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Morphological Study of *Contracaecum rudolphii* (Nematoda: Anisakidae) from White Pelican

Hyeon-Cheol Kim*, Nam-Soo Kim, Hwa-Young Son, Bo-Keun Kwon, Hur Jin, Jeong-Gon Cho** and Bae-Keun Park[1]**

Research Institute of Veterinary Science, College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea
**College of Veterinary Medicine, Kangwon National University, Chuncheon 201-100, Korea*
***College of Veterinary Medicine and Bio-Safety Research Institute, Chonbuk National University, Jeonju 561-756, Korea*

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Abstract : A white pelican, *Pelecanus onccrotalus*, was imported from Tanzania and died 5 days later in Daejeon zoo in Korea. The proventriculus of bird was found to contain parasites. The taxonomic status of the nematodes was studied by morphological and morphometric analysis using light and scanning electron microscopy. Only one species *Contracaecum* occurred in the proventriculus with high values of infection. Morphological characters of the parasite were as follows: The worms have three lips without cuticular dentigerous ridges, and well developed interlabia. The interlabia have a triangular shape, non-bifurcated. Males possess numerous preanal papillae (69-79 pairs) arranged in single subventral rows. Paracloacal papillae were separated 5 postanal papillae and 1 larger pairs. All the characters of the above studied specimens are very similar to those of *C. rudolphii* complex. Accordingly, the parasites from white pelican were identified belong to the *C. rudolphii* complex.

Key words **:** Anisakis, morphology, Nematoda, *Contracaecum rudolphii.*

Introduction

Anisakiasis is a human disease caused by the accidental ingestion of larval nematodes belonging to the family Anisakidae. There are three types of anisakid larvae that have been implicated in human disease: *Anisakis, Pseudoterranova*, and *Contracaecum* species (26). But the number of case reports attributed to the genus *Contracaecum* is rare (7). The genus *Contracaecum* Railliet & Henry, 1912 are found in fish-eating birds both in marine and freshwater areas all over the world, and also in seals. The larvae of the *Contracaecum* species are widely distributed in freshwater and marine fishes (22). *Contracaecum rudolphii* Hartwich, 1964 is a common ascaridoid nematode of fish-eating bird with a global distribution (14). Crustaceans are intermediate hosts and various fishes serve as intermediate or paratenic hosts for *C. rudolphii*. Some papers report that *C. rudolphii* can be also a great cause of mortality in birds (1) and give huge economic damages to the fish industry (29). As many as 63 species are recorded in Yamaguti's (28) monograph. This species has been discovered in many host of birds and its spread is connected to the distribution of shags or comorants, primary definitive host (3). According to Lymbery *et al* (21) the high prevalence of *Contracaecum* in mullet may have implications for the health of the fish.

Many studies have been done on the sectional and external morphology of the genus *Contracaecum* with light microscope (15,17) and Scanning electron microscopy (SEM) studies (1,9,10,11,12). Nevertheless, appropriate identification of parasites often comes into question in adults case. Especially, larvae of both freshwater and marine species of *Contracaecum* sp. are almost alike. SEM provides a means to define the surface topographical features of the species more objectively than light microscopy. Although the number of morphological criteria of taxonomic significance available in the family Anisakidae is low, the diagnosis of the species is still based on a number of different morphological features. To get much more precise information on the anterior and the posterior extremities and on cuticular morphology of the larvae, ultrastructural studies are warranted for the identification. Those data can be used in combination with traditional methods. *Contracaecum spiculigerum* Rudolphi, 1809 (now *C. rudolphii*) occurs in ducks, geese, swans and a wide variety of waterfowl.

Materials and Methods

A white pelican, *Pelecanus onccrotalus*, was imported from Tanzania and found dead 5 days later in Daejon zoo. Because the pelican is not existent species in Korea, we were very restricted to the examination in the parasitological study.

[1]Corresponding author.
E-mail : bkpark@cnu.ac.kr

Standard methods were followed in examining a white pelican, recovering and identifying parasites. We did necropsy the bird and found *Contracaecum* sp. from the proventriculus. The worm burden attached its head part to the mucosa of proventriculus. The proventriculus was preserved in formalin, conventional paraffin sectioned and H-E stained. For light microscopical study, the worms were placed in lacto-phenol solution (glycerin 20 ml, lactic acid 10 ml, phenol 10 g, D.W. 10 ml) for 24 hrs and identified using the characteristics previously described (14). The worms were examined by light microscope. The total number of preanal papillae was established.

For SEM, the parasites were washed with 0.1M phosphate buffer (PB, pH 7.4) and fixed with 2.5% glutaraldehyde solution with a PB at 4°C for 4 hrs. After washed with PB, the specimens were post-fixed in 1% osmum tetroxide at 4°C for 4 hrs. Afterwards, the specimens were dehydrated by serial concentrations of alcohol, dried by CO_2 critical point and coated with 20 *nm* gold palladium. A scanning electron microscopy (Philips Co.) was used and examined.

Fig 1. White pelican, *Pelecanus onccrotalus*.

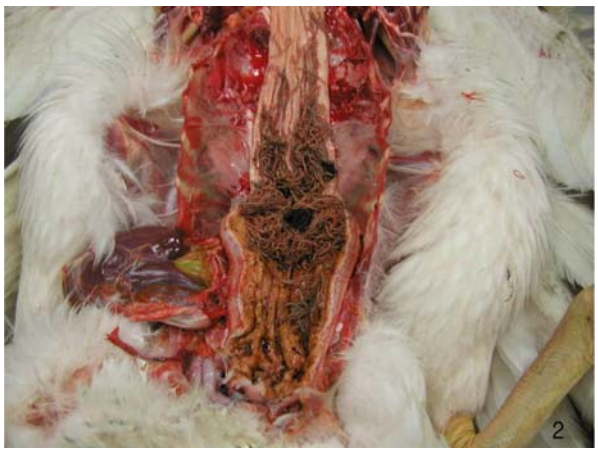


Fig 2. Gross finding of a proventriculus infected with *C. rudolphii*. Showing detachment of mucosal epithelial cells and slight congestion in lamina propria.

Results

The number of parasite was more than a thousand and the worm burden attached its head part to the mucosa of proventriculus (Fig 2). The nematodes were removed easily from the mucosa. But, in histopathological finding of proventricu-

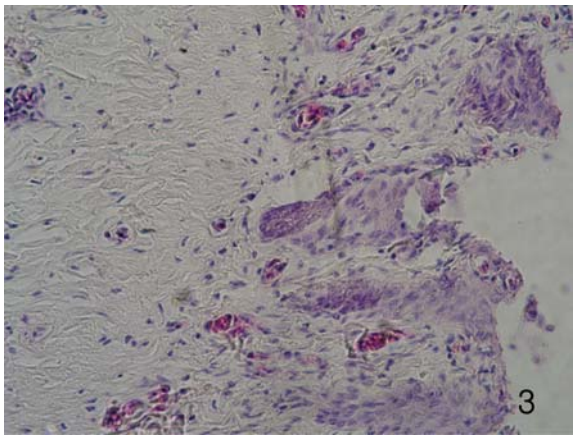


Fig 3. The histopathological finding of proventriculus.

Fig 4. The albumin-coated egg of *C. rudolphii* from uterus.

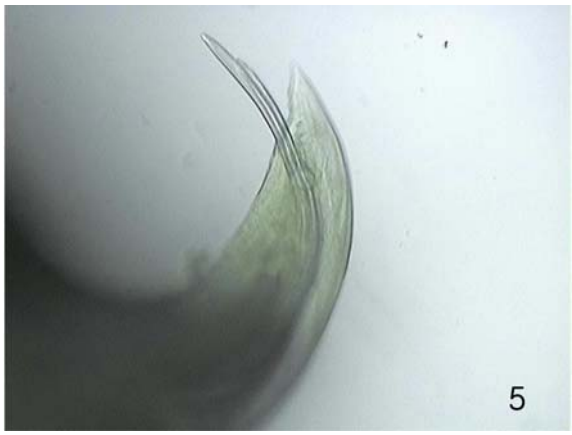
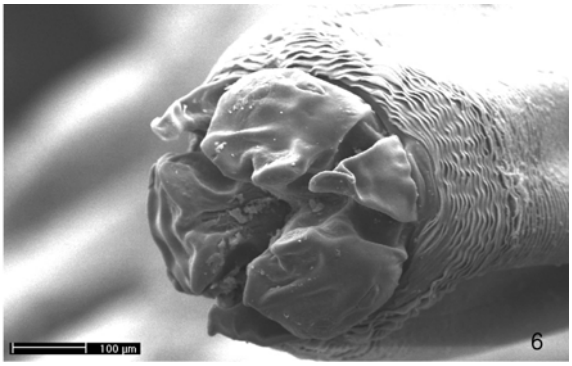


Fig 5. Two spicules are different in length.



Figs 6-13. SEM micrographs of *Contracaecum rudolphii* from the proventriculus of white pelican.
Fig 6. Anterior end of the adult worm. One dorsal and two subventral lips. Between the bases of these lips, three interlabia are seen.

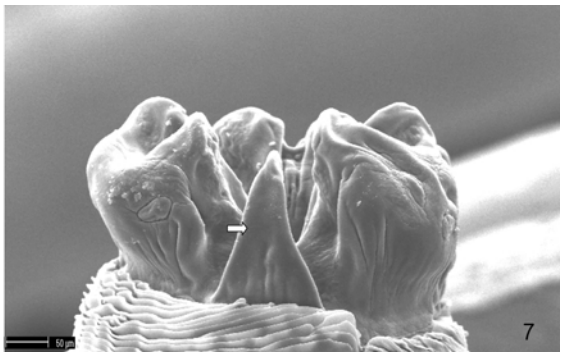


Fig 7. The interlabia have nonbifurcation (arrow).

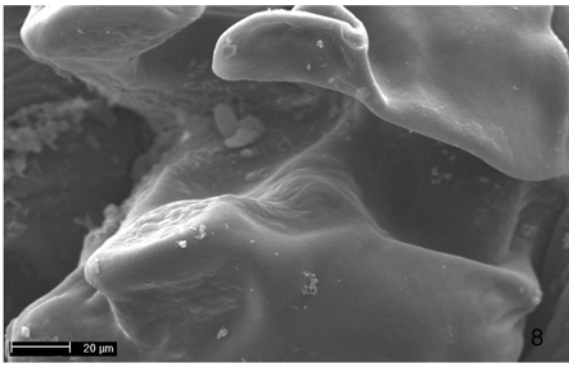


Fig 8. Apical view of the dorsal lip. The dorsal lip has not auricle.

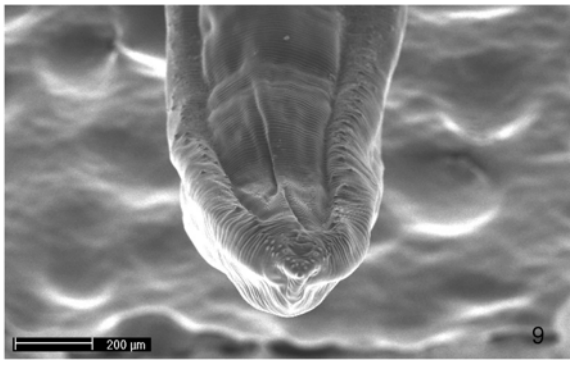


Fig 9. Ventral view. Posterior end of the male.

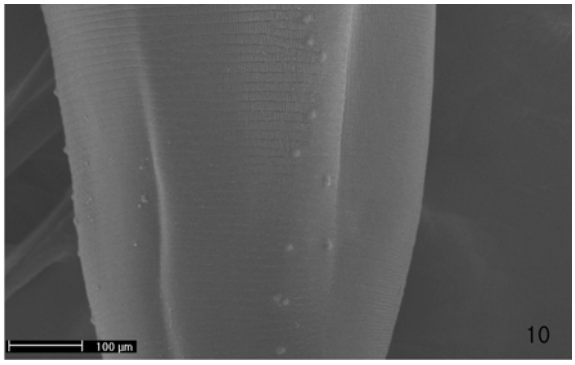


Fig 10. Dorsal view. The tail of male worm.

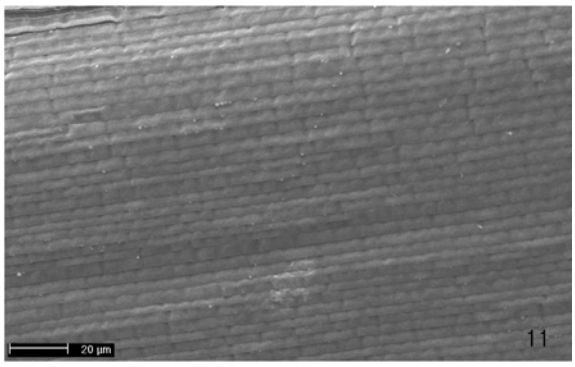


Fig 11. Cuticular transverse striation prominent.

lus, there was slight inflammation and congestion of lamina propria with desquamation (Fig 3).

General description: In both sex, the parasites had three well-developed lips, one dorsal and two subventral, each of them bears two papillae at each corner. Three lips approximately equal in size (Figs 6, 7). Between the bases of these lips, there were smaller lips, the interlabia. Interlabia well developed, almost as long as lips, without bifurcate tips (Figs. 6, 7, 8). The dentigerous ridge is not seen at the outer margin of the internal surface of each of the large lips (Fig 8) and is separated from the outer surface by a groove. The interlabia are seen in lateral view as a triangular outgrowth between each of the three lips (Fig 7). Neck region with transverse striation was interrupted by W-shaped lateral region. Striations are transverse in the whole body cuticle (Fig 11).

Male: Length of parasite was 24-32 mm (n = 20). Two spicules are different in length. The right spicule is longer than the left by 1.5-2.3 mm (Fig 5); the distal end is extended and pointed. Numerous preanal papillae are forming single subventral rows (Figs 9, 10): 69-79 pairs. Paracloacal papillae 6 separate pairs; postanal papillae 5 separate pairs with 1 larger pairs. Tail is relatively pointed, lacking distinct mucron.

Female: Length of parasite was 31-37 mm (n = 20). The albumin-coated eggs from uterus was spherical and 52-57 um

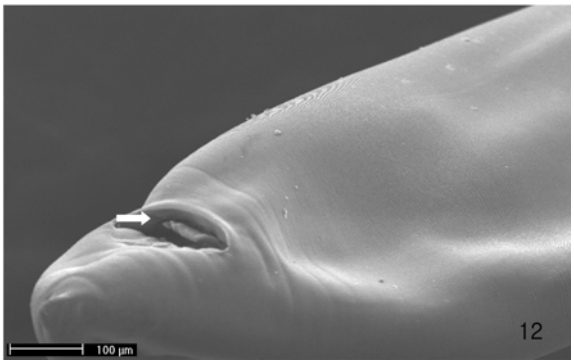


Fig 12. Female tail. Anus (Arrow).

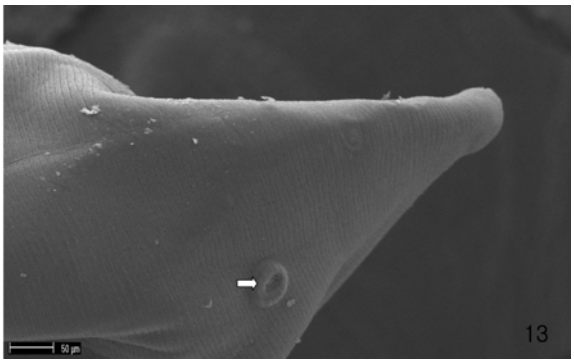


Fig 13. Lateral view of female tail. Phasmid (Arrow).

long, 38-42 um wide (Fig 4). Vulva is located on average 57% of total body length from the anterior end. The conical tail has an anus ventrally and a pair of phasmids situated in a lateral position (Figs 12, 13).

Discussion

Species of the anisakid genus *Contracaecum* Raillet & Henry, 1912 are found mainly seals and piscivorous birds. Although the genus *Contracaecum* are cosmopolitan and have been reported in a wide variety of fishes, birds, and piscivorous mammals (27), identification of species is not yet satisfactory (2). Morphological identification of a parasite at any stage of its development has important implications for studying parasite epidemiology and resolving taxonomic problems. Otherwise, genetic researches based on allozymes have indicated that many genera of ascaridoid nematodes infecting seabirds are composed of not genetically but morphologically similar species (4,5,6). Due to incompletion of formal morphologic descriptions on various species in each genus, distinct names have not been given to the species which instead have provisional titles such as type A, B, etc. D'Amelio *et al* (5,6) and Cianchi *et al* (4) found that *C. rudolphii* sensu lato parasitic on *Phalacrocorax carbo* can be divided into two siblings namely A and B. So the two siblings represent *C. rudolphii* complex.

Ito *et al* (17) reported that the genus *Contracaecum* has interlabia between the lips but the genus *Pseudoterranova* and *Anisakis* have not interlabia. In the present study, the worms have three interlabia between the lips. It's very difficult and often impossible to identify the species only through microscopic examinations because of the morphologic similarities. Indeed, there are no definitive diagnostic morphological characters for their specific identification. In descriptions of *Contracaecum* species the main differentiating criteria considered have been the length of the spicules (10), the morphology of the auricle (19), interlabia (1,15), the distribution of pre- and post-cloacal papillas in the male (12). In the present study, the morphological characters of the parasite had shown differences in the length of spicules, no bifurcation of interlabia, no auricles, and numerous preanal papillas (69-79 pairs). The characters of the parasite that we studied correspond with those of *C. rudolphii* complex (1) and we confirmed them as belonging to this complex. Nevertheless, because of the vague morphological characters available to differentiate several species in this genus (2), gene sequence data currently should provide useful tools to analyse the genus (18,22,23,26).

Huizinga (16) has reported the life-cycle of *C. spiculigerum* involves a copepod (*Cyclops*) in which second-stage larvae grows. Various species of fish ingest the infected copepods and then the third-stage larvae penetrate the intestinal wall of the fish and encyst in the body cavity. Birds acquire infection by eating those infected fish. Dyer *et al* (8) reported that the genus *Contracaecum* was most prevalent helminth in brown pelicans, and the usual infection site was the proventriculus. Hyaline caps and extensive ulcerated nodular lesions have been observed in several different types of hosts by different ascaridoid nematodes. Two natural cases involved caps in lesions caused by *Contracaecum* spp. in the proventriculus of the white pelican, *Pelicanus erythrorhynchus*, and in the stomach of the Steller sea lion, *Eumetopias jubata* (20). In the present study, we found only one species of nematoda from white pelican, the number of parasite was more than a thousand in the proventriculus. However, we did not find clusters of anisakids forming distinct ulcerated nodular lesions as observed in the study of Fagerholm *et al* (13), although mucosal epithelium somewhat was detached and slight congestion was found in lamina propria. Apparently, *C. rudolphii*, although they infect their host in high numbers, may not be the prime cause of death.

Some of *Contracaecum* species have allegedly caused human anisakidosis. Infected humans have consumed raw or poorly cooked fish (24). *C. rudolphii* complex have not as yet linked up with human anisakidosis, but they can be potentially zoonotic since they infect many fishes frequently eaten in China (28). To the best of our knowledge, *C. rudolphii* complex was not reported in Korea. Although there is geographical limits, the population at risk is growing because of developed transportation systems, increased international markets, and demographic changes. Further investigation of helminths of wild birds and fishes in Korea is needed because of the importance of the health of wildlife populations and the potential danger of zoonosis.

Acknowledgements

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페리칸에서 분리한 *Contracaecum rudolphii* (Nematoda: Anisakidae)의 형태학적 연구

김현철*・김남수・손화영・권보근・허진**・조정곤**・박배근[1]

*강원대학교 수의과대학・충남대학교 수의과대학
**전북대학교 수의과대학

요 약 : 대전동물원에 탄자니아로부터 페리칸이 수입되었고 5일 후에 한 마리가 폐사 하였다. 선위에서 다수의 기생충을 분리하였으며 다음과 같은 특징을 가지고 있었다. 3개의 입술이 존재하였고 입술에는 치상돌기가 존재하지 않았으며 입술 사이에는 순간돌기가 잘 발달되어 있었다. 순간돌기는 삼각형으로 분기되어 있지 않았다. 수컷은 다수의 전항문 유두 (69-79)가 아복면에 배열되어 있었다. 부총배설강 유두는 대형의 1쌍과 5개가 존재하였다. 이러한 형태학적 특징을 기초로 페리칸에서 분리한 기생충을 *Contracaecum rudolphii*로 분류하였다.

주요어 : 페리칸, *Contracaecum rudolphii*.