藤女子大学·藤女子短期大学紀要,第24号,第II部:95-120.昭和61年. Bull. Fuji Women's College, No. 24, Ser. II:95-120. 1986.

## REDESCRIPTION OF *PHAGOCATA KAWAKATSUI* OKUGAWA, 1956, WITH SPECIAL REFERENCE TO LOCAL VARIATION OF ITS COPULATORY APPARATUS

(Turbellaria, Tricladida, Paludicola)

by

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#### INTRODUCTION

The original description of *Phagocata kawakatsui* OKUGAWA, 1956, was based upon material collected from a spring-fed stream on the grounds of KAWAKATSU's country residence located in the northern part of the City of Kameoka in Kyôto Prefecture, of the Kinki Region in Honshû, Japan. OKUGAWA (1956) emphasized in his original description that this small-sized, pigmented species with two eyes is unique in having a rounded, thick-walled vagina (i. e., the presence of a mass of the thick musculature on the vagina).

During the past 25 years, *Phagocata kawakatsui* has been collected occasionally from many localities in the following areas: Kagawa Prefecture in Shikoku (cf. Uematsu, 1965; Kawakatsu & Okafuji, 1965; Kawakatsu & Iwaki, 1967; Kawakatsu & Ôgawara, 1968 a, b); Okayama Prefecture in the Chûgoku Region, Honshû (cf. Miyazaki, Kawada & Nishi, In press); Hyôgo and Kyôto Prefectures in the Kinki Region, Honshû (cf. Kawakatsu, Ôgawara & Tarui, 1967; Kawakatsu & Iwaki, 1967; Kawakatsu & Ôgawara, 1968 a, b; Nakano, Yamazaki & Mukaibara, 1976); Ishikawa Prefecture in Hokuriku in the Chûbu Region, Honshû (cf. Kawakatsu & Iwaki, 1967; Kawakatsu, Murayama, Ôgawara & Kishida, 1971); Nagano Prefecture in the Chûbu Region, Honshû (cf. Okugawa & Kawakatsu, 1957; Hara, 1970, 1973 a, b, 1974; Nîmura & Hara, 1975; Nîmura, 1975; Kawakatsu, Nîmura & Aoki, 1975).

KAWAKATSU & IWAKI (1967), who studied the comparative morphology of the animals from 4 localities (Takamatsu in Kagawa Pref., Kameoka in Kyôto Pref., Kanazawa in Ishikawa Pref., and Matsumoto in Nagano Pref.), proved that animals from different localities show some variation in minute details of the copulatory apparatus. After the publication of the paper cited above, additional serial sections of *Phagocata kawakatsui* from various localities were prepared by KAWAKATSU. Close examination of these slides revealed that the degree of the musculature on the vagina in this species shows a rather wide anatomical and histological variations according to locality.

Ecology and life history of *Phagocata kawakatsui* observed in 2 localities (Kameoka and Matsumoto localities) were reported by KAWAKATSU & IWAKI (1967). Recently, AIKAWA & SHIMOZAWA (1986) reported the frequency of occurrence of supernumerary eyes and breeding of this species from the Komatsubara locality in the City of Nagano.

Chromosome numbers of the Matsumoto specimens of *Phagocata kawakatsui* were reported at first by Teshirogi, Nîmura, Ishida & Hasebe (1978) as n = 12 and 2n = 24. Later, Teshirogi, Ishida & Nîmura (1979) reported the chromosomal variations of this species in neoblasts of regenerating pieces (see also Teshirogi & Ishida, 1981); they also reported on regeneration capacity. Recently, Oki and Tamura studied the karyotype of *Phagocata kawakatsui* based upon animals from the Hiruzen locality in Okayama

Prefecture.

The purpose of the present paper is primarily to present a detailed redescription of *Phagocata kawakatsui* using materials from as many localities as possible and giving a new specific definition of this species. Secondly, the karyological description of the species is also given based upon the new material.

#### MATERIALS AND METHODS

The following materials were examined. The numbers designating each sample are those employed by KAWAKATSU in his permanent recording system. Serial sagittal sections (7–8 micrometers) were stained with Delafield's hematoxylin and erythrosin. Some specimens were fed chicken spleen stained with Chinese ink-stick fluid and fixed with Nozawa's fluid; these were prepared as whole mounts (KAWAKATSU's method for tracing the digestive system). For chromosomal examination, usual the squash method was employed (cf. OKI, TAMURA & KAWAKATSU, 1976; and others).

- 1) KAWAKATSU'S Specimen Lot No. 115. Many sexual and asexual specimens fixed with Bouin's fluid and cocoons. Locality: A shallow spring-fed stream at Bukuden (KAWAKATSU'S country residence is there), Hongô, Minoda, Asahi-chô, Kameoka, Kyôto Pref. (the type locality) (alt. 140 m). March 13–20, 1955 (water temp., 8.0 ~ 8.2°C). Coll. M. KAWAKATSU. Cf. OKUGAWA (1956); ICHIKAWA & KAWAKATSU (1961, pl. II, fig. 9); KAWAKATSU & IWAKI (1967, pp. 218–223, figs. 5 and 6); KAWAKATSU, ÔGAWARA & TARUI (1967 b); MIYAZAKI & KAWAKATSU (1980, cover page).
- 2) KAWAKATSU'S Specimen Lot No. 210. Many sexual and asexual specimens fixed with Bouin's fluid. Locality: A spring-fed stream at Genchi, Matsumoto, Nagano Pref. (the type locality of *Dugesia japonica japonica* ICHIKAWA et KAWAKATSU, 1964) (alt. 600 m). March 16, 1957 (water temp., 10.6°C). Coll. M. KAWAKATSU. Cf. ICHIKAWA & KAWAKATSU (1961, pl. II, fig. 10); KAWAKATSU & IWAKI (1967, pp. 220–223, fig. 7).
- 3) KAWAKATSU'S Specimen Lot No. 436. A number of sexual specimens fixed with Bouin's fluid. Locality: The Suribachi-dani River, Ubaga'ike, Miyawaki-chô, Takamatsu, Kagawa Pref. (alt. 10 m). January 8, 1965. Coll. Dr. T. UEMATSU. Cf. KAWAKATSU & OKAFUJI (1965).
- 4) KAWAKATSU's Specimen Lot No. 445. Three sexual specimens (whole mounts). Locality: See Station No. 3.
- 5) KAWAKATSU'S Specimen Lot No. 570. A single sexual specimen fixed with Bouin's fluid. Locality: A stream at Mt. Tomuro-yama in the vicinity of Kanazawa, Ishikawa Pref. (alt. 350 m). March, 1966 (water temp., 11.0 ~ 12.0°C). Coll. Dr. Y. KISHIDA. Cf. KAWAKATSU, MURAYAMA, ÔGAWARA & KISHIDA (1971).
- 6) KAWAKATSU'S Specimen Lot No. 1250. A few sexual and asexual specimens fixed with Boiun's fluid. Locality: A spring-fed stream at Misono, Ina, Nagano Pref. (alt. 670 m). May 5, 1974 (water temp., 12.8°C). Coll. Mr. F. Nîmura. Cf. Nîmura & Hara (1975); Kawakatsu, Nîmura & Aoki (1975).
- 7) KAWAKATSU'S Specimen Lot No. 1251. A number of sexual specimens fixed with Bouin's fluid. Locality: Very near to the Station No. 2. May 5, 1974 (water temp., 13.0°C). Coll. Mr. F. Nîmura. Cf. Nîmura (1975); Nîmura & Hara (1975); Kawakatsu, Nîmura & Aoki (1975).
- 8) KAWAKATSU'S Specimen Lot No. 1254. A number of sexual specimens fixed with Bouin's fluid. Locality: A spring at Babadaira, Takagi-mura, Shimo-Ina-gun, Nagano Pref. (alt. 400 m). June 15, 1974 (water temp., 13.5°C). Coll. Mr. F. Nîmura. Cf. Nîmura & Hara (1975); Kawakatsu, Nîmura & Aoki (1975).
  - 9) KAWAKATSU's Specimen Lot No. 1262. A number of sexual specimens fixed with Bouin's

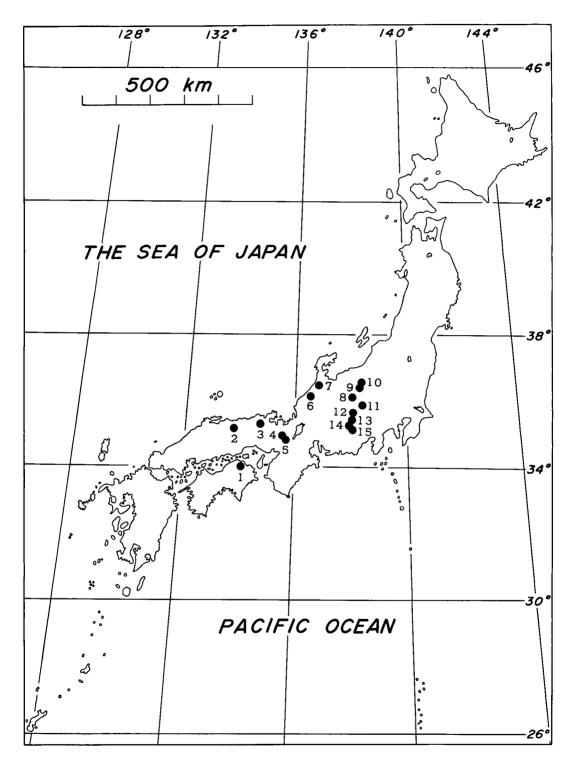


Fig. 1. Sketch-map of Japan, showing the localities of *Phagocata kawakatsui* (see also KAWAKATSU & IWAKI, 1967, p. 223, fig. 8; KAWAKATSU, 1969, pp. 56-57, fig. 5). 1, Takamatsu in Kagawa Pref., Shikoku; 2, Hiruzen in Okayama Pref., Honshû; 3, Toyo'oka in Hyôgo Pref., Honshû; 4, Kameoka in Kyôto Pref., (Continued on page 98.)

- fluid. Locality: A spring at Nishijô, Matsushiro-machi, Nagano Pref. (alt. 410 m). July 31, 1973 (water temp., 13.0°C). Coll. Mr. F. Nîmura. Cf. Nîmura & Hara (1975); Kawakatsu, Nîmura & Aoki (1975).
- 10) KAWAKATSU'S Specimen Lot No. 1269. A number of sexual specimens fixed with Bouin's fiuid. Locality: Gongawa-Shimizu Spring at Zakôji, Îda, Nagano Pref. (alt. 420 m). July 15, 1974 (water temp., 13.5°C). Coll. Mr. F. Nîmura. Cf. Nîmura & Hara (1975); Kawakatsu, Nîmura & Aoki (1975).
- 11) KAWAKATSU'S Specimen Lot No. 1280. Many sexual and asexual specimens fixed with 75% methanol solution. Locality: A shallow pool at Myôrakuji, Toyo'oka, Hyôgo Pref. (alt. 40 m). November 20, 1974 (water temp., 11.0°C). Coll. Messrs. M. NAKANO, Y. YAMAZAKI and Y. MUKAIBARA. Cf. NAKANO, YAMAZAKI & MUKAIBARA (1976, pp. 347-350, fig. 2).
- 12) KAWAKATSU'S Specimen Lot No. 1283. A number of sexual specimens fixed with Bouin's fluid. Locality: See Station No. 11. December 22, 1974 (water temp., 10.5°C). Coll. Messrs. M. NAKANO and N. TAKAHASHI. Cf. NAKANO, YAMAZAKI & MUKAIBARA (1976, pp. 347-350, fig. 2).
- 13) KAWAKATSU'S Specimen Lot No. 1284. A number of sexual and asexual specimens fixed with 75% methanol solution. Locality: A narrow stream running through the Cemetery of the Kyôgoku's (alt. 20 m); Very near to the St. No. 12. December 24, 1974 (water temp., 9.5°C). Coll. Messrs. M. NAKA. NO, Y. YAMAZAKI and Y. MUKAIBARA. Cf. NAKANO, YAMAZAKI & MUKAIBARA (1976, pp. 347-350, fig. 2).
- 14) KAWAKATSU'S Specimen Lot Nos. 1285 and 1289. A number of sexual and asexual specimens fixed with 75% methanol solution. Locality: An old, unused well at Mr. Tomoda's residence (alt. 20 m); Very near to the Station Nos. 11–13. March, 10, 28, and April 2, 1975 (water temp., 6.9 ~ 7.5°C). Coll. Messrs. M. NAKANO, Y. YAMAZAKI, Y. MUKAIBARA, and H. MURAYAMA (on Apr. 2). Cf. NAKANO, YAMAZAKI & MUKAIBARA (1976, pp. 347–350, fig. 2).
- 15) KAWAKATSU'S Specimen Lot No. 1291. A number of sexual specimens fixed with Bouin's fluid. Locality: A stream at Komatsubara, Nagano, Nagano Pref. (alt. 370 m). March 10, 1975 (water temp., 15.5°C). Coll. Mr. F. Nîmura. Cf. Kawakatsu, Nîmura & Aoki (1975).
- 16) KAWAKATSU'S Specimen Lot No. 1327. A number of sexual specimens fixed with Bouin's fluid. Locality: A spring-fed stream near the Hara-no-Chaya, Fujimi-machi, Chino, Nagano Pref. (alt. 960 m). August 10, 1975 (water temp., 10.8°C). Coll. Mr. F. Nimura. Cf. Kawakatsu, Nimura & Aoki (1975).
- 17) KAWAKATSU'S Specimen Lot No. 1815. A number of sexual specimens fixed with Bouin's fluid. Locality: A tributary of the Asahikawa River, Hiruzen Heights, Yatsuka-mura, Maniwa-gun, Okayama Pref. (alt. 450–520 m). December 29 and 30, 1985 (water temp., 7.8 ~ 9.0°C). Coll. Messrs. T. MIYAZAKI, H. KAWADA and H. NISHI. Cf. MIYAZAKI, KAWADA & NISHI (In press, fig. 1).

Live specimens from the Hiruzen locality sent to OKI and TAMURA by Mr. MIYAZAKI were used for chromosomal examination.

The location of each collecting site of Phagocata kawakatsui is shown in Figure 1.

Honshû (the type locality); 5, Saga in Kyôto, Kyôto Pref., Honshû; 6, Yamanakadani Hot Spring in Ishikawa Pref., Honshû; 7, Kanazawa in Ishikawa Pref, Honshû; 8, Matsumoto in Nagano Pref., Honshû (the type locality of *Dugesia japonica japonica*); 9, Matsuhiro in Nagano, Nagano Pref., Honshû; 10, Komatsubara in Nagano, Nagano Pref., Honshû; 11, Fujimi in Nagano Pref., Honshû: 12, Ina in Nagano Pref., Honshû (several localities); 13, Babadaira in Nagano Pref., Honshû (several localities); 14 and 15, Îda in Nagano Pref., Honshû (several localities).

#### SPECIES DESCRIPTION

# Order TRICLADIDA Suborder PALUDICOLA or PROBURSALIA Family Planariidae Stimpson, 1858 Genus *Phagocata* Leidy, 1847

#### Phagocata kawakatsui Okugawa, 1956

External features. This is a rather small, pigmented species with two eyes. The living, fully sexually mature specimens measure 10 to 12 mm in length and 1 to 2 mm in width. The external appearance of 2 live specimens from the Hiruzen locality is shown in Figure 2 (A and B); that of preserved specimens from 4 localities (Takamatsu, Hiruzen, Kanazawa, and Matsumoto) is also shown in Figure 2 (C-I). In life the body is dark gray to grayish brown in color; the ventral side has a lighter coloration than the dorsal. The head has a subtruncated form; the anterolateral corner is slightly projecting, but rounded (a pair of white, auricular sense organs can be seen) (Fig. 2 A and B). The preserved specimens sometimes show a slight protrusion at the central portion of the head (Fig. 2 C-J). Behind the head, the elongated body tapers to the rounded posterior end (Fig. 2 A and B).

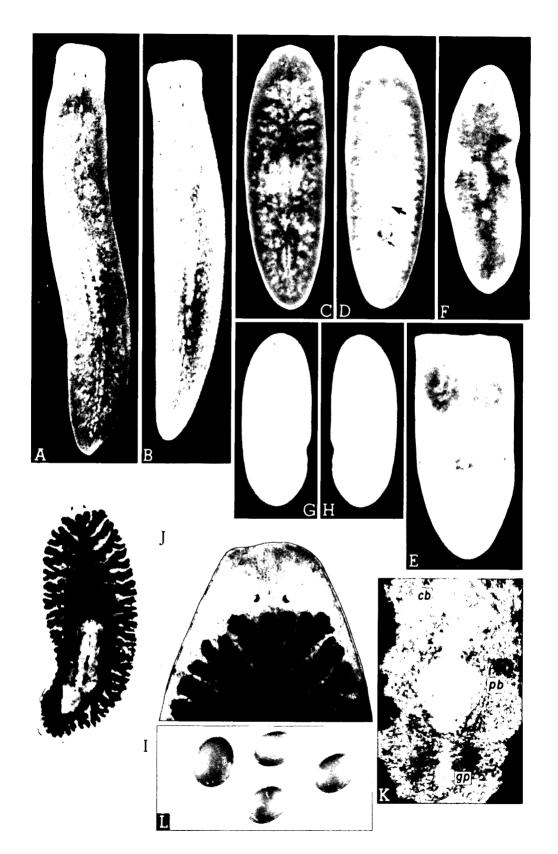
A pair of two eyes, each surrounded by a pigment-free ocular area, is situated on the dorsal side of the head; the distance between them is about one-fifth the width of the head at the level of eyes (Fig. 2 A, C, E-G, I, and J).

The pharynx is inserted somewhat behind the middle of the body and is nearly one-fifth as long as the body (Fig. 2 A-I). The copulatory organs occupy the anterior one-third of the postpharyngeal region (Fig. 2, B, D, E, H, and I). The genital pore opens at about the anterior one-third level of the postpharyngeal region (Fig. 2 B, D, E, H, and K).

The external characters of *Phagocata kawakatsui* (especially, the shape of the head and the position of eyes) have a great resemblance to those of the three Japanese *Phagocata* species: *Phagocata teshirogii* ICHIKAWA et KAWAKATSU, 1962, *Phagocata iwamai* ICHIKAWA et KAWAKATSU, 1962, and *Phagocata suginoi* KAWAKATSU, 1974. The last mentioned species, *Ph. suginoi*, seems to be a subterranean form with two small eyes and low pigmentation. The comparison of external morphology of these 4 species was discussed in detail in the previous paper (cf. KAWAKATSU, MURAYAMA & Nîmura, 1974, pp. 148–150, fig. 1 A-F).

Internal features. ... In histological sections, a distinct caved-in epithelium is found in the center of the ventral anterior tip of the body. The cells here are more flattened than other epithelial cells and without cilia. They are pierced by gland ducts, which provide a coarsely granular, erythrophilic secretion. In live specimens, this part is visible as a short, longitudinal stripe; it is also seen translucently from the dorsal side (Fig. 2 A and B). After preservation, the stripe can usually be seen when the samples are examined under a binocular microscope. This organ and its histological structure are very

<sup>1)</sup> The external appearance of *Phagocata kawakatsui* is also shown in the following papers: figures in OKUGAWA (1965, p. 319, fig. 34; 1970, p. 107, fig. 77C; 1973, p. 226, fig. 12-20 C); a painting in monocolor in KAWAKATSU (1968, p. 42, fig. 4); a painting in color in KAWAKATSU (1969, p. 89, pl. VII, fig. 4); a photograph in Teshirogi, Ishida & Nimura (1979, p. 192, fig. 1A); photographs in Aikawa & Shimozawa (1986, p. 265, fig. 4 A-E). A generalized figure of the genital organs presented by Teshirogi, Ishida & Nimura (1979, p. 193, fig. 2A) is concerned not very useful for taxonomy.



similar to those of *Phagocata vivida* (IJIMA et KABURAKI, 1916) (cf. KAWAKATSU, TESHIROGI & ISHIDA, 1982, pp. 35-36).

The pharynx is structurally typical of the genus *Phagocata* and of the family Planariidae; its inner musculature consists of two distinct layers, a thick circular layer adjacent to the epithelium of the pharynx lumen and a thinner layer of longitudinal fibers. The outer pharyngeal musculature consists of longitudinal fibers and a thin inner layer of circular ones (Fig. 3 A). Duplicated pharynges occur in one animal from the Matsushiro-Nagano locality (Fig. 3 B). The anterior intestinal trunk bears 5 to 10 lateral branches; each posterior trunk bears 13 to 15 or more short lateral branches (Fig. 2 I and J).

The arrangement of the parts of the reproductive system (i. e., ovaries, testes, spermiducal vesicles, penis, copulatory bursa, etc.) is seen translucently from the ventral side of fully mature specimens when preserved (Fig. 2 D, H and E). Photomicrographs of the parts of the genital organs are shown in Figures 3 (C-F) and 4 (B-D). A general view of a sexually mature specimen, reconstructed from whole mounts and serial sections, is shown in a sketch in Figure 4 (A).

A pair of ovaries is situated in the usual ventral space (Figs. 3 G-E, 4 A). Numerous yolk glands (or vitellaria) are distributed throughout the body in the surrounding parenchyma (Fig. 4 B and C).

The testes are numerous, middle- to large-sized, and occupy almost all the entire dorsoventral diameter of the parenchyma of the body (Figs. 3 C-F, 4 B-D). At the prepharyngeal region, testes are arranged in 2 to 3 longitudinal zones on either side of the midline extending from the level shortly behind the ovaries (Fig. 4 B). Behind the level of the pharyngeal base, testes are arranged in one to 2 longitudinal zones extending nearly to the posterior end of the body; they are also found between the two intestinal trunks behind the level of the genital pore (Figs. 2 D and E, 3 G, 4 B-D; see also Fig. 4 A). On examination of the sagittal, transverse and horizontal sections, their total number can be estimated at almost 120 or more. A pair of spermiducal vesicles is developed on either side of the pharyngeal region (Figs. 2 D and E, 3 F).

Figure 5 (A-L) shows sagittal views of the copulatory apparatus of 12 specimens from 11 localities (Takamatsu, Hiruzen, Toyo'oka, Kameoka, Kanazawa, Matsumoto, Matsushiro-Nagano, Komatsubara-Nagano, Fujimi-Nagano, Ina, and Îda). Photomicrographs of the copulatory apparatus of animals from these localities are also shown in Figures 4 (E) and 6 (A-MM). Enlarged photomicrographs of the terminal portion of the bursal canal, or the vagina, of animals from 8 localities are shown in Figure 7 (A-I).

The following anatomical and histological descriptions of the copulatory apparatus are chiefly based upon the slides of animals from the Kameoka locality (the type locality; Specimen Lot No. 115, Figs. 5 E, 6 V). The penis has a very large, spherical bulb embedded in the parenchyma and a very long, finger-shaped papilla of a symmetrical form projecting into the male genital antrum. The penis of Specimen No. 115-i (Figs. 5 E, 6 V) is more elongated than that of the specimen figured in the original description (cf. Okugawa, 1956, p. 20, fig. 3 and photo 2). The bulb is highly muscular; it contains a narrow,

Fig. 2. Phagocata kawakatsui, photographs of live (A and B) and preserved (C-K) specimens from 4 localities and of cocoons (L). A-E: 4 specimens from the Hiruzen locality (Specimen Lot No. 1815). A and B, dorsal views of 2 specimens. C and D, dorsal and ventral views. A large arrow indicates the mouth opening; a small arrow, the genital pore. E, ventral view. F: dorsal view of the specimen from the Kanazawa locality (Specimen Lot No. 570). G and H: dorsal and ventral views of the specimen from the Matsumoto locality (Specimen Lot No. 1251). I-K: 2 specimens from the Takamatsu locality (whole mounts). I and J, No. 445-b. K, No. 445-a. L: cocoons from the Kameoka locality. cb, copulatory bursa; gp, genital pore; pb, penis bulb.

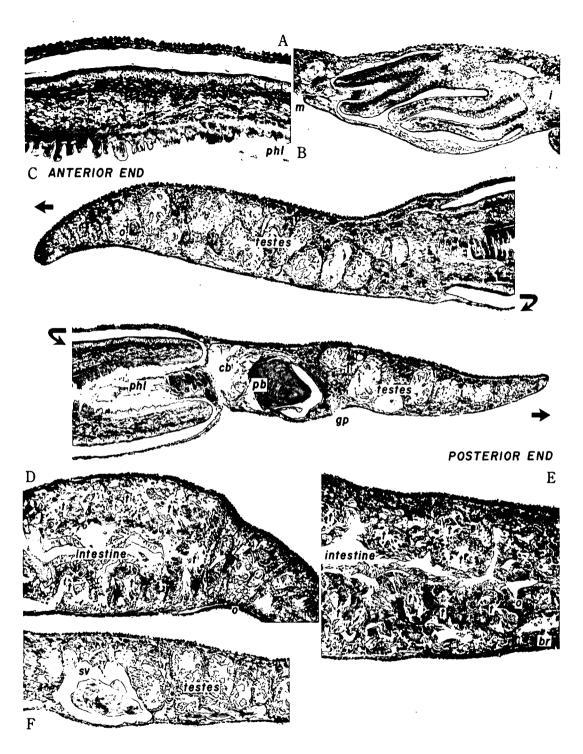
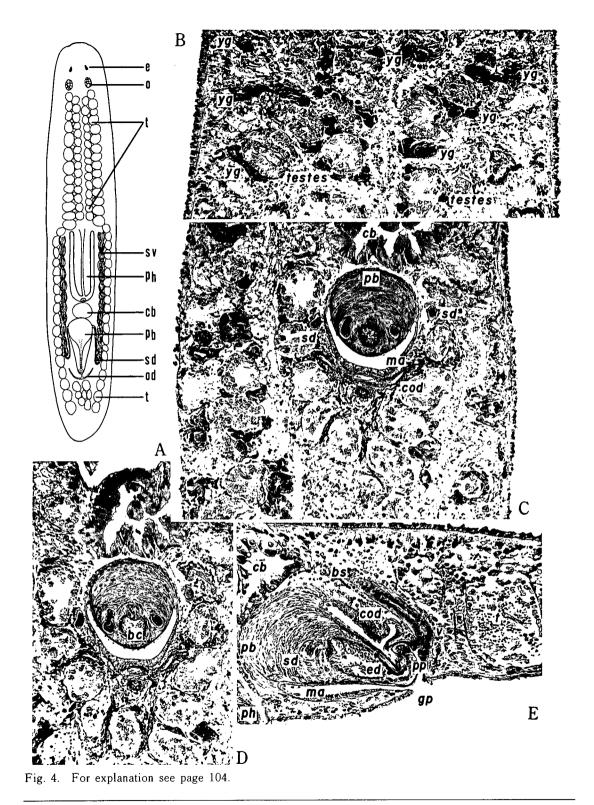


Fig. 3. Phagocata kawakatsui, photomicrographs of near midsagittal sections. A: pharynx of the Takamatsu specimen (No. 436-e). B: duplicated pharynges of the Matsushiro-Nagano specimen (No. 1262-b). C: the Takamatsu specimen (No. 436-e). D: anterior part of the prepharyngeal region of the Takamatsu specimen (No. 436-i). E: anterior part of the prepharyngeal region of the Komatsubara-Nagano specimen (No. 1295-h). F: near the copulatory bursa of the Takamatsu specimen (No. 436-e). br,



brain; **cb**, copulatory bursa; **gp**, genital pore; **i**, intestine; **m**, mouth; **o**, ovary; **pb**, penis bulb; **phl**, pharynx lumen; **sv**, spermiducal vesicle; **t**, testis.

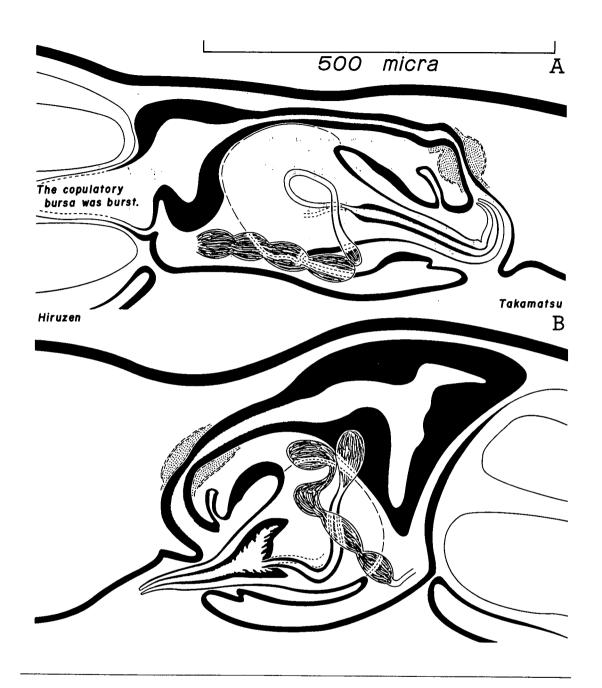
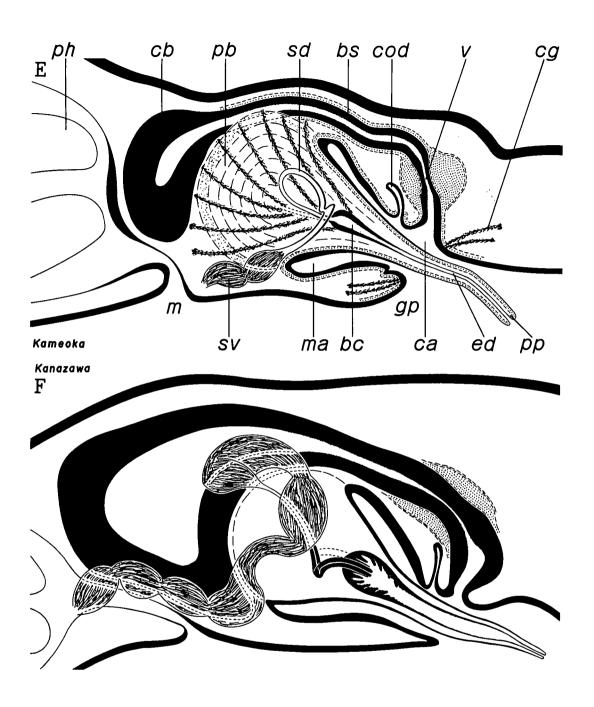


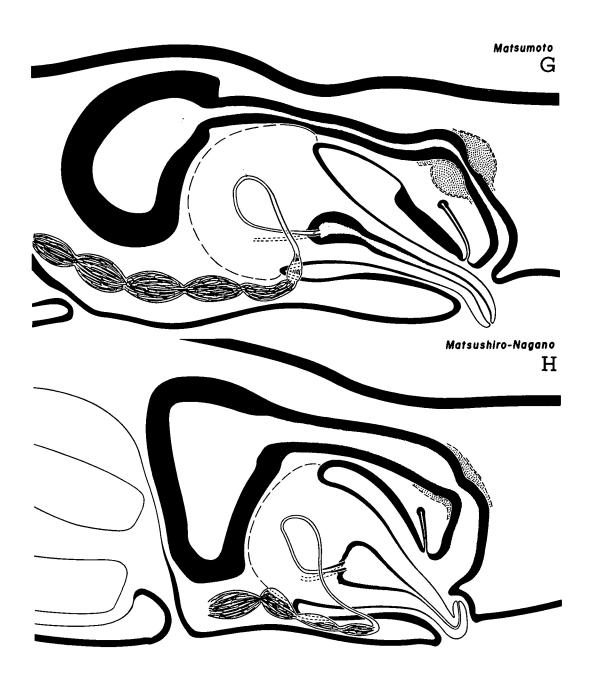
Fig. 4. Phagocata kawakatsui, sketch and photomicrographs. A: general view of a mature specimen from the Takamatsu locality, showing the arrangement of genital organs (after Kawakatsu & Iwaki, 1967, p. 216, fig. 2). B-D: photomicrographs of horizontal sections of the Hiruzen specimen (No. 1285-a). B, a part of the prepharyngeal region. C and D, near the copulatory apparatus. E: photomicrograph of near midsagittal section of the copulatory apparatus of the Toyo'oka specimen (No. 1280-g; see Fig. 6-o). bc, bulbar cavity; bs, bursal stalk; cb, copulatory bursa; cod, common ovoviteline duct; e, eye; ed, ejaculatory duct; gp, genital pore; ma, male genital antrum; o, ovary; od, ovovitelline duct; pb, penis bulb; ph, pharynx; pp, penis papilla; sd, sperm duct; sv, spermiducal vesicle; t, testis; yg, yolk gland.



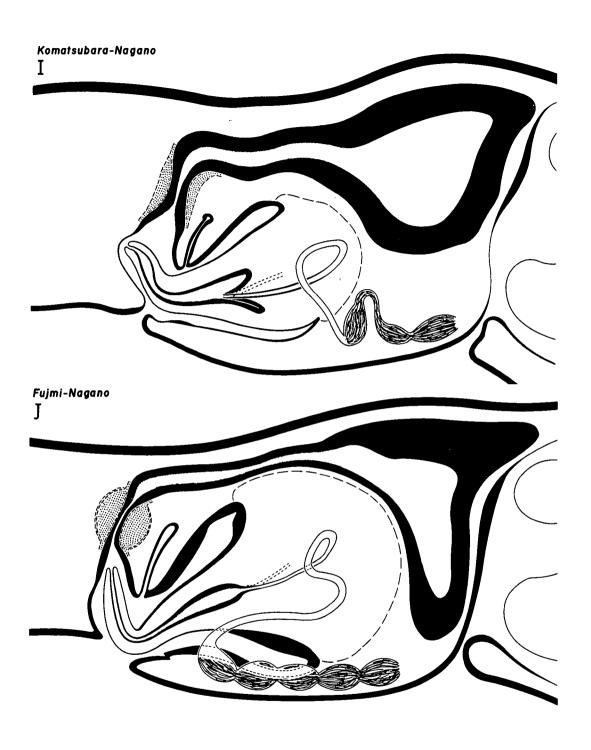
Fig. 5. (on pages 104 - 109). *Phagocata kawakatsui*, semidiagrammatic sagittal views of the copulatory apparatus of 12 specimens from 11 localities. All at the same magnification. A: No. 436-e (Taka-



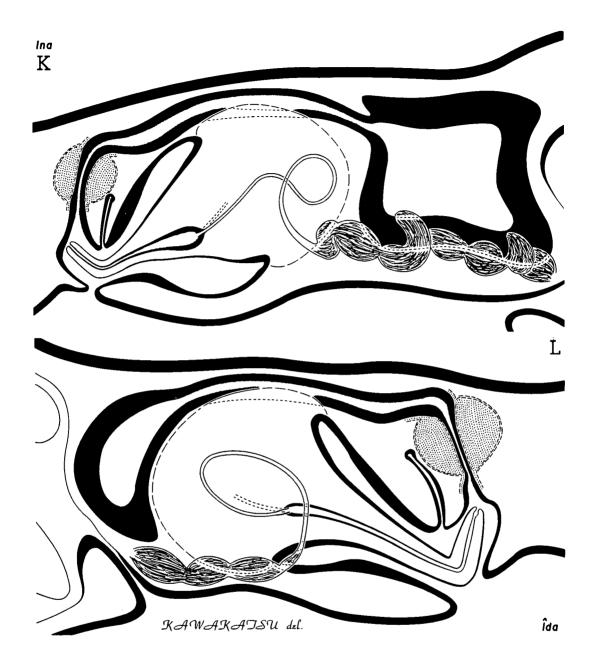
matsu); B: No. 1815-e (Hiruzen); C: No. 1280-l (Toyo'oka); D: No. 1284-a (Toyo'oka); E: No. 115-i (Kameoka); F: No. 570-a (Kanazawa); G: No. 1251-b (Matsumoto); H: No. 1262-b (Matsushiro-Nagano); I: No. 1291-c (Komatsubara-Nagano); J: No. 1327-c (Fujimi-Nagano); K: No. 1254-g (Ina); L: No. 1269-i (Îda). bc, bulbar cavity; bs, bursal stalk; ca, common genital antrum; cb, copulatory bursa; cg, cement gland; cod, common ovovitelline duct; ed, ejaculatory duct; gp, genital pore; m, mouth; ma, male genital antrum; pb, penis bulb; pp, penis papilla; mtmv, a mass of the thick musculature on the vagina; sd, sperm duct; sv, spermiducal vesicle; v, vagina.



club-shaped cavity of smooth outline, the bulbar cavity. It continues to the papilla as a long, narrow, tubular cavity which represents an ejaculatory duct and opens at the tip of the papilla (Fig. 5 E). The bulbar cavity is lined by a rather thick, nucleate, glandular epithelium; the epithelium of the ejaculatory duct is flatter than that of the bulbar cavity. The penis bulb is pierced by many gland ducts (penis gland) which open into the anterior portion of the penis lumen. The outer wall of the penis papilla is covered with a nucleate epithelium; it is thicker at the basal part of the papilla (than at the middle and terminal parts) (Fig. 5 E). Below this epithelium there are two thin layers of muscle fibers, one circular and the other longitudinal (Fig. 5 E).



The bulbar cavity figured in the original description (cf. OKUGAWA, 1956, p. 20, figs. 3 and 4, photo 1) shows a spherical shape (the author used the term "seminal vesicle"). This was undoubtedly due to the penis being in a rather contracted state. Slides examined of several specimens from the Kameoka locality show nearly the same anatomy as OKUGAWA's figures cited above. Each sperm duct recurves on either side of the posterolateral portion of the penis papilla and opens separately into the beginning of the



bulbar cavity (Fig. 5 E). The openings of the sperm ducts are close to each other as shown in the photomicrographs of the horizontal sections of the Hiruzen specimen (No. 1285-a, Fig. 4C and D). This anatomical character was observed in every slide examined of animals from all localities. Regarding the opening positions of sperm ducts in *Phagocata kawakatsui*, OKUGAWA's figure (op. cit., p. 20, fig. 1) should be corrected.

The male genital antrum is an elongate, cup-shaped cavity; its covering epithelium is a nucleate type, below which there are two thin layers of muscle fibers, one circular and the other longitudinal. The posterior part of the roof of the antrum receives the opening of the common ovovitelline duct (Fig. 5 E). It is a narrow, but rather long, tube and is usually curved (Fig. 5 E); its lumen is lined with a flat

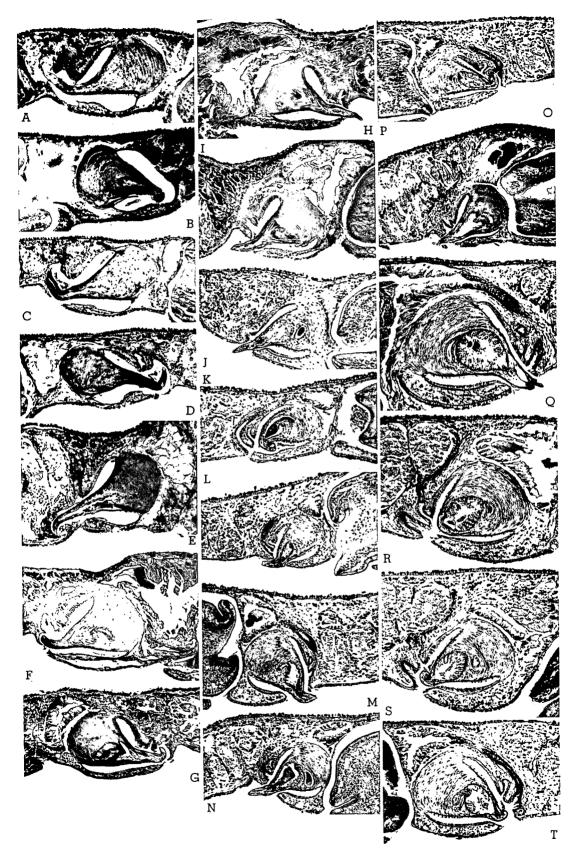
(sometimes slightly cubical according to locality) glandular epithelium of a nucleate type.

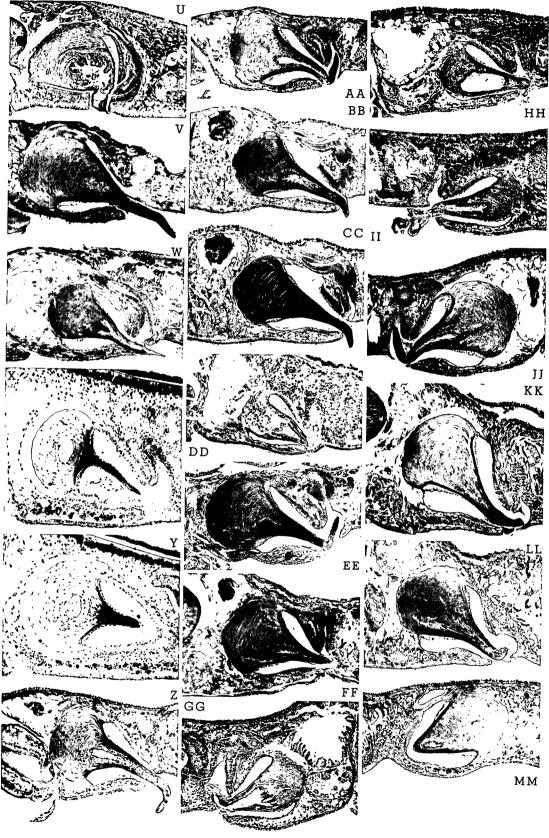
The copulatory bursa of the specimens from the Kameoka locality is a rather small to middle-sized organ with a rounded or an ovoidal outline. From the bursa, a slender and long tube (i. e., the bursal stalk) proceeds posteriorly, then turns ventrally and opens, from the dorsal side, into the roof of the common genital antrum, close to the genital pore (Fig. 5 E). The bursal canal is lined with a thick, glandular, nucleate epithelium, except for the vaginal region. In this region, the canal forms a conspicuous stricture covered with a thin epithelium having insunk nuclei. The subepithelial musculature of the vagina, or the isthmus, consists of three layers of muscle fibers: an inner, thin, longitudinal layer; a middle, very thick, circular layer and an outer, rather thin, longitudinal layer. As seen in Figure 5 (E), a mass of thick musculature surrounding the vagina described above has a rounded outline. The other part of the bursal stalk has a thin muscle coat consisting of an inner, thin layer of circular fibers and an outer, thin layer of longitudinal ones. Numerous erythrophilous glands can be seen at the vaginal region.

Animals of *Phagocata kawakatsui* from different localities show some variation of the copulatory apparatus. The anatomy of the penis lumen shows a rather wide variation. In the specimens from 5 localities (Takamatsu, Fujimi-Nagano, Ina, Babadaira-Nagano, and Îda), the shape and anatomy of the penis lumen are very similar to those of the specimens from the Kameoka locality (Figs. 5 A, E, J-L, 6 A-E, EE, FF, JJ-MM). In the specimens from 2 localities (Kanazawa and Matsumoto), the penis lumen is rather wide (Figs. 5 F and G, 6 W, X-CC). In the specimens from 4 localities (Hiruzen, Toyo'oka, Matsushiro-Nagano, and Komatsubara-Nagano), the penis lumen consists of a wide, cup-shaped bular cavity and a rather wide ejaculatory duct (Figs. 4 E, 5 B-D, H and I, 6 F-U, DD, GG. II). Especially in the specimens from the Toyo'oka locality, the bulbar cavity forms a very wide, elliptical lumen with a highly developed, cubical, glandular epithelium (Figs. 4 E, 5 C and D, 6 J-U). Moreover, the terminal portion of each sperm duct forms a slightly widened, thick-walled tube (Figs. 4 E, 5 C and D, 6 J, N, O-U).

A wide degree of anatomical and histological variations is observed in the structure of a mass of musculature on the vagina. In the specimens from 5 localities (Takamatsu, Matsumoto, Fujimi-Nagano, Ina, and Îda), the vaginal musculature is well developed as in the specimens from the Kameoka locality (Figs. 5 A, E, G, J-L, 7 D and E, G-I). In the specimens from the other 5 localities (Hiruzen, Toyo'oka, Kanazawa, Matsushiro-Nagano, and Komatsubara-Nagano), the epithelial cells of the vagina have nuclei; and the middle, circular muscle fibers of the subepithelial musculature on the vagina are poorly developed (Figs. 5 B-D, F, H and I, 7 A-C, F). Although the degree of differentiation of the vagina varies according to the locality, there does not appear to be pronounced departure in the principal structure of the organ.

Fig. 6. (on pages 111 – 112). Phagocata kawakatsui, photomicrographs of near midsagittal sections of the copulatory apparatus. A-E: 5 specimens from the Takamatsu locality (A, No. 436-a; B, No. 436-c; C, No. 436-d; D, No. 436-e; E, No. 436-i). F-I: 4 specimens from the Hiruzen locality (F, No. 1815-a; G, No. 1815-b; H, No. 1815-c; I, No. 1815-e). J-U: 11 specimens from the Toyo'oka locality (J, No. 1280-e; K and L, 1280-f; M, No. 1280-h; N, No. 1280-r; O, No. 1280-g; P, No. 1283-i; Q, No. 1284-a; R, No. 1284-b; S, No. 1284-c; T, No. 1284-d; U, No. 1289-a). V: one specimen from the Kameoka locality (No. 115-i). W: one specimen from the Kanazawa locality (No. 570-a). X-CC: 5 specimens from the Matsumoto locality (X, No. 210-a; Y, No. 210-b; Z, No. 210-f; AA, No. 210-h; BB and CC, No. 1251-b). DD: one specimen from the Matsushiro-Nagano locality (No. 1262-b). EE-FF: 2 specimens from the Îda locality (EE, No. 1269-i; FF, No. 1269-f). GG-II: 3 specimens from the Komatsubara-Nagano locality (GG, No. 1291-c; HH, No. 1291-d; II, No. 1291-e). JJ-KK: 2 specimens from the Fujimi-Nagano locality (JJ, No. 1327-c; KK, No. 1327-e). LL: one specimen from the Babadaira-Nagano locality (No. 1250-g).





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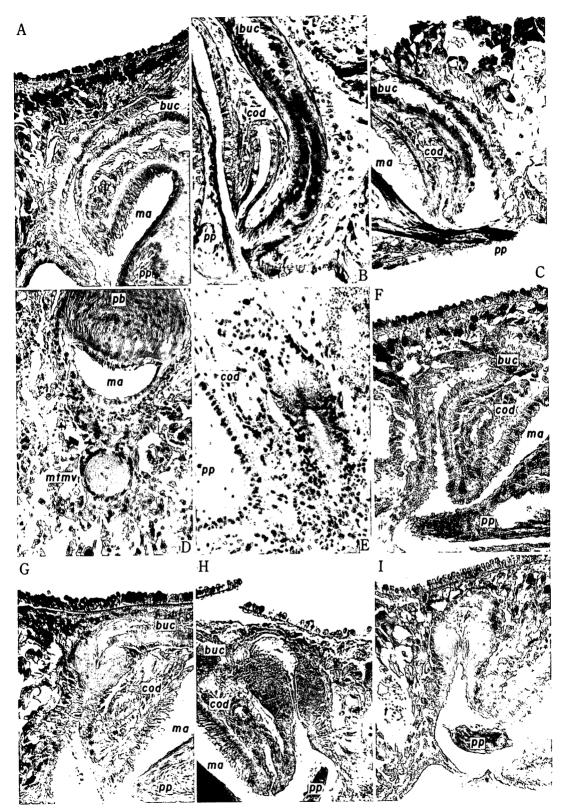


Fig. 7. For explanation see page 114.

The cocoon is ovoid in shape (0.6-1.3 mm in long axis and 0.5-1.0 mm in short axis) (Fig. 2 L). Ecological data on breeding and cocoons of *Phagocata kawakatsui* were described in the previous paper (cf. Kawakatsu & Iwaki, 1967).

Differential diagnosis. ••• Phagocata kawakatsui differs from the other members of the genus in a combination of the following characters: living animal rather small (10 to 12 mm in length) and colored dark gray to grayish brown above, light below; head subtruncated with rounded anterolateral corners; having two eyes situated close together and with a pigment-free ocular area around each eye; adhesive slit well developed at the central part of the ventral side of the head; dorsoventral testes lying in two to three rows on either side and extending close to the posterior end; penis bulb large, spherical and highly muscular with narrow to moderate (sometimes very wide) bulbar cavity into which sperm ducts enter separately, but near to each other, from the posterior side (spermiducal vesicles well-developed); symmetrical penis papilla very long, finger-shaped and with narrow (sometimes rather wide) ejaculatory duct that opens at the tip of the papilla; narrow, but long, common ovovitelline duct entering the roof of the terminal portion of the male genital antrum; copulatory bursa small to moderate in size; bursal stalk long, slender, but with the terminal part highly constricted as a vagina and opening into the common genital antrum near the genital pore; vagina usually accompanied by a mass of thick, subepithelial musculature (wide variation may occur); cocoon ovoid in shape.

Material. ··· Many sets of serial sections, whole mounts and preserved specimens in alcohol from 15 localities (including the type locality; see the section "Materials and Methods") are retained in KAWA. KATSU's laboratory at Fuji Women's College, Sapporo, Japan. Some of the sections separated from this collection will be sent to the Department of Zoology (Nat. Hist.), National Science Museum of Natural History, Tôkyô, Japan.

#### KARYOLOGICAL OBSERVATION

The results of the cytological studies of 3 non-sexual specimens from the Hiruzen locality are shown in Table 1. The idiograms of 2 specimens are shown in Figure 8. Photomicrographs of the chromosomes of 2 specimens are also shown in Figure 9 (A and B).

The chromosome number of the diploid cells of this species is 2x = 24 (Figs. 8, 9 A and B). The karyotype consists of one pair of very large metacentric chromosomes, one pair of large submetacentric chromosomes, and 10 pairs of small meta- and submetacentric chromosomes (Fig. 8). In one of the specimens examined, chromosome gaps were observed on the other one of the largest chromosomes labeled no. 1 as shown in Figure 8 (bottom).

Remarks. · · · The karyotype of *Phagocata kawakatsui* described here agrees with that of specimens from the Matsumoto locality reported by Teshirogi, Nîmura, Ishida & Hasebe (1978) and Teshirogi, Ishida & Nîmura (1979). The karyotype of *Phagocata teshirogii* was reported by Teshirogi & Sasaki (1977 a,

Fig. 7. Phagocata kawakatsui, photomicrographs of near midsagittal (A-C, E-I) and horizontal (D) sections of the vaginal region. All at the same magnifications. A: No. 1815-e (Hiruzen); B: No. 1284-a (Toyo'oka); C: No. 570-a (Kanazawa); D: No. 1251-l (Matsumoto); E: No. 210-a (Matsumoto); F: No. 1291-b (Komatsubara-Nagano); G: No. 1327-c (Fujimi-Nagano); H: No. 1269-i (Îda); I: No. 1250-g (Ina). buc, bursal canal; cod, common ovovitelline duct; ma, male genital antrum; mtmv, a mass of the thick musculature on the vagina; pb, penis bulb; pp, penis papilla; t, testis.

Table 1. Results of the cytological study of *phagocata kawakatsui* from the Hiruzan locality, Okayama Prefecture, Honshû.

No. of non-sexual specimens

examined cytologically

Chromosome nos. & the no. of cells studied in parentheses

Mitosis

24 (62)

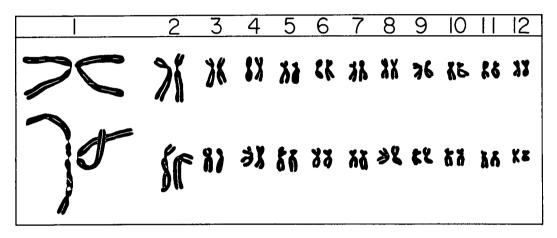


Fig. 8. Idiograms of Phagocata kawakatsui from the Hiruzen locality. For explanation see the text.

b). The other two subterranean species, *Phagocata papillifera* and *Phagocata suginoi*, were also reported by Sugino, Murayama & Horikoshi (1978) and Kawakatsu, Sugino, Oki, Tamura & Horikoshi (1984). All these 4 Japanese species have chromosome numbers of 2x = 24 and n = 12. Differences in their karyotypes are minor. A more detailed discussion will be found in the previous paper (cf. Kawakatsu, Sugino, Oki, Tamura & Horikoshi, 1984, pp. 98–100).

#### **ACKNOWLEDGEMENTS**

For their collection of specimens of *Phagocata kawakatsui* from various localities, the authors wish to thank the following gentlemen: Dr. Tatsumi Uematsu (Takamatsu), Dr. Yoshikazu Kishida (Okayama), Messrs. Fumio Nimura (Kôshoku), Makoto Nakano (Toyo'oka), Yoshihiko Yamazaki (Toyo'oka), Yukio Mukaibara (Toyo'oka), Nobuo Takahashi (Ibaraki), Hitoshi Murayama (Nagaoka), Yoshihiko Hara (Ina), Takeshi Miyazaki (Tamano), Hideo Kawada (Tamano), Hiroaki Nishi (Kurashiki), and the late Mr. Yoshitsugu Tarui. They also thank Professor Dr. Robert W. Mitchell of the Department of Biological Sciences, Texas Tech University, Lubbock, Texas, U. S. A., for his careful reading of this manuscript.

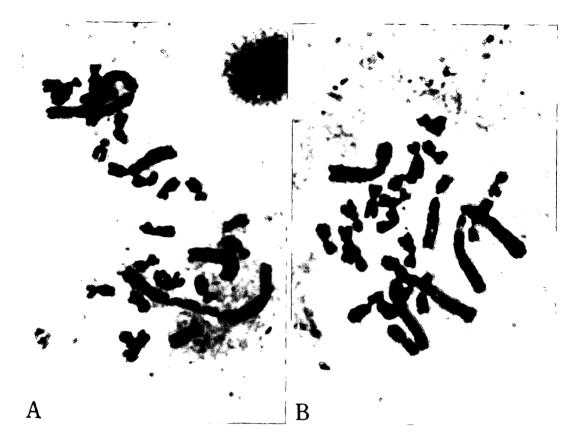


Fig. 9. Photomicrographs of the chromosomes of *Phagocata kawakatsui* from the Hiruzen locality. A and B: 2x = 24 (2 specimens).

#### SUMMARY

Phagocata kawakatsui Okugawa, 1956 (Turbellaria, Tricladida, Paludicola), is redescribed in the present paper based upon materials from 15 localities in Shikoku and Honshû, Japan. This pigmented, epigean species with two eyes shows some variation in the anatomy and histology of the copulatory apparatus according to the locality. A new differential diagnosis for this species is given. The species has 12 chromosomal paris (2x = 24, n = 12).

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#### Note added in proof.

When this paper was in page proof, the authors received a recent published article. It is as follows:

N<sub>I-IMURA</sub>, F. (= N<sub>IMURA</sub>, F.), 1986. Taxonomic consideration about some species of freshwater planarian, genus *Phagocata* and the description of a new subspecies, *Phagocata kawakatsui naganoensis* N<sub>I-IMURA</sub>, subspec. nov. Shinanoji (Report of the Shinano Seibutsukai), No. 43: 12-21. (In Japanese with English abstract.)

Nîmura (1986), who studied materials from 7 localities in Nagano Prefecture, classified animals from 5 localities as *Phagocata kawakatsui* and animals from remaining 2 localities as a new subspecies *Phagocata kawakatsui naganoensis*. Animals from these localities were also reported in the present paper: Matsumoto, Îda, Fujimi-Nagano, Babadaida-Nagano, Ina (Nimura classified them as *Ph. kawakatsui*), Matsushiro-Nagano, and Komatsubara-Nagano (Nimura classified them as *Ph. k. naganoensis*).

According to Nîmura's (op. cit.) article including two diagrammatic figures of the copulatory apparatus of animals from the Matsumoto and Matsushiro-Nagano localities and several photomicrographs of them, Ph. k. naganoensis is separable from Ph. kawakatsui (he did not use the nominate subspecific name) by the following anatomical characters: 1) having the nucleate epithelial cells of the vagina and poorly developed vaginal musculature; 2) having a wide bulbar cabity. These characters in the genital anatomy are, however, insufficient for the establishment of a new subspecies of Phagocata kawakatsui (see a new specific definition of the species given in the present paper). Supplementary, the authors should be pointed out that Nîmura's figures of the copulatory apparatus of Ph. kawakatsui and Ph. k. naganoensis printed in his 1986 article (and his 1975 article under the name of Ph. kawakatsui) show the presence of a common sperm duct. His figures are inaccurate in this point (see the text of the present paper).

Nimura (op. cit.) also emphasized in his 1986 article that Ph. k. naganoensis (2n = 24) has a different karyotype from that of Ph. kawakatsui. According to him, the 5th and 6th paires of chromosomes are submetacentric (he compared the karyotype of Ph. k. naganoensis with that of Ph. kawakatsui from the Matsumoto locality reported by Teshirogi, Ishida & Nimura in 1979). Except for 2 pairs of large chromosomes, differences of the arm ratio of the remaining 10 pairs of small meta- and submetacentric chromosomes in the karyotype of Ph. kawakatsui are minor. Moreover, differences in the karyotypes of 4 Japanese Phagocata species (kawakatsui, teshirogii, papillifera, and suginoi) are also minor. Phagocata kawakatsui cannot be separable into two subspecies based upon the karyological data proposed by Nimura (op. cit.).

In conclusion, *Phagocata kawakatsui naganoensis* N<sub>I-IMURA</sub>, 1986 (type locality: Matsushiro in Nagano City, Nagano Pref.; the type series are retained in the Applied Ecology Laboratory, the Textile Faculty of Shinshû University, Ueda, Nagano Pref., Japan.; No. 853-1 and No. 853-4), is undoubtedly a synonym of *Phagocata kawakatsui* OKUGAWA, 1956.

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December 25, 1986.

### 藤女子大学・藤女子短期大学紀要 第 24 号・第 II 部 昭和 61 年 12 月 20 日 印刷 昭和 61 年 12 月 25 日 発行

発 行 者 淑 子 長 永  $\coprod$ 編集者 要 編 集 委 員 野 表 字 春 夫

発行所 藤女子大学・藤女子短期大学 札幌市北区北16条西2丁目 〒001 電話代表 (011)736-0311 印刷所 凸版印刷株式会社北海道事業部

札幌市西区24軒4条1丁目 5063