Coral Reefs
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RESEARCH

Histopathology of scuticociliate tissue loss associated with *Philaster guamense* (brown band disease) in *Acropora* cf. *muricata* from the Republic of Maldives

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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 vegetative and encysted trophonts intermingled with necrotic coral tissues and saprophytic organisms, whereas, at the tissue loss margin, vegetative trophonts predominated in contact with dissociated tissues and within skeletal spaces adjacent to histologically intact tissues. Occasional vegetative 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 capable 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.

Keywords Coral diseases · Coral reefs · Histology · Indo-Pacific Ocean · Scuticociliatosis

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Introduction

Coral diseases are exacerbating the decline of coral reefs and their associated biodiversity (Weil et al. 2006; Brodnicke 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 of a disease's pathogenesis that can be leveraged to mitigate its occurrence.

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 and Séré 2016; Croquer 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 and 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; Croquer 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, scutiociliatosis 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 and 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 and Willis 2006; Weil et al. 2012; Montano et al. 2016; Bises et al. 2024). Two scuticociliates, *Philaster lucinda* and *Philaster guamense,* were reported from the brown band and advancing front of BrB lesions in *Acropora muricata* from the Indo-Pacific (Bourne et al. 2008; Lobban et al. 2011; Sweet and 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 and 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 and 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 and Back 2009; Nicolet et al. 2018; Montalbetti et al. 2019).

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. 2024). Multidisciplinary approaches to studying coral diseases integrate histopathology with additional diagnostic methods to better connect observed disease processes with specific pathogens or other underlying causes (Sweet et al. 2012; Montano et al. 2020; Becker et al. 2023; Hawthorn et al. 2023). For example, additional techniques can complement histology to identify parasite stages within lesions. Light microscopic and ultrastructural examination provide classical morphological data, while genetic analyses enable molecular taxonomic classification. Together these approaches help identify features unique to pathogenic species or strains. Additionally, histopathology is necessary for accurate disease diagnosis because many coral diseases show similar or overlapping gross visual changes (Work et al. 2008; Work and 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 explore whether the ciliates associated with BrB may initiate tissue loss or simply colonize and feed on tissues already damaged by other potential underlying disease conditions. As such, histology is an 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.

Materials and methods

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. 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 region for comparison.

The remote location of the site (Fig. S1), 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.

Molecular and morphological identification of ciliates associated with the lesion

Ciliates were identified both molecularly and morphologically. For molecular identification, ciliates were collected after treatment with menthol crystals dissolved in seawater for one hour, which helped the detachment of ciliates from the host coral. Ciliates were 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 the outgroups *Porpostoma notata* and *Philasterides armatalis* following previous 18S-based and multi-locus phylogenetic reconstructions (Gao et al. 2012; Liu et al. 2024), 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 and 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. 2020). 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 and Séré 2016). The shape, overall appearance, and median length were measured for 70 individuals.

Histopathology

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) 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 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 h 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 along with the remaining intact coral tissue. 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 bordering apparently healthy 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 up to 100× objective (1000× magnification) with oil immersion with an Olympus BX43 microscope, defining for each biopsy, if present, three areas of investigation: brown band, tissue loss margin, and bordering apparently healthy area. Although care was taken to collect fragments representing all three areas, subgross examination once in the laboratory revealed small foci of tissue loss in the region of the bordering apparently healthy area, suggesting that 'grossly adjacent apparently normal tissues' were actually not normal. 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 bordering apparently healthy 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 10× magnification) was affected, 2 if the percentage was between 25 and 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.

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 10× magnification) of the most affected area of the section. In addition, ciliates were distinguished as vegetative 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, associated non-ciliate organisms were identified based on morphological features from relevant literature, as shown in Table 1.

Results

Ciliate molecular and morphological characterization

The 1300 bp 18S rRNA alignment included 12 *Philaster* sequences plus two outgroups and resulted in a well-supported phylogeny (Fig. 1). 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 and Bythell 2012) and correspond to *Philaster guamense* (Sweet and Séré 2016). Other ciliates isolated from brown band disease and white syndrome on *A. muricata* from Australia, identified as *Philaster lucinda* (Sweet and 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*.

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

Organism	Key morphologic features	References
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 and Poynton 2006
Sponges	Multicellular organisms with siliceous spicules and zooxanthellae	Ereskovsky and Lavrov 2021

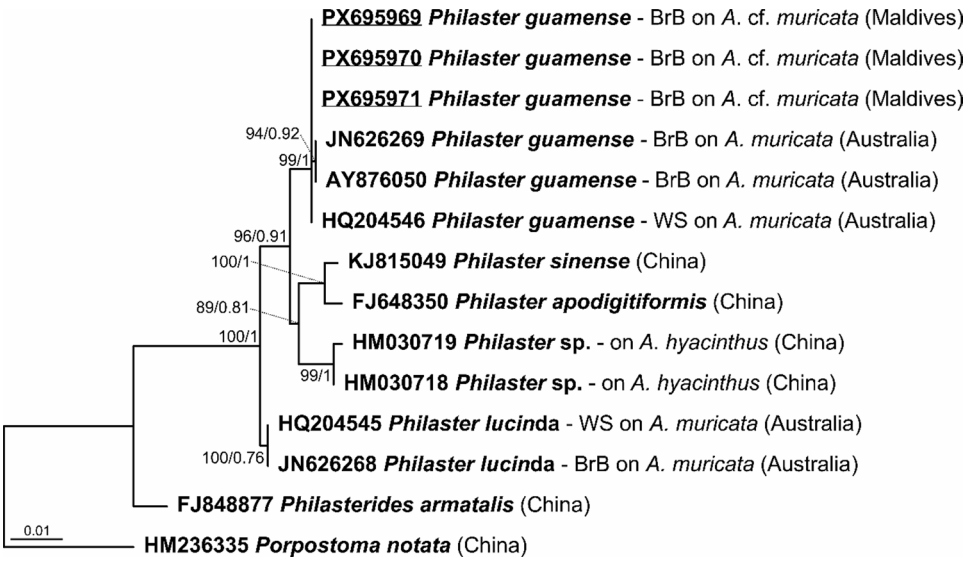


Fig. 1 Maximum likelihood phylogenetic hypothesis of *Philaster* species based on 18S rRNA gene. Numbers at nodes represent maximum likelihood bootstrap support and Bayesian posterior probabilities, respectively. For each sequence, the coral disease and species are indicated when available, together with the sampling locality in brackets. BrB: brown band disease; WS: white spot disease. Underlined sequences were obtained in this study

Ciliates were oval-elongated with a median (SD) length of 336.12 ± 97.76 µm (median ± SD), ranging from 150 to 500 µm with rows of cilia in the vegetative free-swimming phase or encysted in the brown band area (Fig. 2b, c). The cyst could be recognized as a transparent wall surrounding the shrunken and less turgid body of ciliate (or clusters of ciliates) (Fig. 2d). 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.

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 toward 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^{-1} from bare skeleton to intact coral tissue, and their subsequent aggregation and encystment on exposed skeleton as tissue resources become depleted. Moreover, fragments showed no consistent pattern in the intensity or width of the brown band nor its distance from the tissue loss margin.

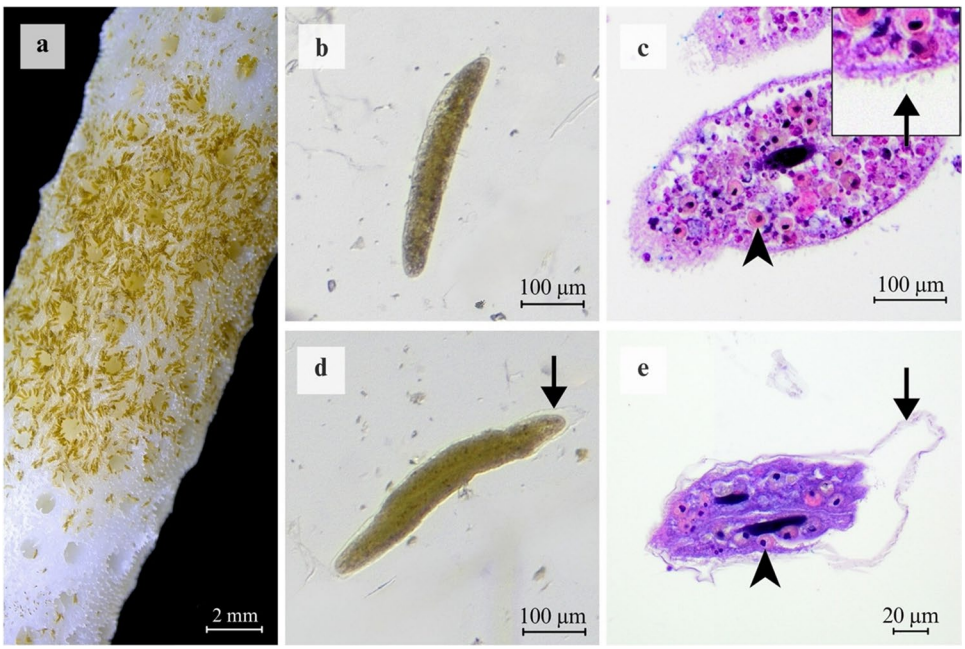


Fig. 2a Dead portion of a diseased *Acropora* branch showing the brown band on the exposed skeleton. **b** Wet mount light microscopy of vegetative trophont. **c** Microscopic section of a vegetative trophont, oval to pyriform with highly basophilic macronucleus, rows of cilia (inset, arrow), and ingested 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** Histology of encysted trophonts encased in the cyst capsule (arrow), with comparatively condensed cytoplasm containing ingested endosymbionts (arrowhead). Section stained with Hematoxylin and Eosin

Histopathology

Histological features of the brown band region were similar across all biopsies (Fig. 3-1 and Table 2), consisting of aggregated and densely packed vegetative and encysted trophonts, and numerous empty cysts (Fig. 3-2). Ciliate morphology was similar across all biopsies, and consistent with features documented with stereoscopic examination. Trophonts in both the encysted and vegetative 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. S2a). In all the biopsies, the mat of micro-organisms contained fragments of variably necrotic dissociated coral tissue (Fig. 3-2a–c), representing basal body wall, surface body wall, and mesentery (necrosis score 3). In five out of the six biopsies, other organisms, including fungi (four cases), platyhelminths (three cases), and Labyrinthulomycetes (one case), were in aggregation or in proximity of the ciliates within the brown band (Fig. S3a). No bacteria were observed.

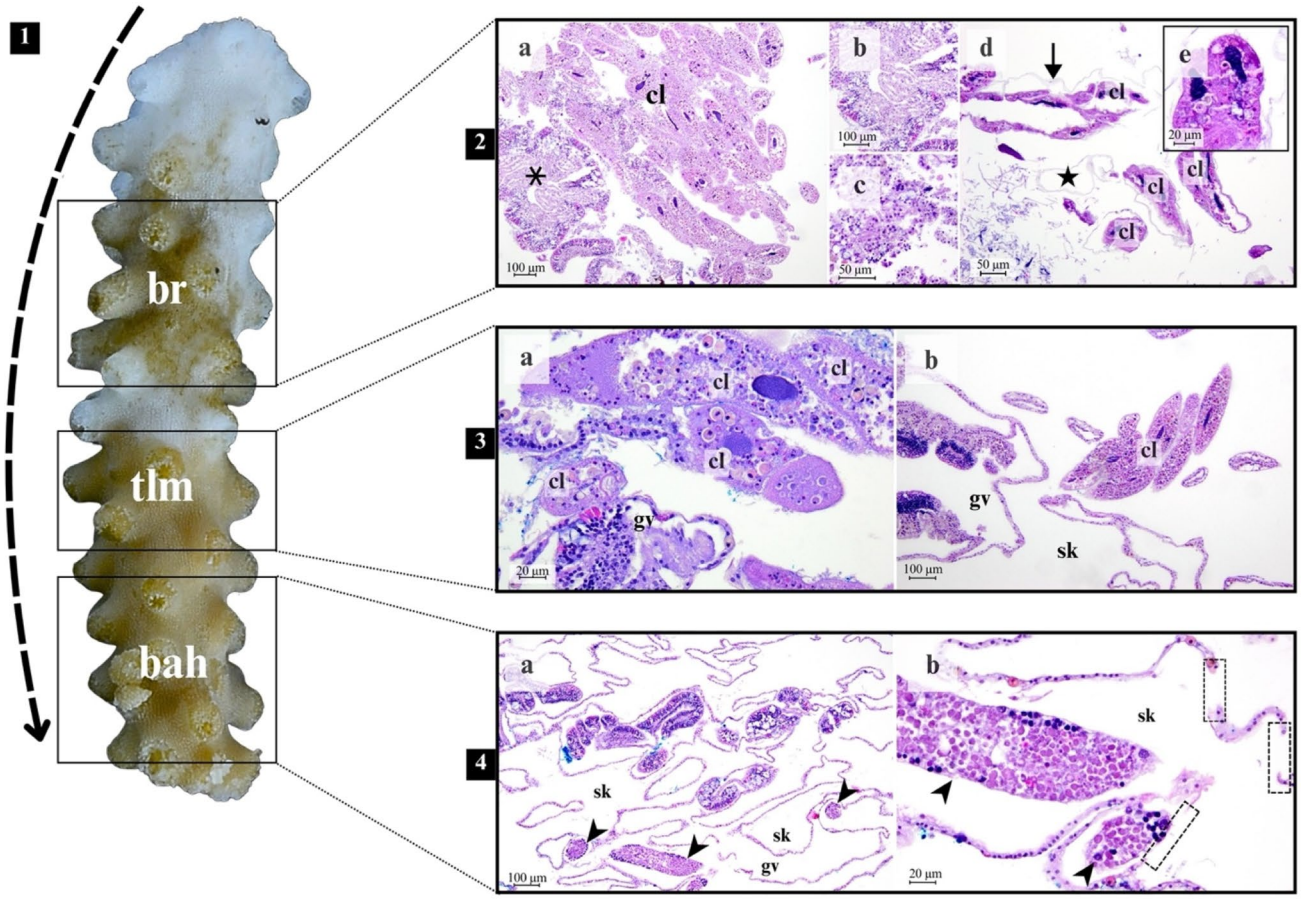


Fig. 3. **1** *Acropora* biopsy showing the three areas of disease progression front (dashed arrow), namely brown band (br), tissue loss margin (tlm), and bordering apparently healthy (bah). **2** Sections of the brown band area stained with hematoxylin and eosin showing: **2a** a mat of densely aggregated vegetative trophonts (cl) and dissociated necrotic coral tissue (asterisk); **2b** higher magnification of dissociated coral tissue; **2c** higher magnification of dissociated coral tissue showing cellular degeneration and necrosis; **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; **2e** higher magnification showing condensed cytoplasm. **3** Sections of the tissue loss margin stained with hematoxylin and eosin showing: **3a** Vegetative 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; **3b** Vegetative trophonts (cl) within deep skeletal spaces (sk), adjacent to intact basal body wall; gv indicates gastrovascular cavity. 3.4) Sections of bordering apparently healthy area stained with hematoxylin and eosin showing: **4a** Vegetative trophonts (arrowheads) within the skeletal spaces (sk). Gv indicates the gastrovascular cavity; **4b** Higher magnification of Sect. 3.4A showing ingested cellular debris within the cytoplasm of vegetative trophonts (cl) and areas of truncated basal body wall (dashed rectangle)

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 bordering apparently healthy tissue), and bordering apparently healthy tissue (grossly normal tissue bordering the tissue loss margin)

		Brown band (n = 6)	Tissue loss margin (n = 6)	Bordering apparently healthy (n = 3)
Histological features				
Vegetative trophonts		5	6	2
Median number[a] of vegetative 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)	0
Dissociated tissue fragments		6	6	0
Median necrosis severity score (range)		3(0–3)	0.5(0–3)	0
Other organisms				
	Fungi	4	1	0
	Platyhelminths	3	0	0
	Labyrinthulomycetes	1	0	0
	CAMAs	n.a	1	1

[a]Enumerated per 2.54 mm^2 field in the most severely affected area

n.a.: not applicable

At the tissue loss margin (Fig. 3-3), the vegetative trophonts, and rarely encysted trophonts (Fig. S4), 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 aggregates (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. S2b). Otherwise, there were no qualitative morphological differences in the coral tissues at the tissue loss margin relative to those in the bordering apparently healthy region.

Bordering apparently healthy areas of the coral biopsy were consistently microscopically healthy, but a few vegetative trophonts were within skeletal spaces in contact with the basal body wall (Fig. 3-4), with the exception of one case in which ciliates were also inside the gastrovascular cavity, in contact with the mesenteries. The bordering apparently healthy 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. S3b). Moreover, no significant loss of endosymbionts in the surface body wall was noted either in the tissue loss margin or the bordering apparently healthy area of the biopsies.

Discussion

Our findings are consistent with the descriptions of BrB in terms of lesion progression (≈1.2 mm h^{-1}), 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 2008; Sweet and Bythell 2012; Randal 2015; Sweet and 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. Future studies could strengthen our

interpretations by demonstrating an absence of these protists within control biopsies from colonies without tissue loss, or from an area far from tissue loss margin.

The present study offered a comparison of the three distinct lesion areas, representing a trajectory of disease progression. Vegetative 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 vegetative 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 bordering 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 endosymbiont 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 and 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 evident 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 and 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 determine whether 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 scuticociliates 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 and Bythell 2012; Sweet et al. 2014, 2018). 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 and 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 and Aeby 2011; Work et al. 2012). Furthermore, the similar invasive nature of *Philaster* spp. in other marine hosts lends further credibility to this hypothesis (Alvarez-Pellitero, et al., 2004; Stidworthy et al. 2014; Jalenques et al. 2021; Hewson et al. 2023; Pilar et al. 2025). While techniques like transmission electron microscopy (TEM) and metagenomics offer higher sensitivity for ruling out bacterial presence, light microscopy remains essential for visualizing bacteria within lesions and establishing their pathogenic role (Work et al. 2012). 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 worthy of further investigations (Bradbury and 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, as endosymbionts presented unremarkable morphology 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 coinfection was noted between *P. guamense* and other microbial agents. Indeed, the organisms colocalized 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. 2004; 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 vegetative trophonts prevail, together with the nonpathogenic 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 cofactor 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 (Miller et al. 2013; Stidworthy et al. 2013; Jalenques et al. 2021; Steverding 2022; Pilar et al. 2025). Although mostly reported in aquaria, 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.

To date, *Philaster* spp. are the main scuticociliates associated with coral disease. *Philaster guamense* and *Philaster lucinda* have previously been identified in Indo-Pacific acroporids with BrB lesions (Bourne et al. 2008; Lobban et al. 2011; Sweet and Séré 2016). *P. guamense* has additionally been associated with acute tissue loss lacking a brown band (Sweet and Séré 2016). While our study helps substantiate a pathogenic role for *P. guamense*, in BrB, the role of closely related *P. lucinda* is unclear. *P. lucinda* was co-occurring with *P. guamense* in some cases of BrB from Great Barrier Reef (Sweet and Séré 2016), but it was not identified in our samples. This result would suggest a potential co-cause or incidental rather than a necessary cause of BrB, although it needs to be substantiated with further samples. Indeed, while *P. lucinda* was not detected in all BrB lesions; it was present in collected samples from diseases associated with acute tissue loss in Caribbean and Indo-Pacific acroporids, known respectively as white band disease and white syndrome (Sweet et al. 2014). The tissue loss observed in these diseases has been attributed, at least in part, to histophagous ciliates that invade and feed upon apparently healthy coral tissues, possibly functioning as secondary pathogens (Sweet et al. 2014). A ciliate of the genus *Philaster* was associated with acute tissue loss diseases (white syndrome and brown jelly syndrome) in aquarium corals and is also observed to invade and ingest seemingly healthy and intact tissues (Sweet et al. 2013). Other histopathological surveys have revealed unidentified histophagous ciliates associated with tissue loss which may represent *Philaster* spp. or other scuticociliates (Work and Aeby 2011; Work et al. 2012, 2016, 2025). In contrast, the ciliates *Holosticha* spp. and *C. verminus*, isolated from tissue-loss lesions in Indian Ocean *Porites* and *Acropora* corals, exacerbated tissue loss but did not induce lesions in healthy colonies, indicating an opportunistic role as consumers of decaying coral tissue (Ravindran et al. 2020). Studies that link morphological and molecular identification of ciliate protists to their histopathology are necessary to disentangle their causal relationship to tissue loss diseases in corals. Specifically, diagnostic tools such as immunohistochemistry (IHC) or in situ hybridization (ISH) could identify and localize pathogens within the lesion.

Interestingly, *Philaster* spp. have also been implicated in tissue loss diseases lacking a brown band (Sweet et al. 2014; Sweet and 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 and Aeby 2006). We propose the following case definition for acroporid scuticociliate tissue loss disease: 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-microscopic 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 would argue against any need for additional sampling for our histopathological observations. Nevertheless, future studies could strengthen our interpretations by deriving control biopsies from colonies without tissue loss, or from an area far from tissue loss margin. This limitation emphasizes the value of collecting samples from healthy areas despite the reluctance to collect destructive samples from endangered species.

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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Author contribution Chiara Bises: writing—review and editing, writing—original draft, investigation, formal analysis, conceptualization. Michelle M. Dennis: writing—review and editing, validation, supervision, investigation, formal analysis. Jacopo Gobbato: writing—review and editing. Davide Maggioni: writing—review and editing, investigation. Paolo Galli: supervision. Simone Montano: writing—review and editing, supervision, conceptualization.

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Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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