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Surface Ultrastructure of *Heterophyes nocens* (Trematoda: Heterophyidae)

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Abstract: The surface ultrastructure of *Heterophyes nocens* (Trematoda: Heterophyidae) was studied by scanning electron microscopy(SEM). The adult worms were recovered from experimentally infected cats and from a naturally infected patient. They were leaf-like, ventrally concave, and ovoid or pyriform in shape. Ciliated knob-like sensory papillae (type I) were observed in single or grouped forms on and around the oral sucker, whereas non-ciliated round swellings (type II papillae) were seen on the lip of the ventral sucker. The tegumental spines around the oral sucker were 5~9 pointed, whereas those between the two suckers were 12~17 pointed. Ventrolaterally, three groups of 5~6 type I papillae were located between the oral and ventral suckers, with single ones alternating between them. The genital sucker was protruded or depressed, depending on the contraction state of the flukes, and the gonotyl spine number ranged 50~60. The number of tip points of tegumental spines was decreased posteriorly; finally they became 1~3 pointed. On the dorsal surface, 4 groups of 4~5 type I papillae were symmetrically located on both lateral sides, and the shape and distribution of tegumental spines were similar to those of the ventral surface. Although the tegumental ultrastructure of *H. nocens* was generally similar to those of other heterophyids, the genital sucker morphology including the number of gonotyl spines and/or the distribution pattern of tegumental spines and sensory papillae were suggested to be the characteristic features of *H. nocens*.

Key words: *Heterophyes nocens*, surface ultrastructure, scanning electron microscopy, tegumental spines, gonotyl spines, sensory papillae

INTRODUCTION

Heterophyes nocens Onji and Nishio, 1916 (Trematoda: Heterophyidae) is an intestinal trematode of dogs, cats or humans in Far Eastern countries such as Japan (Yokogawa *et al.*, 1965) and Korea (Chai and Lee, 1990). Taxonomically, however, there have been debates on the validity of *H. nocens* in comparison with the type species, *H. heterophyes*. Cort and Yokogawa (1921) stressed the validity of *H. nocens*, whereas Lane (1922) and Faust and Nishigori (1926) denied

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it. But Witenberg (1929) retained the validity of *H. nocens*. Asada (1934) compromised this situation putting it as a subspecies, *H. heterophyes nocens*. Nevertheless, Taraschewski (1984) and Chai *et al.* (1986) suggested that the two species should be distinctively different, since the different number of chitinous rodlets on the genital sucker, *i.e.*, 50~60 in *H. nocens* and 75~87 in *H. heterophyes*, is acknowledged as a unique and consistent feature. Further studies on *H. nocens* and *H. heterophyes* including ultrastructural observations would be helpful for taxonomic justification of the two species.

Among the trematodes of the family Heterophyidae, surface ultrastructural studies were done in various species such as *Cryptocotyle lingua* (Køie, 1977), *Metagonimus yokogawai* (Lee *et al.*, 1984), *Heterophyes aequalis* (Taraschewski, 1984) and *Heterophyopsis continua* (Hong *et al.*, 1991). However, the surface ultrastructure of *H. nocens* has not been studied. The present study was undertaken to provide a basic knowledge on the tegumental ultrastructure of *H. nocens* by scanning electron microscopy.

MATERIALS AND METHODS

The metacercariae of *H. nocens* were isolated from the mullets, *Mugil cephalus*, purchased at a local market in Pusan, by artificial digestion at 37°C. They were orally fed to three cats through a gavage needle. Adult worms were recovered from the cats 3 weeks after the infection. Also the adult worms collected from a patient in Sorok Island, Jeonranam-do, after bithionol treatment (Chai *et al.*, 1985) were used for this study.

For scanning electron microscopy, the flukes were washed several times with physiological saline, 2~3 times with 0.2 M cacodylate buffer (pH 7.26), and fixed in 2.5% glutaraldehyde solution. After washing again with 0.2 M cacodylate buffer (pH 7.26), the specimens were dehydrated through graded alcohols (50%, 70%, 80%, 90%, 95% & absolute) and freeze-dried. The dried samples were coated with gold in 30 nm thickness using an ion sputtering coater (Eiko IB-3) and observed using an ISI DS-130C scanning electron microscope (SEM) under accelerating voltage of 10 KV.

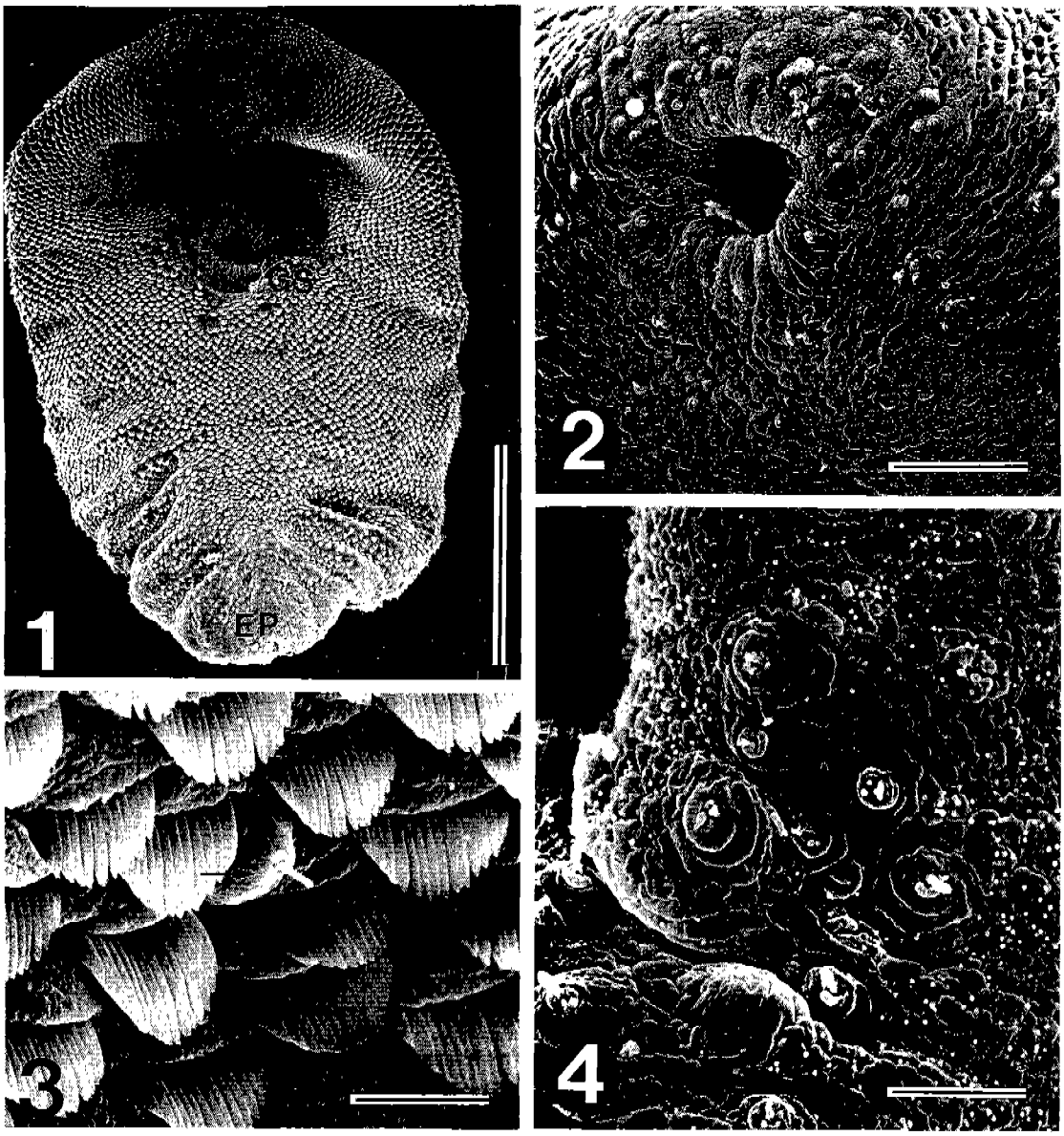
RESULTS

The general shape of *H. nocens* adults was leaf-like, ventrally concave (Fig. 1), and ovoid or pyriform. The oral, ventral, and genital suckers were located on the anterior part of the body. The genital pore was opened on the genital sucker, and the excretory pore was located near the posterior end of the body. The entire surface of this fluke was covered with tegumental spines. The cytoplasmic processes of the anterior body were cobblestone-like, whereas those of the posterior body were fine and velvety. The tegumental spines were regularly distributed on the ventral and dorsal surface.

Ciliated knob-like sensory papillae (Type I) were observed on and around the oral sucker (Figs. 2, 3 & 4); grouped papillae were arranged in an equidistant manner with single ones alternating between the grouped ones (Fig. 2). The tegumental spines around the oral sucker had 5~9 points, while those between the oral and ventral suckers had 12~17 points (Fig. 3). Three groups of type I papillae (Fig. 4), each group consisting of 5 papillae in the right and 6 in the left side, were located between the oral and ventral suckers, with single ones alternating between the grouped ones (Fig. 5). Non-ciliated round swellings (Type II sensory papillae) were seen on the lip of the ventral sucker (Fig. 6).

The genital sucker of the worms recovered from the cats was, in many instances, depressed or only slightly elevated (Fig. 6), in which case it was difficult to observe the gonotyl spines clearly. However, many worms obtained from the patient showed their protruded genital sucker with gonotyl spines, 50~60 in number, although the tegument and spines were destroyed a little by the effect of bithionol (Fig. 7).

The tegumental spines of ventrolateral surface



Figs. 1~4. Scanning electron micrographs of adult *H. nocens* recovered from an experimental cat. 1. Ventral view of a whole worm. CS: oral sucker, VS: ventral sucker, GS: genital sucker, EP: excretory pore. Bar=110 μm. 2. Type I sensory papillae in single or grouped forms on and around the lip of the oral sucker. Bar=13 μm. 3. The tegument between the oral and ventral suckers showing 12~17 pointed tegumental spines and a type I papilla (arrow). Bar=3 μm. 4. Grouped type I papillae around the oral sucker. Bar=3 μm.

and of ventromedial areas posterior to the ventral sucker were less serrated, *i.e.*, 8~12 pointed (Fig. 8 & 9), than those between the oral and ventral suckers. The number of points of the spines was further decreased toward the posterior end of the body, and finally they became 1~3 pointed. The density of the spines was also gradually decreased toward the posterior end of the body.

On the dorsal surface of the body, 4 groups of type I papillae, each group consisting of 4 or 5 papillae, were symmetrically located in the

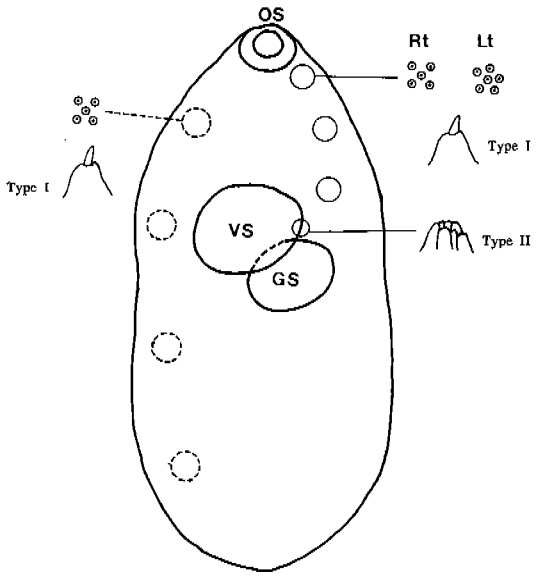


Fig. 5. Schematic view of the distribution pattern of sensory papillae of *H. nocens*. The grouped type I papillae on the ventral surface (Right, large solid circle) and those on the dorsal surface (Left, large broken circle) are depicted. The type II papillae on the lip of the ventral sucker (Right, small solid circle) are also shown. OS: oral sucker, VS: ventral sucker, GS: genital sucker.

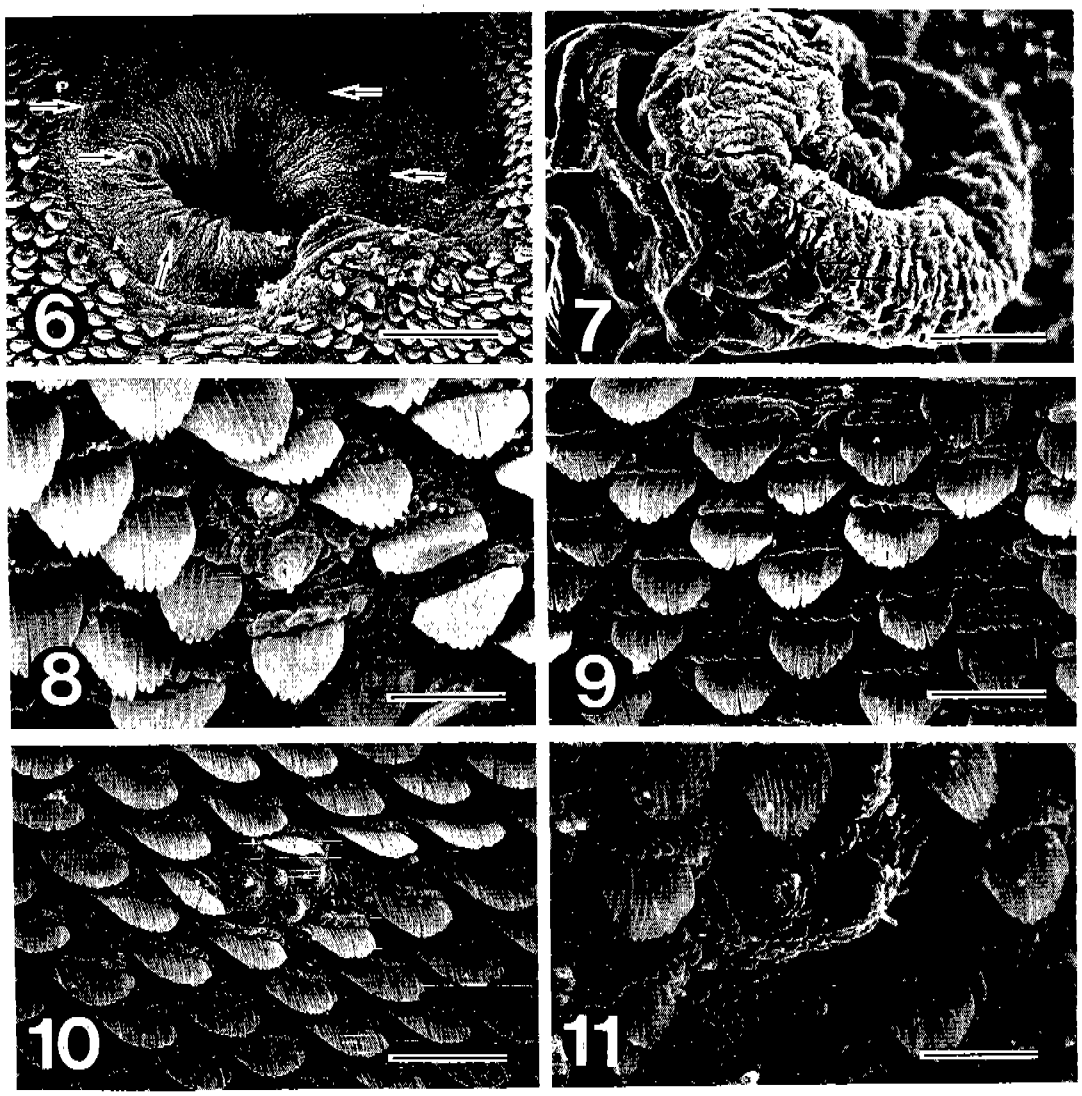
right and left sides (Fig. 5). The spines on the anterior half of the dorsal surface were divided into 15~20 points (Figs. 10) and those on the posterior half into 8~12 points (Figs. 11). The distribution of the tegumental spines on the dorsal surface was similar to that on the ventral surface.

DISCUSSION

The tegument of trematodes is, in general, known to have physiological or biochemical functions essential for their existence and thriving (Lumsden, 1975). As is a common feature of all kinds of trematodes, the whole tegument of *H. nocens* was covered with cytoplasmic processes, together with numerous tegumental spines and sensory papillae. The cytoplasmic processes were cobblestone-like anteriorly, but were fine and velvety posteriorly. The tegument of larval trematodes is generally known to be cobblestone-like, but as they mature it is differentiated into a more fine and velvety one (Lee *et al.*, 1985; Hong *et al.*, 1991). This modification of the surface ultrastructure is regarded as a consequence to suffice the increase of nutrient requirements by the parasites in the host body (Bennett and Threadgold, 1975; Fujino *et al.*, 1979; Font and Wittrock, 1980; Lee *et al.*, 1984).

In trematodes, it is well known that the shape and distribution of tegumental spines are different by species of parasites as well as their developmental stages, habitat, migratory behavior, *etc.* (Bennett and Threadgold, 1975; Fujino *et al.*, 1979; Font and Wittrock, 1980; Lee *et al.*, 1984). In the case of *Clonorchis sinensis*, it was reported that the larval worms excysted in the duodenum have double or triple-pointed tegumental spines in the anterior half of the body and single-pointed ones in the posterior body but the spines gradually disappear as the worms grow to be adults, and the full-grown adults become completely devoid of spines (Fujino *et al.*, 1979; Lee *et al.*, 1982). On the contrary, it was shown in larval worms of *Fasciola hepatica* that the tegumental spines of single-pointed form metamorphose into multi-pointed ones just prior to the entry into the bile duct (Bennett, 1975). Conversion of simple spines into serrated ones during their developmental stages is also known in *Fibricola seoulensis* (Lee *et al.*, 1985) and *P. iloktsuenensis* (Lee *et al.*, 1989). These developmental changes are thought to help parasites to adapt to the changing environment in the host (Bennett and Threadgold, 1975).

The sawtooth or brush-shaped (*i.e.*, multi-pointed) tegumental spines seem to be one of the characteristic features of the family Heterophyidae. The tegumental spines of *H. nocens*, for example, were in most cases multipointed as was also reported in *C. lingua* (Køie, 1977), *H. equalis* (Taraschewski, 1984), *M. yokogawai* (Lee *et al.*, 1984) and *H. continua* (Hong *et al.*, 1991). However, the number of points and especially its changing pattern according to the



Figs. 6~11. Scanning electron micrographs of *H. nocens* from an experimental cat (Figs. 6 & 8~11) or a naturally infected man (Fig. 7). **6.** The lip of the ventral sucker with type II papillae (arrows). The gonotyl of the genital sucker (GS) is slightly protruded, but gonotyl spines are not seen in this specimen. Bar=20 μm. **7.** A protruded genital sucker (gonotyl) with gonotyl spines (arrows). The tegument and spines were destroyed due to the effect of bithionol. Bar=20 μm. **8.** Tegumental spines around the ventral sucker, the tips of which are divided into 8~12 points. Two type I papillae (arrows) are seen. Bar=3 μm. **9.** The tegument posterior to the ventral sucker, covered with 8~12 pointed spines. Bar=5 μm. **10.** Scale-shaped tegumental spines on anterior half of the dorsal surface. Grouped type I papillae are seen. Bar=7 μm. **11.** Tegumental spines and grouped type I papillae on posterior half of the dorsal surface. Bar=3 μm.

maturation of worms seems to be different by the species of parasites. In the case of *H. continua*, the number of points of tegumental spines was increased from 10~14 to 15~17 on the anterior body as the worm matured (Hong *et al.*, 1991), whereas the number of points remained unchanged in *M. yokogawai* during its development (Lee *et al.*, 1984). In *H. nocens*,

the number of points and density of tegumental spines were gradually decreased toward the posterior end of the body. The more pointed spines between the oral and ventral suckers of *H. nocens* seem to be related with abrasion of host intestinal villi for their feeding and anchorage.

It is generally known that the tegumental sensory receptors enable a parasite to become established and maintain itself in a specific site within the host through perception of considerable information from the surrounding environment (Ip and Desser, 1984). In the present study with *H. nocens*, two types of sensory papillae were observed. The sensory papillae with a short cilium (Type I papillae) were observed around the oral sucker, in single or grouped forms. This kind of sensory papillae were also observed in larval and adult stages of other heterophyid flukes. In *M. yokogawai* (Lee *et al.*, 1984) and *H. continua* (Hong *et al.*, 1991), the type I papillae were abundant as clumped forms of 2 or 3 around the oral and ventral suckers, and on the ventral and dorsal surfaces, of metacercariae and adults. Transmission electron microscopic studies (TEM) of the type I papillae showed that they consisted of a bulb bearing an apical cilium originating from a process resembling a nerve dendron (Morris and Threadgold, 1967; Hoole and Mitchell, 1981). The bulb also possesses mitochondria, microtubules, two electron-dense rings and a fan-like rootlet system (Hoole and Mitchell, 1981; Ip and Desser, 1984). The function of these type I papillae has been suggested to be tango-, rheo-, or machanoreceptive (Bennett, 1975; Fujino *et al.*, 1979; Seo *et al.*, 1984; Lee *et al.*, 1984 & 1987; Hong *et al.*, 1991).

The round swellings with no sensory cilium (Type II papillae), found on the lip of the ventral sucker of *H. nocens*, were the same type as observed in *H. continua* (Hong *et al.*, 1991) and in other kinds of trematodes (Bennett, 1975; Lee *et al.*, 1982, 1984 & 1987). These papillae were in many aspects similar to the type I papillae, especially in that they consist of a neuronal bulb containing two electron-dense rings. They differ, however, from the type I papillae, in that no cilium arises from them to penetrate into the overlying tegumental syncytium (Bennett, 1975; Hoole and Mitchell, 1981). This type of sensory receptors would be important in an actively moving or migrating parasite, and those on the suckers would be important in feeding and sucking. Their function was supposed to be tango- and/or pressure-receptive (Bennett, 1975; Fujino *et al.*, 1979; Seo *et al.*, 1984; Lee *et al.*, 1984 & 1987; Hong *et al.*, 1991).

Sensory papillae other than the type I and type II were also observed on the tegument of other trematodes. The round elevations of cytoplasmic ridges with no cilium (type III papillae) were distributed bilaterally on the inner wall of the oral sucker of *M. yokogawai* (Lee *et al.*, 1984). In the hindbody of *Fibricola seoulensis*, type III and type IV papillae (similar to type III but having a cilium) appeared, especially around the excretory pore and genital atrium (Lee *et al.*, 1985). However, the function of these kinds of sensory papillae remain speculative because electrophysiological experiments on the sensory papillae of helminths are lacking (Hoole and Mitchell, 1981; Hong *et al.*, 1991).

Informations on the surface ultrastructure of *H. heterophyes* is not available at present, therefore, no comparison could be made with *H. nocens*. It is inferred that the tegument of *H. heterophyes* would be generally similar to that of *H. nocens* except in the number of gonotyl spines on the genital sucker. However, it should be verified in the future by a comparative SEM study on *H. nocens* and *H. heterophyes*.

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=국문초록=

Heterophyes nocens (Trematoda: Heterophyidae)의 표피 미세구조

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*Heterophyes nocens*의 표피 미세구조를 주사전자현미경으로 관찰하였다. 충체는 실험 감염시킨 고양이에서 얻은 성충과 인체 자연감염자에서 회수한 성충을 재료로 하였다. 충체는 나뭇잎 모양이며 복측으로 약간 굽어 있었고, 유원형 또는 서양배 모양이며 전반부는 둥글고 후반부로 갈수록 충체의 폭이 차츰 감소하였다. 구흡반 주위에는 짧은 섬모가 있는 제 I 형 감각유두가 4~5개씩 모인 군집 감각유두가 관찰되었으며 그 사이 사이에 단일 감각유두가 분포하였다. 구흡반 외연 및 직후방에는 5~9개의 분지를 가진 피극이, 구흡반과 복흡반 사이에는 12~17개의 분지를 가진 피극이 분포하였다. 복흡반의 구순에는 표피가 둥글게 융기한 제 II 형 감각유두가 7~8개 일정 간격으로 위치하였다. 구흡반과 복흡반 사이에는 우측과 좌측에 각각 5개 및 6개의 감각유두로 구성된 군집 감각유두가 3개군씩 대칭적으로 배열되어 있었고 그 사이에 단일 감각유두가 분포하였다. 고양이에서 회수한 성충의 생식반은 흔히 함몰되거나 약간만 돌출되어 있어 gonotyl spine을 관찰하기 어려웠으나, 환자에서 얻은 성충은 대부분의 경우 생식반이 충체 바깥으로 돌출되어 있었고, gonotyl spine의 수는 50~60개이었다. 복흡반 외측 및 후방에는 8~12개의 분지를 가진 피극이 각각 관찰되었으며, 충체의 후반부로 갈수록 피극의 분지수가 점차 감소하여 후단에서는 1~3개의 분지를 가진 피극이 관찰되었고 피극의 밀도도 점차 낮아지는 양상을 띠었다. 충체 배측에는 4~5개의 감각유두가 모인 군집 감각유두 4쌍이 좌우 대칭적으로 배열되어 있었다. 충체 배측의 전반부에는 15~20개의 분지를 가진 피극이 분포하였고 후반부로 갈수록 분지 양상이 감소하여 배측 후단에서는 8~12개의 분지를 가진 피극이 관찰되었다. 또, 후반부로 갈수록 피극의 밀도가 점차 낮아지는 양상을 보였다. 이상의 결과로 보아 *H. nocens* 성충의 표피 미세구조는 다른 이형흡충류의 경우와 비슷하나 생식반 외연에 분포하는 gonotyl spine의 수가 50~60개인 점, 전 표피에서 관찰되는 피극 및 감각유두의 특이한 분포 등은 *H. nocens*의 특징적 소견이라고 생각되었다.

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