



## Gross and microscopic pathology of hard and soft corals in New Caledonia



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### ABSTRACT

We surveyed the reefs of Grande Terre, New Caledonia, for coral diseases in 2010 and 2013. Lesions encountered in hard and soft corals were systematically described at the gross and microscopic level. We sampled paired and normal tissues from 101 and 65 colonies in 2010 and 2013, respectively, comprising 51 species of corals from 27 genera. Tissue loss was the most common gross lesion sampled (40%) followed by discoloration (28%), growth anomalies (13%), bleaching (10%), and flatworm infestation (1%). When grouped by gross lesions, the diversity of microscopic lesions as measured by Shannon–Wiener index was highest for tissue loss, followed by discoloration, bleaching, and growth anomaly. Our findings document an extension of the range of certain diseases such as *Porites* trematodiasis and endolithic hypermycosis (dark spots) to the Western Pacific as well as the presence of a putative cnidarian endosymbiont. We also expand the range of species infected by cell-associated microbial aggregates, and confirm the trend that these aggregates predominate in dominant genera of corals in the Indo-Pacific. This study highlights the importance of including histopathology as an integral component of baseline coral disease surveys, because a given gross lesion might be associated with multiple potential causative agents.

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### 1. Introduction

The Pacific Ocean harbors the vast majority of coral biodiversity in the world; however, these ecosystems face significant threats including global climate change (Hoegh-Guldberg et al., 2007; Pandolfi et al., 2011), land-based sources of pollution (Fabricius, 2005), overfishing (Jackson, 2008), and disease (Harvell et al., 2004). The latter has been responsible for declines of 80% of coral cover in the Caribbean over the past two decades (Gardner et al., 2003). Many causes of coral diseases are unknown, because relatively little attention is paid to examining coral tissues at the light microscope level, and most efforts focus on microbial cultures or molecular biology (Bourne et al., 2009; Work and Meteyer, 2014). This is unfortunate because microscopic examination of coral tissues can help visualize agents associated with lesions in corals as well as host response to these agents (Work et al., 2012). Understanding what is occurring at the tissue level can also

help in designing laboratory tests, and provides a morphological baseline for use in experimental trials to confirm causation of lesions (Work and Meteyer, 2014; Work et al., 2008c).

Diseases in wildlife are interplays between agent(s) (the cause of disease), hosts (animal affected), and the environment where they interact. Corals are a unique case in wildlife disease ecology, because the corals as main frame builders of tropical reef ecosystems are, essentially, the environment. In tropical oceans, coral reefs provide fundamental ecosystem services that underpin tropical marine diversity and generate socio-economic values including income from fisheries, tourism, ocean technologies, and coastal protection (Moberg, 1999). For example, in Hawaii alone, coral reefs are estimated to contribute ca. \$US 10 billion (2004 dollars) annually to the economy (Cesar and van Beukering, 2004). Degradation of corals has myriad adverse effects including loss of three dimensional structure of the substrate, associated fish, invertebrates, and other animals that depend on corals for food and shelter (Paulay, 1996). It is all the more critical, then, to obtain baseline information on potential causes of coral lesions in sensitive habitats before catastrophic disease outbreaks occur.

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One such habitat is the lagoon of New Caledonia, the largest in the world harboring one of the most diverse communities of marine fauna and flora (Roberts et al., 2002). About 60% of the Caledonian reefs were designated as a UNESCO World Heritage Site in 2008. Due to rapid urban and industrial development (the New Caledonian nickel industry is the 3rd largest one in the world), nearshore coral reefs in this region are exposed to anthropogenic inputs (Fernandez et al., 2006) and natural terrestrial run off (Fichez et al., 2010) that in concert with global climate change could adversely affect coral reef health. To get a better sense of coral reef health in the region, we did for the first time baseline coral disease surveys in 14 sites comprising seven inshore reefs, six barrier reefs, and one lagoonal patch reef surrounding the largest island of New Caledonia (Grande Terre) in summers of 2010 and 2013. Our objectives were to systematically describe coral lesions encountered during surveys in the region at the gross and microscopic level.

## 2. Methods

### 2.1. Sampling and gross pathology

We sampled coral colonies from 14 and 12 sites around New Caledonia in January–February 2010 and 2013, respectively (Fig. 1) according to the protocol by Work and Aeby (2006). For *in situ* characterization of gross lesions, entire colony and lesions were photographed and the following data were recorded: date, location (GPS coordinates), and depth of collection. Grossly, lesions were classified into three broad categories including tissue loss, discoloration, and growth anomaly. Tissue loss was subdivided as acute, subacute, subacute with band characterized by healthy

tissue separated from bare skeleton by a colored band, or chronic (Raymundo et al., 2008; Work and Aeby, 2006). Discoloration was categorized as bleaching (white discoloration subdivided as localized, multifocal, or diffuse), dark discoloration comprising variably sized distinct irregular dark brown areas, multifocal pink-to-pale discoloration comprising numerous 2–4 mm pink-to-pale spots, and other discoloration (all other categories). Growth anomalies were categorized as umbonate, exophytic, rugose, or nodular (Work et al., 2008a).

For histopathology, coral fragments (2–5 g) were collected with chisel or bone shears and placed into individually numbered whirlpak bags in seawater. Fragments with lesions were collected ensuring that the border between normal and lesion tissues was incorporated. When available, paired apparently normal fragments were also collected. Coral fragments were processed for histopathology as described (Work and Aeby, 2011). Briefly, fragments were fixed in Z-Fix (Anatech Ltd.) diluted 1:5 with seawater, decalcified in dilute formic acid/formaldehyde solution (Cal-Ex II, Fisher Scientific), tissues dehydrated in alcohol, embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin.

### 2.2. Histopathology and data analyses

Microscopic changes were interpreted in light of findings from paired normal fragments and broadly categorized by agent associated with cell pathology, if present, and host response. Agents were identified according to their microscopic morphology and included sponges or cnidaria (Hyman, 1940a), helminths (Hyman, 1940b), ciliates (Bourne et al., 2008), algae (McCook et al., 2001), fungi (Larone, 1976), cyanobacteria (Stanier and Cohen-Bazire, 1977), and molluscs or crustacea (Ruppert et al.,



**Fig. 1.** Collection sites for coral lesions in 2010 and 2013 around the largest island of New Caledonia, Grande Terre. 1 – Îlot Casy (inshore fringing reef), 2 – Gué reef (barrier reef), 3 – Baie des Citrons (inshore fringing reef), 4 – Sèche Croissant reef (lagoonal patch reef), 5 – Mbere reef (barrier reef), 6 – Banc des Japonais\* (inshore reef), 7 – Chenal de Teremba (inshore reef), 8 – Passe de Ouaraï (barrier reef), 9 – Kreliat reef (inshore reef), 10 – Passe de Koné (barrier reef), 11 – Bouerabate reef (inshore reef), 12 – Passe de la Gazelle (barrier reef), 13 – Neongaon reef\* (inshore reef), 14 – Balade reef (barrier reef in front of the Col d'Amos). Sites with asterisks (\*) were surveyed in 2010 only.

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