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The occurrence, identification and ecological studies of the cactus nematode from dragon fruit crops in Taiwan

Hsiu-Yu Chan[1*], Jyh-Herng Yen[2*], Diann-Yih Chen[3], Tung-Tsuan Tsay[1], Peichen Chen[1**]

[1] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan
[2] Agricultural Extension Center, National Chung Hsing University, Taichung, Taiwan
[3] Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan., Wufeng, Taichung, Taiwan

*The two authors contributed to this research work equally

**Corresponding author, E-mail : janetchen@nchu.edu.tw

ABSTRACT

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Cactodera cacti was first observed on roots of dragon fruit (*Hylocereus* spp.) in central and southern Taiwan in 2011. The brown lemon-shaped cysts showed circumfenestrate and the egg shell were punctuated. Amplifying the rDNA ITS region from the 7 geographical populations all resulted in a 981 bp fragment and in 16 haplotypes. The haplotype 2 (JX051329) was the most prevailed type found in these populations. Eight species of the family Cactaceae tested in this study were the host of *C. cacti*, but *Echinocactus grusonii* and *Rhipsalis cereuscula* were not. *C. cacti* completed the life cycle between 35 to 40 days when inoculated on the dragon fruit and incubated at 32℃, and the egg hatching rates showed no significant differences under the temperatures ranging from 16 to 32℃. The impact of *C. cacti* on the dragon fruit yields is under investigation.

Keywords: *Cactodera cacti*, cyst nematode, *Hylocereus* spp., host range, intra-species variation

INTRODUCTION

The dragon fruit (*Hylocereus* spp.), also known as pitaya, is a climbing epiphytic cacti belonging to the Family Cactaceae. The plants have been found in Mexico, Guatemala, and Costa Rica [16]. Dragon fruit is a perennial cactus with triangular stem shape, and 3-5 spines on the areole curve of the stem. The flowers are white and the plant usually habitats at places with high humidity and low light intensity [8]. There are more than 20 species in the genus *Hylocereus* [2]. The stem and flower are similar in most species but the fruit patterns are different. Dragon fruits became an economic crop in China, Vietnam, Australia, Israel, Malaysia, Nicaragua [9], and recently in Taiwan. The dragon fruits have abundant nutrients in it, such as cellulose, calcium, proteins, vitamins B_1, B_2, B_3, C and mineral contents [14]. Dragon fruits had been introduced to Taiwan in 1645 by the Dutch, and later from Puerto Rico and Vietnam in the 20th century. Dragon fruits are propagated by cuttings, and the planting species in Taiwan are mainly *H. undatus* (Haworth Britton and Rose), *H. polyrhizus*, *H. costaricensis* and their hybrids.

The cactus cyst nematode, *Cactodera cacti*, belongs to the subfamily Heteroderinae, was first described to parasitize the ornamental cactus *Discocactus akkermannii* and *Cereus speciosus*, and was first classified as *Heterodera shachtii* [1]. After Krall and Krall (1978) erected the genus *Cactodera*, *Heterodera cacti* was transferred to *Cactodera cacti* [13]. This nematode has been reported from Asia, Africa, Europe, and South America [12]. The reported host plants of *C. cacti* are mainly the species in the families of Cactaceae, Euphorbiaceae, and Umbelliferae [3, 13]. Typical symptoms such as branched roots and increased numbers of rootlets were found on the infected plants and with high infection the host may die [13]. The objectives of this research are to study the etiology of *Cactodera cacti* on the dragon fruits, and to investigate the parasitize ability of *C. cacti* on the other economic crops.

MATERIALS AND METHODS

Surveying and collecting nematodes

Soil samples were collected between March 2011 and March 2012 at the mainproduction areas of dragon fruits in Taiwan. To isolate the J_2 in the rhizosphere, at each survey site, soils from 5-10 dragon fruits plants at a depth of 5-15 cm were collected using a trowel. All samples from one site were placed and mixed well in an individual plastic bag. Soil samples were store at room temperature (25℃) before processing for isolation. A total of 181 survey sites mainly in the middle and southern Taiwan were investigated in this study. Samples were also collected from Penghu county which is an island in the west of Taiwan. The survey regions were situated between N 22°39'51" to N 24°20'08" and E 121°35' 65" to E 119° 34' 53".

Establishing Nematode isolates and populations

From each survey site, 400 g of the soil were divided into

4 parts, each contained one hundred grams of soil and processed using the modified Baermann funnel method [15] for 24 hours. The J_2 of nematodes were identified and quantified under the dissecting microscope. Because very few nematodes were found from each survey sites, all the available 2nd stage juveniles from one county or city were pooled and inoculated on the dragon fruit plant to establish the geographically different populations for the following experiments (Table 1).

TABLE 1. The location, population codes and number of sampling sites with *Cactodera cacti* on the dragon fruits in Taiwan.

Location	code	# of sites surveyed	# of sites with J_2
Taichung	TC	13	1
Changhua	CH	52	11
Nantou	NT	29	6
Yunlin	YL	5	3
Chiayi	CY[1.]	12	1
Tainan	TN	30	8
Kaohsiung	KH	4	2
Pingtung	PT	6	0
Hualien	HL	11	0
Taitung	TT	8	0
Penghu	PH	11	0
Total		181	32

[1.] means the population failed to establish at the end of the study.

The morphology of second-stage juveniles and the cyst

The 2nd stage juveniles were allowed to reproduce on the dragon fruit plants and later processed for the identification. Specimens were heat-inactive for 3 seconds, and mounted in the distilled water; the morphological traits were photographed using a digital camera (G11, Canon, Tokyo, Japan) under 200 and 400x magnification for juveniles and 70x magnification for the cyst under a light microscope. The values of de Manian formula including stylet length, distance of dorsal gland orifice (DGO) from the stylet base, a, b, b', c, c' values, and the length of the tail hyaline region, along with the width and length of the cysts were measured by AxioVision (Release 4.8.2, Zeiss, Germany) software.

The morphology under Cryo-FESEM

Fifty to one hundred 2nd stage juveniles were collected from the crashed cysts, rinsed and kept in 50 µl distilled water in the 1.5 ml microcentrifuge tube. Ten to fifteen micro liter suspension containing 10-30 juveniles were processed following the method described by Jen et al., [5] and viewed with cryo-field emission scanning electron microscope (6330 Cryo-FESEM, JEOL, Tokyo, Japan). The cysts and the eggs were also processed for observation. The fixation also followed the method described by Jen et al. [5] and the images were saved as bmp file.

Identification using rDNA sequence

Five to ten cysts derived from each isolate were handpicked from the roots, washed with distilled water, and the DNA was extracted using the Tissue & Cell Genomic DNA Purification Kit (GeneMark, Tainan, Taiwan) according to the manufacturer protocol.

The ITS rDNA region was amplified with the primers mTW81 (5'-GTAGCTGTAGGTGAACCTGC-3') and mAB28 (5'-ATATGCTTAAGTTCAGCGGGT-3'), which were modified from Joyce et al.[6]. PCR amplification was performed in a PCR machine. The reaction contained 2 µl of DNA, 2.5 µl 10X polymerase buffer, 2 µl 2.5mM dNTP, 1 ul each of 10 mM mTW81 and mAB28 primer, 0.2 µl DNA polymerase (1U)(TaKaRa, Shiga, Japan), and 16.3 µl MQ water. The PCR started with denaturing at 94℃ for 2 minutes and then followed by 30 cycles of denaturization at 94℃ for 30 sec, annealing at 54℃ for 30 sec, extension at 72℃ for 2 min, and the reaction ended with 72℃ for 5 min. The PCR products was run on a 1.2 % agarose gel stained with ethidium bromide (75 ppm) and the major band was purified using the DNA clean/extraction kit (DP034, Gene Mark, Tainan, Taiwan) and was cloned into pOSI-T vector using the OS01 kit (Gene Mark, Tainan, Taiwan). The transformed bacteria clones were sequenced by the Mission Biotech Company (Taipei, Taiwan). All sequences were alimented using DNASTAR software (DNASTAR Inc., Madison, Wisconsin, U.S.A.). The sequences were compared with the other ITS sequences (#Af 498393 and HQ260422) of *Cactodera cacti* deposited in the National Center for Biotechnology Information (NCBI), and the percentage of sequences similarities were obtained. The sequences obtained from these populations were submitted to NCBI, and the accession numbers were from #JX051329 to JX051344.

The hatching rate of *Cactodera cacti* under 6 temperatures

Cysts from roots of dragon fruit were dry preserved at 4℃ before the experiment. The 16, 24, 28 and 32℃ treatments were conducted in the temperature gradient incubator, and the 20℃ treatment was in another growth chamber. The 4℃ treatment was conducted in a cooling cabinet (CC-3, Firstek scientific co., Ltd, New Taipei city, Taiwan).The cysts were incubated at the treated temperature for 5 days and crushed, the number of eggs and juveniles were then counted. The equation for the hatching rate is as following: second- stage juveniles/unhatched eggs+ hatched eggs. Each treatment had three replicates and each replicate contained a single cyst. The experiment was repeated three times.

The life cycle of *Cactodera cacti* on Hylocereus sp.

Six months old dragon fruit plants were planted in the seedling tray (27 cubic centimeters) with peat moss and sand, and inoculated with approximately 100 juveniles. The experiment was conducted in the growth chamber at 32℃ under a 12-hour daily photoperiod and 70% relative humidity, the plants were only manually given water on the seedling tray every 2-3 days. Three, 7, 14, 21, 28, 35, or 40 days after inoculation (dai), three plants were randomly taken for examination. The roots were fixed in 0.5% bleach (The Clorox Company, USA) for 10 min, and washed with tap water for 10 min, then stained with cotton blue lactophenol (cotton blue 0.24 g,

phenol liquid 60 ml, lactic acid 60 ml, glycerol 120 ml, and distilled water 60 ml) [4]. The roots were immersed in the stain solution and heated in the microwave oven (NE205A, National, Taiwan) for 20 sec at medium power. The roots were allowed to be stained at room temperature for 16-18 hours. They were destained for 2-3 hours, and observed under the dissecting microscope. The destaining solution contained: phenol liquid 60 ml, lactic acid 60 ml, glycerol 120 ml and distilled water 60 ml [4]. The experiment was repeated 2 times.

The host range test of *Cactodera cacti*

Four genus from the Cactaceae tested were bought from a local flower market, including *Hylocereus triangularis*, *Mammillaria spinosiassima*, *Notocactus ottonis*, *Cereus peruvianus*, *Mammillaria sempervivi*. The cactus plants were identified by the host list [3]. A tuber of potato cv. Kennebec (*Solanum tuberosum* L.) was cut into chunks, and planted in the peat moss that was autoclaved. Seeds of *Apium graveolens* var. secalinum, *Apium graveolens* var. dulce, *Beta vulgaris*, *Glycine max* were bought from Known- You seed CO., Ltd.(Kaohsiung, Taiwan) and were grown in the 3 inch diameter pots for 2-3 weeks before the tests. The inoculation concentration was 1 J_2/ cm^3, and the presence of cysts on roots was observed 60 days after inoculation. The plants were maintained in the greenhouse from October 2011 to January 2012. The average temperature of the experimental periods varies from 16.5°C to 25.9°C. Each host had three replicates and the experiment was repeated three times.

Results

A total of 181 sampling sites were surveyed in this study. The second-stage juveniles of *C. cacti* were found at 32 sampling sites, which were mainly in the middle and western Taiwan. Among the 32 sites that had *C. cacti*, 1 located in Taichung City, 11 in Changhua County, 6 in Nantou County, 3 in Yunlin County, 1 in Chiayi County, 8 in Tainan City, 2 in Kaohsiung City. Sites in Pingtung, Hualian, Taitung and Penghu Counties sampled did not have either cysts or J_2 (Table 1). The highest J_2 number recovered from an individual soil sample was 199 per 100 g soil. Eleven sites sampled had scarce J_2, so all the *Cactodera cacti* from one county or city were pooled as one isolate and all the isolates were maintained separately. The Chiayi (CY) population failed to establish because of the low amount of juveniles (average less than 1 J_2 per 100g soil). At the end, 6 populations were established from the survey.

The morphology of second-stage juveniles and cysts

Measurements of 20 J_2 from the 6 populations were listed in the Table 2. The body length (L) of CH population ranged from 482.3-605.5 μm, YL population 480.9- 594.7 μm, NT population 426.5-578.6 μm, TN population 433.0-603.8 μm, KH population 420.9-508.0 μm, and TC population 424.4- 523.1 μm. The body width (K) of CH population ranged from 20.8- 29.9 μm, YL population 20.6-30.5 μm, NT population 19.7- 28.2 μm, TN population 17.4- 29.0 μm, KH population 19.1- 24.6 μm, and TC population 19.35- 23.1 μm. The average stylet length of six populations ranged from 23.1 μm (TN population) to 24.7 μm (NT population).The average tail length of six populations ranged from TC population with 51.2 μm to YL population with 56.4 μm. The average of the tail hyaline region of six populations ranged from 19.4 μm (TN) to 22.4 μm (CH). The distance from the dorsal gland orifice (DGO) to the stylet basal of these populations was in the range of 3.7 μm to 4.3 μm (Table 2). The second stage juvenile are vermiform, with obvious lip region and the stylet, and stylet knobs were well developed (Fig. 1A). The excretory pore was located posterior to the spherical median bulb, but not obvious. Esophageal glands overlap ventrally with the intestine and the lateral field had four incisures (Fig. 1B). The tail was tapering with U or V-shaped hyaline region (Fig. 1C). Males were not observed in this study.

Twenty cysts of the CH population were measured (Table 3). The length of cysts ranged from 358 to 563 μm and the width from 296 to 475 μm. The L/W ratio ranged from 1.1 to 1.6. The fenestral diagram had the average of 23.2 μm (20- 29 μm). The measurements of the CH cysts were compared with the other populations from Netherland, Italy, and Iran [13], and found to be similar (Table 3).

TABLE 2. Morphometrics of J_2 from six *Cactodera cacti* isolates collected from different locations in Taiwan, the measurements were given in micrometers.

Isolates code	CH	YL	NT	TN	KH	TC	Netherlands(1932)
L	547.0 (482.3-605.6)	536.3 (480.9-594.7)	479.7 (426.5-578.6)	535.3 (433.0-603.8)	469.3 (420.9-508.0)	467.5 (424.4-523.1)	486 (428-584)
K	24.93 (20.8-29.9)	25.76 (20.6-30.5)	23.98 (19.7-28.2)	25.03 (17.4-29.9)	21.39 (19.1-24.6)	21.33 (19.4-23.1)	
S2	23.73 (20.0-26.1)	23.87 (21.6-26.7)	24.74 (22.6-27.4)	23.13 (21.1-25.5)	23.24 (20.8-26.5)	23.65 (20.6-25.9)	25.2 (22-28.2)
Tail length	55.48 (49.1-64.5)	56.39 (48.2-61.7)	53.11 (45.6-59.3)	52.15 (40.7-61.4)	53.29 (44.9-59.2)	51.16 (44.3-57.2)	–
Hyaline region	22.41 (15.9-31.1)	20.72 (16.7-24.4)	20.18 (16.1-24.7)	19.40 (14.3-23.2)	21.04 (14.9-26.5)	20.71 (10.5-31.6)	–
DGO	3.73 (2.36-4.9)	3.94 (3.07-4.9)	4.32 (2.4-6.7)	3.61 (2.8-4.9)	3.71 (2.1-4.8)	3.71 (2.4-5.0)	–
Ration a	22.1±1.5	21.0±1.8	20.1±1.7	21.9±1.6	22±1.5	22.0±1.1	22 (10.2-29.4)
b	6.0±0.3	5.8±0.5	4.5±0.4	5.9±0.4	5.4±0.4	5.0±0.6	6 (5.6-6.8)
c	9.9±0.6	9.5±0.7	9.1±0.8	10.3±1.0	8.8±0.6	9.2±0.7	9.4 (8.4-10.5)
b'	3.2±0.3	2.7±0.3	2.4±0.2	2.8±0.2	2.8±0.3	3.1±0.3	–
c'	3.7±0.3	3.5±0.5	3.5±0.3	3.4±0.4	3.9±0.3	3.6±0.3	–

L＝Total body length.
K＝The maximum body width.
S2＝Stylet length
DGO＝the distance of dorsal gland orifice to stylet base
a＝Total body length divided by maximum body width
b＝Total body length divided by oesophageal length
c＝Total body length divided by tail length
b'＝Total body length divided by distance from anterior end of body to posterior end of oesophageal glands.
c'＝Tail length divided by tail width

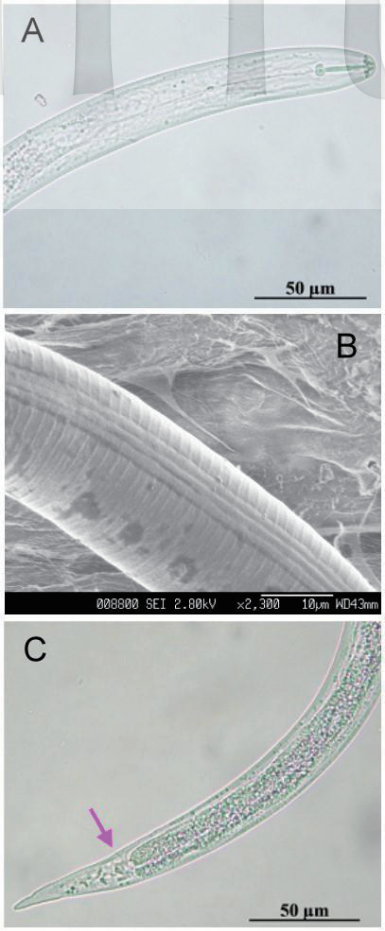

Fig. 1. The juvenile and the tail of *Cactodera cacti* 2nd stage juvenile. A: the anterior region showing strong stylet and stylet knob with the viewed from the lateral side; B: the lateral field had 4 incisures under the SEM ; C: J_2 tail with hyaline region, the arrow indicates the anus.

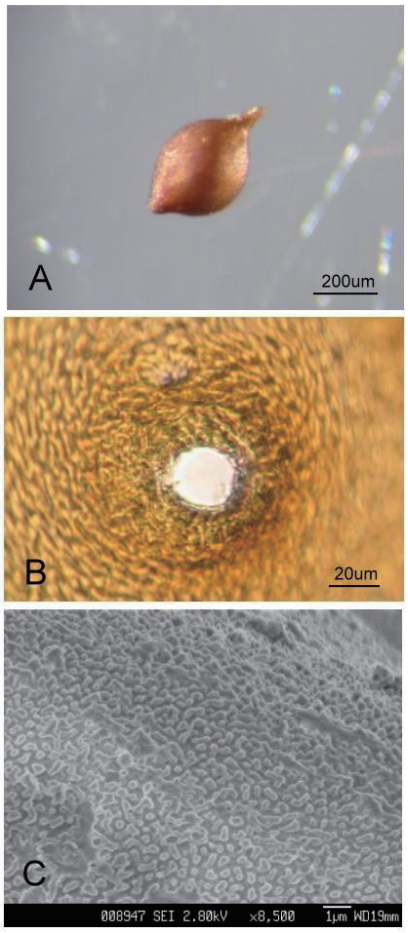

Fig. 2. The cyst and circumfenestrate on the cone top of *Cactodera cacti*. A: the lemon-shaped mature cyst; B: cirumfenestrate located on the cone top; C: the punctuated surface of the eggshell observed under cryo-field emission scanning electron microscope (Cryo-FESEM).

TABLE 3. Morphometrics of *Cactodera cacti* cyst from CH population and three reference populations from the Netherland, Italy, and Iran.

Population characters	The Netherland (Adam, 1932)	Italy (Ambrogioni, 1969)	Iran (Maafi, 2004)	Taiwan
n[1.]	50	25	21	20
L	497 (328-617)	536 (384-780)	490 (380-600)	457 (358-563)
W	447 (309-598)	378 (240-588)	382 (280-490)	359 (296-475)
L/W	1.1	1.4 (1.1-2.0)	—	1.28 (1.1-1.6)
fenestral diameter	—	(26-28)	28±3.3 (23-35)	23.2 (20- 29)

[1.] n = number of cysts measured, L = Length, W = Width, and all measurements were in μm

The color of *Cactodera cacti* cyst was white to light or medium brown with the lemon-like shape. The female cyst had a protruding neck and obvious vulva cone (Fig. 2A). The vulva region with a single circumfenestrate (Fig.2B), and the vulva bridge, underbridge and bullae were absent. All eggs are long oval shape with heavily punctuated eggshell (Fig. 2C).

Identification using rDNA internal transcribed spacer (ITS) sequence

The ITS regions from the seven populations resulted in a 981 bp sequence. The six clones from the CH population were identical and it was named haplotype 2. The haplotype 2 was also found in the TC, CY, TN and KH populations. Three clones from YL population were sequenced and were all different from each other, they were haplotype 3,9,14. The haplotype 9 was 100% identical with the Germany isolate (accession number: AF498393). The 2 clones from TC population were haplotype 2, and the third one was named haplotype 1. The CY population had haplotype 2, haplotype 4 and haplotype 8. In the TN population, three clones were haplotype

TABLE 4. Sixteen ITS region haplotypes of *Cactodera cacti*, their Nactional Center of Biotechnology Information accession number and original isolate codes.

Haplotype	Accession number	Isolate
1	JX051341	TC
2	JX051329	CH, TC, KH, CY, TN
3	JX051343	YL
4	JX051342	CY
5	JX051332	TN
6	JX051340	KH
7	JX051331	KH
8	JX051330	CY
9	JX051344	YL
10	JX051334	NT
11	JX051338	NT
12	JX051339	NT
13	JX051337	NT
14	JX051333	YL
15	JX051335	NT
16	JX051336	NT

2 and one was haplotype 5. The KH population also had haplotype 2, and the last two were haplotypes 6 and 7. The NT population had 6 clones sequenced. They were haplotypes 10-13 and haplotype 16. From the 7 *C. cacti* populations sequenced, 16 different haplotype sequences were submitted to NCBI, and the accession number was from JX051329 to JX051344 (Table. 4).

The 16 haplotypes along with the Germany isolate (AF498393), and the Iranian isolate (HQ260422) were analyzed using Neighbor Joining (NJ) methods with bioNJ parameter to present a distance tree. The 16 haplotypes branched into 3 major clades, haplotypes 1-8 were in clade I, haplotypes 9-13 were in clade II with the Germany isolate (AF498393). Haplotypes 15 and 16 and the Iranian isolate (HQ260422) were in clade III. Haplotype 14 was in a single branch closer to the clade that had haplotypes 15 and 16 (Figure 3).

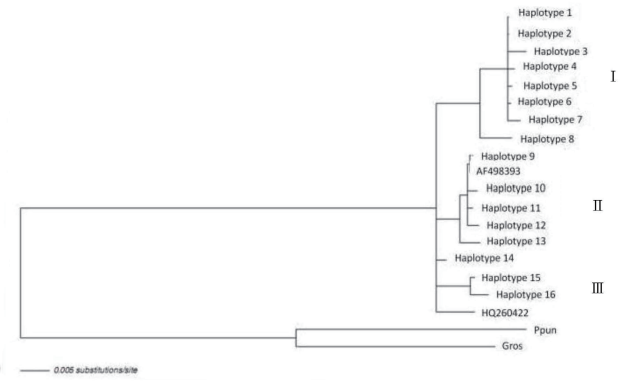

Fig. 3. Neighbor-joining phylogram of 16 internal transcribed spacer (ITS) rDNA sequences obtained from six populations and two reference sequences from NCBI with *Globodera rostochiensis* (Gros) and *Punctodera punctata* (Ppun) served as outgroups.

The egg hatching rate of *Cactodera cacti* under 6 temperatures

The egg hatching rates were obtained at the 5th day after the temperature treatments. The highest egg hatching rate (32.72%) was in the 32°C treatment. In the second experiment, 4°C gave the highest hatching rate which was 30.1%, and the lowest rate was at 16°C (12.98%). In the third experiment, 16°C gave the highest rate (35.75%) and 24 °C the lowest (15.75%). The hatching rate among these three experiments did not differ significantly, and no conclusive results could support an optimal hatching temperature. However, when the eggs were incubated at 32 °C, it gave the most stable hatching rate ranging from 25.47% to 32.72% (Table 5).

TABLE 5. The hatching rate of *Cactodera cacti* obtained at the 5th day after incubated under 6 different temperatures.

Experiment Repeat	Egg hatching rate (%)					
	4°C	16°C	20°C	24°C	28°C	32°C
I	9.57 a[1.]	20.13 a	28.47 a	18.25 a	13.35 a	32.72 a
II	30.1 a	12.98 a	19.22 a	18.01 a	16.69 a	25.47 a
III	26.06 a	35.75 a	22.31 a	15.75 a	17.71 a	26.54 a

[1.] Means (n = 3) in the same row followed by the same letter are not significantly different (α = 0.05) according to the t-test.

The life cycle of *Cactodera cacti* on *Hylocereus* sp.

When the *C. cacti* was inoculated on dragon fruits and cultured under 32°C, the juvenile was observed to develop into the "sausage-stage" at 2-3 dai. The juvenile became larger in width at 7 dai, then a long oval-like shape at 14 dai, and at 21 dai the cyst became lemon-shaped. At 28 dai, eggs were observed inside the female, but the female was still with long, slender neck, and at 35 dai the female was harden into the cyst, and fell off from the roots. Not until 40 dai, the J_2 of *Cactodera cacti* were observed again inside the roots of dragon fruits. In the second repeat of the experiment, the female fell off from the roots at the 35 dai.

The host range test of *Cactodera cacti*

Seventeen plants species within 14 genera were tested in this study (Table 6). Cysts were not found on the roots of *Apium graveolens* var. secalinuma (celery) and *Apium graveolens* var. dulce (celery), *Beta vulgaris* (sugar beet), *Solanum tuberosum* L. (potato), *Glycine max* (soybean), *Coryphantha elephantidens* (Golden barre), and *Rhipsalis cereuscula*. On six cacti; *Hylocereus triangularis* (Honolulu queen), *Mammillaria spinosiassima*, *Notocactus ottonis* (Ball cactus), *Mammillaria sempervivi*, *Cereus peruvianus* (Curiosity plant), *Coryphantha elephantidens* (Variegated Elephant- tooth Cactus), *Echinopsis eyriesii*, *Opuntia stricta* var. dillenii (Prickly-pear) and *Discocactus heptacanthus*, cysts were observed 60 days after inoculation.

Discussion

The cyst nematodes collected from different counties in this

TABLE 6. The classification, scientific name and common name of the hosts used in the host range test.

Family/	Scientific name	common name	Population
Apiaceae	*Apium graveolens* var. secalinum	Celery	CH.YL.TN
	Apium graveolens var. dulce	Celery	CH.YL.TN
Cactaceae	*Cereus peruvianus*	Curiosity plant	CH. YL
	Coryphantha elephantidens	Variegated Elephant- tooth Cactus	YL
	Discocactus heptacanthus	—[1.]	YL
	Echinocactus grusonii	Golden barre	YL
	Echinopsis eyriesii	—	YL
	Hylocereus triangularis	Honolulu queen	CH. YL
	Hylocereus undatus	Dragon fruit	CH. YL
	Mammillaria sempervivi	—	YL
	Mammillaria spinosiassima	—	CH
	Notocactus ottonis	Ball cactus	CH.YL.TN
	Opuntia stricta var. dillenii	Prickly-pear	YL
	Rhipsalis cereuscula	—	YL
Chenopodiaceae	*Beta vulgaris*	Sugar beet	CH.TN
Fabaceae	*Glycine max*	Soybean	CH.YL.TN
Solanaceae	*Solanum tuberosum*	Potato	CH

[1.] "—" means no common name

survey were identified by the morphology and ITS region sequence to the species level, and were all identified to be *Cactoderea cacti*. The *C. cacti* were found at 32 out of 181 sampling sites, and were not found in the dragon fruit plantations located in eastern and the off-shore Penghu County. It is common for the growers to exchange the cutting branches for their own breeding program and might contributed to the dispersal of *C. cacti*.

When the morphometric data from the six *C. cacti* populations collected from different geographical areas were compared, small differences between populations were found. The second stage juveniles from Changhua, Yunlin and Tainan population had larger body length and width. A slight difference on the body width behind the lip region was also found, but this particular difference was not correlated with the geographical distribution. The cysts of *C. cacti* in this study were smaller in size compared to the references [13], indicating the possibility of parasitizing a poor host or developing under a non-optimal environment for the nematodes. On the contrary, J_2 in this study were bigger in size.

In the phylogeny tree, the 16 haplotypes branched into 3 major clades. Haplotype 2 is the most prevail type, generally existing in all the populations in Taiwan. It was grouped in clade I with populations from Taichung, Chunghua, Chiayi, Tainan and Kaohsiung. Haplotypes 9 to 13 which were from Nantou and Yunlin populations were in clade II, with one foreign sequence from Germany (AF498393). Haplotypes 15 and 16 were from Nantou population and was grouped with the Iran foreign sequences (HQ260422) in clade III. Haplotype 14 from Yunlin was itself in a single branch and was the most distant type from the others. But Yunlin population also had the most prevailing haplotype 2, indicating this isolate with the most diverse haplotypes might be the most ancestry form in Taiwan.

In the egg hatching rate experiment, the cysts containing eggs were incubated under 6 different temperatures ranging from 4 to 32°C. The results did not support any of the tested temperature as the optimal one for egg hatching. However, the 2nd stage juveniles were more vigorous when hatched under 32°C in both experiments. *C. cacti* was first reported to parasitize cactus in the greenhouse, and the known hosts of *C. cacti* are mostly grown in the tropical or subtropical area, suggesting that the *C. cacti* J_2 would be more vigorous under high temperature. Sikora and Noel [11] reported the hatching of cyst nematodes *H. glycines* could be regulated by the host factor. Whether the inconsistent hatching rate of the eggs in our study was due to the cysts which were hatched without any host factor needs further investigation.

C. cacti completed its life cycle in 40 days at the 1st experiment and 35 days at the 2nd experiment. Studies showed culturing *C. cacti* under 28°C on Schlumbergera sp. and found that the J_2 became adult female at the 12th day and the cysts were formed 10 weeks after the feeding site formation. *Cactodera cacti* completed its life cycle within 29-34 days at 18 to 26°C on an unknown host [7]. In this study, males were not observed (data not shown), suggesting that *C. cacti* could be parthenogenetic.

C. cacti could parasitize most Cactaceae genera tested in our study, except *Echinocactus grusonii*. *C. cacti* was unable to reproduce on the mature root of *Cereus peruvianus* but was able to reproduce successfully on younger roots, indicating that *C. cacti* prefers to establish the feeding site on the non-lignificated parts of the roots. The same phenomenon was observed on dragon fruit hosts in the surveyed sites. Cysts were clustered on young roots and seldom on the lignified roots.

Celery (*Apium graveolens*) was reported as a host of *C. cacti*, but both celery cultivars (*Apium graveolens* var. secalinum and *Apium graveolens* var. dulce) in this study were not able to support the reproduction of *C. cacti* isolates from Taiwan. The cyst nematode, *H. glycines*, was differentiated into host races by their ability to parasitize on different soybean cultivars [10]. *C. cacti* might have the same intra-species variation on their parasitizing ability on the celery.

Cactodera cacti was not considered as an important nematode because of its narrow host range and insignificant damages on the hosts [3]. The economic crops in Taiwan such as dragon fruits and prickly pears showed no symptoms on the above-ground parts when infected with this nematode. The influences of *C. cacti* on the dragon fruit crop yield need to be tested in the future. To prevent the dissemination of *C. cacti*, the cutting of cactus branches should be soil-free for the breeding program. Hot water treatment at 43 to 45°C of the pot or root ball of cactus, and pesticides such as fensulphothion, prophos, and phenamiphos were reported to be effective in controlling *C. cacti* [7].

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