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Prevalence and Observation of Intestine-Dwelling Gregarines in the Millipede Trigoniulus corallinus (Spirobolida: Pachybolidae) Collected from Shoushan, Kaohsiung, Taiwan【Research report】

壽山地區磚紅厚甲馬陸腸道寄生簇蟲之調查與實驗室觀察【研究報告】

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Abstract

The intestine of a Formosan millipede species, Trigoniulus corallinus, was noted to be heavily infected with gregarine parasites. Millipedes used in this study were randomly collected from Shoushan, Kaohsiung from April 1999 to October 2002. Among 63 millipedes examined, our results showed that the total prevalence rate for cephaline gregarines was 87.30%. The infection in the male millipedes (91.67%) was higher than that in the females (84.67%). In some cases, more than two species of different gregarines were found to have simultaneously parasitized a single millipede. Distribution of gregarines was more concentrated in the anterior and middle parts of the intestine than in the posterior part. The predominant form observed in the millipede' s intestine was free sporadins. The shape, size, and movement of these gregarines are described. This is the first report of the occurrence of gregarines as intestinal parasites in T. corallinus in Taiwan.

摘要

本研究針對磚紅厚甲馬陸 (Trigoniulus corallinus) 腸道內寄生的簇蟲進行調查，所有馬陸均為1999年4月至2002年10月自高雄壽山地區以隨機方式採得。在所檢查的63隻馬陸中，總感染率為87.30%，雄馬陸的感染率 (91.67%) 高於雌馬陸的感染率 (84.67%)。能寄生在磚紅厚甲馬陸腸內的簇蟲可能多達七種，且在同一隻馬陸個體的腸道中可同時被至少兩種以上不同的簇蟲所寄生。簇蟲在馬陸腸道中並非平均分佈，出現於前段與中段的數目遠遠大於後段。馬陸體內出現的簇蟲以在腸中自由游動的sporadin為最多，研究中即以此時期簇蟲的形狀、大小及運動情形作觀察比較。本研究為在台灣磚紅厚甲馬陸腸道寄生簇蟲的第一篇報告。

Key words: Trigoniulus corallinus, millipede, gregarine

關鍵詞: 磚紅厚甲馬陸、馬陸、簇蟲

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Prevalence and Observation of Intestine-Dwelling Gregarines in the Millipede *Trigoniulus corallinus* (Spirobolida: Pachybolidae) Collected from Shoushan, Kaohsiung, Taiwan

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ABSTRACT

The intestine of a Formosan millipede species, *Trigoniulus corallinus*, was noted to be heavily infected with gregarine parasites. Millipedes used in this study were randomly collected from Shoushan, Kaohsiung from April 1999 to October 2002. Among 63 millipedes examined, our results showed that the total prevalence rate for cephaline gregarines was 87.30%. The infection in the male millipedes (91.67%) was higher than that in the females (84.67%). In some cases, more than two species of different gregarines were found to have simultaneously parasitized a single millipede. Distribution of gregarines was more concentrated in the anterior and middle parts of the intestine than in the posterior part. The predominant form observed in the millipede's intestine was free sporadins. The shape, size, and movement of these gregarines are described. This is the first report of the occurrence of gregarines as intestinal parasites in *T. corallinus* in Taiwan.

Key words: *Trigoniulus corallinus*, millipede, gregarine

Introduction

Millipedes are a major group of detritivores in tropical and temperate broadleaf forests. They are important in nature as they play the ecological role of deposit feeders that enhance the rate of decomposition for other smaller deposit feeders as well as decomposers (Hopkin *et al*., 1985; Price, 1988). They are also ideal models in the laboratory for studying arthropod physiology (Marcus *et al*., 1987). However, their own physiological conditions might be distorted if they were to be infected by gregarine parasites.

Gregarines are protozoan parasites found in the intestinal tracts of many invertebrates, especially arthropods and annelids, which often show extremely heavy infections (Manwell, 1977). Although unimportant economically as

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parasites, their generalized life cycle makes them significant objects for study as aids to understanding the important sporozoan parasites of humans, domestic mammals, and birds (Schmidt, 1992). They are frequently found in mosquitoes in Taiwan (Yen *et al*., 1994; Chen and Yang, 1996; Chen *et al*., 1997). Infection by cephaline gregarines is common and widespread in millipedes (Crawford *et al*., 1987). They belong to the family Gregarinidae, suborder Septatina, order Eugregarinida, class Conoidasida, and phylum Apicomplexa (Margulis *et al*., 1993). Levine (1970) depicted the life cycle of gregarines and described as many as 120 species of gregarines from the eight genera that can infect millipedes, *Amphoroides*, *Cnemidospora*, *Fonsecaia*, *Hyalosporina*, *Monoductus*, *Phleobum*, *Spirosoma*, and *Stenoductus*. However, their shape and internal structure vary so much that it is very difficult to select a form that can be considered typical. In fact, the life cycles of gregarines present problems of special interest, for many are correlated with both the life cycle and the external environment of the host (Smyth, 1994).

Due to Taiwan's tropical and subtropical climates, millipedes here are abundant and widespread (Wang, 1961, 1964). However, proper investigations have been lacking for years, and we are currently attempting to bridge systematic gaps in the study of the millipede species which Wang mentioned in the past. A preliminary morphological description of some Formosan millipedes of the order Spirobolida was made in Chinese by Y. C. Liu (pers. commun.). A database of gregarines parasitic in Formosan millipedes has not been established, nor is much known about these millipedes themselves which possess a great deal of variation. This study attempts to begin building up databases of parasitic gregarines found in Formosan millipedes so that further studies can continue to add to these. Since there are many species of millipedes as well as gregarines, the present study focuses on the gregarines parasitizing the alimentary canal of the rusty millipede, *Trigoniulus corallinus*, collected from Shoushan, Kaohsiung.

Materials and Methods

Millipede collection and maintenance

Trigoniulus corallinus (Gervais, 1842), the rusty millipede, was collected randomly from the southern Shoushan area especially on the campus of National Sun Yat-sen University from April 1999 to October 2002 without regard to age, sex, or reproductive status. They were either dissected directly after they were brought back to the laboratory or housed in plastic boxes filled with dead leaves collected from their habitats until use.

Observation of gregarines from millipedes

Millipedes used in this study were distinguished by their sex and anesthetized by low-temperature knock-down at 0ºC. After careful dissection, the intestines were taken out from millipedes and divided into three parts, the anterior, middle, and posterior, with each part comprising one-third of the intestines.

After pounding each part of the intestine into pieces in saline, the stages or forms of the gregarines in the intestine were studied in fresh preparations after extractory or in fixed and stained thin smears. Gregarines were isolated from the residues of intestines by micropipettes, placed in a depression slide, covered with a cover glass and examined with a compound microscope under 40 × and 100 × magnifications.

All different stages of gregarines in the gut were observed and counted. The length and width of the protomerites and deutomerites were measured. The types of movement exhibited by the gregarines were also observed and recorded. Gre-

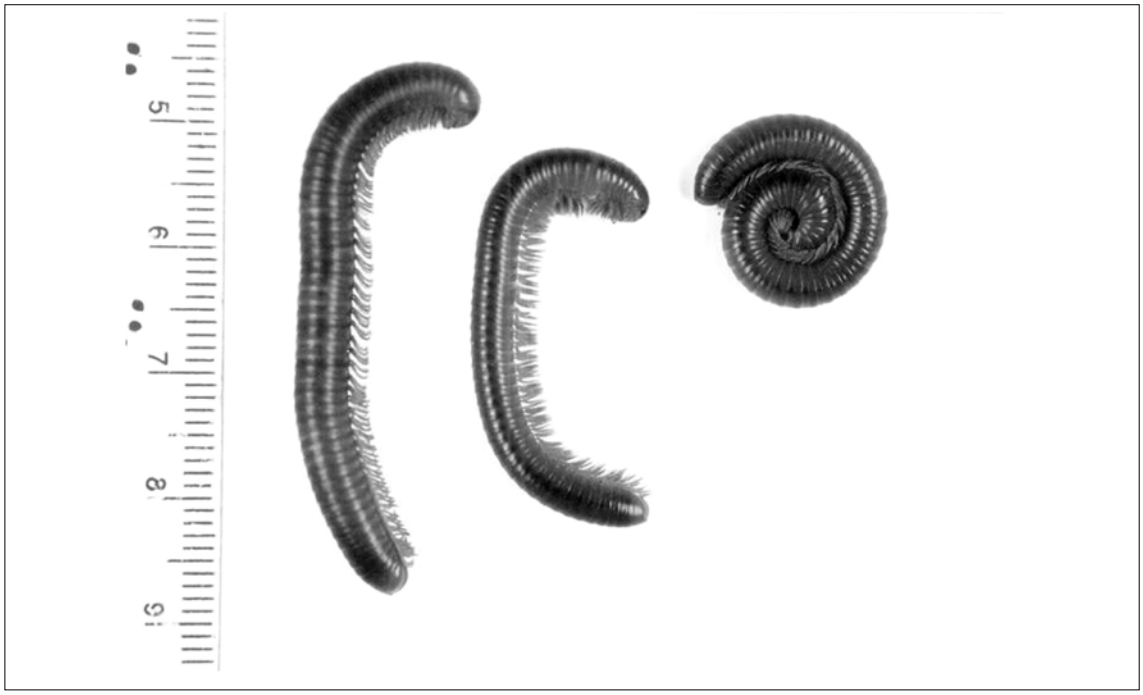

Fig. 1. Rusty millipede, *Trigoniulus corallinus*.

Table 1. Infection rate of gregarines in *Trigoniulus corallinus*

Sex	No. examined	No. positive	Percentage (%)
Male	24	22	91.67
Female	39	33	84.67
Total	63	55	87.30

garines were identified according to their characteristic features as described by Clopton (2002).

Results

In total, 63 rusty millipedes were randomly collected and studied. Fig. 1 shows the host of the gregarines, *Trigoniulus corallinus*. Our results show that *T. corallinus* collected from the Shoushan area was highly infected with gregarines with a total prevalence rate of 87.30% (Table 1). The infection rate in males (91.67%) was higher than that in females (84.67%).

The number of gregarines infecting single millipedes greatly varied (Table 2). One individual millipede might be infected by as many as 535 gregarines or be free of any infection. In some cases, more than two species of gregarines were found to simultaneously be parasitizing a single millipede host. Most of the gregarines were found in the anterior and middle parts of the alimentary canal known as the midgut of the millipede (Table 3). We also observed up to at least six types of gregarines with different morphological characters parasitizing the rusty millipede, *T. corallinus* (Fig. 2). The shapes of the gregarines appeared to be round, egg-like, or stick-like. Lengths ranged from 30 to 1227 μm. The body of

Table 2. Estimated species and number of gregarines from each millipede

Size (cm)	Sex*	No. of gregarine species found In each millipede	Average no. of gregarines per millipede
Large	Female (2)	3	274 ± 185
(≥ 5.0)	Male (1)	4	519
Medium	Female (5)	2	99 ± 40
(4.1 ~ 4.9)	Male (5)	2	17 ± 7
Small	Female (3)	2	133 ± 102
(≤ 4.0)	Male (0)	-	-

* Numbers in parentheses are the number of millipedes examined.

Table 3. Distribution of gregarines in the intestines of *Trigoniulus corallinus* (*n* = 10)

	Part of the intestine			Total
	Anterior	Middle	Posterior	
Number	291	263	7	561
Percentage (%)	51.87	46.88	1.25	100

a complete trophozoite consists of two major parts, a short anterior protomerite and a long posterior deutomerite containing the nucleus. Their epimerites were usually not detected. The two parts are divided by a transverse, membranous septum. The shape of the nuclei was round, crescent, or irregular. The color of the cytoplasm was dark or light under the light microscopes and white or colorless under the dissecting microscope. In the deutomerite, there was usually one nucleus located near the center or posterior end of the parasite body. However, the location of the nucleus varied in certain species (Fig. 2A). The shape of the nucleus was either round or crescent (Fig. 2C).

According to the morphometric measurements and the position of the nucleus as described by Clopton (2002), two families of gregarines could be identified from our collections (Table 4). The family Monoductidae includes one suspected species, TcM1, that ranged from 300 to 500 μm in size with a stick body shape and dark cytoplasm. The family Stenophoridae included six forms (TcS1 to 6). TcS1 measured more than 700 μm in length with dark cytoplasm and had a tadpole-like deutomerite. TcS2 ranged from 250 to 400 μm in length with a stick-shaped body, and a crescent-shaped nucleus with dark cytoplasm. TcS3 ranged from 100 to 250 μm in length with an egg-like shape and light cytoplasm. TcS4 ranged from 100 to 200 μm in length with a short stick body shape and dark cytoplasm. TcS5 ranged from 100 to 250 μm in length with a round body and light cytoplasm. TcS6 was less than 150 μm in length, with a short stick-shaped or rounded body and dark cytoplasm. Species identification of fixed specimens is now in progress.

Most of time the gregarines remained motionless, but two types of gregarine movements were observed. One was a creepy gliding movement, in which gregarines moved forward a rather long distance without changing their shapes. The other was a rapid springing movement, in which the cytoplasm flow produced a protomerite extrusion toward the deutomerite, then they returned to their original shape along with a slight movement forward (Fig. 3). Only few gregarines exhibited this springing move-

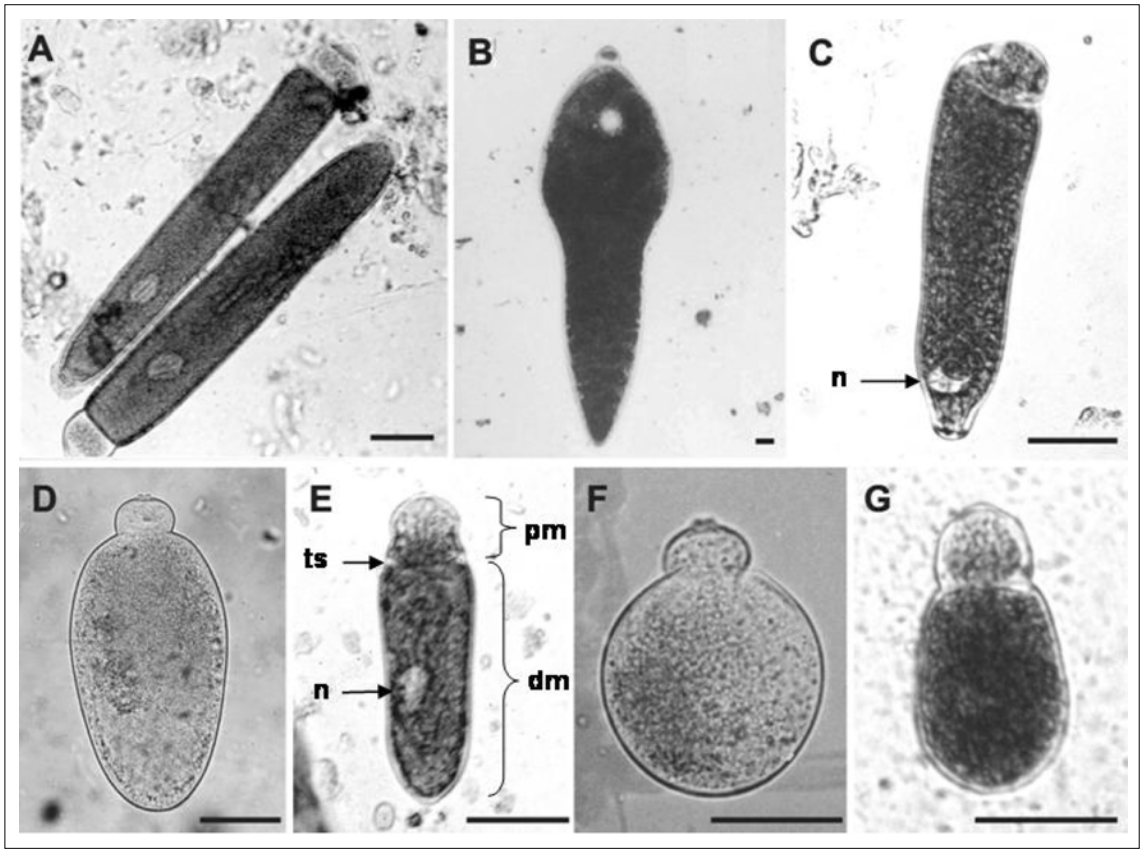

Fig. 2. Morphology of gregarines from Formosan millipedes. A, stick-like gregarines; B, a large gregarine with round nuclei and dark cytoplasm; C, a gregarine with a crescent nucleus (n); D, a small egg-shaped gregarine; E, a small short-stick-like gregarine; F, a round gregarine which appears to have light cytoplasm; G, a small gregarine with dark cytoplasm. dm, deutomerite; n, nucleus; pm, protomerites; ts, transverse septum. Scale bar = 50 μm

ment after a long period of inactivity during the observation.

Discussion

The shape, size, color, and intracellular structures of the sporonts are important characteristics for species identification of gregarines. The ratios of certain parts of gregarines are also helpful in identification (Clopton, 1999). Our results show that at least seven forms in two families could be detected in *T. corallinus*. Among them, TcM1 was likely to be *Monoductus lunatus* as morphologically described by Clopton (2002). TcS3 and TcS5 were similar in size and in the color of their cytoplasm. More-detailed studies on their morphology and life history are required to clarify their taxonomic positions. This is the first report of the millipede gregarines in Taiwan. No comparable survey has been conducted, and no museum collection of millipede gregarines currently exists in Taiwan. Thus, this survey initiates descriptions and specimen collections for the study of gregarines and their arthropod hosts.

According to the intestinal location

Table 4. Measurements of gregarines found in the intestines of *Trigoniulus corallinus*

Family	Code	Morphometric measures*								
		L	W	L/W	PL	PW	PL/PW	DL	DW	DL/DW
Monoductidae	TcM1	368	49	7.51	36	53	0.68	332	49	6.78
Stenophoridae	TcS1	1,227	409	3.00	45	75	0.60	1152	409	2.82
	TcS2	254	60	4.23	40	38	1.05	214	60	3.57
	TcS3	199	100	1.99	23	37	0.62	176	100	1.76
	TcS4	155	49	3.16	35	35	1.00	120	49	2.45
	TcS5	121	86	1.41	17	32	0.53	104	86	1.21
	TcS6	104	55	1.89	27	35	0.77	77	55	1.40

* L, total length; W, maximum width; PL, protomerite length; PW, protomerite width; DL, deutomerite length; DW, deutomerite width. All measurements other than ratios are in micrometers.

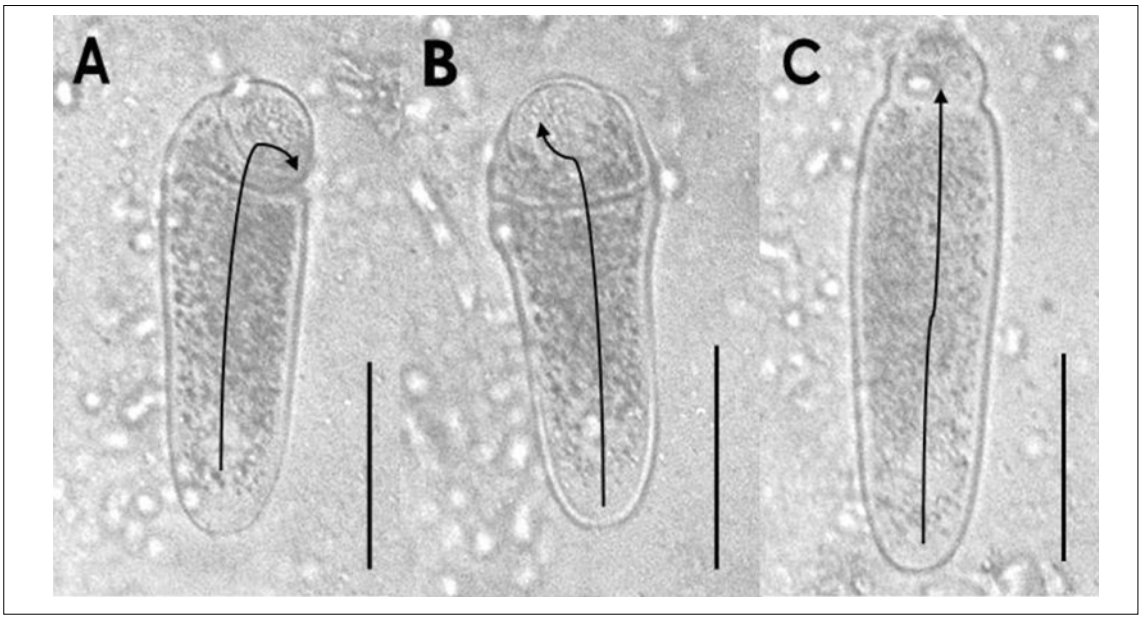

Fig. 3. Springing movement exhibited by some of the gregarines. Cytoplasmic flow causes the protomerite of a gregarine to extrude toward the deutomerite (A and B) and when returning to the original shape, the gregarine moves one step forward (C). The arrows indicate the directions of body movement exhibited by the gregarine. Scale bar = 50 μm

of gregarines parasitizing millipedes and findings of the life cycles of species described, the life history of millipede gregarines may be similar (Jahn, 1979; Janardanan and Ramachandran, 1982, 1983a, b). When a potential host ingests oocysts of gregarines, the oocysts are usually ruptured by digestive fluids, which releases the sporozoites into the gut. The sporozoites then enter epithelial cells of the midgut and initiate infection. After a period of developmental growths, they become trophozoites and later leave the host cell as sporonts, which wander about the coelom looking for partners. Eventually, oocysts are formed after the syzygy of negative and positive sporonts (Manwell, 1977; Smyth, 1994). However, if we are able to breed millipedes without natural infection in the laboratory, iso-

late some gregarines oocysts of a certain species from millipede feces, and infect those millipedes with oocysts, the life cycles could be explored.

In the millipedes *Trigoniulus goesi* and *Xenobolus acuticonus*, infections by the gregarine *Stenoductus trigoniuli* caused cytopathological effects (Janardanan and Ramachandran, 1982). Fat globules were produced in midgut epithelial cells of millipedes by the intracellular stage of gregarines and accumulated in the host-cell cytoplasm, causing a fatty degeneration that facilitated the release of intracellular gregarines into the nutrition-rich gut lumen. According to Hopkin and Read (1992), heavy infections of gregarines may cause the development of gangrene in the legs of millipedes, and the legs may decay leaving stumps. In our study, we observed that the legs of a heavily infected millipede exhibited unusual impaired motion on the central and posterior body parts, and that the millipede eventually died of infection. This is why we consider millipede gregarines to be parasitic rather than symbiotic.

Two types of movement were noticed in this study, although no special organelle for movement was observed in these gregarines. As a matter of fact, they showed few activities. Some cell biologists have reported the gliding movement in the early stage (Mackenzie, 1980), but many questions remain. However, a model of the gliding movement in gregarines was later proposed, and the gliding rate was estimated to be 1~10 μm/s (King, 1988).

The results indicated that *T. corallinus* collected from the Shoushan area was highly infected with gregarines. It may possibly become a laboratory model for the study of host-parasite relationships. This study raises questions with respect to the effects of host age, sex, and reproductive status upon the parasitism. Further studies will be carried out on these host-parasite relationships, and the possibility of using them as biological markers for local millipede distribution will be assessed.

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壽山地區磚紅厚甲馬陸腸道寄生簇蟲之調查與實驗室觀察

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摘　要

本研究針對磚紅厚甲馬陸 (*Trigoniulus corallinus*) 腸道內寄生的簇蟲進行調查，所有馬陸均爲 1999 年 4 月至 2002 年 10 月自高雄壽山地區以隨機方式採得。在所檢查的 63 隻馬陸中，總感染率爲 87.30%，雄馬陸的感染率 (91.67%) 高於雌馬陸的感染率 (84.67%)。能寄生在磚紅厚甲馬陸腸內的簇蟲可能多達七種，且在同一隻馬陸個體的腸道中可同時被至少兩種以上不同的簇蟲所寄生。簇蟲在馬陸腸道中並非平均分佈，出現於前段與中段的數目遠遠大於後段。馬陸體內出現的簇蟲以在腸中自由游動的 sporadin 爲最多，研究中即以此時期簇蟲的形狀、大小及運動情形作觀察比較。本研究爲在台灣磚紅厚甲馬陸腸道寄生簇蟲的第一篇報告。

關鍵詞：磚紅厚甲馬陸、馬陸、簇蟲