

**On Three Species of the Microphallid Metacercariae
Found from a Crab, *Hemigrapsus penicillatus*
with Descriptions of Two New Species
(Trematoda : Microphallidae)**

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It is well known that the microphallid metacercariae parasitize in the decapod crustaceans. We recently examined a number of marine or brackish water crabs, *Hemigrapsus penicillatus* (de Haan) collected in Kunisaki-cho, Oita Prefecture, at the eastern tip of the Kunisaki Peninsula facing to the Japan Inland Sea, and found that those crabs harbored three species of trematode metacercariae in their hepatopancreas and pericardiac tissues. A few days after feeding those metacercariae to kittens, albino rats, and new born chickens, several gravid adult worms of three species of the microphallid trematodes were recovered in small intestines of some of those experimental host animals by autopsy. One species of them is identical to *Levinseniella conicostoma* Bridgman *et al.*, 1972, which has been recently described from Shikoku, and other two are new species of the genera *Maritrema* and *Spelotrema* as described hereinafter.

Materials and Methods

Examination of crabs

All the crabs were collected by hand near the mouth of a small river in Kunisaki-cho, Higashikunisaki-gun, Oita Prefecture, and carried to our laboratory. Those crabs were usually found from beneath small stones in shallow pools of the river. After its transverse length was measured, the dorsal shell

was removed and the viscera such as gill, hepatopancreas, gonad, pericardiac tissues, etc. were separately examined by compression between two glass plates. Metacercariae discovered were accumulated in 0.85% saline and a part of them were subjected to the morphological observations. The rest metacercariae and several unexamined crabs were directly fed to 2 kittens, 6 albino rats, and 6 new born chickens (White Leghorn). Various hours after feeding, these animals were autopsied and their digestive tracts were dissected in saline for the recovery of the grown worms.

Preparation of the adult worms

All the adult worms obtained from the experimental hosts were fixed with Bouin's fluid, stained with alum carmine, and mounted *in toto* with Eukitt.

Results and discussion

Detection of the metacercaria

The crabs were collected four times from August to November, 1971, and identified as *Hemigrapsus penicillatus* (de Haan). They harbored three kinds of the metacercariae which were distinguishable to one another by their sizes, structure of cyst membranes, and habitats in the host crab. Then these three species were tentatively called A, B, and C in order of their sizes. Both the largest species (A) and the medium-sized one

Contributions to the trematode fauna of Japan-IV

(B) were mainly found in pericardiac tissues and gonads, and their discrimination was somewhat difficult only by a glance of the viscera between the glass plates with naked eyes though it was considerable that the cyst wall of the species A was composed of 3 layers whereas that of the species B was of 2 layers by the microscopic observation. Results of the examinations of the crabs are summarized in Table 1. Some crabs were excluded from the examination as they were preserved in alcohol or directly fed to the experimental hosts. Totally 75.5% (77/102) of the crabs examined were found to be parasitized with these metacercariae.

Morphological observation on the metacercaria

The metacercariae obtained are shown in Fig. 1.

Species A. Mainly parasitic in gonad and/or pericardiac tissues. Spherical, 420–430 μ in diameter. Cyst wall composed of three membranes; the outer one fibrous, 12–15 μ , the middle one hyaline, 15–20 μ , and the inner one thin, 8–9 μ , and 30–40 μ in total thickness. In the encysted metacercaria, conical oral sucker, elliptical acetabulum, and excretory bladder variable in shape conspicuous. Excysted metacercaria quite identical with *Levinseniella conicostoma* Bridgman, Otagaki, Shitanda, et Tada, 1972.

Table 1 Parasitization of the microphallid metacercariae in a crab (*Hemigrapsus penicillatus*) collected in Oita Prefecture (1971)

Date of collection	Length of dorsal shell (mm)	examined	No. of crabs parasitized with							Total
			A only	B only	C only	A & B	A & C	B & C	A, B, & C	
Aug. 29	Less than 16	30	2	3	7	1	6		1	20
	16–20	3	1	2						3
	More than 20	4		1	2					3
	Total	34	3	6	7	3	6		1	26
Other 4 exx. not examined (immersed in spirit for specimens)										
Sep. 6	Less than 16	3			3					3
	16–20	26			17		2	3		22
	More than 20	8			4		1	1		6
	Total	37			24		3	4		31
Oct. 10	Less than 16	6				2	2			4
	16–20	12			3	2	3	2		10
	More than 20	1			1					1
	Total	19			4	4	5	2		15
Nov. 21	Less than 16	4					1			1
	19–20	4			1		3			4
	More than 20	1								0
	Total	9			1		4			5
Other 50 exx. not examined (directly fed to 3 albino rats and 5 chickens)										
Grand total		102	3	6	36	7	18	6	1	77

A: Largest species, *Levinseniella*. B: Medium-sized species, *Maritrema*.

C: Smallest species, *Spelotrema*.

Parasitization rates: A: 29/102(28.4%), B: 20/102(19.6%), C: 61/102(59.8%).

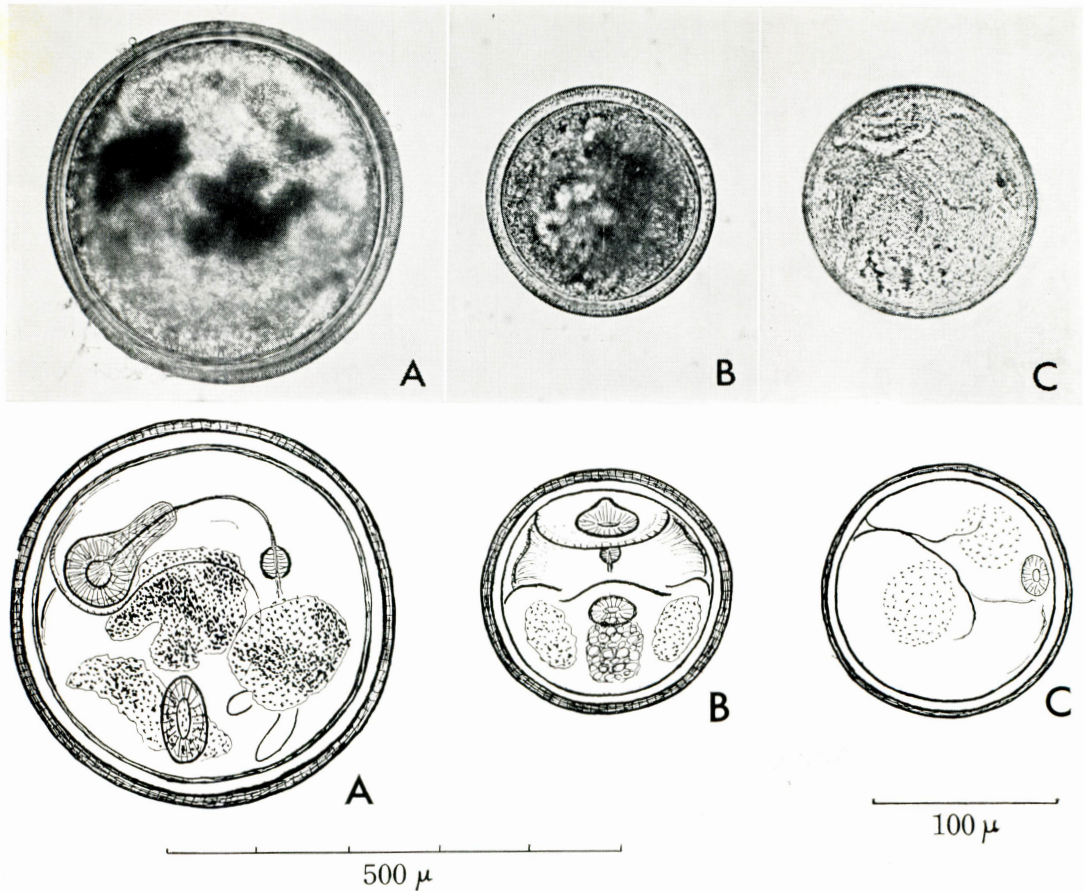


Fig. 1 Metacercariae found from *Hemigrapsus penicillatus*.

- A. Species A, *Levinseniella conicostoma* Bridgman et al.
 B. Species B, *Maritrema longiforme* sp. nov.
 C. Species C, *Spelotrema macrorchis* sp. nov.

Maximum number in a single host was 20.

Species B. Mainly parasitic in gonad or neighboring tissues. Spherical, 270–275 μ in diameter. Cyst wall composed of two membranous layers; the outer one fibrous, the inner hyaline, both 10–12 μ and 20–22 μ in total thickness. Both of elliptical suckers conspicuous, excretory bladder elliptical, longitudinally elongated. Testes also finely conspicuous. Excysted metacercaria characteristic in the distribution of vitellaria encircling the genital organs in the posterior half of the body and easily identified as *Maritrema* sp.

Maximum number in a single host was 14.

Species C. Parasitic in hepatopancreas. Spherical, 135–140 μ in diameter. Cyst wall composed of two thin membranous layers; the outer one fibrous, 2–3 μ , and the inner one hyaline, 5–7 μ , and 5–8 μ in total thickness. Only oral sucker conspicuous, other organs generally inconspicuous except for testes finely visible.

Maximum number in a single host was 118.

Recovery of adults from the experimental hosts

Out of 14 animals fed with the metacercariae, 1 kitten, 2 albino rats, and 1 chicken which were autopsied between 22 and 44 hours after feeding were found to harbor grown adult worms in their small intestines.

Table 2 Number of adult worms recovered from experimental hosts.

Host	No.	Duration till autopsy (hrs)	No. of worms recovered			Total
			<i>Levinseniella</i>	<i>Maritrema</i>	<i>Spelotrema</i>	
Kitten	1	22, 1*	2(1)			2(1)
	2	140				0
Albino rat	1	24	7	2	4	13
	2	40, 19, 17*	6		1	7
	3	115, 88, 86*				0
	4	168				0
	5	"				0
	6	"				0
Chicken	1	19				0
	2	44				0
	3	44, 24*	3			3
	4	52, 32*				0
	5	76, 56*				0
	6	96				0
Total			18	2	5	25

* Metacercariae were fed two or three times.

In the parentheses shows the number of unexcysted metacercaria found from stomach.

Other 10 animals which were sacrificed earlier or later than the above-mentioned durations of the infection were not parasitized. Results are shown in Table 2, in which the hosts are arranged in order of the lengths of the durations. No. 1 of the kittens harbored 2 mature but ungravid adults of *Levinseniella* in small intestine and 1 unexcysted metacercaria of the same species in stomach. The adults apparently originated from those fed 22 hours before and the unexcysted metacercaria was from those fed 1 hour before the autopsy. No. 1 of the albino rats harbored 13 worms of all the three species. From these materials, the species A, B, and C in the metacercarial stage are identified as *Levinseniella conicostoma*, *Maritrema* sp., and *Spelotrema* sp., respectively. *Maritrema* was more abundant than *Levinseniella* in the metacercarial stage but only 2 adults of the former were recovered from the experimental hosts whereas 18 of the latter were obtained. All the adults had a few eggs in their uterine ducts. This may depend on short durations of the infection

till the autopsy of the hosts. In adequate natural hosts, these adults may be able to parasitize for more prolonged durations and to contain much more eggs so as to fill up their hindbody.

Adult

All the adult worms were mounted *in toto*. The species A is quite identical with *Levinseniella conicostoma* Bridgman *et al.* of which the metacercaria is known to be parasitic in the same crab also in Kagawa Prefecture, Shikoku, and the adult was found from knot and curlew. This is harbored by all the experimental host species in the present study. The species B was found only in the albino rat No. 1 (2 exx. only) and is a new species of the genus *Maritrema*. The species C was found from the albino rats Nos. 1 and 2 (5 exx. together) and is also a new species belonging to the genus *Spelotrema*.

Description of the adults

Levinseniella conicostoma Bridgman, Otogaki, Shitanda, et Tada, 1972

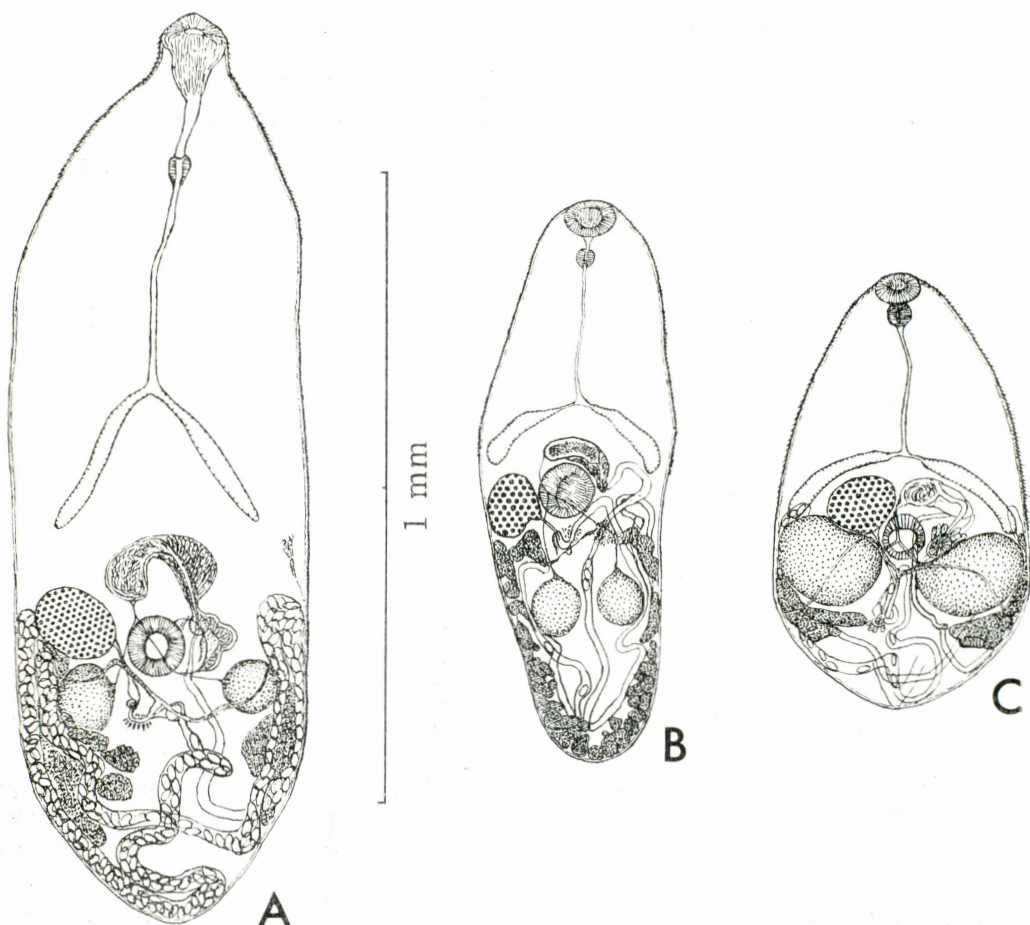


Fig. 2 Adults of the microphallid trematodes obtained from the albino rat no. 1 (exp. host), ventral view.

- A. *Levinseniella conicostoma* Bridgman *et al.* (slide no. 247)
 B. *Maritrema longiforme* sp. nov., holotype (slide no. 256-b)
 C. *Spelotrema macrorchis* sp. nov., holotype (slide no. 257-a)

Shikoku Christ. Coll. Treat. 23: 46, fig. 5, 1972

In the present feeding experiment, totally 18 specimens were obtained; 2 exx. from a kitten (No. 1), 7 and 6 exx. from 2 albino rats (Nos. 1 and 2), and 3 exx. from a chicken (No. 3) as shown in Table 2. All specimens were gravid with comparatively many eggs and are quite identical with the headlined species. This was originally described with 20 specimens obtained from *Calidris canutus* and *Numenius madagascariensis* collected in Kagawa Prefecture, Shikoku. Because the original description is so excellent that the diagnostic

characters are not recapitulated here. Only the result of our measurements is compared with the original one as shown in Table 3. Though both two specimens obtained from the kitten were ungravid, they were equally measured because they seemed to be full grown from the sizes of body and other organs.

Distribution: Shikoku (Kagawa Pref.) (*loc. cit.*) and Kyushu (Oita Pref.) (new record).

Definitive hosts: *Calidris canutus* Linnaeus (probably subsp. *rogersi* (Mathews)), *Numenius madagascariensis* (Linnaeus), mouse (ICR strain) (exp.), albino rat (Wister strain) (exp.)

Table 3 Comparison of measurements in *Levinseniella conicostoma*

(mm)

Authority	Bridgman et al., 1972		Present record
Locality	Kagawa Pref., Shikoku		Oita Pref., Kyushu
No. of specimens examined	20 (from <i>Calidris canutus</i> and <i>Numenius madagascariensis</i>)		18 (2 from a kitten, 13 from 2 albino rats, and 3 from a chicken, exp.)
Body	(L 0.0800 (sic!) -1.120 (0.942) W 0.235 -0.280 (0.250)		0.940-1.450 0.270-0.470
Oral sucker	(L 0.1125-0.1350 (0.1227) W 0.0650-0.0825 (0.0770)		0.095-0.160 0.070-0.110
Prepharynx	L 0.0250-0.0675 (0.0480)		0.017-0.136
Pharynx	(L 0.0300-0.0375 (0.0333) W 0.0250-0.0325 (0.0297)		0.023-0.047 0.025-0.043
Oesophagus	L 0.225 -0.350 (0.274)		0.250-0.400
Ceca	L 0.150 -0.180 (0.178)		0.160-0.290
Acetabulum	D 0.0660-0.0725 (0.0670)		(L 0.065-0.110 W 0.050-0.100
Testes	(L 0.0425-0.0750 (0.0608) W 0.0625-0.0900 (0.0762)	(Left Right	(L 0.060-0.170 W 0.060-0.120 L 0.055-0.120 W 0.055-0.130
Ovary	(L 0.0450-0.0625 (0.0527) W 0.0550-0.0950 (0.0725)		0.060-0.130 0.065-0.135
Uterine eggs	(L 0.0225-0.0250 (0.0237) W 0.0125-0.0150 (0.0141)		0.021-0.028 0.014-0.018
Metacercaria	D 0.390 -0.415		0.420-0.430
Thickness of outer layer	0.0190-0.0250		0.012-0.015
middle layer	0.0050-0.0075		0.015-0.020
inner layer	0.0010-0.0020		0.008-0.009

D: Diameter L: Length W: Width

(*loc. cit.*); *Felis catus* Linnaeus (juvenile) (exp.), and *Gallus gallus v. domesticus* (Brisson) (white leghorn, juvenile) (exp.) (new records).

Habitat: Small intestine.

..First intermediate host: Unknown.

Second intermediate host: *Hemigrapsus penicillatus* (de Haan) (*loc. cit.*)-habitat: hepatopancreas (*loc. cit.*) and pericardiac tissues and gonad (new records).

There are two species of the genus *Levinseniella* in Japan and those are distinguished by the following key.

Key to the Japanese species of the genus *Levinseniella* Stiles et Hassall

1. Oral sucker ordinary, not protruded anteriorly. Ceca longer than esophagus
.....*L. bucephalae* (Yamaguti, 1935)
Oral sucker distinctly protruded anteriorly,

cone-shaped. Ceca shorter than esophagus.
.....*L. conicostoma* Bridgman et al., 1972

Remarks: There is another apparently distinguishable unnamed species reported by Miyazaki from Japan. It is allied to *L. bucephalae* but differs from the latter by its larger sizes and more elliptical shapes of testes and ovary. Komiya (1965) transferred this species to the genus *Gynaecotyla* but it is incorrect. He may be affected by the transfer of *Levinseniella squatarolae* to *Gynaecotyla* by Yamaguti (1939). *G. squatarolae* and *Levinseniella* sp. of Miyazaki are undoubtedly not congeneric.

Maritrema longiforme sp. nov.

Measurements are based on two gravid specimens obtained from the albino rat No.

1, which was autopsied 24 hours after feeding the metacercariae. No other animals harbored this species.

Definitive host: albino rat (Wister strain) (exp.).

Habitat: Small intestine.

Body elongate fusiform, 0.89–1.04 mm in length, broadest at level of cecal terminations, 0.25–0.405 mm. Integument of the anterior half of body spined. Oral sucker elliptical, broader than long, $60 \times 65\text{--}80 \mu$. Prepharynx short but conspicuous, $21\text{--}25 \mu$ in length. Pharynx globular, $26\text{--}29 \times 22\text{--}27 \mu$. Esophagus slender, long, $210\text{--}270 \mu$ in length. Ceca shorter than esophagus, $160\text{--}210 \mu$ in length; cecal terminations located at level of the anterior margin of acetabulum. Acetabulum larger than oral sucker, $105\text{--}125 \times 85\text{--}90 \mu$, located medially in the middle of body. Ovary elliptical, near the right lateral margin of body and at just side of acetabulum, $100\text{--}110 \times 70\text{--}85 \mu$. Testes symmetrical, elliptical, almost equal to each other in size and shape, left testis $100\text{--}110 \times 85\text{--}90 \mu$, and right one $90\text{--}100 \times 80\text{--}90 \mu$. Cirrus pouch arched, anteriorly convex, located just anterior to acetabulum. Vitellaria distributed near the margin of hindbody and encircling the uterine and testicular area, not closing at the anterior part of it on the median line. Egg small, elliptical, $23\text{--}29 \times 11\text{--}14 \mu$, operculated.

This is allied to but distinguishable from the following congeneric species: *M. linguilla* Jägerskiöld by its much smaller size; *M. opisthometra* Lenov by its much larger cirrus pouch and testes; long prepharynx, and location of ovary being behind acetabulum; *M. prosthometra* Deblock et Heard by its long prepharynx and ceca, elongated cirrus pouch, and large testes; *M. setoense* Bridgman by its long prepharynx and large cirrus pouch; and *M. laricola* Ching by its small size, long prepharynx, anteriorly distributing vitellaria.

Type materials: 1 holotype (Fig. 3B) and 1 paratype (slide no. 256).

Type depository: The type materials are preserved in the Department of Parasitology, Kurume University School of Medicine.

There are at least 7 species of the genus *Maritrema* in Japan and those are distinguished by the following key.

Key to the Japanese species of the genus
Maritrema Nicoll

1. Ovary more or less lobed. 2
– Ovary not lobed, usually elliptical. 4
2. Posterior part of vitellaria not interrupted medially. *M. eroliae* Yamaguti, 1939
Posterior part of vitellaria interrupted medially. 3
3. Large species, body length more than 1.0 mm. Metacercaria parasitic in crab.
. *M. urayasense* Ogata, 1951¹⁾
– Small species, body length less than 0.6 mm. Metacercaria parasitic in shrimp.
. *M. kitanense* Shibue, 1953
4. Body pyriform, testes separated to each other by a distance longer than their own diameter.
. *M. macravestibulum* Ogata, 1947²⁾
– Body elongated, longer than twice of breadth. 5
5. Prepharynx conspicuous; vitellaria completely encircling testicular and uterine area. 6
– Prepharynx short; vitellaria clearly interrupted in the anterior part.
. *M. longiforme* sp. nov.
6. Ovary at the right side of acetabulum and subequal to testes in size.
. *M. setoense* Bridgman, 1971
– Ovary behind acetabulum and much larger than acetabulum; testes greatly larger than ovary.
. *M. opisthometra* Leonov, 1958³⁾

***Spelotrema macrorchis* sp. nov.**

Measurements and description are based

- 1) This was synonymized with *M. eroliae* Yamaguti by Bridgman (1971). We agree with his synonymization but reserve to seject this species in the present paper.
- 2) This has been often incorrectly cited as 1946 by many authors including Ogata himself, but the publication date on the cover of the reprint is read as 30 March 1947.
- 3) This has been added to the Japanese fauna by Bridgman *et al.* (1972).

on five specimens obtained from the albino rats nos. 1 and 2, which were autopsied 24 and 17-19-40 hours after feeding the metacercariae. No other animals harbored this species.

Definitive host: albino rat (Wister strain) (exp.).

Habitat: Small intestine.

Body oval to fusiform, 0.61-0.735 mm in length; broadest at level of testis, 0.36-0.42 mm in breadth. Integument of the anterior two-thirds of body spined. Oral sucker terminal, elliptical, slightly transversely elongated, 50-70×70-80 μ . Prepharynx short, occasionally inconspicuous, 0-7 μ in length. Pharynx slightly transversely elongated, elliptical, 25-32×36 μ . Esophagus slender, long, 140-190 μ in length. Ceca slightly longer than esophagus, 190-200 μ in length, slightly anteriorly anteriorly convex. Acetabulum subequal to oral sucker, 60-70×65-75 μ . Testes symmetrical, large, elliptical, obliquely situating in the third quarter of the body; right testis 110-150×90-170 μ ; left testis 130-165×100-170 μ . Genital pore cuticularized so as to form a sucker-like structure, 29-34×36-38 μ . Ovary elliptical, dextroanterior to acetabulum, 70-95×70-120 μ . Seminal vesicle elliptical, sinistroanterior to acetabulum, 60-77×27-41 μ . Prostatic cells not so numerous, located near genital pore. Uterus occupying the posterior space of hindbody; a part of it distributed a little front of testis through a narrow gap between testis and lateral margin of body in both sides. A few eggs contained in uterus probably because of a short duration of the experimental infection. Egg small, elliptical, 21-25×9-11 μ , operculated.

This is allied to *S. pygmaeum* (Levinsen), the generotype, but differs from it by large sizes of body and testes, on the contrary small seminal vesicle.

Type materials: 1 holotype (Fig. 3C) (slide no. 257) and 4 paratypes (slide nos. 257-259; each slide contains two specimens together).

Type depository: The type materials are preserved in the Department of Parasitology, Kurume University School of Medicine.

According to Yamaguti (1971), there have

been at least 3 species and 1 subspecies of the genus *Spelotrema* in Japan. Adding the species described in the present paper, the 5 species of the genus occurring in Japan are distinguishable by the following key.

Key to the Japanese species of the genus
Spelotrema Jägerskiöld

1. Seminal vesicle very large, larger than acetabulum, almost completely occupying the intercecal space in front of acetabulum. 2
 - Seminal vesicle subequal to acetabulum in size. 3
2. Ovary transversely elongated.
 - *S. capellae* Yamaguti, 1939
 - Ovary almost circular.
 - *S. asadai* (Kaji, 1957)¹⁾
3. Body elongated, prepharynx longer than the half length of esophagus.
 - *S. longicollis* Yamaguti, 1939
 - Body pyriform, prepharynx short. Anterior part of uterus reaching in front of testes, overlapping the cecal terminations. 4
4. Moderate-sized species, length about 0.7 mm. Metacercaria parasitic in crab.
 - *S. macrorchis* sp. nov.
 - Small species, length less than 0.6 mm. Metacercaria parasitic in shrimp.
 - *S. minus* (Ochi, 1928)

1) This species was originally described as *Microphallus minus* var. *asadai* by Kaji (1957). The nominotypical species, *M. minus*, was later transferred to the present genus, *Spelotrema*, by Yamaguti (1958), and subsequently var. *asadai* was also transferred to the same genus with an alteration to the subspecific category of *S. minus* by Yamaguti (1971). According to the original description of the variety, however, it is sympatric with the nominotypical species and has enough differences to be separable specifically with the latter. We, therefore, elevate the variety (or subspecies by Yamaguti) to the specific rank as follows.

Spelotrema asadai (Kaji, 1957)

Microphallus minus var. *asadai* Kaji, 1957

Tokyo Iji Shinshi, 74: 270, figs. 1-2.

Spelotrema minus asadai Yamaguti, 1971

Synopsis of digenetic trematodes of vertebrates, 1: 781.

Summary

Three species of the microphallid trematodes are obtained from a few experimental host animals orally infected with metacercariae found from a crab, *Hemigrapsus penicillatus* (de Haan), collected in Kunisaki-cho, Oita Prefecture, Kyushu. Two of them are new to science and described on the basis of several specimens obtained from albino rats under the names of *Maritrema longiforme* sp. nov. and *Spelotrema macrorchis* sp. nov. Another one is identical as *Levinseniella conicostoma* Bridgman *et al.*, 1972. This is a new distributional record and the domestic cat (kitten) and the new born chicken (White Leghorn) are added as its experimental hosts.

M. longiforme is closely related to *M. setoense* Bridgman, 1971, but differs from it by elongated body, anteriorly interrupted vitellaria, short prepharynx, etc. *S. macrorchis* is allied to the generotypical species, *S. pygmaeum* (Levinsen, 1881), but easily distinguished with large sizes of body and testes. Keys to the Japanese species of the above-mentioned three genera are given at the ends of the descriptions of each species. *Spelotrema minus asadai* (Kaji, 1957) is elevated to the specific rank because it is quite sympatric to and different from the nominotypical species, *S. minus* (Ochi, 1928).

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ケフサイソガニに見出された新種を含む Microphallidae 被囊幼虫 3 種について

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大分県東国東郡国東町で採取したケフサイソガニ *Hemigrapsus penicillatus* (de Haan) に見出した被囊幼虫を数種の実験動物に経口感染させて Microphallidae 吸虫 3 種の成虫を得た。うち 2 種は新種と認められたので、ラットより得た標本をもとに、それぞれ *Maritrema longiforme* および *Spelotrema macrorchis* と命名記載した。残る 1 種は最近香川県から発見された *Levinseniella conicostoma* Bridgman et al., 1972 と同定されたが、大分県は新しい分布記録となり、また同時に子猫と初生雛 (白色レグホン) とが新しい実験の終宿

主として追加された。

M. longiforme は *M. setoense* Bridgman, 1971 に似るが、体形はより細長く、卵黄腺は前半中央部で明瞭に分離すること、前咽頭が甚だ短いことなどで区別できる。*S. macrorchis* は属の模式種 *S. pygmaeum* (Levinsen, 1881) に似るが、はるかに大型で、体内各器官とくに睾丸が著しく大きいことで容易に区別できる。なお上記 3 属の邦産種のための検索表をそれぞれの種の記載の末尾に添えた。また *Spelotrema minus asadai* (Kaji, 1957) を種に昇格せしめた。