemical compound is taken into account in the phylogenetic analyses. Alternatively, by assessing the phylogenetic signal of chemical compounds, metabolomes can be interpreted in an evolutionary context.

Introduction Phylogenetics is the science concerned with the evolutionary relationships among taxa (Wiley & Lieberman, 2011). Traditionally, phylogenetics was based on morphological data (Sokal, 1986), but developments in gene, and more recently genome sequencing largely superseded the use of morphological data matrices (Nei & Kumar, 2000). As a result the term phylogenetics gradually became synonymous with molecular phylogenetics, which strictly speaking assesses evolutionary relationship between species based on molecular differences (Nei & Kumar, 2000). Technically, however, the term "molecular" encompasses all biomolecules (e.g. proteins, polysaccharides, lipids, nucleic acids, primary metabolites, secondary metabolites, etc.) (Fig. 3.Box2.1).

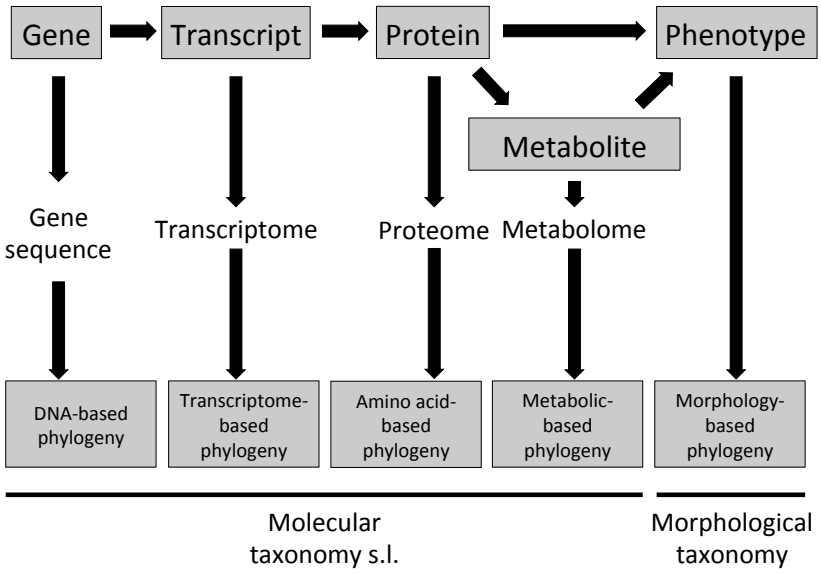

Figure 3.Box2.1. From gene to phenotype levels of phylogenetic studies.

While molecular phylogenetics is considered superior for evolutionary studies since the actions of evolution are ultimately reflected in the gene sequences, chemotaxonomy has found its utility in taxonomic classification by complementing DNA-based approaches in several taxa, e.g. plants, fungi, sponges (Gibbs, 1974; Erpenbeck & van Soest, 2007; Aliferis *et al.*, 2013). Metabolites are the products of interrelated biochemical pathways and changes in metabolic profiles can be regarded as the ultimate response of biological systems to genetic or environmental changes (Fiehn, 2002). Secondary metabolites are organic compounds that are not directly involved in the growth, development, or reproduction of an organism (Fraenkel, 1959). In plants for instance, they play an important role in defense against herbivory and often function as signaling molecules (Wink, 2003) and also have physiological roles (Rhodes 1994). The biosynthesis of secondary metabolites is growth phase-dependent and can be triggered by a wide variety of environmental and physiological signals (Koricheva *et al.*, 1998; Fox & Howlett, 2008). For example, reduction in growth rate or nutrient limitation can trigger secondary metabolism (Scheible *et al.*, 2004; Bibb, 2005). Consequently, at specific developmental stages and through particular environmental conditions an organism present a specific secondary metabolism that reflects the pathways that are being actively expressed at that moment. While the number of secondary metabolites is finite for a given organism, a specific qualitative and quantitative set of secondary metabolites are expressed at a given time. Consequently, in theory, an organism has a multitude of possible secondary metabolisms (Fig. 3.Box2.2).

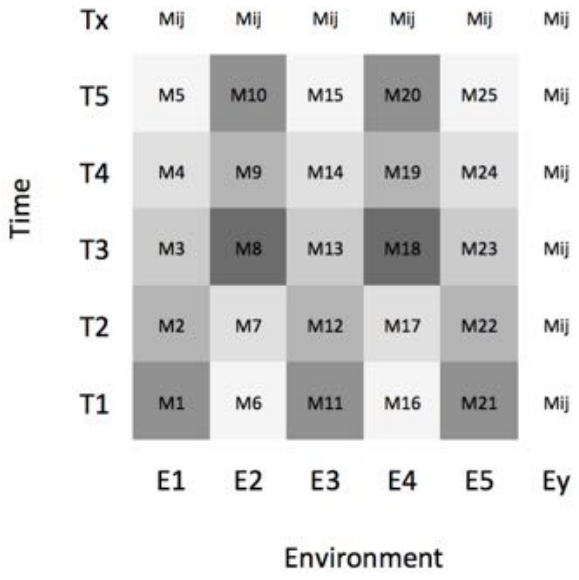

Figure 3.Box2.2. Possible secondary metabolites as expressed in function of time and space. Ey: Environment. Tx: Time. Mxy: Metabolome at time x and environment y.

Metabolite profiling refers to a qualitative and quantitative evaluation of metabolite collections (Oliver *et al.*, 1998). Metabolic fingerprinting is a high-throughput method that focuses on collecting and analyzing data from crude extracts to classify whole samples rather than separating individual metabolites. Metabolomic profiling (metabolomics/metabonomics) characterizes the secondary metabolism of an organism expressed at a given time. Since secondary metabolites are often restricted to a narrow set of species within a phylogenetic group (Wink, 2003), they lend themselves well to phylogenetics (chemotaxonomic) studies. Chromatography techniques are used to generate metabolomic profiles. The resulting chromatogram or profile displays a succession of different compounds by the presence of peaks, at specific retention times, and their quantity by the height of these peaks. It is used as a pattern or fingerprint for the analyzed sample. A chromatogram is characterized by a succession of an important number of peaks. In early practice, similarity between metabolomic profiles was assessed visually, and therefore somewhat subjective. Later, multivariate analyses were advocated, offering more objective and consistent results. The use of multivariate data analysis techniques and chemometrics has become a commonly used strategy to analyze metabolic differences. Multivariate data analysis techniques reduce the complexity of datasets and enable more simplified visualization of metabolomic results. These include principle-components analysis (PCA), hierarchical clustering analysis (HCA), K-means clustering, and self-organizing maps (SOM).

Given that organisms have unique metabolomic profiles, which diverge in time and space, we are raising the following question: can metabolomics be used to infer phylogenetics? If yes, at which taxonomic level (generic, specific, sub-specific), can metabolomics differentiate individuals? However, a preliminary question is whether or not relationship between species metabolomics reflects the molecular phylogenetic relationship. Secondly, since species metabolism is variable in time and space, if we compare the metabolomics profile of different species at different developmental stages and environment conditions, do they still have a strong enough phylogenetic signal to associate individuals from the same taxon? In other words, can we compare different species at any time and space and still get similar phylogenetic results? Or in other words, the question is whether the intra-specific diversity is significantly less important than the inter-specific diversity (Fig. 3.Box2.3 and 3.Box2.4).

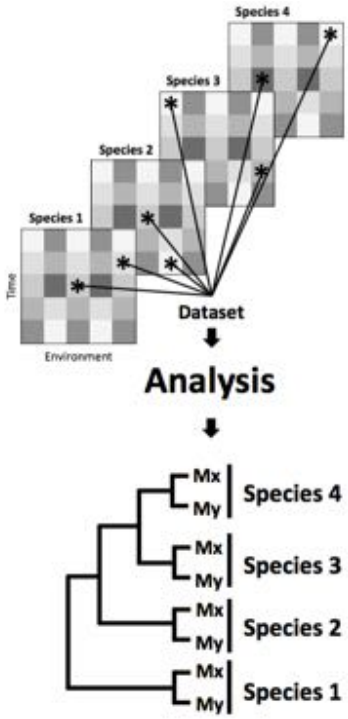

Figure 3.Box2.3. Comparison of secondary metabolites resulting from differnt time and space from different species

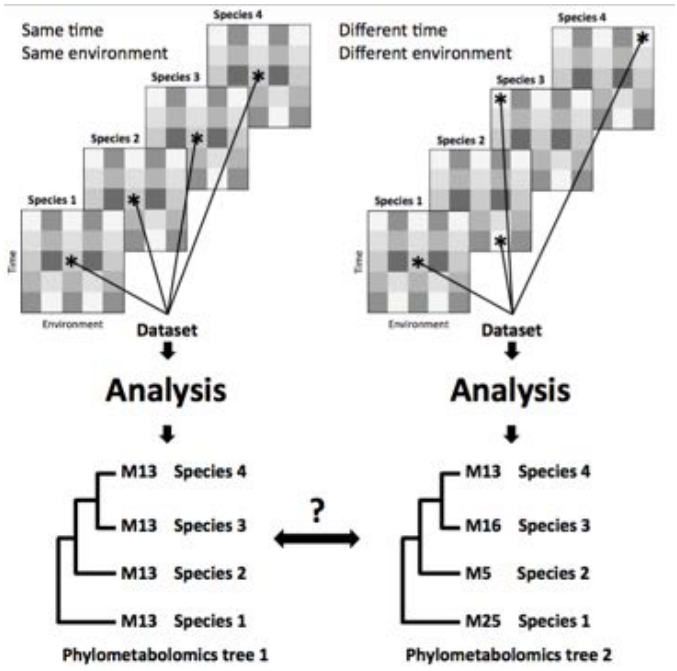

Figure 3.Box2.4. Comparison between phylogenies made with secondary metabolites issued from different place an time.

We presently propose the use of statistical inference (maximum likelihood and Bayesian inference) to generate metabolomics-based phylogenies. We coin this approach 'phylometabolomics'. We compare metabolomic profiles of individuals from different species, and found in contrasting environment, to test the strength of this

approach in phylogeny. The brown algal genus *Lobophora* (Dictyotales, Phaeophyceae) is presently used as a case study to test this phylogenetic approach.

Method Eight *Lobophora* species growing in habitats with contrasting biotic interactions (e.g. direct contact or not with corals) and environmental conditions (e.g., high vs low hydrodynamics, different depths, etc.) (Table 3.Box2.1) were collected. One species, *L. rosacea*, grows in two clearly distinct habitats, and will therefore be used to compare intraspecific differences resulting from environmental conditions (Table 3.Box2.1).

Table 3.Box2.1. *Lobophora* species morphological, anatomical and ecological descriptions.

Species	Morphology	Thickness (µm)	Habitat	Substrate
L. crassa	Crustose	291.6 ± 39.8	Shallow exposed reefs	Dead coral, coral rubble, bedrock, rock
L. dimorpha	Procumbent	101.2 ± 12.8	Branching coral fields	Dead coral basal part
L. hederacea	Shelf-like	188.6 ± 26.1	Branching coral fields	Dead coral basal part, live coral branches
L. monticola	Shelf-like	152.9 ± 24.4	Branching coral fields	Dead coral basal part, live coral branches
L. nigrescens	Stipitate	211.2 ± 8.2	Macroalgae beds	Bedrock, rock
L. rosacea	Fasciculate	146.5 ± 16	Branching coral fields	Dead coral basal part
L. rosacea	Decumbent	146.5 ± 16	Macroalgae beds	*L. nigrescens, Sargassum* spp.
L. undulata	Shelf-like	214 ± 52.3	Branching coral fields	Dead coral basal part

Samples were analyzed by liquid chromatography coupled to mass spectrometry (LC-MS) to obtain metabolomic profile data. The resulting LC-MS chromatograms were aligned using the peak picking open-source software for mass-spectrometry data processing MZmine 2 (Pluskal *et al.*, 2010). The chromatograms were then converted from continuous to discretized, nominal variables (Fig. 3.Box2.5). Transformation of chromatograms into discretized data and data conversion was performed in R (R Development Core Team, 2013) using the R package "reshape2" (Wickham, 2007). Statistical inferences, Maximum Likelihood and Bayesian Inference, are applied to the resulting data matrix to generate metabolomical phylogenetic trees. A flow chart of the method is shown in Fig. 3.Box2.6. Character-based inference methods (e.g. Parsimony, Maximum Likelihood (ML), and Bayesian) generate trees with the minimum number of changes needed to explain the data, or the highest likelihood of occurring with the given data and assuming the simplest substitution model.

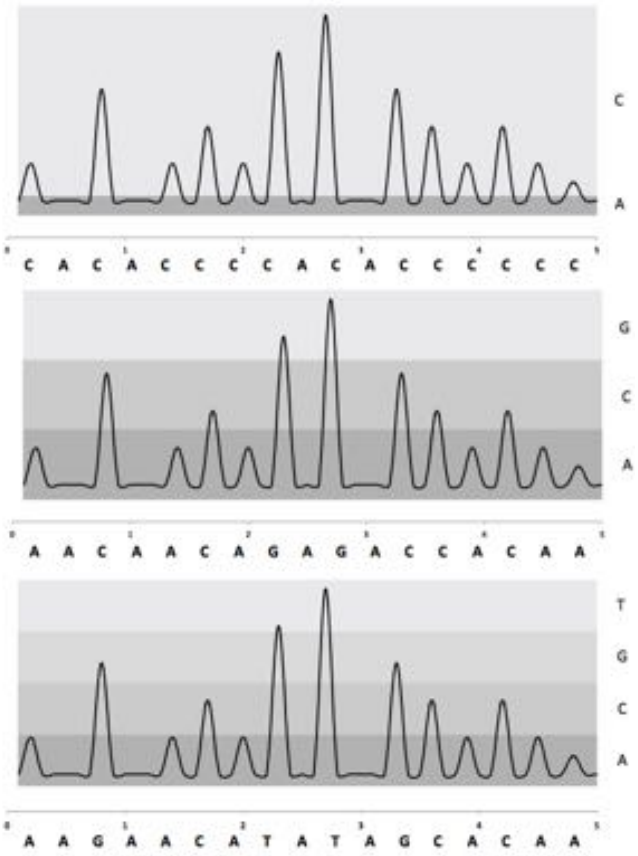

Figure 3.Box2.5. Peak coding to discretized variables. From top to bottom: binary, ternary and quaternary variables.

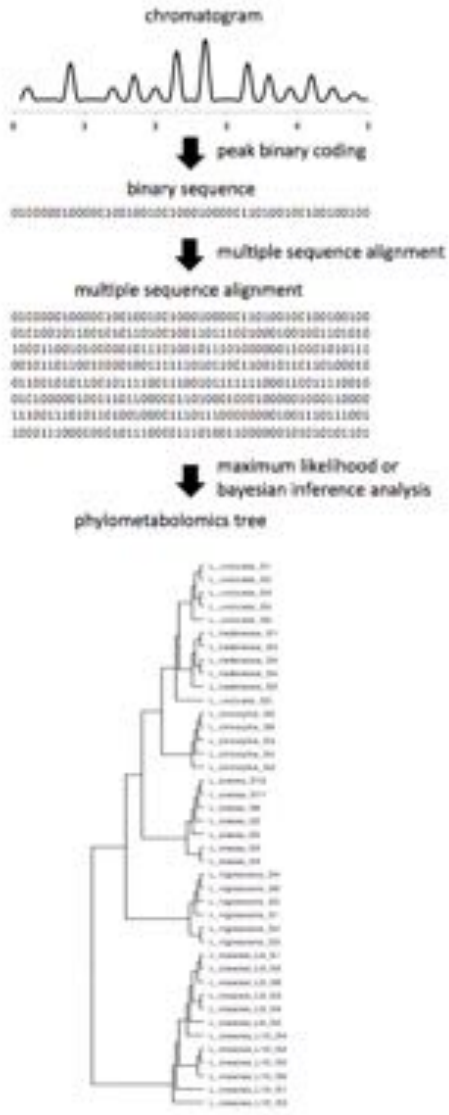

Figure 3.Box2.6. Phylometabolomic methods rundown.

Results The resulting tree topology highly mirrors the molecular phylogenetic tree (Fig. 3.2.6). The phylogenetic positions are respected except for two species (*L. crassa* and *L. dimorpha*). However, the positions of these two species in the molecular phylogenetic tree change whence additional sequences of species related to those two species are added (Vieira *et al.*, 2014b). Therefore this only difference is not questionable based on the metabolomic approach. The results suggest that independently of the conditions, intraspecific diversity is less important than inter-specific diversity, and thus that the phylogenetic signal transcends infra-specific diversity. Indeed, the species *L. rosacea* sampled from different environments were closer to each other than to the other species. The metabolomics profiles of *L. rosacea* slightly diverged between the two habitats. While, the metabolome-based phylogenetic tree configuration matched the one from the molecular phylogenetic

tree, we observe rather low bootstrap values at the nodes. Low bootstrap values result from the low similarity between all the sequences, what phylogeneticists called the "twilight zone" or "midnight zone" of sequence similarity (Ponting & Russell, 2002; Chang *et al.*, 2008; Bhardwaj *et al.*, 2012). To improve bootstrap values, alternative methods may be considered. Recent novel multiple sequence alignment methods (e.g. PHYRN; Bhardwaj *et al.*, 2012) have shown to return high-resolution phylogenies, and may consequently be considered for phylometabolomics.

In conclusion phylometabolomics comes as a promising new approach to not only study phylogenetic relationship between species, but even beyond at the subspecies level.

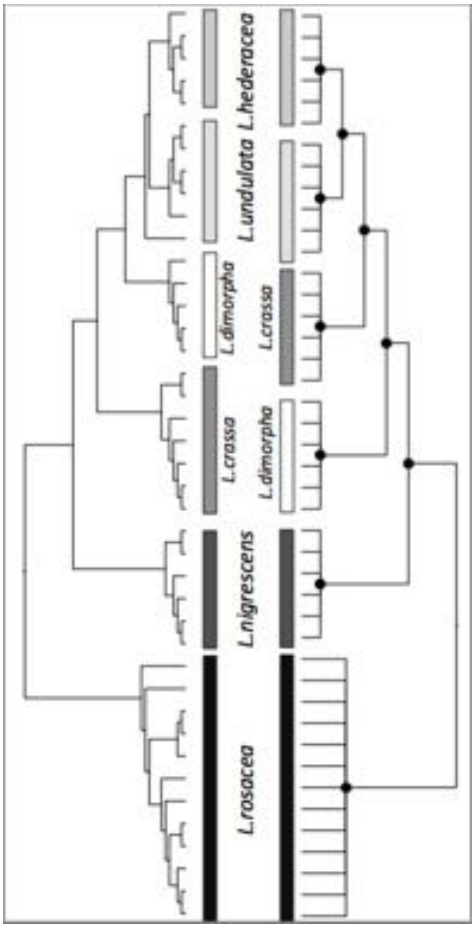

Figure 3.Box2.7. Comparison of phylometabolomic (right) and phylogenetic (left) trees.

Chapter 4: Macroalgal-coral chemical warfare

Part 1. Biological activities associated to the chemodiversity of brown algae belonging to the genus *Lobophora* (Dictyotales, Phaeophyceae).[10]

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[10] Submitted as a Review in Phytochemistry Review

Abstract

This review summarizes the current state-of-the-art of the brown alga *Lobophora* (Dictyotales, Phaeophyceae) natural products and associated bioactivities. All bioactivities are reported, including studies for which the active substance was described as well as studies limited to extracts or enriched fractions. *Lobophora* exhibits a wide array of bioactivities such as antimicrobial, biopesticidal, medical, as well as allelopathic towards herbivores and competitors. To date and since the early 80s, thirty-three publications were written, among which 30 have reported bioactivities. Only four studies, however, have identified and tested 13 bioactive compounds (a membered cyclic lactone, three sulfolipids, a sulfated polysaccharide, one polyketide, one Tocopherol, three fatty-acids and three C_{21} polyunsaturated alcohols). The majority of those studies have been conducted for their benefits for human health and well-being. Although *Lobophora* belongs to one of the richest marine algal family (Dictyotaceae) it has received lesser attention than other genera such as the genus *Dictyota* in terms of natural compounds characterization. The present review intends to trigger the interest of chemists, biologists and pharmacists given the recent significant taxonomical progress of this brown algal genus, which holds a plethora of natural compounds yet to be discovered with ecological and pharmacological properties.

1. Introduction

The brown marine algal genus *Lobophora* J. Agardh (Dictyotales, Phaeophyceae) is distributed worldwide in tropical to temperate waters and represents an important algal component in coral reef ecosystems (De Ruyter van Steveninck & Breeman, 1987a; Diaz-Pulido *et al.*, 2009; Bennett *et al.*, 2010; Vieira *et al.*, 2014b). *Lobophora* belongs to the Dictyotaceae family, which has proven to be a particularly rich and diverse source of natural products and predominantly diterpenes (Vallim *et al.*, 2005; Maschek & Baker, 2008; Blunt *et al.*, 2015). These natural products have been particularly studied for their bioactivity for human health but also for their putative ecological role in nature. The terpenoids isolated from the Dictyotaceae exhibit bioactivities such as feeding deterrence and antifungal, cytotoxic, antibiotic, anti-inflammatory, insecticidal and antiviral activities. However, while some genera have received much attention, notably some *Dictyota* and *Dictyopteris* species (Hay & Steinberg, 1992; Paul *et al.*, 2006; Paul & Ritson-Williams, 2008), others like *Lobophora* raised less interest and a very limited number of natural products have

already been described from algae of this genus. This limited attention may be explained by the taxonomic deficiency this genus has suffered until recently. Indeed, only three *Lobophora* species were recognized until the end of the last century, with *Lobophora variegata* (Lamouroux) Womersley ex Oliveira being by far the most commonly reported species, apparently distributed in all Oceans. This species has been cited in virtually all the chemical studies on the genus *Lobophora*. However, the recent DNA-based studies of Sun *et al.* (2012) and Vieira *et al.* (2014b) have shaded a new light on *Lobophora* taxonomy. Nowadays, 20 species are currently taxonomically recognized (Guiry & Guiry, 2015). The high genetic diversity recently shown in this genus underpins a richer chemical diversity yet to be discovered as shown in a recent study by Vieira *et al.* (in revision).

Note that the recent taxonomical progress of the genus *Lobophora* naturally questions the validity of what has been nearly always reported as *L. variegata* based on external morphological criteria. Therefore, although referred in the literature as *L. variegata* we will presently simply refer to *Lobophora*.

2. Antibacterial, antifungal and antiprotozoal activities

Antimicrobial (anti-bacteria, -viruses, -fungi or -protozoan) activities of extracts, fractions or compounds isolated from *Lobophora* species have been by far the most explored type of bioactivities searched for this genus. It was recently shown that, like corals or sponges, algae harbor a large and diverse microbial community which may play important roles for the host (Egan *et al.*, 2013). The selection of associated or symbiotic bacteria may be related to the production of specialized metabolites that play important functions against harmful marine microorganisms.

2.1. Antibacterial activities

Hydrophilic and lipophilic extracts of *Lobophora* species have shown a broad-spectrum of antibacterial activities (Engel *et al.*, 2006; Manilal *et al.*, 2010; Morrow *et al.*, 2011; Manilal *et al.*, 2012). Engel *et al.* (2006) considered two morphotypes of *Lobophora*, crustose and ruffled. Lipophilic and hydrophilic extracts from both types of *Lobophora* resulted in growth inhibition of the bacteria *Pseudoalteromonas bacteriolytica*. However, both extracts, which we strongly suspect to be from two distinct species, yielded contrasting IC_{50} values: the lipophilic extracts showed an IC_{50} of 1 and 0.24 $\mu g.mL^{-1}$ for the crustose and ruffled types respectively; and the hydrophilic extracts exhibited an IC_{50} of 0.51 and 0.67 $\mu g.mL^{-1}$ respectively. It is

therefore evident that these different types/species have contrasting chemical production.

Manilal *et al.* (2010) and Manilal *et al.* (2012) showed that *Lobophora* methanolic extract exhibit a strong antibacterial activity against the biofilm-forming bacteria *Vibrio* sp., *Colwellia* sp. SW125 and *Pseudoalteromonas bacteriolytica*, and the pathogenic bacterial strains *Bacillus cereus*, *Micrococcus luteus*, *Salmonella typhimurium*, *Aeromonas hydrophila* and *Escherichia coli*. Manilal *et al.* (2012) characterized seven fatty acids (palmitic, lauric, stearic, alpha linolenic, oleic, myristic and hexadecatrienoic acids) from *Lobophora* by gas chromatography, thus suggesting that the antibacterial bioactivity could be attributed to the synergistic effects of these fatty acids. In fact, fatty acids, such as oleic, lauric and palmitic acids have already demonstrated antibacterial activity (Kabara *et al.*, 1972). But while lauric acid and myristic acid presented inhibitory effect on the 11 bacterial strains tested by the authors, the effect of oleic acid was restricted to only one strain (*Streptococcus* group A) (Kabara *et al.*, 1972). Morrow *et al.* (2011) showed that *Lobophora* crude extract induced a shift in the assemblage of bacteria associated to corals. Gerwick and Fenical (1982) tested the *in vitro* antibacterial activity of a new aromatic polyketide identified from this species, 1-(2,4,6-trihydroxyphenyl)hexadecane-1-one (**1**), against a panel of six bacteria (*Staphylococcus aureus*, *Bacillus subtilus*, *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Vibrio anguillarum*) but did not observe any effect.

2.2. Antiviral activities

Lobophora aqueous extracts presented interesting bioactivities against a wide range of viruses. Some polysaccharides isolated from this species exhibited antiviral activities against the herpes simplex virus types 1 and 2 (EC_{50} 18.2 and 6.25 $\mu g.mL^{-1}$, respectively), and a very low cytotoxicity to Vero, HEp-2, and MDCK cell lines as well as a moderate activity against respiratory syncitial virus (RSV) (Wang *et al.*, 2008b). The same aqueous extract also exhibited anti-HSV properties (EC_{50} 18.5 and 9 μg.mL-1 for HSV-1 and HSV-2, respectively) and a moderate anti-RSV activity (Wang *et al.*, 2008a; Soares *et al.*, 2012). Queiroz *et al.* (2008) showed that a sulfated polysaccharide isolated from *Lobophora* (a galactofucan of 1400 kDa, with fucose, galactose, glucose and sulfate at molar ratio of 1:2:3:0.5), exhibited antiretroviral effect by inhibiting reverse transcriptase activity of human immunodeficiency virus. Kremb *et al.* (2014) showed that *Lobophora* aqueous extracts also inhibited HIV-1 infection at the level of virus entry into cells.

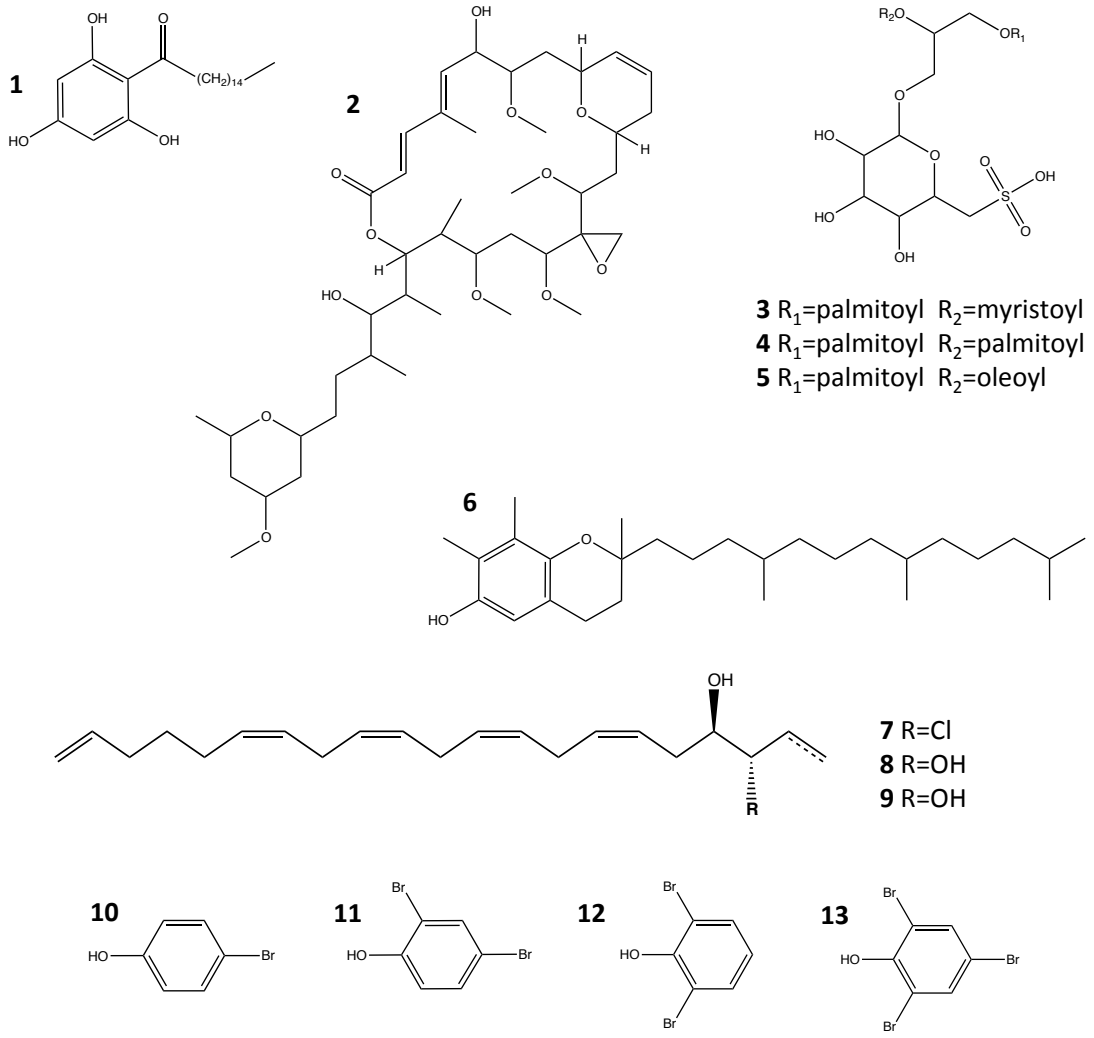

Fig. 4.1.1 Structure of natural compounds in *Lobophora*.

2.3. Antifungal activities

Some *Lobophora* extracts showed antifungal activities against a broad spectrum of fungi. The lipophilic extract of the crustose type induced 100% growth inhibition of *Dendryphiella salina* (ascomycete) and the fungi-like *Halophytophthora spinosa* (oomycete), but no effect on *Lindra thalassiae* (ascomycete). On the other hand, the lipophilic extract of the ruffled type did not inhibit the growth of any of the three tested fungi. The hydrophilic extracts of both *Lobophora* types resulted in the growth inhibition by ca. 70% of only the oomycete *H. spinosa*. We can conclude here again that the different morphotypes of *Lobophora* have contrasting bioactivities against different micro-organisms. Gerwick and Fenical (1982) tested the antifungal

activity of the polyketide (**1**) against *Candida albicans*, a causal agent of opportunistic oral and genital infections in humans, but did not observe any effect. Kubanek *et al.* (2003) identified a new macrolactone polyketide named lobophorolide (**2**), which exhibited sub-micromolar activity against pathogenic and saprophytic marine fungi (*Dendryphiella salina, Lindra thalassiae* and *Candida albicans*) with IC_{50} values ranging from 0.034 to 1.3 μg.mL-1. Lobophorolide is structurally related to tolytoxin, scytophycins, and swinholides, macrolides previously isolated from terrestrial cyanobacteria, marine sponges and gastropods (Kubanek *et al.*, 2003). These structural similarities raise the question of its origin, and the authors suggested that the molecule is more probably biosynthesized by *Lobophora* associated-bacteria.

2.4. Antiprotozoal activities

Lobophora extracts presented antiprotozoal activities against six protozoan parasites, namely *Trichomonas vaginalis* (a common and worldwide parasite which infects the urogenital tract of men and women), *Entamoeba histolytica* (parasite infecting humans and other primates), *Giardia intestinalis* (responsible for enteric protozoan infections), *Schizochytrium aggregatum* (marine fungi), *Leishmania mexicana* (one of the causative species of leishmaniasis) and *Trypanosoma cruzi* (causative species of trypanomiasis). The organic extract exhibited anti-trichomonal activity with an IC_{50} of 1.39 μg.mL-1 (Moo-Puc *et al.*, 2008), an IC_{50} of 3.2 μg/mL against *Trichomonas vaginalis* (Cantillo-Ciau *et al.*, 2010), and anti-leishmanial *in vitro* properties against *Leishmania mexicana* promastigote forms with a LC_{50} value of 49.9 μg/mL (Freile-Pelegrin *et al.*, 2008). The same extract exhibited a moderate *in vitro* antiprotozoal activity against *Trypanosoma cruzi* with an IC_{50} of 9.72 μg/mL (León-Deniz *et al.*, 2009). Cantillo-Ciau *et al.* (2010) identified three sulfoquinovosyldiacylglycerols (SQDGs; 1-*O*-palmitoyl-2-*O*-myristoyl-3-*O*-(6‴-sulfo-α-D-quinovopyranosyl)glycerol (**3**), 1,2-di-*O*-palmitoyl-3-*O*-(6‴-sulfo-α-D-quinovopyranosyl)glycerol (**4**) and 1-*O*-palmitoyl-2-*O*-oleoyl-3-*O*-(6‴-sulfo-α-D-quinovopyranosyl)glycerol (**5**) with antiprotozoal activity from the lipophilic fraction. SQDGs were shown to exhibit an *in vitro* antiprotozoal activity against *Entamoeba histolytica* with an IC_{50} of 3.9 μg/mL, and a moderate activity against *T. vaginalis* trophozoites with an IC_{50} of 8 μg/mL. Engel *et al.* (2006) observed differences in the antiprotozoal activities of both *Lobophora* types presented earlier. While both hydrophilic and lipophilic extracts of the crustose type inhibited the growth of *Schizochytrium aggregatum*,

only the lipophilic extract of the ruffled type showed a significant inhibition (Engel *et al.*, 2006).

3. Additional pharmacological bioactivities

In addition to the antimicrobial activities presented above, *Lobophora* presented several additional bioactivities with some pharmacological potential, including antioxidant, anti-inflammatory and cytotoxic (including antitumoral) activities. Some *Lobophora* extract and sulfated polysaccharides were shown to exhibit antioxidant (Zubia *et al.*, 2007; Paiva *et al.*, 2011; Castro *et al.*, 2014) as well as anti-inflammatory activities (Medeiros *et al.*, 2008; Paiva *et al.*, 2011; Siqueira *et al.*, 2011; Castro *et al.*, 2014). The same extract demonstrated low cytotoxic properties on human breast carcinoma MCF-7 cell lines, at a concentration of 200 μg/mL (Wang *et al.*, 2008b), and against the human nasopharyngeal carcinoma (KB) cell line (Moo-Puc & Robledo, 2009). Semi-purified fractions of *Lobophora* also exhibited potential cytotoxic activity on a cultured human melanoma cancer cell line (Rocha & Ribeiro Soares, 2007). Lobophorolide (**2**) also showed antineoplastic activity (IC_{50} 0.03 μg/mL) on the human colon tumor cell line HCT-116 (Kubanek *et al.*, 2003) and sulfated polyscaccharides presented anti-tumoral effects on human colon adenocarcinoma cell line HT-29 (Castro *et al.*, 2014). Gerwick and Fenical (1982) isolated one form of vitamin E (γ-tocopherol (**6**)) from *Lobophora*, which has distinct propertiesfrom the more common α-tocopherol (Jiang *et al.*, 2001), the form of vitamin E that is preferentially absorbed an accumulated in humans (Rigotti, 2007). Sousa *et al.* (2008) measured the content in β-carotene, retinol equivalent (vitamin A) and γ-tocopherol (vitamin E) in *Lobophora*: 4.185±1.559 of β-carotene, 0.697±0.260 of retinol equivalent and 4.722±2.062 of γ-tocopherol. *Lobophora* presented the lowest γ-tocopherol concentration amongst other Phaeophyceae (i.e. *Dictyopteris delicatula*, *Dictyota dichotoma*, *Padina gymnospora* and *Sargassum cymosum*).

3.1. Biopesticidal

Only one study assessed the biopesticidal activities (i.e. pupicidal, nematicidal and phytotoxic activities) of *Lobophora* (Manilal *et al.*, 2012). The authors showed a bioactive potential of *Lobophora* as pupicidal against the urban mosquito *Culex quinquefasciatus,* as nematicidal against *Meloidogyne javanica* and phytotoxic activities against several plant seeds (*Cicer arietinum*, *Vigna radiate* and *Cajanus*

cajan). They have attributed these biopesticidal bioactivities to a synergistic effect between the fatty acids they have identified (see above).

3.2. Bromophenols

Lobophora have been shown to produce bromophenols, a group of key flavor compounds in seafood. Chung *et al.* (2003) found four bromophenols in *Lobophora* sp. namely 4-bromophenol (**9**), 2,4-dibromophenol (**10**), 2,6-dibromophenol (**11**), and 2,4,6-tribromophenol (**12**). These authors also showed that comparatively to two other brown algae, *Padina arborescens* and *Sargassum siliquastrum*, *Lobophora* presented the highest amount of bromophenols. Bromophenols have demonstrated a variety of biological activities including antioxidant, antimicrobial, anticancer, anti-diabetic, and anti-thrombotic effects (Liu *et al.*, 2011). Nevertheless, to our knowledge no study has yet shown bioactivities for any of the four bromophenols isolated from *Lobophora.*

4. Ecological roles

Fewer are the studies targeted towards understanding the ecological roles of *Lobophora* metabolites. Three main ecological roles have been investigated, namely the antifouling, feeding deterrence properties, and negative as well as positive effects on benthic competitors.

4.1. Antifouling

As an evolutionary response to the ecological disadvantages of epibiosis, most if not all macroalgae have developed antifouling chemical defenses. However, these antifouling defenses are not equally efficient across different algal taxa, and some may harbor a significant community of epiphytes. Such is the case of *Lobophora*, which blades act as an important living substratum (Fricke *et al.*, 2011). Yet, interestingly the upper-side blade surface is generally less epiphytized than the underside surface. Two studies have been performed to assess the antifouling properties of compounds produced by this species, against mussels, barnacles and bacterial biofilm (Da Gama *et al.*, 2008; Manilal *et al.*, 2010). The methanolic extracts showed considerable antifouling activity against biofilm forming bacteria, i.e. *Vibrio* sp. (11±2.5 mm zone of inhibition (MZI)), *Colwellia* sp. SW125 (6±2.1 mm MZI) and *Pseudoalteromonas* sp. SW124 (9±1.5 mm MZI) (Manilal *et al.*, 2010). On the other hand, some *Lobophora* extract stimulated the attachment to the

algal surface of the brown mussel *Perna perna*, and apparently did not show significant activity against the barnacle *Balanus amphitrite* and mussel *Mytilus edulis* attachment (data not presented; Manilal *et al.*, 2010). Although not clearly demonstrated, antifouling activities might be attributable to phlorotannins, a class of molecules present in *Lobophora*, that have been reported to present antifouling activity (Amsler & Fairhead, 2005).

4.2. Defense/offence against benthic competitors

As a consequence of natural or anthropogenic perturbations of their environmental conditions, some coral reefs have shifted from a coral- to a macroalgal-dominance. *Lobophora* has been reported in such events and allelopathy has been suggested as a possible mechanism allowing the alga to outcompete corals in damaged reefs by causing bleaching and suppressing photosynthetic efficiency. Some authors (e.g. Antonius & Ballesteros, 1998; Longo & Hay, 2014; Vieira *et al.*, 2015) observed that *Lobophora* contacting some corals (e.g. *Agaricia, Porites, Seriatopora*) was associated with more or less important bleaching. While an allelopathic mechanism has been suggested in the late 90s (Antonius & Ballesteros, 1998), it has only recently been experimentally tested (Rasher & Hay, 2010; Slattery & Lesser, 2014; Vieira *et al.*, in revision). Those latter studies clearly demonstrated that *Lobophora* possesses chemicals potentially adverse to several corals (*Porites cylindrica, Porites porites*, *Montastrea cavernosa*, *Acropora muricata*, *Stylophora pistillata* and *Montipora hirsuta*), although their actual efficiency *in situ* remains to be proven (Vieira *et al.*, in revision). Slattery and Lesser (2014) and Vieira *et al.* (in revision) identified four molecules with bleaching properties: SQDG (**3**) identified by Cantillo-Ciau *et al.* (2010) (Slattery & Lesser, 2014), and three new C_{21} polyunsaturated alcohols (**6**-**8**) (Vieira *et al.*, in revision). Slattery and Lesser (2014) demonstrated that the **3** presented bleaching activity against the coral *M. cavernosa*, and Vieira *et al.* (in revision) showed that the all lobophorenols exhibited bleaching activities against the coral *A. muricata*. In Vieira *et al.* (in revision) a significant number of semi-purified fractions also exhibited a more or less significant activity against corals.

Lobophora natural compounds adversity towards corals may be indirect, by affecting the coral-associated bacterial community and notably by causing community shifts on *Montastraea faveolata* and *Porites astreoides* colonies (Morrow *et al.*, 2012) and also causing a sublethal stress. No compounds with such effects have yet been identified, but only the aqueous extract has been tested.

4.3. Inhibitory and enhancing role in coral larvae recruitment

Lobophora has contrasting effects on coral larvae recruitment. Birrell *et al.* (2008a) showed that *Lobophora* is able to enhance larvae settlement of *Acropora millepora* by 40%. On the contrary, Kuffner *et al.* (2006) showed that *Lobophora* causes either recruitment inhibition or avoidance behavior in *P. astreoides* larvae. Diaz-Pulido *et al.* (2010) also showed that *Lobophora* presented either no effect on 2-days-old larvae or inhibitory effects on settlement of coral larvae. Similarly, Baird and Morse (2004) showed that *Lobophora* inhibited metamorphosis in coral larvae. Morse *et al.* (1996) found that larvae of several Acroporids species did not settle in assays that included *Lobophora* plants presence. Nevertheless, no compound, either acting as enhancers or inhibitors, has already been identified.

4.4. Deterrence function

Lobophora has been the subject of contradictory observations in terms of susceptibility to herbivory. For example, while De Lara-Isassi *et al.* (2000) showed ichthyotoxicity (from ethanol and acetone extracts) against the goldfish (*Carassius auratus*), Slattery and Lesser (2014) concluded that *Lobophora* chemical defenses (*Lobophora* crude extract and a purified SQDG) were inactive against the omnivorous pufferfish (*Canthigaster rostrata*). De Lara-Isassi *et al.* (2000) experiment, which aimed at testing the ichthyotoxicity of phlorotannins, is nonetheless ecologically poorly relevant since the goldfish is a freshwater fish. *Lobophora* feeding deterrence potential has been suggested to be based on the presence of phlorotannins and terpenes (Targett and Arnold 1998, Amsler and Fairhead 2005). Stern *et al.* (1996) isolated phlorotannins from *Lobophora* and suggested several explanations to explain why the biological activity of phlorotannins may vary as a function of the gut environment of marine herbivores. In addition, Bolser and Hay (1996) concluded that the greater consumption of temperate (North Carolina) versus tropical (the Bahamas) *Lobophora* by the sea urchin *Arbacia punctulata* was likely due to the higher concentrations of secondary metabolites such as pholorotannins in *Lobophora* from the temperate regions than in tropical regions. Weidner *et al.* (2004) showed that while *Lobophora* exhibited inducible defenses following direct consumption by amphipods, the repulsive effects of the non-polar extracts were overridden by counteracting effects of non-extracted chemicals, making live plants more nutritive. Nevertheless, toxicity of *Lobophora* extracts towards fish has only been suggested, but not rigorously tested (De Lara-Isassi *et al.*, 2000).

5. Conclusion and prospects

The chemical content and associated bioactivities of *Lobophora* species really started to be explored in the early 80s. *Lobophora* exhibits a wide array of bioactivities such as biopesticidal, pharmacological including antimicrobial, as well as negative and positive allelopathic effects towards benthic organisms (e.g. herbivores, space competitors, epiphytes). Most of these studies were performed with extracts and mainly focused on their pharmacological potential, whereas only few chemicals have been characterized. Only four studies have identified and tested a total of 13 bioactive compounds (an aromatic polyketide, a macrolactone polyketide, three SQDG, a sulfated polysaccharide, a tocopherol, three fatty acids and three C_{21} polyunsaturated alcohols). Additional chemical studies are urgently required in order to fully characterize the compounds responsible for the large array of biological activities encountered. Furthermore, recent major progress in the taxonomy of this brown algal genus, suggest that a plethora of natural compounds is yet to be discovered with an estimated 110 species.

The review is written in this pivotal moment in the chemical knowledge of *Lobophora*, and will aim at triggering the interest of chemists, biologists and pharmacologists in exploring this mine of natural compounds still unexplored.

Table 4.1.1. Review of all the publications on *Lobophora* natural compounds and associated activities.

Bioactivity	Sp.	Biological target	Molecule	MW (Da)	Reference
Antimicrobial					
Antibacterial					
		Bacillus cereus, Micrococcus luteus, Salmonella Typhimurium, Aeromonas hydrophila, Escherichia coli	Seven fatty acids ranging from C-12 to C-18.		(Manilal *et al.*, 2012)
	a	*Pseudoalteromonas bacteriolytica*	Lipophilic and hydrophilic extracts		(Engel *et al.*, 2006)
	a	Broad-spectrum antibacterial	Hydrophilic extract		(Morrow *et al.*, 2011)
	a	Biofilm forming bacteria	Methanolic extracts		(Manilal *et al.*, 2010)
	b	No effect on bacteria tested	2-(1-oxo-hexadecyl)-1,3,5-trihydroxybenzene	364.519	(Gerwick & Fenical, 1982)
	b	No effect on bacteria tested	γ-tocopherol (vitamin E)	416.680	(Gerwick & Fenical, 1982)
Antiviral					
	a	Herpes simplex virus type 1 and 2 (HSV-1 and -2), respiratory syncitial virus	Polysaccharides		(Wang *et al.*, 2008a)
	a	Herpes simplex virus type 1 and 2 (HSV-1 and -2),, respiratory syncitial virus (RSV)	Water crude extract		(Wang *et al.*, 2008a)
	a	Anti-HSV-1	Crude extract		(Soares *et al.*, 2012)

	a	Anti-HIV	Galactofucan	1400	(Queiroz *et al.*, 2008)
	a	Anti-HIV	Aqueous extract		(Kremb *et al.*, 2014)
Antifungal					
	a	*Dendryphiella salina, Halophytophthora spinosa* and *Schizochytrium aggregatum*	Lipophilic extract		(Engel *et al.*, 2006)
	a	*Halophytophthora spinosa* and *Schizochytrium aggregatum*	Hydrophilic extract		(Engel *et al.*, 2006)
	a	*Dendryphiella salina, Lindra thalassiae, Candida albicans*	Lobophorolide ($C_{42}H_{70}O_{12}$)	767	(Kubanek *et al.*, 2003)
Antiprotozoal					
	a	*Trichomonas vaginalis*	Crude extract		(Moo-Puc *et al.*, 2008)
	a	*Trichomonas vaginalis*	Crude extract		(Cantillo-Ciau *et al.*, 2010)
	a	*Entamoeba histolytica, Giardia intestinalis*	Non-polar fractions containing: $C_{45}H_{84}O_{12}S$, $C_{43}H_{82}O_{12}S$, $C_{41}H_{78}O_{12}S$	849.21, 823.17, 795.12	(Cantillo-Ciau *et al.*, 2010)
	a	*Leishmania mexicana*	Organic extracts		(Freile-Pelegrin *et al.*, 2008)
		Schizochytrium aggregatum	Lipophilic and hydrophilic extracts		(Engel *et al.*, 2006)
	a	*Trypanosoma cruzi*	Crude extract		(León-Deniz *et al.*, 2009)
Medical					
Anti-inflammatory					
	a	Rats	Sulfated polysaccharide		(Siqueira *et al.*, 2011)
	a	Human plasma	Sulfated heterofucan		(Medeiros *et al.*, 2008)
	a	Rats	Sulfated polysaccharides (fucans) containing fucose, galactose and glucose		(Paiva *et al.*, 2011)
	a	Mice	Sulfated polysaccharides (fucans) containing fucose glucose and xylose		(Castro *et al.*, 2014)
Anti-coagulant					
	a	Human plasma	Sulfated heterofucan		(Medeiros *et al.*, 2008)
Antioxidant					
	a	Rats	Sulfated polysaccharides (fucans) containing fucose, galactose and glucose		(Paiva *et al.*, 2011)
	a	Chemical test	Crude extract		(Zubia *et al.*, 2007)
	a	Chemical test	Sulfated polysaccharides (fucans) containing fucose glucose and xylose		(Castro *et al.*, 2014)
Cytotoxic					
	a	Melanoma cells	Semi-purified fractions (named XAD LOB I and XAD LOB II)		(Rocha *et al.*, 2007)
	a	Human nasopharyngeal carcinoma (KB) cell line)	Organic extract		(Moo-Puc *et al.*, 2009)
	a	Vero, HEp-2, MDCK cells	-		(Wang *et al.*, 2008b)
anticancer	*a*	Human breast carcinoma MCF-7 cells	Crude extract		(Wang *et al.*, 2008b)
anticancer		Human colon tumor cell line HCT-116	Lobophorolide ($C_{42}H_{70}O_{12}$)		(Kubanek *et al.*, 2003)
anticancer	a	Human colon tumor cell line HT-29	Sulfated polysaccharides (fucans) containing fucose glucose and xylose		(Castro *et al.*, 2014)
Biopesticidal					
Pupicidal					

		Culex quinquefasciatus (mosquito)	Mixture of fatty acids ranging from C-12 to C-18.		(Manilal *et al.*, 2012)
Nematicidal					
		Meloidogyne javanica	Mixture of fatty acids ranging from C-12 to C-18.		(Manilal *et al.*, 2012)
Phytotoxic					
		Cicer arietinum, Vigna radiate and Cajanus cajan seeds	Mixture of fatty acids ranging from C-12 to C-18.		(Manilal *et al.*, 2012)
Ecological functions					
Antifouling					
	a	*Perna perna* (mussel)	Crude extract		(Da Gama *et al.*, 2008)
	a	*Balanus amphitrite* (barnacle), *Mytilus edulis* (mussel)	Methanolic extracts		(Manilal *et al.*, 2010)
Ichtyotoxic					
phlorotannins-proteins interactions	*a*	Herbivores	Phlorotannins		(Stern *et al.*, 1996)
	a	*Carassius auratus* (goldfish)	Rthanol, acetone and water extracts		(De Lara-Isassi *et al.*, 2000)
Negative effect on corals					
bleaching and suppression of photosynthetic efficiency	*a*	*Porites cylindrica*	Lipid-soluble extract		(Rasher & Hay, 2010)
shift on coral-associated bacteria		*Montastraea faveolata* and *Porites astreoides*	Aqueous extract		(Morrow et al., 2012)
sublethal stress response of corals		*Montastraea faveolata* and *Porites astreoides*	Aqueous extract		(Morrow et al., 2012)
bleaching	*a*	*Montastrea cavernosa*	Crude extract and SQDG		(Slattery & Lesser, 2014)
bleaching	*c,d, e,f,g ,h,i*	*Acropora muricata, Porites cylindrica, Stylophora pistillata, Montipora hirsuta*	Crude extract		(Vieira *et al.*, in revision)
bleaching	*c*	*Acropora muricata*	Lobophorenol A	352.24	(Vieira *et al.*, in revision)
bleaching	*c*	*Acropora muricata*	Lobophorenol B	334.27	(Vieira *et al.*, in revision)
bleaching	*c*	*Acropora muricata*	Lobophorenol C	336.29	(Vieira *et al.*, in revision)
Positive effect on corals					
Settlement enhancement	*a*	*Acropora millepora*	Waterborne effects of algae		(Birrell *et al.*, 2008a)
Bioactivity not tested					
	a	*n/t*	β-carotene		(Sousa *et al.*, 2008)
	a	*n/t*	Retinol equivalent (vitamin A)	(Sousa *et al.*, 2008)	(Sousa *et al.*, 2008)
	a	*n/t*	*g*-tocopherol (vitamin E)		(Sousa *et al.*, 2008)
n/s	*b*	*n/t*	(+)-7,8-dimethyltocol	416.68	(Gerwick & Fenical, 1982)
n/s	*b*	*n/t*	$C_{22}H_{36}O_4$	364.52	(Gerwick & Fenical, 1982)
n/s	*a*	*n/t*	4-bromophenol	173.01	(Chung *et al.*, 2003)
n/s	*a*	*n/t*	2,4-dibromophenol	251.90	(Chung *et al.*, 2003)
n/s	*a*	*n/t*	2,6-dibromophenol	251.90	(Chung *et al.*, 2003)
n/s	*a*	*n/t*	2,4,6-tribromophenol	330.80	(Chung *et al.*, 2003)
n/s		*n/t*	Polyphenol		(Arnold *et al.*, 1995)

n/s: not studied, *n/t*: no target, a : *L. variegata,* b : *L. papenfusii,* c : *L. rosacea,* d : *L. crassa,* e : *L. nigrescens*, f : *L. monticola*, g : *L. hederacea*, h : *L. dimorpha*, i : *L. undulata*

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Author contributions

CV and JG wrote the manuscript. OT, GC, ODC and CP commented on the manuscript.

Part 2. *Lobophora* allelopathy against scleractinian corals[11]

Publication title:

Cold war in the tropics: allelopathic interactions between the brown algal genus *Lobophora* (Dictyotales, Phaeophyceae) and scleractinian corlas?

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Abstract

Negative allelopathy has been recently suggested as a mechanism by which macroalgae may outcompete corals in damaged reefs. Members of the brown algal genus *Lobophora* are commonly observed in close contact with scleractinian corals and have been considered responsible for the negative effects of macroalgae to scleractinian corals. While their adversity has been initially attributed to smothering, recent field assays have suggested the potential role of chemical mediators in this interaction. Until recently, ecological studies have erroneously referred to a single species, *Lobophora variegata*, with a circumtropical distribution. Recent taxonomical studies, however, have shown that *Lobophora* is a highly diverse genus and that some but not all species are associated to live corals. We performed *in situ* bioassays testing the negative allelopathy of crude extracts and isolated compounds of several *Lobophora* species against specific corals in New Caledonia. Our results showed that, regardless of their natural association with corals, organic extracts from species of the genus *Lobophora* are intrinsically capable of bleaching some coral species (*Acropora muricata, Stylophora pistillata*) upon direct contact. Additionally, three new C_{21} polyunsaturated alcohols named lobophorenols A-C (**1-3**) were isolated and identified on the basis of MS and NMR data. Significant allelopathic effects against *A. muricata* were identified for these compounds. Nevertheless, *in situ* observations in healthy reefs indicated that, although potentially chemically armed, *Lobophora* spp. did not or rarely bleached their coral hosts, thereby raising the issue of the location of these bioactive components and the environmental factors enabling their putative release by the alga. We hypothesize that either the confinement of adverse compounds to the endometabolome, or the combination of coral defense and herbivory may result in macroalgae such as *Lobophora* naturally associated with corals to have limited negative allelopathic effects on their coral hosts.

1. Introduction

Allelopathy is defined as the positive or negative effects on growth, health or population biology, that organic compounds produced and released by an organism may exert on another one (Rice, 1984). For the most part, early studies on macroalgal allelopathy have been dedicated to deleterious effects, and have mainly focused on four main categories of effects: (1) regulation of algal populations, (2) regulation of invertebrate colonization, (3) lethal and sublethal effects on fishes, and (4) antimicrobial activities (see Harlin & Rice, 1987 for review). By far, allelopathic

defensive functions against herbivores have been the most extensively studied role for macroalgal secondary metabolites over the past 30 years (Paul & Puglisi, 2004). More recent studies also revealed the role of negative allelopathy in the competition with benthic competitors other than algae and notably with corals in damaged reefs (Bonaldo & Hay, 2014). A series of studies demonstrated that some macroalgae possess allelochemicals with bleaching properties on specific coral species (De Nys *et al.*, 1991; Rasher & Hay, 2010; Rasher *et al.*, 2011; Andras *et al.*, 2012). Although representing a much lesser body of work, macroalgal positive allelopathy has been evinced in the form of facilitation for the recruitment of other benthic organisms (Morse, 1992; Walters *et al.*, 1996; Williamson *et al.*, 2000; Steinberg & De Nys, 2002; Birrell *et al.*, 2008a). These antagonistic effects demonstrate the complex role of allelopathy in phycochemical ecology. Furthermore, although experimental studies may suggest a specific role for a given allelochemical, it does not in itself prove that it is the primary ecological function of the compound, and ecological conclusions should be carefully drawn.

The brown algal genus *Lobophora* J.Agardh (Dictyotales, Phaeophyceae) is an important benthic component of tropical coral reefs and species of this genus are commonly observed interacting with scleractinian corals in the Caribbean (De Ruyter Van Steveninck & Bak, 1986; Mumby *et al.*, 2005) and in the Pacific (Jompa & McCook, 2002a; Diaz-Pulido *et al.*, 2009). Among the macroalgae present in the southwestern lagoon of New Caledonia, *Lobophora* is most commonly encountered in association with scleractinian corals. A review on the species diversity in New Caledonia indicated that the genus is a lot more diverse than reported in the literature (Vieira *et al.*, 2014b) with at least 31 lineages, representative of biological species, present in New Caledonia. Furthermore, species closely associated with scleractinian corals predominantly belong to a specific clade. In fact, *Lobophora* species have apparently developed very specific ecological niches together with morphologies. For instance, four species of *Lobophora* with decumbent to encrusting growth forms are in direct contact with corals (i.e. *L. hederacea*, *L. monticola*, *L. rosacea*, *L. undulata*), while other species with different morphotypes were found growing in different habitats and substrates (Vieira *et al.*, 2014b). Furthermore, association with corals in New Caledonia, except in some rare cases (Vieira *et al.*, 2015), did not represent an apparent threat for corals, but rather a shelter for algae from herbivores (Bennett *et al.*, 2010). Nevertheless, *Lobophora* has been considered as a potent competitor against corals, particularly following the dramatic regime shift in the Caribbean (Hughes, 1994). Subsequently, several studies have aimed at

studying *Lobophora*-coral interactions and understanding the mechanisms by which species of *Lobophora* may outcompete corals. Dead coral surface is generally a prerequisite for the algal settlement while only a limited number of living coral species seem vulnerable to *Lobophora* overgrowth (De Ruyter van Steveninck *et al.*, 1988b; Jompa & McCook, 2002b; Diaz-Pulido & McCook, 2004; Nugues & Bak, 2006). However, two studies also showed that *Lobophora* allelochemicals presented bleaching properties against three coral species, *Porites astreoides*, *P. cylindrica* and *Montastraea cavernosa* (Rasher & Hay, 2010; Slattery & Lesser, 2014). Conversely, a study demonstrated that *Lobophora* waterborne compounds enabled coral recruitment (Birrell *et al.*, 2008a). Overall, *Lobophora* association with corals has been largely stigmatized as negative, even though only a limited number of studies convincingly demonstrated that *Lobophora* could pose an important threat to corals.

Taking into account that: (1) some *Lobophora* species are naturally occurring associated with coral species on healthy reefs without apparent signs of competition towards their coral "hosts", and; (2) that *Lobophora* organic extracts displayed negative allelopathy against some coral species in bioassay experiments, we address the following questions: Do *Lobophora* species naturally found in association with corals present negative allelopathy against the latter; are all *Lobophora* species, regardless of their association with corals, equally susceptible to bleach corals; and last, if allelopathic interactions are at play, which compounds mediate these interactions?

To tackle these questions, we implemented a multi-level approach of allelopathic bioassays starting from a multi-species and crude extract level to a single species and isolated compounds level. We first tested and compared the negative allelopathy of several species of *Lobophora* crude extracts against several species of corals. Then, we compared the negative allelopathy of numerous semi-purified fractions and purified compounds from a single *Lobophora* species on the most vulnerable coral.

2. Material and methods

2.1. Quantification of *Lobophora* – corals association

Eight species of *Lobophora,* commonly encountered in the southwest lagoon of New Caledonia were selected to quantify their association with corals and for the bioassays, i.e. *L. abscondita, L. crassa, L. dimorpha, L. hederacea, L. monticola, L. nigrescens*, *L. undulata*, and *L. rosacea* (Figure 4.2.1).

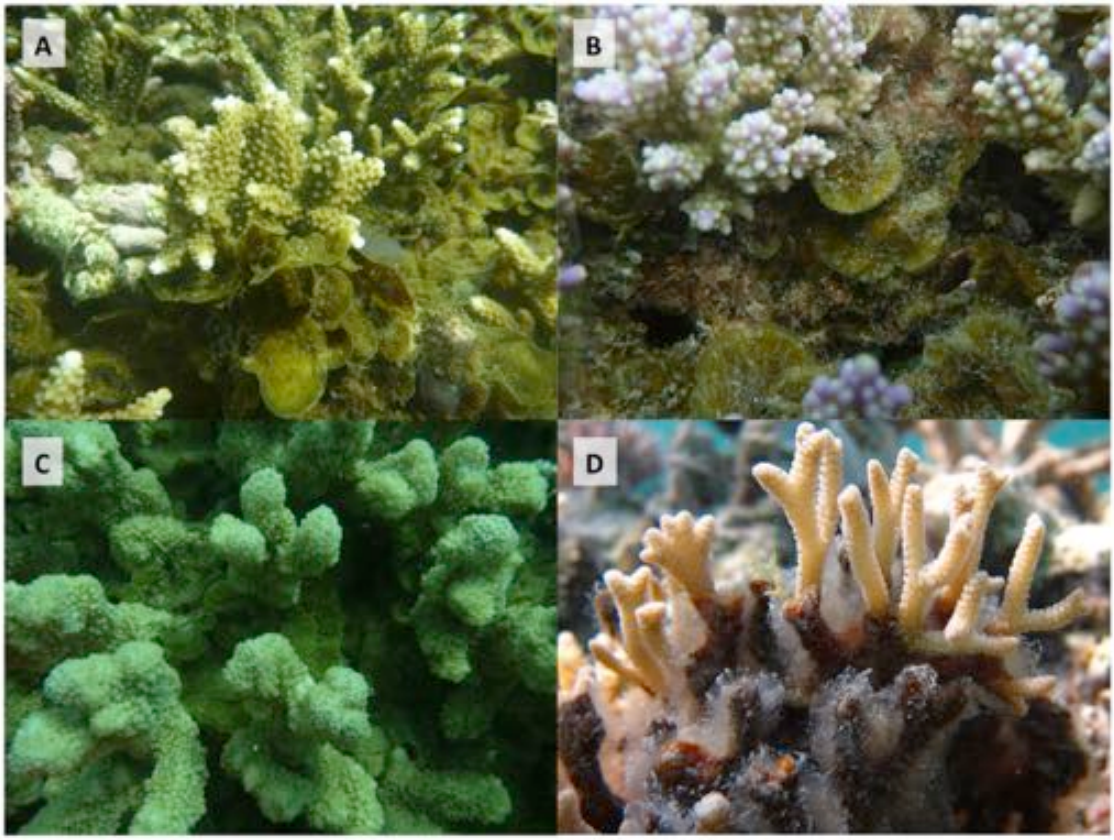


Figure 4.2.1. Pictures of natural association between *Lobophora* spp. and coral species in New Caledonia. (A) *L. rosacea* at next to *Acropora* sp., (B) *L. undulata* at the base of *Acropora* sp., (C) *L. rosacea* at the bases of *Acropora lobata*, (D) *L. hederacea* on *Seriatopora caliendrum* branches. Photo credit: Christophe Vieira

78 belt transects, as described by English and Baker (1994), each 10 m long, were deployed across coral dominated reefs in the southwest lagoon of New Caledonia. Within a belt transect, a 2500 cm^2 quadrat (50 × 50 cm) was placed consecutively left and right along a defined line, and photographs were taken directly above each quadrat using a Lumix Panasonic digital camera (12 megapixels) mounted on a photoquadrat framer. In each quadrat, the frequency of *Lobophora* – coral associations was assessed using a stratified random point count method (CPCe; Kohler & Gill, 2006). Details of the sampling locations and quantification methods are outlined in Supplementary material. From these data we calculated the percentage of transects in which *Lobophora* was associated with corals. The average percentage of associations of each species was calculated per transect where the species was observed.

2.2. Preparation of the extracts and fractions of *Lobophora* for bioassays

Algal samples for bioassays were collected by SCUBA in January 2013 in the southwest lagoon of New Caledonia (Supplementary material). Samples were cleaned from epiphytes and stored at -20°C until freeze-drying. Four coral species were selected as targets of the bioassays, i.e. *Acropora muricata* (Linnaeus, 1758; Acroporidae), *Montipora hirsuta* (Nemenzo, 1967; Acroporidae), *Stylophora pistillata* (Esper, 1797; Pocilloporidae) and *Porites cylindrica* (Dana, 1846; Poritidae). Specimens were identified at species-level using mitochondrial *cox*3 gene sequences (see Vieira *et al.*, 2014b). The area of each individual was estimated. Then, the specimens were freeze-dried and the dried samples were ground with a mortar and pestle using liquid nitrogen. One gram of ground powder was exhaustively extracted, by adding consecutively three times 10 mL of a 1:1 mixture of dichloromethane/methanol (CH_2Cl_2/MeOH) (v/v), leaving it 5 min in an ultrasonic bath and 5 min to decant, and then retrieving the supernatant liquid (upper phase) using a 100 mm in diameter and 10 µm in porosity qualitative filter paper folded (Whatman, UK). The resulting supernatant was concentrated under *vacuum* and the extracts were weighted and divided by the algal surface area to obtain a mass of extract per surface area ($\mu g.cm^{-2}$).

Crude extracts of *L. rosacea* were then submitted to fractionation in order to gain information on the polarity of the compounds responsible for the allelopathic activity. The dried extract was resuspended in MeOH/CH_2Cl_2 (1:1; v/v), mixed with an equal amount *of C_{18} silica powder (Polygoprep® 60-50, Macherey-Nagel, France) and concentrated under vacuum. The resulting powder was deposited on a solid phase extraction (SPE) cartridge (Strata® C18-E, 500 mg / 6 mL; Phenomenex, USA) and then fractionated using five solvent mixtures (10 mL for each) of decreasing polarity: H_2O, H_2O/MeOH (1:1; v/v), MeOH,* MeOH/CH_2Cl_2 (3:1; v/v), and then MeOH/CH_2Cl_2 (1:1; v/v). *The five resulting fractions (F1 to F5) were evaporated under a fume hood during 48h, weighted,* and divided by the algal surface area to obtain a quantity of fraction per surface area ($\mu g.cm^{-2}$).

2.3. Isolation and structure identification of specialized metabolites

Since no *Lobophora* species stood out in terms of bioactivity against *A. muricata* or any of the other corals (cf. results), *L. rosacea* was chosen for subsequent analytical identification of purified allelopathic compounds as it is the most common and abundant species in the southwest lagoon of New Caledonia, thus allowing collection

of enough material for subsequent analytical identification of purified allelopathic compounds. The biomass (209 g) of *L. rosacea* was exhaustively extracted, by adding consecutively five times MeOH/CH_2Cl_2 (1:1, v/v; 1.2 L of solvent), leaving it 10 min in an ultrasonic bath and 5 min to decant, and then retrieving the supernatant liquid (upper phase). The resulting extract was concentrated under vacuum to yield a homogeneous dry powder (8.3 g). The extract was then mixed with an equal amount of C_{18} silica powder (Polygoprep® 60-50) and fractionated by Vacuum Liquid Chromatography (VLC) into five fractions (F1-F5), eluting with the five organic solvents aforementioned for SPE. An additional elution was done with CH_2Cl_2 in order to ensure exhaustive compounds extraction from the crude extract, and was additionally tested as a sixth fraction (F6). The resulting filtrates were evaporated under vacuum, resuspended into MeOH to reach a concentration of 10 mg·mL^{-1}, filtered through 0.22 μm PTFE syringe filters (Phenomenex, UK) and filled into HPLC vials for subsequent Ultra-High Performance Liquid Chromatography-Diode Array Detection (UHPLC-DAD) analyses and High Performance Liquid Chromatography (HPLC) purification.

According to the results on the ecological activity (cf. next paragraph), F3 and F4 were selected for compounds isolation and purification. The HPLC purification was performed on a Jasco (Groß-Umstadt, Germany) preparative HPLC system (pump PU-2087 plus; diode array detector MD 2018 plus; column thermostat CO 2060 plus; autosampler AS 2055 plus; LC Net II ADC Chromatography Data Solutions; sample injection loop: 250 µL) on a *phenyl-hexyl* reversed phase column (XSelect CSH™, 5 μm, 19 × 250 mm; Waters, France), using for F3 an isocratic elution mode [acetonitrile (CH_3CN) + 0.1% trifluoroacetic acid (TFA)/H_2O + 0.1% TFA; 69/31, v/v] and a flow rate of 10 mL/min. Fourteen sub-fractions (from F3P1 to F3P14, Table S4.2.1) were obtained. Fraction F4 was fractionated on the same column with a CH_3CN/H_2O + 0.1 % TFA gradient on a 30 min run (0-5 min: 90% CH_3CN; 5-10 min: 90 at 100% CH_3CN, 10-25 min: 100% CH_3CN) at 10 mL/min, leading to five sub-fractions (F4P1 to F4P5). The purification of compounds from four sub-fractions of F3 (F3P10, F3P11, F3P13, and F3P14, Table S4.2.1) were performed on a C_{18} semi-preparative column (XSelect CSH™ C_{18}, 5 µm, OBD, 19 × 250 mm; Waters, France) with a CH_3CN/H_2O + 0,1% TFA gradient (UV detection: 210 nm, flow rate: 10 mL/min).

Among all the fractions and sub-fractions only the three major, pure and bioactive compounds **1**-**3**, corresponding to fractions F3P13a (18.4 mg), F3P10a (3.8 mg) and F3P11b (3 mg) respectively, were identified on the basis of NMR and MS data.

NMR analyses were performed in CD_3OD on a Bruker Avance 500 spectrometer using signals of the residual peaks of the solvent for calibration of the chemical shifts in ppm (δ_H 3.31 for ^1H NMR and δ_C 49.0 for ^{13}C NMR). LC-DAD-ELSD-ESI/MS^n analyses were carried out on a LaChrom Elite HPLC (VWR-Hitachi) composed of a L-2130 quaternary pump, a L-2200 autosampler, and a L-2300 column oven. Detection was performed with a L2455 DAD and an ELSD (Chromachem model, Eurosep) coupled to an Esquire 6000 spectrometer. UHPLC-HRMS were performed on a UHPLC U3000 (Dionex) coupled to a QqToF Impact II (Bruker).
Conformational analyses were performed along the O-C4-C3-R (with R = Cl for **1** or OH for **2**) dihedral angle in order to find the most stable conformer using the Hartree-Fock theory at the 6-31g level for both *like* and *unlike* configurations. These conformers were then subjected to geometry optimization and frequency calculation at the same level of theory. NMR shielding tensors calculation from which chemical shifts are derived were computed using DFT at the B3LYP/6-311g level. Comparison between experimental and theoretical chemical shifts was realized by calculation of the mean average error (MAE) and the corrected mean average error (CMAE).

2.4. *In situ* allelochemicals assays

Field experiments, conducted *in situ* were designed to keep the coral under natural field conditions, thus limiting pre-experimental stress usually resulting from cutting, gluing and transplantation. The bioassays were conducted in Sainte Marie Bay (22° 17.863' S, 166° 28.898' E) with three of the coral genera, i.e. *Acropora muricata, Porites cylindrica* and *Montipora hirsuta* and on genus in Maitre Islet Reef for *Stylophora pistillata* (22° 20.446' S, 166° 24.108' E). A series of three bioassay experiments were successively performed. The first experiment evaluated the bioactivity of the crude extract of the *Lobophora* species previously selected (*L. abscondita*, *L. crassa*, *L. dimorpha*, *L. hederacea*, *L. monticola*, *L. nigrescens*, *L. undulata*, and *L. rosacea*) on four coral species (*Acropora muricata*, *Porites cylindrica*, *Stylophora pistillata* and *Montipora hirsuta*). The second experiment tested the bioactivity of the five fractions obtained from the extracts of *L. rosacea* on *A. muricata*. The final experiment tested the bioactivity of the sub-fractions and compounds from two of the most bioactive fractions of *L. rosacea* identified in the previous experiment (F3 and F4). All bioassay experiments were performed *in situ* directly on coral colonies at approximately natural concentration (i.e. concentration per surface area previously estimated), the latter being critical for bioassays assessing allelopathic interactions. Thereto, we determined the amount of crude extracts,

fractions, sub-fractions, and pure compounds per unit of algal surface area (i.e. 1 cm^2) and reported it to the surface of the agarose patch applied on the coral (i.e. 2 cm^2).

A replicate was defined by one colony of coral on which all the extracts, fractions or sub-fractions (including in some cases pure isolated compounds) were tested. A total of 10 replicates were implemented. The chemical samples (crude extracts, fractions, sub-fractions or pure compounds) were resuspended in 1 mL MeOH and added at natural concentration into a 4% agarose gel (Conda Pronadisa, Spain). The mix chemical sample/agarose was poured into a polyvinyl chloride mold, composed of 10 times 2-cm^2 wells. Before that, tulle bands, of 20 × 2 cm, were disposed at the bottom of the wells onto which the gel mixture will adhere while gelifying. The strips were prepared the day before field application and refrigerated until then at 5°C. They were applied onto the coral by knotting the tulle bands to the branches, and removed after 24 h of exposure. Agarose strips with and without MeOH were additionally made as controls, to ensure the non-effect of either the agarose strips itself or the solvent on the coral. Gel strips were applied on the corals between 09:00 and 11:00 AM.

2.5. Coral photosynthesis measurements

Pulse Amplitude Modulated (PAM) fluorometry measurements were performed with a Diving-PAM (Walz) right after removal of the strips. PAM fluorometry measures the photosynthetic efficiency of photosystem II within the endosymbiotic *Symbiodinium* spp. that may be used as a quantitative measure of photo-inactivation during coral bleaching (Warner *et al.*, 1999). PAM fluorometry values of healthy corals are ranging between 0.5 to 0.8, depending on the coral species and time of the day. Values between 0 to 0.2 are indicative of severe bleaching or mortality (Fitt *et al.*, 2001). PAM fluorometry measurements were performed where the strips were applied and 5-cm next to it, as a spatial control to have a coral health baseline for comparison.

2.6. Statistical analyses

Normality of distribution of the coral responses for all the bioassay experiments was tested with the normality Shapiro-Wilk test. If the responses violated parametric assumptions, coral responses were evaluated using the Kruskal-Wallis H test followed by the Tukey honestly significant difference (HSD) post hoc comparisons test for

significant Kruskall-Wallis findings. If the data respected the parametric assumptions, a one-way ANOVA was performed followed by the Tukey post hoc HSD test for significant ANOVA findings. Statistical analyses were performed using the computing environment R (R Development Core Team, 2013).

3. Results

3.1. Importance of *Lobophora* – coral associations in New Caledonia

Association between *Lobophora* and corals occurs in a variety of habitats, ranging from coral-dominated to algal-dominated communities. We monitored 78 transects in the southwest lagoon and detect *Lobophora* species associated with corals in 54 transects (69 %) (Table 4.2.1). Restricting ourselves to transects in which a specific *Lobophora* was present, the average percentage of associations of this species ranged from 7 to 24 %. Three species, *L. abscondita*, *L.crassa* and *L. nigrescens* were never associated with corals. Instead these species grew on a variety of substrates such as dead coral rubble and bedrock (Table 4.2.1).

Table 4.2.1. Association of *Lobophora* species with corals in the southwest lagoon of New Caledonia

	Percentage of transects [a]	**Average associations** [b]	***Acropora***	***Montipora***	***Stylophora***	***Porites***	***Seriatopora***	***Turbinaria***	**Non-coral substrate**
Lobophora abscondita	9	0	0	0	0	0	0	0	100
Lobophora crassa	9	0	0	0	0	0	0	0	100
Lobophora dimorpha	11	15	100	0	0	0	0	0	0
Lobophora hederacea	23	23	15	0	6	22	42[c]	15	0
Lobophora monticola	11	24	82	12	0	6	0	0	0
Lobophora nigrescens	9	0	0	0	0	0	0	0	100
Lobophora rosacea	42	22	45	22	15	12	0	0	0
Lobophora undulata	19	7	50	42	8	0	0	0	0

[a] percentage of transects where associations of the species with corals were observed.

[b] average percentage of associations as assessed by the stratified random point count method in transects where the species was present

[c] *Lobophora* - coral associations with visible deleterious effects (bleaching and or overgrowth)

Lobophora species are associated to a limited number of coral genera. Association between *Lobophora* and *Acropora* is by far the most important. Except in the case of *L. hederacea* where the alga appears to have deleterious effects on the *Seriatopora*

coral. Living parts of other corals were not overgrown by *Lobophora* nor presented evident traces of bleaching. *Lobophora* predominantly grew at the dead basal parts of branching coral colonies. In the case of *L. rosacea*, the alga forms dense rosettes niched within the coral branches. In the case of *L. hederacea* and *L. monticola* the alga attaches itself to the coral base and adopts decumbent forms, while *L. dimorpha* adopts a procumbent form.

3.2. Effects of *Lobophora* spp. extracts on corals

All extracts prepared from *Lobophora* species caused significant visual bleaching on the corals *A. muricata* and *S. pistillata* and suppression of photosynthetic efficiency *in situ*, relative to controls ($p < 0.001$), while no significant bleaching effects were detected in *P. cylindrica* and *M. hirsuta* (Figure 4.2.2). In general, *A. muricata* was more pronouncedly bleached than *S. pistillata* (Figure 4.2.2). No significant difference was observed between the *Lobophora* species (Figure 4.2.2). In consequence, *A. muricata* was selected as a target coral for the identification of allelopathic compounds, and the alga *L. rosacea* was chosen as it is the most common and abundant species in the southwest lagoon of New Caledonia, allowing collection of enough material for subsequent analytical identification of purified allelopathic compounds.

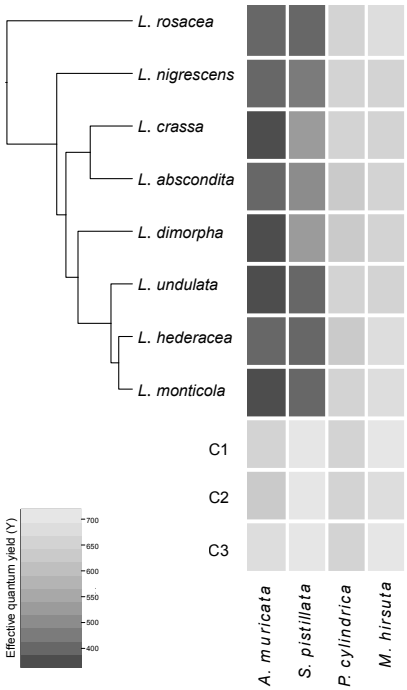


Figure 4.2.2 Heatmap representation of the bioassay results of eight species of *Lobophora*, viz. *L. rosacea*, *L. nigrescens*, *L. crassa*, *L. abscondita*, *L. dimorpha*, *L. undulata*, *L. hederacea* and *L. monticola*, crude extracts tested against four coral species, viz. *Acropora muricata*,

Stylophora pistillata, *Porites cylindrica* and *Montipora hirsuta*. The color is indicative of the coral effective quantum yield (Y) measurement under the patch surface after 24 h of exposure. C1 (no patch), C2 (patch without solvent) and C3 (patch with solvent) are the three controls. The phylogenetic tree is the maximum clade credibility tree obtained from BEAST analysis of the concatenated alignment of four genes (*rbc*L, *cox*3, *psb*A and LSU) from (Vieira *et al.*, 2014b)

3.3. Bioguided fractionation

The *L. rosacea* extract was fractionated by VLC into five fractions of contrasting polarity. Out of the five fractions tested against the coral A. muricata, the less polar ones (F3 to F5) caused significant visual bleaching and *suppression of photosynthetic efficiency* relative to controls (Figure 4.2.3), with a decrease of the photosynthetic efficiency of ca. 50% for F3 and F4, and of 70% for F5. The most polar fractions (F1 and F2) significantly suppressed coral photosynthetic efficiency (25% decrease) but less than F3-F5. F4 and F5 displayed very similar HPLC-DAD-ELSD-MS profiles and consequently only F3 and F4 were chemically studied.

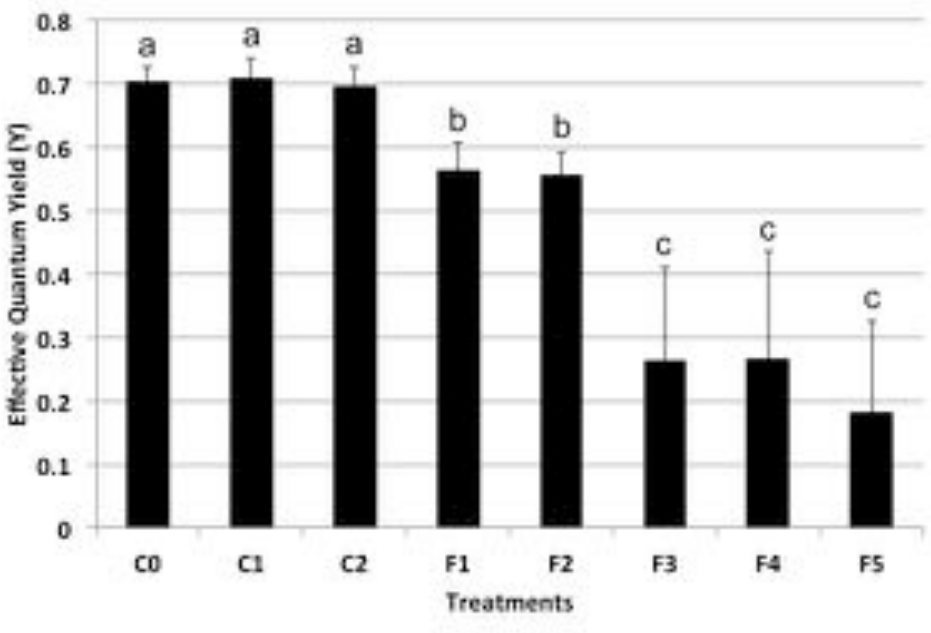
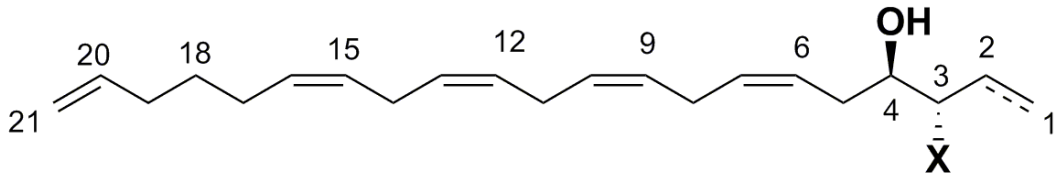


Figure 4.2.3 Barplot representation of the bioassays results with the five fractions of *L. rosacea* on *A. muricata*. The statistical analyses, comparing the fractions treatment patchs to MeOH-treated patch and untreated patch controls, were performed using Kruskal-Wallis and Tukey's HSD post-hoc test. Letters indicate distinct groupings based on post-hoc statistical comparison among sub-fractions. Asterisks indicate significance in relation to controls (MeOH-treated or untreated, accordingly) with $P<0.001$, $n=10$ assays, ≥ 5 fractions per assay for all experiments. Error bars represent standard deviation of the mean.

A first fractionation of F3 by reversed phase HPLC resulted in 14 sub-fractions named F3P1 to P14. Because most of them were still identified as mixtures of compounds by ^{1}H NMR, the most bioactive sub-fractions were further purified to identify compounds responsible for the bioactivity. Therefore, the final purification of F3P13, F3P10 and F3P11 led to the pure compounds **1** (F3P13a), **2** (F3P10a) and **3** (F3P11b) respectively (Figure 4.2.4). The structure of the chemical components of the other sub-fractions was not identified due to the low amount available or complexity of the mixture. Reversed phase HPLC fractionation of F4 resulted in five sub-fractions (F4P1-F4P5) from which no pure compound was identified.



	X	
Lobophorenol A (**1**)	Cl	$\Delta_{1,2}$
Lobophorenol B (**2**)	OH	$\Delta_{1,2}$
Lobophorenol C (**3**)	OH	

Figure 4.2.4 Chemical structure of Compounds 1-3. Compound 1 (F3P13): (6Z,9Z,12Z,15Z)-nonadeca-1,6,9,12,15,18-hexaene-3,4-diol; Compound 2 (F3P10a): (6Z,9Z,12Z,15Z)-nonadeca-6,9,12,15,18-pentaene-3,4-diol; Compound 3 (F3P11b): (6Z,9Z,12Z,15Z)-4-chlorononadeca-6,9,12,15,18-pentaen-3-ol.

Sub-fractions and pure compounds caused contrasting effects, with ca. 80% of them causing significant bleaching and suppression of photosynthetic efficiency relative to controls (Figure 4.2.5). Photosynthetic efficiency suppression ranged from ca. 40 to 80%, relative to the coral effective quantum yield baseline, depending on the sub-fractions. Based on the Tukey HSD post hoc test results, six significantly different groups of allelopathic sub-fractions or pure compounds stood out. Three allelopathic compounds were selected for structure identification, as they were considered sufficiently pure.

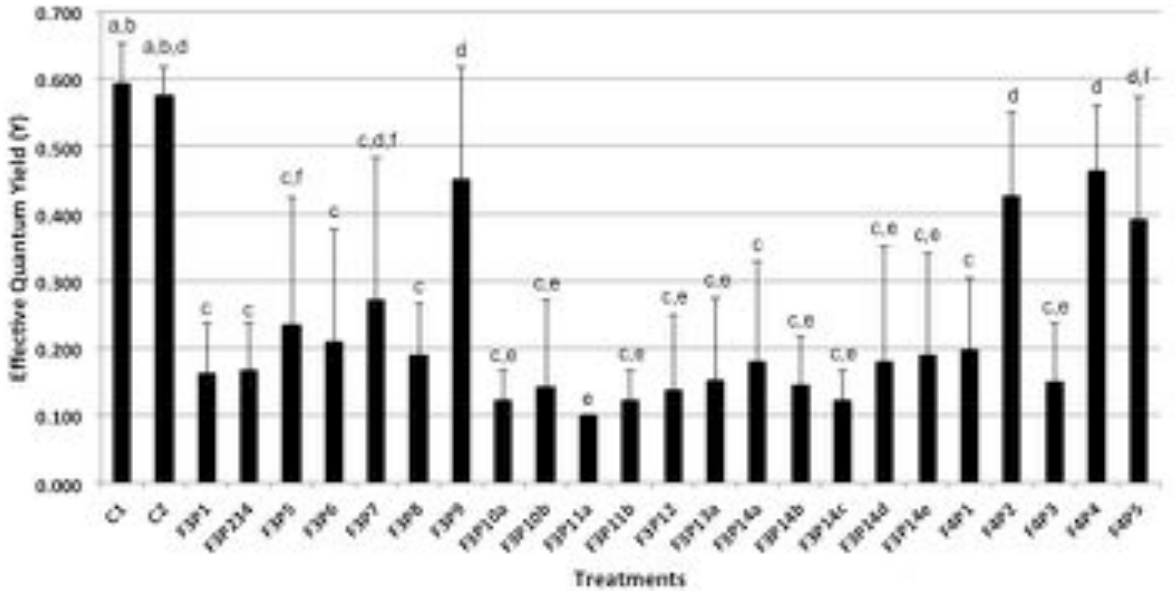


Figure 4.2.5 Barplot representation of the allelopathic bioassay results with the 23 compounds isolated from the fractions 3 and 4 of *L. rosacea* on *A. muricata*. The statistical analyses, comparing the compounds-treated patchs to MeOH-treated patch and untreated controls, were performed using Kruskal-Wallis and Steel-Dwass-Critchlow-Fligner post-hoc test. Letters indicate distinct groupings based on post-hoc statistical comparison among sub-fractions. Asterisks indicate significance in relation to controls (MeOH-treated or untreated, accordingly) with $P<0.001$, n=10 assays, 23 sub-fractions per assay. Error bars represent standard deviation of the mean. Letters indicate significant differences (Kruskal–Wallis test, $P<0.01$; Steel–Dwass post-hoc test, $P<0.05$, mean+s.d., n=10).

3.4. Structure identification of compounds 1-3

Compound **1** was isolated as colorless oil and its molecular formula was proposed as $C_{21}H_{31}ClO$ by HRESIMS analysis ($[M+NH_4]^+$ at *m/z* 352.2407 and 354.2382 with isotopic ratio 3:1). The ^1H NMR analysis started with a terminal vinyl group at δ_H 5.34 (dt, H-1a), 5.21 (dt, H-1b) and 6.02 (ddd, H-2) which was COSY coupled to a deshielded methine at δ_H 4.38 (ddt, H-3) (Table 4.2.2). Even if we first suspected the presence of a secondary alcohol at this position, the chemical shift of the corresponding carbon was more shielded than expected at δ_C 67.8 (C-3) for an allylic alcohol. In agreement with MS data, we then deduced the presence of a chlorine atom at this position, which was COSY correlated to a oxygenated methine (δ_H 3.70 ddd, H-4; δ_C 75.2, C-4). The spin coupled system was then extended to an ABXM system at δ_H 2.49 (H-5a) and 2.25 (H-5b) which was further coupled to an alternate polyunsaturated carbon chain composed of four double bonds separated by three methylenes. The configurations of the double bonds were assigned as *Z* by interpretation of the chemical shifts of allylic carbons. All these connections were later confirmed using HSQC and HMBC spectra. The other end of the compound

was deduced to be composed of a second terminal vinylic system coupled to the polyunsaturated core trough three COSY correlated methylene units. Unfortunately no similar allylic chlorohydrine was found in the literature that could allow us to conclude on the relative configuration of **1**. We then decided to compare the ^{13}C NMR experimental values with the calculated values obtained on the most stable conformers of the *like* and *unlike* diasteroisomers. Working on the most stable conformer, the Mean Absolute Error (MAE) was found to be lower for the *unlike* configuration (Figure 4.2.6).

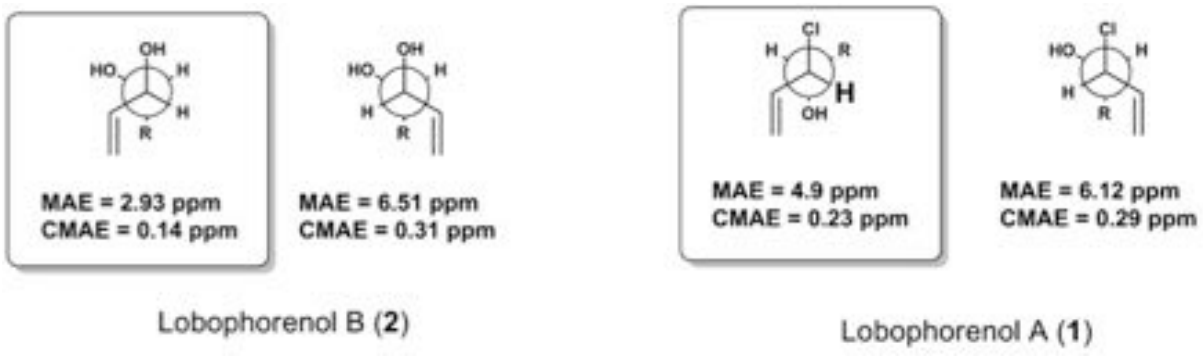


Figure 4.2.6 Mean Absolute Errors (MAE) and Corrected Mean Absolute Errors (CMAE) obtained between the ^{13}C NMR experimental and theoretical values for the two possible diasteroisomers of compounds 1 and 2.

For **2**, the isotopic pattern of the HRESIMS spectrum evidenced the absence of a chlorine atom in this molecule and the molecular peak at *m/z* 334.2744 ($[M+NH_4]^+$) suggests the replacement of this atom by an alcohol. Inspection of the ^1H and ^{13}C NMR spectra allowed us to localize the structural changes in the vicinity of the first vinylic system. Indeed, the methine signals at δ_H 4.38 (ddt, H-3) and δ_C 67.8 (C-3) in **1** were replaced by signals at δ_H 3.94 (H-3) and δ_C 76.6 (C-3) that are reminiscent of an allylic secondary alcohol. Therefore, the chlorine atom placed at C-3 in **1** was replaced by a second alcohol in **2** at this position. The relative configuration of compound **2** was deduced to be *unlike* applying the same method as for **1** (Figure 4.2.6). In this case, hydrogen bonds between the two vicinal alcohols render the *gauche* conformer more stable than the *anti* obtained for **1**. Both compounds may be produced by an *anti* opening of a common epoxide intermediate with water or a chloride ion.

The HR-(+)ESIMS data obtained for compound **3** with a molecular peak at *m/z* 336.2895 ($[M+NH_4]^+$) suggested that this natural product corresponds to a dihydrogenated derivative of **2**. The location of the reduced double bond was unambiguously deduced from ^1H NMR data that showed the lack of a terminal vinylic system. The appearance of a methyl at δ_H 0.97 (t, H-1) definitely placed the

new ethyl group at the beginning of the chain. We assume the same relative configuration for this compound as those previously proposed for **1** and **2**, being linked biosynthetically. The low amounts of compounds isolated prevented any attempts to assign their absolute configuration at C-3 and C-4.

Table 4.2.2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) chemical shifts (in ppm) for compounds 1-3 in CD_3OD

	Lobophorenol A (1)		**Lobophorenol B (2)**		**Lobophorenol C (3)**	
Compound	**δ_C**	**δ_H, mult. (*J* in Hz)**	**δ_C**	**δ_H, mult. (*J* in Hz)**	**δ_C**	**δ_H, mult. (*J* in Hz)**
1a	118.4	5.34, dt (17.0, 1.0)	116.7	5.31, dt (17.0, 1.0)	10.8	0.97, t (7.5)
1b		5.21, dt (10.0, 1.0)		5.18, dt (10.0, 1.0)		
2	137.3	6.02, ddd (17.0, 10.0, 8.5)	139.4	5.92, m	26.8	1.57, m 1.47, m
3	67.8	4.38, ddt (8.5, 4.5, 1.0)	76.6	3.94, m	76.2	3.32, m
4	75.2	3.70, ddd (9.5, 5.5, 4.5)	75.5	3.48, m	74.9	3.45, m
5a	32.8	2.49, br dt (14.5, 5.5)	31.8	2.36, m	32.2	2.36, dt (14.5, 7.0)
5b		2.25, ddd (14.5, 8.0, 5.5)		2.14, m		2.24, dt (14.5, 7.0)
6	126.4	5.48, m	127.3	5.51, m	127.6	5.52, m
7	131.4	5.49, m	130.8	5.45, m	130.6	5.45, m
8	26.8	2.87, t (6.0)	26.7	2.85, m	26.8	2.87, t (6.0)
9	128.7	5.37, m	128.7	5.37, m	128.7	5.37, m
10	129.4	5.37, m	129.4	5.37, m	129.4	5.37, m
11	26.6	2.86, t (6.0)	26.6	2.86, m	26.6	2.86, t (6.0)
12	129	5.37, m	129	5.37, m	129	5.37, m
13	129.1	5.37, m	129.1	5.37, m	129.1	5.37, m
14	26.6	2.82, t (6.0)	26.6	2.82, m	26.6	2.82, t (6.0)
15	129.3	5.37, m	129.3	5.37, m	129.3	5.37, m
16	130.8	5.38, m	130.8	5.38, m	130.8	5.38, m
17	27.6	2.10, br q (7.0)	27.6	2.07, m	27.6	2.08, m
18	30.1	1.46, quint (7.0)	30.1	1.46, quint (7.0)	30.1	1.46, quint (7.0)
19	34.4	2.07, br q (7.0)	34.4	2.06, br q (7.0)	34.4	2.07, m
20	139.8	5.82, ddt (17.0, 10.0, 7.0)	139.8	5.82, ddt (17.0, 10.0, 7.0)	139.8	5.82, ddt (17.0, 10.0, 7.0)
21a	115.1	5.00, dq (17.0, 2.0)	115.1	5.00, dq (17.0, 2.0)	115.1	5.00, dq (17.0, 2.0)
21b		4.94, dt (10.0, 1.0)		4.94, dt (10.0, 1.0)		4.94, dt (10.0, 1.0)

4. Discussion

4.1. *Lobophora* – coral associations

Proliferation of *Lobophora* in coral reef environments has caused recent concern among biologists (De Ruyter van Steveninck & Breeman, 1987b; Diaz-Pulido *et al.*, 2009). Yet the presence of *Lobophora* does not necessarily represent a threat to corals. Our survey of *Lobophora* – coral associations in the southwest lagoon of New Caledonia demonstrates that not all species of *Lobophora* are found associated with corals. Out of eight *Lobophora* species, three were never associated with a coral, but instead grew attached to scattered hard substrate in seagrass beds, shallow wave-washed habitats or coral rubble. The other five species were associated with living coral colonies, but grew at the dead bases adopting procumbent to decumbent forms (e.g. *L. dimorpha, L. undulata, L. hederacea, L. monticola*), or a fasciculate morphology niched within coral branches such as *L. rosacea*. The latter species is also the most commonly encountered, being observed in 42% of the transects. Most *Lobophora* species, however, were only observed in 10 to 20% of the transects. Even then, these numbers tend to overestimate the prevalence of *Lobophora* on the reef since the sites where the transects were laid out were precisely those locations where *Lobophora* – coral interactions were most conspicuous during initial surveys. Per transect, the presence of *Lobophora* never surmounted 25%. *Acropora* species were clearly the preferred partner, but all but one *Lobophora* species displayed a broader range of hosts. Furthermore, corals associated with *Lobophora* did not present traces of bleaching (Fig. 4.2.1), except in the case of *L. hederacea* associated with *Seriatopora caliendrum*.

4.2. Negative allelopathic interactions?

Based on the ecological niche and the morphological differentiation between *Lobophora* species we investigated if the species found in direct contact with corals have developed specific allelochemicals capable of impairing corals. Our results demonstrate that all *Lobophora* species, usually found in contact or not with corals, displayed similar bleaching effects on the tested corals. In other words, naturally found in contact or not, extracts of the eight *Lobophora* species show similar effects on corals: they are equally capable or not of bleaching specific corals. These results are of significant importance as they mean that species of the genus *Lobophora* are intrinsically capable of bleaching some corals upon direct contact. Evolutionary

speaking, it either means that: (1) *Lobophora* spp. have developed allelopathic compounds targeted towards competing benthic organisms or (2) allelopathy against corals, or other benthic organisms, appears as a side-effect (i.e. secondary effect, unintentional effect) of secondary metabolites with different ecological roles, such as herbivore deterrence or antimicrobial properties (antibiofilm for example). Indeed, Rasher and Hay (2014) and Longo and Hay (2014) showed that the allelochemicals released during competition with corals maybe the same used for herbivore deterrence. The latter idea is further supported by the multiplicity of allelopathic compounds (80% of the isolated compounds from two fractions) resulting in a significant suppression of photosynthetic efficiency on corals. Nevertheless, as discussed further below, the negative allelopathy demonstrated may not be ecologically relevant.

4.3. Differential susceptibility to bleaching across coral species

Among the four coral species tested with the eight species of *Lobophora, A. muricata* and *M. hirsuta* were the most significantly bleached corals. These results indicated a differential susceptibility to *Lobophora* allelopathy depending on the coral species. In this aspect our results echoed those of Rasher *et al.* (2011) who also noticed differential susceptibility across coral species to algal allelopathy. Rasher *et al.* (2011) found that *A. millepora* and *P. damicornis* were more sensitive to macroalgal allelopathic damage than *M. digitata* and *P. cylindrica*. We shared three genera (*Acropora, Montipora,* and *Porites*) and one same species (*P. cylindrica*) with Rasher *et al.* (2011). Also, we had similar results across those genera, although in our case *M. hirsuta* and *P. cylindrica* were not damaged at all. *P. damicornis,* which belongs to the same family as *S. pistillata,* i.e., Pocilloporidae, was also quite sensitive (Rasher *et al.*, 2011). However, *Acropora* and *Montipora,* which belong to the same family (Acroporidae), were differentially susceptible in both studies. Partly agreeing with our findings, Lesser *et al.* (2007) showed that Acroporids are not as resilient in the face of environmental perturbation compared to other species on the same reef. Nugues and Bak (2006) also showed that Caribbean corals had differential competitive abilities against *Lobophora*. These results would indicate that sensitivity is to some extent taxonomically related, but need to be strengthened with the studies showing a differential host susceptibility to opportunistic pathogens. In fact, some corals taxa are more susceptible to disease, and this reflects different levels of "immunity" between the corals species (Palmer *et al.*, 2010; Hawley & Altizer, 2011). These different levels of immunity to thermal bleaching and disease, which are

largely taxonomically related, could probably also be underlying corals vulnerability to chemical bleaching.

4.4. New allelopathic compounds with bleaching properties

We then proceeded to the isolation and structure identification of the chemicals from *L. rosacea* exhibiting bleaching properties against *A. muricata,* the most susceptible coral out of the four tested. Results from the bioassays with the five fractions showed that allelopathy against coral correlates with the polarity of the compounds, with the less polar fractions displaying the highest allelopathic activity. These results concur with the findings of Rasher and Hay (2010), showing that lipidic extracts from several algal species, including *Lobophora variegata*, resulted in significant bleaching, while hydrophilic compounds from *Chlorodesmis fastigata* (Udoteaceae, Chlorophyta) and *Galaxaura filamentosa* (Galaxauraceae, Rhodophyta) were not active. These results corroborate the importance of direct contact, which is preferable for hydrophobic allelochemicals transfer.

Most of the purified compounds from *L. rosacea* displayed a significant bleaching effect on *A. muricata*. The three new C_{21} polyunsaturated alcohols, named lobophorenols A-C (**1**-**3**) were among the most active fractions and sub-fractions were identified after NMR and MS analyses. These compounds were identified as three new C_{21} polyunsaturated alcohols. All these compounds may originate after opening of a common epoxide intermediate formed from a polyene. Similar C_{21} apolar polyenes have been reported only once from the alga *Fucus vesiculosus* (Halsall & Hills, 1971). It is worth highlighting the presence of a chlorinated analogue **1**, which is particularly rare and represent less than 1% of all the secondary metabolites isolated from species of the Phaeophyceae family (Cabrita *et al.*, 2010; La Barre *et al.*, 2010). Although, we may point out that De Nys *et al.* (1991) also isolated halogenated allelochemicals, the presence of the chlorine atom may however not be related to the bleaching properties of the molecule, since both compounds **2** and **3**, deprived of this halogen atom, present similar adverse properties. Furthermore, the isolated allelochemicals do not belong to the terpene family of natural products, as somewhat expected from De Nys *et al.* (1991) and Rasher *et al.* (2011) but polyunsaturated alcohols. It shows that allelopathy against corals may involve a variety of families of compounds as already reported by Slattery and Lesser (2014), and strongly supported by the diversity of compounds displaying bleaching properties in this study. It is worth pointing out that we were expecting to find

terpenes, given the richness in terpenoids of the Dictyotaceae family in which *Lobophora* belongs (Paula *et al.*, 2011). However, much to our surprise this family of compounds was not detected by NMR. Yet, the genus *Zonaria,* which is sister to *Lobophora*, did not present terpenes either (authors' unpublished data).

Lobophora bioactivity against corals does not come as a surprise as in the literature *Lobophora* extracts (crude, hydrophilic or hydrophobic extracts) and isolated compounds have been shown to display a broad spectrum of activities and in particular antimicrobial (e.g. fungi, bacteria, protozoa) bioactivities (e.g. Kubanek *et al.*, 2003; Engel *et al.*, 2006; Cantillo-Ciau *et al.*, 2010). The exact bleaching mechanisms are unaddressed here and could very well be targeting either the polyp or the *Symbiodinium*. Nonetheless, it is worth mentioning that after two weeks following the bioassays, the surface area which bleached in contact with the patches, recovered their original coloration.

4.5. Ecological implication: armed but not necessarily dangerous

Present field assays would suggest that *Lobophora* has the potential to chemically impair some coral species by direct contact. Nevertheless, *in situ* observations indicate that although apparently chemically potent, *Lobophora* do not or rarely bleach coral hosts in a natural setting (this study). Slattery and Lesser (2014) also questioned if *Lobophora* presented allelopathic effects on corals in the Bahamas. Yet, while *Lobophora* extracts and a purified compound bleached the coral *Montastrea cavernosa*, contact experiments between *Lobophora* and the coral did not (Slattery & Lesser, 2014). Furthermore, no claim of coral bleaching as a result of contact with *Lobophora* in natural setting was made by the authors (Slattery & Lesser, 2014). Even though it would be tempting to conclude that allelopathy is ecologically important in the competition between *Lobophora* and corals, there is no strong evidence from field observations. Herbivory on the other hand, clearly appears as an important factor preventing competition to occur. Therefore, the question remains: what explains the inconsistency between field observations and bioassay experiments?

A possible explanation for this discrepancy would be the localization of the bioactive compounds within the endometabolome. In bioassays, corals are artificially exposed to chemicals with negative allelopathic effects, a situation that would only occur as a result of abrasion or herbivory under natural conditions. Alternatively, the compounds may be part of the exometabolome, present on the surface of the alga, but external factors (e.g. herbivory) or a defense system by the coral itself may ward

off allelopathic interactions, thus preventing *Lobophora* from outcompeting corals. Indeed, field experiments demonstrated that most coral species prevented the overgrowth of crustose *Lobophora* species owing to a set of defense mechanisms (De Ruyter van Steveninck *et al.*, 1988b; Nugues & Bak, 2006). Additionally, field observations and experiments showed that herbivory is a major factor preventing increase in *Lobophora* abundance (De Ruyter van Steveninck & Breeman, 1987b; Jompa & McCook, 2002b; Slattery & Lesser, 2014). Nevertheless, in New Caledonia, close contact is being observed between *Lobophora* and corals, thus questioning what is actually preventing negative allelopathic actions. Since hydrophilic compounds are quickly diluted, dispersed and degraded in seawater, it limits the putative allelopathic effects to lipophilic compounds (Lewis Jr, 1986), which exhibited significant negative allelopathy in field assays. However, their adverse action requires direct and continuous contact between the macroalgae and corals, which necessitates from the algae to either be crustose or to form dense mats or canopies.

In New Caledonia, only one species of *Lobophora*, *L. hederacea*, is actually capable of overgrowing a coral species, *Seriatopora caliendrum* (Vieira *et al.*, 2015). In this latter case, negative allelopathic action and subsequent coral overgrowth appears to be possible owing to a combination of multiple factors including the coral vulnerability and the inhibition of grazing (Vieira *et al.*, 2015), supporting the important role of coral defense and herbivory in preventing negative allelopathic interactions. In the Great Barrier Reef, Jompa and McCook (2002a) showed that a *Lobophora* species, adopting a crustose morphotype, was capable of overgrowing the coral *Porites cylindrica* when herbivory was reduced. In our experiments, *P. cylindrica* was not damaged at all by any of the *Lobophora* species crude extract, thus suggesting that allelopathy may not be a major mechanism allowing the overgrowth of this species.

Finally, the confinement of adverse compounds to the endometabolome, or the combination of coral defense and herbivory may result in macroalgae such as *Lobophora* naturally associated with corals to have limited negative allelopathic effects on their coral hosts. Negative allelopathy evidenced from bioassays does not prove that the extracts and isolated allelochemicals have for primary role to regulate/inhibit competitors.

In damaged reefs, however, coral morbidity and mortality in addition to shifts in herbivory pressure result in whole different setting where macroalgal allelopathy may have harmful effects on corals. Although not yet explored, it is possible that negative allelopathy in damaged reef may results from the synergetic effects of macroalgal

exudates/allelochemicals acting in combination with a number of environmental parameters/stressors such as seawater pH, oxygen depletion, and or temperature maxima (Harlin & Rice, 1987).

5. Conclusion and perspective

The role of chemical interactions between macroalgae and corals initially evinced in the early 90s in form of positive allelopathy (Morse, 1992), has regained interest only recently, yet this time in form of negative allelopathy (Rasher & Hay, 2010). The limited number of studies on the subject has basically disclosed deleterious effects (e.g. bleaching, recruitment inhibition) in damaged reefs and beneficial effects (e.g. recruitment facilitation) in healthy reefs. The present work concluded that although potentially chemically adverse, macroalgae might in fact not be harmful to the corals they are interacting with. This questions the importance of negative allelopathy in the interaction between macroalgae and corals in healthy coral reefs. Whole macroalgal metabolome bioassays may finally not be ecologically relevant, and future studies should specifically implement surface compounds analyses in order to corroborate the hypothesis that those bioactive compounds are part of the endometabolome and thus explain their inactivity *in situ*.

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Author contributions

C.V., O.D.C, C.P., O.T. and G.C designed research; C.V., J.G. and F.H. performed bioassays experiments; C.V. and F.H. analyzed bioassays data; O.T., J.G. performed extraction and fractionation; G.C., O.T. and G.G.-J. performed the NMR characterization and mass spectrometry; C.V. prepared Figs. 1-3 and 5; O.T. prepared Figs. 4 and 6; C.V., O.D.C., C.P., G.C. and O.T. wrote of the manuscript.

Chapter 5: General discussion or *Lobophora* success story: when the algae bow down to the Red Queen

Alternative title: ecological speciation in marine brown algae

"Tropical environments ... provide an example of the Red Queen hypothesis (Van Valen, 1973), where dynamic species interactions and coevolution continually drive phenotypic change (Stenseth, 1984)." -Schemske (2009)-

Abstract

The advent of molecular taxonomy has increased species discovery at an unprecedented rate (Blaxter, 2004). Especially, taxa suffering from an important taxonomic deficit, such as algae, have greatly beneficiated from DNA-taxonomy (De Clerck *et al.*, 2013; Leliaert *et al.*, 2014). For example, a recent DNA-based taxonomical study of the brown alga genus *Lobophora* (Dictyotales, Phaeophyceae) disclosed an outstanding diversity unforeseen before, leaping our taxonomical knowledge of this genus from only three species at the beginning of the century to presently over 100 species (Vieira *et al.*, in prep.-c). This spectacular species richness uncovered from a single genus in turn raises several evolutionary questions related to the drivers of diversification in the marine environment. In this final chapter, we recapitulate the major findings of this PhD, and discuss the speciation processes, which could have contributed to the hyper-diversity presently observed. We suggest that founder events and ecological speciation must have represented important speciation modes.

1. An overview of our key findings

In this thesis, I studied *Lobophora*, an algal taxon for which our taxonomical knowledge was evidently severely deficient. DNA-based taxonomy revealed a remarkable number of species, bringing our taxonomical knowledge from eleven to over 100 species in the time-span of only two years (Vieira *et al.*, 2014b; Vieira *et al.*, in prep.-c). We implemented morphological, ecological and metabolomic analyses and investigated to which extent genetic diversity was morphologically, ecologically and metabolomically paralleled. However, we were able to discriminate only a limited number of morphotypes and ecological habits in the field (Vieira *et al.*, 2014b), implying that the bulk of *Lobophora* species diversity is cryptic. On the other hand, we noticed that genetic diversity within this taxon was echoed in its metabolome. This led us to explore the potential roles of secondary metabolisms and to perform interspecific comparisons. More specifically, we explored the roles of secondary metabolisms in two types of biotic interactions: defense and offense. We tested if different species of *Lobophora* were differentially capable of damaging corals, with which they compete for space (Vieira *et al.*, in revision). Much to our surprise, in our experiments we did not observe differences between the *Lobophora* species on the targeted corals (Vieira *et al.*, in revision). We then tested the susceptibility of different *Lobophora* species to grazing. Here again, against all expectations based on

the literature, we did not observe major differences in grazing susceptibility between *Lobophora* species, which were all consumed without outstanding differences (Vieira *et al.*, in prep.-b). From the results of allelopathic bioassays and grazing experiments we can conclude that: 1. corals and *Lobophora* maintain a chemical-mediated *status quo* on healthy reefs; 2. chemical defense apparently does not deter grazing of *Lobophora* by prominent herbivores; 3. it is more likely that *Lobophora* avoids being grazed by escape strategies such as growing under the coral canopy.

2. Hidden diversity in a hyper-diverse brown algal genus

2.1. Hyper-genetic-diversity

We reassessed the species diversity of *Lobophora* locally, in New Caledonia, and globally at the geographical distribution scale of the genus. To do so, we implemented a DNA-based taxonomic approach using multi-organelle markers, the mitochondrial marker *cox*3, the chloroplast markers *rbc*L and *psb*A, and the nuclear marker LSU. We used three DNA-based species delimitation algorithms: the maximum likelihood (ML) as well as the Bayesian Implementation of the General Mixed Yule Coalescent model (GMYC; Pons *et al.*, 2006; Reid & Carstens, 2012), and the Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.*, 2012). We interpreted consensual results between the four markers as well as delimitation algorithms to delimit species. In New Caledonia, analyses resulted in 31 – 39 molecular taxonomic units (MOTUs), and 10 species were described de novo (Vieira *et al.*, 2014b). On a global scale analyses of the *cox*3 data set resulted in 98 – 120 MOTUs (Vieira *et al.*, in prep.-c). In order to assess the global diversity of the genus *Lobophora* we implemented a rarefaction analysis and calculated several richness estimators, which resulted in estimates of 140 – 215 MOTUs. In conclusion, *Lobophora* is at least 40-fold more diverse than a morphology-based taxonomy seemed to indicate as recent 5 years ago. Such hyperdiversity identified from molecular taxonomy within an algal group, is nevertheless not an isolated case. Several other taxa (e.g. Dumontiaceae, *Portiera*, *Sellaphora, Porphyra*) have demonstrated a remarkable diversity explosion, with diversity estimates up-scaled to a factor of 10 to 100 (e.g. Stiller & Waaland, 1993; Evans *et al.*, 2007; Saunders, 2008; Payo *et al.*, 2013), highlighting the high level of cryptic diversity within algae in general. The magnitude of algal diversity, which remains largely uncertain (Guiry, 2012; De Clerck *et al.*, 2013), does not presently represent a significant part of the global eukaryotic diversity (Mora *et al.*, 2011; Sweetlove, 2011; Appeltans *et al.*,

2012; Costello *et al.*, 2013). It remains to be seen by how much our knowledge of algal diversity will increase with the help of molecular taxonomy. Will the magnitude of algal diversity reach a comparable level to other mega-diverse groups such as fungi or even beetles?

Nevertheless, high genetic diversity is not really astonishing, since there are infinite genome possibilities. What is more intriguing on the other hand is why and how such genomic diversity is maintained, whether this variation is largely adaptive or neutral, and why these distinct individuals can coexist. Of course one may question the validity of what we call species. But we do not intend to enter the 'species definition' debate. For the moment we must accept that molecular taxonomy will remain controversial until better founded in existing theory of evolutionary biology and phylogenetics (Vogler & Monaghan, 2007).

2.2. Hypo-morphological-diversity

Four *Lobophora* morphotypes were previously discerned in the literature, namely crustose, ruffled, decumbent and stipitate (Womersley, 1967; Littler & Littler, 2000). Our morphological analyses resulted in the identification of three additional morphotypes in New Caledonia (i.e. procumbent, anastomosing, conk-like) (Vieira *et al.*, 2014b). Consequently, we observe significantly less morphological than genetic diversity. Low morphological diversity, however, does not imply that genetic-based species entities are not valid. Evidently, phenotypic expression possibilities are far lesser than genetic possibilities, which result in inevitable and extensive cryptic diversity. Developmental and environmental constraints are such that the possibilities of morphological expressions are limited. This is why we observe remarkable morphological convergences between the three major algal divisions which are phylogenetically far apart (Rhodophyta, Chlorophyta, Chromophyta), and which led marine ecologists to define algal functional groups (Littler, 1980; Littler & Littler, 1980; Littler *et al.*, 1983).

2.3. High-ecological-diversity

Lobophora can be found in all ocean basins, and although chiefly a tropical genus it is also encountered in warm-temperate waters (Vieira *et al.*, in prep.-c). *Lobophora* grows from the surface down to 140 m (Markager & Sand-Jensen, 1992), inhabiting almost any habitat with hard substrate, from intertidal pools to subtidal areas, including shallow and sheltered parts of the coast, exposed reef faces, offshore coral

reefs, bedrock in lagoons, and rocky outcrops surrounded by sand. *Lobophora* grows on a variety of substrates encompassing mangrove prop roots, sunken logs, dead, unhealthy or live corals, at the bases of branching and massive corals, epilithic, epiphytic to other algae (e.g. crustose coralline algae, other *Lobophora* species), in habitats ranging from seagrass and macroalgal beds to coral fields (Littler & Littler, 2000; Payri *et al.*, 2000; Abbott & Huisman, 2004; De Clerck *et al.*, 2005a; Coppejans *et al.*, 2009; Kraft, 2009; Sun *et al.*, 2012; Vieira *et al.*, 2014b). In conclusion, *Lobophora* has successfully colonized a high diversity of habitats and is consequently highly ecologically diverse. In New Caledonia, *Lobophora* species were clearly associated to specific habitats. It remains to be seen if this pattern holds true for other *Lobophora* species across the globe.

2.4. Hyper-metabolomic-diversity

While possibilities to diverge morphologically are constraint compared to the genetic diversity, metabolomic possibilities are on the other hand much more diverse, as supported by our results. But hyper-metabolomic-diversity is neither really surprising. In fact, unlike the morphology, which offers a unique possibility per entity – without the consideration of phenotypic plasticity – the secondary metabolism is composed of a multitude of secondary metabolites. Not only may a secondary metabolite slightly vary between two individuals, but also the combination of secondary metabolites offers an infinite possibility of secondary metabolites without considering spatial and temporal variation.

The ecological roles of secondary metabolites of *Lobophora* have received relatively little attention. Studies that did focus on the ecological roles of natural compounds in *Lobophora* investigated the roles of feeding deterrence, antifouling and allelopathic interactions. Generally speaking, most of the research has focused on the roles metabolites in defense against generalist consumers, mainly fishes, sea urchins, and gastropods (Amsler, 2008). While several studies performed grazing experiments with *Lobophora*, only two actually tested the ichyotoxicity. De Lara-Isassi *et al.* (2000) showed ichtyotoxicity against a freshwater fish, the goldfish, which is ecologically irrelevant since *Lobophora* is a strictly marine alga. Slattery and Lesser (2014) concluded that purified sulfolipids and whole crude extracts were not ichtyotoxic against omnivorous and herbivorous fishes. In other words, today we have no evidence that *Lobophora* possesses ichtyotoxic compounds targeted towards its natural predators. We still know relatively little about the allelopathic, antifouling, and antipathogenic bioactivities of macroalgal natural products (Amsler, 2008). Only

two studies investigated the antifouling (Da Gama *et al.*, 2008; Manilal *et al.*, 2010) and only three studies tested the role of natural compounds as allelopathic agents against space competitors (Rasher & Hay, 2010; Slattery & Lesser, 2014; Vieira *et al.*, in revision). Finally, our knowledge of the chemical ecology of *Lobophora* remains largely scarce. Future studies should be targeted towards exploring the role of secondary metabolites as (1) chemical defenses against herbivores, competitors, epibionts, pathogens, and as (2) cues in the chemical communications with other organisms, to finally (3) determine what their exact role is in structuring marine communities.

3. Successful diversification of *Lobophora* in coral reef ecosystems

The spectacular species richness uncovered from a single genus in turn raises the question, what drives speciation to such high level of biodiversity? Speciation processes are nonetheless a disputed area of research (Fitzpatrick *et al.*, 2009). The traditional classification of speciation into discrete geographical categories (allopatric, parapatric and sympatric) has become progressively obsolete (Fitzpatrick *et al.*, 2009). Alternatively, classifications centering on mechanisms that drive the evolution of reproductive isolation (ecological speciation, speciation by divergence under uniform selection, speciation by genetic drift and polyploidy speciation) is becoming gradually more accepted (Schluter, 2001). Ecological speciation refers to the evolution of reproductive isolation between populations or subsets of a single population by adaptation to different environments or ecological niches (Schluter, 2001). An archetypical example of ecological speciation is that of the cichlids of East Africa in the Rift Valley Lakes, particularly Lake Victoria, Lake Malawi and Lake Tanganyika (Schliewen *et al.*, 2001). Recent studies on marine speciation, particularly in the tropics, pointed towards the importance of ecological speciation (Bowen *et al.*, 2013), especially in marine fishes (Schluter & Rambaut, 1996; Rocha *et al.*, 2005). Gentry (1989) suggested that explosive and essentially sympatric speciation has played an important role in the production of high species richness in the tropical forests, which appears to be equally true in coral reefs (Bowen *et al.*, 2013). Ecological/sympatric speciation has been demonstrated in several marine taxa including marine fishes (Schluter & Rambaut, 1996; Rocha *et al.*, 2005; Crow *et al.*, 2010), snails (Pfenninger *et al.*, 2003; Johannesson *et al.*, 2010) and sponges (Duran & Rützler, 2006). Nevertheless, to our knowledge virtually no study has investigated the role of ecological speciation in marine benthic algae yet. Numerous evidences are nevertheless directing towards the importance ecological speciation in marine benthic

algae, and should be given consideration by marine evolutionary phycologists. Similar to the formation of animal and terrestrial plant species, algal speciation is characterized by the evolution of barriers to gene flow between previously interbreeding populations. Both, prezygotic and postzygotic barriers may contribute to reproductive isolation (Rieseberg & Willis, 2007). However, today little is known on algal speciation, and the relative importance of prezygotic vs. postzygotic barriers is virtually unknown. Here, we will only consider the geographical and ecological factors leading to divergence. We will discuss the roles (1) founder events and (2) ecological speciation in driving the hyper-diversity of this genus in coral reef ecosystems. In the course of this discussion, we will pay special attention to the roles of phenotypic plasticity, abiotic and biotic interactions in enabling this mode of speciation in marine algae.

3.1. Allopatric speciation: founder events

Using a combinations of calibrated phylogenies and model-based historical biogeography we concluded that the major historical geological marine barriers (i.e. closure of the Tethys Sea, closure of the Panama Isthmus, Benguela Upwelling formation) did not represent important vicariance events in the diversification *Lobophora* (Vieira *et al.*, in prep.-c). On the other hand, long-dispersal over evolutionary time-scales appears to have contributed to the observed diversity of this genus. Founder event speciation is the process by which a small group of individuals disperse and colonize a new area that is geographically or physically separated form the original population. Through time, divergence in the genetic composition of the small colony from the large population that occurs by genetic drift eventually results in reproductive isolation and hence speciation. Although, not the only speciation mechanism to leave an inprint in the diversity pattern of *Lobophora*, founder event speciation appears to be responsible for a non-negligible part of the sampled species diversity.

Long- vs. short-distance dispersal

The global distribution of *Lobophora* provides circumstantial evidence, that regardless of the mechanism, long-distance dispersal took place over long evolutionary time scale. The restricted distribution of most *Lobophora* species, on the other hand, provides an indication that over short time-scales long-distance dispersal is not common enough to prevent diversification.

Both Santelices (1990) and Norton (1992) reviewed the processes of dispersal in macroalgae and discussed the different mechanisms favoring or preventing long-

distance dispersal. Dispersal is achieved not only by propagules, i.e. zygotes and spores, but also by adult plants (Norton, 1992). *Lobophora,* which is a small macroalgae, releases its propagules close to the substratum, which by finding themselves in quiet waters, within the parents plants canopy, will passively sediment (Norton, 1992). Finally, propagules of small macroalgae such as *Lobophora* will not achieve far dispersion (Norton, 1992). Such was the conclusion of De Ruyter van Steveninck and Breeman (1987a) who showed in his study on deep water populations of *Lobophora* on the coral reef of Curaçao, that dispersal and spread of *Lobophora* does not reach any considerable distance. This has major repercussions, the most important one being that local populations will be genetically relatively isolated, with limited connectivity (i.e. gene flow) among populations. Valero *et al.* (2011) commented that the limited dispersal of macroalgae "*suggests a system of self-sustaining, "closed" populations*". Since dispersal abilities are limited, we consequently expect *Lobophora* to have little genetic structuring at small spatial scales. And clearly, gene flow will not occur at large geographic scales. The second obvious implication being that the long-dispersal by propagules will practically be inexistent. But even if *Lobophora* propagules were to disperse far away, they might not survive the journey, since while they are capable of photosynthesis, macroalgal propagules life-span has been shown to be rather short lasting at sea several days at most (Santelices, 1990). Consequently, long-distance dispersal is most likely achieved by drifting adult plants that have been dislodged, as they can travel further than propagules. Among the Phaeophyta, *Saragassum* species, e.g. *S. muticum*, are capable of covering major distance reaching up to 7000 km (Deysher & Norton, 1981; Van den Hoek, 1987). *Lobophora*, however, does not possess structures allowing flotation, and consequently requires hitching a lift on floating objects that can be transported far away. *Lobophora* grows on a variety of hard substrata including potentially floating rafts such volcanic pumices and trees, but also other macroalgae such as *Sargassum*. In fact, *Lobophora* is capable of epiphytizing other organisms such as corals, gorgonians, seagrasses, *Sargassum*, and even other *Lobophora*. Nevertheless, long-dispersal by adult plants are only speculations, and no study has yet investigated long-distance dispersal in *Lobophora*.

3.2. Ecological speciation

Marine species diversity reaches a maximum in tropical regions in coral reefs (Connell, 1978). While, some algal groups (e.g. Rhodophyta and Chlorophyta) dominate in tropical climates, globally, marine algal diversity reaches its maximum

in temperate regions (Silva, 1992; Kerswell, 2006). Nevertheless, some algal groups, such as the Dictyotales order, are chiefly tropical, and thus reach its species diversity peak in tropical regions (Bold & Wynne, 1985; De Clerck *et al.*, 2006). Three major hypotheses have been suggested to explain this high level of species diversity in tropical waters in comparison to temperate waters, including (1) historical hypotheses, (2) ecological hypotheses and (3) higher speciation rate hypothesis (reviewed in Mittelbach *et al.*, 2007). Additionally, Mittelbach *et al.* (2007) suggested that among seven evolutionary mechanisms, biotic interactions play a major role in increasing speciation rates in tropical regions. To quote Schemske (2009): *"The biotic interactions hypothesis suggests that natural selection in temperate regions is governed primarily by abiotic factors, particularly low temperature, while in tropical regions, a greater role of biotic interactions may increase the opportunity for evolutionary novelty (Dobzhansky, 1950) and a rapid diversification (Schemske, 2002)"*. Together with abiotic interactions, biotic interactions are the selective agents acting in ecological speciation. According to Rundle and Nosil (2005) "*Selection is ecological when it arises as a consequence of the interaction of individuals with their environment during resource acquisition"*. Therefore, ecologically-based divergent selection arises from the interaction of an individual with its physicochemical and biological environment, and most likely a combination of both. Simply put, two conspecific individuals that have colonized two different habitats will undergo contrasting biotic and abiotic interactions and thus experience divergent selection. Three ecological causes have been acknowledged: environmental differences, sexual selection and ecological interactions (see Rundle & Nosil, 2005). Here, we will only consider environmental differences. First, we will look into the notion of algal functional groups, which is a clear evidence for ecological specialization. We will also discuss the importance of phenotypic plasticity in ecological speciation. Then we will discuss the abiotic and biotic interactions contributing to divergent selections.

3.2.1. Function groups: evidence for ecological speciation

It is remarkable to observe evolutionary convergence in representatives of most groups in the three major algal divisions (Chlorophyceae, Rodophyceae, Phaeophyceae), which are phylogenetically far apart. Morphological traits capture important variation in a species ecological strategy and function (McGill *et al.*, 2006).

The observations of manifest correlation between morphological traits and ecological features (Norton *et al.*, 1981) led marine ecologists to introduce the notion of "algal function groups" (Littler, 1980; Littler & Littler, 1980). Classification of marine algae into functional groups aimed at determining ecological characteristics based on the morphology (Littler, 1980; Littler & Littler, 1980). These studies evidenced a link between algal anatomy, morphology, physiology and their ecology (i.e. morphological traits are correlated through shared responses to ecological strategy), and showed that in a given environment morphological adaptations play a critical role in the survival and reproduction of macroalgal individuals. In other words, in an environment characterized by a combination of multiple biotic and abiotic factors, an alga is specifically ecologically adapted. Ecological factors have induced through time phenotypic changes, like strategies enabling algae to persist in habitats under conditions of high disturbances such as high herbivory (Lewis *et al.*, 1987), high hydrodynamism (Norton *et al.*, 1981) or water-flow (Stewart, 2008). We do not only observe morphological adaptations between distantly related algal taxa, but also at the intraspecific level, e.g. morphological plasticity in response to herbivory (Lewis *et al.*, 1987). Ecological diversification within a clade can in turn drive the adaptive evolution of morphological traits. It is the observation of morphological adaptations to specific habitats between algal groups and intraspecifically that highlights the important role of ecological speciation in marine algae. Local adaptation heavily relies on phenotypic plasticity.

3.2.2. Role of adaptive phenotypic plasticity in diversification

Morphological adaptation results from natural selection, which operates on other evolutionary factors – mutation, drift and gene flow. In addition, the idea of plasticity as a source of novelty and a factor in evolution is gradually becoming more appreciated (Fitzpatrick, 2012). Phenotypic plasticity, the ability of a single genotype to produce multiple phenotypes in response to variation in the environment, is increasingly regarded as an important player in local adaptation (West-Eberhard, 1989), although its evolutionary significance remains controversial (Pfennig *et al.*, 2010). While, phenotypic plasticity can represent a buffer against divergent selection (Levin, 1988; Van Kleunen & Fischer, 2005), it can facilitate colonization of new niches and rapid divergent evolution (Schlichting, 2004). Here we will focus on the role of adaptive plasticity in promoting diversification in marine algae. Morphological plasticity is common in red, brown and green algae (Lubchenco & Cubit, 1980; Collado-Vides, 2002; Benedetti-Cecchi *et al.*, 2006; Stewart, 2006).

Plasticity has several advantages including the exploitation of a variety of habitats and resources. Several morphological features of macroalgae are plastic with respect to their size and shape. These morphological traits may vary in response to biotic and abiotic factors (Norton *et al.*, 1981). Abiotic factors include water-depth, desiccation, substratum, light, water motion, temperature, salinity, nutrients and damage (e.g. storm, sand abrasion) (reviewed in Norton *et al.*, 1981). Biotic factors include for example predation (Lubchenco & Cubit, 1980; Diaz-Pulido *et al.*, 2007) and bacterial association (Singh *et al.*, 2011). Macroalgae not exposed to desiccation, receiving enough light, growing in relatively low temperature and high salinity, on stable substratum, protected from herbivory will generally present a well-developed thallus, much bigger in size than macroalgae growing in contrasting conditions (Norton *et al.*, 1981). Opposite conditions will usually result in stunted plants (Norton *et al.*, 1981). Also, we argue that algal morphological plasticity played a significant role in the process of ecological speciation. Phenotypic plasticity is one major means by which benthic macroalgae can cope with environmental variability. We will now look into the environmental factors that may act as selection agents, leading to phenotypic adaptation.

3.2.3. Ecological selective agents

The biotic and abiotic environmental factors that presently control marine benthic macroalgal distribution and abundance are very likely the same ones that acted as selective pressures over evolutionary time-scales. The most important abiotic factors controlling the growth and distribution of algae are temperature, salinity, nutrients, light, substrate and water motion (Norton *et al.*, 1981). Ecological selection can thus arise as a consequence of the interaction of individuals with their physicochemical environments. A striking example is given by the morphological adaptation of macroalgae to wave-washed habitats (Norton, 1991). Ecological selection can also arise as a consequence of the interaction of individuals with other organisms. Any biological interaction along the full spectrum of biotic interaction (i.e. competition, amensalism, antagonism, neutralism, commensalism, mutualism) may act as selective agents by affecting growth rate and reproductive outputs. Marine macroalgae interact with various organisms, which we will categorize within three groups: (1) predators, (2) competitors and (3) epibionts. We suggest that the strength of these three types of biotic interactions acted as important evolutionary mechanisms driving tropical marine macroalgal diversification.

Predation pressure. Herbivore pressure represents the most important biotic agent exerting an intense and constant pressure on macroalgae (Hay, 1997). Herbivory has been shown to play a major role in determining diversity, abundance, and species composition of seaweeds in shallow water benthic communities (Hay et al 1988c, van Alstyne 1989). It represents a major selective force in the natural selections of plants (Fritz & Simms, 1992). In tropical marine communities, we except a more intense grazing pressure on macroalgae than in temperate regions, since the marine fauna reaches a diversity peak at low latitudes (Floeter *et al.*, 2005),. Over evolutionary time scales, this herbivory pressure selected macroalgal phenotypic and ecological traits that allowed survival in tropical habitats. Macroalgae evolved all sorts of strategies against herbivores in forms of escape (e.g., spatial, associational) or defense (e.g., morphological, structural, chemical) (Lubchenco & Gaines, 1981). For instance, greater deterrence of tropical macroalgae in comparison with temperate macroalgae has been interpreted as a macroevolutionary response to an intensification in herbivory rates (Hay, 1991). Herbivores are engaged in evolutionary arms-race with macroalgae, as they themselves continuously evolve counter-strategies allowing the consumption of macroalgae (e.g. feeding tolerance for chemically-defended macroalgae) (Hay, 1997). Comparably to herbivore-plant coevolution, herbivore–macroalgal coevolution may very well represent a major factor promoting the escalation of herbivores and macroalgae diversification in coral reefs.

Competition pressure. According to Mitarai *et al.* (2014): *"The species self-organize their spatial distribution through competitive interactions to create many patches, implicitly protecting each other from competitively superior species, and speciation in each patch leads the system to high diversity".* Competition is undeniably an important structuring factor in coral reef benthic communities (Hughes, 1989). It has been suggested that competition generally represents an important driver of diversification, especially when individuals share very similar resources (Dieckmann & Doebeli, 1999). Coexistence between competitors, such as terrestrial plants, is largely possible thanks to niche separation along environmental axes (Silvertown, 2004). In coral reef ecosystems, competition for space between macroalgae and other benthic organisms has also played an important role in macroalgal adaptation and diversification. Competition for space between benthic organisms may contribute to diversification in several ways, for instance, by affecting the selection of life history traits that will either permit to outcompete or to coexist

with other benthic organisms. In coral reefs, competition between macroalgae and other competitors such as other macroalgae (Carpenter, 1990), seagrasses (Davis & Fourqurean, 2001), sponges (Preciado & Maldonado, 2005; González-Rivero *et al.*, 2011), octocorallia (De Nys *et al.*, 1991) and scleractinian corals (McCook *et al.*, 2001) can be intense. Several mechanisms by which macroalgae compete with their competitors have been identified such as allelopathy, overgrowth, epithelial soothing, shading and abrasion (Harlin & Rice, 1987; Carpenter, 1990; McCook *et al.*, 2001; Rasher & Hay, 2010). For instance, some macroalgae have demonstrated the potential to inhibit the growth of competiting macroalgae, e.g. *Ascophyllum nodosum* deter algal competitors such as *Laminaria* and *Fucus* (Walker & Smith, 1948). Coexistence of macroalgae with other benthic sessile organisms is also possible, for instance by using them to their advantages. For instance, since coverage of the primary substrata by benthic sessile organisms often reaches 100%, macroalgae may find additional space and refuge from predators under the branching coral canopy (Bennett *et al.*, 2010). Finally, competitive interaction has certainly played an important role in macroalgal diversification in coral reefs.

Epibionts pressure. Heavy epibiosis on macroalgae may be adverse to the host by impeding photosynthesis or causing tissue necrosis. It is therefore indispensable for macroalgae to develop antifouling strategies e.g. epithallial shedding or antifouling chemicals (Harlin & Rice, 1987; Keats *et al.*, 1997; Steinberg & De Nys, 2002). Some macroalgae, such as coralline algae excel in antifouling strategies (Keats *et al.*, 1997). Nevertheless, while heavy epibiosis may be harmful to the macroalgal host, some epibionts may strongly affect herbivore consumption of the macroalga by acting as feeding repellent (Wahl *et al.*, 1997). Selection and regulation of specific epibionts may therefore be beneficial to the macroalgal host, and may be selected as a defensive strategy against predators. This may possibly explain why certain macroalgae such as *Lobophora* have the ventral-side more epiphytized than the dorsal-side. While, the dorsal-side needs to be clear from epiphyte to efficiently perform the photosynthesis, the ventral-side on the other hand can be epiphitized without being adverse to the alga. Presence of unpalatable epibiont will consequently be beneficial to the algal host. Some *Lobophora* species are generally epiphyte-free e.g. *L. rosacea*, whereas others are heavily epiphytized *e.g. L. monticola*, at least on the ventral surface.

Recently, scientists started appreciating the role of epibiotic bacterial community in maintaining the health of their macroalgal host (Armstrong *et al.*, 2001; Wahl *et al.*,

2012; Egan *et al.*, 2013). In terrestrial plants, the role of host-microbe interactions in shaping the evolution of the plant immune system is well established (Chisholm *et al.*, 2006), and certainly represented another important driver of diversification in macroalgae.

3.2.4. Process of ecological speciation

Individuals of a certain species will disperse stochastically and successfully or not colonize new habitats. If habitats are colonized with comparable conditions, individuals will not experience divergent selection and will therefore normally survive without the need to adapt. On the other hand, in habitats with contrasting biotic and abiotic conditions, in order to survive, individuals will have to adapt phenotypically. Evidently, biotic and abiotic features act in concert as selective pressures. It is the reduction and finally the absence of gene flow between two populations experiencing contrasting divergent selection that will eventually allow reproductive isolation and subsequently speciation. A schematic representation of ecological speciation process is given in Fig. 5.1.1.

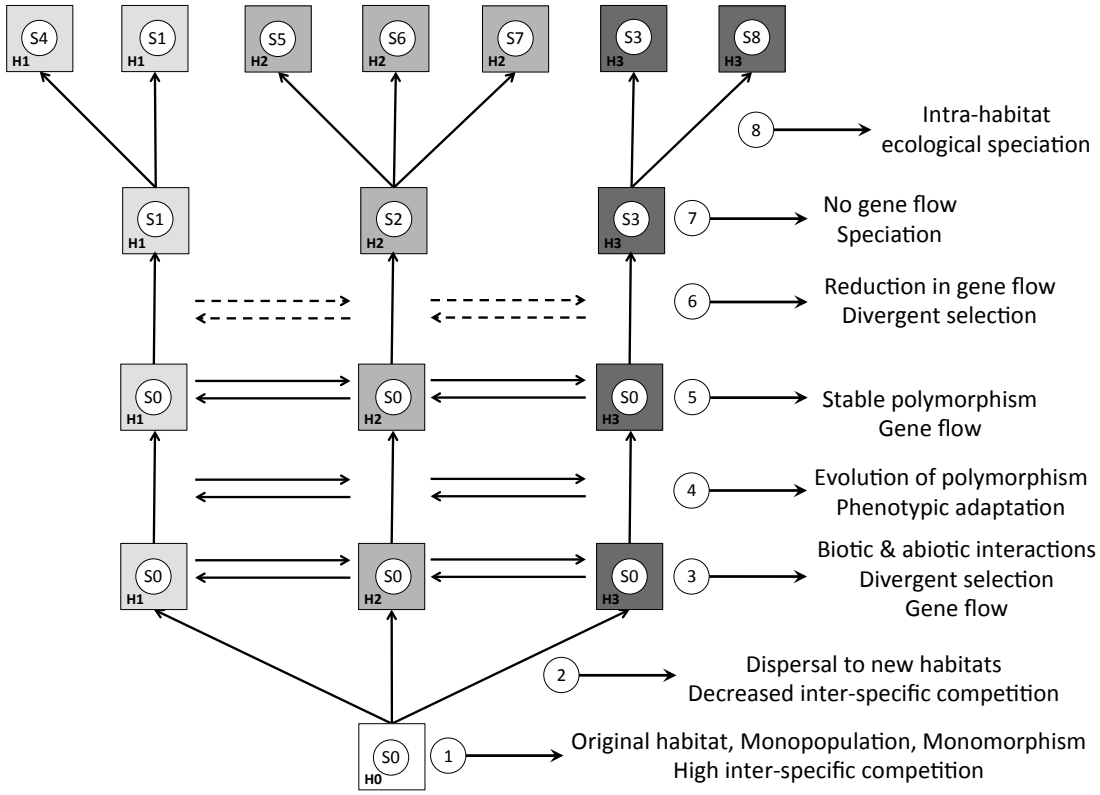

Figure 5.1.1. Schematic representation of ecological speciation as a result of environmental differences between habitats, displaying the different step from dispersal to speciation. The habitats are represented by squares labeled 'Hx', and the species by the circles labeled 'Sx'.

4. Phenotypic adaptations of *Lobophora*

Reciprocal transplant experiments are classically used to study local adaptation of divergent forms (e.g. Waser & Price, 1985). De Ruyter van Steveninck *et al.* (1988a) implemented a reciprocal transplant experiment with two distinct populations of *Lobophora* with divergent morphologies, and noticed that while differences between growth forms decreased after transplantation, significant differences still persisted between these two different populations. Their transplant experiments showed that traits enhancing fitness in one environment reduce it in the other, implying divergent selection between the two environments. Vieira *et al.* (2014b) confirmed that while species morphology may vary to some extent, *Lobophora* predominantly adopts a specific growth form, which reflects the adaptation of *Lobophora* species to specific habitats (Fig. 5.1.2). For instance, *L. hederacea* commonly presents a decumbent morphology, but in some cases it adopts a crustose morphotype (Vieira *et al.*, 2014b; Vieira *et al.*, 2015). The experiment of Jompa and McCook (2002b) showed that herbivore exclusion resulted in the change of the growth of a *Lobophora* species from creeping to foliose. We suspect that the *Lobophora* species associated to *Porites cylindrica* studied by Jompa and McCook (2002b) is none other than *L. hederacea* which is also found growing on the same coral species, and which can be aggressive towards other coral species (Vieira *et al.*, 2015). It appears that herbivory is maintaining *L. hederacea* crustose form. In habitats where grazing intensity is low, *L. hederacea* adopts a shelf-like form. Similarly the crustose species *L. crassa* occasionally adopts decumbent forms, particularly when it is found in deeper habitats hidden from herbivores (personal observation). In this latter case, it is the intense hydrodynamism in the shallow wave-washed habitats that caused the adoption of the crustose growth form. In conclusion, *Lobophora* morphotypes are closely related to their habitats. Crustose species generally grow in shallow and wave-washed waters on bedrocks or coral rubbles or in habitats with intense grazing. Decumbent species e.g. *L. monticola, L. hederacea, L. undulata* commonly grow among branching corals. It is probably the partial protection provided by the coral branches that allows the adoption of shelf-like forms and not crustose forms. Stipitate species like *L. sonderii* grow in habitats where hydrodynamism and herbivory are not intense. We may also point out that a correlation exists between anatomical traits and morphology (Vieira *et al.*, 2014b) (Fig. 5.1.2). Basically, crustose species have the thickest thalli and the prostrate species have the thinnest

thalli. Shelf-like species have rather thick thalli and stipitate species have intermediate thickness thalli.

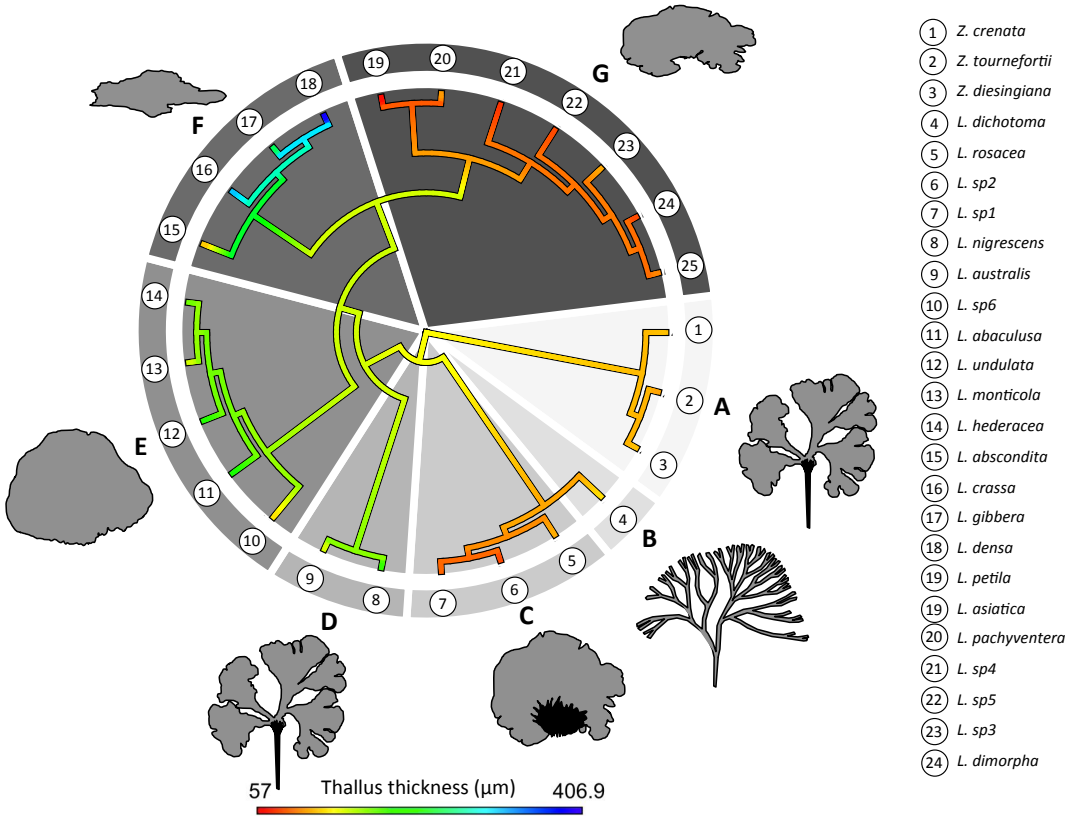

Figure 5.1.2. Comparison between *Lobophora* molecular phylogeny, morphological traits and ecological habits. The external morphology is represented by the pie chart with each slice representing a grouping of species sharing the same morphology; clockwise from the *Zonaria* species: stipitate, dichotomate, shelf-like, stipitate, shelf-like, crustose and prostrate. The ecological habitat is represented by the donut chart with each slices representing a grouping of species sharing the same ecology; A: algae beds, B: low reef, C: coral-dominated habitats, D: algae beds, E: coral-dominated habitats, F: shallow wave-washed habitats, G: coral dominated habitats. The continuous anatomical trait thallus thickness is represented within the phylogenetic tree. Thallus thickness values have been reconstructed along the ancestral branches using the function contMap form the R package phytools (Revell, 2013), which uses a Maximum Likelihood method to estimate the states at internal nodes and Felsenstein (1985) equation to interpolate the states along each edge.

5. Morphological evolution: down to the ground

Recently, Sun *et al.* (2012) and Vieira *et al.* (2014b) deduced that the ancestral growth form of *Lobophora* was *Zonaria*-like, i.e. stipitate, with thalli organized in dense erect blades, with fronds composed of several lobes. *L. sonderii* probably is the extant species resembling a *Lobophora* ancestor the most. While the majority of the

extant species have lost the ancestral stipe, we observe several species across the tree still presenting a stipe e.g. *L. sonderii*, *L. dichotoma*, *L. dimorpha*. Remnants of the ancestral stipe is observed in *L. rosacea* in the characteristic basal mounds of hairs by which it attaches itself to hard substratum. The growth form of *Lobophora* progressively evolved towards a crustose form firmly attached to the substrate by ventral rhizoids (Fig. 5.1.3).

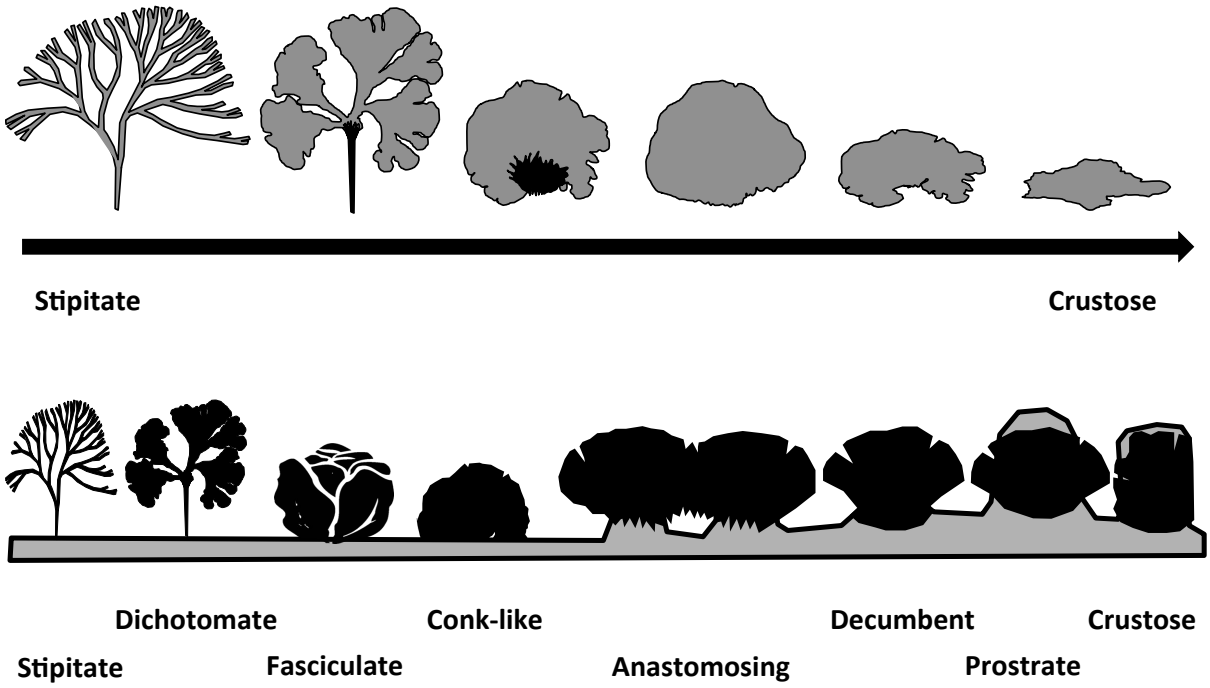

Figure 5.1.3. Schematic representation of *Lobophora* morphological and growth-form evolutions.

6. Evolutionary scenario

We propose a plausible evolutionary scenario of the genus *Lobophora* based on ecological, morphological evidences and the phylogenetic tree of *Lobophora*.

6.1. *Lobophora* ancestor

The *Lobophora* ancestor (*L*A) may have possessed a *Zonaria*-like morphology, characterized by thalli organized in dense erect blades, with fronds composed of several lobes, stipitate, anchored on hard substratum by an obvious holdfast made of slender fibers, which retain sand particles and present a distinct dark orange to dark brown/black color (Vieira *et al.*, 2014b). Given the common habitat of *Zonaria* species and of the *Zonaria*-like *Lobophora* species, *L. sonderii*, *L*A was most likely

epilithic mixed among other stipitate and foliose Dictyotales (e.g. *Stypopodium* and *Padina*) forming understory in dense beds of larger Fucales (e.g. *Sargassum*) protected from herbivore grazing.

6.2. Dispersal to new habitats

From this original habitat, the hypothetical ancestor may have dispersed into a wide range of habitats, allopatrically or sympatrically, with high to low grazing pressure and settled the best they could on hard substratum. In those newly colonized habitats, environmental factors (e.g. hydrology, herbivory and diminution of the light) fostered preferential phenotypes. For those that settled in shallow and wave-washed waters, they adopted crustose morphotypes in response to the hydrology. For those that reached out coral-dominated habitats, they most likely settled under the branching corals canopy protected from herbivory. All species of *Lobophora* associated to living coral have developed shelf-like morphologies (e.g. decumbent, conk-like) instead of an erect and stipitate form probably in response to the shading of coral branches and herbivory, since the stipitate morphology would be penalizing since the algae would stick out of the coral branches and be easily accessible to herbivores. Shelf-like morphotypes were favored in this new habitat through phenotypic plasticity. Consequently, loss of the stipe and diminution in size occurred. *Lobophora* probably evolved chemosensory receptors allowing the systematic settlement within corals. Chemical evolution in some *Lobophora* species may have led to the capacity to impair their coral hosts (Vieira *et al.*, 2015). Association with living corals has occurred in several lineages belonging to different clades (Vieira *et al.*, 2014b). Individuals that colonized surrounding but contrasting habitats, morphologically adapted to their new habitats owing to the divergent selective pressures, and progressively formed divergent ecotypes. For individuals that dispersed into far-distant habitats, the geographic distance acted as a natural barrier in the gene flow between these divergent ecotypes, eventually resulting in reproductive isolation and to speciation. The progressive reduction in gene flow between sympatric ecotypes, eventually also led to reproductive isolation. In summary, *L*A has diverged into multiple ecologically different populations (i.e. ecotypes), as the habitats and its associated level of grazing favored either a shelf-like or a crustose form. Thus, parallel populations of shelf-like and crustose evolved independently by adaptation and divergent selection and eventually, the divergence led to reproductive isolation between the former ecotypes.

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Concluding remarks and future directions

This study confronts us with our limited knowledge of algal diversity. It remains to be determined by how much our knowledge of algal diversity will increase with the help of molecular taxonomy. Will the magnitude of algal diversity reach a comparable level to other mega-diverse groups such as fungi or even beetles? This study also highlights our limited knowledge of algal chemical ecology. While algae hold a plethora of secondary metabolites, we know still very little about their exact ecological functions. It reflects not only a major lack of interest towards phyco-chemical ecology, but also the inadequacy or insufficiency of the available experimental designs.

Lobophora comes out as a remarkable algal genus to investigate chemical ecology and evolutionary questions. The major contribution of this doctoral research work was the unveiling of the high taxonomical diversity of this genus. And correct taxonomical identification is essential to properly address ecological and evolutionary questions. Future studies could be targeted towards exploring the role of secondary metabolites as (1) chemical defenses against herbivores, competitors, epibionts, pathogens, and as (2) cues in the chemical communications with other organisms, to finally (3) determine what their exact role is in structuring marine communities.

Table des illustrations

Table des tableaux

Supplementary figures

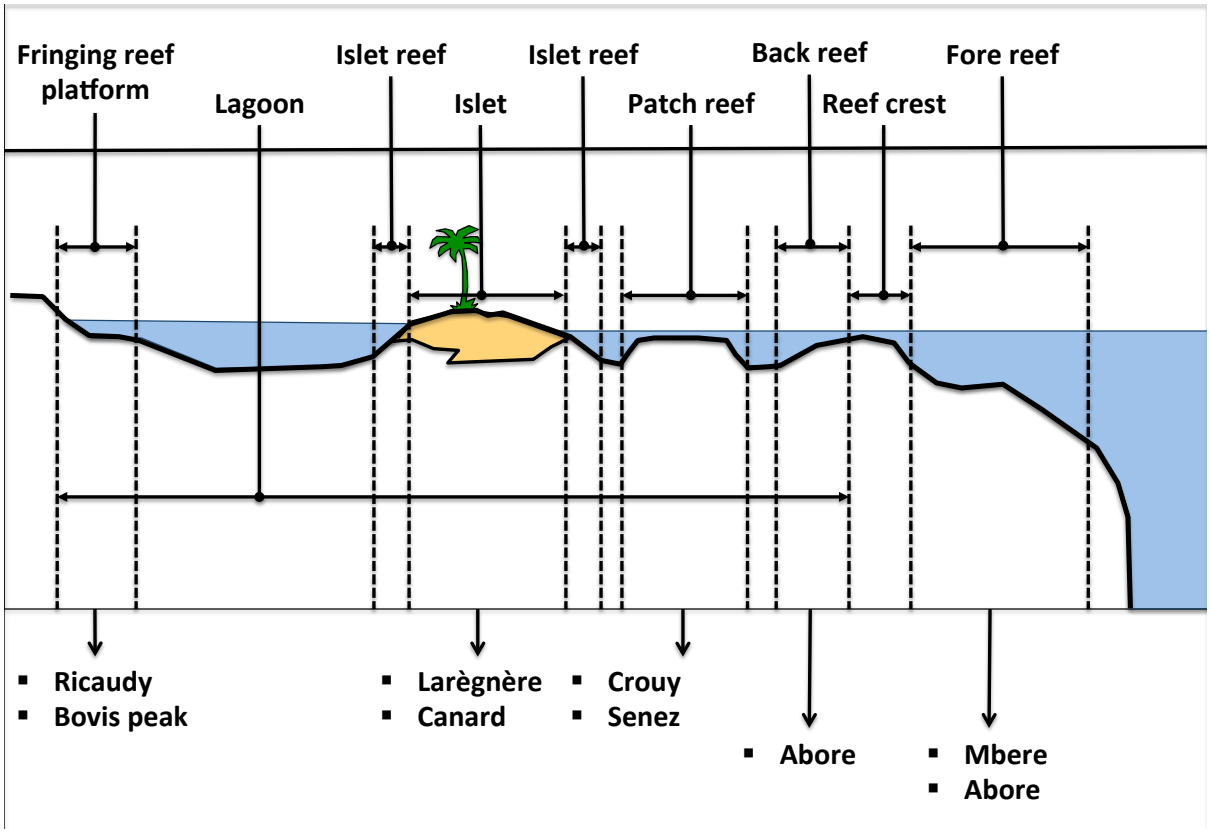

Figure S2.1.1. Schematic representation of the reef types encountered and investigated during the qualitative and quantitative surveys in the southwest lagoon of New Caledonia.

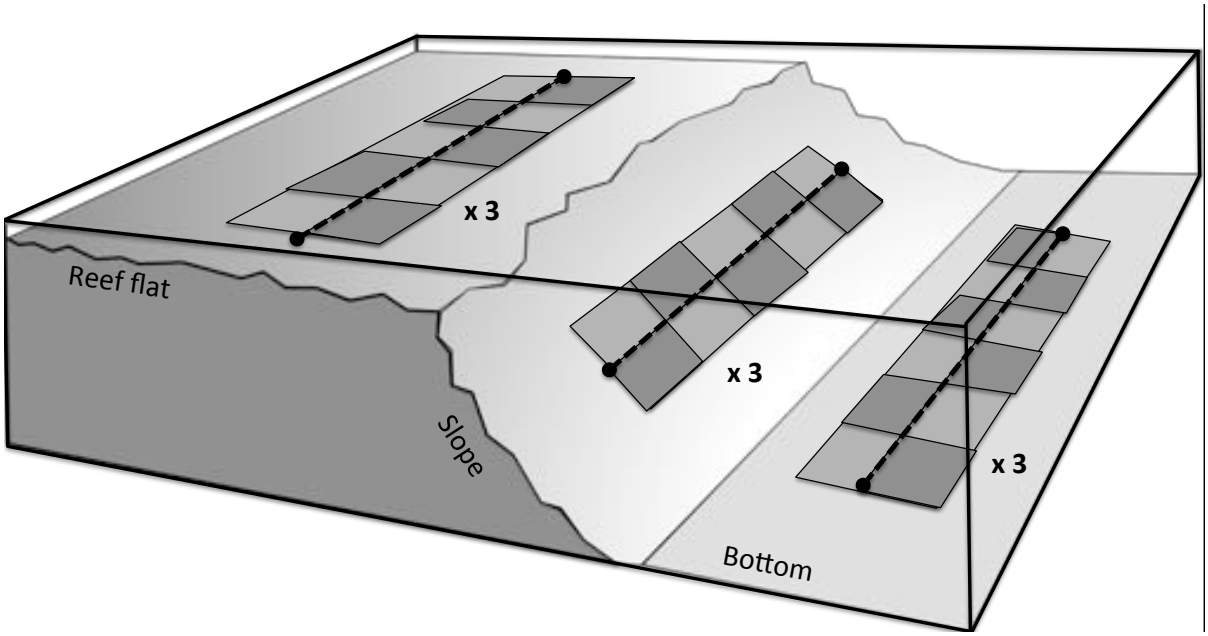

Figure S.2.1.2. Schematic representation of the reef zones and of the belt transects conducted to quantify MCI in habitats where MCI are conspicuous.

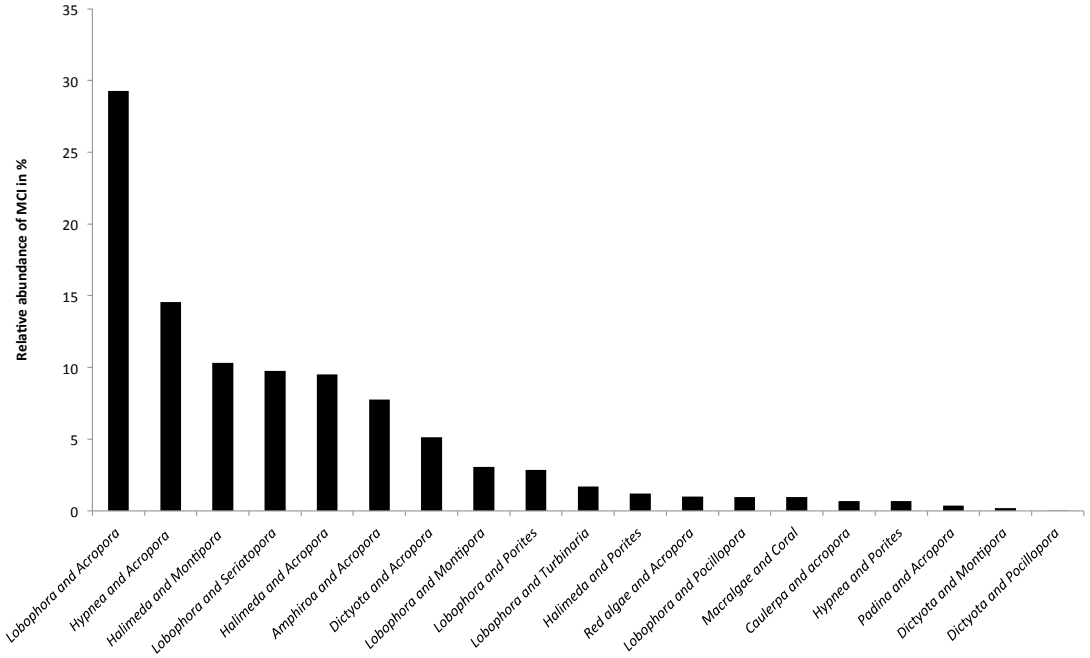

Figure S2.1.3. Relative abundance in percentage of the MCI encountered in the southwest lagoon of New Caledonia.

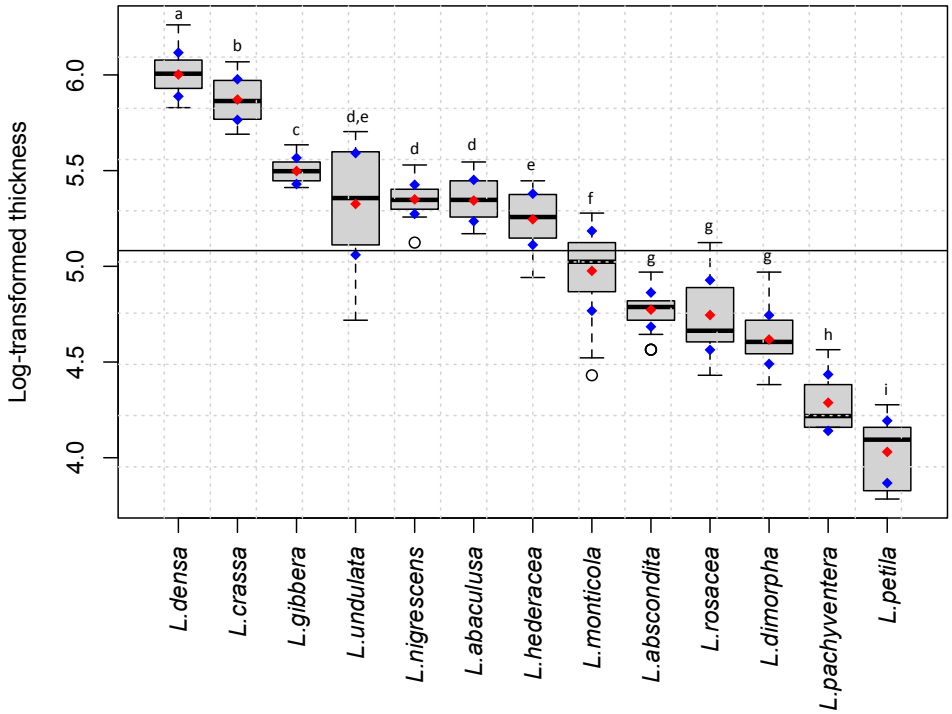

Figure S3.1.1. Boxplots representing the log-transformed thickness values of New Caledonian *Lobophora* species. One Way ANOVA; Df = 20, F value = 788 and p < 2.2e-16. A Tukey HSD post-hoc test revealed significance groups, represented by letters. Rectangles and whiskers bound 25-75 percentiles and the 10-90 percentiles respectively, horizontal lines show the median, circles are extreme values, red and blue points show the mean and standard deviation respectively.

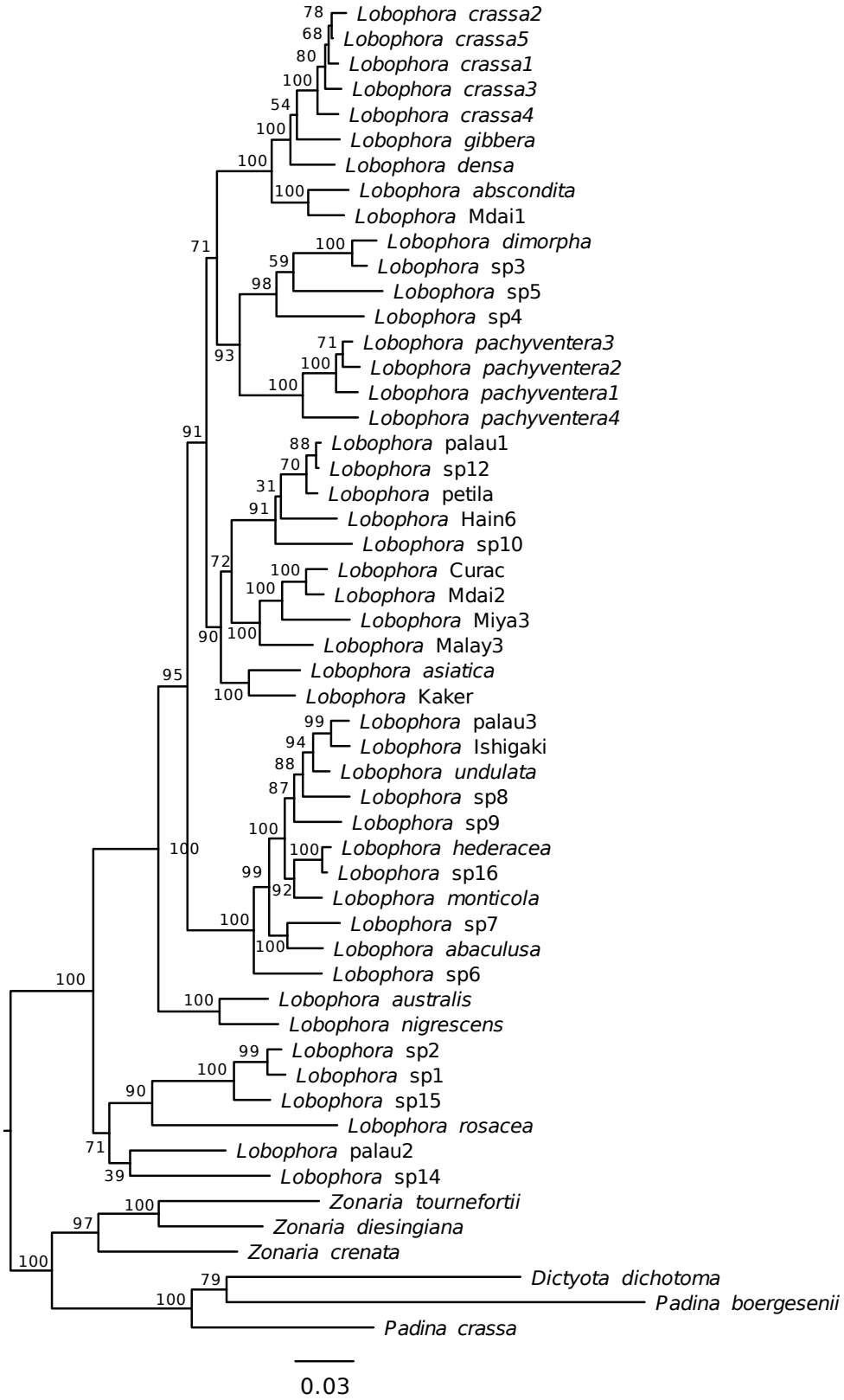

Figure S3.1.2. Maximum likelihood tree, generated with RAxML, based on the concatenation of *rbc*L, *cox*3, *psb*A and LSU sequences. The values shown at each node represent ML boostrap values.

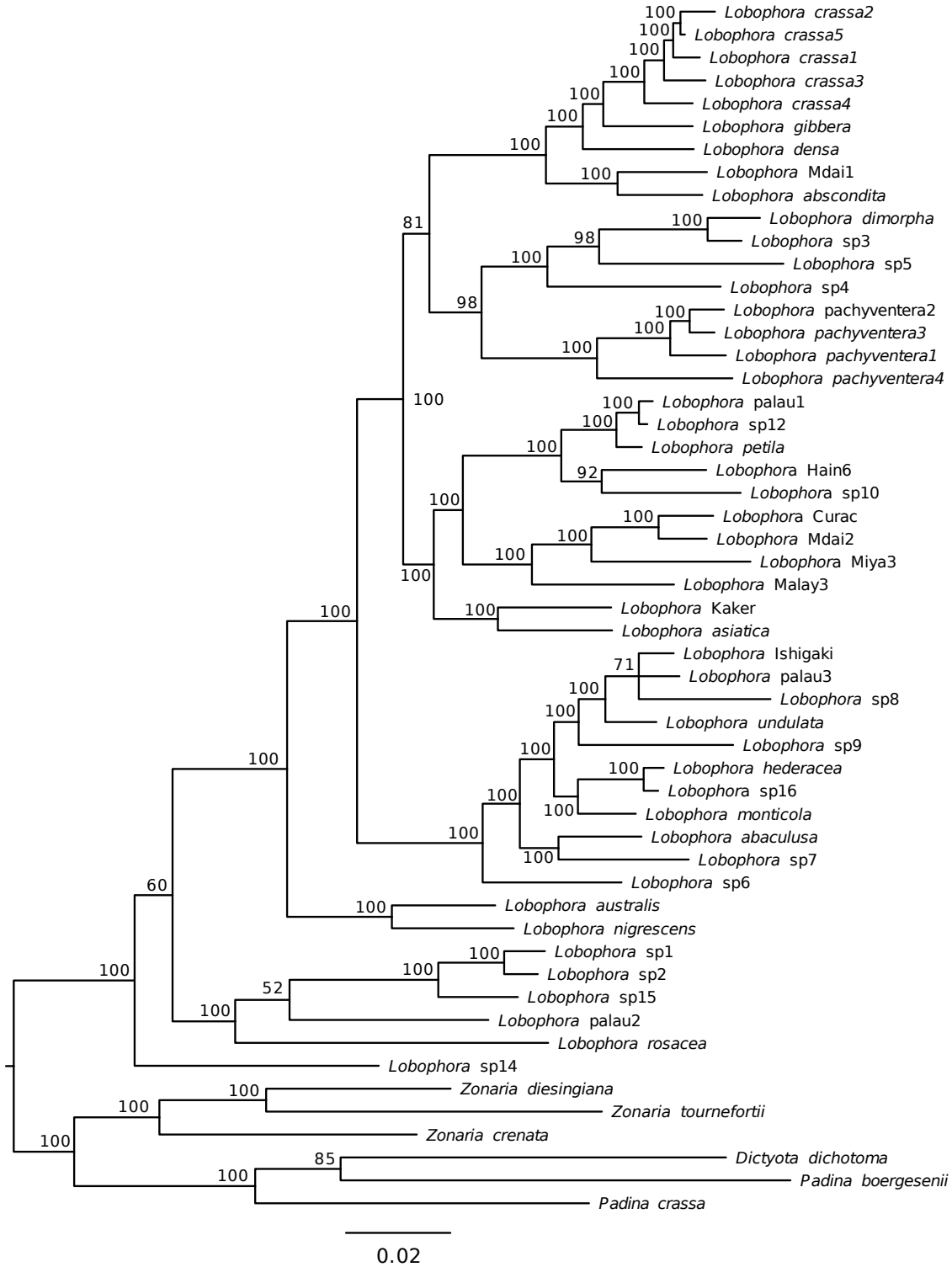

Figure S3.1.3. Bayesian tree, generated with MrBayes, based on the concatenation of *rbc*L, *cox*3, *psb*A and LSU sequences. The values shown at each node represent the posterior probability values.

Interactions biotiques et diversification du genre *Lobophora*

Résumé

L'algue brune *Lobophora* représente une composante benthique importante au sein des récifs coralliens tropicaux, et a à attirer dès le début des années 80 l'attention des écologistes marins en proliférant de façon remarquable au détriment des coraux. Les écologistes marins ne s'accordent toujours pas pour conclure si le changement de communauté au profit des macroalgues représente une conséquence ou une cause de la dégradation des coraux. Alors que *Lobophora* a fait l'objet d'observations contradictoires en termes de susceptibilité à l'herbivorie et des effets sur les coraux, les preuves suggèrent que sa prolifération dans les récifs coralliens est opportuniste et symptomatique de la dégradation des récifs. Taxonomiquement, *Lobophora* ne représentait que 11 espèces décrites au début de cette étude, et pratiquement toutes les espèces de *Lobophora* rapportées dans le monde avaient été assignées à *L. variegata*, décrite dans les Caraïbes. Cette étude vise à apporter un nouvel éclairage taxonomique et écologique sur ce taxon algal sujet de controverses écologiques. En utilisant une approche taxonomique basée sur l'ADN nous avons réévalué la diversité des espèces de ce genre en Nouvelle-Calédonie et au niveau mondial. Les résultats dévoilent une remarquable diversité, multipliant par 10 le nombre d'espèces jusque là reconnues. Nous avons testé si les différentes espèces de *Lobophora* étaient chimiquement différentes et si elles variaient (1) en toxicité envers différents coraux, et (2) en susceptibilité aux herbivores. Nous avons montré que le genre *Lobophora* était intrinsèquement capable de blanchir certains coraux, et nous avons isolé trois nouveaux alcools polyinsaturés C_{21} nommés lobophorenols A-C avec des propriétés de blanchissement. Néanmoins, les observations *in situ* en Nouvelle-Calédonie indiquent que, bien qu'elles soient potentiellement armées au plan chimique, les espèces de *Lobophora* n'induisent pas ou rarement le blanchissement de leurs hôtes coralliens, soulevant ainsi la question de l'emplacement de ces composants bioactifs dans l'algue et des facteurs environnementaux permettant leur libération supposée. Nous avons également montré que les herbivores consomment indistinctement les espèces de *Lobophora*. D'après ces résultats nous pouvons conclure que : (1) au sein de récifs en bonne santé, les coraux et les *Lobophora* maintiennent un *statu quo* résultant probablement d'une médiation chimique ; (2) la défense chimique ne dissuade apparemment pas le broutage des *Lobophora* par les principaux herbivores ; (3) il est probable que *Lobophora* échappe au broutage en se développant par exemple entre les branches des coraux. Le genre *Lobophora* représente un excellent modèle pour étudier le rôle de la spéciation écologique des algues au sein des récifs coralliens. Aussi, les études futures devront étudier le rôle des métabolites secondaires des *Lobophora* et enquêter sur les facteurs écologiques responsables de la diversification impressionnante de cette algue.

Mots clés : *Lobophora*, New Caledonia, biogeographie, diversification, herbivorie, interactions macroalgues-coraux, taxonomie, spéciation

Abstract

The brown alga *Lobophora* represents a notable benthic component in tropical coral reefs, and began drawing the attention of marine ecologists by achieving impressive blooms at the expense of corals since the early 80s. Marine ecologists are still debating whether or not macroalgal dominance represents a consequence or cause of coral degradation. While *Lobophora* has been the object of contradictory observations in terms of susceptibility to herbivory and effects on corals, evidence tends to suggest that episodes of reef take-over are opportunistic and symptomatic of reef degradation. From a taxonomic point of view, only 11 species of *Lobophora* were recognized at the beginning of this study, and virtually all species of *Lobophora* reported around the world had been assigned to *L. variegata*, originally described from the Caribbean. This study intends to shed new taxonomical and ecological insights on this algal taxon. Using a DNA-based taxonomical approach we reassessed the species diversity of this genus at a local scale in New Caledonia and subsequently on a global level. Results disclosed a remarkable global diversity, increasing our taxonomic knowledge of this genus by no less than 10 folds. From an ecological perspective, we tested if different species of *Lobophora* were differentially (1) capable of chemically damaging scleractinian corals, and (2) susceptible to herbivory. We showed that the genus *Lobophora* was inherently capable of bleaching certain coral species, and we isolated three new C_{21} polyunsaturated alcohols named lobophorenols A-C with bleaching properties. Nevertheless, *in situ* observations in New Caledonia indicated that although potentially chemically armed, *Lobophora* species did not or rarely bleached their coral hosts, thereby raising the issue of the location of these bioactive components and the environmental factors enabling their putative release by the alga. We also showed that herbivores indiscriminately consumed *Lobophora* species. From the results of allelopathic bioassays and grazing experiments we conclude that: (1) corals and *Lobophora* maintain a chemical-mediated *status quo* on healthy reefs; (2) chemical defense apparently does not deter grazing of *Lobophora* by prominent herbivores; (3) it is more likely that *Lobophora* avoids being grazed by escape strategies such as growing under the coral canopy. The genus *Lobophora* represents an excellent model to study the role of ecological speciation in macroalgae within coral reefs. Therefore, future studies should be targeted at investigating the role of *Lobophora* secondary metabolites and exploring the ecological factors responsible for the impressive diversification of this alga.

Keywords: *Lobophora*, New Caledonia, biogeography, diversification, herbivory, macroalgal-coral interaction, taxonomy, speciation, chemical ecology