Florida's mystery coral-killer identified

An unusual coral disease appeared on the Florida Reef Tract in June 1995. It was distinct in its microbiology, its pattern of tissue degradation, the species susceptible to it, and its regional distribution. Symptoms included a sharp line between healthy and diseased tissue, as occurs with other coral diseases, but the pathogen responsible for the new outbreak seemed more virulent, affected a wider variety of species, and destroyed tissue much more rapidly than these other 'line' or 'band' diseases. We have identified the pathogen responsible for this new disease as a new species of *Sphingomonas*.

The disease first targeted the elliptical star coral, *Dichocoenia stokesi* Milne-Edwards and Haime. Affected colonies[1] exhibited a sharp line between apparently healthy tissue and a thin zone of bleached tissue grading into exposed coral skeleton (Fig. 1a). Similar lines are associated with other coral diseases[2], but these colonies of diseased *D. stokesi* exhibited an unusually rapid rate of tissue degradation: up to two centimetres per day. Also, and equally unusually, tissue loss started from the base of the colony.

Within four months of its discovery, the disease had spread about 200 kilometres along the Florida Keys (Fig. 1b). Mortality rates in the *D. stokesi* population, determined from repeated surveys of 20-metre-diameter plots[3], averaged 26% over an 11-week period (with a range of 0 to 38% per plot).

Surveys of *D. stokesi* populations on five reefs ($n = 1{,}196$ colonies) revealed a clumped distribution of infected colonies (Morisita's index of dispersion[4], $I = 1.84$, $\chi^2 = 49.76$, d.f. = 27, $P < 0.005$; comparison to Poisson distribution, $\chi^2 = 33.1$, d.f. = 4, $P<0.001$). Overall disease incidence was 20.1% (with a range of 0–33.3% per site). There was a correlation with water depth ($r = 0.42$, d.f. = 24, $P<0.05$), with the highest incidence of disease at 14 metres (with a range of 1.8–16 m). The number of diseased *D. stokesi* colonies was strongly correlated with the density of all colonies of this species regardless of depth ($r = 0.88$, d.f. = 24, $P<0.001$), suggesting that the disease is contagious.

In the 1995 outbreak, 16 further species of coral and a hydrocoral were observed with symptoms identical to those of the diseased *D. stokesi*. The other corals affected were *Agaricia agaricites, A. lamarcki, Colpophyllia natans, Dendrogyra cylindrus, Diploria labyrinthiformis* (Fig. 1c), *D. strigosa, Eusmilia fastigiata, Madracis decactis, M. mirabilis, Manicina areolata, Meandrina meandrites, Montastrea anularis* (species complex), *M. cavernosa, Siderastrea siderea, Solenastrea bournoni* and *Stephanocoenia michelinii*, and the hydrocoral *Millepora alcicornis*.

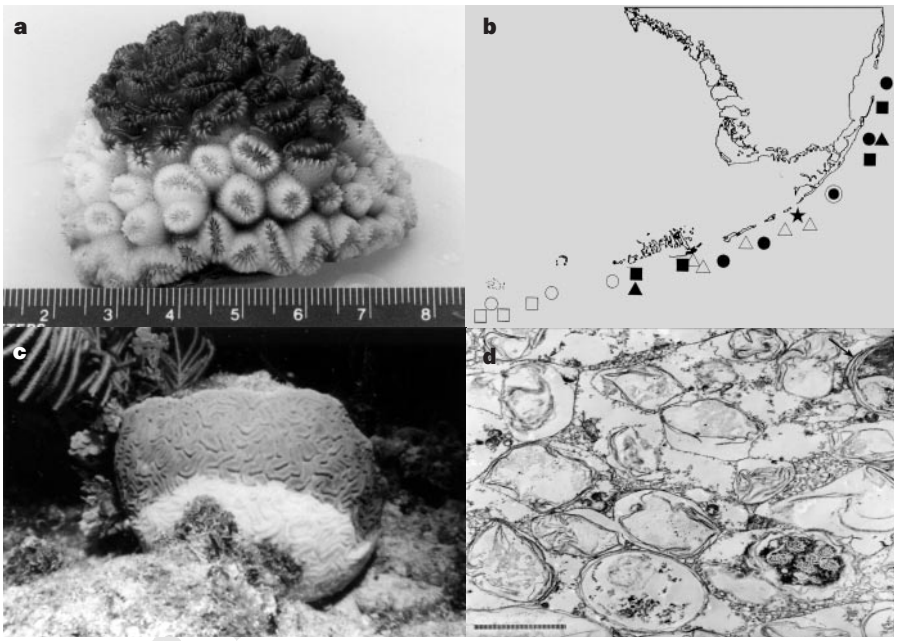

Figure 1 Diseased corals. **a,** Freshly collected diseased colony of *Dichocoenia stokesi* (scale in cm). **b,** Regional survey of diseased *D. stokesi* on reefs of the Florida Reef Tract. Data were collected during three research cruises in August (circles), September (squares) and October (triangles) of 1995. Black symbols, reefs with active disease; white symbols, disease-free reefs; star, site of the first disease observation (7 June 1995, Alligator Reef; K. Nedimyer, personal communication); double circle, site of highest disease incidence (Conch Reef). **c,** *In situ* photograph of diseased *Diploria labyrinthiformis*. **d,** Transmission electron micrograph of gastroderm from the diseased tissue zone of *D. stokesi*. The zooxanthellae have collapsed and degenerated, with multiple periplast membranes (arrow, upper right) and remnants visible within host cells. Methodology has been described[10]. Scale bar, 5.0 μm.

No microbial population was visible on the surface of diseased corals. Transmission electron microscopy of the bleached tissue zone revealed degenerated coral tissue with remnants of zooxanthellae, which accounted for the bleached appearance, but no demonstrable pathogens (Fig. 1d).

Samples collected from the surface of the disease line of three infected coral species (*D. stokesi, Dendrogyra cylindrus*, and *Diploria labyrinthiformis*) exhibited a predominance of small, motile, Gram-negative rods (measuring 1 × 1.5 micrometres) at concentrations of 10^5 cells per millilitre. Fewer bacteria were present in samples from apparently healthy tissue and denuded skeletal areas (10^3 and 10^4 cells per millilitre, respectively). Bacterial colonies from diseased tissue were uniform in colour, size and texture, but non-disease-line samples gave rise to mixed bacterial populations.

A bacterial isolate from diseased *D. stokesi* was characterized metabolically by obtaining profiles based on cluster analysis of patterns of carbon-source use[5] (95 different carbon sources) using Biolog GN plates, and genetically by using the polymerase chain reaction to amplify 16S ribosomal RNA sequences.

The metabolic profiles obtained placed the disease isolate as being most closely related to the bacterial genus *Sphingomonas*, a genus first described in 1990 (ref. 6). Genetic analysis placed the isolate in the α-subclass of the *Proteobacteria*, matching most closely the *Erythromicrobium/Erythrobacter/Sphingomonas* groups (exhibiting an identity of 89, 87 and 86%, respectively). The genera *Erythromicrobium* and *Erythrobacter* (not in the Biolog database) are facultative aerobic photoheterotrophs, but extracts from a culture of the disease isolate incubated under light yielded no photosynthetic pigments. This suggested that the isolate may be a new species of the genus *Sphingomonas*.

Laboratory experiments suggested that the bacterial isolate is the disease pathogen: two healthy colonies of *D. stokesi* placed on marine agar plates inoculated with the isolate became infected at their bases and lost all tissue within three days, whereas control colonies remained healthy. This identification of a single bacterium as pathogen contrasts with the results from microbiological studies of other coral diseases.

Further experiments suggested that the pathogen is readily transmissible: freshly collected, healthy colonies placed in the same aquaria with freshly collected, diseased ones also died.

In the two years following 1995, the reefs

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of the Florida Keys were virtually free of disease recurrence. However, in 1996 we recorded new outbreaks in the western portion of the Florida Reef Tract (Key West to the Dry Tortugas), and in 1997 on the reefs north of Miami. In each year (1995–1997) the disease incidences were seasonal, occurring from June to October.

Such repeated, severe infestations may restructure Florida's reef communities, as has occurred as a result of disease in other coral reefs[7–9].

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Termites fumigate their nests with naphthalene

Termite nests provide a controlled environment and physical defence for the colonies of insects they house[1]. Another important factor involved in colony defence may be volatile chemicals present in the nest: we have found the hydrocarbon naphthalene in extracts of the nest material produced by Formosan subterranean termites. This is the first time naphthalene has been found naturally associated with any insect species.

Figure 1 Soldier termites following a naphthalene trail. Naphthalene solution (4 μl) in hexane (2.03 mg ml^{-1}) was streaked on the left-hand pencil circle (final concentration of naphthalene was 0.045 μM cm^{-1}). The right-hand circle was streaked with 4 μl hexane as a control.

Formosan subterranean termites (*Coptotermes formosanus* Shiraki (Rhinotermitidae)) use saliva and excrement to cement soil and masticated wood into a maze of enclosed galleries to make nests[1,2]. We found between 50.56 and 214.6 micrograms per kilogram of naphthalene in extracts of this nest material, which is known as 'carton'.

Naphthalene acts as a general arthropod fumigant which is used against clothes moths and carpet beetles, a microbial inhibitor and an anthelmintic agent[3,4]. The chemical is also used as a repellent against mammals and birds: bats, squirrels, rabbits, pigeons, sparrows and starlings[3].

The volatility of this compound would allow it to permeate the closed system of the termite nests and shelter tubes[5]. In this system, it may be used for chemical defence against natural enemies such as ants, pathogenic microorganisms and nematodes. One group of these enemies, pathogenic fungi, cause mortalities that limit the establishment of termite colonies in the laboratory[5]. Ants are able to inhibit microbial pathogens, an important attribute that enables them to flourish in the soil habitat[6]. In termite nests, naphthalene as an antiseptic agent, as well as other nest fumigants, may inhibit the growth of such pathogenic microorganisms.

We have shown that naphthalene can inhibit natural fungi from proliferating and also compared the effect of naphthalene on *C. formosanus* and the red imported fire ant, *Solenopsis invicta* Buren, one of the most dominant predators. We found that *C. formosanus* had higher tolerance to naphthalene than did *S. invicta*; ants were paralysed at concentration levels that had no visible effect on termites.

The use of naphthalene as a fumigant by Formosan subterranean termites is unique. However, the function of naphthalene may not be limited to defence. We also discovered that naphthalene elicits trail-following behaviour in Formosan subterranean termite soldiers (Fig. 1).

Kaib[7] has argued that, in the adaptive evolution of termite defence strategies, the most important driving force is predation pressure from ants. Predator–prey interactions between ants and termites are constant, unlike occasional or seasonal predation by vertebrates[7]. Ants are social insects with well developed recruitment systems enabling them to use and defend food sources rapidly. These characteristics can create constant pressure on a termite colony. To repel such continued predation pressure, nest fumigation may represent an optimal defence strategy.

Previously, petroleum, coal and the products of incomplete combustion of organic matter were considered to be the only sources of naphthalene in nature[8]. However, the compound has been found as a constituent of *Magnolia* flowers and in the forehead region of male white-tailed deer, *Odocoileus virginianus*[9,10]. But how termites incorporate naphthalene in their nests and the mechanism of their tolerance to the chemical remain a mystery.

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