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CORAL DISEASE ECOLOGY AND PATHOLOGY IN THE MALDIVIAN ARCHIPELAGO: CHARACTERIZATION OF GROWTH ANOMALIES AND BROWN BAND DISEASE

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ABSTRACT

Coral diseases, although historically overlooked, are increasingly recognized as critical drivers of reef decline; however, their ecology and pathology remain insufficiently investigated, particularly in biodiversity hotspots such as the Maldives. This thesis presents an integrated, multi-scale investigation of coral disease ecology, morphology, and pathology in the Maldives, focusing on two understudied lesions: coral growth anomalies (GAs) and brown band disease (BrB).

By combining ecological observations with detailed morphological, skeletal, and histopathological analyses, this research provides a comprehensive investigation of GAs' pathologic features within a region of high ecological importance.

GAs, found with widespread distribution across the Maldives, affected multiple genera, including *Acropora*, *Montipora*, and *Pachyseris*. Analyzed GA lesions were consistently associated with severe alterations in both skeletal and tissue architecture. Coupled microscopic and gross morphology analysis with scanning electron microscopy (SEM), showed that GAs are primary characterized by hyperproliferation of basal body wall tissues, strictly associated with excessive extension of the coenosteum. Affected skeleton exhibited increased porosity, fragile and haphazard structures, and a higher incidence of skeletal defects, consistent with altered growth and possibly calcification processes. Histopathological analyses confirmed the complex and multifactorial nature of the disease, with no clear or consistent etiological agent across samples or genera. However, in Maldivian corals, GAs were uniquely associated to diverse microbial assemblages not observed in GA lesions elsewhere, suggesting regional ecological drivers despite consistent core pathological features. Concurrently, this study provides the first histopathological characterization of GAs in *Pachyseris* and identified a possible genus-specific pathological process.

In parallel, this research delivers the first comprehensive histopathological characterization of BrB. Gross lesion assessment, genetic analyses, and microscopic observations consistently identified the ciliate *Philaster guamense* in association with active tissue loss in *Acropora* cf. *muricata* from the Maldives, supporting its identification as the primary pathogen. Histopathology revealed ingestion of host cellular material by the ciliate and an absence of pathological features indicative of alternative microbial agents, underscoring the diagnostic value of histological analysis. This research aims to highlight the limitations and inaccuracies of relying solely on gross visual assessment for coral disease identification, emphasizing the essential role of histopathology in building solid case definitions, especially in corals with lesions displaying overlapping external features.

This thesis also presented a long-term monitoring study of coral disease prevalence at Thudufushi Island over a twelve-year period (2010–2022), which revealed an overall increase in disease prevalence, a trend consistent with global observations. The temporal analysis confirmed that the Maldives is not exempt from the global intensification of coral disease events under climate change scenario.

Collectively, this thesis emphasizes the urgent need for standardized, pathology-informed frameworks for coral disease classification, monitoring, and management. This work contributes to establishing a baseline for understanding coral disease dynamics in the Maldives and offers insights that may support reef conservation and mitigation strategies. As mitigation efforts expand, there is both a research imperative and an ecological necessity for comprehensive and accurate disease risk assessment and health screening prior to any intervention. Ultimately, enhancing the ability to diagnose, characterize, and manage coral diseases is crucial for developing evidence-based conservation strategies and ensuring reef resilience in a rapidly changing ocean.

CHAPTER 1

INTRODUCTION

1.1 CORAL REEF ECOSYSTEMS

Tropical coral reefs are among the most diverse and productive ecosystems on Earth, often referred to as the "rainforests of the sea." They are primarily formed by reef-building corals, colonial organisms belonging to the phylum Cnidaria. Each polyp, the basic structural and functional unit of the coral, secretes calcium carbonate ($CaCO_3$) forming a rigid exoskeleton that contributes to the complex three-dimensional structure of the reef (Graham & Nash, 2012; McLaughlin et al., 2023). This structural complexity plays a fundamental role, among other functions, in providing habitat for the richest biodiversity on Earth. Indeed, despite covering less than 0.1% of the ocean floor, coral reefs support nearly 25% of all known marine species (Plaisance et al., 2011; Fisher et al., 2015), which lives in and around and which survival depends upon it. They provide critical habitat, food, and nursery grounds for numerous fish and invertebrate species, supporting complex food webs and promoting high productivity (Hoegh-Guldberg et al., 2017). Reefs also influence global biogeochemical cycles contributing to nutrient cycling, water filtration, and carbon sequestration (Zhang et al., 2022).

Besides the crucial ecological value of coral reefs for marine life, coral reefs deliver substantial ecosystem services to humankind (Stoeckl et al., 2011; Giglio et al., 2023). They provide coastal protection acting as natural barriers of coasts from erosion, storm events, and flooding. Reef-based fisheries and tourism industries provide employment, income, and food security to millions of people worldwide. According to global estimates, the economic value of coral reef services amounts to billions of dollars annually (Fezzi et al., 2023; United States Environmental Protection Agency, 2025).

Beyond their material benefits, coral reefs hold cultural, spiritual, and educational significance for many coastal communities (Woodhead et al., 2019). Coral reefs are distributed mainly in tropical and subtropical regions, typically between latitudes 30°N and 30°S, where environmental conditions such as temperature, light, and salinity favor coral growth. Scleractinian corals, the

dominant order of tropical reefs, develop optimally in shallow, clear waters with temperatures ranging from 23°C to 29°C, mainly due to their obligate symbiosis with unicellular algae of the family Symbiodiniacea, which provide the majority of coral species most of its nutritional need throughout photosynthesis. Because of the functional limitations of the photosynthetic apparatus of the dinoflagellate, the abundance of zooxanthellatae scleractinian corals generally decreases with depth beyond 20-40 m, depending on the turbidity of the water column (Kirk et al., 2016; Liao et al., 2019).

The reefs can be classified on the base of the morphology and their geological formation in three main types as fringing reefs, barrier reefs, and atolls. Fringing reefs, the most common one, grow directly along coastline, while the barrier reefs are separated from the shore by a lagoon. Instead, atolls are ring-shaped coral reefs that encircle a central lagoon after the subsidence of a volcanic island (Stoddart, 1969).

The majority of global coral reefs are found in shallow and warm waters of the Indo-Pacific region, particularly within the so called "Coral Triangle", which comprises Indonesia, Malaysia, Papua New Guinea, the Philippines, the Solomon Islands, and Timor-Leste. However, significant reef systems also occur in the Caribbean, the Red Sea, and the western Indian Ocean, such as the Maldivian archipelago (Spalding et al., 2001).

1.1.1 THREATS TO CORAL REEFS ECOSYSTEMS

In the last decades, the resilience of coral reef ecosystems has been increasingly threatened by multiple anthropogenic stressors, ranging from global drivers such as climate change to local disturbances including overfishing, sedimentation, pollution, destructive coastal development and recurrent coral disease outbreaks (Riegl et al., 2009; Burke et al., 2023; Goldberg & Wilkinson, 2023). Factors that result in overall significant decline in coral cover and coral resilience (Souter et al., 2021). Globally, climate change impacts the oceanic system primarily by raising water temperatures and increasing acidification, which together represent some of the most serious threats to coral survival.

The growing concentration of carbon dioxide (CO_2) in the atmosphere, largely due to human activities, leads to greater CO_2 absorption by the ocean, forming carbonic acid and thereby lowering seawater pH. As pH decreases, the availability of carbonate ions, essential for the formation of calcium carbonate skeletons, also declines, resulting in slower reef growth and structurally weaker corals (Armstrong & Bahr 2025). At the same time, the ocean also absorbs

excess atmospheric heat, causing a rise in sea surface temperatures. Elevated temperatures trigger coral bleaching, during which corals expel their symbiotic algae (endosymbionts), losing both coloration and a crucial source of energy (Helgoe et al., 2024). This compromised physiological state can ultimately lead to coral mortality or significantly increase susceptibility to disease. Since the first documented bleaching events, episodes of prolonged heat stress have become increasingly frequent and intense, leading to mass coral mortalities worldwide, including the most recent event in 2025 (Mellin et al., 2025; Mies et al., 2025). Furthermore, disease outbreaks, often linked to environmental stressors, have become more frequent and severe, compounding the stress on already vulnerable coral populations (Vega et al., 2025).

As global stressors intensify, local human pressures continue to escalate. Overfishing, coastal pollution from multiple sources, and expanding coastal infrastructure all severely undermine coral resilience (Hughes et al., 2003). Together, these interconnected factors create a cascading effect, amplifying each other's impacts and making it increasingly difficult for coral reefs to recover, thrive, and sustain marine biodiversity relying upon the ecosystem.

1.1.2 MALDIVIAN CORAL REEFS

The Republic of Maldives, an island nation situated in the central Indian Ocean, consists of 26 natural atolls (21 administrative ones) stretching across 823 kilometers from north to south and 130 km east to west (Gischler et al., 2013).The Archipelago consists of low- lying 1190 islands, of which only 198 inhabited, with average elevation of just 1.5 meters above sea level. The country's unique geography is almost entirely defined by its marine spaces (99%), with less than 1% of its total area comprising land (**Fig.1**).

Coral reefs are the core of marine life in the Maldives, covering a total area of 21,370 km^2. These reefs form one of the largest and most biologically diverse reef complexes in the world, providing essential ecosystem services, sustaining high biodiversity, and are deeply intertwined with the lives and culture of local communities, as the small, low-lying and isolated nature of the islands, makes inhabitants highly dependent on reef ecosystems (Galli et al., 2021; Hilmi et al., 2023). The reef ecosystem also underpins the nation's tourism industry, which constitutes a major component of the Maldivian economy. However, as experienced by all other reef systems, increasing anthropogenic pressures, including coastal development, overfishing, and climate-induced coral bleaching and disease outbreaks, have raised significant concerns regarding the long-term health and sustainability of these ecosystems (Dhunya et al., 2017; Perry & Morgan, 2017; Saliu et al., 2019; Pancrazi et al., 2025).

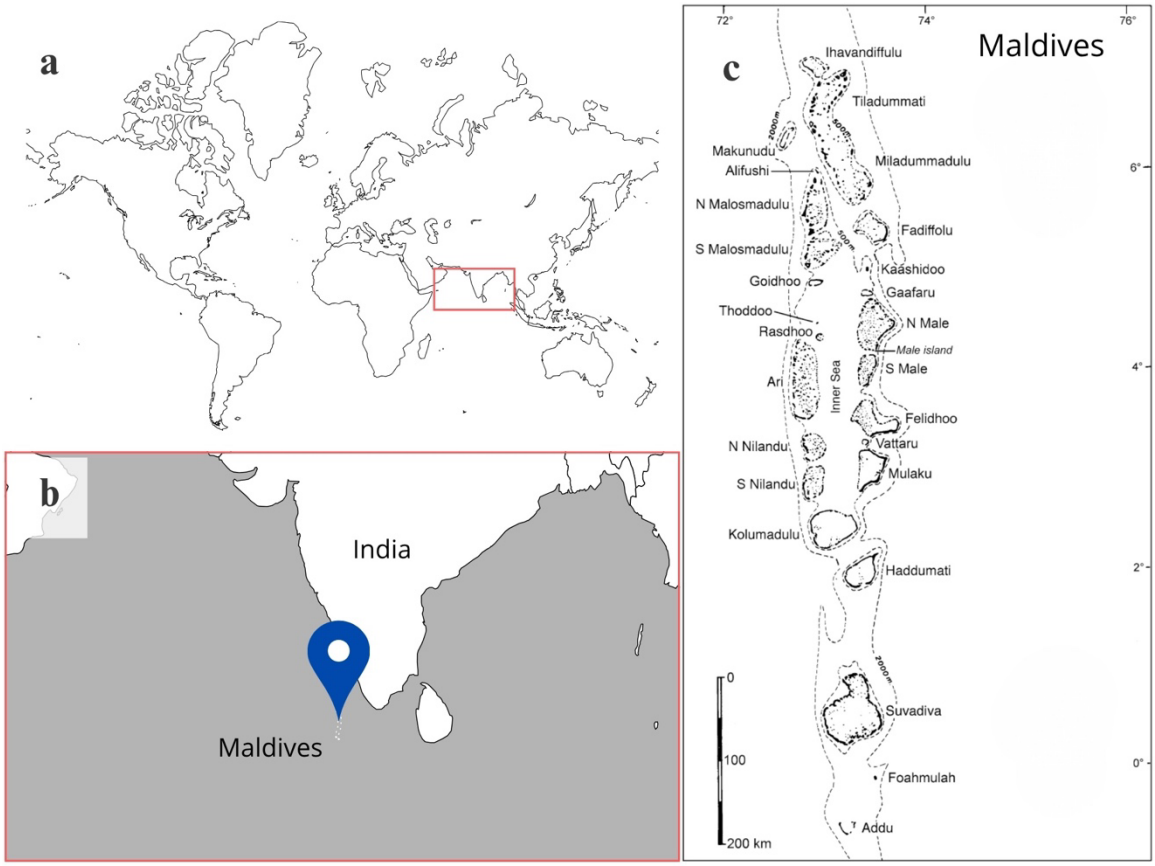

Figure 1. World map and location of the Republic of Maldives **(a**,**b)**. **c)** Overview map of Maldivian atolls. modified from Gischler et al. (2013).

Compared to many continental shelf-associated reef systems, Maldivian coral reefs exhibit a distinct ecological configuration shaped by their atoll-dominated, open-ocean setting and strong monsoon-driven hydrodynamics (Kench et al., 2006; Naylor, 2015; Su et al., 2021). While features such as atolls, channelized flow, and thermal fluctuations are present in other Indo-Pacific reef systems, in the Maldives these processes occur at high intensity and across the tightly connected archipelago. The interaction between seasonal currents and numerous reef channels (*kandu* in Divehi) generates distinct fine-scale heterogeneity in environmental conditions and biodiversity among outer reef slopes, channel reefs, and lagoonal habitats, promoting spatially structured coral and fish assemblages (Dryden et al., 2020). High larval connectivity among atolls supports regional biodiversity and recovery potential, while exposure to localized thermal stress may favor resilient coral taxa, thereby supporting functional diversity and ecosystem resilience. Together, this combination of strong connectivity, habitat heterogeneity, and environmental variability

distinguishes Maldivian reefs from many other reef systems and underpins their high biodiversity and ecological relevance in the global coral reef context.

Similar to the challenges faced by other low-lying island nations, such as Vanuatu, the Maldives' small, low-elevation islands are extremely vulnerable to the impacts of climate change. The combination of rising sea levels, increased frequency of extreme weather events, and coastal erosion, poses severe threats not only to the physical integrity and resilience of these islands but also to the livelihoods and long-term survival of islands communities (Magnan et al., 2022; Schmidt & Malatesta, 2022). To a limited extent, land reclamation is increasingly adopted in small island nations as a strategy to address the impacts of sea level rise, although it is primarily driven by the need to sustain population growth and support tourism infrastructures (Duvat, 2020). However, the extensive implementation of such measure rises serious environmental concerns, including the disruption of marine ecosystems, sedimentation impacts on surrounding reefs, and potential long-term disastrous ecological consequences on reef systems (Riyaz & Ali, 2004; Zubair et al., 2011). This increased vulnerability underscores the critical interdependence between ecological stability and socio-economic wellbeing in small island states, where environmental changes can rapidly and largely translate into profound impacts on the communities.

1.2 FUNDAMENTALS OF SCLERACTINIAN BIOLOGY

1.2.1 PHYSIOLOGY AND GROSS ANATOMY

Scleractinians are members of the Phylum Cnidaria, class Anthozoa, subclass Hexacorallia. They are usually characterized by a modular body structure composed of thousands of individuals repeated units, with exception of solitary corals, known as polyps. The entire colony is supported by a structure made of aragonite (calcium carbonate), which is secreted by the coral and constitutes a hard exoskeleton. Each polyp is a cylindrical, soft-bodied and sac-like organism possessing only one oral opening, functioning as both mouth and anus, which leads to a body cavity known as gastrovascular cavity. The oral opening is surrounded by a circular arrangement of tentacles, which are equipped with specialized cells, cnidocytes (nematocysts or spirocysts), condensed in areas called acrospheres and are devoted to prey capture and defense. Polyps occupy individual skeletal depressions known as corallites, into which they can fully retract in response to disturbance or environmental stress (Avian & Ramšak, 2021; Berzins et al., 2021). Reef- building coral species employ both heterotrophy and autotrophy to meet their metabolic needs. The autotrophic input (up to 90% of coral nutrition needs) comes from the activity of the symbiotic single-cell dinoflagellates of the family Symbiodiniacea, hosted in cytoplasmic vacuoles inside coral gastrodermal cells. The endosymbionts supply the host with organic carbon compounds produced through photosynthesis, which support energy-demanding processes such as skeletal deposition and contribute to the coral's gross coloration. In return, the coral provides the dinoflagellate with nitrogenous waste and carbon dioxide necessary for their photosynthetic activity (Davy et al., 2012; LaJeunesse et al., 2018).

The polyps of a colony are interconnected by an overlying sheet of living tissue termed the coenenchyme, which extends across the surface of the skeletal structure. This tissue continuity and network enable the exchange of nutrients, metabolites, and signaling molecules among polyps, effectively integrating them into a single functional unit despite their modular organization (Li et al., 2023).

Scleractinian corals reproduce sexually by producing gametes that develop into planktonic larvae. Most species are broadcast spawners, releasing eggs and sperm in synchronized mass-spawning events, while others are brooders that release fully developed planulae (Baird et al., 2009; Harrison, 2010). After dispersal, larvae settle, metamorphose into primary polyps, and initiate new colonies, promoting genetic diversity and population connectivity.

At the colony level, scleractinians exhibit a considerable diversity of macromorphological forms, including branching, massive, laminar, and encrusting. These forms are species-specific depending upon growth modes and environmental influences such as hydrodynamics and light availability (Pratchett, 2015). Regardless of colony morphology, the fundamental gross anatomical organization remains consistent throughout species.

1.2.2 SKELETAL MORPHOLOGY

The skeleton of scleractinian corals forms a complex, hierarchically organized aragonitic framework composed of both macrostructural and microstructural elements that together provide the mechanical stability, growth pattern, and overall colony architecture. At the macroscopic level (**Fig.2**), each polyp occupies a skeletal cup termed the corallite, which consists of several distinct components, including the theca (outer wall), the calyx (the inner cup-shaped depression housing the polyp), the columella (central axial structure), and radially arranged septa that project inward from the thecal wall (Stolarski 2003; Zhao et al. 2021). Externally, adjacent corallites are connected by the coenosteum, a shared skeletal structure that provides support and integration of modular units within the colony. Vertical digitate protrusions covering coenostial areas are called spinules and have different ornamentations.

Moreover, this skeletal network architecture creates intra- trabecular spaces that are often colonized by endolithic organisms (i.e. algae, fungi, sponges) with uncertain role in coral ecology to date (Pernice et al., 2019). Each element presence or morphological features are species- specific (Peters, 2015; Berzins et al., 2021). Moreover, the arrangement of corallites, whether plocoid, cerioid, meandroid, or phaceloid, varies markedly among taxa and influences gross colony morphology such as branching, massive, foliaceous, or encrusting growth forms (Veron, 2000).

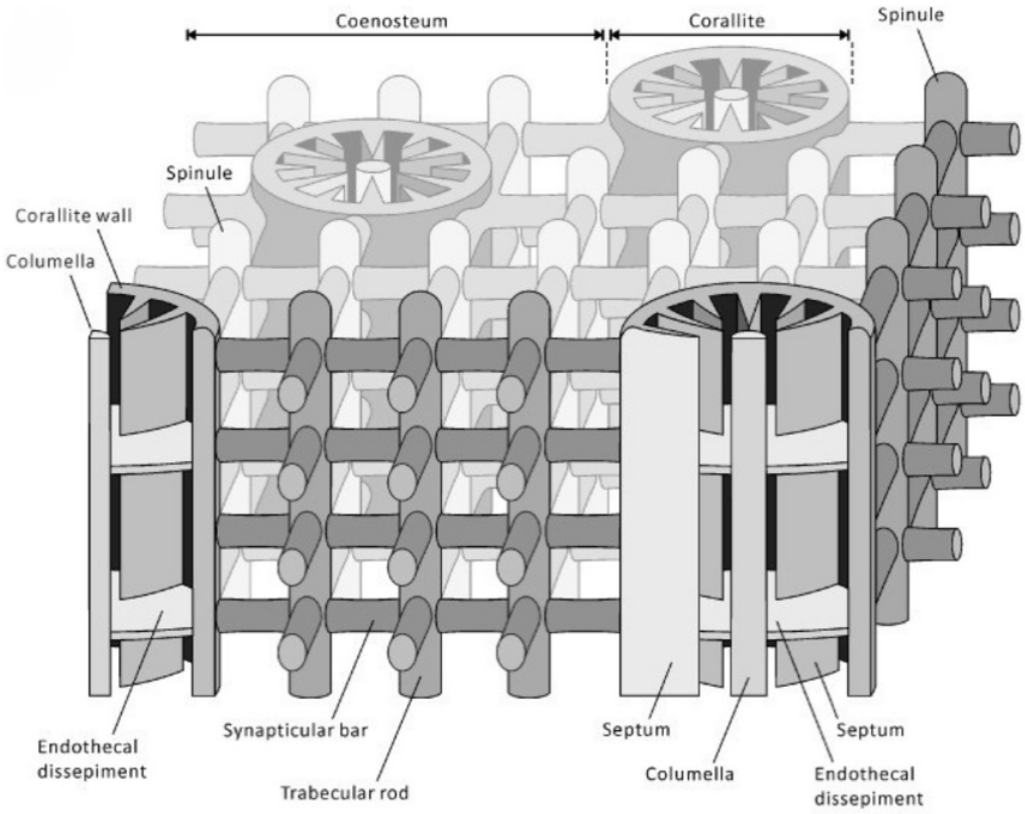

Figure 2. Graphical representation of main skeletal macrostructures (Modified from Humblet et al. 2014).

At the microstructural scale, the skeleton is composed of an intricate network of aragonite fibers, spherulites, and center of calcification (COC) zones. Early mineral deposition begins at discrete COCs, from which bundles of needle-like aragonite fibers radiate outward, progressively forming the fibrous framework that constitutes septa, walls, and coenosteum (Stolarski, 2003). These fibers are embedded within and guided by an organic matrix composed of acidic proteins, polysaccharides, and lipids that regulate nucleation, crystal orientation, and mineral accretion (Mass et al., 2017). The resulting microarchitecture often displays distinct growth bands, skeletal increments, formed in response to diel or seasonal cycles in physiology and environmental conditions (Cohen & McConnaughey, 2003). Additionally, an intrinsic material property of the skeleton is its variable porosity, including micro- and macro-pores that facilitate internal water movement and influence mechanical properties such as stiffness and resistance to breakage (Enríquez et al., 2017; Tambutté et al., 2015).

On longer temporal scales, the vertical and lateral accretion by repetition of skeletal elements leads to the progressive expansion of the reef framework and implementation of reef tridimensionality.

1.2.3 MICROSCOPIC ANATOMY: HISTOLOGY

Stony corals are structurally soft-bodied diploblastic metazoans characterized by two primary tissue layers, the outer and inner epithelial layers, derived from embryonic ectoderm and endoderm, known as epidermis/calicodermis and gastrodermis (Peters, 2015, Berzins et al. 2021). Separating the two epithelial surfaces is the connective layer known as the mesoglea.
Microscopically these epithelial layers are divided in two regions, the surface body wall which faces the external seawater environment, and the basal body wall which is in contact with the skeleton (**Fig.3**).

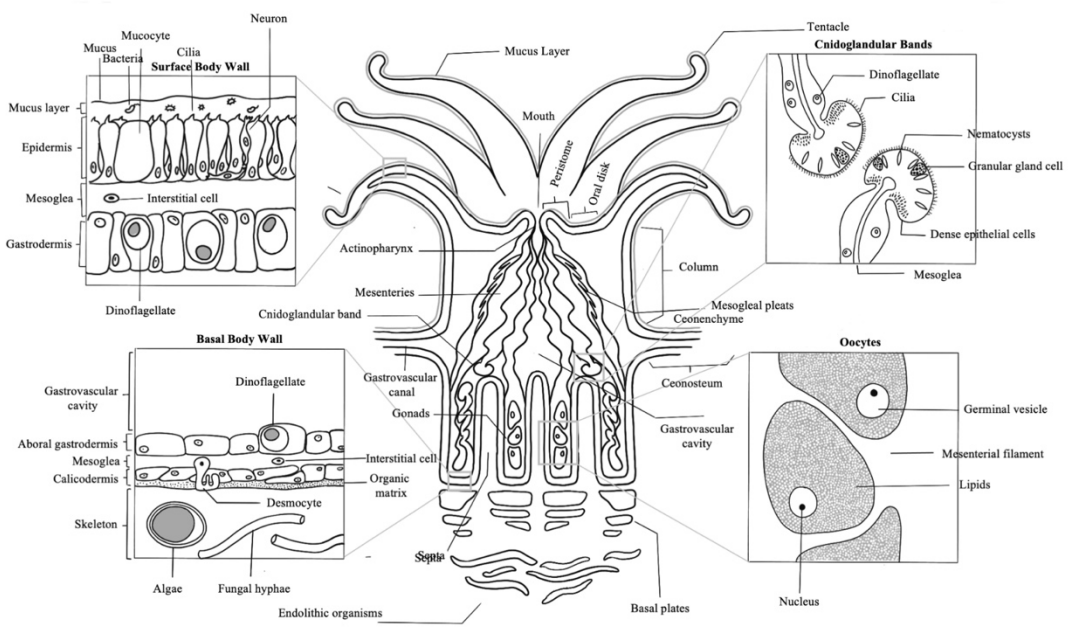

Figure 3. Schematic representation of polyp anatomy of an imperforate coral (https://cdhc.noaa.gov).

The surface body wall is constituted of three tissue types, the epidermis, the mesoglea and the gastrodermis (**Fig.4**). The epidermis is directly in contact with seawater and is formed by a layer of simple columnar or pseudostratified columnar epithelium equipped with cilia to facilitate mucus transport or sediment removal. This superficial layer, along with columnar supporting cells, displays a variability of cell types including mucocytes, cnidocytes (nematocysts or spirocysts), neurons, pigment cells, epitheliomuscular cells, each serving different functional roles. Finally, the gastrodermis lines gastrovascular cavity and interconnecting gastrovascular canals, and constitutes the digestive system of the coral. The supporting cells exhibit phagocytic activity for nutrient absorption, are usually cuboidal with

apical nuclei and house endosymbionts within cytoplasmic vacuoles. In addition to supporting cells, the gastrodermis layer may contain other cell types including mucocytes, cnidocytes, acidophilic granular grand cells, epitheliomuscular cells and chromophores (with melanin or green, fluorescent pigments).

The gastrovascular canals arrangement in the skeleton of stony corals, with subsequent differences in the histology, determines the distinction between imperforate and perforate corals. Imperforate corals, such as *Orbicella curta* and *Montastrea* spp., have polyps with fused corallite walls and therefore the gastrovascular canals are only connected on the surface of the coenosteum at coenenchyme level. Whereas, in perforate corals, such as *Acropora* spp. or *Porites* spp., gastrovascular canals connect adjacent polyps throughout corallite walls and are found all along skeletal depth.

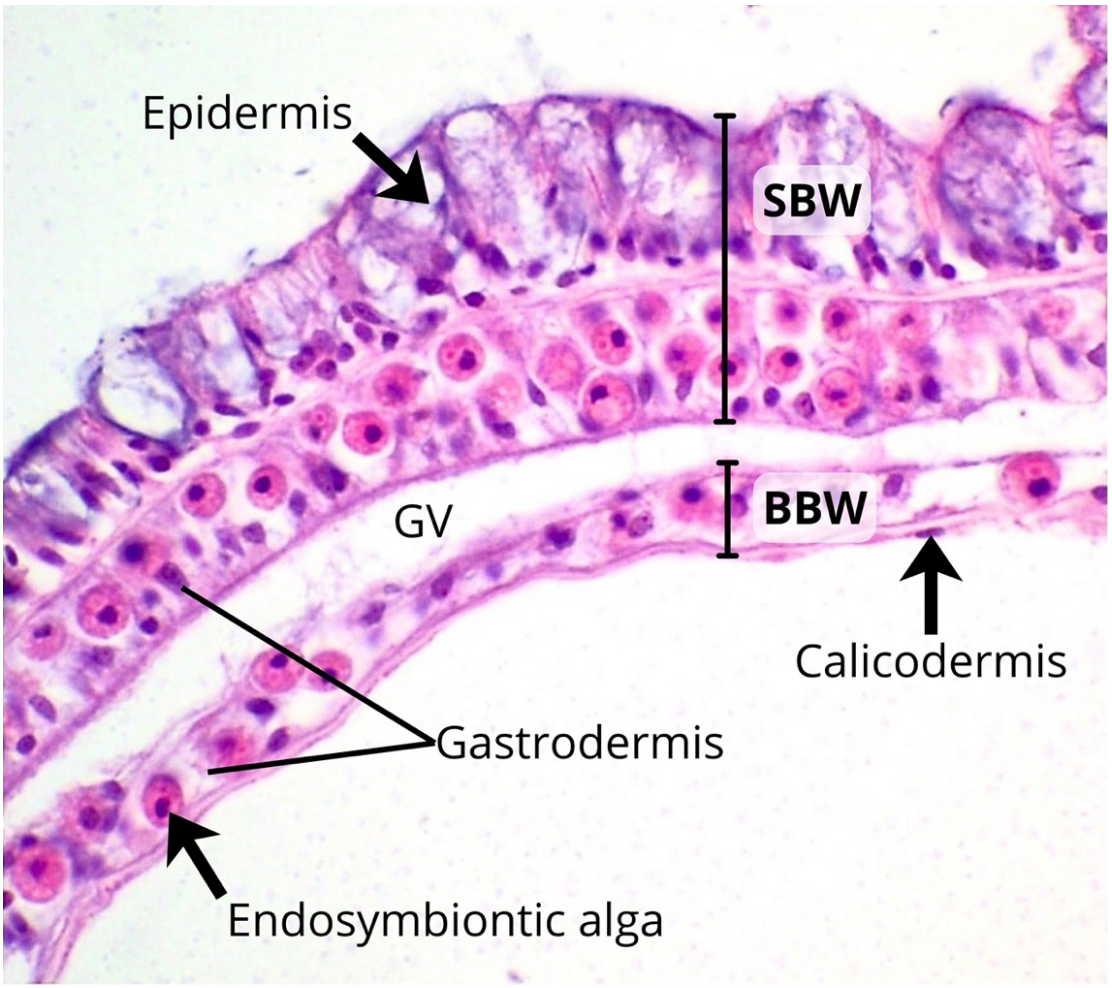

Figure 4. Histological section of *Acropora cervicornis* showing basic structure of surface body wall (SBW) and basal body wall (BBW). GV indicates the gastrovascular cavity. (Credits to Michelle M. Dennis).

The basal body wall, facing the skeleton, is composed of tissue layers as gastrodermis, mesoglea and calicodermis. Depending on the species and location within the organism, the gastrodermis may be more vacuolated and have fewer endosymbionts with respect to more superficial gastrodermis of the surface body wall.

The calicodermis is the tissue layer lining the basal skeletal surface of the coral and is composed of a very thin layer of cuboidal to squamous cells, the calicoblasts. Calicoblasts are responsible for skeletal deposition actively secreting organic matrix and coral acid-rich proteins (CARPs) involved in the crystallization process of calcium and carbon dioxide into aragonite crystals (Mass et al. 2017).

In addition to calicoblasts, the calicodermis also contains desmocytes, specialized epithelial cells that mediate the attachment of the calicodermis to the underlying skeleton, thereby maintaining skeletal integrity and facilitating continued skeletal growth.

Going deeper into specialized coral structures, the mouth opens into a short, muscular tube called the actinopharynx, which leads to the gastrovascular cavity. Within the gastrovascular cavity, partitions called mesenteries extend from the body wall towards the center and are classified as "complete" or "incomplete" depending on whether they connect with the actinopharynx. Complete mesenteries, which are paired and in multiple of six in Hexacorallia, enhance the gastrodermis surface area and provide structural support. The free edges of mesenteries develop into thickened and convoluted structures known as mesenterial filaments, which play a major role in digestion. To fulfill the function, the localize median tract of mesenterial filaments, known as cnidoglandular band, which exhibits lobes and is lined by columnar ciliated epithelial cells, is interspersed with cnidocytes, mucocytes, granular (pigmented) gland cells and phagocytic cells, often with ciliated surface (Peters, 2015; Berzins et al. 2021).

The mesoglea is the intermediate collagen matrix of variable thickness between two epithelial surfaces. Within the mesoglea, there may be present fibroblasts, which secrets ground substance and collagen fibers, and in some species migratory amoebocytes (Young, 1973). In superficial regions of the polys, the mesoglea may become pronounced and vertically folded like an accordion, supporting sheets of myonemes from epitheliomuscular cells. Those regions are known as mesogleal pleats and serve for further contraction and relaxations of the polyp. In the deeper part of the polyp, the mesoglea also serves as the site of gonadal development in corals. Germ cells originate in the gastrodermis and then migrate into the mesoglea for maturation. Depending on the reproductive strategies of the different species, as

hermaphroditic (monoecious) or gonochoric (diecious), corals may develop oocytes and spermaries in the same polyp or in two distinct individuals (Richmond, 1997).

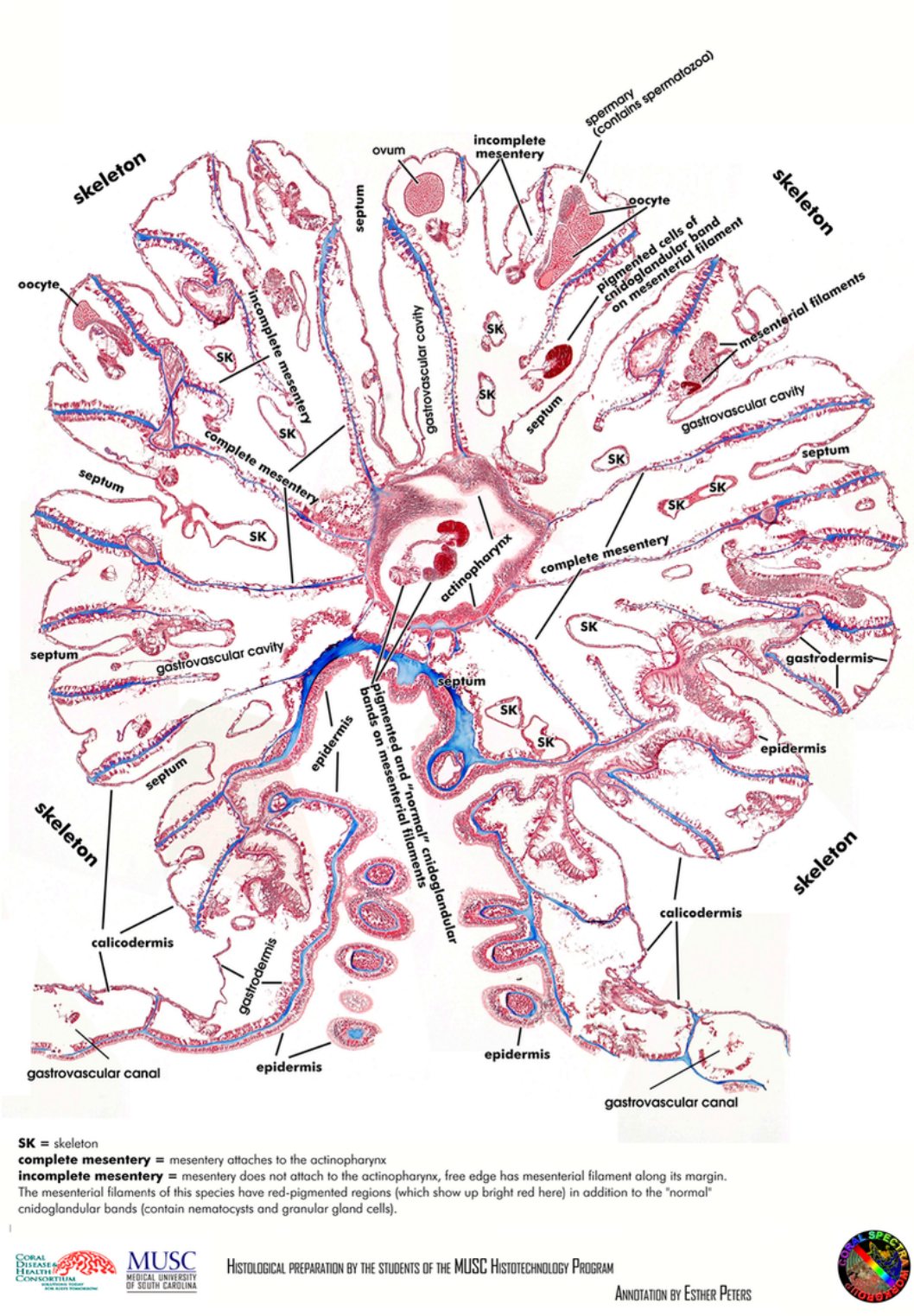

Figure 5. Microscopic anatomy of scleractinian coral.

1.3 INNATE IMMUNE SYSTEM OF SCLERACTINIA

Cnidarians represent one of the earliest and structurally simplest metazoan lineages and therefore lack many of the organ systems characteristic of higher animals, including fully developed circulatory, muscular, nervous, and excretory systems. Nevertheless, they have evolved a few specialized morphological and physiological adaptations that effectively compensate for the absence of these systems and allow them to perform the essential functions (Palmer et al., 2012; Snyder et al., 2021). Corals are constantly exposed to external microbial agents, and, despite their simplicity, they developed some innate immune mechanisms to cope with harmful pathogens (Bosch & Rosenstiel, 2015). Firstly, most cnidarian species possess amoebocytes, which serve as the principal cells of their innate immune system (Mydlarz et al., 2008). Those highly plastic and pleomorphic cells with phagocytic and biochemical properties, play crucial roles in various physiological processes, including antimicrobial and antioxidant functions. Amoebocytes can phagocytize and digest particulate matter and transport nutrients and waste materials throughout the organism (Thies et al., 2025). Indeed, they also exhibit considerable mobility, which allows them to easily migrate between cells and across epithelial layers (Palmer & Traylor-Knowles, 2011). Moreover, the production of mucus by mucocytes is critical to provide the first protective layer and anatomical barrier, the surface mucus layer (SML), in addition to other functions including gas and metabolites exchange or sediment removal (Piggot et al., 2009; Bythell & Wild, 2011). From the molecular point of view, the epithelial cells seem to mediate most innate immune responses, throughout induction of antimicrobial signaling activity (Reed et al., 2010; Bosch & Rosenstiel, 2015). Nevertheless, increasing body of evidence suggests that corals not only eliminate harmful micro-organisms, but also structure tissue-associated microbial communities which may contribute to the overall animal's health and resilience (Bourne et al., 2016; van Oppen & Blackall, 2019; Voolstra et al., 2024). Building this complex relationship, the "coral probiotic hypothesis" proposes that corals maintain a dynamic association with their symbiotic microorganisms, whereby shifts in environmental conditions select for microbial communities that enhance the overall fitness of the holobiont under prevailing circumstances (Reshef et al., 2006; Rosenberg et al., 2007). Therefore, the microbial assemblages associate with coral tissue, which are usually species-specific, may be considered as an additional important component of coral immune defense.

1.4 CORAL DISEASES

Coral diseases constitute one of the primary drivers of coral reef decline worldwide, posing a major threat to biodiversity, ecosystem function, and the livelihoods of millions of people who depend on reef systems.

Diseases represent a key regulatory factor in coral population dynamics, yet they have historically been overlooked in reef research. In marine environments, disease processes are often complex, pervasive, and highly virulent, and the majority of coral diseases exhibit poorly resolved etiologies that, in addition to changing through time, do not conform to the classical terrestrial model of one-pathogen, one-disease. Climate change has exacerbated epizootic outbreaks by increasing the pathogenicity and virulence of disease agents and increasing the susceptibility of corals to infection (Burke et al., 2023). The investigation of coral diseases should therefore be regarded as an urgent research priority.

Outbreaks such as white band (WBD), white plague (WP), black band diseases (BBD) and newly-described stony coral tissue loss disease (SCTLD), coupled with increased frequency of heatwaves, have caused coral cover losses exceeding 80% in some areas (Aronson & Precht, 2001; Bruno et al. 2007; Precht et al., 2016; Muller et al., 2020; Emslie et al. 2024). Over the past four decades, both the number and distribution of coral disease have increased significantly, with about 40 distinct diseases now reported globally affecting about 200 coral species (Weil et al., 2006; Bruckner, 2009; Aeby et al., 2020; Morais et al. 2022). However, such number likely underrepresents the global diversity of coral diseases and is likely due to further increase. Disease prevalence is expected to rise as environmental changes enhance host vulnerability and pathogen virulence (Sokolow, 2009; Burke et al. 2023; Hawthorn et al. 2023). Indeed, global climate change represents the most incisive driver of coral disease emergence. Rising sea surface temperatures and ocean acidification weaken coral responses to external agents and disrupt symbioses with dinoflagellates, leading to increased disease susceptibility (Maynard et al., 2015; Heron et al., 2016). Repeated temperature anomalies exceeding local bleaching thresholds can double or triple disease incidence, while local stressors such as eutrophication, sedimentation, and overfishing further enhance coral vulnerability by destabilizing microbial communities and reducing coral resilience (Bruno & Selig, 2007; Vega Thurber et al., 2013).

The etiology of coral diseases, as identification of causative agents, is highly complex and multifactorial. A broad range of pathogens, including bacteria, fungi, protozoans, and viruses, have been associated with coral tissue degeneration and mortality, often interacting with environmental stressors to trigger disease manifestations (Bruckner, 2015; Sweet & Bulling, 2017; Meyer et al.,

2025). Moreover, opportunistic infections by secondary microorganisms, such as *Vibrio* spp. or *Aspergillus sydowii*, occur frequently, particularly following coral bleaching or physical injury, when the coral's defense mechanisms are compromised (Rosenberg & Ben-Haim, 2002; Ainsworth et al., 2016). Ultimately, similar to other animals, coral diseases represent a complex interplay between host, pathogen, an environment, demanding multidisciplinary approaches to unravel their pathogenesis and ecological implications (**fig.6**).

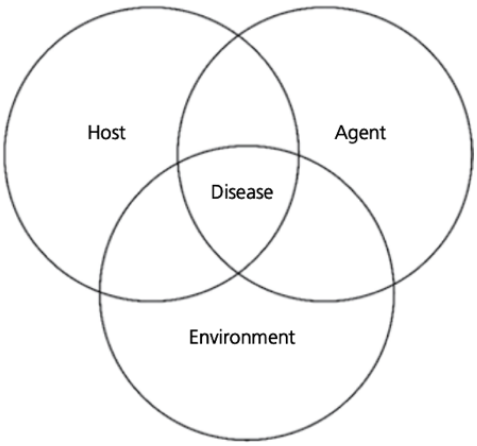

Figure 6. The disease triad (Thrusfield, 2015). The "epidemiological triad" model proposes that pathogenesis of a disease is the product of an interaction between factors related to the host, the causal agent and the environment.

1.4.1 PRINCIPLES OF WILDLIFE DISEASE INVESTIGATION

Pathology is the study of a disease and constitutes the detailed investigation of abnormalities in the structure and function of an organism. Etiological factors may be multiple and intertwined and are divided in infectious and non-infectious. Bacteria, fungi, viruses and parasites are considered infectious agents and can induce a disease by infecting and multiplying inside the host. Whereas, non-infectious agents are abiotic and environmental factors, including toxins, trauma, competition, nutritional, genetic, metabolic and immune-mediated. The investigation of diseases in animals follow a standardized and deductive process, which typically includes field investigations, gross and microscopic pathology, biodetection techniques, experimental infection studies or disease progression observations, and trials to fulfill Koch's postulates (Wobeser, 2007). Field

investigations involve photo documentation and detailed descriptions of visible lesions, conducting monitoring to assess disease prevalence or progression in natural settings, and developing models to better understand disease dynamics. Microscopic pathology focuses on the systematic examination and characterization of tissue and cellular lesions using light or electron microscopy to identify structural changes associated with disease. Biodetection methods comprise a wide range of analytical tools, including microbial culture, molecular assays, and various biochemical or physiological tests. In the case of corals, the essays involve multiple parameters including assessment of endosymbionts' physiology or abundance, protein and lipid quantification, measurements of skeletal density or endolithic organisms, or measures of immune mediators. The next steps involve experimental inoculation and disease transmission studies. These consist of trials in which healthy specimens are exposed to cultured pathogens or diseased tissues, allowing disease progression to be monitored within controlled aquaria or *in situ* field conditions. Laboratory-based experiments are typically conducted in aquaria, whereas field trials are performed directly in natural habitats. Lastly, the application of Koch's postulates (Koch, 1893) includes the detection of the infectious agents with cell pathology from field specimens, the culture of the putative pathogen, experimental infection reproducing the lesions observed in the field, and re-isolation of the organism (Work & Meteyer, 2014). The fulfillment of the Koch's postulates increases the likelihood of the organism being the etiological agent. However, the rigid framework of such postulates holds some neglections and limitations related, such as the importance of environmental factors in the disease process, the challenges in isolating certain pathogens in the aquatic environment, and the inability to extend its application to non-infectious diseases. As a result, Koch's postulates are mostly feasibly applied to diseases caused by a single, clearly identifiable agent, and have since been adapted through the incorporation of molecular tools and revised guidelines that reflect modern concepts of causality (Evans, 1976; Fredericks & Relman, 1996; Ritchie et al., 2001).

Given the intrinsic complexity of polymicrobial infectious diseases and the close association between corals and diverse microbial communities, alternative models are required to better describe coral disease pathogenesis. One widely accepted model in coral disease ecology is the multifactorial causal model (Rothman, 1976), which defines a "sufficient" cause as a combination of factors that inevitably leads to a specific effect (if no external interruption occurs). A cause is considered "necessary" if it is a component of every sufficient cause. This model has been demonstrated to be applicable to coral diseases and is now commonly used in coral disease research (Work et al., 2008; Sweet et al., 2011; Thrusfield, 2015).

1.4.2 CORAL DISEASE INVESTIGATION: CHALLENGES AND MISSING POINTS

Despite the biological simplicity of corals relative to other animals, the investigation of coral diseases has long presented substantial challenges. In contrast to higher animals, corals exhibit only a limited set of pathological processes, disorders of growth, cellular injury, and abnormal tissue deposits or pigments. These processes manifest grossly as three primary lesion types: growth anomalies, tissue loss, and tissue discoloration (Work & Meteyer, 2014; Woodley et al., 2015). The restricted range of possible visible disease signs often results in overlapping gross presentations among different coral diseases, and in some cases, lesions may lack any externally perceptible changes (Work et al., 2008; Page et al., 2024). For such reasons, microscopic examination is an essential component of a disease case definition and is often referred to as "gold standard" (Page et al., 2024).

By concept, the case definition represents the complete body of evidence that defines a particular disease, encompassing all relevant morphologic and laboratory information (Cummings et al., 2001). As new data are acquired and accumulated, the case definition is refined and may change over time. The foundation of any case definition is the morphological description, with includes both gross and microscopic one.

Pathology provides a standardized language for describing lesion morphology and follows four ordinal steps that are well established in animal disease investigations: naming the disease, describing the lesion, formulating a morphologic diagnosis, and identifying an etiologic diagnosis. Failure to correctly identify a disease at any of these stages undermines the validity of the case definition (Work & Aeby, 2006; Work et al., 2008).

In corals, however, disease nomenclature remains inconsistent and confusing, as these diagnostic steps have often been frequently conflated or restricted solely to initial observations. This highlights the need for explicit and standardized methodologies that emphasize as objective the lesion description rather than premature inference of causality.

Indeed, coral disease research has historically focused predominantly on documenting *in situ* signs, prevalence patterns, and outbreak dynamics, while devoting far less, if any, attention to microscopic investigation (Willis et al., 2004; Williams & Miller, 2005; Work & Meteyer, 2014). This imbalance has led to substantial gaps in histopathological nomenclature, diagnostic criteria, and standardized investigative protocols (Page et al., 2024). Recent attempts to apply microscopic diagnosis to specific coral diseases have at times, generated controversy and confusion with previous studies, particularly regarding disease identity and the nature of putative causal agents (Ainsworth et al., 2007; Work et al., 2008). In contrast, histopathology is a well-established gold

standard in veterinary and medical sciences, providing indispensable insights into disease mechanisms that can inform management and prevention strategies.

The absence of a systematic, pathology-based framework for the study of coral lesions, such as those routinely applied in wildlife health, has contributed to the current situation in which the etiologies of most of the approximately 40 described coral diseases remain unresolved (Weil et al., 2006). Furthermore, the ecological complexity of reef environments, the scarcity of baseline data on coral–microbe associations in healthy colonies, and remaining gaps in our understanding of coral physiology and stress responses collectively hinder the development of detailed and accurate descriptions of coral diseases (Sweet & Bythell, 2012).

1.4.3 CORAL DISEASE MANAGEMENT EFFORTS

To date, coral disease treatments have been applied largely for experimental or diagnostic purposes, spanning at least seven different diseases and yielding variable levels of success. Existing interventions can be broadly categorized into mechanical (removal of diseased tissue, shading, smothering), chemical (drugs, often coupled with mechanical) and biological (bacteriophage probiotic therapy) approaches. Mechanical methods represent the most widely used strategies, though their effectiveness has been highly variable (Aeby et al. 2019; Neely et al., 2021; Walker et al., 2021). Chemical treatments typically involve the topical application of antibiotics or antiseptics, often incorporated into clay or putty matrices to increase contact time (Contardi et al., 2020; Scribano et al., 2025). More recently, biological interventions, including phage-probiotic treatments, have targeted specific microbial pathogens. Notably, phage-based approaches have successfully slowed tissue loss in Red Sea corals affected by white plague and, in some cases, halted progression when applied early or prophylactically (Efrony et al., 2006; Atad et al., 2012). While not all coral diseases have been subjected to treatment trials, and not all interventions have succeeded in stopping lesion progression, these efforts demonstrate that targeted colony-level mitigation is achievable under certain circumstances (Neely et al., 2021). However, treatment success remains highly dependent on the underlying etiological agent, environmental conditions, and species-specific coral responses (Forrester et al., 2022; Studivan et al., 2023; Vega Thurber et al., 2025). Despite increasing interest in disease mitigation, evidence for effective population-level interventions remains sparse and inconclusive. Large-scale response programs, especially those addressing stony coral tissue loss disease (SCTLD) in Florida, show that antibiotics and physical barriers can temporarily slow lesion progression on individual colonies. Yet these benefits rarely translate into measurable improvements at the reef-community level, in part because the treatments are not prophylactic and do not prevent new infections (Florida DEP, 2021; Neely et

al., 2025). This represents a core concept in wildlife disease management, where treatment is rarely curative at the population level; instead, it is employed for educational purposes, including rehabilitation and *ex situ* conservation, or to protect the final individuals of critically endangered species from extinction.

Treatments are resource-intensive, require repeated application, and show declining efficacy over time, thereby limiting their practicality for sustained and widespread management. Additional concerns in coral disease treatment include the potential for antibiotic resistance, unintended ecological side effects and the introduction of pharmaceuticals into reef environments, which are well-documented issues in terrestrial wildlife disease management but insufficiently investigated in marine systems (Vega Thurber et al., 2025). Furthermore, treatment-induced shifts in coral-associated microbial communities may indirectly influence future disease susceptibility. Coral microbiomes vary among populations, and these differences correlate strongly with differential vulnerability to disease (Mohamed et al., 2023; Voolstra et al., 2024). Consequently, interventions that alter microbiome composition may have unintended long-term consequences. Population-level monitoring frequently indicates that disease prevalence continues to rise even on reefs where intensive treatments are applied (Miller et al., 2014) and treated colonies often develop new lesions over time (Shilling et al., 2021).

As considered before, this pattern mirrors challenges in terrestrial wildlife disease management, where interventions directed at individual animals rarely change population-scale outcomes without concurrent attention to environmental conditions, pathogen reservoirs, or vectors (Wobeser, 2002). For corals, major knowledge gaps persist concerning disease etiology, pathogen transmission pathways, environmental reservoirs, and host susceptibility. These gaps limit the identification of control points for effective targeted mitigation. Consequently, no comprehensive management or treatment protocols currently exist for coral disease outbreaks that parallel those available for certain terrestrial wildlife (*National Guidelines for Management of Disease in Free-Ranging Australian Wildlife*, 2020), underscoring the need for integrated approaches that consider both host ecology and environmental drivers.

Ultimately, coral disease research should be framed within the broader context of disease ecology rather than therapeutic development, even though interventions may be beneficial in limited contexts, particularly for corals maintained in captivity. Emphasis on host-pathogen-environment interactions allows for the identification of ecological control points that influence disease dynamics, mitigating disease occurrence. Such control points typically involve environmental modification, underscoring the importance of ecosystem-level approaches to disease mitigation in coral health management.

1.4.4 GROWTH ANOMALIES (GAS)

Growth Anomalies (GAs) represent chronic lesions characterized by discrete masses having tissue and underlying skeleton significantly different in morphology with respect to the surrounding tissue and skeleton (Work et al., 2015) (**Fig.7**). These masses result from abnormal skeletal accretion and exhibit varying morphologies, largely determined by the coral's growth form (Work et al., 2008; Ricci et al., 2022) (**Fig.8**).

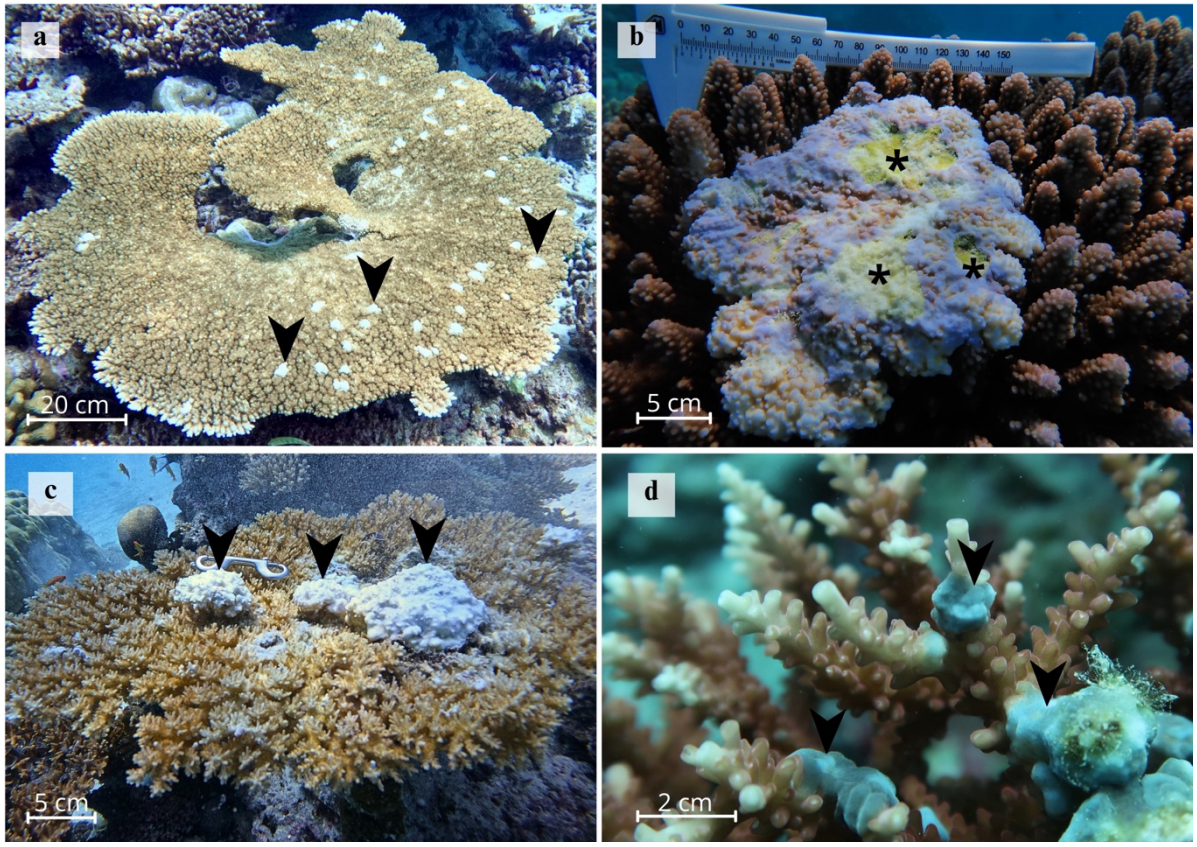

Figure 7. GAs on *Acropora* colonies (arrowheads) in various Maldivian reefs. The asterisks indicate chronic tissue loss areas of the lesion colonized by algae. (Photo **b**,**c**,**d** credits to Inga Dehnert)

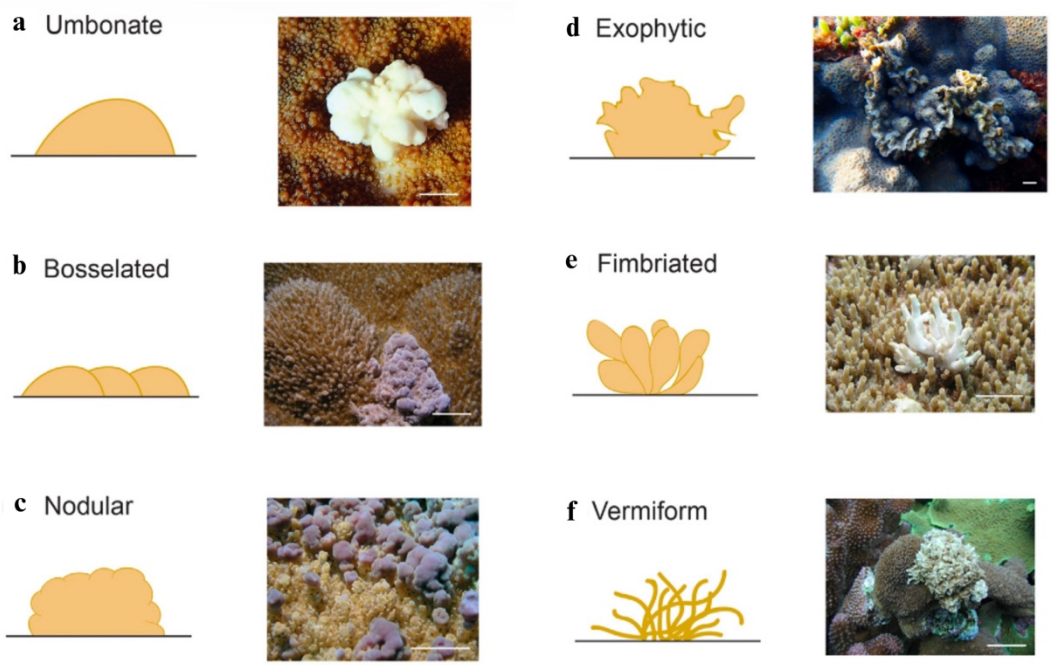

Figure 8. Classification of GAs morphologies. Scale bar 1 cm. (modified from Ricci et al., 2022)

Their prevalence was reported ranging from low (<5%) to high (>70%) depending on the region, with most observations from the Pacific Ocean (Gateño et al., 2003; Work et al., 2008). However, as the impacts of climate change intensify, GAs are being reported with increasing frequency and severity on stony corals. The diseased reported genera are multiple, including *Montipora*, *Fungia* and *Favia*, but *Acropora* and *Porites* are disproportionately affected. In addition, they appear to manifest on larger and older coral colonies compared to their smaller counterparts and, mostly in shallow waters (Caldwell et al. 2020). This observation stems from the nature of GAs as chronic progressive conditions that tend to increase in size and accumulate over time, as indicated by previous research (Caldwell et al. 2020), despite in certain coral species, they may exhibit transient characteristics (Stimson et al. 2011) (**Fig.9**).

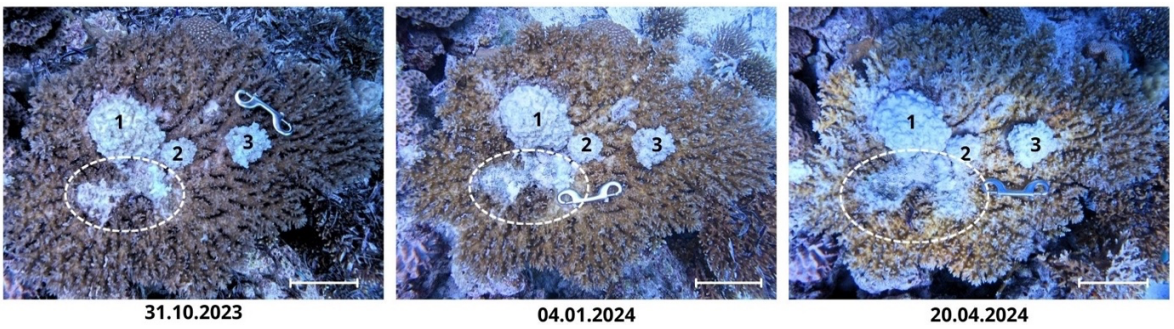

Figure 9. Time series of multifocal GA lesions (1,2,3) on *Acropora*. Note the extending area of necrotic tissue loss (dashed circles). Scale bar 10 cm. Falhumaafushi, Maldives. (Photo credits to Inga Dehnert)

To date, the etiology of GAs is unknown, even though genetic predisposition, UV radiation, environmental degradation and infectious agents (Peters et al., 1986; Coles & Seapy 1998; Domart-Coulon et al., 2006; Aeby et al., 2011) have all been suggested as possible causes of the disease onset. Nevertheless, GA lesion appears to compromise colony fitness causing multiple impairments and often resulting in constrained reproductive abilities (Kelly et al., 2016; Palmer & Baird, 2018; Preston & Richards, 2020), reduced feeding efficiency (Burns et al., 2011, 2013), and compromised defenses against external threats (Rich et al., 2021; Andersson et al., 2020). These impairments are the direct consequences of the aberrant growth of the skeleton and tissues associated with the lesion. Indeed, the circumscribed affected area appears structurally compromised both in the tissue and skeleton architecture (Work et al., 2015). Over longer time scales, mortality rates vary among coral genera, with Acroporidae experiencing the most severe decline, though it is unclear whether this is related to an increase in the size, number, or both, of the masses on the same colony.

1.4.5 BROWN BAND DISEASE (BRB)

Brown band disease (BrB) is characterized by lesions involving progressive tissue loss.

In the field, generally it can be grossly identified by a distinct brown band that precedes the advancing tissue-loss margin, from which is often separated by a zone of recently exposed white skeleton (**Fig.10**).

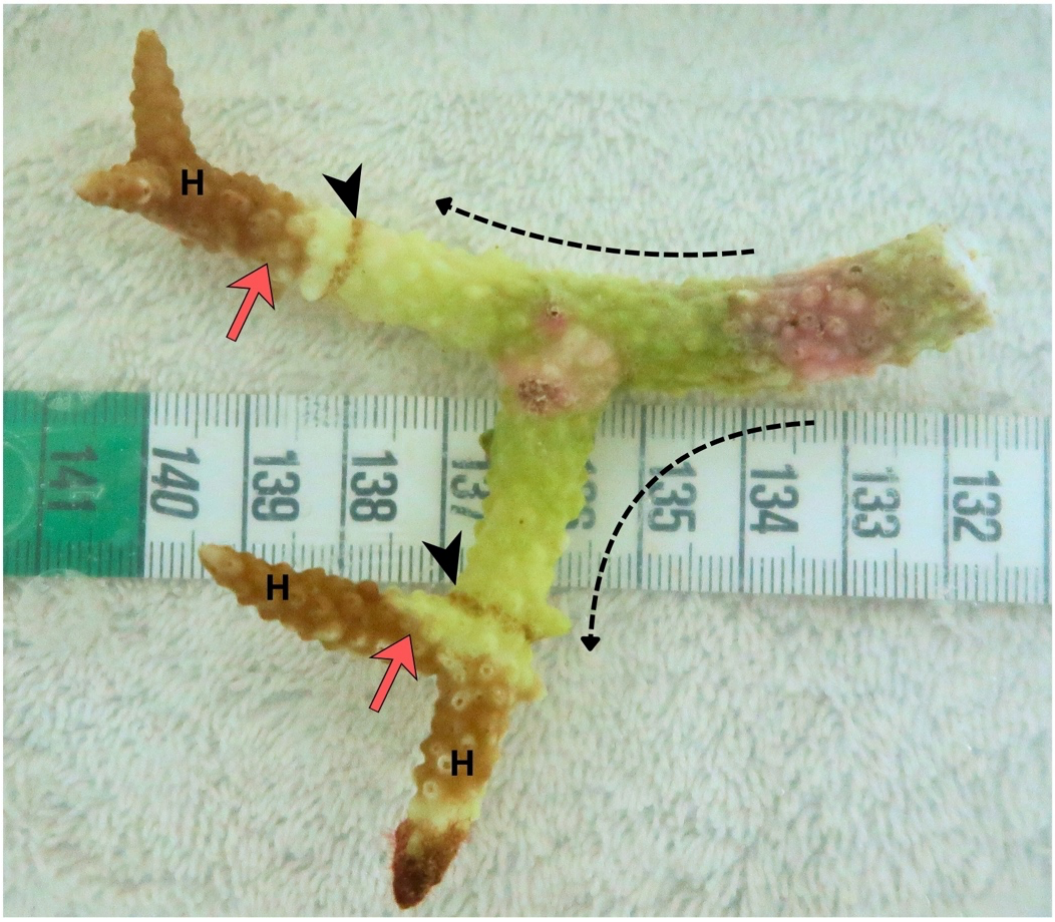

Figure 10. Fragment of *Acropora* branches multifocally affected by BrB. The lesion (acute tissue loss) progresses from the base of the colony to branches' tips (dashed arrow). The arrowheads point at brown ciliate bands, which progress with tissue loss margin (red arrow). H indicates the apparently healthy regions ahead of tissue loss.

This band, which varies in width and pigmentation intensity, tracks the progression of the lesion and consists predominantly of a dense aggregation of ciliates (approximately 120 cells per square millimeter) (Lobban et al., 2011; Raymundo & Weil, 2015). Scuticociliates of the genus *Philaster* are considered the putative etiological agents, as they have been observed actively consuming coral tissue and engulfing and harvesting the host endosymbionts (Ulstrup et al., 2007; Lobban et al., 2011; Sweet & Bythell, 2012). Although, their role in disease development, whether as primary

pathogens versus opportunistic agents, is still debated, as comprehensive histopathological analysis is still lacking.

BrB has been reported throughout the Indo-Pacific and affects several coral families, with species of Acroporidae appearing particularly susceptible (Willis et al., 2004; Haapkylä et al., 2009). Although its prevalence at the reef scale is typically low (generally <1%), the disease exhibits a rapid progression rate, at times advancing several centimeters per day (Haapkylä et al., 2009; Page et al., 2009; Weil et al., 2012). Despite more than two decades since its original description (Dinsdale, 1994), the definitive causative agent has not been conclusively identified, and the underlying pathogenesis remains insufficiently understood and studied, with notable gaps in the histopathological characterization of the disease.

1.4.6 CORAL DISEASES IN THE MALDIVES

Reports of coral diseases in the Indo-Pacific region remain comparatively lower than those documented in the Caribbean (Bruckner et al., 2015; Morais et al., 2022). This discrepancy is partly attributable to the vast spatial extent of the Indo-Pacific area and the remoteness of most reef systems, which limit long-term monitoring and systematic disease surveys. Within this broader context, studies investigating coral diseases in the Maldivian Archipelago are particularly scarce and are characterized by a relatively recent history of observations (Montano et al., 2020; Morais et al., 2022; Dehnert et al., 2024).

The first documented report of coral diseases in the Maldives dates back to 2012, when Montano et al. described the presence of five pigmented lesions affecting scleractinian corals (Montano et al., 2012). These included black band disease (BBD), skeletal eroding band (SEB), brown band disease (BrB), and white syndrome (WS).

Subsequent studies in the region have further investigated the ecology of these lesions (Montano et al., 2013, 2014, 2015, 2016); however, research efforts have largely focused on disease prevalence, spatial occurrence, and macroscopic morphology. Notably, there has been a consistent lack of histopathological characterization of coral diseases in the Maldives, a limitation that reflects a broader gap in coral disease research at the global scale.

More recently, the occurrence of growth anomalies (GAs) has also been reported across multiple sites in the Maldivian archipelago, affecting several genera of scleractinian corals (Bises et al., 2023; see **Chapter 2**). Despite the overall lack of data and limited research coverage, available evidence suggests that coral disease prevalence in the Maldives is increasing. This trend appears to be associated with the intensification of climate-driven stressors and local human activities, consistent with global trends (Sokolow, 2009; Montano et al., 2013; Bises et al., 2024, see **Chapter 6**).

1.5 RESEARCH AIM

This research aimed to enhance the understanding of coral disease ecology, diversity, and pathology within the Republic of Maldives, a region of high ecological importance where coral health remains insufficiently documented. The research focused on two comparatively understudied coral diseases: coral growth anomalies (GAs) and brown band disease (BrB), with the overarching goal of providing an integrated characterization of their ecological and pathological features.

Given the high biodiversity of coral species in the Maldives and the potential for taxon-specific disease processes, this study further aimed to characterize lesion features and pathological processes that may differ from those described in other reef systems worldwide. By addressing critical knowledge gaps regarding coral disease presence, ecology, pathology, and temporal dynamics in the Maldives, this research contributes to a baseline framework important for evaluating long-term trends in coral disease prevalence, evolution, and their implications for reef health under ongoing environmental change.

1.6 RESEARCH OBJECTIVES

The primary objective was to conduct a comprehensive investigation of coral growth anomalies, examining their ecology, spatial distribution, and pathological characteristics across multiple biological scales. This included macroscopic assessments of lesion morphology, detailed analyses of alterations in key skeletal morphological structures, and histopathological examination of tissue-level changes associated with GA lesions.

A secondary objective was to expand current knowledge of brown band disease (BrB) ecology in the Maldives through gross lesion characterization, genetic identification of associated and putative pathogenic organism, and the first comprehensive histopathological characterization of Brb.

An additional objective of this thesis was to investigate the temporal patterns of coral disease prevalence at Thudufushi Island, Maldives, over a twelve-year period (2010–2022). This long-term analysis aimed to assess changes in disease prevalence and composition through time, providing insights into potential trends, variability, and shifts in coral disease dynamics in relation to environmental stressors and increasing anthropogenic pressures.

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CHAPTER 2

WIDESPREAD OCCURRENCE OF CORAL GROWTH ANOMALIES IN THE REPUBLIC OF MALDIVES

This work is published as:

Bises, C., Dehnert, I., Aeby, G., Dennis, M., Gobbato, J., Hodge, J., Staiger, M., Siena, F., Galli, P., & Montano, S. (2023). Widespread Occurrence of Coral Growth Anomalies in the Republic of Maldives. *Diversity*, 16(1), 15. https://doi.org/10.3390/d16010015.

2.1 Abstract

In the last decades, there has been a concerning increase in the frequency and severity of coral disease outbreaks on a global scale, resulting in significant damage to the coral reef ecosystem and biodiversity. Growth anomalies (GAs) have been increasingly observed, with significant higher occurrence in larger and older coral colonies, compared to their smaller counterparts. However, there is a notable lack of knowledge and reports regarding growth anomalies in the Maldivian region. Here, we provide the first evidence of four distinct growth anomalies on three coral species, respectively on *Acropora* sp., *Montipora* sp. and *Pachyseris speciosa*, observed across four different locations across three atolls within the Maldivian Archipelago.

2.2 Growth Anomalies in the Maldives: Geographical Locations, Corals Affected, and Gross Pathology

Coral reefs are experiencing a rapid and significant decline in biodiversity, primarily attributed to the consequences of climate change, disease outbreaks, and pollution resulting from human activities (Hughes et al., 2017). In recent times, there has been an alarming increase in the frequency and intensity of coral disease outbreaks worldwide, causing significant damage to coral reef community and diversity. This phenomenon has resulted in enhanced susceptibility among coral hosts, expanded pathogen range, increased pathogen survival and disease transmission (Maynard et al., 2015; Pinzón et al., 2015). Among the most widespread, but less known, coral diseases are the anomalous growth forms, commonly known as growth anomalies (GAs) (Work et al., 2015), observed with increasing frequency and severity on stony corals (Preston & Richards, 2020; Das et al., 2022).

The etiology of growth anomalies is still under investigation (McClnahan et al., 2008; Ruiz-Moreno et al., 2012). However, studies have established that, in some coral species, prevalence of growth anomalies is correlated to anthropogenic stressors and extreme temperature conditions (Aeby et al., 2020). Even though the causes are still unknown, genetic predisposition, UV radiation, and infectious agents have all been suggested (Work et al., 2015).

Generally, GAs have been observed on larger and older colonies relative to smaller colonies, as growth abnormalities usually are a chronic disease which can accumulate through time (Caldwell et al., 2020), although can be transitory in some coral species (Stimson, 2010).

Growth anomalies have significant implications for the morphology (Yamashiro et al., 2000; Gateño et al., 2003) and biological functioning of coral colonies, leading to restricted reproductive capabilities (Kelly et al., 2016; Palmer & Baird, 2018; Preston & Richards, 2020), diminished feeding capacities (Burns & Takabayashi, 2011; Burns et al., 2013), and compromised defences against external agents (Burns et al., 2013; Andersson et al., 2020). Furthermore, *Acropora*, *Montipora*, and *Porites* are the genera most frequently reported as being affected by GAs (Aeby et al., 2011; Burns et al., 2013), suggesting a notable susceptibility of these genera to the development of growth abnormalities. Nonetheless, it remains unclear whether the prevalence of GAs within these genera is primarily influenced by their dominance within coral reefs or if it signifies a distinct susceptibility inherent to each genus.

Herein we report a widespread occurrence of growth anomalies found in the Republic of Maldives. While tumours seemed uncommon in the region, coincidental observations by SCUBA during other underwater studies or activities, such as the ones here reported, began to occur more frequently. In particular, GAs on coral colonies were observed between 2021-2023 on different islands‘ reefs: Adanga (3°08'19.9"N 73°00'30.6"E) and Magoodhoo (3°4'49.08"N, 72°57'57.19"E) in Faafu Atoll; Falumafushi house reef in the Gaafu Alif Atoll (0°40'07.1"N 73°26'05.3"E); Olhuveli house reef in the Laamu Atoll (1°48'57.7"N 73°24'15.6"E). The growth anomalies were found on three coral species (*Acropora* sp., *Montipora* sp. and *Pachyseris speciosa*) at different depth and zonation on the reefs: at the edge of the flat and front reef of in the first 3 – 4 m (Figure 1 a, b); and different depth on the slope, respectively, 5 – 6 m (**Fig.1c,d**), 12 – 13 m (**Fig.1e,f**) and 9 – 10 m (**Fig.1g,h**).

Grossly, the GAs appear as a discrete mass of abnormal skeleton and thin tissue of various shapes, with colour different to the rest of the colony. Following the classification of Ricci et al. (2022), shape was further characterized as nodular formation (**Fig.1a,b,g,h**), isolated to coalescing and rounded; bosselated shape (**Fig.1c,d**) with undulating surface; and umbonate (**Fig.1e,f**) with smooth and rounded shape. The location of growth anomalies on colonies varied, having no obvious common position on the colony.

Notably, this is the first report of GAs on *Pachyseris speciosa.*

Finally, despite coral diseases having been extensively studied and found in the Maldives (Montano et al., 2015; Montano et al., 2016), no growth anomalies were previously reported. There is still lack of information regarding the prevalence of these anomalies in the reef, however, the growing interest in the subject may suggest an increasing frequency or an enhanced ability to recognize them.

Therefore, the lack of knowledge of growth anomalies in this region of the Indo-Pacific emphasizes the need for conducting investigations to assess their presence, prevalence, distribution, and pathology to better understand their ecology and potential causes.

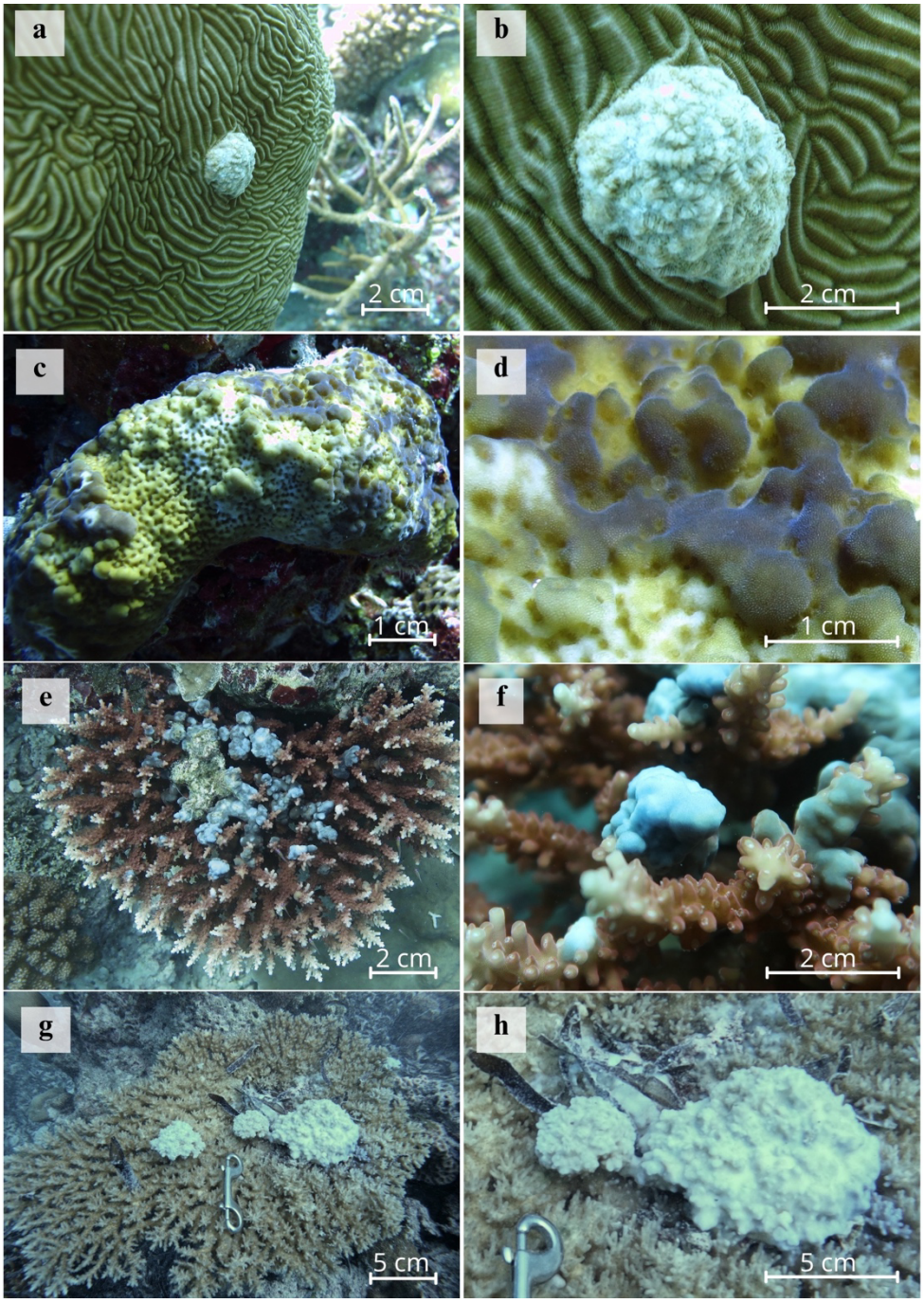

Figure 1. **a)** Nodular growth anomaly on *Pachyseris*; **b)** Closer view of growth anomaly on *Pachyseris*; **c)** Bosselated growth anomalies on *Montipora*; **d)** Closer view of growth anomalies on *Montipora*; **e)** Umbonate growth anomalies on *Acropora*; **f)** Closer view of growth anomalies on *Acropora*; **g)** Nodular growth anomalies on *Acropora*; **h)** Closer view of growth anomalies on *Acropora*.

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CHAPTER 3

COMPARATIVE SEM ANALYSIS OF SKELETAL MACRO-MORPHOLOGY IN *ACROPORA* AND *PACHYSERIS* GROWTH ANOMALIES

Part of this work is currently under review as:

Bises C., Dennis M.M, Gobbato J., Aeby S.G., Galli P. and Montano S. Pathology of Coral Growth Anomalies in the Maldives: Insights from *Acropora*, *Montipora*, and *Pachyseris*. *Diseases of Aquatic Organisms*. Manuscript: DAO-2025-10-004.

3.1 ABSTRACT

Coral growth anomalies (GAs) are chronic skeletal lesions that locally alter coral skeletal morphology and may impair coral fitness. This study examines GA masses in two reef-building coral genera with contrasting morphologies, *Acropora* and *Pachyseris*, using scanning electron microscopy (SEM). Eight GAs were collected from reefs in Faafu Atoll (Republic of Maldives), analyzed, and compared with adjacent healthy skeleton. Across both genera, GA-affected regions exhibited severe disruption of primary skeletal organization.

In *Acropora*, lesions were associated with malformed or absent corallites, enlarged calices, and expanded coenosteum characterized by inconsistently spaced trabeculae and abnormal spinule development.

In *Pachyseris*, GAs resulted in the loss of regular calices arrangement, disorganized radial elements, and reduction or absence of the columella. Despite taxon-specific skeletal architectures, similar patterns of structural aberration were observed, suggesting a shared pathological response involving dysregulated calcification and impaired skeletal patterning. These findings indicate that GAs profoundly alter coral skeletal construction, likely reducing mechanical integrity and functional efficiency. This study highlights the importance of detailed skeletal morphological characterization for understanding coral disease processes and underscores the need to integrate skeletal and tissue-level analyses to improve knowledge of pathogenesis under ongoing climate change.

3.2 INTRODUCTION

Coral skeleton provides a permanent record of growth conditions, integrating biological responses to both environmental and pathological stressors over time (Barnes & Lough, 1996; Lough, 2010; Thompson, 2021). Among all the threats undermining coral resilience and survival, climate change alters the ocean chemistry, notably through ocean acidification, which affects coral calcification rates and consequently impacts the skeletal growth (Langdon et al., 2000; Erez et al., 2010; Madin et al., 2012). Beyond these environmental influences, coral skeletal growth and macro-morphology are also altered by lesions and injuries arising as pathological responses to various stressors (Work & Aeby, 2006; Bozec et al., 2014; Andersson et al., 2020). Such structural modifications can compromise the mechanical stability of coral colonies, disrupt their ecological functions, and diminish their capacity to recover from disturbances (Foster et al., 2016; Putnam et al., 2017; Mollica et al., 2018).

Growth anomalies (GAs) represent one of the most prominent chronic skeletal lesions affecting reef-building corals. These lesions manifest as localized, abnormal skeletal accretions that form discrete, protuberant calcified masses on coral colonies (Work & Aeby, 2006; Work et al., 2015). Over time, these masses can increase in both size and number within individual colonies (Kelly et al., 2016; Ricci et al., 2022). Detailed analyses of the skeletal structure underlying such lesions are essential for understanding disease mechanisms and the intrinsic alterations to coral skeletal architecture that influence polyp physiology and overall colony health. These investigations help clarify whether GAs reflect accelerated but disorganized skeletal growth, disruptions in calcification regulation, or fundamental breakdowns in skeletal patterning.

To accurately characterize and describe skeletal lesions in their three-dimensional structure, the application of non-conventional morphological techniques is required. Scanning Electron Microscopy (SEM) provides a powerful tool for high-resolution examination of coral skeletal architecture, enabling detailed visualization while preserving spatial relationships among corallites and across colony-scale features (Ducklow & Mitchell, 1979). Understanding how chronic stress and disease affect coral skeletal architecture is critical for predicting reef resilience in the face of ongoing climate change. Focusing on two coral genera with distinct growth forms and skeletal architectures, this study analyzed the macromorphological changes associated with GAs in the primary skeletal structures of *Acropora* and *Pachyseris*.

3.3 MATERIALS AND METHODS

Between February 2022 and May 2023, a total of eight growth anomalies (GAs) were collected from coral genera *Acropora* (n=7) and *Pachyseris* (n=1) through SCUBA diving across various reef sites around Faafu Atoll in the Republic of Maldives. Sampling depths ranged from 7 meters to a maximum of 20 meters. The GA samples were sampled using hammer and chisel, collecting skeletal masses of varying sizes, each including an adjacent section of apparently healthy skeleton to serve as a control. Upon collection, all samples were labelled and immersed in a 10% commercial hypochlorite solution to ensure complete removal of all soft tissues and rinsed with tap water.

To prepare the skeleton fragments for imaging, they were cut with an electric saw if needed, ground lightly and subjected to sonication in ethanol, to remove any adhering dust or particulate residues that could interfere with high-resolution imaging. Each cleaned fragment was then mounted on aluminum specimen stubs using conductive silver glue. Subsequently, the samples underwent sputter-coated with conductive gold to enhance surface conductivity and image quality during electron microscopy. Scanning Electron Microscopy (SEM) observations were performed using a Vega Tescan 5136XM and Zeiss Gemini 500 microscopes at the SEM Laboratory, University of Milano-Bicocca.

The SEM analysis was performed to compare key macromorphological characteristics between the skeletal growth anomaly masses and the adjacent healthy skeleton in both *Acropora* and *Pachyseris* samples. In *Acropora*, morphological features of the corallites were examined, including the structure of the theca walls and septa, as well as the coenosteum comprising trabeculae and spinules. For each affected *Acropora* corallite, the maximum diameter was measured with the measuring tool of the Tescan software and compared with measurements taken from healthy corallites. These measurements were then statistically analyzed by calculating the arithmetic mean of corallite diameters, with differences assessed for significance using the non-parametric Mann-Whitney U-test performed in SPSS 28.0.1.1 (IBM, New York).

In *Pachyseris*, the analysis focused on documenting general macromorphological features such as the shape and arrangement of calices and radial elements, and the presence or absence of the columella, to detect deviations from normal skeletal morphology in the growth anomaly samples compared to healthy controls.

3.4 RESULTS

All growth anomalies (GAs) examined in this study (n=8), across both *Acropora* and *Pachyseris*, exhibited missing, abnormal, or confounded primary skeletal macromorphological structures.

Acropora. Marked differences were observed between healthy and GA-affected skeletal regions of *Acropora*, in many cases with distinguishable interface regions between the two (**Fig.1**).

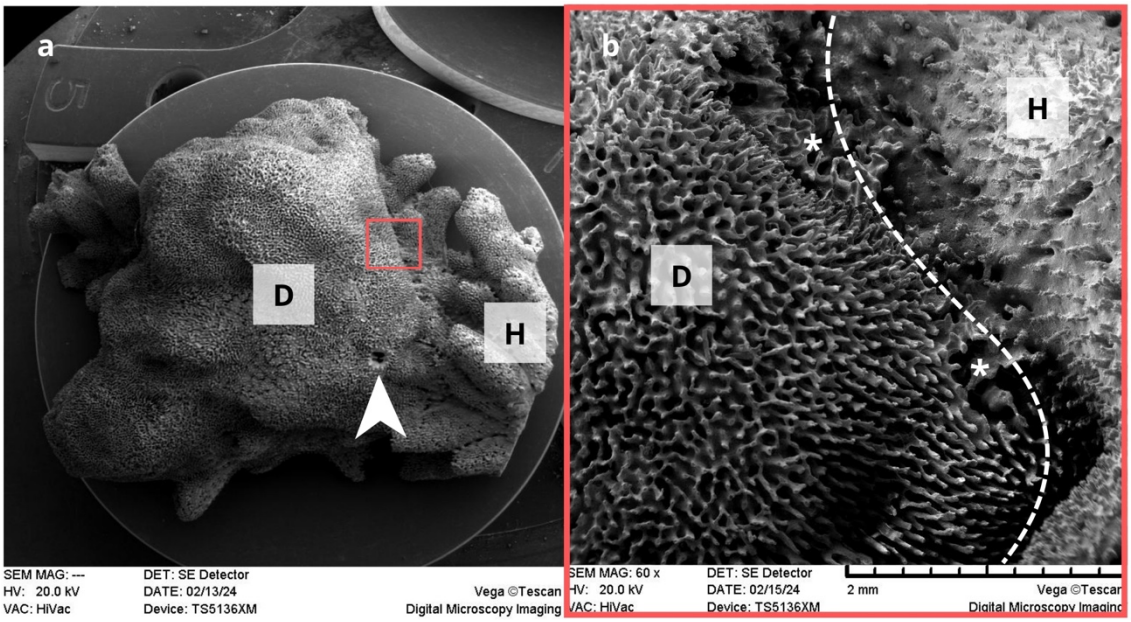

Figure 1. SEM imaging of growth anomaly in *Acropora*. **a)** Growth anomaly (D) with only one corallite (arrowhead) and bordering normal skeleton (H). **b)** Higher magnification of interface area between diseased skeleton (D) and healthy skeleton (H). The dashed line marks the distinct boundary of the mass, along which abortive corallites are present (asterisks).

In only one case, the GA mass did not present any corallite. Nonetheless, in all other samples (n=6) the GA-affected areas presented corallites, but sparse and displaying pronounced deviations from normal skeletal morphology. The typical corallite architecture was largely absent. Corallite walls (theca), typically prominent and well- defined against the coenosteum as circular and well-defined, were offset by overgrown coenosteum (**Fig.2**).

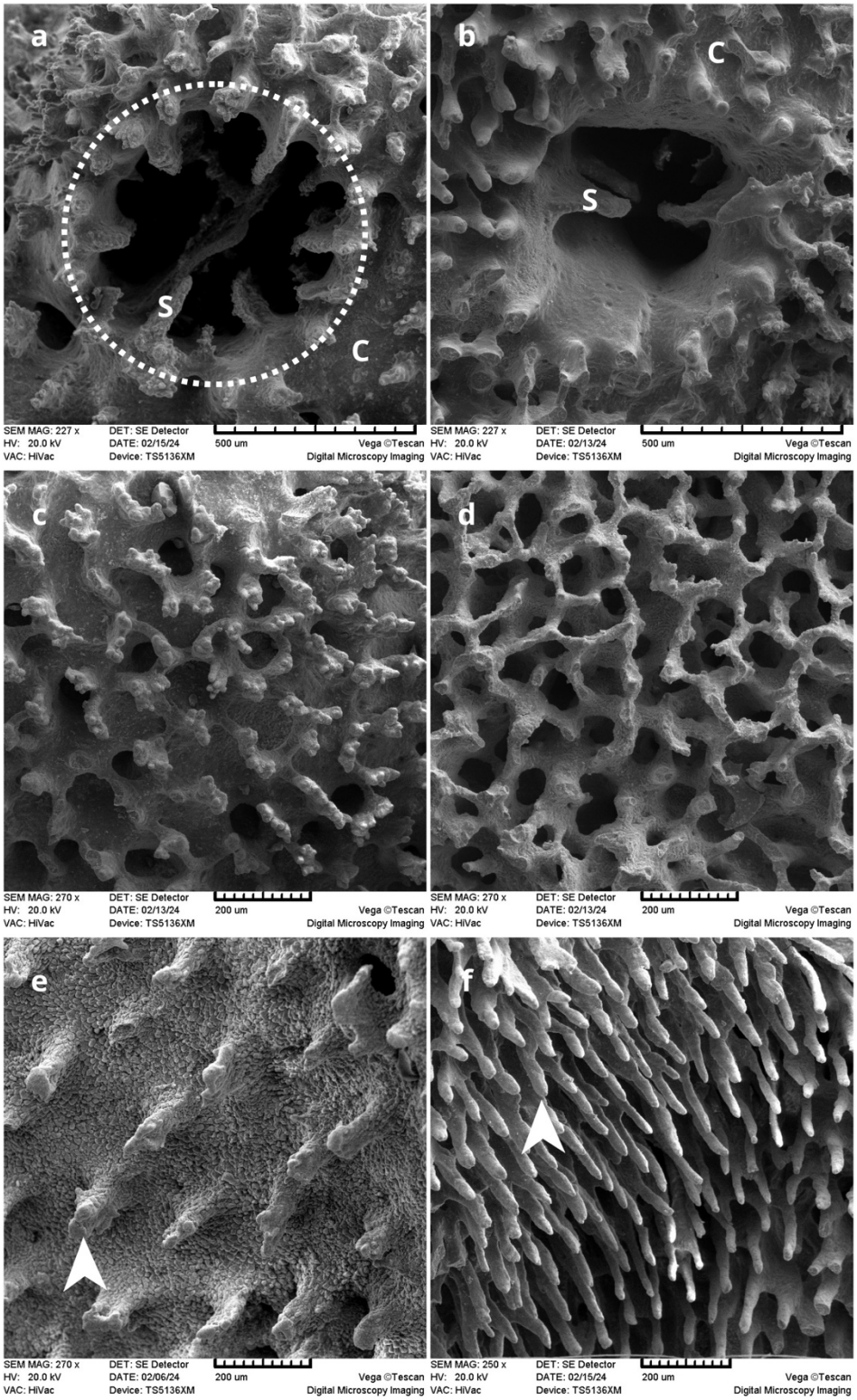

Figure 2. Main morphological characters in *Acropora* GA skeleton versus healthy skeleton. **a)** Corallite in healthy area with distinct corallite wall (Dashed circle) slightly protruding on the surface of coenosteum (C), and calyx with normally developed and complete septa (S). **b)** Corallite in diseased area with abortive septa (S) and obscured corallite wall. **c)** Coenosteum of healthy area, compact and well organized. **d)** Coenosteum of diseased area inhomogeneous and lax. **e)** Thick and well-ornamented spinules (arrowhead) protruding from the coenosteum in healthy area. **f)** Densely packed and thin spinules (arrowhead) in diseased areas.

Septa projections towards the center of corallite were underdeveloped or absent. In addition to structural changes, calices in affected regions were significantly enlarged, with a diameter of 0.681 ± 0.14 mm (arithmetic mean ± standard deviation), compared to 0.605 ± 0.12 mm in healthy regions ($p < 0.001$).

The coenosteum in GA-affected regions exhibited a highly inconsistent trabecular arrangement, characterized by increased spaces and a lax skeletal framework.

This contrasted sharply with the well-organized, reticular coenosteum observed in apparently healthy skeletal regions. Spinules in GA-affected regions protruded prominently from the skeletal surface as densely packed, bristle-like structures with sharp and irregular ornamentation. In healthy skeletons, by contrast, spinules were thicker, regularly spaced, and displayed rounded, well-developed, tree-like projections emerging from a smooth coenosteal surface.

Pachyseris. Clear structural changes were also observed in *Pachyseris* skeletons between healthy and GA-affected fragments. Healthy specimens exhibited a regular arrangement of calices organized in long, continuous rows running parallel to one another. In GA-affected regions, calices were randomly distributed, lacking the orderly alignment in valleys of calices characteristic of healthy skeletons (**Fig.3** & **Fig.4**).

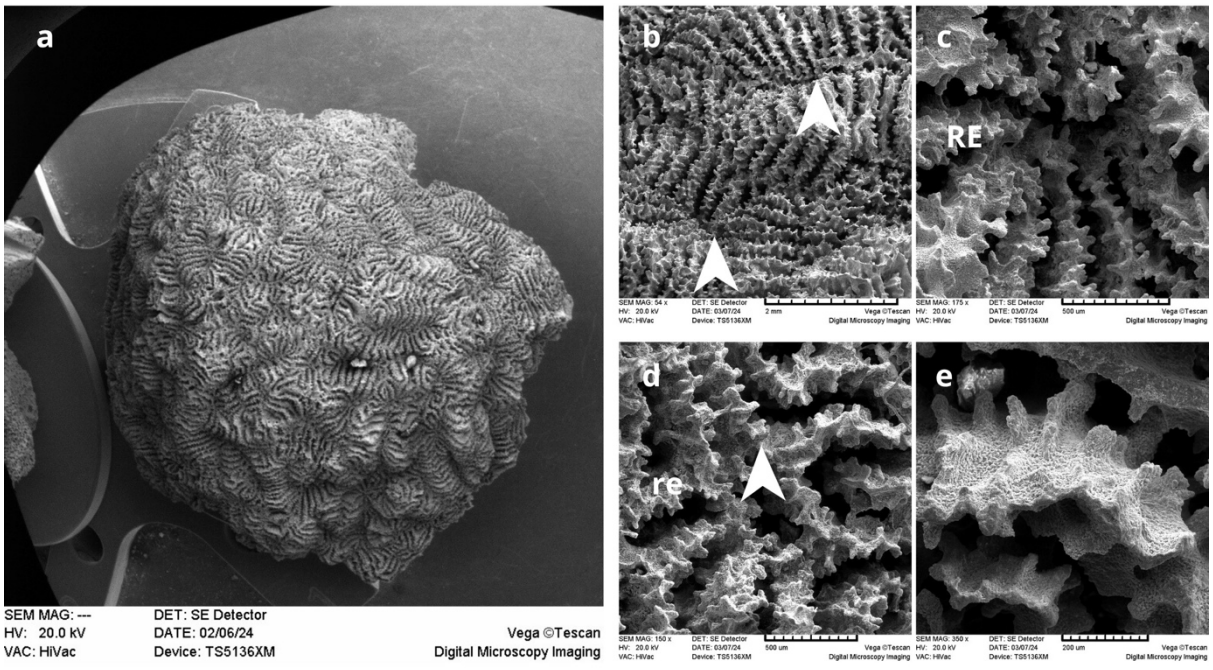

Figure 3. SEM imaging of GA skeleton in *Pachyseris*. **a)** Fisheye-view image of diseased skeleton fragment. **b)** Calices are randomly arranged and difficult to detect (arrowheads) **c)** Higher magnification of a confounded calyx, with unparallel radial elements (RE). **d)** Radial elements randomly arranged. Note the point of convergence between radial elements of calices (arrowhead). **e)** Higher magnification of radial elements.

Radial elements in diseased regions were irregularly branching and anastomosing, deviating from the straight parallel rows which were arranged perpendicular to and connected rows of calices in the bordering healthy skeleton. Furthermore, the columella was well-defined and developed in healthy skeletons, displaying distinct dash-like processes. In contrast, GA-affected regions showed a rudimentary or completely absent columella.

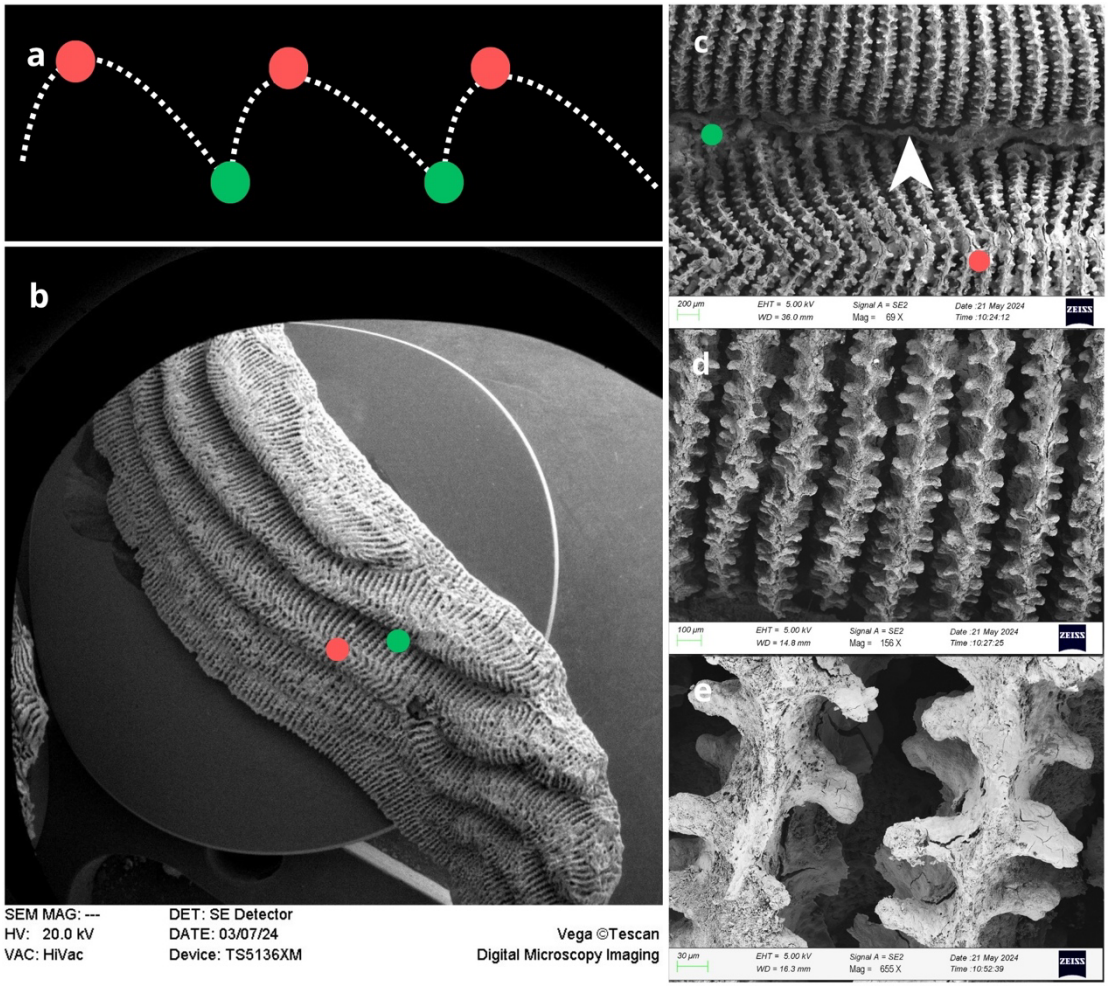

Figure 4. *Pachyseris* healthy skeleton. **a)** Graphical representation showing calices arrangement in cross section: calices are organized in rows inside valleys (green dots), which are connected by ridges (red dots) of radial elements (white dashed lines). **b)** Fisheye-view SEM image of healthy skeleton. **c)** Calices in line inside a valley (green dot). Note the well-developed columella of calices (arrowhead). Radial elements of different calices orderly converge over ridges (red dot) **d)** Well-organized, parallel radial elements exhibiting a distinct zigzag pattern. **e)** Higher magnification of radial elements.

3.5 DISCUSSION AND CONCLUSION

Despite clear species-specific differences in skeletal architecture, both *Acropora* and *Pachyseris* exhibited pronounced and convergent disruptions of primary skeletal macromorphology in GA affected regions. In both genera, these alterations align with previous studies linking GAs to pathological calcification and skeletal malformations (Cheney, 1975; Gateño et al., 2003; Frazier et al., 2017; Andersson et al., 2020), suggesting a common pathological process underlying GA development across contrasting coral growth forms.

In *Acropora*, GAs were primarily expressed through localized distortion or loss of corallite architecture, including malformed or absent corallites and reduced septal development. These features are consistent with impaired biomineralization and altered organic matrix protein regulation reported in lesioned areas of diseased corals (Zhang et al., 2017; Wong et al., 2021). In contrast, *Pachyseris* exhibited disruption at the colony-scale organizational level and architectural inconsistency, indicating failure to maintain skeletal patterning rather than localized corallite deformation.

Despite these taxon-specific manifestations, both genera shared key pathological traits, including enlarged skeletal elements and loss of fine-scale organization, likely driven by abnormally rapid and poorly regulated calcification (Domart-Coulon et al., 2006; Yasuda et al., 2012; Andersson et al., 2020). The resulting lax trabecular frameworks and altered surface ornamentation suggest compromised mineral deposition and matrix organization, potentially reducing mechanical integrity and increasing vulnerability to environmental stress and microbial colonization (Cheney, 1975; Rich et al., 2021).Together, these comparative patterns indicate that while GAs manifest differently according to skeletal architecture, they converge toward a shared outcome of disrupted skeletal construction. This underscores the need for detailed microstructural analyses to resolve the mechanistic pathways driving GA formation and to better understand how chronic stress and disease interact to shape coral skeletal responses under ongoing climate change.

In conclusion, growth anomalies in *Acropora* and *Pachyseris* cause distinct skeletal malformations indicative of possibly disrupted biomineralization. However, ultrastructural morphology alone do not fully capture the complexity of these anomalies. Integrating assessments of overlying tissue condition is essential for accurate diagnosis and understanding of their development (See **chapter 4**). This combined approach is critical for improving coral health monitoring and guiding conservation efforts in the light of escalating environmental challenges.

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CHAPTER 4

PATHOLOGY OF CORAL GROWTH ANOMALIES IN THE MALDIVES: INSIGHT FROM *ACROPORA*, *MONTIPORA* AND *PACHYSERIS*

This work is currently under review as:

Bises C., Dennis M.M, Gobbato J., Aeby S.G., Galli P. and Montano S. Pathology of Coral Growth Anomalies in the Maldives: Insights from *Acropora*, *Montipora*, and *Pachyseris*. *Diseases of Aquatic Organisms*. Manuscript: DAO-2025-10-004.

4.1 ABSTRACT

Coral growth anomalies (GAs) are chronic skeletal lesions that alter coral morphology and functionality, yet their etiology remains poorly understood, particularly in understudied regions such as the Maldives.

This study, intended to deepen our understanding on these lesions, provides the first detailed morphological and histopathological characterization of 15 GAs from the Maldivian Archipelago across three coral genera, *Acropora*, *Montipora*, and *Pachyseris*, with the last lacking a histopathological characterization to date.

Common histological findings included calicoblast hypertrophy (74%), endosymbionts depletion in the surface body wall (60%) corresponding to grossly observed discoloration of the GA, absence of gonadal development (80%), and diffuse mucocyte hyperplasia (seen only in *Pachyseris*). Most GAs (60%) contained centrally located masses of organisms that corresponded to regions of skeletal cavitation observed on sub-gross examination. Organisms included fungi, algae, cyanobacteria, diatoms and labyrinthulomycetes, which in 90% of cases were invading surrounding tissues, questioning their role in pathogenesis. Additionally, some GAs had solitary endolithic sponges (26%) or degenerate sponge-like debris (13%) in gastrovascular cavities. These findings support a multifactorial etiology for the lesion, reinforcing the complexity of GAs and the limitations of current diagnostic frameworks.

4.2 INTRODUCTION

Coral diseases significantly threaten coral reef ecosystems, contributing to declines in coral biodiversity and community worldwide, (Weil et al., 2006; Willis et al., 2019) a trend exacerbated by increasingly frequent and severe heatwaves that cause mass bleaching and coral mortality (Camp et al., 2018; Emslie et al., 2024). To date, at least 40 coral diseases have been reported affecting 200 coral species; however, those accounts represent a fraction of coral species and geographical locations (Bruckner, 2015; Morais et al., 2022). Indeed, the number of reported diseases is expected to increase as influenced by changes in host susceptibility, pathogen virulence, and environmental stress related to climate change (Sokolow, 2009; Burke et al., 2023; Hawthorn et al., 2023). The minority of known coral diseases have comprehensive pathology descriptions alongside identification of a single causative agent.

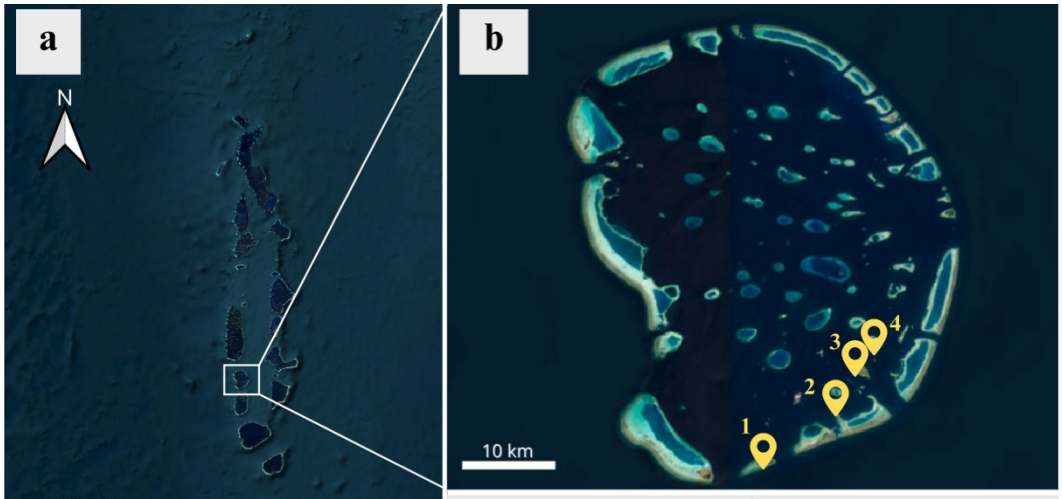
Growth anomalies (GAs) are a distinct lesion type in corals characterized by solitary to multifocal aberrant skeletal masses with various morphologies with reduced to absent corallites or corallite gigantism typically overlaid by hypopigmented tissues (Work & Aeby, 2006).

The etiology of GA remains uncertain, but various causes include UV radiation (Coles & Seapy, 1998), genetics (Peters et al., 1986), environmental degradation (Kaczmarsky, 2006), infectious agents (Domart-Coulon et al., 2006), senescence (Irikawa et al., 2011), endolithic organisms (Work et al., 2008) or host response to corallivory (Kaufman, 1977). Histopathology of growth anomalies includes basal body wall proliferation, necrosis of basal body wall and mesenteries, depletion of endosymbiotic algae (i.e., Symbiodinacea), absence of polyps or gonads (Work et al. 2008), or polyp gigantism (Rich et al., 2021). GA are thought to lead to reduced reproductive capacity and feeding efficiency (Burns & Takabayashi, 2011; Preston & Richards, 2021).

Most reported cases involve Indo-Pacific corals from the families Acroporidae and Poritidae, but histological data from this region remain limited (Yamashiro et al., 2000; Gateño et al., 2003; Work et al. 2008; Williams et al., 2011). In the Maldives, where no histopathological studies have been conducted, growth anomalies were only recently documented (Bises et al., 2024). To address this gap, we systematically describe the pathology of GAs in Maldivian corals belonging to the genera *Acropora*, *Montipora*, and *Pachyseris*, integrating both gross morphological observations and microscopic pathological analyses.

4.3 MATERIALS AND METHODS

Between February 2023 and November 2024, 15 GA biopsies were collected from three coral genera, *Acropora*, *Pachyseris*, and *Montipora*. The samples were taken by scuba diving from reef sites around Faafu Atoll (03°03'20"N, 72°53'28"E), Republic of Maldives, at depths of 7–20 meters (**Fig.1**). Biopsies were sampled with the use of hammer and chisel collecting mass of lesion of various sizes and the surrounding grossly unaffected tissue as control tissue.



SITES	GPS coordinates
1- Dharamboodhoo	3.059690° N, 72.924664° E
2- Maghoodhoo	3.090273° N, 72.967601° E
3- Bileiydhoo	3.117843° N, 72.980033° E
4- M&Ms	3.123731° N, 72.991487° E

Figure 1. Geographical contextualization and sampling sites in the Faafu atoll **(b)**, in the Republic of Maldives **(a)**. The table shows name of sites pinned on map **b** and corresponding GPS coordinates.

The samples were labelled and the tissues were fixed immediately after collection in a 1 to 4 ratio of zinc-formaldehyde concentrate diluted in seawater (Z-Fix, Anatech) for 1:5 final concentration with approximately a 10 to 1 ratio of fixative to tissue. The biopsies were then transferred to the College of Veterinary Medicine at the University of Tennessee for histological processing. Photographs were taken of biopsied lesions before decalcification and under the stereomicroscope to help define lesion type and aid in trimming tissues for histology.

Before trimming, the epidermal surface of the GA was marked using tissue dye (Bradley products, TMD) to facilitate microscopical discrimination of the grossly affected coral tissue, the biopsy margin, and the apparently healthy tissue. Coral fragments were decalcified in formic acid (Formical-2000, StatLab medical products inc., United States) for about 4 hours. Tissues were

trimmed sagittally to include the interface with the bordering grossly normal tissue. Sections were later placed into histology cassettes, wrapped in filtering paper to help maintain the proper orientation of the tissue. Care was taken not to stretch or squeeze the tissue to avoid artifacts that may modify the structure and size of the decalcified skeletal ridges for microscopy observation. Tissues were briefly rinsed with tap water to remove any residues of Formical and then kept in Z-Fix until they were routinely processed for histology. Tissues were embedded in paraffin wax, surface decalcified if needed, trimmed in a rotary microtome at 4 μm, mounted onto microscope slides, and stained with hematoxylin and eosin. In certain cases, further sections were made and stained with special stains, such as Giemsa to better accentuate protists and Grocott methenamine silver stain (GMS) for fungi. The lesion was qualitatively assessed for each GA sample with an Olympus BX43 microscope while comparing to tissues in apparently healthy areas. Control tissues were defined as entire control biopsies or grossly normal areas bordering the GA in the same biopsy.

The following coral tissue changes were noted on microscopy: necrosis (Hawthorn et al., 2023), endosymbiont loss was recorded as a clear depletion to absence of endosymbionts in the surface body wall compared to paired control tissues, calicoblast hypertrophy was defined as a change from squamous to cuboidal or columnar with or without hyaline membranes, and basal body wall hyperplasia (Work et al., 2008). Gonadogenesis was recorded.

Specific microscopic lesions were also scored for severity based on the percent area coverage in a field area, which was representative of the most severely affected regions across all slides evaluated. The field diameter was calculated as the field number of the ocular/objective magnification, and the diameter was used to determine field area (Meuten et al., 2016). Severity of calicoblastic hypertrophy, mucocyte hyperplasia, hyaline lamellae deposition and necrosis were scored as 1 if <25%, 2 if ≥25 to 75% and 3 ≥75% of the basal body wall in the field area of 2.54 mm^2 (10x) was affected.

Microscopic organisms were classified and identified based on morphological features as reported in **Table 1**. In addition, we annotated the presence of cell-associated microbial aggregates (CAMAS) in the tissues of the biopsies.

Lastly, calicoblast hypertrophy and focal masses of endolithic organisms were tested for possible correlation with Pearson's rank using SPSS ver. 30.0.0.0 (IBM, New York).

Table 1. List of microscopic organisms and their morphological features used for identification.

Microscopic organism	Key morphologic features	Reference
Fungi	Filamentous hyphae, possibly with septa or branches	Larone, 1994
Algae	Thick cell walls; filamentous or mosaics of cuboidal to rectangular cells	Work et al., 2015
Cyanobacteria	Filamentous, chain-like aggregations of oval to rectangular cells	Kramarsky-Winter et al., 2014
Helminths (i.e. nematodes or platyhelminthes)	Vermiform multicellular organisms, acoelomate or pseudocoelomate, lack true segmentation	Gardiner et al., 1998
Sponges	Multicellular organisms with siliceous spicules and zooxanthellae	Ereskovsky & Lavrov, 2021
Crustaceans	Cuticle, segmented appendages, striated muscle, gut, hepatopancreas and reserve inclusion cells	Halanych, 2004
Annelid (i.e. segmented worms)	Segmented body with body cavity, Alimentary, nervous and circulatory systems, setae anchored in epidermis	Molnár et al., 2021
Diatoms	Radially to bilaterally symmetric, geometrically shaped to elongate cells, surrounded by clear cell wall (frustule)	McLaughlin, 2012
Labyrinthulomycetes	Elongate to ovoid single-celled organisms, embedded in a network of fine, transparent filaments (ectoplasmic net)	Burge et al., 2012 Polglase, 2019

4.4 RESULTS

4.4.1. GROSS PATHOLOGY

The GAs affected three different genera, *Acropora*, *Montipora,* and *Pachyseris*, each representing distinct colony growth forms. While *Montipora* and *Pachyseris* exhibited encrusting form, *Acropora* colonies displayed both tabular and branching morphologies. GAs gross morphologies were categorized into four distinct morphological types building on previously established nomenclature (Work & Aeby, 2006; Work et al., 2008b). In particular, bosselated GAs characterized by sessile, slightly raised masses with a smooth undulating surface, filling space between calices, principally located at the base of branches, and having reduced to vestigial polyps. Fimbriated were recognized as protruding diverging finger-like projections with a smooth surface and rare polyps. Nodular GAs were recognized by raised spherically to pedunculated, smooth-surfaced masses, notably lacking calices. Lastly, umbonate GAs characterized by broad-based smooth-surfaced convex protuberances (**Fig. 1**). Among the 12 *Acropora* GAs analyzed, 11 exhibited a bosselated gross morphology (92%) and only one was fimbriated. Grossly, they were either blue-pink (n = 3), grey (n = 5), or white (i.e. bleached; n = 4). The GA in *Montipora* were bosselated and bleached. The two GA in *Pachyseris* had normal color and were umbonate and nodular respectively.

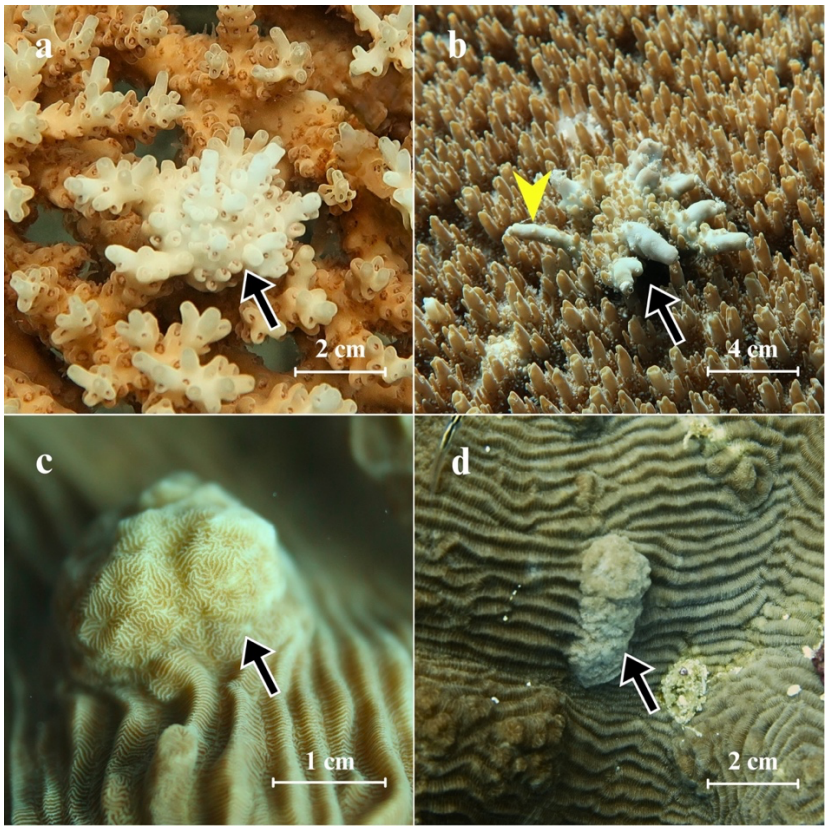


Figure 2. Growth anomaly morphotypes (arrows and arrowhead) in *Acropora* **(a-b)** and *Pachyseris* **(c-d)**. **a**) bosselated, **b)** fimbriated, **c)** nodular, **d)** umbonate.

4.4.2 SUB-GROSS PATHOLOGY

The cut surface of the undecalcified biopsies analyzed under the stereomicroscope highlighted sub-gross morphological characteristics, facilitating correlations with microscopic changes. In *Montipora*, *Pachyseris*, and *Acropora*, the cut surface of undecalcified GAs contained scattered empty spaces of varying size that were lacking in the bordering coral tissue. Often, the small cavities coalesced into a larger one, variably containing organic matter (**Fig. 3a**). Moreover, one case of *Acropora* presented in the center of the GA a large mass of non-skeletal matter surrounded by coral tissue and skeleton (**Fig. 4a-c**). In another case, the mass exhibited a discrete central area of acute tissue loss at its tip.

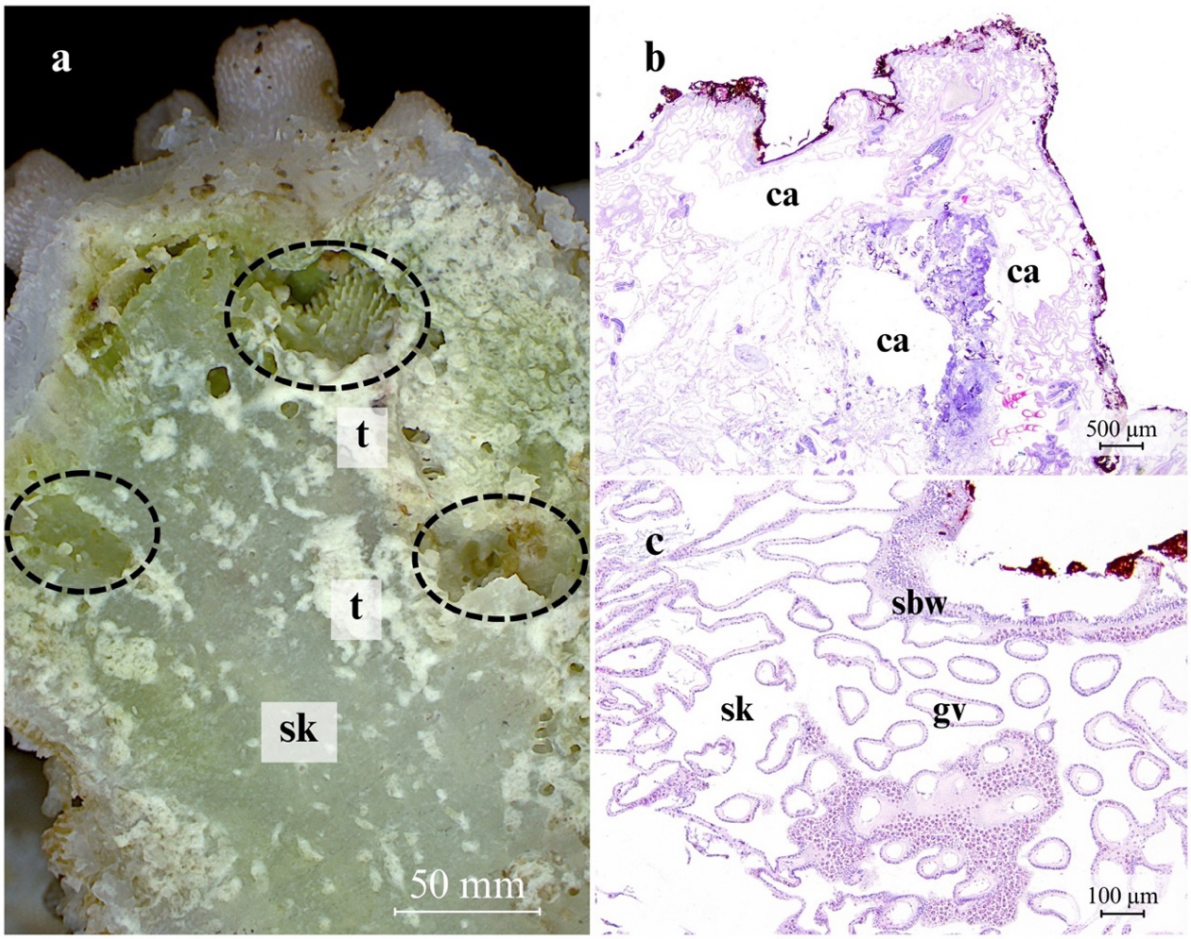


Figure 3. Overview sections of growth anomaly (GA) in *Acropora*. **a)** Cut surface of a bosselated growth anomaly mass examined by stereomicroscopy. Note the large empty cavities in the coenosteum (circles). t: tissue; sk: skeleton. **b)** Photomicrograph of **a** highlighting the large cavities (ca) filled with organic matter. Hematoxylin and eosin. **c)** Photomicrograph of **a** showing basal body wall hyperplasia as indicated by increased profiles of skeletal spaces (sk) and gastrovascular canals (gv). Note lack of polyp structure including mesenterial filaments. sbw: surface body wall. Hematoxylin and eosin

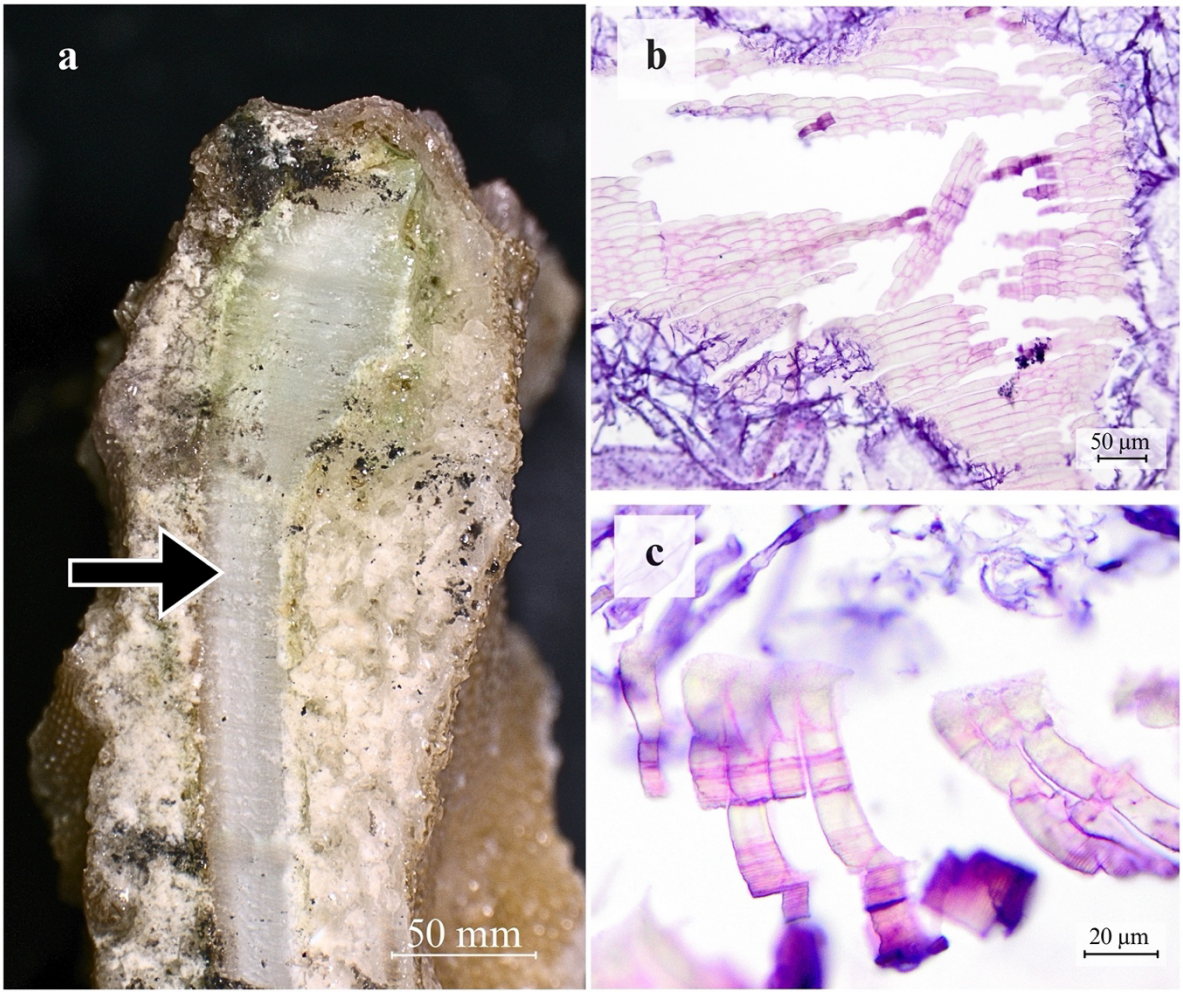


Figure 4. Cut surface and microscopic sections of *Acropora* growth anomaly (GA). **a)** Cut surface of a bosselated GA examined by stereomicroscope in *Acropora* with core of skeleton containing a non-skeletal matter (arrow). **b)** Microscopic section of the area denoted in **a** showing a degenerate alga. Hematoxylin and eosin. **c)** Closer view of B showing absence of nuclei but striated structure consistent with the remnant cell wall of putative crustose coralline degenerate alga. Hematoxylin and eosin.

4.4.3 MICROSCOPIC PATHOLOGY

Microscopically, all GAs across all the three genera represented various degrees of basal body wall hyperplasia (**Table 2**). This was microscopically evident by increased profiles of gastrovascular canals and variably sized trabeculae of skeleton with absence of polyps.

Table 2. Histopathological characterization (N(%)) of GAs in *Acropora*, *Montipora* and *Pachyseris*.

	Acropora		***Montipora***		***Pachyseris***	
Sample area	GA	control	GA	control	GA	control
N	12	4	1	1	2	1
Basal body wall hyperplasia	12(100)	-	1(100)	-	2(100)	-
Calicoblast hypertrophy	9(75)	-	-	-	2(100)	-
Median (range) calicoblast hypertrophy score	2				3	
Necrosis	9(75)	-	-	-	1(50)	-
Median (range) necrosis score	2				3	
Surface body wall gastrodermis endosymbionts loss	6(50)	-	1(100)	-	2(100)	-
Focal masses of organisms	8(58)[a]	-	-	-	1(50)	-
Fungal hyphae*	8(100)	-	-	-	1(50)	-
Filamentous algae	6(75)	-	-	-	-	-
Sponge	6(75)	-	-	-	-	-
Helminths	5(60)	-	-	-	-	-
Cyanobacteria	6(75)	-	-	-	-	-
Helminths	5(60)	-	-	-	-	-
Diatoms	2(25)	-	-	-	-	-
Labyrinthulomycetes	2(25)	-	-	-	1(50)	-
Hyaline lamellae deposition	6(50)	1(25)	-	-	1(50)	-
Median (range) hyaline lamellae deposition score	1.5	2			2	

Polyp atrophy/loss	4(33)	-	1(100)	-	1(50)	-
Gonadogenesis	2(16)	4(100)	-	1(100)	1(50)	1(50)
Mucocyte hyperplasia	-	-	-	-	2(100)	-
Other organisms[b]	10(83)	-	-	-	2 (100)	-
Gastrodermal cell-associated microbial aggregates (CAMA)	3(25)	3(75)	-	-	-	-
Endolithic sponge**	2(16)	-	-	-	1(50)	1(50)
Gastrovascular cellular debris***	2(16)	-	-	-	1(50)	-
Gastrovascular crustacea	1(8)	-	-	-	1(50)	-
Endolithic annelid	1(8)	-	-	-	-	-
Endolithic putative crustose coralline alga (CCA)	1(8)	-	-	-	-	-
Endolithic crustacea	-	-	-	-	1(50)	-

[a] Organisms were invasive in 7/8 and associated with tissue necrosis in 5/8 instances.
* Organisms invasive and associated with necrosis of basal body wall.
[b] Organisms not included within masses of endoliths.
** Diffusely present within GA skeleton with absence of other endolithic organisms.
*** Thought to represent degenerative aspiculate sponge.

The hyperplastic basal body wall was lined by well-differentiated tissues normally comprising the basal body wall, calicodermis, and gastrodermis. Thus, lesion identification was facilitated by correlation of histological sections with gross sample preparation, microscopic identification using tissue ink, and identification of a transition between normal tissue and the GA as indicated by polyp loss and surface contour distortion **(Fig. 4b,c)**. In areas where convex protuberances were observed grossly, basal body wall hyperplasia **(Fig. 5)** formed masses that distorted the surface contour of the coral, sometimes covering the polyp, with deep displacement of the actinopharynx or tentacles **(Fig. 5a,c)**.

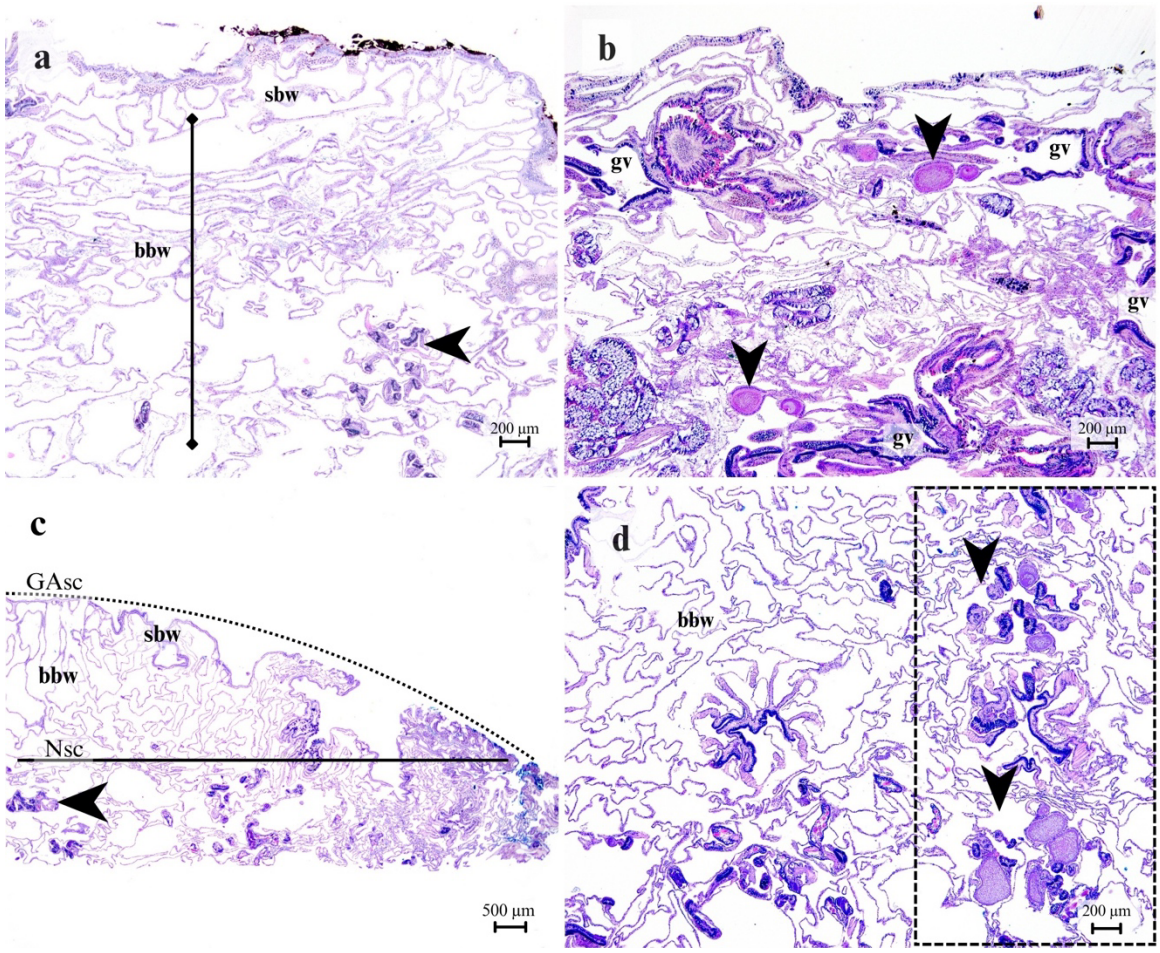


Figure 5. Basal body wall hyperplasia in *Acropora* and *Montipora*. sbw: surface body wall; bbw: basal body wall. Hematoxylin and eosin. **a)** Basal body wall hyperplasia in *Acropora* growth anomaly (GA). Note rare polyp structures deeply displaced (arrowhead). **b)** Control *Acropora* region with numerous polyp structures noted by gastrovascular cavities (gv). Note presence of numerous developing ova (arrowheads). **c)** Basal body wall hyperplasia in *Montipora* growth anomaly. Note the surface contour deviation (GAsc dashed line) from the colony normal surface contour (Nsc full black line). Note deep displacement of polyp structures in the mass (arrowhead). **d)** Control region (rectangle) next to growth anomaly region in *Montipora*. Note different presence of polyp structures and numerous developing ova (arrowheads) in the healthy region.

The majority of GAs (9/15) in this study also had central masses of densely aggregated organisms within a region lacking coral tissue, corresponding to skeleton and skeletal cavities observed within GAs sub-grossly. These were bordered by basal body wall hyperplasia, and sometimes the overlying coenenchyme was thinned relative to its thickness in the tissue bordering the GA (n=6; **Fig. 6a**).

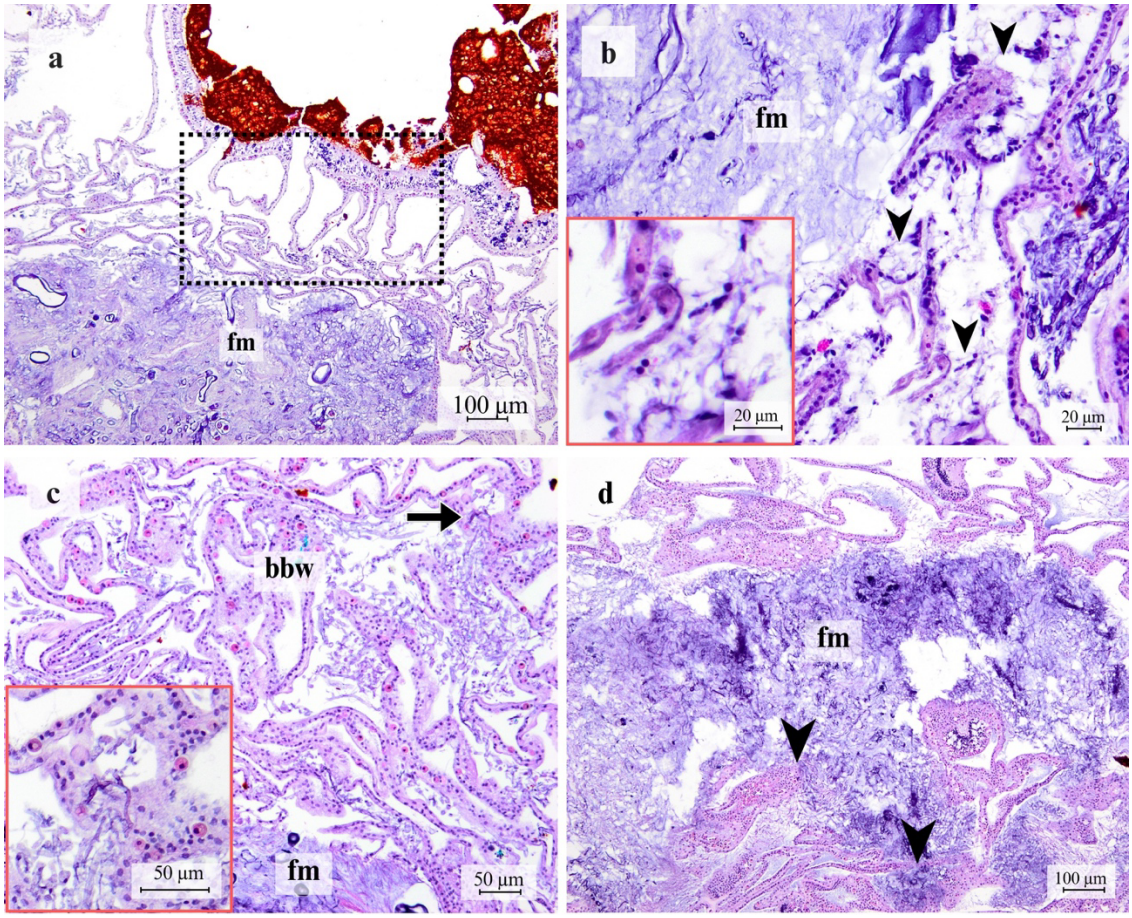


Figure 6. Growth anomalies (GAs) with focal masses of organisms. fm: focal mass of organisms; sk: skeleton; sbw: surface body wall. bbw: basal body wall. Hematoxylin and eosin. **a)** Growth anomaly in *Acropora* with a focal mass of mixed algae, fungi and cyanobacteria with an area of coenenchymal thinning (rectangle). **b)** Focal mass of algae and fungi in *Acropora* extends into necrotic tissues (arrowheads and inset) of the host in the mass. **c)** Focal mass of algae, fungi and cyanobacteria adjacent to the hyperplastic basal body wall in *Acropora* GA. Fungal hyphae focally invade host tissues (arrow, inset). **d)** Mass of mixed algae and fungi in *Acropora* GA, focally invading host tissues (arrowheads).

The composition of most focal masses of organisms was variable, including fungal hyphae, filamentous algae, cyanobacteria, sponges, helminths, labyrinthulomycetes, and diatoms (**Table 2**). Fungal hyphae within these masses focally invaded the host basal body wall (n = 7), associated with necrosis of the affected basal body wall (n = 5) (**Fig. 6b,c**). Within most masses, there were also scattered rare structures that could not be morphologically assigned to any unique taxonomical group using previously described methods. Moreover, the organic matter noted sub-grossly in one of the masses in *Acropora* was morphologically consistent with a dead crustose coralline alga (CCA) (Quéré et al., 2015) **(Fig. 4b,c)**. Endolithic organisms, including algae, fungi, annelids, crustaceans and sponges were also present in a more dispersed distribution within the GAs of both *Acropora* and *Pachyseris*, but not in *Montipora* (**Fig. 7**).

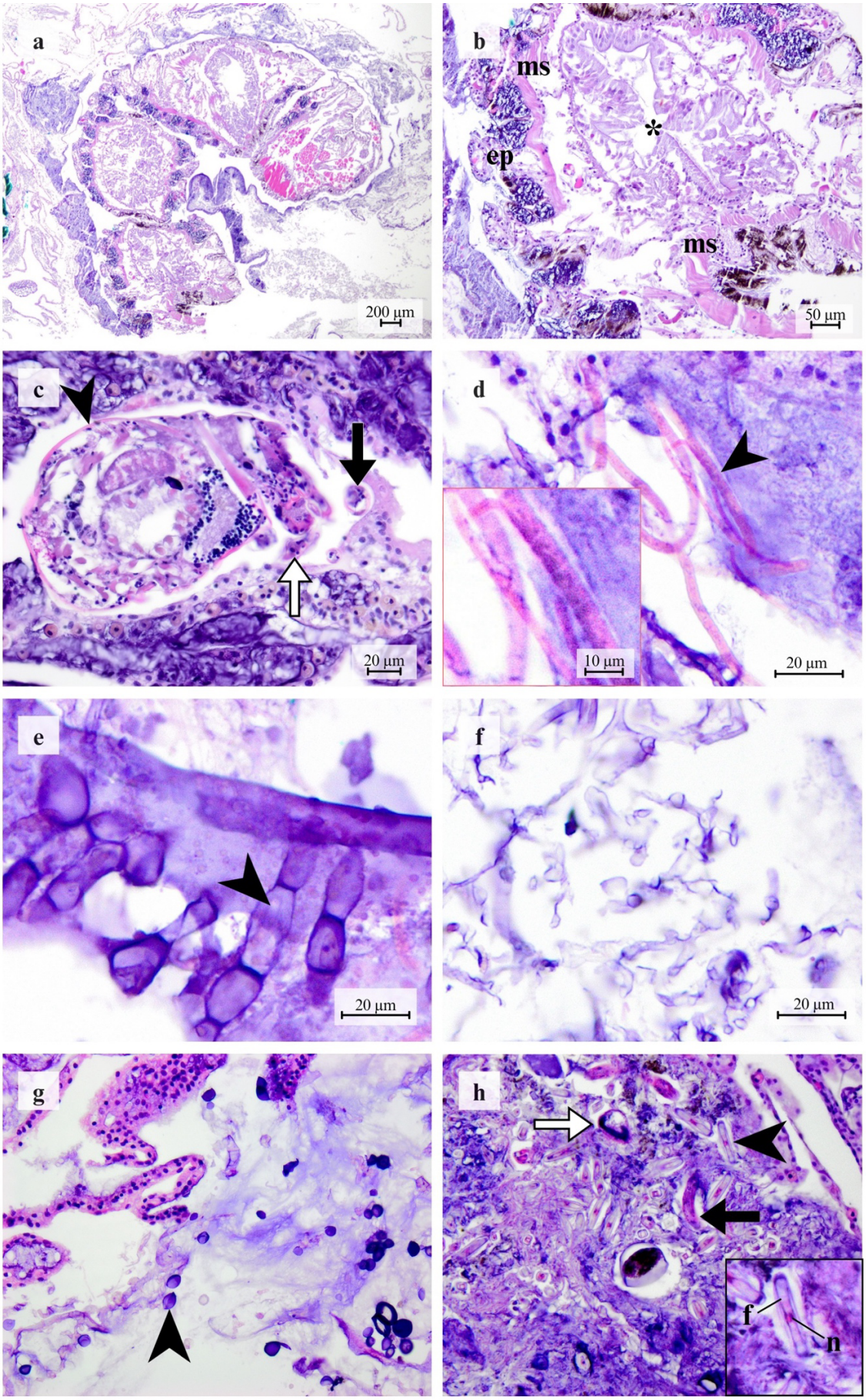

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Figure 7. Other organisms in growth anomalies. Hematoxylin and eosin. **a)** Annelid in *Acropora*. Note segmented body. **b)** Closer view of a body segment of the annelid in **a**. Note the gut (asterisk), muscles (ms) and epidermis (ep). **c)** Crustacean in *Pachyseris*. Note appendages (white arrow), the cuticle (arrowhead), and the eye (black arrow). **d)** Cyanobacteria (arrowhead and inset). Note linear chains of rectangular cells. **e**) Algae (arrowhead) with thick cell wall. **f)** Filamentous fungal hyphae. **g)** Labyrinthulomycetes (arrowhead) within fine ectoplasmic net next to necrotic basal body wall in *Pachyseris*. **h)** Multiple organisms inside focal mass. Diatoms (arrowhead) with inset showing higher magnification of fusiform frustule (f) and nucleus (n); ciliate (white arrow); helminth (black arrow).

In contrast, these did not aggregate to form masses that replaced the coral tissue or skeleton. In three GAs of *Acropora* (n = 2) and *Pachyseris* (n = 1), where no focal mass of organisms was present, an endolithic sponge was abundant diffusely throughout the skeleton without any other endolithic organisms (**Fig. 8**).

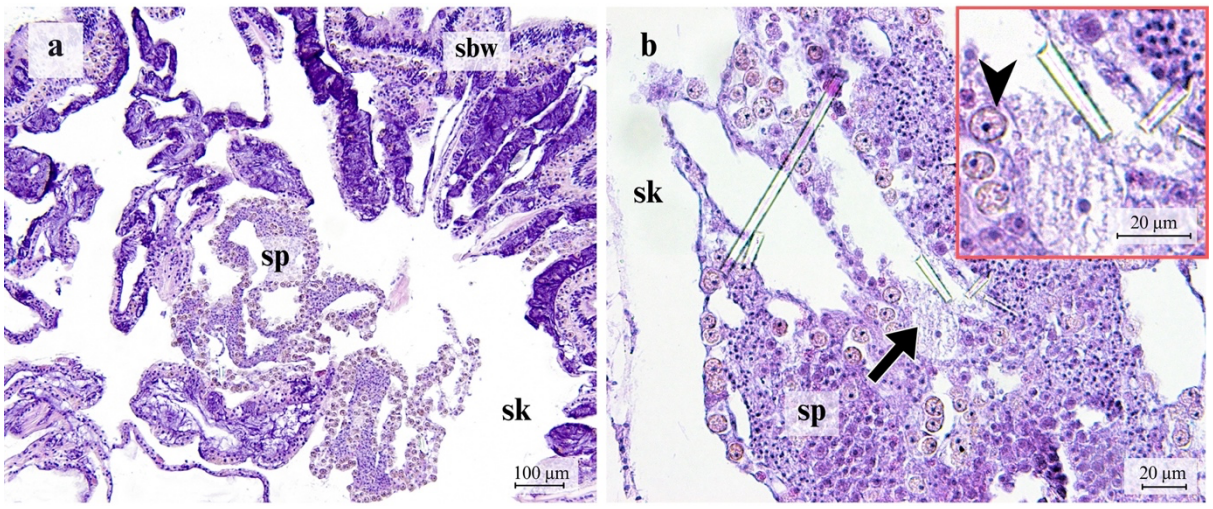

Figure 8. Endolithic sponge in *Acropora* and *Pachyseris* growth anomalies (GA). sk: skeleton. **a)** Endolithic sponge (sp) in a *Pachyseris* GA. sbw: surface body wall. **b)** Endolithic sponge (sp) in an *Acropora* GA with refractile spicules (arrow and inset). Arrowhead indicates zooxanthellae within the sponge. Note the absence of other organisms in the skeleton.

These sponges were widely extended throughout skeletal spaces, being more numerous within the GA than the bordering tissue. In Moreover, in two cases of *Acropora* GAs, the gastrovascular canals and, to a lesser extent, the skeleton were variably occupied by masses of bright eosinophilic material containing oval-shaped basophilic bodies presumptively representing cytoplasmic cellular debris and nuclear remnants (**Fig. 9a-c**). Discrete clear oval spaces were interspersed within these, suspicious for a degenerate aspiculate sponge (**Fig. 9c**). These putative gastrovascular degenerate sponges were substantially more numerous than observed in the bordering healthy tissue.

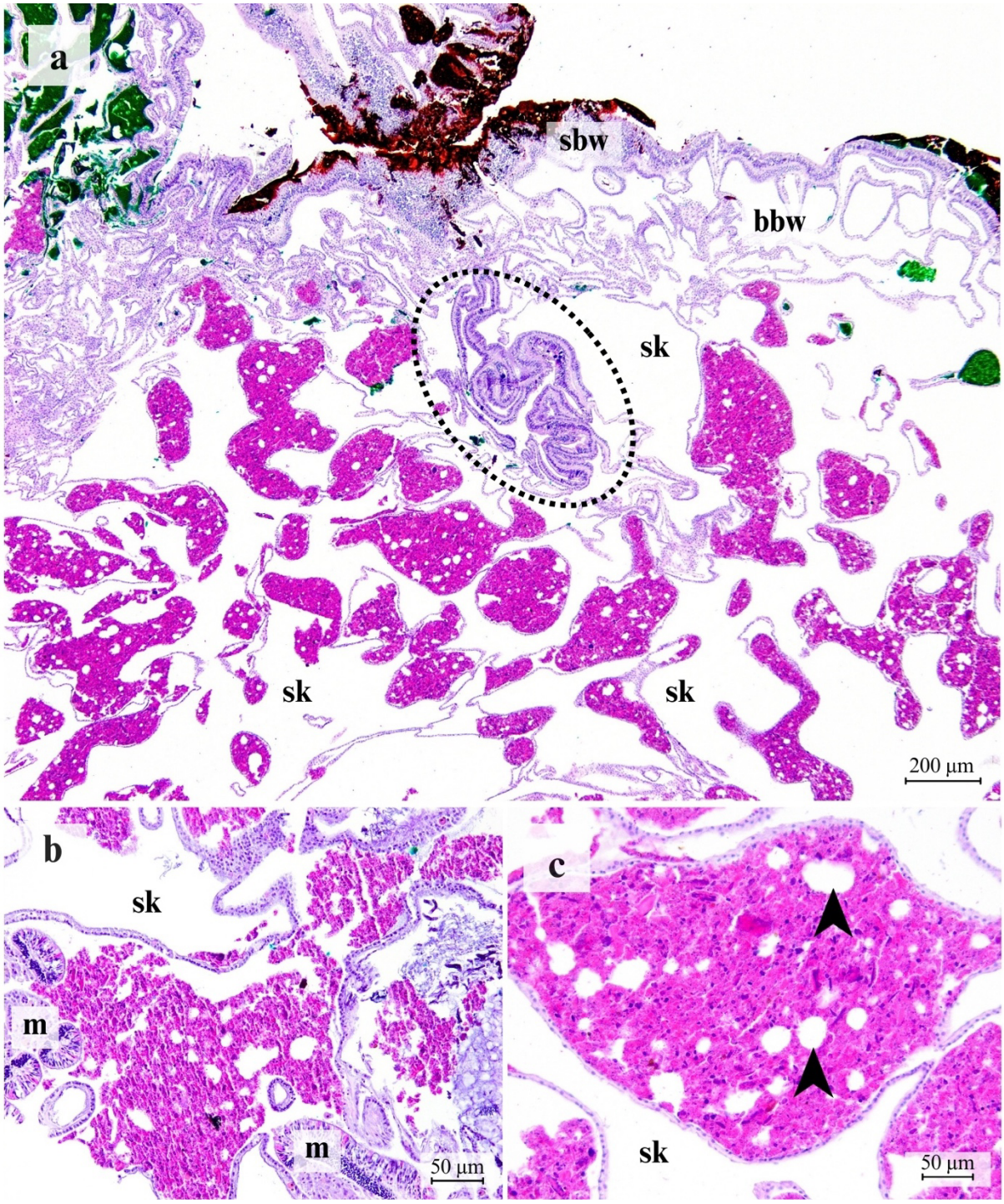

Figure 9. Gastrovascular debris in *Acropora* growth anomalies (GA). Hematoxylin and eosin. sk: skeleton **a)** Cellular debris in the gastrovascular cavities. Note the displacement of the polyp structure (circle) overlain by thickened region of basal body wall (bbw); sbw: surface body wall. **b)** Cellular debris next to mesenterial filaments (m). **c)** Closer view of the cellular debris composed of cytoplasmic and nuclear material, and oval to circular-shaped clear spaces (arrowheads).

Crustaceans were in the skeleton and in the gastrovascular spaces of one *Acropora*, and one *Pachyseris*. Cell-associated microbial aggregates (CAMAs) were found in the surface body wall gastrodermis only in the *Acropora* genus, in both control areas (n = 3) and in the GAs (n = 3). Calicoblast hypertrophy (**Fig. 10**) occurred with moderate severity in 11 cases, typically with hyaline lamellae deposition (n = 7) (**Fig.10a**), and was present in all GA cases with focal masses of organisms (n = 9). Indeed, a strong positive correlation was found between calicoblast hypertrophy and the presence of endolithic organisms (Pearson's Test, ϱ (13) = .74, p = .02*).

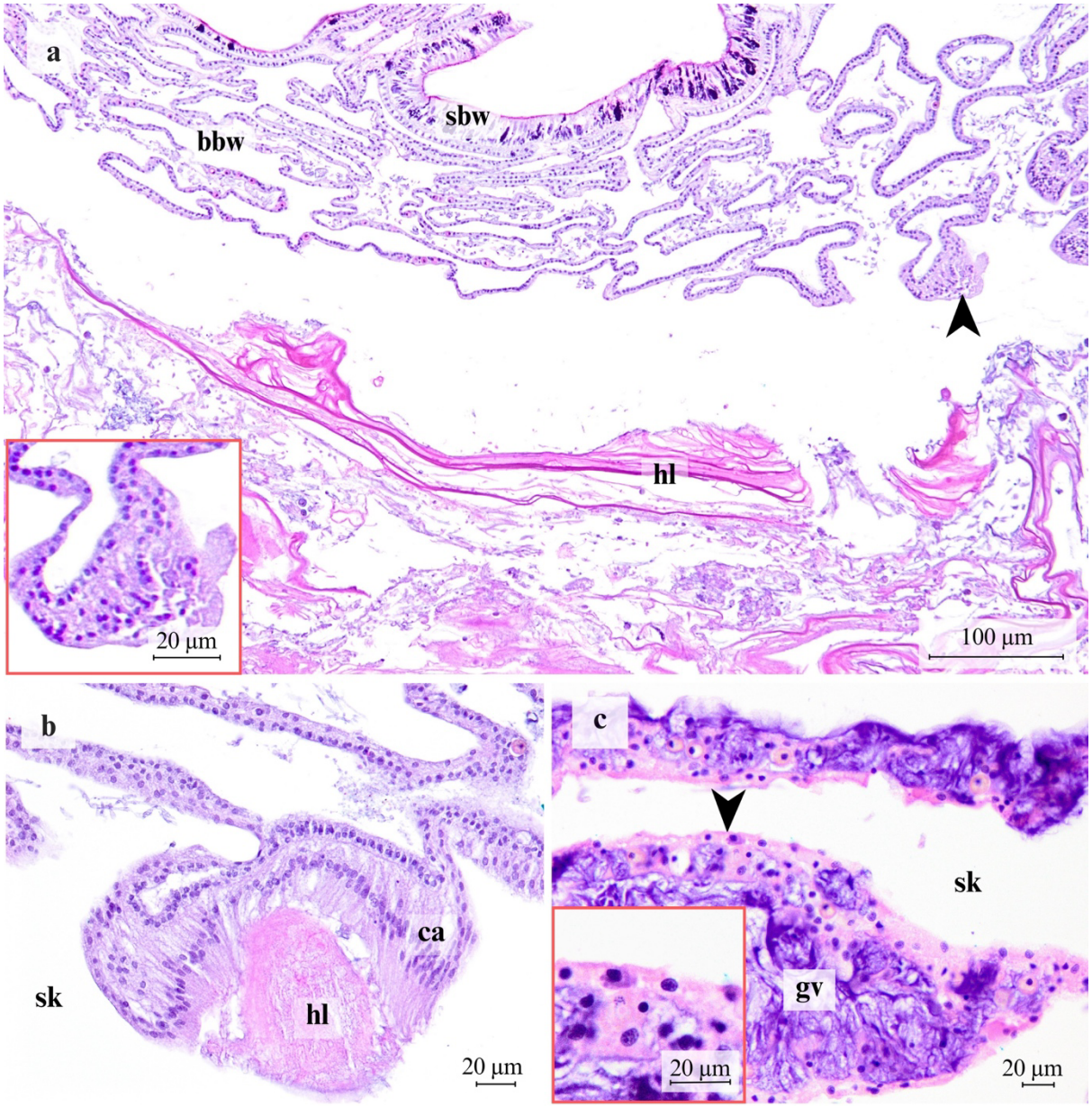

Figure 10. Calicodermal changes in the basal body wall of growth anomalies (GA). sk: skeleton. Hematoxylin and eosin. **a)** Severe hyaline lamellae deposition (hl) in *Acropora* growth anomaly associated with calicoblast hypertrophy (arrowhead and inset). Note the absence of polyp structures. **b)** Calicoblast hypertrophy (ca) with hyaline lamellae deposition (hl) in *Acropora*. **c)** Diffuse calicoblast hypertrophy (arrowhead and inset) in *Pachyseris*. gv: gastrovascular cavity.

Necrosis was observed in 10 cases, often involving multiple tissues, including surface body wall (n = 9), basal body wall (n = 6), and mesenterial filaments (n = 8), and was moderate in severity. Endosymbiont depletion in the gastrodermis of surface body wall was noted in all three genera in correspondence with the grossly bleached appearance of GAs. *Pachyseris* was the only genus presenting diffuse mucocyte hyperplasia (in all samples n = 2) in GAs, consisting of an increase in both the number and size of mucocytes in the gastrodermis of surface and basal body walls and mesentery, typically >50% of cells lining these structures, along with mucus-filled gastrovascular cavities, in contrast to neighboring healthy tissue. (**Fig. 11**).

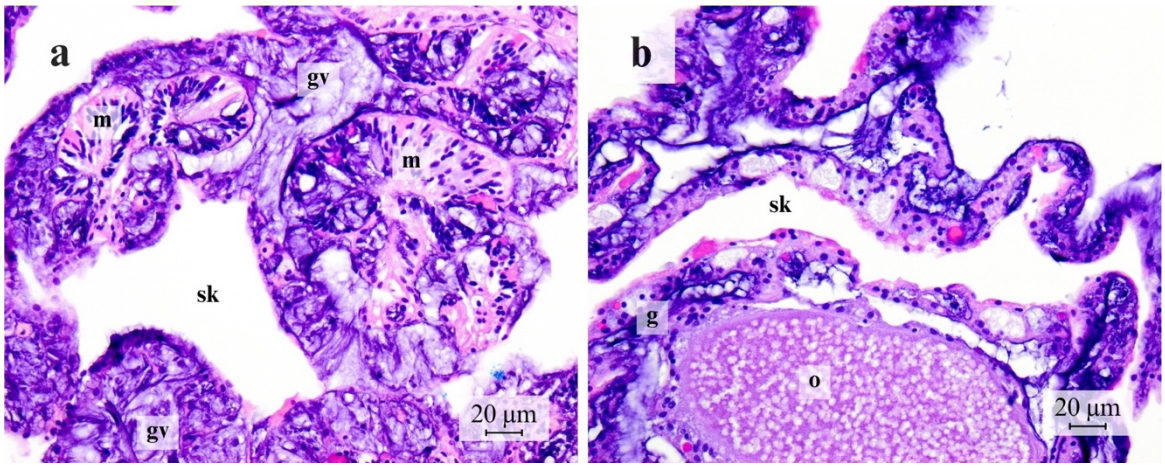

Figure 11. Mucocyte hyperplasia in *Pachyseris*. sk: skeleton. **a)** Increased mucocytes (>50%) in the basal body wall of a growth anomaly (GA) with excessive mucus filling gastrovascular cavities (gv). m indicates mesenterial filaments. **b)** Basal body wall in a control region; mesentery with developing ova (o) and gastrodermis (g) lined by <50% mucocytes.

Presence of gonads in GAs was noted in 20% of cases, whereas all the control areas (n=6) of all three investigated genera contained gonads. Both oocytes (n = 4) and spermaries (n = 2) in the lesion did not show prominent pathological features compared with gonadal tissues in control areas but were subjectively rare and less numerous.

4.5 DISCUSSION

Coral growth anomalies (GAs) have been documented globally with prevalence varying across regions and reefs (Gateño et al., 2003; Willis et al., 2004). While mortality rates differ among species, in Acroporidae, GAs often manifest as a chronic and progressive condition that could potentially result in colony death (Work et al., 2008; Das et al., 2022). Despite this, few studies have provided comprehensive gross and microscopic descriptions of their pathology (Williams et al. 2011; Work et al., 2015; Rich et al., 2021). However, the recent report about their occurrence in the Maldivian Archipelago (Bises et al., 2024) has emphasized their widespread distribution. It also highlights the need to unravel etiology and pathogenesis of GAs, ideally through long-term studies assessing their consequences on coral health and survival.

Despite their different gross presentations and coral genera examined, all GAs had a common microscopic lesion, basal body wall hyperplasia. This diagnosis is consistent with reports from other coral genera across different regions, including *Pocillopora*, *Colpophyllia*, *Diploria*, and *Pseudodiploria* (Work & Rameyer, 2005; Rich et al., 2021). The consistency of this microscopic lesion across multiple genera suggests that the genesis of GAs lie with altered gastrodermal or calicodermal cells of the basal body wall. Indeed, basal body wall hyperplasia is the term currently used for this overgrowth of the basal body wall, composed of well-differentiated calicodermis and gastrodermis together (Williams et al., 2011; Rich et al., 2021; Hawthorn et al., 2023). In pathology of vertebrates, hyperplastic tissues are typically expanded with a predominance of single cell types that have cytomorphological features of cellular immaturity (such as cytoplasmic basophilia, enlarged nuclei, or higher nuclear-cytoplasmic ratios), all of which are lacking in the basal body wall hyperplasia seen in coral GAs. As the understanding of GA pathogenesis improves, the term "basal body wall hyperplasia" will likely require refinement to reflect whether these GAs represent a malformation (e.g., coenenchyme-coenosteum hamartoma, or supernumerary developmental anomaly involving gastrovascular canals and skeletal trabeculae), a type of adaptive (e.g., hyperplasia or dystrophy) or uncontrolled growth (i.e., neoplasia), or other pathological process (Cockerell & Cooper, 1982). Similar basal body wall proliferations in Caribbean *Acropora palmata* were considered neoplastic and termed calicoblastic epithelioma; however, nuclear atypia, increased mitotic figures, or cellular homogeneity indicative of clonal growth habit are histological features of neoplasia that were lacking in Maldivian GAs and have yet to be convincingly demonstrated in any GA. Accordingly, the term "tumor" is often avoided for GAs to reduce misinterpretation of their occurrence. Similar to coral GAs, mammalian hamartomas can involve multiple well-differentiated tissue lineages and be acquired later in life with progressive growth

patterns that disrupt adjacent anatomical structures. Meaningful refinement of nomenclature will probably require cytogenic or molecular studies to determine cellular clonality, associated chromosomal or genetic abnormalities, and cell-cycle alterations.

Microscopically, GAs of Maldivian corals also frequently had foci of calicoblast hypertrophy, but this feature was inconsistent; for instance, calicodermis in *Montipora* GA was normal. Calicoblast hypertrophy is most likely confounded by the concurrent presence of endolithic organisms (Hawthorn et al., 2023), a relationship that demonstrated a strong positive correlation. This suggests that localized skeletal or microbial interactions may influence calicoblastic morphology rather than representing a primary driver of GA development.

Histologically, Maldivian GAs commonly had depletion of endosymbionts in the surface body wall, consistent with previous studies (Kaczmarsky, 2006; Work et al., 2015; Preston & Richards, 2021) and the associated gross presence of bleaching or altered pigmentation. Endosymbiont depletion likely reduces energy availability for routine physiological processes, including calcification and reproduction. The loss of endosymbionts generally represents a coral response to diverse stress conditions, often related to environmental disturbances or infectious agents that compromise colony health (Helgoe et al., 2024). In addition, the cells within GA lesions are considered as more rapidly dividing and putatively less differentiated (i.e. immature) compared to normal cells. As a result, they may either not yet have developed the capacity, or may be functionally incapable, of endocytosing the dinoflagellates (Work et al., 2015). Further transcriptomic essays together with ultrastructural analysis of GA-affected tissues using transmission electron microscopy (TEM) could help elucidate about defects in uptake mechanisms from alterations in downstream intracellular processing. Nonetheless, GA was consistently associated with a reduction of endosymbiotic algae and altered gross pigmentation of affected areas (Gateño et al., 2003).

The focal masses of organisms present within GAs is a unique aspect of their pathology in Maldivian corals. These large aggregations of diverse organisms (including algae, fungi, and cyanobacteria) or else, the presence of dispersed endolithic organisms (sponges, annelid, and crustaceans), suggest the possibility that they may contribute to the formation of GAs. Alternatively, they may simply opportunistically occupy distorted deposits of skeleton or pockets left by boring organisms. In all cases these masses invaded adjacent tissues, suggesting a potentially injurious role capable of triggering localized cellular responses. However, invasion remained relatively shallow despite high organismal abundance and diversity.

Regardless, endolithic organisms represent a recurring feature of GAs, with differences in both abundance and composition (Peters et al., 1986; Domart-Coulon et al., 2006; Work et al., 2008;

Rich et al., 2021). Being considered part of the coral holobiont, endolithic microscopic assemblages are influenced by physicochemical parameters of the microhabitat they inhabit, the coral skeleton. Their diversity and quantity depend upon multiple factors such as light, oxygen, and pH, and may also be species-specific (Voolstra et al., 2024). They have been studied as playing a significant role in primary productivity and supporting coral health and survival during stress events, such as bleaching (Work et al., 2015; Pernice et al. 2020). However, there is also evidence that some organisms may be pathogenic for the host, such as fungi, or bioeroders, as in the case of phototrophic endoliths (Bentis et al., 2000; Fordyce et al., 2021). Moreover, cyanobacterial mats are involved in the pathogenesis of black band disease and pink line syndrome, and endolithic fungi are associated with endolithic hypermycosis, sometimes manifesting grossly as focal pigmentation referred to as dark spots disease (Richardson, 2004; Work et al., 2008; Ravindran et al., 2015). The complexity and mutability of the microbial composition may lead to difficulties in assessing and studying their pathogenic potential. However, a more detailed characterization of microbial assemblages, along with an assessment of any deviations from typical composition and of endolithic organism density within the coral skeleton, is needed. These observations are essential to confirm or rule out the involvement of consistent microbial or endolithic contributors in the development of GAs. To enhance diagnostic resolution, more extensive histological investigations of healthy colonies are essential to establish robust diagnostic baselines for pathological investigations, especially in peculiar Maldivian coral species.

Some Maldivian GAs in *Acropora* and *Pachyseris* contained a solitary endolithic sponge that did not form a mass and was not associated with other endolithic organisms. The presence of endolithic sponges, typically alongside other endolithic organisms, has been associated with tissue loss in Pacific corals (Work & Aeby, 2011; Work et al., 2014), and observed in GAs from the Caribbean (Rich et al., 2021). Boring sponges bioerode coral skeleton both mechanically and chemically (Zundelevich et al., 2007; Nava & Carballo, 2008), and their invasion, activity, and growth may impose stress on the coral colony and often cause the death of the coral (Chaves-Fonnegra et al., 2008). In the case of coral GAs, sponges may potentially contribute to abnormal tissue development. Furthermore, chemical compounds released by the sponge may inhibit the proliferation of other endolithic organisms to reduce competition (Pomponi, 1980), possibly explaining their absence in the masses where the boring sponge was present. In addition, two cases of *Acropora* GAs had gastrovascular cavities debris putatively representing degenerate aspiculate sponge (Ereskovsky & Lavrov, 2021), which may have contributed to GA pathogenesis prior to its death. Further studies should aim to couple the morphological identification of potential agents

in GAs, such as the observed invasive sponges, with samples suitable for DNA analysis to confirm their identification and lesion-developing potential.

The histopathological analysis of the 15 samples of GAs did not identify any clear, obvious, or single unifying causative agent(s) involved in the development of the GAs. This finding highlights the inherent complexity and multifactorial nature of the disease, consistent with conclusions across the GA literature. We hypothesize that abnormalities in the growth of coral skeleton may be determined by multiple factors, ranging from genetic factors to encapsulation-type responses to injury or injurious organisms, or possible pathogens not yet observed. Findings in *Acropora*, such as the putative degenerate crustose coralline algae in the bosselated GA, the cavities with focal masses of organisms, the endolithic annelid-associated GA, as well as the solitary endolithic sponge also present in *Pachyseris*, suggest that GA development may be a consequence of certain injurious organisms invading the coral skeleton.

Pachyseris was the only genus histologically showing diffuse mucocyte hyperplasia, which has been previously noted only in *Porites* GAs (Kaczmarsky, 2009). Mucus production, typically occurring in the surface body wall, is a fundamental mechanism used by corals to mediate interactions between the epithelium and the external environment (Brown & Bythell, 2005). However, corals may also increase mucocytes and mucus production as an adaptive response to stress to cope with wound repair and microbial infection (Vargas-Ángel et al., 2007; Piggot et al., 2009). Although *Pachyseris* is recognized as a mucocyte-rich genus, GA lesions contained a notably higher proportion of mucocytes compared to controls, indicative of pathological change rather than a baseline genus characteristic. Nonetheless, the absence of histopathological baseline for this genus necessitates further histological analyses on GA masses from *Pachyseris* to validate the hypothesis.

In addition to clarifying the pathological processes and potential etiological factors underlying GAs, our findings highlight the broader ecological implications of these lesions in corals. Our pathological characterization indicates that GA lesions consist of excessive coenosteal and basal body wall growth that engulfs and distorts polyp architecture. The remaining rare polyp structures had enlarged corallites, hypertrophied tissues, and gonadal structures largely absent. The absence of reproductive elements indicates substantial energetic reallocation from reproduction toward sustained tissue growth, potentially compromising overall colony fitness (Preston & Richards, 2021; Rich et al. 2021).

Integrated microscopic and sub-gross analysis on GAs in this study confirmed key microscopic features, supporting the consistency of our observations and the diagnostic value of established pathological traits. Our findings reinforce the need for histopathological examination of grossly identified GAs to determine whether such lesions meet the criteria for neoplastic processes as

defined in animals. Our integrated characterization further demonstrates the complexity and multifactorial nature of GA pathology, with evidence suggesting involvement of single or mixed invasive organisms. Notably, the potential pathogenic role of invasive sponges should be investigated further, along with their identification.

Although potential genus-specific pathological differences were observed, limited sample availability necessitates validation through broader sampling. Future studies should also aim to clarify the progression of GA lesions and examine their long-term implications for coral health.

As coral diseases continue to increase in both prevalence and severity, deepening our understanding of existing lesions is of critical importance.

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CHAPTER 5

HISTOPATHOLOGY OF SCUTICOCILIATE TISSUE LOSS ASSOCIATED WITH *PHILASTER GUAMENSE* (BROWN BAND DISEASE) IN *ACROPORA* CF. *MURICATA* FROM THE REPUBLIC OF MALDIVES

This work is currently under review as:

Bises C, Dennis M.M, Gobbato J., Maggioni D., Galli P. and Montano S. Histopathology of scuticociliate tissue loss associated with *Philaster guamense* (brown band disease) in *Acropora* cf.*muricata* from the Republic of Maldives. *Coral Reefs*.

5.1 ABSTRACT

Corals with rapidly progressing tissue loss marginated by a brown band on exposed skeleton are typically given a field diagnosis of brown band disease (BrB). It is associated with scuticociliates, protists capable of causing epidemic mortality in a wide range of marine organisms, but with uncertain pathogenic role in BrB.

This study presents the first comprehensive pathological description of BrB affecting *Acropora* cf. *muricata* from the Maldives. Diseased coral fragments were examined using integrated gross and subgross lesion assessments, histology, and morphological and molecular (18S rRNA) identification of the associated ciliate, comparing three lesion areas: the brown band, tissue loss margin, and adjacent apparently healthy tissue. Molecular and morphological analyses confirmed *Philaster guamense* as the ciliate associated with the lesion. The brown band comprised dense aggregations of motile and encysted trophonts intermingled with necrotic coral tissues and saprophytic organisms, whereas, at the tissue loss margin, motile trophonts predominated in contact with dissociated tissues and within skeletal spaces adjacent to histologically intact tissues. Occasional motile trophonts were also found within deep skeletal spaces of apparently healthy regions. No other infectious agents or abnormalities of coral tissues were observed at the tissue loss margin or within adjacent apparently healthy tissue.

These findings suggest that *P. guamense* is able of invading otherwise healthy coral tissues and is closely associated with active tissue loss, supporting its potential primary role in disease initiation and progression. This study provides a histopathological framework for BrB progression and underscores the value of histology-based diagnostics in coral disease investigation.

5.2 INTRODUCTION

Coral diseases are exacerbating the decline of coral reefs and their associated biodiversity (Weil et al., 2006; Willis et al., 2019), already threatened by climate-driven heat stress (Camp et al., 2018; Emslie et al., 2024). To date, over 40 diseases have been reported affecting about 200 coral species (Morais et al., 2022), though this likely underrepresents the global diversity of coral diseases. Disease prevalence is expected to rise as environmental changes enhance host vulnerability and pathogen virulence (Sokolow, 2009; Burke et al., 2023; Hawthorn et al., 2023; Bises et al., 2024). Given these circumstances, accurate diagnoses are fundamental to comprehend disease ecology and identify control points, which are critical aspects for disease mitigation.

The etiology of many coral lesions is poorly understood and suspected to be complex, involving multiple contributing factors, including environmental stressors and pathogens (Bruckner, 2015). Ciliated protists are increasingly recognized to play a role in various coral diseases characterized by acute tissue loss in captive and wild corals (Sweet et al., 2012; Hawthorn et al., 2023). Their relationships with the host coral, affected by acute tissue loss, range from colonization of necrotic tissue and exposed skeleton to histophagy (Sweet & Séré, 2016; Cróquer et al., 2006; Gobbato et al., 2024). In the colonization of necrotic tissue, ciliates primarily feed on coral-associated microorganisms, such as bacteria and other protozoans, and are not thought to cause the primary injury to the coral host. In contrast, histophagous ciliates are considered more aggressive pathogens that can potentially ingest living coral tissue of the host, directly contributing to lesion development and disease pathogenesis (Sweet & Séré, 2016). The best-recognized ciliate-associated coral diseases involve histophagous ciliates, including skeletal eroding band (SEB) linked to folliculinid ciliates (*Halofolliculina* spp.; Page et al., 2015; Cróquer et al., 2006; Montano et al., 2020), brown band disease (BrB), and various white syndromes linked to scuticociliates (Sweet et al., 2014; Bruckner et al., 2015). However, the distinction between their roles as primary versus opportunistic pathogens remains a subject of considerable debate among experts. Regardless, scuticociliatosis can cause epidemic mortality in a variety of marine organisms, especially in stressed captive populations (Munday et al., 1997; Iglesias et al., 2001; Stidworthy et al., 2014), but also occasionally in free-living populations under poorly understood circumstances (Small et al., 2005; Retallack et al., 2018; Hewson et al., 2023)

From the first record in 1994 by Dinsdale (Dinsdale et al., 1994), gross lesions consistent with brown band disease have been documented in multiple areas in the Pacific and Indian ocean (Page & Willis, 2008; Haapkylä et al., 2009; Montano et al., 2016) and affecting a variety of scleractinian corals (Willis et al., 2004). Despite the variability of reported hosts, coral species of the genus

Acropora appear to be primarily susceptible (Willis et al., 2004; Lobban et al., 2011), which was also the first genus reported in the Maldives to be affected by the disease (Montano et al., 2012). BrB has a visually distinct appearance, with dense aggregations of ciliates forming a characteristic brown band bordering rapidly progressing tissue loss, in some cases adjacent to a band of denuded skeleton. The progression of the BrB lesion is quite rapid (lesion growth approximately >5 cm/day; Willis et al., 2004; Lobban et al., 2011), while the prevalence in the studied areas is low relative to other diseases (Page et al., 2008; Weil et al., 2012; Montano et al., 2016; Bises et al., 2024). Two ciliates were identified from the brown band and advancing front of BrB lesions in *Acropora muricata* from the Great Barrier Reef: *Philaster lucinda* and *Phylaster guamense* (Bourne et al., 2008; Lobban et al., 2011; Sweet & Séré, 2016).

Pathogenesis of BrB remains uncertain. Endosymbionts and other cellular debris identified within the cytoplasm of BrB ciliates were determined to most likely originate from the coral host, leading to a debate regarding whether the ciliates were consuming live or decaying (already dead) coral tissue (Bourne et al., 2008; Ulstrup et al., 2007; Lobban et al., 2011). The observation that ciliates were aggregated some distance from the tissue-loss margin further called into question their role in causing the tissue loss (Bourne et al., 2008). Stereoscopic observations confirmed that ciliates burrowed into tissue that was not grossly abnormal, suggesting that they were largely responsible for tissue loss (Lobban et al., 2011; Sweet & Bythell, 2012). On the other hand, consistent similarities in the bacterial community composition of BrB could indicate primary bacterial injury of coral tissue with secondary ciliate feeding on necrotic tissue (Sweet & Bythell, 2012). Additionally, BrB lesions are often observed in colonies predated by corallivores, including the mollusk *Drupella* and the starfish *Acanthaster plancii*, suggesting that predation wounds may facilitate ciliate infection (Nugues & Bak, 2009, Nicolet et al., 2018).

Histopathology is a fundamental step in coral disease diagnosis and the current gold standard for coral health classification. It allows accurate identification of pathological processes at the tissue level and often elucidates associated pathogens, facilitating the understanding of pathogenesis and causative factors (Page et al., 2023). Multidisciplinary approaches to studying coral diseases integrate histopathology with additional diagnostic methods, such as morphological and molecular tools for identifying pathogens, to better connect observed disease processes with specific pathogens or other underlying causes (Sweet et al., 2011; Montano et al., 2020; Becker et al., 2023; Hawthorn et al., 2023). Additionally, it is necessary for accurate disease diagnosis because many coral diseases show similar or overlapping visual changes (Work et al., 2008; Work & Meteyer, 2014). By integrating documented pathological processes, associated pathogens, clinical observations, and ancillary diagnostic findings, a case definition can be established to standardize

disease classification and improve consistency in BrB research and surveillance (Pollock et al., 2011; Training manual on wildlife diseases outbreak investigations, fourth cycle Workshop for OIE National Focal Points for Wildlife, 2021). The histopathology of BrB has not been characterized yet, but it would help determine whether the ciliates associated with BrB initiate tissue loss or simply colonize and feed on tissues already damaged by other potential underlying disease conditions. As such, histology is the essential approach for the accurate evaluation of the pathogenicity of BrB ciliates.

The present study, therefore, aims to investigate and describe the histopathology of *Acropora* cf. *muricata* affected by brown band disease in the Republic of Maldives.

5.3 MATERIALS AND METHODS

5.3.1 SAMPLE COLLECTION

Samples were collected by SCUBA diving during May 2022 in the reefs around Magoodhoo Island, in Faafu Atoll, in the Republic of Maldives (13°24'45" N 103°52'00" E) at a depth of 7-15 m (**Fig.1**). A total of three *Acropora* cf. *muricata* colonies showing gross features of brown band disease (BrB) were identified in the field, and two small diseased branches were collected from each colony. Specifically, the gross lesion was identified in the field by the presence of a variably intense brown band parallel to a discrete annular area of acute tissue loss, evident by bright white exposed but not eroded skeleton. For histological analysis, care was taken to collect biopsies that represented three regions: the brown band, the tissue loss margin, and the bordering apparently-healthy (grossly non-diseased) region as a control.

The remote location of the site, the low prevalence of the disease, the difficulty in finding active lesions and the willingness to reduce undue pressure on the local benthic community already under stress, posed significant limitations on the number of samples available for this study.

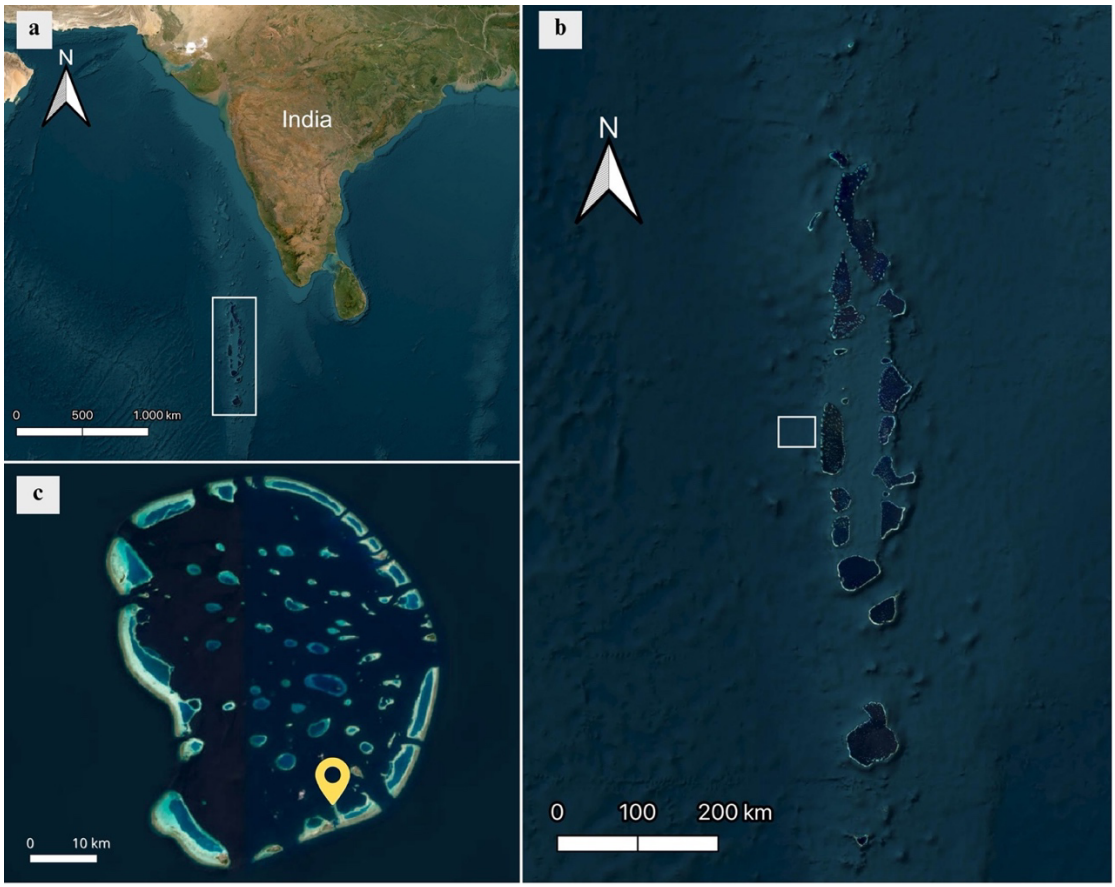

Figure 1. Geographical contextualization and sampling sites in the Faafu atoll **(c)**, in the Republic of Maldives **(b)**. The pin indicates the sampling site in the house reef of Magoodhoo.

5.3.2 MOLECULAR AND MORPHOLOGICAL IDENTIFICATION OF CILIATES ASSOCIATED WITH THE LESION

Ciliates were identified both molecularly and morphologically. For molecular identification, ciliates were detached from the three living colonies using menthol crystals, then fixed in 99% ethanol and stored at -20°C until further analyses. Total genomic DNA was extracted from the isolated ciliates using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The obtained DNA was used to amplify a portion of the 18S rRNA gene using the primer pair BrB-F-171 and BrB-R-1721, following the protocols described by Bourne et al., (2008). Amplification results were checked with a 2% agarose electrophoretic run and subsequently sequenced with both forward and reverse primers using an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA). The obtained chromatograms were visually checked and assembled using Geneious Prime (Biomatters, Auckland, New Zealand). The obtained sequences were deposited in GenBank under the accession numbers PX695969-PX695971. Sequences were initially compared with those deposited in NCBI GenBank (Sayers et al., 2021) using blastn to assess the presence of contamination and to check the closest identity matches. All available 18S rRNA sequences of *Philaster* were then downloaded, together with *Porpostoma notata* and *Philasterides armatalis* as outgroups, to reconstruct a phylogenetic hypothesis to better characterize the identity and phylogenetic relationships of the ciliates studied in this work. All sequences were aligned using MAFFT 7.110 (Katoh & Standley, 2013) with the E-INS-i option and the resulting alignment was used to infer a maximum likelihood phylogenetic hypothesis using RAxML 8.2.12 (Stamatakis, 2014) with 1000 non-parametric bootstrap replicates, after identifying the appropriate substitution model with ModelTest-NG (Darriba et al., 2019). Similarly, a Bayesian phylogenetic hypothesis was reconstructed using MrBayes 3.2.6 (Ronquist et al., 2012), for which two independent runs for four Markov chains were conducted for ten million generations, with trees sampled every 1000th generation. In this latter analysis, parameter estimates and convergence were checked using Tracer 1.6 (Rambaut et al., 2014), setting a burn-in at 25%.

For the morphological identification, *Acropora* fragments and detached ciliates were observed under a microscope (Leica Stemi 508), and ciliates were identified according to relevant literature (Lobban et al., 2011; Sweet & Séré, 2016). The shape, overall appearance, and median length of the organisms were assessed. The typical length was determined by calculating the median length across 70 individuals.

5.3.3 HISTOLOGY

The six biopsies, two for each of the three *Acropora* cf. *muricata* colonies sampled, were fixed immediately after collection in a 1 to 4 ratio of zinc-formaldehyde diluted in seawater (Z-Fix, Anatech) with approximately a 10 to 1 ratio of fixative to tissue.

The biopsies were then transferred to the College of Veterinary Medicine at the University of Tennessee for histological processing. Photographs were taken of biopsied lesions before decalcification and under the stereomicroscope to help define lesion type and aid in trimming tissues for histology. Before trimming, the coral tissue identified as tissue loss margin and the biopsy margin were marked using tissue dye (Bradley products, TMD) to facilitate microscopic orientation of lesion progression. Coral fragments were decalcified with Formical-2000 (StatLab) for 3 hours in different containers. For each sample, the dispersed material, including coral tissue fragments and any other organisms detached from the colony, was collected in a mesh histology cassette, while the remaining intact coral tissue was trimmed before being placed into the cassette. Samples were trimmed sagittally along the branch length to obtain sections that captured the disease progression, from the ciliates brown band through the tissue loss margin to the unaffected control tissue. Tissues were briefly rinsed with tap water to remove any residual Formical-2000 and then kept in Z-Fix until processed for histology. Tissues were embedded in paraffin wax, surface decalcified if needed, sectioned in a rotary microtome at 4 μm, mounted onto microscope slides, and stained with hematoxylin and eosin. In some cases, additional sections were prepared and stained with special stains, such as Alcian blue (pH 2.5), periodic acid Schiff, and Grocott methenamine silver stain (GMS), to better characterize microorganisms and specific ciliate structures. The lesion for each BrB sample was qualitatively assessed with an Olympus BX43 microscope, defining for each biopsy, if present, three areas of investigation: brown band, tissue loss margin, and control area. Although care was taken to collect fragments representing all three areas, sub-gross examination once in the laboratory revealed small foci of tissue loss in the region of the control area; these biopsies (n=3) were considered to lack a proper control area. For histological assessment, the tissue loss margin was defined as the area showing tissue loss strictly adjacent to the recently exposed skeleton, as indicated by tissue dye. The control region corresponded to the terminal portion of the biopsy, consisting of the grossly normal tissue most distant from the tissue-loss margin. Lastly, the brown band was defined as the area presenting a skeletal surface deposit over the exposed skeleton. Histological parameters were evaluated to describe the BrB lesion microscopically. Tissue necrosis was identified and evaluated for severity with a score of 1 if less than 25% of the basal body wall or dissociated coral tissue in the field area (2.54 mm^2 at 10x magnification) was affected, 2 if the percentage was between 25 to 75% and 3 if

higher than 75%. In particular, dissociated coral tissue was identified as detached and disoriented fragments of coral tissue, not contiguous with the polyp or coenenchyma, and tissues were recognized whenever possible.

Moreover, endosymbiont loss was recorded as a clear reduction or absence of endosymbionts, as indicated by fewer than two endosymbiont cells deep within the surface body wall. The presence of ciliated protists was evaluated as the number of trophonts (as delineated by nuclei or cell borders) in the field area (2.54 mm^2 at 10x magnification) of the most affected area of the section. In addition, ciliates were distinguished as motile and encysted trophonts based on the presence or absence of the resting cyst, respectively, where the cyst is a protein-polysaccharide external wall involved in cryptobiosis processes during unfavorable conditions (Verni et al., 2011; Li et al., 2022). Finally, all observed organisms were recorded to evaluate potential co-infectors, and they were identified based on morphological features from relevant literature, as shown in **Table 1**.

Table 1. List of organisms and their key morphological features used for identification.

Organism	Key morphologic features	Reference
Fungi	Filamentous hyphae, possibly with septa or branches	Larone, 1994
Labyrinthulomycetes	Elongate to ovoid single-celled organisms, embedded in a network of fine, transparent filaments (ectoplasmic net)	Burge et al., 2012 Polglase, 2019
Platyhelminths	vermiform multicellular organisms	Gardiner & Poynton, 2006
Sponges	multicellular organisms with siliceous spicules and zooxanthellae	Ereskovsky & Lavrov 2021

5.4 RESULTS

5.4.1 CILIATE MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION

The 1300 bp 18S rRNA alignment included 12 *Philaster* sequences plus two outgroups and resulted in a well-supported phylogenetic hypothesis (**Fig. 2**). Our samples had identical sequences and clustered in a highly supported clade (bootstrap = 99, Bayesian posterior probabilities = 1) together with other ciliates isolated from diseased *Acropora muricata* colonies from Australia. These latter sequences were obtained from ciliates isolated from both brown band disease and white syndrome (Bourne et al., 2008; Sweet & Bythell, 2012) and correspond to *Philaster guamense* (Sweet & Séré, 2016). Other ciliates isolated from brown band disease and white syndrome on *A. muricata* from Australia, identified as *Philaster lucinda* (Sweet & Séré, 2016), were sister to all other *Philaster* species. Finally, two *Philaster* species not isolated from corals, namely *P. apodigitiformis* and *P. sinensis*, both from China, were sister to another clade isolated from *Acropora hyacinthus* from China, reported to be very similar to brown band ciliates (Qiu et al., 2010), and these sequences altogether formed a sister group to *Philaster guamense.*

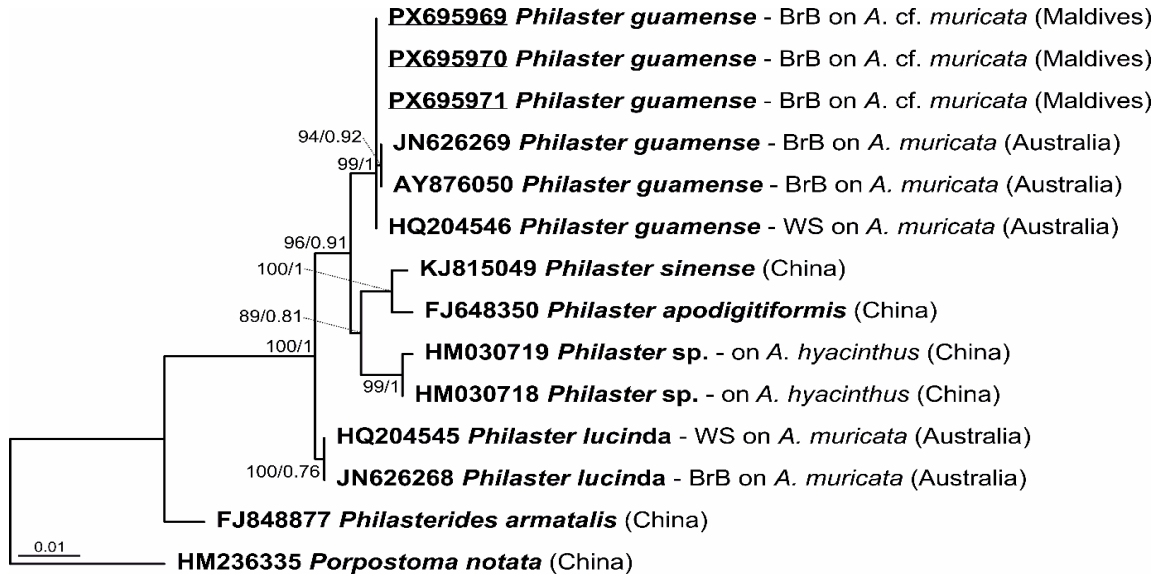

Figure 2. Maximum likelihood phylogenetic hypothesis of *Philaster* species based on 18S rRNA gene. Numbers at nodes represent maximum likelihood bootstrap support and Bayesian posterior probabilities, respectively. For each sequence, the coral disease and species are indicated when available, together with the sampling locality in brackets. BrB: brown band disease; WS: white spot disease. Underlined sequences were obtained in this study.

The typical length of the ciliates was on average 336.12 ± 97.76 µm (median ± SD), ranging from 150 to 500 µm. Ciliates were oval-elongated in shape and surrounded by rows of cilia in the motile free-swimming phase or encysted in the brown band area (**Fig. 3b,c**). The cyst could be recognized as a transparent wall surrounding the shrunken and less turgid body of ciliate (or clusters of ciliates) (**Fig. 3d**). When visible, the macronucleus appeared elongated and positioned centrally along the main axis. Ciliates were colorless to brownish yellow, in the latter case due to the ingested coral cells and endosymbionts.

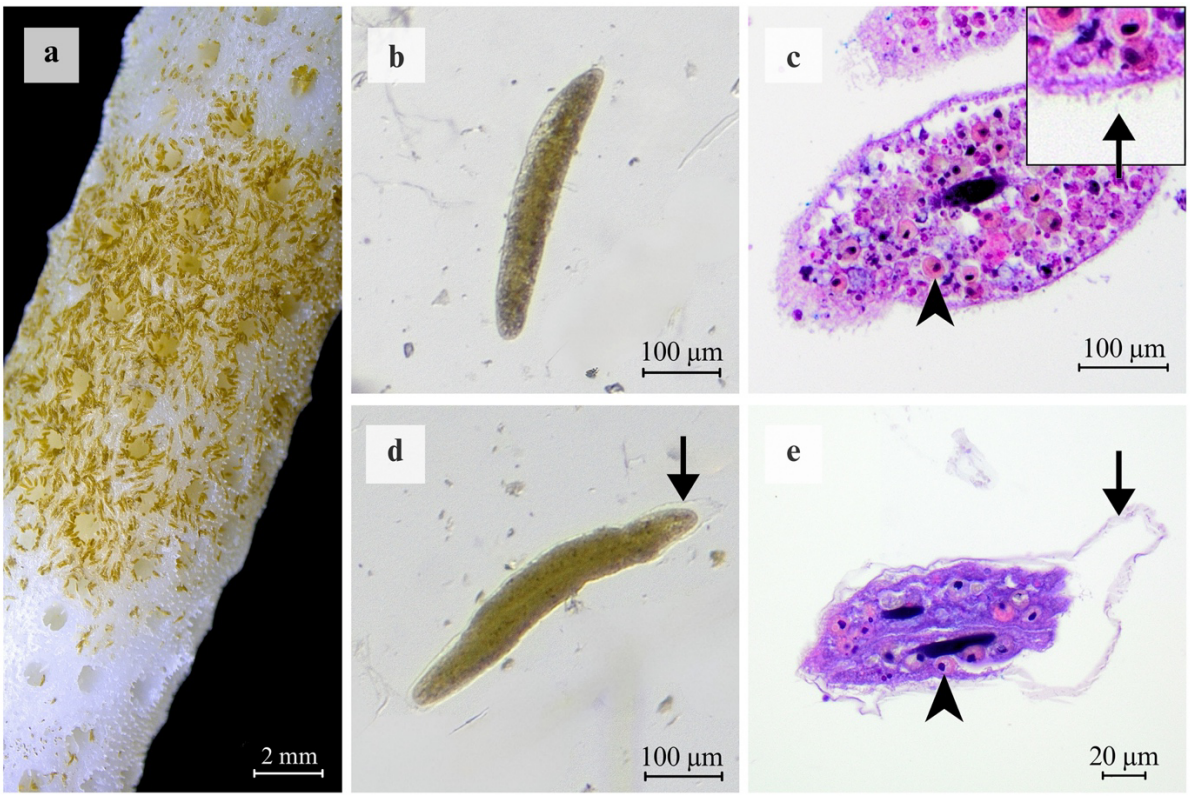

Figure 3. a) Portion of a diseased *Acropora* branch showing the brown band on the exposed skeleton. **b)** Wet mount light microscopy of motile trophont. **c)** Microscopic section of a motile trophont, oval to pyriform with highly eosinophilic macronucleus, rows of cilia (inset, arrow), and ingested endosymbionts (arrowhead). Section stained with Hematoxylin and Eosin. **d)** Wet mount light microscopy of encysted trophont with shrunken body with condensed cytoplasm and cyst capsule (arrow). **e)** Microscopic image of encysted trophonts encased in the cyst capsule (arrow), with comparatively condensed cytoplasm containing ingested endosymbionts (arrowhead). Section stained with Hematoxylin and eosin.

Observation of *Acropora* fragments and ciliates under the stereomicroscope provided the opportunity to describe disease progression *ex situ*. Tissue loss proceeded from the base of a branch towards its tip. As shown in the supplementary time-lapse video (**Video S1**), the typical progression is illustrated, showing the rapid ciliate advancement of approximately 1.2 mm/h from skeletal cavities toward intact coral tissue, and their subsequent aggregation and encystment on exposed skeleton as tissue resources become depleted. Moreover, fragments showed variability in the brown band intensity, width, and distance from the tissue loss margin, showing no consistent pattern of such features.

Video S1. The media is available in the repository "Bicocca Open Archive Research Data" as Bises, Chiara (2026), "Supplementary video S1_ Chapter 5: PhD thesis", DOI: 10.17632/g87rkk4gz3.1

5.4.2 HISTOPATHOLOGY

Histological features of the brown band region were similar across all biopsies (**Fig.4.1** and **Table 2**), consisting of a mat of aggregated organisms dominated by densely packed motile, encysted trophonts, and numerous empty cysts (**Fig.4.2**).

Table 2. Summary of the main histological features observed in the Maldivian acroporids affected by brown band disease, with comparison among the three investigated areas: brown band (brown surface deposit on skeleton), tissue loss margin (tissue interfacing the brown band and the control tissue), and control tissue (grossly normal tissue bordering the tissue loss margin).

		Brown band	Tissue loss margin	Control
N		6	6	3
Motile trophonts		5	6	2
Median number[a] of motile trophonts (range)		20 (0-160)	19.5 (9-81)	4(0-8)
Encysted trophonts		6	2	1
Median number[a] of encysted trophonts (range)		19.5 (0-180)	0 (0-4)	-
Dissociated tissue fragments		6	6	-
Median necrosis severity score (range)		3(0-3)	0.5(0-3)	-
Other organisms				
	Fungi	4	1	-
	Platyhelminths	3	-	-
	Labyrinthulomycetes	1	-	-
	CAMAs	n.a.	1	1
Surface body wall gastrodermis endosymbiont loss		n.a.	-	-

[a] Enumerated per 2.54 mm^2 field in the most severely affected area.
n.a.: not applicable.

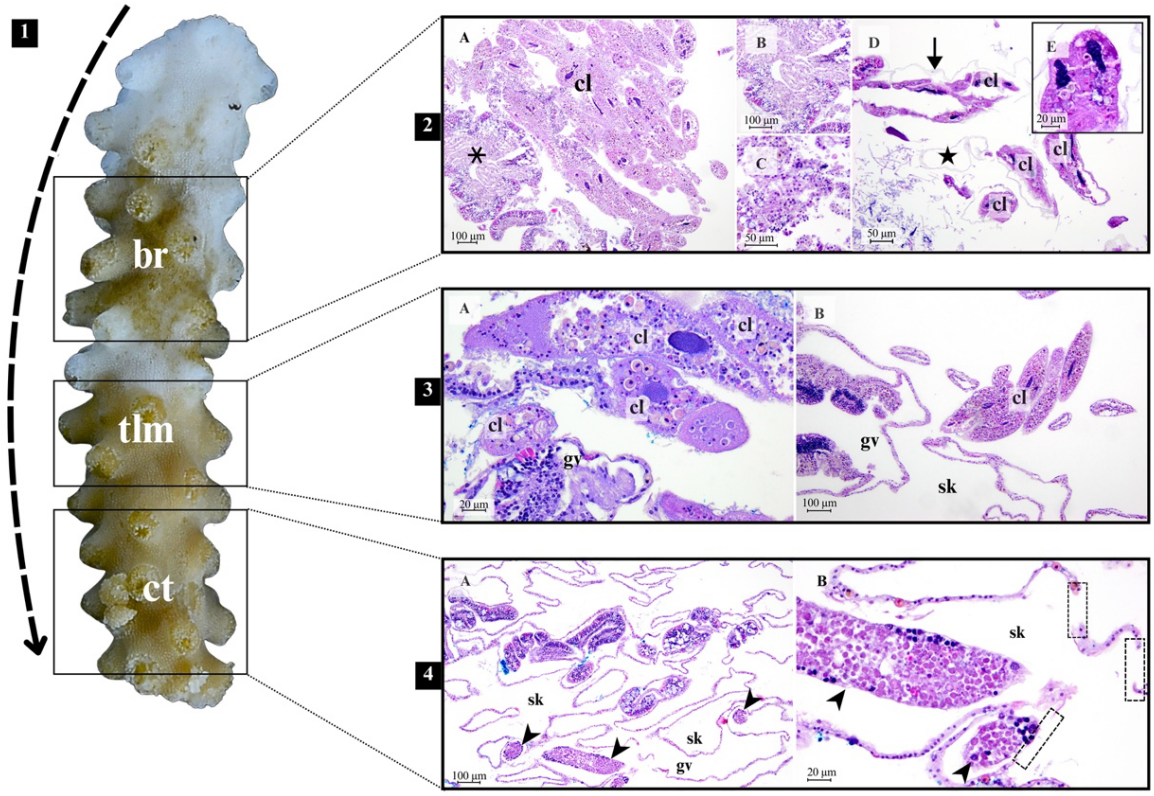

Figure 4. 1) *Acropora* biopsy showing the three areas of disease progression front (dashed arrow), namely brown band (br), tissue loss margin (tlm), and control (ct).
4.2) Sections of the brown band area stained with Hematoxylin and Eosin showing: **4.2a)** a mat of densely aggregated motile trophonts (cl) and dissociated necrotic coral tissue (asterisk); **4.2b)** higher magnification of dissociated coral tissue; **4.2c)** higher magnification of dissociated coral tissue showing cellular degeneration and necrosis; **4.2d)** The brown band area presented the greatest number of encysted trophonts (cl) along with empty cyst capsules (star). The arrow points out the cyst capsule; **4.2e)** higher magnification showing condensed cytoplasm.
4.3) Sections of the tissue loss margin stained with hematoxylin and eosin showing: **4.3a)** Motile trophonts (cl) aggregated at the coral surface, disrupting and in direct contact with surface body wall and mesentery, and containing phagocytosed endosymbionts and cellular debris. gv indicates the gastrovascular cavity; **4.3b)** Motile trophonts (cl) within deep skeletal spaces (sk), adjacent to intact basal body wall; gv indicates gastrovascular cavity.
4.4) Sections of control area stained with hematoxylin and eosin showing: **4.4a)** Motile trophonts (arrowheads) within the skeletal spaces (sk). Gv indicates the gastrovascular cavity; **4.4b)** Higher magnification of section 3.4A showing ingested cellular debris within the cytoplasm of motile trophonts (cl) and areas of truncated basal body wall (dashed rectangle).

Ciliate morphology was similar across all biopsies, and consistent with features documented with stereoscopic examination. Trophonts in both the encysted and motile forms were often aggregated to form clusters, but no prominent intercellular junctions were noted. The ciliate cytoplasm contained coral endosymbionts and was condensed, resulting in an overall reduction in cell size in encysted trophonts. The cyst capsules were recognized as strongly positive in the sections stained with Alcian blue (pH 2.5)-periodic acid Schiff (PAS), and did not stain with PAS, Gomori methenamine silver, or Masson's trichrome, consistent with acidic mucopolysaccharides (mucins) (**Fig.5**).

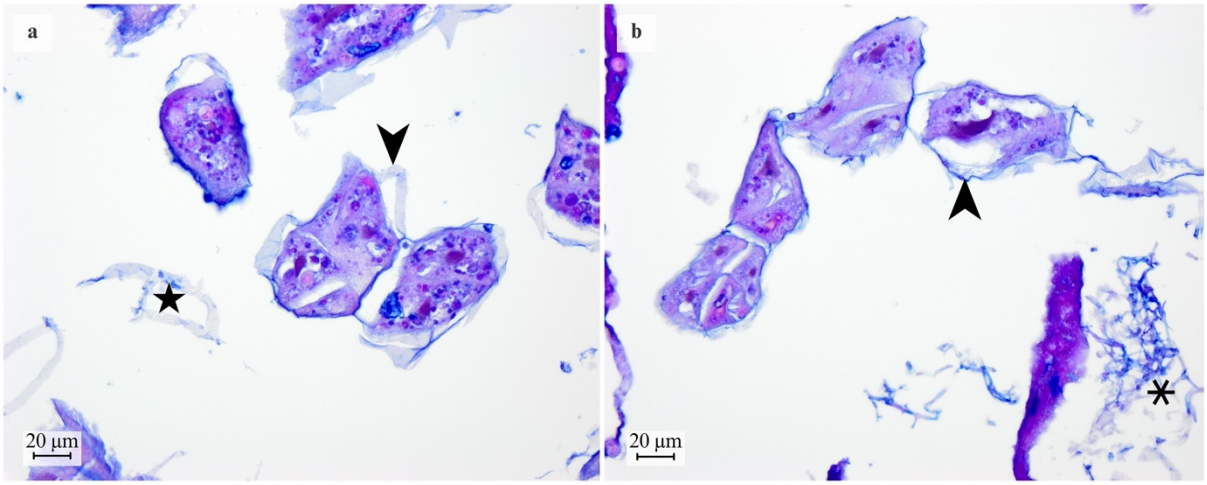

Figure 5. Sections of tissue loss margin in *Acropora* biopsy stained with Alcian blue (pH 2.5)-periodic acid Schiff. **a)** Encysted trophonts surrounded by highly positively stained cyst capsules (arrowhead). Note the empty cysts (star); **b)** Encysted trophonts with clustered fungi hyphae (asterisk). The arrowhead points out the cyst capsule.

In all the biopsies, the mat of micro-organisms contained fragments of variably necrotic dissociated coral tissue (**Fig.4.2a-c**), representing basal body wall, surface body wall, and mesentery (necrosis score 3).

In five out of the six biopsies, other organisms, including fungi (4 cases), platyhelminths (3 cases), and Labyrinthulomycetes (1 case), were in aggregation or in proximity of the ciliates within the brown band (**Fig.6**). No bacteria were observed.

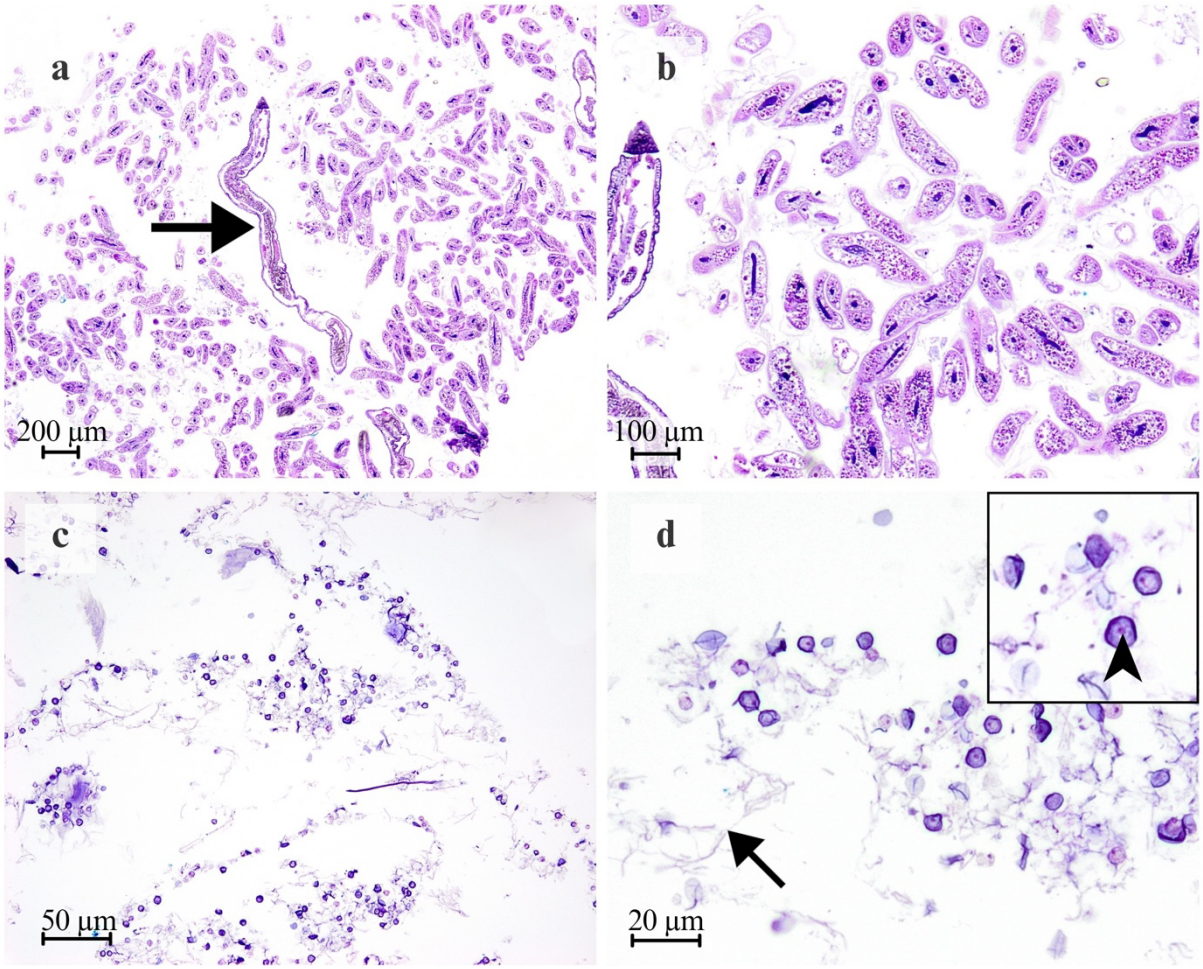

Figure 6. Sections of an *Acropora* biopsy at the brown band deposit on the skeletal surface, microscopically composed of a mat of aggregated organisms dominated by ciliates. Hematoxylin and Eosin **a)** Predominance of encysted trophonts with sparsely motile trophonts, and a central vermiform metazoan organism (arrow, platyhelminth); **b)** Encysted trophonts predominate and are haphazardly oriented, but often in contact with one another and intermingled with necrotic cell debris; **c)** Area containing Labyrinthulomycetes. **d)** Higher magnification of C showing the single-celled organisms with visible nuclei (arrowhead in inset), inside an ecotoplasmic net of fine transparent filaments (arrow).

At the tissue loss margin (**Fig.4.3**), the motile trophonts, and rarely encysted trophonts (**Fig. 7**), which were morphologically identical to those of the brown band region, were associated with intact and healthy coral tissues or fragments of variably necrotic dissociated coral tissue. Where coral tissues were intact, trophonts were mainly within skeletal spaces, but also in the gastrovascular cavity, in contact with basal body wall (n=6), surface body wall (n=2), or mesentery (n=2). Dissociated coral tissues included basal body wall, surface body wall, and mesentery (necrosis score 0-1). No instances of tissue necrosis were identified in non-dissociated (intact) coral tissues, nor were bacteria, endolithic organisms, or other potential pathogens, apart from cell-

associated microbial agents (CAMA), in the surface body wall of one coral. In one coral, encysted trophonts aggregated with dissociated coral tissue at the tissue loss margin were accompanied by clusters of fungal hyphae, forming a mat similar to the brown band region (**Fig.5**). Otherwise, there were no qualitative morphological differences in the coral tissues at the tissue loss margin relative to those in the control (apparently healthy) region.

Control areas of the coral biopsy were consistently microscopically healthy, but a few motile trophonts were within skeletal spaces in contact with the basal body wall (**Fig. 4.4**), with the exception of one case in which ciliates were also inside the gastrovascular cavity, in contact with the mesenteries. The control area presented intact polyp structures and tissue architecture, regular cell morphology, no pathological features including necrosis and degeneration, and no pathogens such as bacteria or other microbial agents, apart from one instance of cell-associated microbial aggregates (CAMAs) in the surface body wall (**Fig.7**). Moreover, no significant loss of endosymbionts in the surface body wall was noted either in the tissue loss margin or the control area of the biopsies.

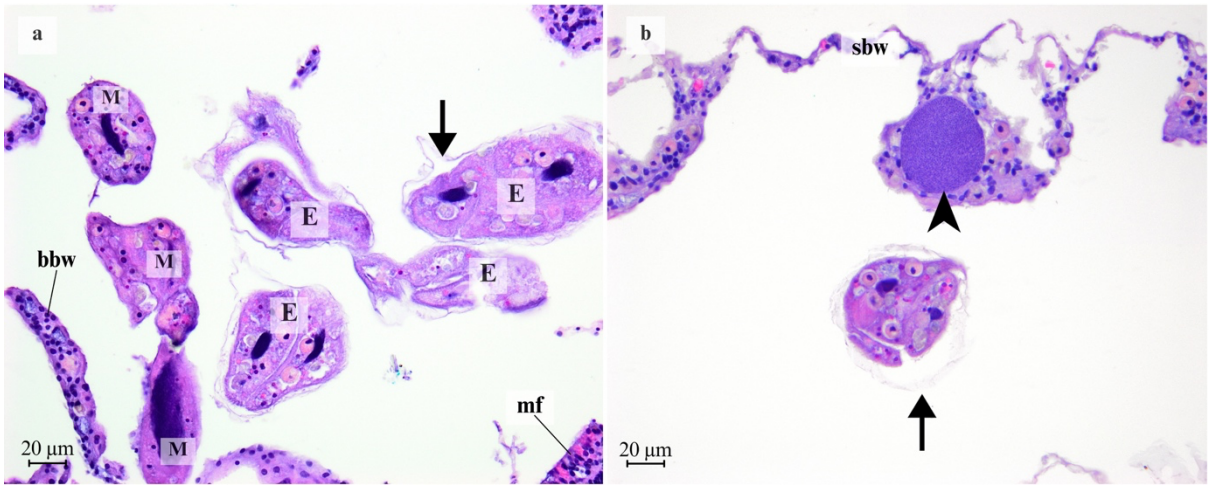

Figure 7. Sections of an *Acropora* biopsy at the tissue loss margin located at the tip of the branch of the colony. Hematoxylin and eosin. **a)** Motile (M) and encysted (E) trophonts next to dissociated tissue including basal body wall (bbw) and mesenterial filaments (mf). The arrow indicates the cyst capsule. **b)** Encysted trophonts encased in a cyst capsule (arrow) within the skeleton. The surface body wall (sbw) contains cell-associated microbial aggregates (CAMA; arrowhead).

5.5 DISCUSSION AND CONCLUSION

Our findings are consistent with the descriptions of BrB in terms of lesion progression (≈1.2 mm/h), gross pathology, and morphologic and genetic profile of the scuticociliate *Philaster guamense*, which is the prominent ciliate species associated with brown band diseased corals (Bourne et al., 2008; Sweet & Bythell, 2012; Randall et al., 2015; Sweet & Séré, 2016). We present the first comprehensive histopathological description of BrB, helping address uncertainty about the role of ciliates in its pathogenesis. Our work demonstrates that trophonts, morphologically and molecularly consistent with *Philaster guamense*, are able to invade histologically normal coral tissues in advance of the tissue loss margin, supporting the hypothesis that ciliates promote tissue loss in BrB independent of other disease processes. These results contribute to a growing body of evidence supporting their role as a primary pathogen, although definitive confirmation will ultimately require experimental infection trials in healthy corals.

The present study offered a comparison of the three distinct lesion areas, representing a trajectory of disease progression. Motile and encysted histophagous ciliates predominated in the brown band region, variably admixed with other organisms. Toward the tissue loss margin, they were less aggregated, but numerous motile trophonts were in contact with fragments of variably necrotic dissociated tissue.

Notably, trophonts frequently formed discrete clusters despite the absence of any histologically detectable prominent adhering or bridging structures between individuals. Transitioning to the control (apparently healthy) region of the biopsy, ciliates were few and not clustered, but they were found deep within the skeleton, where polyp tissues were otherwise histologically unremarkable. In particular, histophagous ciliates were in close contact with coral tissues with no evidence of any pathological features, such as prior symbiont injury, bacterial infection, polyp necrosis or degeneration, preceding ciliate invasion. This feature strongly indicates that *P. guamense* does not target compromised or necrotic areas but instead consumes healthy host tissue (**Fig. 3.3**).

A sequence of invasion, proliferation, aggregation, and encystment seems to be the most probable dynamic pattern of lesion progression from the documented in our supplementary time-lapse video (**Video S1**), which visually captures the advancement of the lesion front and the behavioral transitions of the ciliates during disease progression, similar to stereoscopic observations recorded by others (Lobban et al., 2011; Sweet & Bythell, 2012). Encysted trophonts predominantly observed in the brown band region of biopsies, where tissue has been completely consumed, represent a resting or dormant phase. Encystment is thought to be triggered by unfavorable conditions such as nutrient deficiency or population density threshold, and is accompanied by

biological adjustments, including reduced cell volume and cytoplasmic dehydration (Li et al., 2022). These changes were histologically evidenced by increased cytoplasmic basophilia and shrunken bodies when enclosed within a cyst capsule. It is unclear why encystment occurs in subacute regions of *Philaster*-associated tissue loss, and which are the decisive triggering factors that might help to explain the observed variation in the width and intensity of the brown band (Raymundo & Weil, 2015). Trophonts, which are inactive in the encysted state, can excyst when the conditions allow. Indeed, in our observations, the brown band area can present numerous empty cysts and may explain the intensity change in pigmentation of the brown band, which would grossly appear lighter. Clarifying the factors that trigger en/excystment is required to determinewhether the brown band is truly a defining diagnostic feature of the disease, or if it is only present in a proportion of cases. In any case, the identification of a brown band of encysted scuticocilates adjacent to a tissue loss margin can be useful for determining the stage of disease (subacute to chronic). Additionally, diagnosticians should be aware of the morphological distinctions to avoid confusion between scuticociliate cysts and loricae observed in folliculinid ciliates (Hawthorn et al., 2023). The lorica forms an open, flask-shaped dark-colored "pseudochitinous" envelope that houses the active trophont, whereas the scuticociliate cyst is defined by a completely closed mucinous capsule that fully encloses the dormant organism, distinctions that would be evident on subgross examination (Dovgal et al., 2018; Li et al., 2022). To the authors' knowledge, the clustering behavior and encysted forms of ciliates observed in this study have not been documented in other scuticociliatoses, including in crustacea, bivalves, echinoderms, and finfish (Elston et al., 1999; Small et al., 2005; Miller et al., 2013; Hewson et al., 2023; Pilar et al., 2025). These aspects suggest a unique pathogenesis deserving of further investigation.

One hypothesis for the pathogenesis of BrB and other ciliate-associated tissue loss diseases proposes that bacteria act as the primary causal agents, invading healthy tissue and causing damage that subsequently allows ciliates to consume the compromised coral (Sweet & Bythell, 2012; Sweet et al., 2014; Sweet & Bythell, 2015). Histological findings of this study do not support this hypothesis; specifically, bacteria were not microscopically evident within coral tissues, nor were host tissue degenerative changes indicative of injury by microbes. The only bacteria detected were cell-associated microbial aggregates (CAMAs), which were confined to the healthy surface body wall in one coral and were not associated with any pathology; moreover, these structures are considered potentially symbiotic rather than pathogenic (Work & Aeby, 2014). Additionally, these findings argue against a heterotrophic role where ciliates are simply feeding on decaying tissue (McClanahan et al., 2004; Work et al., 2025). Instead, they are consistent with the presence of invasive ciliates in the tissues of corals, proceeding tissue loss and similarly lacking evidence for

primary bacterial-induced tissue necrosis (Work & Aeby, 2011). Furthermore, the similar invasive nature of *Philaster* spp. in other marine hosts lends further credibility to this hypothesis (Stidworthy et al, 2014; Jalenques et al, 2021; Hewson et al., 2023; Vilanova-Cuevas et al., 2025). Our observations would support the hypothesis that *Philaster* spp. invade coral tissues from the underlying skeleton, causing injury to deep tissues. This pattern may mirror the deep invasion documented in shrimps and sea urchins, where ciliates were within calcified matrix, suggesting a comparable pathogenic strategy across hosts, a subject worth of further investigations (Bradbury & Goyal, 1976; Hewson et al., 2023). The use of controlled inoculation experiments and electron microscopy paired with histology will more definitively exclude the role of other microbes in BrB. Other hypotheses surmise the role of stress or primary endosymbiont injury predisposing the coral host to ciliate invasion, which could not be substantiated in this study by the presence of normal endosymbiont quantity and unremarkable morphology observed in the investigated corals.

In almost the totality of analyzed biopsies, the brown band was also characterized by the concurrent presence of other organisms. However, unlike other diseases caused by a consortium of organisms, such as black band disease (BBD) (Richardson, 2004), no consistent co-infection was noted between *P. guamense* and other microbial agents. Indeed, the organisms co-localized with ciliate aggregations were rarely in contact with host tissues, nor did they invade the polyp or skeleton, indicative of little direct role in the pathogenesis of tissue loss in BrB. It is unclear if the other organisms in brown band deposits represent a form of tissue decay or are a convergence with ubiquitous skeleton-befouling organisms in the form of a biofilm. Ciliates are recognized as important regulators of biofilm microbial composition through their grazing activity on a wide range of microorganisms (Huws et al., 2005; Dopheide et al., 2009; Früh et al., 2011; Watson et al., 2015). Consequently, when ciliates encyst and become inactive, their reduced grazing pressure may permit the proliferation of other saprophytic taxa, which can subsequently exploit the remaining coral tissues. This dynamic may account for the absence of such saprophytes in other regions of the lesion where motile trophonts prevail, together with the non-pathogenic nature of such saprophytes. Notably, previous work investigating fungal communities in BrB lesions (Yarden et al., 2007) also failed to detect a consistent association with specific fungal taxa, reinforcing the lack of evidence for a mycotic co-factor in this disease. Based on our findings, researchers of BrB should be aware that investigations of the brown band consortium are unlikely to reveal alternative primary pathogens, which would best be identified in the apparently healthy tissue adjacent to the tissue loss margin, where early stages of ciliate invasion are occurring.

Scuticociliatosis is often reported in marine taxa, where, under favorable conditions, it becomes invasive without requiring a primary infectious trigger (Álvarez-Pellitero et al., 2004; Miller et al.,

2013; Stidworthy et al., 2013; Jalenques et al., 2021; Steverding, 2022; Vilanova-Cuevas et al., 2025). Although mostly reported in aquaria as critical in debilitated populations, scuticociliates are fully capable of causing epidemic-scale mortality in the wild, as seen in recent sea urchin die-offs (Ritchie et al. 2024). However, the host, pathogen, and environmental factors conducive to epidemics in free-living animals remain poorly understood. Our observations suggest that similar dynamics may operate in corals, where scuticociliates may function as primary pathogens under the right circumstances.

Interestingly, *Philaster* spp. have also been implicated in tissue loss diseases lacking a brown band (Sweet et al., 2014; Sweet & Bythell 2015). The confusion around the diagnosis of scuticociliate-associated tissue loss diseases in corals emphasizes the need for robust case definitions, that include histology, together with other methods such as molecular identification, rather than solely relying on visual diagnosis. The described sequence of histological findings in our study will help diagnosticians discern the potential pathogenicity and need for molecular identification of ciliate pathogens associated with tissue loss syndromes, which would ideally be named according to cause and lesion (scuticociliate tissue loss) rather than visual appearance (brown band disease) (Work & Aeby, 2006). We advocate for acroporid scuticociliate tissue loss disease to be defined by: rapidly-progressing tissue loss, presence or absence of brown-pigmented deposit on recently denuded skeletal surface, microscopic confirmation of invasive ciliates in the skeleton of deep polyp that is otherwise morphologically normal, microscopically evident dissociation of coral tissues in contact with histophagous trophonts, and molecular or morphological confirmation of scuticociliate features. These features should be confirmed in other locations and other susceptible coral host species.

Finally, the study highlights a fundamental well-known challenge in coral disease diagnostics: tissues that appear grossly normal may nonetheless exhibit substantial microscopic pathology, resulting in a considerable gross-microscospic discrepancy (Pollock et al,. 2011; Landsberg et al., 2020). While our study used a limited sample size because of restrictions around destructive sampling of endangered species and low prevalence of the lesion, the consistency in pathological findings argued against any need for additional sampling for our histopathological observations.

Overall, this work establishes a clear histopathological narrative for BrB progression and positions *P. guamense* as a credible primary driver of the lesion, offering a foundation for future diagnostic, experimental, and etiological investigations into ciliate-mediated coral diseases.

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CHAPTER 6

TEMPORAL PATTERNS IN CORAL DISEASE PREVALENCES AT THUDUFUSHI ISLAND, MALDIVES, 2010-2022

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6.1 ABSTRACT

Coral reefs are lately suffering a fast decline in biodiversity due to the coupled effect of climate change and disease outbreaks, which in recent decades have been reported with higher frequency and shorter intervals. Limited studies have been conducted on coral diseases in the Maldives resulting in the impossibility to assess the temporal trend in their dynamics.

In this context, we evaluated the change in the distribution, prevalence, and host range of four diseases, namely black band disease (BBD), brown band bisease (BrB), skeletal eroding band (SEB) and white syndrome (WS), in the reef system around Thudufushi island after twelve years from the previous report published in 2015 by Montano et al. In this period, the overall disease prevalence increased, except for BrB, with SEB showing the most severe increase in 2022 with respect to 2010. The overall average prevalence of coral diseases is approximately 2%, indicating an increase of about 0.7% from 2010. Diseased coral colonies were found in all the investigated sites, with the east site being the most affected and SEB emerging as the most prevalent disease across all the investigated sites. The affected colonies belong to 13 genera, with *Psammocora* genus showing the highest overall mean disease prevalence.

This study depicted a basic temporal trend in disease prevalence that confirms an increase of coral diseases in the region and calls for a dedicated national monitoring protocols to better understand and predict future coral disease dynamics at regional scales.

6.2 INTRODUCTION

Coral reefs, despite covering just 0.2% of the Earth surface, are known as one of the most important and biodiverse ecosystems, providing habitat for countless marine species and associations between them (Fautin et al., 2004; Stella et al., 2010; Plaisance et al., 2011; Fisher et al., 2015). They are of outmost importance not only due to their unique ecological role in several aspects of marine life, such as providing food and shelter for different species, nitrogen fixation, and carbon sequestration (Wild et al., 2011), but also for their provision of ecosystem services yielding economic value (Cesar, 2002; Pascal et al., 2016; Woodhead et al., 2019; Fezzi et al., 2023). Unfortunately, coral reefs are lately experiencing a fast-progressing decline in biodiversity because of climate change and disease outbreaks, strictly related to pollution and anthropogenic pressure (Pandolfi et al., 2005; Hughes et al., 2017). In particular, the prolonged presence of a high level of sedimentation, nutrient and chemical runoff (Kuta & Richardson, 2002; Owen et al., 2002; Kaczmarsky et al., 2005; Kuntz et al., 2005; Voss & Richardson, 2006; Baker et al., 2007; Danovaro et al., 2008; Sekar et al., 2008; Sokolow, 2009; Miller & Richardson, 2014), combined with the impact of ocean warming (Green & Bruckner, 2000; Bruno & Selig, 2007; Harvell et al., 2007), is causing an extreme amount of stress on corals that consequently are becoming more susceptible to disease infection (Mera & Bourne, 2018). Indeed, there are several studies and models to predict trends in infection and mortality of the coral reefs, some of which predict that possibly 76.8% of coral reefs worldwide will be infected and decline by 2100 (Burke et al., 2023). Corals may respond to thermal and mechanical stress by reducing the functionality of their immune system (Mydlarz et al., 2010; Toledo-Hernández et al., 2014) and/or experiencing a shift in their microbiome composition, resulting in a higher prevalence of disease-causing organisms, including both primary and opportunistic pathogens (Egan & Gardiner, 2016; Mera & Bourne, 2018; Van der Loos et al., 2019). In the last twenty years, outbreaks of coral diseases, such as the stony coral tissue loss disease (SCTLD), have been registered with higher frequency and with shorter intervals between them, leading to an increase in disease prevalence, amplification of host susceptibility, host range, pathogen survival and disease transmission (Myers & Raymundo, 2009; Maynard et al., 2015; Pinzón et al., 2015; Estrada-Saldívar et al., 2021).

Most of the world's coral reefs are in the Indo-Pacific Ocean, with the Maldivian reefs being particularly significant due to their biodiversity and abundance of species. Indeed, these reefs are home to over 180 zooxanthellate coral species belonging to 51 genera (Pichon & Benzoni, 2007), covering a total surface of 8920 km^2, which constitutes approximately 5% of the world's reef area and makes it the seventh largest coral reef system globally (Spalding et al., 2001). Nevertheless, in 1998 and again in 2016, the Maldives experienced a mass bleaching event that resulted in extensive

coral mortality, with mortality rates up to 90% in many reefs in the central region of the archipelago, especially in Ari Atoll (Ibrahim et al., 2017), while the northern region was impacted to a lesser extent, followed by the southern region (Cowburn et al., 2019; Pisapia et al. 2019). This event caused a qualitative shift in the biodiversity of species among the coral community, leading to a strong dominance of *Acropora* and *Porites* and a significantly less diverse reef structure (McClanahan, 2004; Hughes et al., 2018; Pisapia et al., 2019). The synergic effect of biodiversity reduction and coral bleaching has led also to the increase of coral susceptibility to infectious diseases (McClanahan et al., 2009), such as brown band disease (BrB) or skeletal eroding band (SEB) (Cróquer et al., 2006; Page & Willis, 2007; Montano et al., 2016), as seen in the first report of coral diseases in the Maldives (Montano et al., 2012). From 2010, coral diseases have been thoroughly studied in the area starting from black band disease (BBD) (Montano et al., 2013) to the investigation of prevalence and occurrence of BrB, SEB, white syndrome (WS), and ulcerative white spot (UWS) (Montano et al., 2014a, 2014b, 2015). Moreover, molecular analyses investigating the stress response of corals to different stressors were made searching for a possible specific resistance of some genera which may be the cause for the shift in the coral reef community composition (Seveso et al., 2012, 2015, 2016; Montano et al., 2014b).

Despite these studies, there remains still a considerable lack of essential information regarding the temporal trends and potential negative effects of diseases on reef-building corals in the Maldives. In this context, this study aimed to thoroughly re-evaluate the occurrence, prevalence, and host range of four diseases, namely black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB), and white syndrome (WS), and compare the findings with a previous assessment conducted on the same four diseases twelve years ago in the reef system around Thudufushi island. This comparison could provide a first basic assessment of temporal trends regarding coral diseases in this area and may provide insight into the evolution of the situation within this understudied geographical area.

6.3 MATERIALS AND METHODS

From December 2021 to February 2022, underwater surveys were conducted to investigate the occurrence of diseases in the reef system surrounding the resort island of Thudufushi (3°47'10.6"N; 72°43'53.2"E), located on the western side of the South Ari Atoll in the Republic of Maldives (**Fig. 1a,b**)

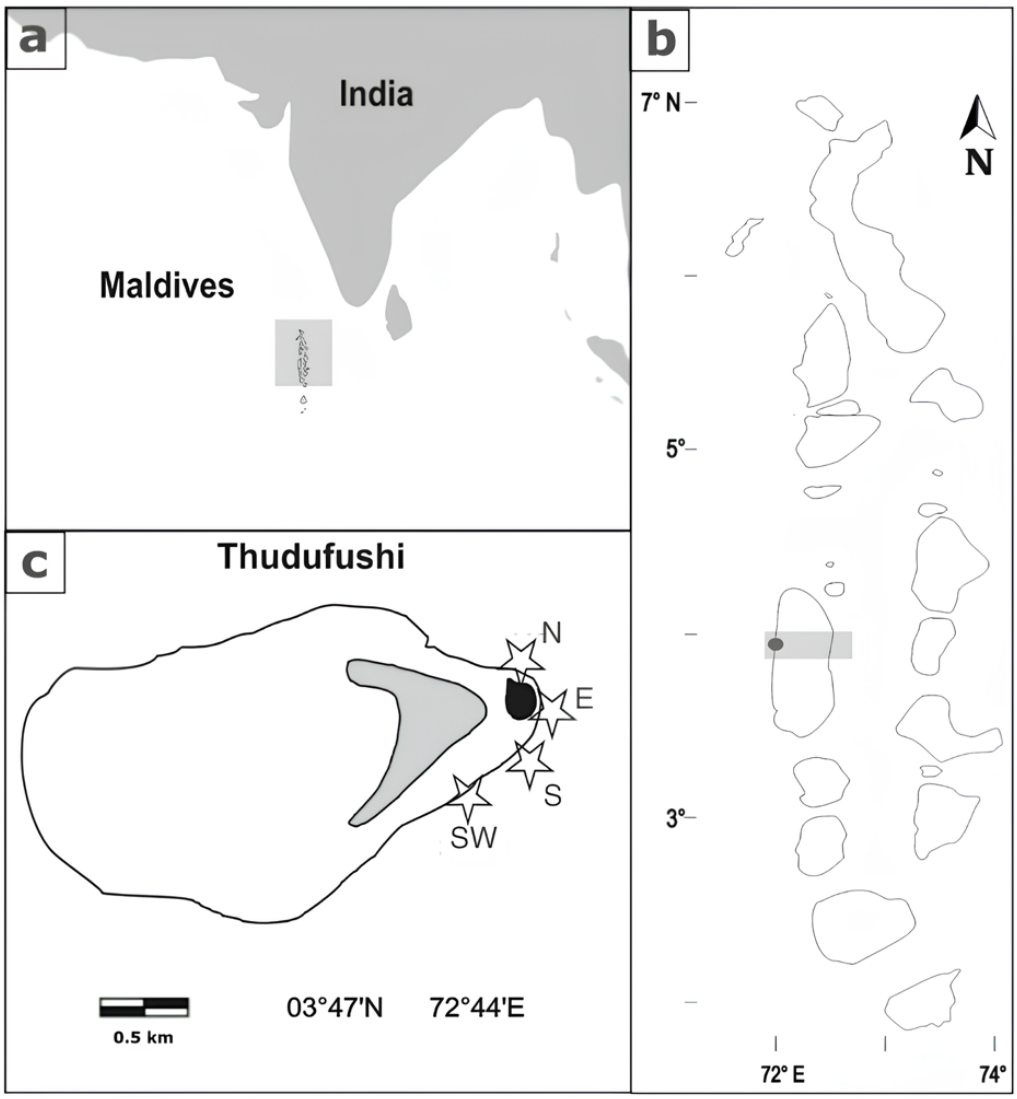

Figure 1. Geographical contextualization and overview of the study area: **a)** Indian Ocean; **b)** Maldivian Archipelago with the study area location highlighted; **c)** Thudufushi island with the four sampling sites indicated by stars: North (N); East (E); South (S); and Southwest (SW). The black area represents the island, the grey area signifies the deeper lagoon, and the black line marks the edge of the reef system surrounding the island

The island has a maximum diameter of approximately 300 m and it is surrounded by a lagoon that is almost 3.5 km in length and 1.7 km in width. This island was selected for the surveys due to a previous assessment conducted at this location by Montano in 2010 (Montano et al., 2015). The initial choice was influenced by the close relationship with the management, which facilitated easy access to the dive site and monitoring activities. Additionally, this collaboration allows for the possibility of long-term monitoring of the potential impact of human presence on the natural reefs. Since this island is located at the occidental external edge of the atoll, three out of four reef sides are situated inside the atoll, while the western side approaches the open ocean. The present assessment has been conducted in the same four sites analyzed in the study of Montano et al. 2015, which were located to cover all the sides of the Thudufushi island, namely North (N), East (E), South (S), and Southwest (SW) (**Fig. 1c**). The dive sites were originally chosen randomly based on the easy accessibility and the presence of low-energy reef habitats on the gentle slope of the island, but also to compare possible differences in disease prevalence due to environmental factors (wave action and currents) and direct human impacts (proximity to water-villas or other human infrastructure) in the different island sides. .

Quantitative analyses were conducted by snorkelling or SCUBA diving, depending on depth, placing three belts transects of 25 m x 1 m at two different depths for each site (total=24 transects), where shallow and deep transects were between 0−5 m and 12−15 m respectively. Each belt transect was randomly placed along the reef, with at least 10−20 m distance from each other, according to the method used by Montano et al. 2015. During the survey, each coral colony encountered within the transect was counted and evaluated for disease signs to compile a comprehensive list of the hard coral genera and diseases occurring in the study area. Only coral colonies in which 50% or more of the colony laid within the belt transect area were included in the analyses. Coral colonies were identified *in situ* at genus level according to relevant literature: Acroporidae (Wallace et al., 2012), Fungiidae (Gittenberger et al., 2011; Benzoni et al., 2012), Lobophylliidae (Budd et al., 2012; Arrigoni et al., 2014), Merulinidae (Budd et al., 2012; Huang et al., 2014), Poritidae (Kitano et al., 2014), Psammocoridae (Benzoni et al., 2007, 2010), Scleractinia *incertae sedis* (Budd et al., 2012; Benzoni et al., 2014) and the others by Veron (2002). The same four diseases considered by Montano et al. 2015, namely black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS), were identified by looking at macroscopic signs and according to relevant literature (Willis et al., 2004; Beeden et al., 2008, Montano et al., 2012, 2015a, 2015b; Séré et al., 2015).

The total disease prevalence has been computed as the ratio between the number of infected colonies and the total number of colonies observed. In addition, the taxon-specific prevalence for

each coral genus was calculated by dividing the number of infected corals for each genus by the total number of counted coral belonging to the same genus, according to the method used by Montano et al. 2015. Moreover, the potential relationship between disease susceptibility and colony size was evaluated through the comparison of the prevalence observed in three size classes (small <15 cm; medium 15–30 cm; large >30 cm). The size of the colonies was estimated by placing a measurement reference close to the specimen.

All the data obtained were tested for normality with Kolmogorov-Smirnov test. As the assumption for normal distribution and homogeneity of variance were violated, the comparison of the mean value of prevalence between sites, depths, and size classes were performed using the Mann–Whitney U-test and Kruskal–Wallis test. Moreover, Spearman's rank correlation was used to assess possible relationships between overall and taxon-specific prevalence, and density or size classes. Unless stated otherwise, data are presented as the arithmetic mean ± standard deviation (SD). All statistical analyses were performed using SPSS ver. 28.0.1.1 (IBM, New York).

6.4 RESULTS

In each of the four sites examined around Thudufushi island, six belt transects (three shallow and three deep) were performed covering approximately 600 m^2. In all the transects, at least one diseased colony was found at both depths. A total of 3443 colonies belonging to 32 different genera were counted, with 6.36% (219 colonies) identified as diseased colonies. Notably, out of the 32 genera observed, only 13 genera have been found affected by diseases. In all the investigated sites, SEB was the most prevalent disease with a prevalence of 1.31 ± 0.37% in E, 0.46 ± 0.51% in N, 1.08 ± 0.74% in S and 0.98 ± 0.95% in SW, followed by the WS (**Tab. 1**). Moreover, the E site experienced the highest total disease prevalence of 2.75 ± 0.56%, followed by 0.53 ± 0.52% in the S site, 0.51 ± 0.33% in the SW site and lastly the N site with a prevalence of 0.26 ± 0.21% (**Tab. 1**).

Table 1. Summary of the prevalence (%) of the four diseases analyzed and total overall prevalence per site in the surveys of 2022.

	EAST	NORTH	SOUTH	SOUTHWEST
black band disease (BBD)	0.68 ± 0.62	0.00 ± 0.00	0.86 ± 0.44	0.43 ± 0.42
brown band disease (BrB)	0.00 ± 0.00	0.19 ± 0.30	0.00 ± 0.00	0.46 ± 0.45
white syndrome (WS)	0.75 ± 0.67	0.40 ± 0.32	0.17 ± 0.27	0.19 ± 0.32
skeletal eroding Band (SEB)	1.31 ± 0.37	0.46 ± 0.51	1.08 ± 0.74	0.98 ± 0.95
Total	**2.75 ± 0.56**	**0.26 ± 0.21**	**0.53 ± 0.52**	**0.51 ± 0.33**

Regarding taxon-specific prevalence, the highest mean overall disease prevalence was observed for *Psammocora* (8.58 ± 12.98%), with a maximum value of 33.33% and higher prevalence in the N site, while the lowest mean disease prevalence was detected for *Favites* (0.46 ± 2.26%), which was observed affected only in the S site (**Tab. 2**). There was no significant difference in the overall mean prevalence of diseases between depths (Mann-Whitney U-test, $p > 0.05$), as well as among the different sites (Kruskal-Wallis test, $p > 0.05$). From the correlation analysis between disease prevalence and genera, a positive correlation was detected for *Acropora* (Spearman's $\varrho = 0.714$, $p= 0.47$, $p < 0.05$).

Table 2. Summary of the relative abundance, total disease prevalence, and coral density for each genus in the surveys of 2022.

Genus	Relative abundance (%)	Prevalence (%)	Coral density (m^2)
Acropora	40.00	2.67 ± 2.77	1.89 ± 0.53
Pocillopora	16.92	2.78 ± 3.62	0.72 ± 1.40
Psammocora	13.85	8.85 ± 12.98	0.13 ± 0.05
Porites	7.69	1.05 ± 2.21	0.78 ± 0.12
Astreopora	4.62	2.6 ± 7.35	0.07 ± 0.05
Isopora	4.62	1.9 ± 5.20	0.15 ± 0.05
Dipsastrea	3.08	1.42 ± 4.91	0.17 ± 0.07
Diploastrea	1.54	1.38 ± 6.80	0.09 ± 0.05
Favites	1.54	0.46 ± 2.26	0.27 ± 0.11
Galaxea	1.54	1.38 ± 6.80	0.1 ± 0.02
Leptoria	1.54	1.38 ± 6.80	0.05 ± 0.02
Pavona	1.54	0.52 ± 2.55	0.27 ± 0.05
Pectinia	1.54	0.52 ± 2.55	0.09 ± 0.06

Generally, there was a significant difference in coral density between shallow and deep transects in the four sites (Mann-Whitney U-test, $p < 0.05$), with the deep sites (~ 4 colonies per m^2) resulting in a two-fold lower coral density than shallow ones (~ 8 colonies per m^2). However, no significant correlation was found between overall coral density and prevalence of the diseases (BBD, BrB, WS and SEB), with the only exception of a significant positive correlation between disease prevalence and host density for the genus *Astreopora* (Spearman's, $\varrho = 0.737$, $p= 0.37$, $p < 0.05$).

The distribution of diseases among different size classes (small <15 cm; medium 15−30 cm; large >30 cm) showed statistical difference towards the corals belonging to the medium size class (Kruskal-Wallis test, $p < 0.001$). In particular, medium size corals were present at higher densities in all the sites, especially in the SW (3.12 ± 1.63 colonies per m^2), followed by the S site (2.74 ± 1.01 colonies per m^2), the N site (2.67 ±0.72 colonies per m^2) and the E site (1.04 ±0.59 colonies per m^2). Moreover, small corals were present at higher densities compared to larger corals in all the sites. This trend in the density of size classes is equally evident across shallow and deep sites. Although there are more diseased colonies of medium size compared to other size classes at both depths, the proportion of large colonies affected by diseases in deeper sites (4%) is higher than that of medium-sized colonies (2%). Finally, a comparison was conducted between the data obtained in this study and the findings presented by Montano et al. 2015 in the corresponding sites around Thudufushi island. The overall prevalence of the four diseases in the 2022 was significantly higher compared to the disease prevalence twelve years prior (Kruskall-Wallis test, $p =0.049$, $p <$

0.05; **Figs. 2 & 3**). In addition, there was an increase in prevalence in all the diseases analysed, with the only exception of BrB (**Fig. 3**). In particular, the prevalence of SEB was 75% higher in 2022 compared to 2010 (Mann-Whitney U-test, $p = 0.04$, $p < 0.05$). Moreover, the overall coral density was significantly higher (30.93 versus 5.73 colonies per m^2) in 2010 compared to 2022 (Mann-Whitney U-test, $p < 0.001$). Since *Psammocora* was the most affected genus in 2010 and 2022, a comparison between *Psammocora* densities was conducted and showed that the density in 2010 was significantly higher than the density in 2022 (Mann-Whitney U-test, $p = 0.014$, $p < 0.05$).

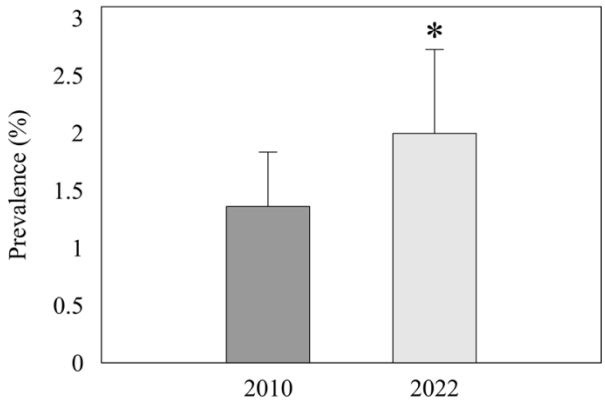

Figure 2. Comparison of the overall mean of the four diseases (black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS)) prevalence between 2010 and 2022. The results are presented as mean ± standard deviation (SD; showed as bars); the asterisks mark where statistical significance is present ($p < 0.05$).

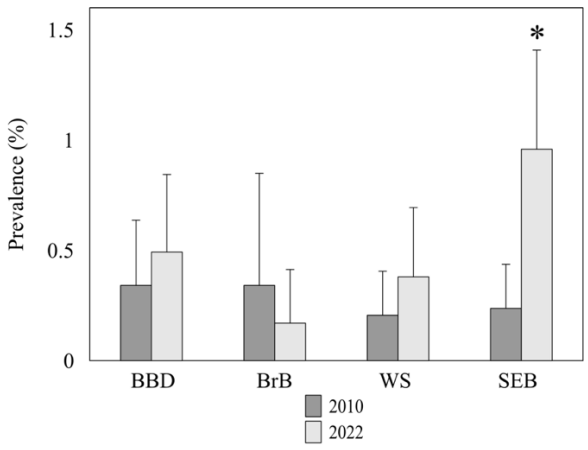

Figure 3. Comparison of mean prevalence for each disease (black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB), and white syndrome (WS)) between 2010 and 2022. The results are presented as mean ± standard deviation (SD; showed as bars); the asterisks mark where statistical significance is present ($p < 0.05$).

6.5 DISCUSSION

In recent decades, coral diseases have gained increasing research attention as a major contributor to the decline of coral cover and causative agent of mortality of Scleractinian corals worldwide (Ruiz-Moreno et al., 2012; Morais et al., 2022). Several studies have demonstrated that in the Indo-Pacific coral diseases are becoming more frequent, extensive, and severe due to the intensification of thermal stress and human activities, which are the major drivers of coral disease transmission (Harvell et al., 2007; Muller et al., 2008; Ruiz-Moreno et al., 2012; Randall & van Woesik, 2015).
Despite the major role that coral diseases play in the decline of coral reefs worldwide (Bruno & Selig, 2007; Osborne et al., 2011), there is a lack of studies thoroughly investigating the possible temporal trend in coral diseases, particularly through long-term monitoring conducted at the same locations over years (Randazzo-Eisemann et al., 2022). This gap is especially notable in the Maldivian region where, to our knowledge, no similar studies are present. A recent report from Morais et al. (2022) identified six major and most common coral diseases in the Maldives: black band disease, dark spot disease, white plague, white syndrome, skeletal eroding band, and yellow band disease, which all together account for 78.6% of all the disease records in the region.
Therefore, the present study, after exactly 12 years since the initial report by Montano et al. (2014b), re-evaluated the abundance and prevalence of four diseases in the same reef system surrounding Thudufushi island, aiming to identify potential changes in disease prevalence and distribution within an ever-changing coral community. Consistent with the previous study, WS and SEB were identified in all the investigated sites, while BrB was absent from the East and South sites, and BBD was not found in the North site, confirming different spatial distributions of diseases around the island. This current study highlights an increase in all disease categories compared to 2010 with the exception of BrB, which decreased by approximately 30% over the last twelve years. This deviation against the trend of BrB, showing a lower prevalence, may be attributed to the notable decrease in the density of its primary host, the genus *Acropora*, which underwent a five-fold reduction in density since 2010 (5.87 versus 1.89 colonies per m^2). Indeed, a positive correlation between the presence of *Acropora* and BrB was found, indicating a propensity of the ciliate responsible for the infection *Porpostoma guamense* (class Oligohymenophorea; subclass Scuticociliatia) (Sweet & Sèrè 2016, Ravindran et al. 2023) towards this specific genus (Boyett et al., 2006; Sweet & Bythell, 2012; Ashraf et al., 2023). These findings confirm once again how coral disease, exacerbated by global warming and changes in environmental conditions, significantly impacts the decline of the hermatypic corals, consequently reshaping the composition coral reefs. Indeed, it is well known that climate change has altered sea surface temperature and other critical connected parameters such as salinity, current and nutrient availability (Hoegh-Guldberg et al.,

2017; Gattuso et al., 2018). These changes have contributed to shifts in the relative abundance of species on coral reefs, especially following the mass bleaching event in the region in 2016 (Perry & Morgan, 2017; Ibrahim et al., 2017; Cowburn et al., 2019). Our results confirm that these changes in the distribution and abundance of coral species are reflected in the presence, occurrence, and distribution of coral diseases. In fact, each disease targets specific hosts, and therefore their ability to spread has aligned with availability of these hosts and their susceptibility to infection (Palmer et al., 2010; Muller et al., 2018).

In the study area, the overall disease prevalence was approximately 2%, which is a lower value compared with other Indo-Pacific regions (Myers & Raymundo, 2009; Ruiz-Moreno et al., 2012). However, over the past twelve years, there has been a significant increase in the overall coral disease prevalence of about 40%, possibly attributed to ocean warming and other human-induced stressors, known to increase the coral susceptibility to pathogens (Aronson et al. 2003; Harvell et al. 2007, Bourne et al., 2009, Ruiz-Moreno et al., 2012). In particular, elevated sea surface temperatures may increase the pathogens' growth rate and virulence, increasing their density and infectivity (Kushmaro et al., 1998; Toren et al., 1998; Rosenberg et al., 2007), as well as compromise the coral immune system increasing the host susceptibility to infection (Ritchie, 2006; Lesser et al., 2007; Muller et al., 2008; Reed et al., 2010). From the results obtained, SEB exhibited the highest prevalence with a significant 60% increase compared with 2010. This higher prevalence may be explained by the wide host range of SEB associated with the ciliate *Halofolliculina corallasia* (Antonius, 1999; Willis et al., 2004; Bruckner, 2015; Page et al., 2015). Indeed, in the present study, SEB affected 13 coral genera, particularly *Pocillopora* and *Acropora*, in accord with the results of the study by Montano et al. (2012), where *Acropora* and *Pocillopora* were the most affected genera by SEB and hosted the highest number of coral diseases. This supports the hypothesis that fast-growing coral may have higher susceptibility to infection rather than slow-growing corals (Willis et al., 2004; Palmer et al., 2008; Mydlarz et al., 2010), where the thinner tissues of fast-growing corals have been suggested to be more sensitive to bleaching and diseases possibly due to lower energy investment in the immune system (Willis et al., 2004; Palmer et al., 2008). In this case, the statistical analysis revealed a positive relationship between the overall disease prevalence and the disease prevalence in *Acropora*, which can be associated with higher susceptibility of fast-growing corals (Willis et al., 2004) or may be a consequence of the dominant presence of *Acropora* in Maldivian coral reefs, as well as in Thudufushi where it has the highest relative abundance (40%). Interestingly, *Psammocora* was the genus mostly affected by diseases, in accord with the data provided by the previous study by Montano et al. (2015). This could be explained by two reasons.

First, the relative abundance of the host increased from 3.47% in 2010 to 13.85% in 2022. Second, this genus might be more susceptible to disease compared to other coral genera.

Moreover, the study revealed a trend indicating higher susceptibility to diseases among medium size class colonies (15−30 cm). This result was unexpected since in the study by Montano et al. (2016) the large colonies were more susceptible. In general, older and larger colonies are considered more vulnerable to infection and multiple stressors than younger and smaller colonies, as they have greater surface area and longer exposure to the environment (Caldwell et al., 2018). However, the 2016 bleaching event, together with other stressor affecting the coral reef, have potentially induced a shift in the coral reef community towards lower biodiversity and smaller average colony size (Hughes et al., 2017; Cowburn et al., 2019; Pisapia et al., 2019). Therefore, further studies are needed to determine whether this pattern is related to higher density of the medium size colonies, or if this class size is effectively more susceptible to diseases due to specific biological processes or environmental conditions, which have been also demonstrated to influence disease susceptibility (Vega Thurber et al., 2020).

In the current study, a minimal variation in temperature was observed since 2010. However, we noted an increase in coral coverage at the deepest sites, coinciding with a higher prevalence of the considered diseases. These findings suggest that various abiotic factors may be at play, impacting the coral's ability to resist disease and influencing the growth and virulence of pathogens responsible for infections. Specifically, high turbidity conditions may foster pathogen proliferation, weaken host defenses, and impair photosynthetic efficiency, ultimately leading to reduced oxygen levels in the water (Talke et al., 2009; Vega Thurber et al., 2014; Chaves-Fonnegra et al., 2021). Moreover, a high coral coverage may lead to multiple colonies growing in close proximity, providing an easy pathway for contagious pathogens to spread among different coral colonies and therefore enhancing the possible transmission and presence of diseases (Ladd et al., 2016; Moulding & Ladd, 2022).

Furthermore, there was variation in disease prevalence trends among sites, with the North site showing the lowest incidence of diseases. This result may be explained by the impact of human activities that may alter the reef environment by increasing nutrient availability (Bruno et al., 2003; Voss & Richardson, 2006), organic matter (Kuntz et al., 2005; Baker et al., 2007), sewage effluent (Kaczmarsky et al., 2005; Sekar et al., 2008) and introduced chemicals (Owen et al., 2002; Danovaro et al., 2008). Indeed, the most impacted sites are the ones closer to human structures (South and Southwest sites), in proximity of gas pipelines departing from the resort island, or in the inner side of the atoll (East site) where artificial walls have been built to protect the beach from the wave erosion. In addition, all these sites are impacted by snorkelling activities offered to

tourists, therefore to mechanical or chemical stress due to sunscreen (Danovaro et al., 2008; Mitchelmore et al., 2019). On the contrary, the North site does not face any accommodation/resort facility and is excluded from snorkelling activities reducing the impact on coral health. Moreover, this site is exposed to conditions that differ from the other sites, such as strong currents and waves, being located at the outer edge of the atoll, which may also positively influence the disease prevalence.

In conclusion, the observed decline in coral density and variation in reef composition since 2010 may indicate that changes in environmental conditions may have influenced the spread of coral disease, potentially elevating both infectivity rates and coral susceptibility to stressors. Indeed, coral diseases are threatening health and survival of the corals in the Maldives and all around the world. For this reason, it is fundamental to study the progression of the infection in relation to the variation of environmental conditions, such as rising sea temperature, which could possibly exacerbate the situation. Moreover, determining a possible trend in disease dynamics is important for future predictions for both the shift in the local coral reef communities and the impact of diseases on a temporal scale. In this context, the present study is the first study that surveyed the same area over different years in the Maldives and it highlights the need for monitoring the progression of coral diseases. Further studies and monitoring projects are needed to fully understand the temporal variation and evolution in coral diseases spreading in sensitive areas, such as the Maldives.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

A comprehensive, multi-scale investigation of coral growth anomalies (GAs) was conducted, integrating ecological, spatial, morphological, and pathological analyses. GAs were found to be widespread across multiple locations in the Maldivian Archipelago, affecting several coral genera, including *Acropora*, *Montipora*, and *Pachyseris*. Lesions were associated with severe alterations in both skeletal and tissue morphology.

Hyperproliferation of basal body wall cells results in excessive skeletal extension of the coenosteum, which consists in the skeletal connecting structure between polyps.

Although it remains uncertain and debated whether calcification is primarily governed by physicochemical or biological processes, evidence suggests that it is predominantly biologically driven (Von Euw S. et al., 2017). Calicoblasts - specialized cells of the basal body wall - secrete the skeletal organic matrix (SOM), which is essential for coral skeleton deposition (Tambutté et al., 2011; Falini et al., 2015).

In this context, the coupling of skeletal and histological analyses presented in this research (**Chapter 3** & **4**) proved highly informative, confirming the consistency of findings, enabling a detailed pathological characterization, and providing important insights for future investigations.

Consistency with previous findings was observed in both the scanning electron microscopy (SEM) and histological analyses of GAs (Work & Rameyer, 2005; Domart-Coulon et al., 2006; Andersson et al., 2020; Rich et al., 2021).

The skeleton of growth anomalies (GAs), compared to healthy counterparts, exhibited higher porosity and a greater incidence of skeletal defects, indicative of changes in the abundance of centers of calcification (COC), which are fundamental microstructural elements linked to skeletal pathophysiology (Von Euw S. et al., 2017; Andersson et al., 2020). These skeletal defects, characterized by underdeveloped and abortive elements, such as columella and septa, are consistent with the observed reduction in COC abundance, which are normally concentrated in these structures. In GAs, skeletal extension occurs without corresponding thickening; the skeleton remains porous and less dense, reflecting shifts in the overall energy allocation and calcification balance.

Basal body wall cells in all examined GAs showed hyperproliferation, a clear and consistent pathological feature, leading to an increased profile of basal body wall tissues and a higher number

of gastrovascular canals. While calicoblasts actively deposit skeleton material, this occurs at the expense of skeletal quality. Furthermore, the affected tissues are largely depleted of endosymbionts, resulting in reduced energy availability for routine physiological processes.

In GAs, energy is reallocated to sustain cell proliferation, compromising other energy-demanding functions such as the pH regulation of the extracellular calcifying fluid (ECF) (Andersson et al., 2020). Under normal conditions, corals elevate the pH of the ECF during calcification to favor aragonite precipitation at optimal saturation states. In contrast, although skeletal extension in GAs is increased due to calicoblast hyperproliferation, aragonite precipitation occurs under lower saturation state conditions, resulting in a skeleton that is less dense and irregular in morphology, as observed in SEM analysis (Mollica et al., 2018).

The affected skeleton not only becomes more fragile and prone to injury but also exhibits significant alterations in its overall architecture. SEM analysis revealed that, while the number of structural elements in the coenosteum of GA masses - such as trabeculae and spinules - remained unchanged, their thickness, arrangement, and refinement were noticeably altered.

As a result, the coenosteum displayed increased intertrabecular spaces, a feature also evident in sub-gross and histological analyses, where large skeletal cavities are observed, often filled with dense aggregations of organisms.

Overall, combined SEM and microscopic analyses indicate that GA lesions consist of a mass characterized by excessive growth of the coenosteum and basal body wall, which engulf, overgrow, and distort polyp structures. The remaining polyps within affected areas are enlarged - confirmed by increased corallite diameter - and exhibit hypertrophied tissues, often accompanied by a near-complete absence of gonads. This lack of gonadal development is a distinctive feature of GA lesions and aligns with a reallocation of energetic resources away from reproduction toward sustained tissue growth (Domart-Coulon et al., 2006; Andersson et al., 2020).

Gonad presence may further depend upon the developmental stage of the lesion. Early-stage GA lesions may retain some reproductive capacity, whereas advanced or larger lesions may surpass a critical size threshold beyond which reproductive investment becomes energetically unfavorable. Furthermore, this may represent an adaptive mechanism to limit the spread of pathological traits within the colony, analogous to the selective sacrifice of non-functional tissues.

The long-term consequences of growth anomalies on coral health and survival remain uncertain, largely due to the lack of long-term studies, particularly over the past two decades during which the impacts of climate change have intensified. This research did not include a long-term study of growth anomalies; however, an effort was made to monitor an *Acropora* colony presenting multiple

GA masses. As shown in Figure 8 (**Chapter 1**), the colony exhibited several masses and was monitored for nearly one year, until it died during the 2025 heatwave.

This outcome highlights how coral diseases, when coupled with thermal stress, may accelerate colony mortality. Under climate change scenarios in which coral diseases are expected to increase in both prevalence and severity, lesion etiologies may shift over time (Joyner et al., 2015; Burke et al., 2023; Vega Thurber et al., 2025). Consequently, principles and methodologies from veterinary science should be more routinely integrated into coral disease ecology, and health monitoring frameworks should be implemented with a stronger focus on pathological assessment.

The etiology of growth anomalies was confirmed to be highly complex, multifactorial, and still unresolved. However, this research identified potential contributing factors, including the involvement of endolithic organisms such as sponges, fungi, and algae, which may initiate or exacerbate lesion development.

Given the complexity of the lesion, further analysis employing different approaches are needed. Trasmission electron microscopy (TEM), along with investigations of cellular pathways and proteomic profiling of basal body wall cells from affected areas, would provide valuable insights. TEM analysis could help elucidate, and potentially rule out, the presence of viruses or other etiological agents that remain undetectable with regular light microscopy. Additionally, examining changes in intra- and intercellular pathways involved in the calcification process, as well as molecular analyses to detect consistent upregulation of proteins commonly associated with tumorigenic or hyperplastic tissues in other metazoans, would deepen our understanding of the lesion's pathology.

This research highlights the importance of identifying consistency across studies of the same disease in order to build a robust case definition supported by clear and precise nomenclature, thereby reducing confusion in coral disease classification. Comparing lesions across different coral genera is particularly important for identifying shared pathogenic traits; features that are consistently observed among multiple genera are likely to represent fundamental character of the lesion rather than genus-specific responses.

Histopathological analyses on GAs revealed pathological features consistent with previous reports, while also identifying genus-specific characteristics, particularly in *Pachyseris*, representing the first histopathological description for this genus. In Maldivian corals, analyzed GAs were also uniquely associated with a diverse assemblage of microorganisms not reported in GA lesions from other regions, highlighting an aspect that warrants further investigation. Together, these findings suggest that disease etiology may not only change over time but also involve different drivers and host responses across geographic contexts, where species composition and local adaptations may vary.

In parallel to comprehensive analysis of GA lesion, this research provides an integrated study of brown band disease (BrB) combining gross lesion characterization, genetic identification of associated and putative pathogen, providing the first comprehensive histopathological description of the disease. The lesion was consistently associated with the ciliate *Philaster guamense*, providing strong evidence for its involvement in initiating coral tissue loss.

Indeed, despite the well-established principle in pathology that association does not imply causation, our results - when considered alongside consistent evidence from the literature regarding morphology and genetic identification of the ciliate - support its role as the primary pathogen of BrB. This interpretation is further strengthened by microscopic evidence of host cellular debris within the ciliate and the absence of pathological features indicative of involvement of other microbial agents.

The BrB lesion exemplifies a key principle emphasized throughout this research: relying solely on gross visual assessments - especially during field monitoring - for case definition can lead to misidentification and confusion. In **Chapter 6**, the prevalence of four coral diseases was recorded over a 12-year period at Thudufushi Island, including both brown band disease (BrB) and white syndrome (WS). However, lesions initially identified in situ as WS may in fact have been BrB lesions that did not clearly display the characteristic brown ciliate band. This example highlights the critical role of histopathology in achieving accurate and precise disease diagnosis, especially in corals where gross disease manifestations are limited and often overlapping.

The possibility of misidentification of some lesions in the field, does not nullify the meaning of the presented study, as the overall prevalence of coral disease in the first long-term assessment of coral disease trends in the Maldives, revealed an increase consistent with global observations and projections. The overall increase of disease prevalence demonstrated that, despite its remoteness and ecological uniqueness, the Maldivian Archipelago is not exempt from the ongoing global degradation of coral reefs.

Despite having persisted for hundreds of millions of years and surviving past environmental upheavals, corals are now facing an unprecedented global decline driven by a big part by interacting climatic and anthropogenic stressors. Coral restoration has emerged as a key mitigation strategy, with hundreds of projects implemented globally. However, restoration practices that propagate genetically similar corals at high densities may inadvertently increase disease susceptibility and facilitate epizootic outbreaks, events widely reported in multiple restoration sites (Moriarty et al., 2020). This raises a critical question: "Is it worth farming and propagating thousands of corals at the risk of losing them all to disease?"

In wildlife conservation, disease-driven mass mortality events are treated with urgency and ethical concern; yet similar outbreaks in coral nurseries and restoration sites are often uninvestigated or insufficiently addressed.

Established wildlife translocation guidelines emphasize the need for feasibility assessments, careful planning, and comprehensive disease risk evaluation, particularly in the context of epizootic events (IUCN/SSN, 2013; *National Guidelines for Management of Disease in Free-Ranging Australian Wildlife*, 2020). Effective reintroduction or restoration strategies cannot be developed without a solid understanding of disease ecology, and health screening prior to translocation is essential to minimize the risk of introducing unknown pathogens into recipient ecosystems. The same principle applies to disease treatment, which must be preceded by a careful and robust understanding of disease ecology before any intervention is attempted.

This issue is particularly critical for corals propagated in captivity, which may develop uncharacterized or subclinical diseases that are not readily detectable through gross visual assessment. Reintroducing such corals to natural reefs without adequate disease evaluation may trigger epizootic events with severe ecological consequences. In line with *IUCN Guidelines for Reintroductions and Other Conservation Translocations* (IUCN/SSN, 2013), treatment is not recommended when population decline is associated with diseases that have not been clearly characterized, as empirical or prophylactic interventions do not substitute for diagnosis and may simply mask symptoms, fail to eliminate causative agents, or promote resistance.

Ultimately, the answer to whether coral restoration is "worth the risk" may be yes - doing something may be preferable to doing nothing - but only with full awareness of the potential costs involved. Restoration practices must therefore be supported by rigorous disease-focused research aimed at identifying and characterizing the diseases contributing to coral decline. Establishing robust case definitions represents a necessary first step before implementing disease management or mitigation strategies, ensuring that restoration efforts do not inadvertently exacerbate disease-related risks.

In conclusion, there is an urgent need for a systematic framework to address coral disease outbreaks, incorporating regular health monitoring, disease risk assessment, standardized disease characterization, and a deeper understanding of disease transmission processes. These measures should be formally integrated not only into coral reef routine monitoring, but also into restoration and mitigation practices, alongside outplanting strategies that explicitly consider host density and disease dynamics. Without such an approach, restoration efforts risk undermining their own long-term effectiveness.

Taken together, the findings presented in this thesis reinforce the view that coral diseases are not isolated phenomena but emergent outcomes of interacting biological, environmental, and anthropogenic stressors. By combining long-term ecological observations with detailed pathological and microstructural analyses, this work contributes to a more holistic understanding of coral disease processes and their implications for reef resilience in the Republic of Maldives.

Ultimately, improving our capacity to diagnose, monitor, and manage coral diseases is essential not only for refining mitigation practices, but also for safeguarding the ecological integrity of coral reefs. In a rapidly changing ocean, the persistence of coral reef ecosystems will depend on our ability to move from reactive interventions toward informed, evidence-based conservation strategies grounded in robust scientific evidence.

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