Extract of the Natural Botanical Melia Toosendan Prevents and Treats Coral Ciliate Disease

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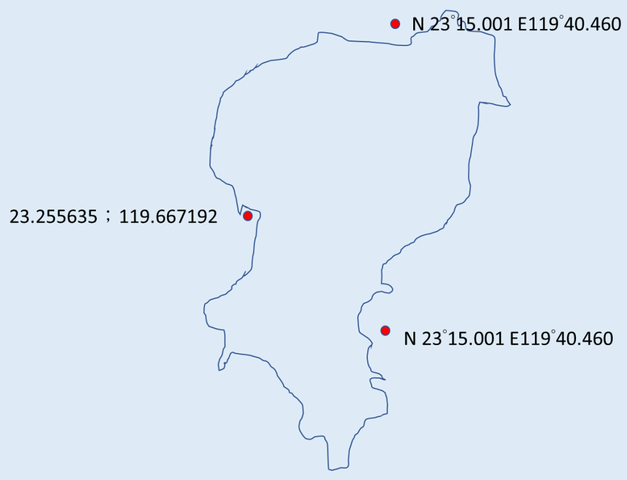
Abstract:

In recent years, coral diseases have posed serious threats to large-scale coral aquaculture and global coral reef resources. Corals infected with ciliate disease rapidly develop ulcerations or die. This study focuses on the diseased corals in the northernmost part of the Coral Triangle, isolated and identified ciliates, and large-scale aquaculture and prevention research. Two experiments were performed. The first was the isolation, identification, and cultivation of coral lesions. Six sampling points were established in dongjiyu, southwest Taiwan, to sample coral lesions. Infected lesions were found in Hydnophora microconos, Echinopora gemmacea, Acropora anthocercis, Pachyseris speciosa, and Pocillopora grandis. The second experiment evaluated the natural herb Melia toosendan extract for treating coral ciliate disease. The half-lethal concentration (LC50) of M. toosendan extract against ciliates was 2068.05 ppm. Extract concentrations of 0 (control), 1500, 2500, 5000, and 7500 ppm were applied to coral infected with ciliates. Uninfected corals not treated with extract were used as a negative control. Genetic analysis of 18S rRNA revealed infection with Glauconema sp. Treatment with 1500 and 7500 ppm M. toosendan extract significantly increased the levels of the antioxidant enzymes (superoxide dismutase, malondialdehyde and catalase). The stress response of the 2500 ppm treatment was lower. Morphological observations and histological examination revealed that the morphology of cells using 2500 ppm polyp was intact, with no vascular or tissue ulceration. The collective results indicate that ciliate disease can be treated with 2500 ppm M. toosendan extract in large-scale coral aquaculture.

Keywords: ciliates, coral, disease identification, Penghu Southern Islands National Park

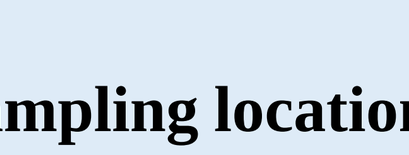
Sampling location:

Location	Latitude (N)	Longitude (E)	Depth (m)
(1)東吉嶼燈塔外海東北側	23˚15.808	119˚40.406	7-8
(2)東吉嶼薰衣草森林南側	23˚15.001	119˚40.460	7-8
(3)東吉碼頭北堤防	23.255635,	119.667192	2~3

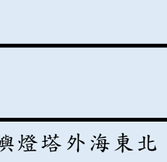


Dongjiyu Coral Monitoring and Sampling Point

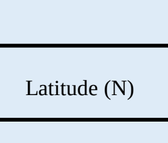
Result:



P. Lucinda (Ding *et al.*, 2022)

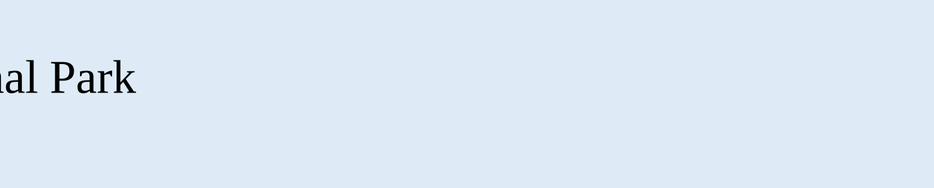


Diophrys sp. (Ding *et al.*, 2023)



Nudibranchia sp.

Lesions after coral infection by ciliates:



Lobophyllia sp. *Porites* sp. *Montipora* sp *Porites* sp. *Porites lobata*

In the second season, signs of coral disease were observed. The corals initially showed localized bleaching with noticeable tissue detachment along the edges, and as the condition progressed, the coenosarc gradually shrank, the amount of tissue significantly decreased, and eventually the entire colony became fully bleached and lost all living tissue. In some cases, small white patches first appeared and continued to expand until the whole coral turned white. In certain situations, the early symptoms were mistaken for fish bite marks, but ciliates were later collected from the wound area, and during the mid-stage the coral developed characteristic gelatinous tissue decay, with only small surviving portions remaining in the later stage. However, not all cases were caused by ciliates; some were the result of nudibranch grazing, which is particularly harmful because the nudibranchs lay eggs on the underside of the coral, and once the eggs hatch after approximately three to seven days, the coral may rapidly disappear.

Micromussa sp. *Montipora* sp. *Micromussa* sp. *Acropora* sp. *Montipora* sp.

In the third season, after being grazed by fish, ciliates were collected from the wound area. In another case, ciliates were found on coral wounds caused by aggression between neighboring colonies, with surrounding tissue initially showing signs of repair. Ciliates were also collected at the boundary between healthy and bleached tissue. In areas where sediment and algae accumulated, tissue ulceration occurred, and ciliates were detected within the ulcerated regions. Some colonies exhibited multiple central injuries, with ciliates feeding on the tissue and creating small white patches. In other cases, the corallite mouths were consumed, leaving only the exposed skeleton.

TotalPCR identification results

The ciliates discovered in this experiment were identified as P. lucinda and Diophrys sp. using 18S rRNA gene sequencing and the ciliate-specific primers 384f-cil and 1147r-cil, and their morphology was further confirmed through microscopic observation. *Nudibranchia sp.* has not yet been identified by PCR; currently, only morphological characteristics observed under the microscope are used for preliminary confirmation. Further molecular identification will be conducted to determine the species.

Nudibranchia sp. individuals grew to 2–9 mm within ten days, and began to lay eggs when reaching 6–9 mm in body length. Each egg mass contained approximately 150–250 eggs. The eggs hatched after about 7 days, and the nudibranchs were able to spawn again after 14 days. Egg masses were deposited on the underside of the coral, likely to avoid predation by other organisms.
Morphologically, the nudibranch had a body length of about 10 mm, with a pale yellow to milky-white coloration and a slightly translucent appearance. The body was elongated, with a more pointed anterior end and a rounded posterior end. The anterior region featured two pairs of protruding structures; the front pair resembled elongated tentacle-like appendages extending forward and upward. The boundary between the head and body was indistinct, giving the animal a soft, streamlined profile. On the posterior portion of the body, there were multiple branched appendages (approximately 1–2 mm), arranged in a radial or clustered pattern. Each appendage displayed fine surface textures or granules, with slightly pointed tips. These branched structures appeared to function as cerata (branched gills).

SecondQuarter:

水樣編號	檢測項目	水質數據
東吉嶼燈塔外海東北側	KH	5.3 dKH
	Ca	420 mg/L
	Mg	1230 mg/L
	pH	7.6
	氨氮	0 mg/L
	亞硝酸	0.1 mg/L
	NO_3^-	5.0 mg/L
	PO_4^{3-}	0.2 mg/L
	水溫	27 ℃
東吉嶼薰衣草森林南側	KH	6.1 dKH
	Ca	320 mg/L
	Mg	1125 mg/L
	pH	8.3
	氨氮	0 mg/L
	亞硝酸	0.3 mg/L
	NO_3^-	5.0 mg/L
	PO_4^{3-}	0.3 mg/L
	水溫	27℃
東吉碼頭北堤防(內側台階出去)	KH	6.1 dKH
	Ca	380 mg/L
	Mg	1245 mg/L
	pH	8.1
	氨氮	0 mg/L
	亞硝酸	0.2 mg/L
	NO_3^-	1.0 mg/L
	PO_4^{3-}	0.1 mg/L
	水溫	28℃

Third Quarter:

水樣編號	檢測項目	水質數據
東吉嶼燈塔外海東北側	KH	6.1 dKH
	Ca	440 mg/L
	Mg	1200
	pH	mg/L
	氨氮	8.1
	亞硝酸	0 mg/L
	NO_3^-	0.1 mg/L
	PO_4^{3-}	5.0 mg/L
	水溫	0.2 mg/L
		29 ℃
東吉嶼薰衣草森林南側	KH	6.7 dKH
	Ca	340 mg/L
	Mg	1170 mg/L
	pH	8.2
	氨氮	0 mg/L
	亞硝酸	0.3 mg/L
	NO_3^-	5.5 mg/L
	PO_4^{3-}	0.3 mg/L
	水溫	29℃
東吉碼頭北堤防(內側台階出去)	KH	6.1 dKH
	Ca	380 mg/L
	Mg	1080 mg/L
	pH	8.0
	氨氮	0 mg/L
	亞硝酸	0.2 mg/L
	NO_3^-	2.0 mg/L
	PO_4^{3-}	0.1 mg/L
	水溫	28℃

Conclusion:

I. Current Status of Ciliate Infections

1.In Year 113, ciliate infections of *Porites lichen* and *Diophrys* sp. were first recorded on Dongji Islet, Penghu.

2.In Year 114, infections reappeared, indicating that corals in the area are experiencing continuous and severe threats from ciliates.

3.This situation highlights the importance of long-term monitoring and prevention of coral diseases.

II. Appearance of Nudibranchs and Ecological Imbalance

1.During SCUBA surveys in Year 114, nudibranchs (*Nudibranchia* sp.) were observed for the first time.

2.The presence of nudibranchs may be related to ecological imbalance, changes in the food web, or environmental stress.

III. Water Quality Assessment and Coral Growth Inconsistency

1.Water quality suitability assessments showed the following ranking: South side of Lavender Forest > Northeast offshore area near the lighthouse > North breakwater at Dongji Wharf.

2.However, actual coral growth conditions exhibited the opposite trend: the northeast offshore lighthouse area had the best coral growth, while the south side of Lavender Forest and the north breakwater showed poorer growth.

V. Inference: Coral Health Is Influenced by Multiple Factors

1.Water quality is not the only factor affecting coral growth.

2.Potential interfering factors include changes in currents and sea conditions, sediment coverage or abnormal turbidity, substrate structure alterations, fishing activities, and anthropogenic pressures.

3.These factors may collectively contribute to coral decline and mortality in the area.