Nasitrema lagenorhynchus n. sp. from the Larynx and Lungs of a Pacific Striped Dolphin (Nasitrematidae, Trematoda)

SHIGERU KIKUCHI¹⁾, YOSHITERU OKUYAMA¹⁾ AND MASAYUKI NAKAJIMA²⁾

(Received for publication; December 17, 1985)

Abstract

Nasitrema lagenorhynchus n. sp. was described from a Pacific striped dolphin, Lagenorhynchus obliquidens, which died in the aquarium on February 17, 1984 after captured off the coast of Iki Island, Nagasaki Prefecture, Japan. At postmortem examination 86 flukes containing adults and larvae were collected from the larynx. The flukes and eggs were also found from the lungs by histopathological examination. The present species differs from all the other known species of Nasitrema by the smaller sizes of body and suckers, not lobed ovary, and parasitism in the larynx and lungs. The dolphin may have died of bronchopneumonia by the parasitism.

Key words: Trematoda, Nasitrema lagenorhynchus n. sp., Pacific striped dolphin, morphology, pathology

Introduction

Several papers of morphological studies papers have on the flukes of the genus *Nasitrema* been published from Japan (Ozaki, 1935; Yamaguti, 1951, 1958, 1971) and USA (Neiland *et al.*, 1970; Dailey and Brounell, 1972; Sweeney and Ridgway,1 975; Skrjabin *et al.*, 1964).

A Pacific striped dolphin, *Lagenorhynchus* obliquidens, 193.5 cm in body length and 99 kg in body weight, was captured off the coast of Iki Island, Kyushu on February 17, 1984, and died after introduced into the Izu-Mito Sea Paradise Aquarium. On postmortem examinination, a total of 86 flukes containing adults and larvae were obtained from the mucosal rugae of the larynx, and histopathological examination revealed the presence of the flukes and eggs in the lungs.

Materials and Methods

For photomicroscopic examination, the mature flukes flattened between the glass slides were fixed in Schaudinn's fixative and placed in iodine-alcohol for 20 min. to remove $HgCl_2$ and then in 70% ethanol. The specimens were stained with Delafield's hemato-xylin, dehydrated in ethanol and mounted with Canada balsam after clarified in xylol.

For histopathological examination, the specimens were stained with hexatoxylin and eosin or PAS reaction. The specimens for scanning electron microscopy were prepared by critical point drying.

Results

Description is based on 10 mature flukes. All the measurements are expressed in mm with their means in parentheses.

Description of species

Nasitrema lagenorhynchus n. sp. (Figs. 1 and 2) Host: Lagenorhynchus obliquidens Habitat: Larynx and lungs Locality: Off the coast of Iki Island, Nagasaki-ken, Japan

¹⁾Kikuchi Biomedical Laboratory, 2-19-10, Rinkan, Yamato-shi, Kanagawa 242, Japan.

菊池 滋 奥山義光 (菊池生物医学研究所)
 ²⁾Izu-Mito Sea Paradise Aquarium, 3-1, Nagahama, Numazu-shi, Shizuoka 410, Japan.
 中島将行(静岡県伊豆三津シーバラダイス)

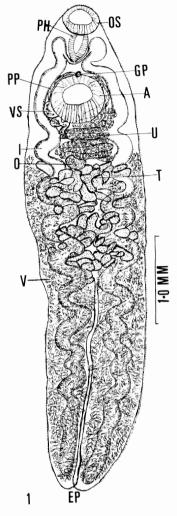


Fig. 1 Nasitrema lagenorhychus n. sp., holotype, ventral view.

Date: February 18, 1984

Type specimen: Deposited at the Kikuchi Biomedical Laboratory

Abbreviations used in Figures

A: Acetabulum, C: cirrus, EP: excretory pore, GP: genital pore, I: intestine, O: ovary, OS: oral sucker, OT: ootype, PA: papilla, PH: pharynx, PP: pars prostatica, PR: prostate cell, S: spine, T: testis, U: uterus, V: vitellaria, VS: vesicula seminalis.

Body 5.0-8.13 long and 1.15-1.50 wide, thick, subcylindrical, widest at level of ovary and testes, slightly attenuated posteriorly, end-



Fig. 2 Anterior part of body, ventral view. x10.

ing in obtuse angle. Body surface covered with spines 0.050-0.060 lung at middle portion of ventral surface (Figs. 3, 5). Subcircular oral sucker situated at anterior end of body, directed anteriorly, measuring 0.200–0.275 $(0.237) \times 0.225 - 0.300$ (0.262) short spines on thick outer wall, papillary processes along inner rim, minute longitudinal rugae all over lining of lumen. Prepharynx short. Pharynx pyriform, long, measuring 0.25-0.33 (0.29) x 0.12-0.20 (0.16) (Figs. 1-3). Esophagus lacking in some specimens. Intestine bifurcated immediately posterior to pharynx, then runs anteriorly along pharynx, twists posteriorly, and ends in ceca near posterior end of body (Fig. 1). Acetabulum situated on midline of ventral surface 1.15-1.30 (1.25) from anterior end of body, swollen at its rim, densely covered with short cuticular spines on outer surface, short spines and papillary processes on inner surface of suckers, 0.225-0.400 (0.313) ×

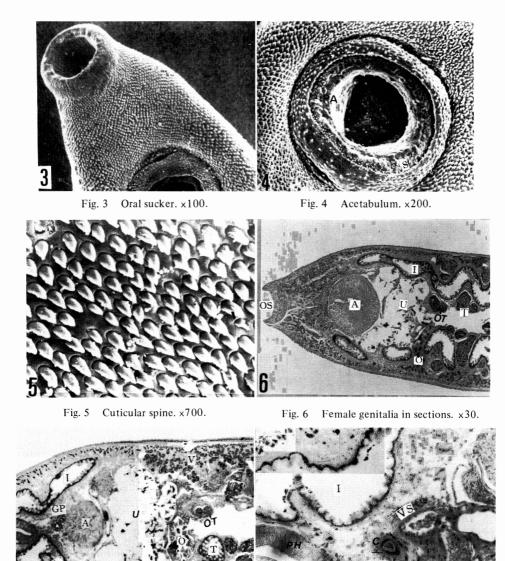


Fig. 7 Female genitalia in sections. x50.

0.275-0.500 (0.388), 1.4 times larger than oral sucker (Figs. 3, 4). Testes situated tandem between anterior 1/4 and 1/2 of body, multilobate, overlap with ceca. Vas efferens originates from middorsal portion of anterior testis and runs anteriorly along ventral side of ovary (Figs. 1, 2). Vas efferens of posterior testis runs along leftsid of ovary, and unites with that of anterior testis on dorsal side of coiled

Fig. 8 Section showing seminal vesicle area dorsal to acetabulum. x100.

VS

uterus to give rise to vas deferens entering into posterior end of seminal vesicle. Seminal vesicle swollen at posterior part, composed of thin, muscular envelope and epithelial endothelium, measures 0.23–0.35 in maximum width, runs dextrodorsal to acetabulum. Pars prostatica sylindrical, muscular, surrounded by prostatic cells, 0.25 long and 0.07 wide. Cirrus very short and shorter than pars prostatica,

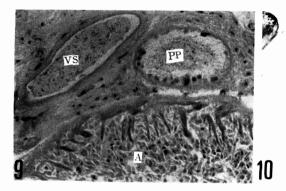


Fig. 9 Section showing seminal vesicle and pars prostatica dorsal to acetabulum. x200.





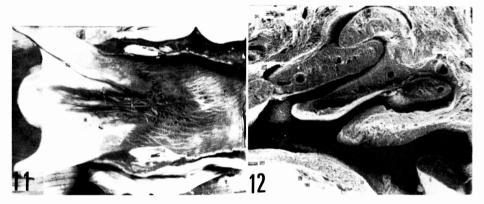


Fig. 11 Flukes on arytenoid cartilage. x30.

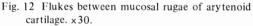




Fig. 13 Lung lesions caused by flukes. x30.

0.018 in width, composed of thick cuticules, and opens into genital pore. Cirrus pouch completely lacking (Figs. 1, 8, 9). Ovary laterally longer, elliptical, not lobed, measuring 0.25-0.38 in diameter, located somewhat dextrally to midline in front of anterior testis (Figs. 1,



Fig. 14 Pulmonary nodular lesion, showing a worm and infiltrating cells in central portion. ×100.

6, 7). Oviduct originates from dorsolateral side of ovary, runs towards right side of body, gives off Laurer's canal, and unites with vitelline reservoir to form ootype. Laurer's canal runs posteriorly, opens on midline of dorsum at level of ovary. Vitelline reservoir pyriform,



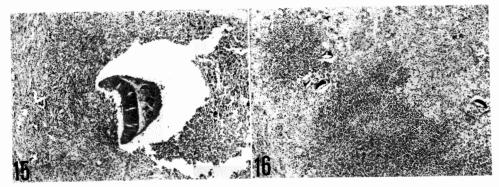


Fig. 15 Pulmonary nodular lesion, showing a worm and infiltrating cells in central portion. ×100.

Fig. 16 Abscess-like pulmonary nodule containing infiltrating cells and exudate together with disseminated eggs. x50.

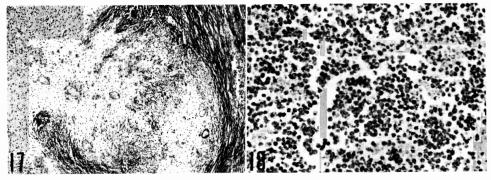


Fig. 17 Obsolete egg nodule in pulmonary tissue. x50.

0.15-0.20 in diameter. Ootype and shell glands situated on left side of ovary on midline. Uterus swollen with eggs and sperm, passes anteriorly along dorsal side of acetabulum after twisting between two intestinal ceca (Figs. 6, 7). Metraterum composed of well-developed inner layer of annular muscles, outer layer of longitudinal muscles and accessory cells. Genital pore situated immediately anterior to acetabulum on midline. Vitellaria divided into numerous clusters of highly dendritic acini, commence anteriorly at level of ovary and proceed to near posterior end of body, occupying dorsolateral area of ovary zone, dorsal area of testis zone and peripheral area of both intestines. Eggs elliptical, thick-shelled, somewhat obtuse at operculated pole and somewhat swollen at opposite pole with a process resulting from thickening of shell, nearly triangular in cross section, measuring $0.068 - 0.072 \times$

Fig. 18 Granulation tissue of lungs. x200.

0.040-0.050 (Fig. 10). Excretory bladder tubular, thick, extending from orifice at posterior end of body about midway between two testes.

Pathological findings

Pathological and histopathological changes were observed in the habitats of the flukes. The flukes atrached themselves deeply or superficially between the annular mucosal rugae of the larynx or were free in the lumen. The mucous membrane of the parasitized region was hyperemic, swollen, dotted with many petechial or miliary hemorrhages, and covered with mucous exudate (Figs. 11, 12). The lungs were mildly emphysematous, and scattered with blackish-red hemorrhagic nodules or somewhat hard protuberances on the bilateral surfaces (Fig. 13). These protuberances were especially abundant on the apical lobes, but scarcely seen on the diaphragmatic lobes. Nodules of various sizes were observed on the cross section of the lung lesions. There were new and old nodular lesions surrounding parasites and eggs in the affected area of the lungs. In the new lesions the parasites were surrounded by inflammatory granulomatous tissues, and infiltrated by cells including lymphocytes, plasma cells, eosinophils and histiocytes (Fig. 18). The central portions of nodular lesions gave rise to caverna which involved masses of decaying infiltrating cells and the parasites (Figs. 14, 15). Some lesions contained a lot of effusion, resulting in abscess-like appearance. New eggs were scattered in this effusion (Fig. 16). Degenerative or disintegrated eggs or egg shells were observed in the portions scarce in cell components in the nodules. The obsolete nodular lesions had little infiltrating cells, were necrotic from the middle layer to the central portion, which stained deeply and had cracks. Fibrosis or hyalinization was observed within the nodules of the markedly obsolete lesions. There was also the coalescence of several egg nodules (Fig. 17).

Discussion

A total of nine species of the genus Nasitrema have so far been described: four species from Japan (N. spathulatum Ozaki, 1935, N. gondo Yamaguti, 1951, N. sunameri Yamaguti, 1951 and N. dalli Yamaguti, 1951) and five species from USA (N. attenuata Neiland, Rice et Holden, 1970, N. lanceolata Neiland, Rice et Holden, 1970, N. delphini Neiland, Rice et Holden, 1970, N. globicephalae Neiland, Rice et Holden, 1970 and N. stenosoma Neiland, Rice et Holden, 1970).

All the known species of Nassitrema are different from the present species in habitat and the morphological characters and measurements (Tablw 1) as follows: N. spathulatum in abruptly tapering posterior half of body, larger suckers, deeply lobed oaary, and vitellaria occupying zone above acetabulum; N. gondo in largest body, larger suckers, testes occupying spacious region, markedly multilobed ovary, and larger eggs; N. sunameri in larger body and suckers, and vitellaria commencing at preacetabular level; N. attenuata in lanceolate body, markedly slender posteriro body,a and not highly dendritic testes occupying anterior 1/3 to 1/6 of body; N. lanceolata in larger body, two larger suckers, and vitellaria occupying zone above genital pore; N. delphini in spatulate body being widest at level of acetabulum, tapering more or less rapidly posterior to acetabulum, and pointed at terminal end of body; N. stenosoma in slender body being nearly parallel-sided posterior to widest point

Species	Body (mm)	Oral sucker (µm)	Acetabulum (µm)	Egg (µm)
Spathulatum	19-26×2. 3-3. 1	$350-500 \times 600-660$	$850 - 1000 \times 1050$	$70-95 \times 40-57$
Gondo	$28 - 35 \times 5.0$	$750-850 \times 950-1100$	1150	$90 - 105 \times 54 - 63$
Sunameri	$15-20 \times 3.8-4.0$	$550-600 \times 620-750$	800-1050	$81 - 90 \times 42 - 60$
Dalli	$8 - 15 \times 2 - 3$	$320-550 \times 550-700$	650-950	$72 - 84 \times 42 - 48$
Attenuata	18. 9–23. 0×2. 4	508-581 imes 552-627	846890×853865	$71 - 81 \times 46 - 47$
Lanceolata	$\begin{array}{c} 14.\ 619.\ 5\times3.\ 44.\ 6\\ (16.\ 8\times4.\ 0) \end{array}$	$\begin{array}{c} 667-878 imes 657-897\ (801 imes 798) \end{array}$	$\begin{array}{c} 821 - 1100 \times 815 - 1060 \\ (987 \times 972) \end{array}$	$75-89 \times 47-56$ (83 × 52)
Delphini	8. 9-14. 3×1. 6-2. 3 (12. 2×1. 9)	$408-533 \times 477-545 \ (457 \times 507)$	$\begin{array}{c} 583-633\times571-655\\(621\times623)\end{array}$	$72-78 \times 47-51$ (75 × 49)
Stenosoma	9. 6-12. $2 \times 1.$ 6-1. 9 (11. $1 \times 1.$ 7)	$344-408 \times 383-452 \ (374 \times 422)$	$552-608 \times 552-640 \ (587 \times 595)$	$74-82 \times 42-55$ (79 × 47)
Globicephalae	9. 5-22. 0×1.75 -4. 2 (15. 0×3.0)	$\begin{array}{c} 452 - 897 \times 414 - 966 \\ (583 \times 638) \end{array}$	$\begin{array}{c} 646 1166 \times 564 1166 \\ (823 \times 806) \end{array}$	$74-86 imes 48-53 \ (79 imes 49)$
Lagenorhynchus	$5.0-8.13 \times 1.15-1.50 (6.57 \times 1.32)$	$200-275 \times 225-300 \ (237 \times 268)$	$\begin{array}{c} 225 - 400 \times 275 - 500 \\ (313 \times 388) \end{array}$	$68-72 \times 40-50$ (70 × 45)

Table 1 Measurements of the species of the genus Nasitrema

at level of acetabulum, ovary consisting of 2 to 5 lobes, and vitellaria commencing at level of posterior margin of acetabulum; and N. *globicephalae* in larger suckers and ovary with 5 to 10 short, blunt outgrowths. The present species, on the other hand, has the closest resemblances to N. *dalli*, but differs from it in the following characters: pharynx with cylindrical lumen in anterior portion and triradiate lumen in posterior bulbous portion, intestine winding not so strongly at level of uterus and testes, larger suckers, and ovary with short digitiform outgrowths.

As a result, the present species differs from all the known species of the genus *Nasitrema*. Thus, *N. lagenorhynchus* n. sp. was proposed for it.

The affected dolphin was not vividly swimming, anorectic, and occasionally coughing when captured. The animal fell inti dyspnea immediately before death. From the above observations the death was diagnosed to be caused by bronchopneumonia resulting from the parasitism.

Acknowledgements

The authors wish to express their gratitude to Prof. H. Itagaki, School of Veterinary Medicine, Azabu University for his kind advice in preparating the manuscript, to Prof. W. Shirai, College of Agriculture and Veterinary Medicine, Nihon University for his kind advice in pathological study, and Associate Prof. Shiro Chinone, School of Veterinary Medicine, Azabu University and Instructor Akihiko Uchida, College of Environmental Health, Azabu University for their kind cooperation in the preparation of specimens.

References

- Dailey, M. D. and Brounell, R. L. (1972): A checklist of marine mammal parasites. *In*: Mammals of the Sea, Biology and Medicine, Ridgway, S. H., ed., C. C. Thomas, 11, 528-589.
- Neiland, K. A., Rice, D. W. and Holden, B. L. (1970): Helminths of marine mammals. 1. The genus *Nasitrema*, air sinus flukes of dephinid Cetacea. J. Parasitol., 56, 305-316.
- Ozaki, Y. (1935): Trematode parasites of Indian porpoise *Neophocaena phocaenoides* Gray, J. Sci. Hiroshima Univ., Ser. B, Div. 1, 3, 115-138.
- Skrjabin, K. E. (1964): Keys to the Trematodes of Animals and Man. Univ. Illinois Pr., Urbana, 351 pp.
- Sweeney, J. C. and Ridgway, S. H. (1975): Procedure for the clinical management of small cetaceans. J. Am. Vet. Med. Assoc., 167, 540-546.
- Yamaguti, S. (1951): Studies on the helminth fauna of Japan. Part 45. Trematodes of marine mammals. Arb. Med. Fak. Okayama, 7, 283-294.
- Yamaguti, S. (1958): Systema Helminthum. Vol.
 1. The Digenetic Trematodes of Vertebrates. Parts 1 and 2. Interscience Publ., New York, 852-853.
- Yamaguti, S. (1971): Synopsis of Digenetic Trematodes of Vertebrates. Vol. 1. Keigakusha Shuppan, Tokyo, 728-829.