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Validity and Redescription of *Contracaecum himeu* (Nematoda, Anisakidae), a Parasite of Cormorants in Japan

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Abstract Nematode specimens taken from the stomach of Japanese cormorants (*Phalacrocorax capillatus*) collected on the coast of the Japan Sea in central Japan were examined by light and scanning electron microscopy and identified as *Contracaecum himeu* Yamaguti, 1941. Although the taxonomic validity of this nematode species remained uncertain, the present study shows that it is a valid species and definitely differentiated from a closely related species, *C. rudolphii*, specimens of which were also examined from Europe. *Contracaecum himeu* is redescribed, and the shape of the interlabium, i.e., its massive construction and a markedly rounded distal end, is important to differentiate the species from its congeners. The Japanese cormorant is a new host. The body length-weight relationship is also shown for both sexes of *C. himeu*.

Key words: Parasitic Nematoda, Anisakidae, *Contracaecum himeu*, *Contracaecum rudolphii*, cormorants, *Phalacrocorax capillatus*.

Introduction

Three nematode species of the genus *Contracaecum* have been reported from cormorants (genus *Phalacrocorax*) in Japan (Yamaguti, 1941): *C. rudolphii* Hartwich, 1964, as *C. spiculigerum* (Rudolphi, 1809) from common cormorants *P. carbo hanedae*, *C. himeu* Yamaguti, 1941 from pelagic cormorants *P. pelagicus pelagicus*, and *C. umiu* Yamaguti, 1941 from Japanese cormorants *P. capillatus*. *Contracaecum rudolphii* is a widely distributed parasite of fish-eating birds and is morphologically well defined (Hartwich, 1964, 1975; Baruš *et al.*, 1978). However, the taxonomic validity of *C. himeu* and *C. umiu* remains uncertain, and there are some different opinions: Skrjabin *et al.* (1951) regarded *C. himeu* as a junior synonym of *C. spiculigerum* (=*C. rudolphii*), but Yamaguti (1963, footnote of p. 239) did not agree to their opinion. Mozgovoy (1953) and Baruš *et al.* (1978) thought that both *C. himeu* and *C.*

umiu are conspecific with *C. spiculigerum* (=*C. rudolphii*). Hartwich (1964) left this problem open for further study.

Recently we had an opportunity to study the nematode material from Japanese cormorants and concluded that *C. himeu* is definitely differentiated from *C. rudolphii*. We herein redescribe *C. himeu* as a valid species.

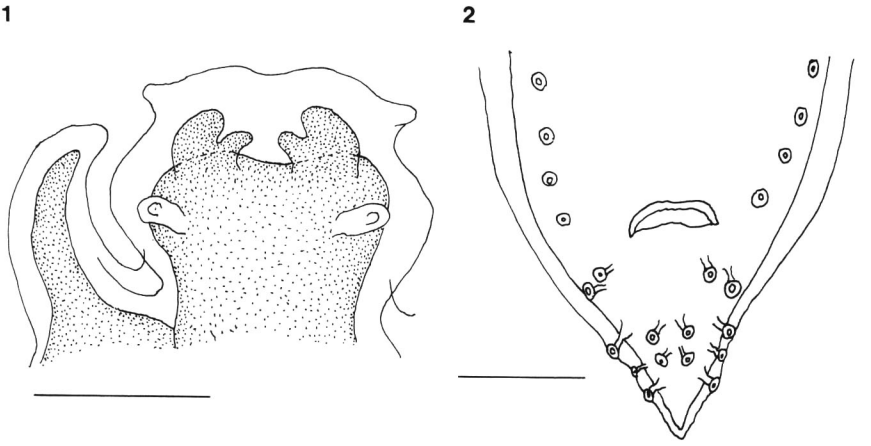
Materials and Methods

Seven specimens (73.3–83.2 cm in total length [TL]) of the Japanese cormorant, *Phalacrocorax capillatus* (Temminck and Schlegel, 1850), were used for parasitological examination. They were found dead, mostly due to oil spill, at various locations in Akita (N=1), Niigata (N=3), Fukui (N=2) and Shimane (N=1) prefectures, Japan, along the coast of the Japan Sea in January and February 1997. Additional seabird samples for examination included the Pacific diver, *Gavia pacifica* (Lawrence, 1858) (N=1, 59.8 cm TL), the red-necked grebe, *Podiceps grisegena holboellii* (Reinhardt, 1854) (N=4, 44.6–46.7 cm TL), and the black-necked grebe, *P. nigricollis nigricollis* (Brehm, 1831) (N=2, 28.1–28.8 cm TL) found on the coast of the Japan Sea in Niigata and Fukui prefectures in January 1997. The seabirds were brought frozen to the laboratory, where a necropsy was conducted. All nematodes found were removed from the stomach (proventriculus and/or gizzard), fixed in 10% formalin and then preserved in 70% ethanol. For light microscopy (LM), specimens were cleared in glycerin and later stored in vials in 70% ethanol. After the LM observation, 20 adult specimens (10 males and 10 females) were selected for scanning electron microscopy (SEM) and processed using the method described by Wiger *et al.* (1978). The worms were examined and photographed with a Tesla BS-300 electron microscope. Measurements are given in millimeters as ranges.

For a comparative SEM study, 20 adult specimens (10 males and 10 females) of *Contracaecum rudolphii* were used. The worms were taken from common cormorants (*P. carbo*) sampled in the Czech Republic and Germany. Morphometric and morphological data by Hartwich (1964, 1975) also were used for comparison.

To determine the relationship between the body length and weight of *C. himeu*, all adult worms (N=106) taken from two birds were examined. Each worm was measured for body length (to the nearest 0.1 mm) using an Olympus BX-50 microscope and weighed (to the nearest 0.0001 g) using a Mettler AJ150 balance. The relationship was analyzed using the powered regression analysis ($y=ax^b$).

Throughout the text, prevalence and intensity of infection are defined as the percentage of infected hosts and the number of worms found in a host, respectively (Bush *et al.*, 1997).



Figs. 1–2. *Contracaecum himeu*. 1. Adult female, lip and interlabium (dorsal view). Scale bar: 0.1 mm. 2. Adult male, posterior end of body (ventral view). Scale bar: 0.1 mm.

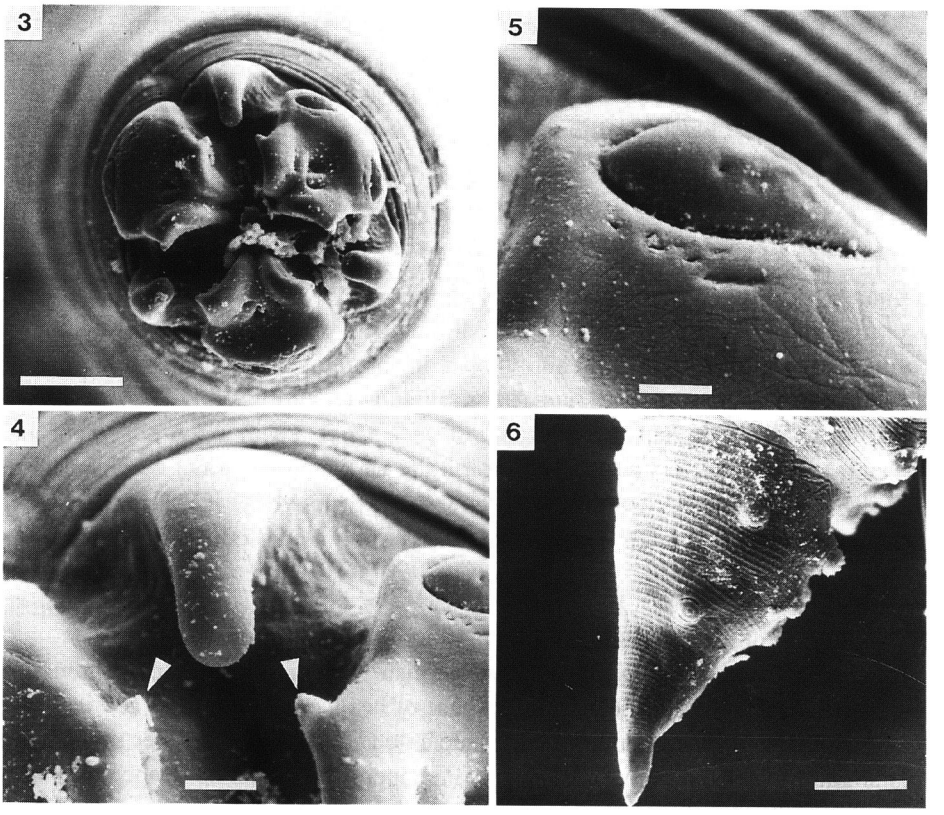
Results

Redescription of *Contracaecum himeu* Yamaguti, 1941 (Figs. 1–10)

General description: Nematodes of whitish to yellowish color, medium-sized, with fine transversely striated cuticle. At base of head, cuticle forming cervical collar with marked transverse striation, clearly separated from head and other part of body. Lips slightly wider than long, of hexagonal shape, with moderate deepening in middle of upper margin. Short rounded processes (auricles) present on lateral side near upper margin. Lip papillae well visible. Interlabia at basis broad, moderately tapering to apex with clearly rounded distal ends in both sexes.

Male (10 adult specimens): Head 0.14–0.21 in diameter. Lips 0.084–0.132 long, interlabia 0.061–0.097 long. Nerves ring and cervical papillae 0.32–0.69 and 0.63–0.85, respectively, from anterior extremity. Ventriculus 0.22–0.30 long and 0.18–0.26 wide. Posterior end of body always curved ventrally, tail conical, without distinctly separated peak. Spicules equal or subequal, with longitudinal alae. Distal end of spicules without alae 0.025–0.027 long; peak slim and rounded. Width of free distal end of spicules without alae 0.010–0.014, with alae 0.035–0.038. Seven postcloacal papillae (three lateral and four subventral) present on tail; 40–56 pairs of precloacal papillae arranged in two longitudinal rows on ventral body surface, reaching distance of 6.84–8.33 from upper margin of cloaca.

Female (10 adult specimens): Head 0.17–0.26 in diameter. Lips 0.096–0.146 long, interlabia 0.072–0.115 long. Nerves ring and cervical papillae 0.49–0.78 and 0.73–0.99, respectively, from anterior extremity. Ventriculus 0.27–0.39 long and 0.22–0.30 wide. Posterior end of body conical, anal opening without distinct margins. Phasmids located 0.026–0.039 from posterior extremity. Eggs subglobular, egg shell



Figs. 3–6. *Contracaecum himeu*. SEM. 3. Adult female, head (apical view). Scale bar: 50 μm. 4. Adult female, interlabium and lip auricles (arrowheads) (apical view). Scale bar: 10 μm. 5. Adult female, lip papilla (dorso-lateral view). Scale bar: 5 μm. 6. Adult male, tail (ventro-lateral view). Scale bar: 20 μm.

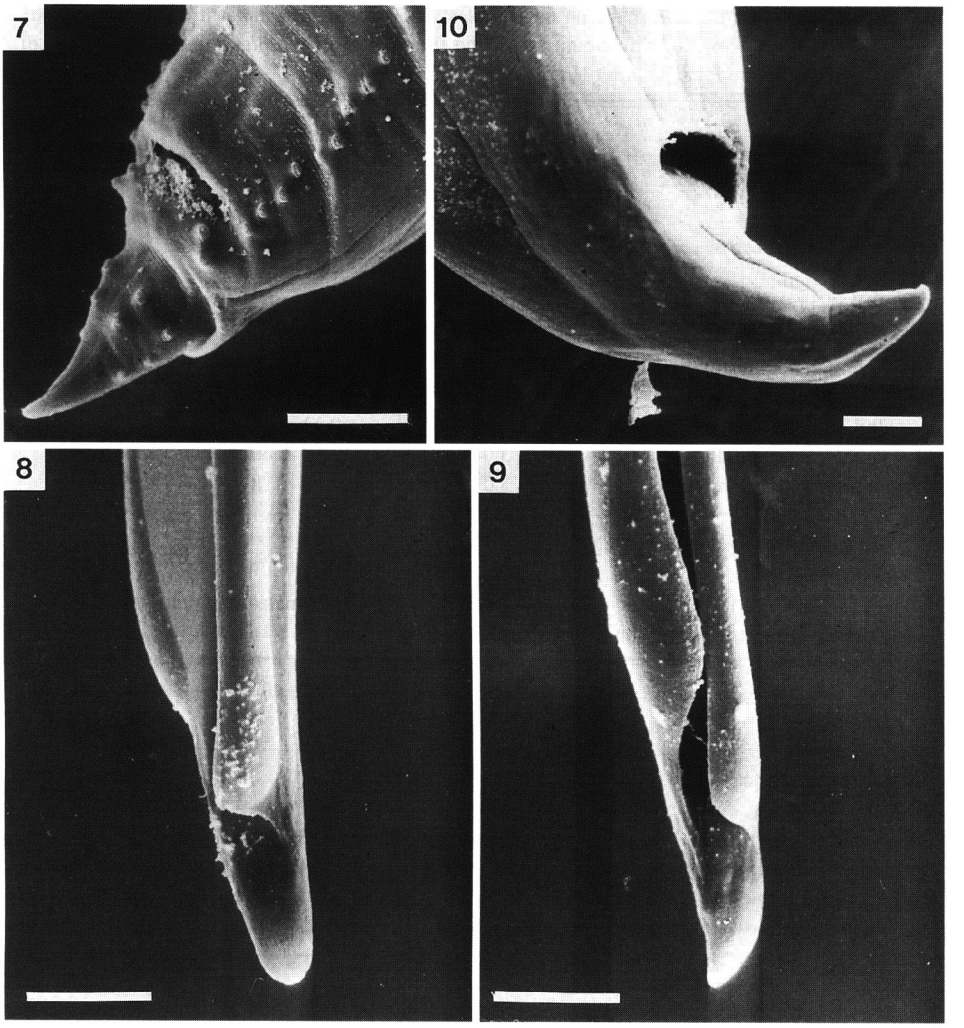
0.003–0.006 thick.

Other measurements and length ratios ("indecies" by Hartwich, 1964) of *C. himeu* together with those of *C. rudolphii* are shown in Tables 1–2 for males and in Tables 3–4 for females.

Host: Japanese cormorant, *Phalacrocorax capillatus* (Temminck and Schlegel, 1850) (Pelecaniformes: Phalacrocoracidae) (new host).

Site of infection: Stomach (proventriculus and/or gizzard).

Localities: along the coast of the Japan Sea, Honshu, Japan: near the mouth of the Aso River on the Seki Beach in Kisakata-machi, Akita Prefecture (January 28, 1997), at Kita-ebisu in Aikawa-machi (January 23, 1997), on the Hagenohama Beach in Awashima-ura-mura (February 4, 1997), at Konagare in Ogi-machi (February 3, 1997), Niigata Prefecture, at Saki in Mikuni-machi (January 17, 1997) and on the



Figs. 7–10. *Contracaecum himeu*. SEM. 7. Adult male, posterior end of body (ventro-lateral view). Scale bar: 50 μm. 8. Adult male, distal part of left spicule (lateral view), Scale bar: 20 μm. 9. Adult male, distal part of left spicule (lateral view), intraspecific variability or slight deformation in preparation for SEM, Scale bar: 20 μm. 10. Adult female, posterior end with anal opening (latero-ventral view), Scale bar: 50 μm.

Azo Beach in Tsuruga-shi (February 4, 1997), Fukui Prefecture. No infection was found in a bird from Fuse-mura, Oki Islands, Shimane Prefecture (January 21, 1997).

Specimens deposited: National Science Museum, Tokyo (NSMT-As-2854).

Table 1. Morphometric data (mm) on adult males of *Contracaecum himeu* from *Phalacrocorax pelegicus pelagicus* (after Yamaguti, 1941) and from *P. capillatus* (present study) and those of *C. rudolphii* from *P. carbo* (after Hartwich, 1964).

	C. himeu		*C. rudolphii*
	Yamaguti (1941)	Present study N=10	Hartwich (1964) N=39
Body length	17.0–27.0	18.3–36.3	12.1–33.9
Body width	0.50–0.82	0.65–1.37	0.24–0.95
Esophagus length	2.1–3.3	2.16–3.68	2.03–4.26
Ventricular appendix length	0.7–0.9*	0.79–1.24	0.58–1.37
Intestinal caecum length	1.6–2.9	1.68–3.31	1.53–3.68
Tail length	0.12–0.18	0.13–0.21	0.14–0.24
Right spicule length	3.0–5.1	3.62–5.60	4.46–9.19
Left spicule length	3.0–5.1	3.70–5.78	4.05–9.98

*The length of the ventriculus included.

Table 2. Length ratios (="indecies" by Hartwich, 1964) of adult males of *Contracaecum himeu* (present study) and *C. rudolphii* (after Hartwich, 1964).

	C. himeu N=10		*C. rudolphii* N=39	
Body length/Body width	21.84–44.27	(30.5±4.52)*	29.40–98.10	(52.3±2.35)
Body length/Esophagus length	6.95–11.27	(8.98±1.03)	5.25–10.78	(8.01±0.21)
Body length/Tail length	101.66–206.25	(149.4±27.93)	74.10–197.20	(130.8±5.17)
Esophagus length/Intestinal caecum length	1.06–1.30	(1.17±0.084)	1.11–1.54	(1.30±0.016)
Esophagus length/Ventricular appendix length	2.57–3.71	(3.04±0.30)	1.82–4.25	(3.25±0.09)
Body length/Mean spicule length	3.65–7.09	(5.54±0.92)	2.06–5.69	(3.86±0.14)

*range (mean±standard deviation).

Prevalence and intensity of infection

Six (85.7%) of the seven Japanese cormorants examined were infected with adult and larval *C. himeu*. One bird without *C. himeu* was taken in the southernmost site (Shimane Prefecture) among seven sampling locations. Adults and larvae were found in six (85.7%) and four (57.1%) birds, respectively. The intensity of infection ranged from 24 to 70 (mean 49.0) worms per infected host. Of 294 worms found, adults were 263 (89.5%) and 31 (10.5%) fourth-stage larvae. The intensity of infection with adult females (range: 19–37, mean: 25.2) were slightly higher than that of adult males (4–30, 18.7). The intensity by larval worms ranged from 1 to 14 (mean: 7.8). The sex ratio was 1.35 : 1 (adult females: adult males).

For the three species of seabirds (i.e., Pacific diver, red-necked grebe, and black-

Table 3. Morphometric data (mm) on adult females of *Contracaecum himeu* from *Phalacrocorax pelegicus pelagicus* (after Yamaguti, 1941) and from *P. capillatus* (present study) and those of *C. rudolphii* from *P. carbo* (after Hartwich, 1964).

	C. himeu		*C. rudolphii*
	Yamaguti (1941)	Present study N=10	Hartwich (1964) N=36
Body length	30–43	19.3–48.0	10.10–57.60
Body width	0.60–1.75	0.37–1.92	0.29–1.51
Esophagus length	3.5–4.0	2.77–4.93	1.62–5.48
Ventricular appendix length	1.1–1.2*	1.40–1.72	0.62–1.58
Intestinal caecum length	2.60–2.85	2.00–4.25	1.28–4.12
Tail length	0.26–0.40	0.23–0.52	0.19–0.63
Head–vulva length	—	6.28–13.54	5.12–17.70
Egg length	0.066–0.080	0.060–0.072	0.059–0.072
Egg width	0.050–0.069	0.048–0.059	0.041–0.059

*The length of the ventriculus included.

Table 4. Length ratios (="indecies" by Hartwich, 1964) of adult males of *Contracaecum himeu* (present study) and *C. rudolphii* (after Hartwich, 1964).

	C. himeu N=10		*C. rudolphii* N=39	
Body length/Body width	25.00–57.62	(38.62±8.00)*	34.60–70.80	(49.7±1.75)
Body length/Esophagus length	6.97–9.94	(8.63±0.54)	6.23–13.50	(9.34±0.31)
Body length/Tail length	83.91–105.78	(95.49±4.62)	38.00–165.99	(90.15±3.86)
Esophagus length/Intestinal caecum length	1.07–1.40	(1.23±0.097)	1.13–1.87	(1.32±0.024)
Esophagus length/Ventricular appendix length	1.86–3.74	(2.45±0.40)	2.03–4.36	(3.18±0.10)
(Vulva–Head end×100)/Body length	24.21–32.54	(27.3±1.83)	24.24–50.69	(32.22±0.91)

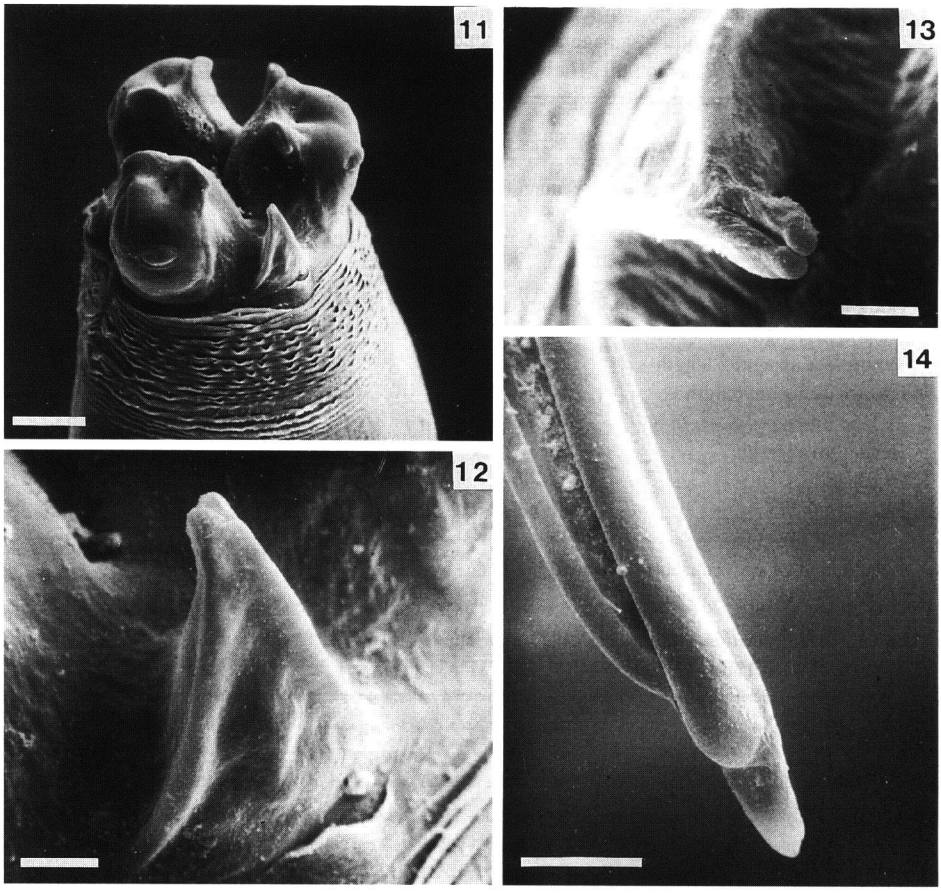
*range (mean±standard deviation).

necked grebe) other than Japanese cormorants, three worms of *Contracaecum variegatum* (Rudolphi, 1809) were found in a single Pacific diver (Nagasawa *et al.*, 1999) but no infection with *C. himeu* was found in all birds of the three species.

Parasitic load and length-weight relationship of *Contracaecum himeu*

When the gizzards were opened, nematodes were found firmly attached to the mucosa. Scars were seen at attaching sites when the worms were removed.

As usual in most species of nematodes, the body length and weight of adult female worms are higher than those of adult male worms (Table 5). The total wet weight of all adult worms found in each of two hosts was 1.5977 g (N=42) and



Figs. 11–14. *Contracaecum rudolphii*, SEM. 11. Adult male, head (ventro-lateral view). Scale bar: 50 μm. 12. Adult male, interlabium with excretory pore at base (ventral view), Scale bar: 10 μm. 13. Adult female, interlabium (ventro-apical view), Scale bar: 10 μm. 14. Adult male, distal part of left spicule (ventro-lateral view), Scale bar: 20 μm.

1.3370 g (N=64). When mean body weights of adult male and female worms were used to estimate the parasitic load in each of four other infected hosts, the total wet weights of adult worms in individual hosts were calculated as 1.2730 g (N=59), 1.4406 g (N=60), 1.0726 g (N=39), and 0.8216 g (N=24).

The relationships between the body length and weight of *C. himeu* are shown in Figs. 15–16. The equations are $y=5\times10^{-5}x^{1.76}$ ($R^2=0.75$) for males and $y=2\times10^{-5}x^{2.06}$ ($R^2=0.87$) for females. The power of female length is higher than that for males, indicating that at the same size a female has a heavier body than a male.

Table 5. Body length and weight of adult males and females of *Contracaecum himeu* from *Phalacrocorax capillatus*.

Males Host Number	Number of worms	Body length (mm) range (mean±standard deviation)	Body weight (g) range (mean±standard deviation)
2	23	25.4–36.3 (29.8±3.09)	0.0114–0.0311 (0.0221±0.0053)
5	27	11.5–34.5 (20.0±4.90)	0.0029–0.0306 0.0115±0.0060)
Total	50	11.5–36.3 (24.5±6.44)	0.0029–0.0311 (0.0164±0.0078)
Females Host Number	Number of worms	Body length (mm) range (mean±standard deviation)	Body weight (g) range (mean±standard deviation)
2	19	24.8–57.2 (48.0±8.02)	0.0099–0.0965 (0.0574±0.0211)
5	37	14.8–42.3 (26.6±8.45)	0.0052–0.0732 (0.0277±0.0210)
Total	56	14.8–57.2 (33.9±13.09)	0.0052–0.0965 (0.0378±0.0253)

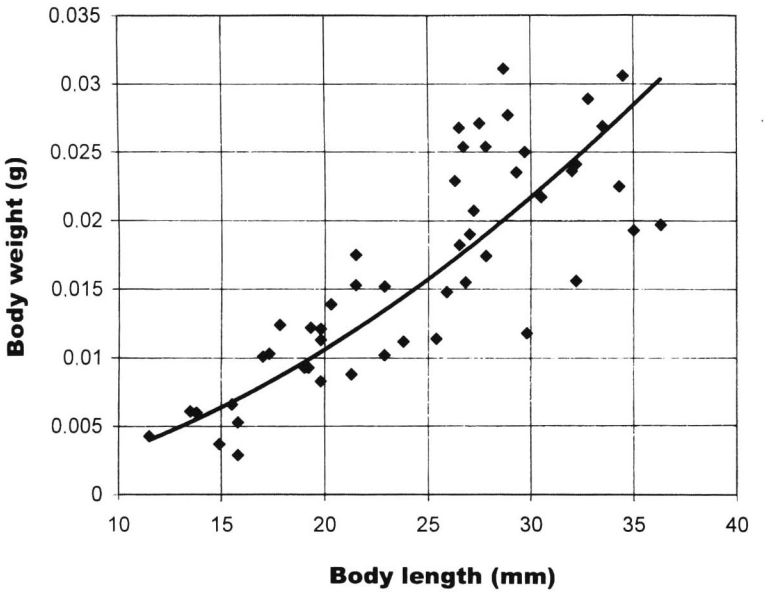


Fig. 15. Relationship between the body length and body weight of male *Contracaecum himeu*.

Discussion

When Yamaguti (1941) originally described *Contracaecum himeu*, he stated that "this species must have been confused by previous authors with *Contracaecum spiculigerum* (Rud.), owing to their very close resemblance." The latter species is currently identified as *C. rudolphii*. For differentiation of these species, he mentioned

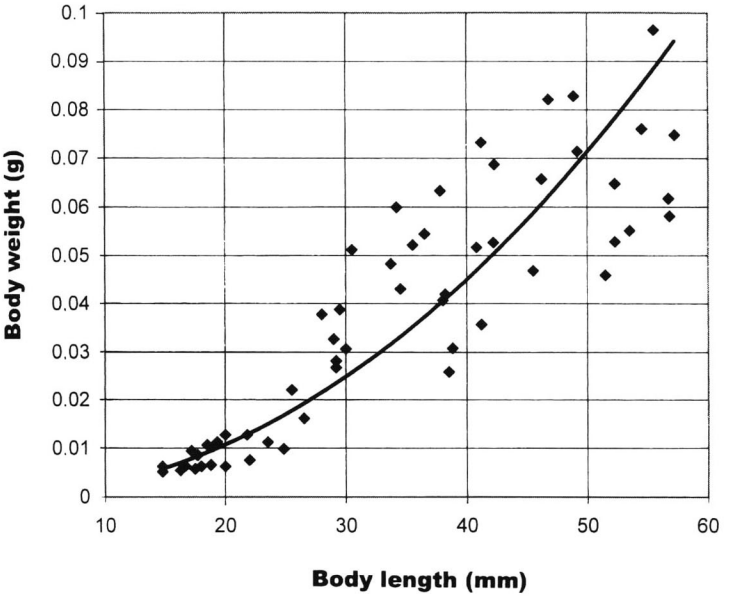


Fig. 16. Relationship between the body length and body weight of female *Contracaecum himeu*.

that there was "a constant difference in the length of the spicules and the thickness of the egg shell." However, these characters are not considered to be of significant importance: the length ranges of the spicules of both species are considerably overlapped although the spicular length of *C. himeu* is shorter than that of *C. rudolphii* (Table 1); the thickness (0.003–0.004 mm) of egg shells of *C. rudolphii* falls within a range (0.003–0.006 mm) of the thickness of those shells for *C. himeu*. As shown in Tables 1 and 3, the values of measurements of both species are quite similar and mostly overlapped. These facts may be a main reason why *C. himeu* was erroneously regarded as a junior synonym of *C. rudolphii* (=*C. spiculigerum*) by Skryabin *et al.* (1951), Mozgovoy (1953) and Baruš *et al.* (1978).

Some species of the genus *Contracaecum* are known to have morphological characters that are useful for differentiation from their congeners. According to Hartwich (1964, 1975) and Fagerholm (1989), the following morphological characters are useful for identification: the shape of the lips, interlabia, and distal end of the spicules, and the number and distribution of the postcloacal papillae. The actual use and high taxonomic vales of these characters were documented in a study on *C. magnipapillatum* Chapin, 1925 and *C. variegatum* (Fagerholm *et al.*, 1996; Nagasawa *et al.*, 1998).

One of the morphological characters that differentiate *C. himeu* from its congeners is the shape of the interlabium, which has a relatively massive construction and a markedly rounded distal end (Figs. 1, 4). The latter character was illustrated in

the paper by Yamaguti (1941, Fig. 19), although he did not mention it in the original description. The interlabium of *C. rudolphii* has a slimmer construction and its distal end is always bifurcated (Figs. 11–13).

There are further differences between *C. himeu* and *C. rudolphii* in the shape of lateral processes (auricles) of the lips: *C. himeu* has a rounded peak (Fig. 4), whereas *C. rudolphii* possesses a peak with sharper end. Moreover, although the shape of the spicule shows certain variability in both species, small differences are present: *C. rudolphii* (Fig. 14) has a little more fleshy spicules than *C. himeu* (Figs. 8–9). As pointed out by Yamaguti (1941), the number and distribution of the postcloacal papillae are nearly identical in both species.

The length ratios shown in Tables 2 and 4 are of almost no use for differentiating *C. himeu* from *C. rudolphii*. Only the length ratio of the mean spicular length and the body length may be used for differentiation of the two species, although the values are considerably overlapped.

Nonbifurcated interlabia are found on some other species of the genus *Contracaecum*, such as *C. microcephalum* (Rudolphi, 1809) and *C. magnipapillatum* (cf. Baruš *et al.*, 1978; Fagerholm *et al.*, 1996). These species are differentiated from *C. himeu* by other morphological characters (the construction of the interlabium, the shape of the distal end of the spicule, the shape of the lip, and others). Bifurcated interlabia are found on *C. micropapillatum* (Stossich, 1890) and *C. variegatum* as well as *C. rudolphii* (cf. Baruš *et al.*, 1978; Nagasawa *et al.*, 1998, 1999). Although there was no description of the shape of the distal end of the interlabium for *C. umiu*, the interlabium illustrated by Yamaguti (1941, Fig. 21) has a slightly bifurcated distal end. It is thus likely that *C. umiu* differs from *C. himeu* and belongs to a group of nematodes with bifurcated interlabia. Further study based on the type and additional specimens is required to elucidate the taxonomic relationship of *C. umiu* with others.

The intensity of infection with *C. himeu* in Japanese cormorants examined in the present study was high, ranging from 24 to 70 (mean 49.0) worms per infected host. Although the total wet weights of adult worms in individual hosts were quite low (0.8276–1.5977 g), their pathogenic effects on the host is likely severe, as reported by Sarashina *et al.* (1987) for *C. rudolphii* (as *C. spiculigerum*) in common cormorants, Greve *et al.* (1986) for *C. multipapillatum* in black pelicans (*Pelecanus occidentalis*), and Fagerholm *et al.* (1996) for *C. magnipapillatum* in black noddies (*Anous minutus*). Potential mortality of birds infected with nematodes of *Contracaecum* has been suggested (Fagerholm, 1996).

The observed differences in the body length and weight between both sexes of *C. himeu* manifest the sexual dimorphism, which is common in most nematodes. The differences are thought to have resulted from different growth rates of individual sexes, particularly associated with the development of sexual organs and the creation of their products. For some nematodes, differences in relative abundance of amino acids between sexes have been reported as one aspect of the sexual dimorphism

(Baruš *et al.*, 1998).

In summary, *C. himeu* is taxonomically a valid species and definitely differentiated from a closely related species, *C. rudolphii*. *Contracaecum himeu* is redescribed, and the shape of the interlabium, i.e., a relatively massive construction and a markedly rounded distal end, is an important feature for differentiation.

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