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Building living systematic reviews and reporting standards for comparative microscopic analysis of white diseases in hard corals.

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Abstract

1. Over the last four decades coral disease research has continued to provide reports of disease states, the occurrence and severity of disease outbreaks, and associated disease signs however nomenclature and protocols for microscopic disease descriptions have yet to be implemented. Histopathology using systematic protocols is a gold standard for the microscopic assessment of diseases in veterinary and medical research. Increasing disease outbreaks on coral reefs as human impacts intensify highlights the need to also establish standardised diagnostic terminology and methodology for coral disease research. 2. Here we apply a systematic approach to collating, reviewing, and evaluating histopathological methods used to study white diseases in hard coral taxa and map research effort in this field spanning study design, sample processing and analysis in the 33 publications identified between 1984 and 2022. 3. We find that studies to-date have not uniformly detailed methodologies, and terminology associated with histopathological reporting and disease description is inconsistent between studies. Combined these limitations reduce study repeatability, limiting the uptake of histopathology in coral disease research and the capacity for researchers to compare disease reports. 4. A primary outcome of this study is revision of the most widely used terminology applied to coral tissue and development of recommendations for standardised reporting procedures with the aim of increasing accessibility and uptake of histopathology in addition to allowing for ongoing comparative analysis through living systematic reviews for the coral disease field.

Building living systematic reviews and reporting standards for comparative microscopic analysis of white diseases in hard corals.

Running headline: A systematic review of histopathology methods

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Abstract

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Keywords

Coral disease, disease description, histopathology, living review, systematic review

Introduction

Coral diseases pose an immediate threat to coral reef systems because they can contribute to the reduction in abundance and cover of calcifying habitat forming corals (Estrada-Saldívar et al., 2021; Heres et al., 2021; J. Miller et al., 2009; Precht et al., 2016), and ecosystem phase shifts (Harvell et al., 2007; Norström et al., 2009). Given the ongoing impact of climate change and anthropogenic stressors on coral reefs (MacNeil et al., 2019; Maynard et al., 2015; Sokolow, 2009) it is critical we develop and apply standardised tools to understand coral health and to allow for comparative analysis. Histopathology is a standard method used many fields to diagnose and assess disease pathologies at a microscopic level. Despite being highlighted as critical to the assessment of coral diseases, standardised reporting protocols have yet to be taken up likely contributing to a majority of coral disease studies not including histopathological assessment in disease reports (Galloway et al., 2007; T. Work et al., 2008; T. M. Work & Meteyer, 2014). Coral disease research has predominantly focused firstly on assessing disease at epidemiological scales (disease occurrence and prevalence within a reef ecosystem), and secondly on gross pathological assessment (disease description without microscopic assessment) (T. M. Work & Meteyer, 2014). Assessment of disease at epidemiological scales is a rapid way to gain information on disease dynamics and impacts within a population, but provide no information on host condition, possible causes of lesions (disease aetiology) and other non-visible impacts of stress (T. M. Work & Meteyer, 2014).

Scleractinian corals (hard corals) are simple, multicellular organisms where interconnected polyps form the colonial organism. Individual polyps are formed from a surface body wall which provide the outer most barrier between the organism and its surroundings (Figure 1) and a basal body wall that anchors coral tissue to its skeleton (Figure 1) (Galloway et al., 2007). These tissue layers are made up of an epidermis, connective tissue termed the mesoglea, and gastrodermis which hosts the photoendosymbiotic dinoflagellates (Figure 1) (Galloway et al., 2007). The epidermis comprises of the epithelium with columnar cells, mucocytes and cnidocytes (specialised stinging cells) (Figure 1) (Galloway et al., 2007). The basal body wall is comprised of the epithelial calicodermis, desmocytes and mucocyte cells, and is the layer responsible for secreting a soluble organic matrix facilitating calcium carbonate secretion (Figure 1) (Galloway et al., 2007). Histological analysis of coral tissues involves fixation of coral colony fragments and decalcification to remove the network of calcium carbonate skeleton, tissues can then be processed with standard techniques to allow microscopic assessment of coral tissues and cell structure (Galloway et al., 2007; Greene et al., 2020). Histology has also been used to identify microbes, parasites (T. Work et al., 2008; T. M. Work & Meteyer, 2014), assess reproductive state (Ward, 1995), and undertake cellular assessment of endosymbiosis breakdown (bleaching) (Brown, 1997; Brown et al., 1995; Gates et al., 1992; Weis, 2008). Studies have also applied a variety of stains, imaging and analysis techniques to assess coral tissue integrity, thickness, cell death, necrosis and algal symbiont characteristics (Ainsworth, Kramasky-Winter, et al., 2007; Ainsworth et al., 2008; Gierz et al., 2020; T. M. Work & Rameyer, 2005). Histology is also key to marrying colony scale gross observations of disease lesions to coral condition (Ainsworth, Kramasky-Winter, et al., 2007; Ainsworth, Kvennefors, et

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al., 2007a; Ainsworth et al., 2008). Often, differences at the cellular level are apparent before visible signs of stress manifest at the whole colony level (Ainsworth et al., 2008) and similar visual signs of diseases in corals can have very different pathologies at a tissue level (Ainsworth, Kramasky-Winter, et al., 2007).

Despite increased risk of disease emergence on coral reefs and continued calls for establishment of standardised terminology and pathological tools (Galloway et al., 2007; Pollock et al., 2011; Weil et al., 2006; T. M. Work & Meteyer, 2014) uptake has remained slow (T. M. Work & Meteyer, 2014). Work and Meteyer (2014) called for increased multi-disciplinarity to address a disconnect between those who study and elucidate the causes of animal disease (i.e. veterinary scientists and pathologists), and those who study coral biology and ecology (T. M. Work & Meteyer, 2014). Doing so will address ambiguous communication of findings and a deficiency in consistent and comprehensive protocols within the literature. Increasingly there is a growing need for reliable synthesis of research to address challenges facing the environment. Systematic reviews for evidence synthesis use transparent methodology to overcome problems of bias commonly associated with narrative reviews for summarising research and are now gold standard in many fields (Pussegoda et al., 2017). Systematic reviews are commonly used to inform practice, policy and management because they are assumed to be unbiased, comprehensive and reproducible syntheses (Bilotta et al., 2014; Pullin & Stewart, 2006). Additionally, review methodology can be tailored for the research questions at hand and can vary in the depth of synthesis from mapping research areas, to rapid reviews or more comprehensive qualitative and quantitative protocols including meta-analysis (Lagisz et al., 2022). Living systematic reviews are an emerging approach that involves continual updating to include the most recent research with the aim of making relevant evidence available to users, including managers and decisions makers (Khamis et al., 2019).

Here we provide a systematic protocol for collating and reviewing studies that have used histology to microscopically investigate white diseases in hard coral taxa over the past four decades. White diseases have been reported as some of the most damaging and widespread diseases on coral reefs, and include white plague, white band disease and white syndromes (Willis et al., 2004) (Bourne et al., 2015; Bythell et al., 2004; Willis et al., 2004) of hard coral taxa that all can cause significant coral mortality (Aronson & Precht, 2001; Estrada-Saldívar et al., 2021; Heres et al., 2021; Willis et al., 2004). These diseases are characterized by a white tissue lesion of partial mortality or symbiosis breakdown before loss of tissue, leading to exposed white skeleton (Bourne et al., 2015).

The primary research question addressed in this study is:

What histological methods have studies used to microscopically assess white diseases in hard coral taxa? Secondary questions we also address in this review are:

a) What is the research effort in this field?

b) What is the study design of publications that have used histology to investigate disease?

c) What methods have publications used in preparation and processing for histology?

d) What is the standard of reporting for methodology of publications?

In doing this we hope to provide 1) transparent and repeatable protocols for systematically reviewing literature associated with white diseases of hard coral taxa, and 2) standard reporting recommendations for future coral disease histopathology studies to aid uptake of histopathology and continued synthesis and appraisal of research through establishing living review procedures.

Methods

Systematic review

We conducted a systematic identification of literature using histological methods to assess white diseases in hard coral taxa. This review protocol follows the reporting guidelines set out by the PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses - Protocols) *(Shamseer et al., 2015)* and is

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amended with the reporting standards of ROSES (Reporting standards for Systematic Evidence Syntheses)*(Haddaway et al., 2018)*. A conceptual diagram showing systematic protocol development is detailed in Figure S1.

Specifically, we used an initial pilot phase to identify relevant key words, exclusion, and inclusion criteria, in addition to defining a detailed search and data collection plan (Supplemental Materials, Figure S1). Scopus was selected for this study as it offers high coverage of subjects relevant to life sciences (Mongeon & Paul-Hus, 2016). Search terms included those referring to organism (hard corals), disease (white diseases and tissue loss) and method (histopathology) (Table 1). The final list of search terms for application in academic data bases Scopus and Web of Science were applied on the 24/2/23 (Table 1). The returned lists of references in each database were exported into reference management software Zotero (Vanhecke, 2008) and reference lists were merged and duplicates removed.

The pilot screening phase developed exclusion and inclusion criteria to identify literature for full-text review (see Figure S1, Supplemental Information S1). The final criteria (Table 2) formed a decision tree, which was then applied to all studies returned by the searches and were carried out by a single reviewer (CP) in Rayyan (Ouzzani et al., 2016). A final search was completed in Google Scholar to screen for any relevant publications not returned through Scopus or Web of Science. To do this, inclusion and exclusion criteria were applied to the first 10 pages returned on seven search strings by a single reviewer (CP): "coral histo* stony coral tissue loss*", "coral histo* white syndrome", "coral histo* plague ", "coral histo* white band" and "coral histo* lesion" on the 24/2/23.

For each of the publications included in this study we systematically recorded information (i.e. data extraction) through filling in a pre-coded data sheet in GoogleForms for general data extraction (see https://forms.gle/4enApHx2qtJMeQX5A) and a quality of methods reporting appraisal (see https://forms.gle/t3s4L35LUkXc93um7). Data categories followed the research questions presented in the introduction. Our approach followed PICO elements commonly used in evidence synthesis*(Livoreil et al., 2017)*. The functioning of the datasheet was piloted on randomly selected 10 articles. Data extraction allowed mapping of research effort through collected information on first, second and last authors of publications including country of institution (further classified as low-and middle-income countries based (OECD, 2020) and when stated online (through self-report) pronouns used following guidelines for gender outlined by SAGER (i.e. he/she/them, (Heidari et al., 2016)) and year of publication.

Information was collected on study scope, including location of the study, study type (field or experimental), diseases studied, taxa studied and other methods utilised unrelated to histopathology. Location of the study was classified as the primary region of coral sample collection and grouped into major biogeographical regions (as per Crisp et al., (2022)). We extracted data for fields relevant to histological methods and sample preparation including information on sample types, decalcification, embedding, staining, and imaging. We extracted data on methods for histological analysis including the type of data collected (i.e. qualitative, quantitative or semi-quantitative), and specific tissue structures/conditions/agents that were examined. Additional comment fields were used to capture relevant information including the main findings of each study.

We used the STAR (Structure, Transparent, Accessible Reporting) Methods protocol outlined by Cell Journal (Marcus, 2016) in addition to work by Gibson-Corley et al., (2013) presenting best practice for scoring histopathological tissues to develop criteria in which to appraise the quality of methods reporting in each of the studies. General instructions for STAR methods include reporting methods in sufficient detail so readers do not need to refer to other papers to understand how procedures were performed. The developed criteria consisted marking studies based on categories: resource availability, method details and quantification and statistical analysis. Here we also collect information on reporting of sample sizes. Table S1 presents criteria for data extraction for methods reporting appraisal.

Data visualisation and statistical analyses

We used R version 4.1.1 (2021-08-10) (R Development Core Team, 2010) for analyses and data visualisation.

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All code and raw data are available at https://github.com/CharlotteEPage/Histology_methods_systematic_review.git .When multiple methods and/or data fields were used in a study, each category was counted independently for summary statistics and visualisation. Diseases studied were grouped hierarchically by the affected taxa and the disease name (e.g *Montipora* White Syndrome). If a study included investigation of multiple species and diseases each combination was separately accounted for. Spatial illustration were made using Google My Maps (Google, 2023) to record latitude and longitude of sampling regions and QGIS version 3.26 (Team QD, 2022)World map was downloaded from Natural Earth (https://www.naturalearthdata.com/) and coral reef locations from UNEP-WCMC (https://data.unep-wcmc.org/datasets/1) (UNEP-WCMC, et al., 2021).

Results

Systematic identification of literature

Searches of Scopus and Web of Science using the final search terms yielded 71 and 162 articles respectively. After removal of 59 duplicates, 174 articles were screened on Rayyan. 30 articles were deemed relevant to this study and included for full-text screening. 144 articles were excluded from the study because they were not in English, did not use histology, did not consider hard coral, or considered other coral diseases. Five articles were further deemed relevant for this study through Google Scholar searches, leading to a total of 35 papers reviewed at full-text for data extraction. At full-text review, a further two publications were removed from the review because they used only Transmission Electron Microscopy methods which is out of scope for this review. This left a final list of 33 publications for data extraction. A ROSES flow chart detailing results of our systematic protocol can be found in Figure S2.

Overview of systematic review data

Of the 33 studies included in this review, 28.7 % (n = 27) of first, second and last authors were identified as female and 42.5 % (n = 40) were identified as male (Figure 2A). The remaining authors (28.7 %, n = 27) did not clearly have gender specified online (Figure 2A). Of the 27 first, second and last authors with no gender identified, ten authors were from countries classified as low and middle-income countries. Of the last authors only, 57.6 % (n = 19) were identified as male, 18.2 % (n = 6) identified as female and 24.2% (n = 8) did not clearly have gender specified online. First, second and last authors were affiliated with 16 countries. In total the highest proportion of authors were affiliated with institutions in the United States (46.8 %, n = 44), followed by Australia (16 %, n = 15) (Figure 2B). Mexico and the New Zealand had the same representation of authors (6.4 %, n = 6) (Figure 2B). The remaining countries were affiliated with five or less authors. In total 67 % (n = 63) of authors were affiliated with institutions in countries classified as high-income countries with coral reefs, 19 % (n = 18) from high-income countries with no coral reefs, and 13.8 % (n =13) from low- and middle-income countries with coral reefs (Figure 2B). We recorded no instances of authors with affiliations from low- and middle-income countries with no coral reefs.

Of those studies that had more than two authors (n = 29), authors with affiliation at institutions in Australia, United States and Denmark commonly formed collaborations between first, second and last authors (Figure 2B). Authors with affiliation at institutions in developing countries with coral reefs (i.e. Mexico and Brazil) formed within country collaborations, whereas authors in institutions in Venezuela, South Africa and Kenya formed collaborations with authors in the United States, United Kingdom and France (Figure 2B). 82 % (n = 56) of first, second and last authors co-authored a single paper (Figure 2C). A single author each co-authored seven and nine publications respectively (Figure 2C). The 33 studies included in the review ranged in publication years from 1984 to 2022 (Figure 2D). There is a steady increase in the number of studies published over time following a gap in relevant publications from 1985 – 2002 (Figure 2D). There is a spike in publications using histological methods in 2021, where a maximum of five relevant publications were returned (Figure 2D).

Scope of studies

In total 32 of the 33 included studies specified the region of sampling of coral colonies for histology analysis

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(Figure 3A,B). The single study that did not specify a sampling location was experimental. Studies represent sampling locations across all major biogeographic regions with coral reefs (Figure 3A,B). A single study included sampling locations of corals from two distinct bioregions (Indo-West Pacific and Central Pacific). Eleven studies studied corals sampled from Western Atlantic (Figure 3A,B), five of these studies were based in a single state of the United States (Florida), and the remaining studies were located on Caribbean reefs including US Virgin Islands, Virgin Islands, Dominica and a single study was located on a Brazilian reef (Ponta do Seixas) (Figure 3A). Eleven studies studied corals sampled from Central Pacific (Figure 3A,B), including three studies located in a single offshore state of the United States, Hawaii, and other sampling locations were American Samoa, Micronesia, New Caledonia and Palmyra Atoll National Wildlife Refuge (Figure 3A,B). A further five studies (15 %) were in the Indo-West Pacific and the Western Indian Ocean respectively. Studies in the Indo-West Pacific included Australian coral reefs, Heron Island and surrounding reefs, and Lizard Island on the Great Barrier Reef, in addition to Montebello and Barrow Island in Western Australia (Figure 3B). Two of studies located in the Western Indian Ocean sampled corals in the Red Sea and one in the Persian Gulf (Figure 3A). Two studies sampled corals from coral reefs on islands surrounding Madagascar (Mayotte, Reunion) (Figure 3A). A single study was recorded as sampling corals from the Tropical Eastern Pacific, Mexican Pacific (Figure 3A,B).

The identified studies included eight separately named diseases or disease states: Bleaching with tissue loss, Lesion, Stony Coral Tissue Loss Disease (SCTLD), White band, White disease (WS), White patch, White plague and White syndrome (Figure 3C). In total studies assessed 109 disease, taxa combinations. The most highly cited diseases investigated was termed a "Lesion" (43 %, n = 47), followed by WS (23 %, n = 25) and SCTLD (16.5 %, n = 18) (Figure 3A). In total 39 hard coral genera were studied (Figure 3D). The most studied genera were *Acropora* ,*Porites* and *Montipora* (Figure 3D). 21 genera were studied in the context of a single disease name (Figure 3D). Studies ranged in the number of species included from 1 to 52. 16 studies focused on a single species.

In total 82 % (n = 27) of publications investigated coral diseases in the field (i.e. samples were taken *in situ* at a reef site) and 15 % (n = 5) investigated disease in an experimental context (i.e. samples were taken from *in situ* and placed *ex situ* where corals were kept in aquaria before samples were taken) (Figure 3E). A single publication investigated coral disease in the field, and in an experimental context (Figure 3E). Of the 27 studies that investigated coral disease in the field, 15 did not report on any environmental variables taken at the site of collection, eleven studies reported depth and four studies reported temperature (Figure 3F). A single study reported other environmental information of rainfall and stream flows. Experimental studies included use of other methods to investigate disease including lesion progression, lesion transmission, analysis of microbial communities and host physiology (Figure 3G). Studies that investigated coral disease in the field also used other methods including ecological surveys, measures of host physiology, measures of endosymbiont physiology, microbial community analysis, lesion progression and testing of disease treatments (Figure 3H). 20% (n = 7) of field studies used histology only to study coral disease. 46 % (n = 16) (Figure 3H) of field studies reported various ecological survey techniques which allowed for identification of coral disease through description of gross characteristics and quantification of disease prevalence (i.e. the proportion of the community impacted). A single study used ecological surveys to investigate the presence of organisms directly associated with causing tissue loss (e.g. *Drupella*).

Sampling and tissue processing methodologies

Studies conducted sampling from uncompromised (also termed "healthy") colonies with no disease signs and sampling from colonies with active signs of disease (Figure 4A). Three types of sample were recorded as being taken from colonies with active disease signs: 1) the disease lesion, 2) the disease lesion border and 3) non-diseased samples. Eight publications sampled uncompromised colonies and diseased lesions from colonies with active disease signs (Figure 4A). Twelve studies did not include analysis of samples from an uncompromised colony (Figure 4A). Of these twelve studies, eight included analyses of non-diseased samples from colonies with active disease signs (Figure 4A).

Once sampled, specimens are placed in liquid fixing agents. In total eight fixatives were reported as being

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used by studies: formalin, helly's fixative, paraformaldehyde, glutaraldehyde and Z-fix. The most common fixatives reported was paraformaldehyde (n = 12) and Z-fix (n = 15). Removal of the coral skeleton from coral samples to allow slicing of soft tissue samples occurs during a process called decalcification. Publications reported several reagents used for this process: Cal-ex II (Fisher Scientific), EDTA, EtOH, Formic acid, HCl and a combination of HCl and EDTA. The most common reagents for decalcification was EDTA (n = 12) followed by formic acid (n = 9). A single study did not report the reagent used for decalcification as another study was referenced in place of giving methodological detail. Enrobing of samples in agarose can help preserve the placement of material that might be present on the denuded skeletal surface, or in corallite or gastrovascular canal crevices (Bythell et al., 2002). Ten publications enrobed coral samples in agarose prior to decalcification. Once fully decalcified, specimens are processed in ethanol, xylene and paraffin washes for tissue dehydration, wax infiltration and embedding before the sections can be cut from the specimen. Thirty studies used paraffin wax for this stage, the remaining studies used resin for embedding (n = 3) and conducted transmission electron microscopy, in addition to light microscopy. Following embedding specimens are sectioned, and tissue sections are mounted on glass slides. In total 28 of the 33 studies reported the thickness of tissue sections cut. Tissue section thickness varied between 0.5 µm - 7 µm, and 5 µm was the median thickness reported. Nine of the 33 studies reported details on number of tissue sections examined per sample. Sections per sample examined varied between 1 – 10. Once mounted onto slides, tissue sections are stained with routine stains to visualise cellular structures in addition to stains for identifying microorganisms, cellular components, and structures. 28 of the 33 studies reported use of Hematoxylin and Eosin (H & E) with eleven studies applying only an H & E stain to visualise tissue sections. A table of all the stains reported in more than two publications visualised using light microscopy is presented in Table 3. All stains and descriptions are presented in Table S2.

Coral histopathology methodologies

Across all studies histology/histopathology was used to assess tissue condition, reproduction and to identify agents associated with disease pathology (Figure 4B). 61 % of studies (n = 20) assessed for tissue condition and identification of agents associated with disease pathology (Figure 4B). Seven studies used histology to assess tissue condition only, two studies focused on agents associated with tissue pathology and a single study assessed reproduction only (Figure 4B). Three studies used histology to investigate all three focuses.

Publications used qualitative (descriptive), quantitative (ratio data), and semi-quantitative (transformation of qualitative into quantitative data that is nominal or ordinal) analysis methods (Figure 4C). Two types of semi-quantitative data formation were used: 1) ordinal scoring of tissue based on ordered categories of severity that yield a discrete value and 2) nominal data that forms proportions of samples (i.e. incidence) with presence/absence of pathologies or organisms (Figure 4C). Two publications used semi-quantitative scores applied to samples to assess group differences. Twelve studies presented qualitative analysis of tissue sections, whilst other studies combined qualitative observations with either semi-quantitative measures (incidence, n = 5) or quantitative measures (n = 8) (Figure 4C). Four studies used purely quantitative assessment of histological sections. Publications that qualitatively or semi-qualitatively assessed tissue used terms to describe tissue condition and reproductive state (Figure 4D).

Use of terminology for describing histopathological observations

Of the 30 studies that reported on tissue conditions, we found 16 terms that were used to qualitatively and quantitatively described histological features (Figure 4D). Publications most consistently made statements associated with the following terms to describe tissue condition: necrosis (n = 23), tissue fragmentation (n = 15), endosymbiont abundance (n = 9), cellular integrity of the host (n = 8), swelling and/or lysis of host cells (n = 6) and wound repair (n = 6) (Figure 4D). Four publications made statements about stages of gonad development (Figure 4D). See Table 5 for re-definition of these terms to describe tissue condition and references for examples. Nine tissue parameters were assessed quantitatively in publications (Figure 4E). Quantitative measures specific to reproduction were measures of oocyte size (i.e. volume) (n = 3), oocyte numbers per polyp (n = 2) and the number of reproductive polyps in a tissue section (n = 1) (Figure 4E). Other quantitative measures were associated with tissue condition and include counts of

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endosymbiont abundance (n = 2), endosymbiont vacuole ratios (vacuolisation) (n = 2), tissue thickness (n = 2), counts of exocytosed endosymbionts (n = 1), measurement of the separation distance between mesoglea and gastroderm (n = 1) and counts of apoptotic nuclei (n = 1) (Figure 4E). 25 total publications examined tissue sections for eleven possible disease-associated agents (Figure 4F). Bacteria were the most commonly cited organisms tissue sections were examined for (n = 20). Publications also examined tissue for fungi (n = 15) and ciliates (n = 13), helminths (n = 7), sponges (n = 7), algae (n = 6), cyanobacteria (n = 5), crustacea (n = 2), molluscs (n = 1), polychaetes (n = 1) and other (n = 1) which refers to invasive gastrovascular multicellular structures (IGMS see (T. M. Work et al., 2012). See Table 6 for morphological characteristics associated with each of these agents, and references to publications for examples.

Critical appraisal of coral histopathology methodologies

Appraisal of resource availability showed that 94 % (n = 31) of publications specified a lead contact and 36 % (n = 12) of studies released raw data with the publication. 12 % (n = 4) of studies did not release raw data but specified release on contact with the authors. None of the publications included within this review used code for statistical analysis or data visualisation (Table 4). 18 % (n = 6) of publications cited a separate publication in replacement of providing complete details of the methodology used (Table 4). 91 % (n = 30) of studies specified study organisms to the species level (Table 4). Of those studied that used aquaria facilities (n = 6), four provided details of aquaria maintenance however none of the four these studies provided information necessary to replicate culture conditions, whereas two publications provided all details for aquaria maintenance (Table 4). Information on time of sampling was most often provided as a month of sample collection (36 %, n = 12), a single study stated year of collection, and others reported sampling over a specific time period (27 %, n = 9). 18 % of publications did not specify a sampling time point and only four provided the exact date of sampling (Table 4). No studies reported time of day for collection of samples. 48% (n = 16) of studies reported the longitude and latitude location of the sampling/study locations, and 39% reported a reef location name (Table 4). Three publications reported only a country of sampling, and one publication did not clearly state a site of sampling (Table 4).

When reporting sample sizes 51 % (n = 17) of publications were unclear, or reported unequal sample sizes, for each type of sample group (e.g. diseased, uncompromised), and for different taxa studied for each disease (Table 4). Biological replicates at the level of a colony accounts for variation within individuals but reporting on biological replicates was also inconsistent. The number of biological replicates per sample group (i.e. fragments per colony) was reported by 91 % (n = 30) of publications and one publication specified a range (1 – 2 fragments per colony) (Table 4), while 28 studies sampled one fragment of each sample type per replicate colony, one study took two sample types per replicate, and one study sampled four sample types (replicates) per colony. Two studies in total reported on using masking or randomisation during histology methods (Table 4). 67 % of studies did not provide clear, repeatable definitions of the terms or categories used to assess tissue (Table 4). Seven studies in total provided in depth repeatable, methodology for how tissue was assessed for each sample, six of which were quantitative and two were semi-quantitative. Each of these studies took different approaches to assessment of tissue. This included statements of units of cell types or tissues assessed per sample. Two studies divided analysis based on tissue type: surface body wall and basal body wall. Of the 22 studies that used statistical tests, only four reported an explanation for how statistical significance was tested.

Discussion

Ongoing climate change, increasing anthropogenic impacts and growing reports of coral disease within the scientific literature highlights the urgency for developing standardised, comparative approaches for microscopic diagnosis of coral disease states. This information is key for elucidating disease-causation, disease impacts and for assessing management options during outbreaks. The importance of doing so is evident by the rapid increase in reports within the scientific literature of disease related decline on coral reefs and other marine ecosystems (Groner et al., 2016; Heres et al., 2021; J. Miller et al., 2009). However standardised reporting guidelines and ongoing, transparent means to compare research has not yet been developed for the field of coral disease research. In this study we provide:

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a systematic review protocol for repeatable identification of research into white diseases affecting hard coral, and

a systematic review of literature identified from 1984 to April 2023.

From our systematic review we propose:

reporting standards for microscopic studies of white diseases to support future comparative analysis including required reporting information within methods for inclusion in future systematic reviews, and

the need for living reviews of coral disease literature overall, and specific disease states including the microscopic assessment of white diseases in hard coral.

Together these are required to improve understanding of coral diseases, establish trends in disease events and disease literature, in addition to allowing comparative assessment of disease outbreaks overtime. We further argue that doing so provide support for researchers on coral reefs observing disease outbreaks to plan and implement studies investigating disease causation and impacts, access information of gross and microscopic disease signs and coral pathologies, and ultimately increase the body of work that uses histology to assess and compare coral health.

Overall findings of systematic review .

The application of histological and histopathological studies to understanding coral white diseases has yielded compelling insights into diagnosis and disease impacts on coral function. However, we show uptake of standardised methods and subsequent comparative assessment of coral white disease events has been slow to come to fruition. The earliest publication identified applied histopathology to assess white disease in 1984 (Peters, 1984). White band disease was examined in *Acropora*colonies collected from St Croix and Puerto Rico, Caribbean and ten stains were applied to examine reproduction, coral-algal interactions, bacteria, cell structure and microparasites (Peters, 1984). The authors concluded that histopathology allowed accurate assessment of the coral colony, presence of microorganisms and the study correlated tissue state, microparasite infestations with apparent disease signs (Peters, 1984). However, despite the conclusions reached by Peter, 1984 the next publication our systematic review methodology returned was 17 years later, published in 2002. In 2014 a coral disease literature review also highlighted the disparity in methodology used in coral disease studies (T. M. Work & Meteyer, 2014) reporting that prior to 2013 only 12% of the total publications of coral disease applied standard histopathology and light microscopy to investigate microscopic pathology, compared to 65% of reports undertaking ecological surveys to report disease (T. M. Work & Meteyer, 2014). We did not collate literature on white diseases using methods (such as ecological surveys) without applying histopathology. We identified only one study prior to 2002, 17 studies from 2002 to 2013 and then 18 studies in the ten years following. While there is an increase in the use of histology in the coral disease over the last decade these results suggest that further development of comparative techniques is needed to encourage the uptake of histology methods.

We find women contribute substantially to leadership (herein defined as first, second and last author roles) within this field of coral reef science with 28.8% of authors identifying (in open access online resources) as female and 42.5% as male. In terms of last author role we found 18 % female and 56% male, compared to the study of Ahmadia et al (2018) who reported last authorship across the coral science field is dominated by men (80% in 2018). Similarly, as seen across the coral reef research field (Ahmadia et al., 2021) in this study author representation from high income, developed countries dominated the leadership of research effort to date with some evidence of collaboration between developing and developed nations. Ahmadia et al., (2018) highlight there is an ongoing disconnect between geographic origins of scientific knowledge for coral research and the locations of the world's coral reefs. Coral histopathology does require specialised equipment, and research coming from a limited number of dedicated research laboratories is to be expected. As identified by several studies molecular tools for assessment of coral health (e.g. microbiome, or other 'omics' (Traylor-Knowles et al., 2022) can be inaccessible to reef managers due to the need for specialist equipment in addition to sample processing, high cost analysis and specialist data analysis (Donner &

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Potere, 2007). Histopathology can be accessible method for assessment of coral health assessment due to ease of sample collection, storage, and analysis if methods are accessible and openly available. However, our results emphasize the need for continued efforts for standardised tools and reporting, interdisciplinary collaboration, and knowledge-sharing to facilitate the use of these methods in coral disease research. This is particularly evident when considering outbreaks of white disease. Early identification of disease signs and sampling during disease emergence can enable identification of potential causative agents of the disease to be more easily differentiated from secondary colonisers (Lesser et al., 2007), of which reef managers are most likely to be the first observers of outbreaks.

Across the studies identified through the systematic literature search 26 separate stains were used to visualise various structures and organisms. Yet, eleven studies reached conclusions applying only a single routine stain, haematoxylin and eosin. Studies included in this review also used histology to assess reproduction and four studies in total made assessment of reproductive parameters in diseased tissue. Different research aims were also stated across the studies including assessment of tissue condition, reproduction, and identification of other non-coral organisms. Our review finds that for the studies undertaken to-date, white diseases of hard corals are associated with a wide array of different bacterial and ciliate associates, as well as numerous other microorganisms such as fungi, viruses and parasites (Luna et al., 2010; Sweet & Bythell, 2015; T. M. Work & Aeby, 2011). Additionally, studies identified endolithic algae (Fine et al., 2006), fungi (e.g.(Howells et al., 2020), bacterial structures (e.g (Sere et al., 2013)) and ciliates (e.g. see (Smith et al., 2020) as possible secondary invaders to diseased tissue.

We find only eleven out of the total 33 papers included in this study were classified as having repeatable or comparable definitions for tissue terms. The lack of definable terms used in studies are likely reflective of the challenges of describing unresolved and understudied coral cell types and cell functions, limited knowledge of coral tissue microstructure abnormalities, and the complexities of definitions provided by publications. For example a widely assessed pathology in studies is necrosis and has been defined in two separate studies as "swelling and lysis of cells, disruption of cell structure" (Ainsworth, Kramasky-Winter, et al., 2007) and "cytoplasmic hypereosinophilia or fragmentation coupled with nuclear karyorrhexis, karyolysis or pyknosis" (Sudek, Aeby, et al., 2012). Both definitions being correct with the latter definition using specialised pathological terminology requiring morphological descriptions for correct and comparative tissue scoring. Resolving the terminology with examples of tissue classification (Table 5) will aid in addressing these challenges while future research into cell function and standardising histological and histopathological methods continues.

We find that there are several types of tissue scoring that have been applied by authors to assess disease pathology and group differences between sample types. 25 publications out of the 33 reviewed in total provided some qualitative description of tissue, with twelve of these providing qualitative descriptions only. Qualitative descriptions can be useful in providing initial descriptive changes observed between samples, but there are limitations for more rigorous insight to group comparisons. Here, the formation of quantitative or semi-quantitative data from tissue is recommended following methods outlined in (Gibson-Corley et al., 2013). Specifically, formation of semi-quantitative ordinal data is a commonly used practice in medical pathology (references). We find that there are two studies that use semi-quantitative approaches to disease. These studies assigned tissue a score from 0 – 5 based on severity (0 = excellent, 5 = very poor) for endosymbiont condition and abundance, and six parameters of polyp health (both cell and tissue) and bacteria (Gignoux-Wolfsohn et al., 2020; M. W. Miller et al., 2014). Gibson-Corley et al., (2013) also suggest limiting 'diagnostic drift' (where assignment of scores vary slightly in consistency through the scoring process) by slides being examined over a reasonable amount of time by one person, and additionally scoring by independently by multiple assessors. There are also several methods for validating scoring systems including observer repeatability and tissue pathobiology. Details of these can be found in Gibson-Corley et al., (2013) in addition to (Cross, 1996; Germolec et al., 2004; Landis & Koch, 1977).

Recommendations for standardising coral histology

Reporting Quality. In this study we find quality of reporting across sampling, processing, and analysis of

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tissue sections has not consistently met reporting standards in similar fields. In this study we used the STAR (Structure, Transparent, Accessible Reporting) Methods protocol outlined by Cell Journal (Marcus, 2016) in addition to work by Gibson-Corley and colleagues (2013) presenting best practice for scoring histopathological tissues to develop criteria in which to appraise the quality of methods reporting in each of the studies. Poor usability, defined as a difficulty in evaluating what was done by a study, leads to an inability to reuse methodology, making cross comparisons between studies and in incorporating evidence into systematic reviews and meta-analysis difficult (Munafò et al., 2017). Reproducible research in contrast results in faster methodological development and innovation because research is accessible to more scientists (Alston & Rick, 2021). On reviewing reporting of practices in methodology for coral disease histology alongside STAR protocols (Marcus, 2016) and best practice for histological tissue scoring (Gibson-Corley et al., 2013) we are able to recommend guidelines for study design, sample processing, analysis and publication (Figure 5).

Study design . A critical step in reproducibility of histopathological methods is the experimental design which can reduce biases in the analysis of tissue (Gibson-Corley et al., 2012). Key considerations to address include appropriate description of sample collection sites, including latitude and longitudes, and dates and times of sample collection, and for coral reef research collection of environmental metadata where possible (such as temperature conditions, water quality metrics and site descriptions to aid in the replication and comparative analysis of studies). In this study we find 85 % of publications sampled coral colonies from the field and 15 % were purely experimental (i.e. corals were collected in the field and then sampled after *ex situ* experimentation), and a single experimental study did not provide any reference for a collection country or reef site, with corals reported as obtained from aquaria facilities but source location not stated in the methodology. For experimental studies reporting of culture conditions is also valuable, including details of acclimation periods following collection, aquaria temperature regimes, light conditions and if possible flow rates (see (Grottoli et al., 2021) for other aquaria recommendations). Although it is well recognised that environmental factors like temperature, depth, turbidity, and flow (C. E. Page et al., 2019) can all drive the biology of the coral holobiont we found that 49 % of articles did not provide metadata to support contextualising the environment of sourced samples. In addition to depth, temperature and flow conditions, contextual information on drivers of coral health and disease like pollution levels and rainfall from sourced populations is also important to understanding coral health (Haapkylä et al., 2011; C. Page et al., 2023)). For example given corals have been found to respond differently to stressors within reef habitats (Ainsworth et al., 2021) including 'reef habitat' following classification by the Allen Coral Atlas (Kennedy et al., 2020) can allow researchers to later consider reef locations in comparative studies.

Host taxa, gross disease descriptions and nomenclature . An important consideration for coral disease research is the challenge of taxa identification particularly in understudied regions where taxonomic classification can be uncertain, classifications remain unresolved and taxonomic sources/records are unclear. Online resources such as World Register of Marine Species (www.marinespecies.org), the World List of Scleractinia (http://www.marinespecies.org/scleractinia) and reporting databases such as redmap.org (Pecl et al., 2014) and iNaturalist (Matheson, 2014) can be useful for access to species observations in addition to museum resources and specialists. Work & Aeby, 2006 (and followed by Bourne et al., 2015; Rogers, 2010; T. M. Work et al., 2008) provide reccomendations for standardised and systematic observations of disease signs to aid in halting the proliferation of distinct names for similar disease signs, with often a lack of defined and distinguishable aetiologies. For example, tissue loss diseases with no clear distinguishable features (i.e. a brown or black band) is recommended to be referred to as "White Syndrome" (Bourne et al., 2015) and diseases named in reference to the taxa they impact (e.g. "*Acropora* White Syndrome") as it is understood that diseases that present similarly in different taxa can have different underlying drivers and therefore should be treated as separate diseases (Aeby et al., 2011; Rogers, 2010; Sussman et al., 2008).

Controls. Uncompromised and healthy individuals . Critically selection of the appropriate controls and calculation of sufficient sample sizes needed for statistical tests are also widely recommended for histopathological research to discern abnormal pathology (Meyerholz & Beck, 2018a). Here we find twelve studies of hard coral white diseases did not include samples of uncompromised colonies (i.e. apparently heathy /not infected by the described disease). Including 'healthy' controls within studies can be a challenge during outbreaks as

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visually healthy colonies may at the cellular level be stressed however consistent terminology to described uncompromised and metadata on environmental drivers can assist in differentiating disease states (Ainsworth et al., 2008). To overcome this a single study by M. W. Miller et al., 2014 used reference to samples taken from apparently healthy samples a year before the study period and disease outbreak to assess comparison to assess tissues, highlighting the value on continuous site assessment where possible (M. W. Miller et al., 2014).

Histopathological tissue scoring . We also find that few studies clearly specified the method for how sample tissue were scored. Ideally the scoring procedure should be clearly stated and clear language should be used when defining terms, or categories samples will be scored by (Gibson-Corley et al., 2013; Meyerholz & Beck, 2018b, 2018a). The foundational concepts for assessment of diseased tissues are similar across scientific areas (Gibson-Corley et al., 2013; Meyerholz & Beck, 2018b) and include principles associated with bias control in tissue evaluation (Meyerholz & Beck, 2018a). For example bias can be reduced through steps at sample processing stage including randomisation through labelling individual samples without reference to treatment group (e.g, 1, 2, 3, 4, 5, etc) (Gibson-Corley et al., 2013), or masking of sample identification prior to scoring to ensure the scorer is aware of tissue grouping and background information (e.g. A1, A2. . . ; B1, B2. . . (Gibson-Corley et al., 2013)). At the processing stage, authors should also be sure to provide information on the number of sections examined per sample, and the number of sections examined per stain, per sample, this biological replication is important for accounting for variation within a single sample.

Nomenclature for microscopic assessment. Inconsistencies in nomenclature has also been identified as an ongoing issue in coral disease research with various reports of disease signs referring to the same disease symptoms (e.g. "black band disease" and "black aggressive band") (Moriarty et al., 2020). In addition to a lack of standardised nomenclature, there also remains lack of information to distinguish diseases as distinct from each other (highlighted by Moriarty et al., 2020). Standardised microscopic assessment of tissue through histology is therefore a critical step towards more clear delineation of disease signs and symptoms. The use of consistent diagnostic terminology is strongly recommended when assessing tissue, as the use of none-definable criteria limits the conclusions that can be drawn from analysis (Gibson-Corley et al., 2013; Meyerholz & Beck, 2018b). Therefore, a key output of this is review is redefinition of most widely used terms to describe tissue condition: necrosis, tissue fragmentation, hyperplasia, inflammation, suspect wound repair, atrophy and hypertrophy (Table 5). While this list is not exhaustive, we suggest that authors continue to provide clear definitions and examples of other terms used to aid in building comparative resources for the field. We also provide morphological characteristics of agents associated with coral diseases identified by publications (Table 6). In these tables we provide citations to publications included in the review where images of each pathology and associated organisms have been provided. Understanding the types of data as well as their constraints helps can also in planning analysis, we highlight these in Table 7.

Reporting standards. STAR protocols for methods reporting (Marcus, 2016), best practices for reproducible research (Munafò et al., 2017), and increasing knowledge-sharing through providing author/data contact information and accessible raw data are all now becoming norms across research disciplines. A field-wide development of reporting standards specific to coral disease reports would also aid in comparative and collaborative research. Towards tackling systemic inequalities present in STEMM fields including coral reef science (Ahmadia et al., 2021; Runnels et al., 2014; Tricco et al., 2023), increasing representation and collaboration within the field, including through the use of evidence syntheses are important ways forward in building the research field as the coral reef crisis continues globally.

Conclusions

As coral diseases and coral bleaching have become an increasing threat to coral reefs in the last few decades, knowledge of the microscopic structure, composition of coral tissue and what drives poor coral health has accumulated but there remains many crucial knowledge gaps and inconsistencies in the field. Histology to look at coral cells is a flexible tool that can help assess coral holobiont function and aid in disease diagnostics. In this review we use a systematic protocol to map publications that have used histology to study white diseases in corals, and review the methodological design and procedures used. The results of this study highlight

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advantages to histology, however we find that studies tend to lack detailed methodological information reducing the useability of research. Using this body of literature, we present re-definition of the most widely used tissue terminology and recommendations for methodology and reporting of future histological studies, with the aim of increasing accessibility, comparability and encouraging uptake of histology in future studies of coral disease.

Author contributions

CP and TA conceived the ideas and designed methodology; CP and EA collected the data; CP analysed the data; CP led the writing of the first draft of the manuscript. All authors contributed critically to the drafts and gave final approval for publicatio n.

Data availability

Raw data collected during data extraction of the systematic review, and code used for analysis and visualisation is available for download at https://github.com/CharlotteEPage/Histology_methods_systematic_review.git .

Tables

Table 1. Final search terms used for systematic review of the literature.

Category	**Key word**
Organism	"coral*" OR "scleractini*" OR "cnidaria*" "Hard coral"
Disease reference	"white-band" OR "white band" OR "tissue loss" OR "white syndrome" OR "white disease" OR
Method	"histo*"

Table 2. Final inclusion and exclusion criteria for screening of the literature.

Inclusion criteria	The study likely considers hard coral taxa
	The study likely considers a tissue loss disease
	The study likely conducts histological analysis
	The study is in English
	The study is peer-reviewed
Exclusion criteria	The study likely refers to some other disease type that does not involve tissue loss (e.g. Growth Ano
	The study likely refers to some other disease type that involves other disease signs that are not white
	The study is secondary (i.e. a review of the literature)

Table 3. Stains for light microscopy used in two or more publications and description of what the stain is used to visualise. A full list of stains and visualisation techniques applied in this study are found in Table S2.

Stains	**What does the stain visualise and how**
Hematoxylin and Eosin (H&E)	H&E is a routine stain that helps differentiate cell and tissue t
Grocott's methenamine silver	Used to visualise fungi. The fungal cell wall is stained black/b
Periodic Acid Schiff (PAS)-hematoxylin	Fungal hyphae in addition to polysaccharides and mucosubsta
In situ end labelling (ISEL) Programmed Cell Death assay	Used to visualise programmed cell death (apoptosis). Apoptot
Trichrome (Mallory's, Masson's & Gomori)	A routine stain that helps differentiate cell and tissue types. C
Giemsa	The giemsa stain can be used to look for parasites such as pro
Phyloxine B	Phyloxine B is a dye typically added to H&E to enhance the r
Alcian blue	Mucopolysaccharides in mucus. Mucus is stained blue and sur

Stains	What does the stain visualise and how
Metanil yellow	Metanil yellow is a counterstain that targets collagen in conne
Thionin	DNA in is stained green/blue and other tissue components ap
Brown and Brenns	Visualises gram-positive and gram-negative bacteria in tissues
Fuelgen	The DNA is stained a red/pink and the background is stained
Gram stains (general)	Confirm the presence of gram-positive and gram-negative bact

Table 4. Critical appraisal of methodology detailed as the number of publications out of all total publications included within the review.

Resource availability	Lead contact: is the lead contacts details specified?	Yes = 31/33 No = 2/33
	Is the raw data released with the study?	Yes = 12/33 No = 17/33 Contact author = 4/33
	If code is used is it released with the study?	No code specified in any studies
Method details	Is another paper cited in replacement of providing adequate details of the procedure applied?	Yes = 6/33 No = 27/33
	Are the species studied listed?	Yes = 30/33 No (genus level only) = 3/33
	If experimental are full details on aquaria maintenance provided?	NA (no aquaria used) = 27/33 Some details but not enough to repeat the experiment = 4/33 All details have been specified = 2/33
	Is information on timing of sampling provided?	Date (d//m/y) = 4/33 Month = 12/33 Year = 1/33 Time frame = 9/33 Not specified = 6/33
	Is information on sampling location provided?	GPS coordinates = 16/33 Reefs specified = 13/33 Country only = 3/33 No location = 1/33
	Are sample sizes provided?	
	Colonies per sample group	Not specified = 2/33 Different per taxa, disease type or sample group= 17/33 Consistent sample sizes = 13/33
	Fragments per sample group	Not specified = 2/33 Specified = 30/33 Range given = 1/33
	Is masking applied during evaluation of tissue?	Yes = 1/33 Not specified = 32/33
	Is randomisation applied during evaluation of tissue?	Yes = 1/33 Not specified = 32/33
	Are repeatable definitions of tissue assessment terms provided?	Yes = 11/33 No = 19/33 For some = 3/33
	Are repeatable definitions of how tissue were assessed provided? (e.g. the tissue area assessed per sample, the tissue layers assessed per sample)	Yes = 8/33 No = 25/33

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Resource availability	Lead contact: is the lead contacts details specified?	Yes = 31/33 No = 2/33
Quantification and statistical analysis	If statistics are used, are all test completed stated?	NA = 11/33 Yes = 22/33
	If statistics are used, is explanation for how significance is assessed defined?	Yes = 4/22 No = 18/22

Table 5. Morphological description and characters of general terms used to describe conditions of coral tissue identifiable under routine staining (H & E).

Term	Morphological description and characteristics under routine staining (i.e. H & E)
Necrosis	Necrosis is the accidental or passive death of cells (Syntichaki & Tavernarakis, 2002) characteristic
Tissue fragmentation	Variable sized clumps of intact cells (T. M. Work et al., 2016).
Hyperplasia	Widespread proliferation of cell types (gastrodermis, epidermis, mesoglea, calicodermis) alongside re
Inflammation	A process aimed at destroying, diluting or walling off infectious agents, characterised by infiltrates c
Suspect wound repair	Fragmented tissues with evidence of regeneration of undifferentiated epidermal tissue (e.g. no clear
Atrophy	Generalised shrinking of the epidermis or gastrodermis.
Hypertrophy	The increase in the volume of an organ or tissue due to the enlargement of its component cells. Als

Table 6. Morphological characteristics and references for agents associated with coral disease.

Organism	Morphological description and characteristics under routine staining (i.e. H & E)
Helminths	None-segmented, worm shaped (vermiform) metazoan with or without gut
Sponge	Metazoa with connective tissue matrix containing spicules and choanocytes. There may be presence of end
Algae	Metazoa with cell walls.
Fungi	Elongate branching, irregular filamentous structures with or without septa.
Crustacea	Metazoa with gut, muscle, reserve inclusion cells, cuticle, hepatopancreas, and segmented appendages.
Ciliates	Allantoid (round, slightly elongated) unicellular ciliate covered organisms. Can be found invading gastrova
Cyanobacteria	Parallel walled, filamentous striated structures.
Bacteria	Diverse structures that very in size including coccoid like and coccobacilloid like, in addition to larger bact
Mollusc	Large metazoan with gills, and striated muscle. Eyes and radula sometimes visible.

Table 7. Types of semi-quantitative and quantitative data that can be obtained from histological tissue. Adapted from (Gibson-Corley et al., 2013)

Type	Definition
Semi-quantitative, Nominal	Samples assigned to a category without references to severity. Examples include binary p
Semi-quantitative, Ordinal	Samples assigned to a category showing an ordered progression in severity.
Quantitative, Ratio	Data is quantified on a scale with a true zero value.

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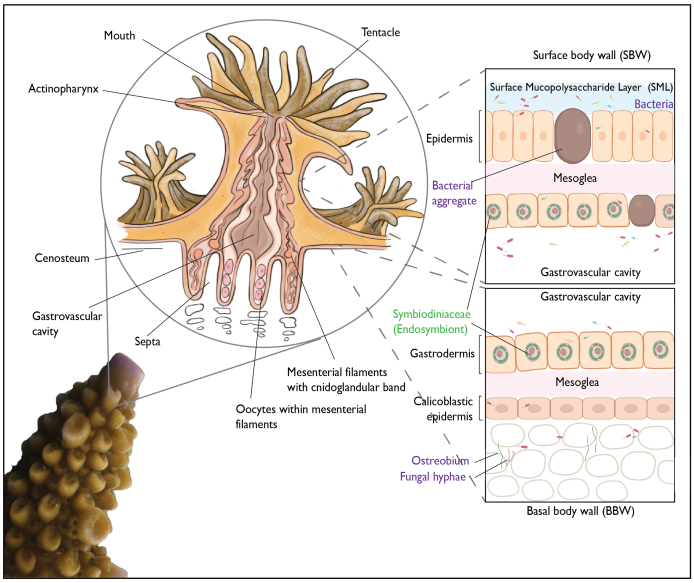

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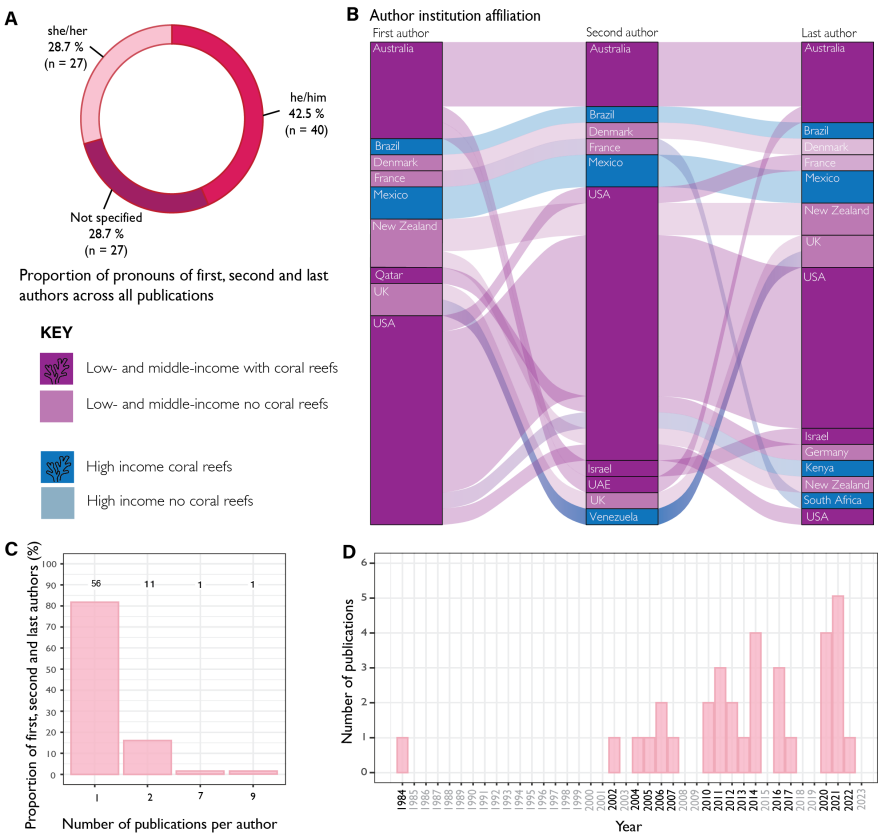

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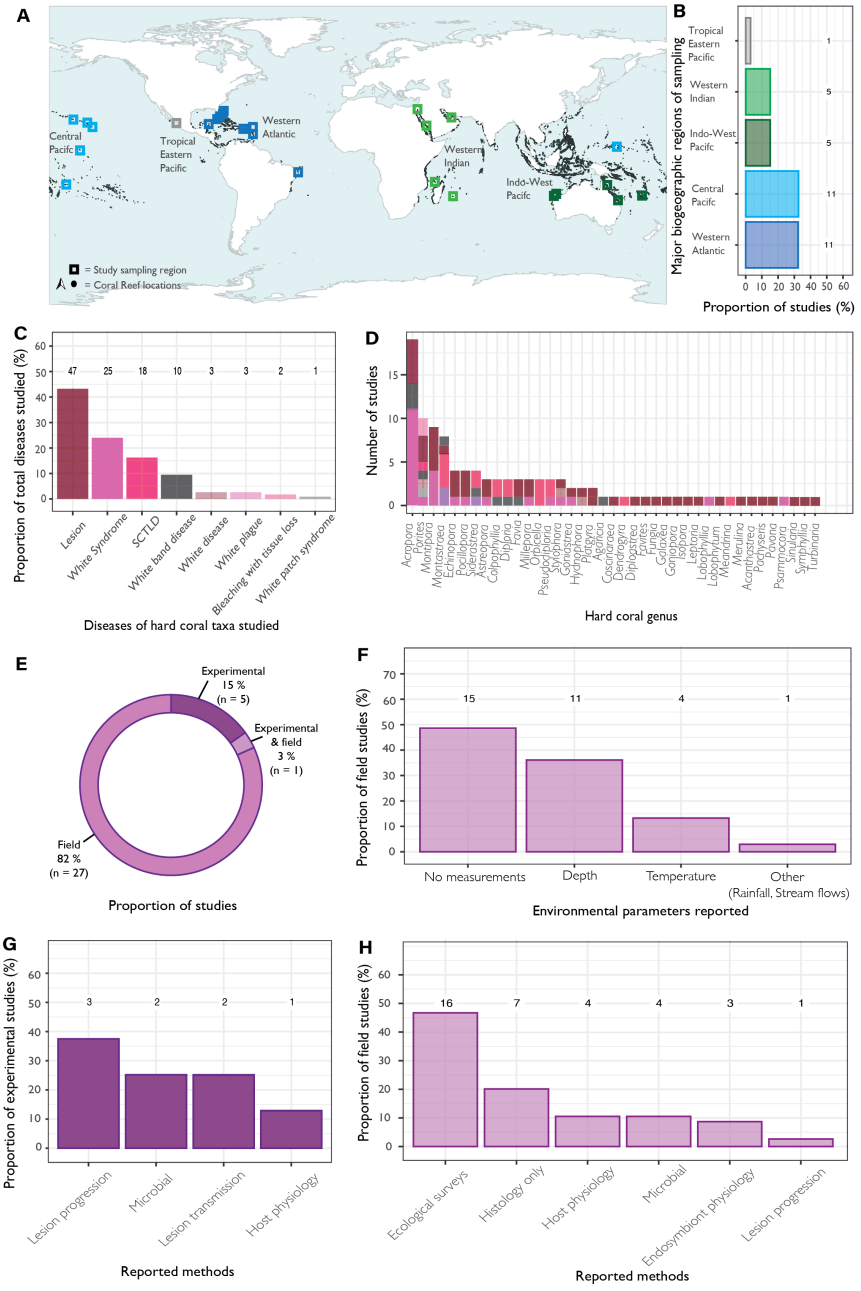

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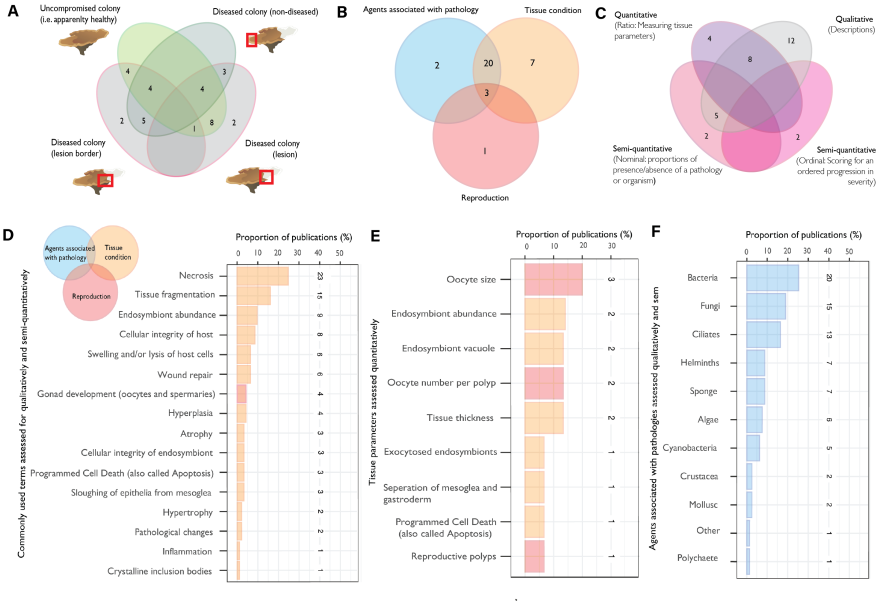

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