STUDIES ON THE MORPHOLOGY, KARYOLOGY AND TAXONOMY OF
THE JAPANESE FRESHWATER PLANARIAN *Dugesia japonica*
ICHIKAWA ET KAWAKATSU, WITH A DESCRIPTION OF
A NEW SUBSPECIES, *Dugesia japonica ryukyuensis*
SUBSPEC. NOV. 1)

by

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I. INTRODUCTION

*Dugesia japonica* ICHIKAWA et KAWAKATSU, 1964, is the commonest Japanese freshwater planarian found everywhere both in running and standing waters including shallow underground habitats. When the species was originally described, the specimens collected from a spring-fed stream in Matsu-moto City in Central Japan, Honshū, were employed as the type-series (cf. ICHIKAWA et KAWAKATSU, 1964). During the past twelve years, the morphological variation of the genital anatomy of this polymorphic species from various localities in the Japanese Islands including the Southwest Islands of Japan (i.e., the Satsunan Islands and the Ryūkyū Islands) and the neighbouring countries in the Far East (i.e., Taiwan, China, Hong Kong, and Korea) was studied by KAWAKATSU and his co-researchers. The principal literature on the taxonomy of this species are: ICHIKAWA & KAWAKATSU, 1967; KAWAKATSU, 1971; KAWAKATSU, HOKUSHI & AKAMA, 1972; KAWAKATSU & IWAKI, 1967 b, 1968; KAWAKATSU, IWAKI & KIM, 1967; KAWAKATSU & KANG, 1969; KAWAKATSU & KIM, 1966, 1967; KAWAKATSU & MACK-FIRX, 1975; KAWAKATSU & MIYAZAKI, 1972; KAWAKATSU, MORITA & IWAKI, 1967; KAWAKATSU & TANAKA, 1971, 1976; KAWAKATSU & WONG, 1975; MACK-FIRX & KAWAKATSU, 1972. The distributional ecology of this species was discussed and reviewed in the serial articles by KAWAKATSU (1965, 1967, 1970, 1974).

DAHM (1963) who studied the karyotype of *Dugesia japonica* from the type locality (he mentioned the species as *Dugesia* species, "Matsumoto stock"; cf. pp. 60 - 62) demonstrated that the chromosome number of the sexual specimens was found to be 2n = 16 and during meiosis 8 completely paired bivalents appeared. He also mentioned the differences of the karyotypes between the Japanese species and European *Dugesia gonocephala* (Duges). DAHM'S (op. cit.) observation was ratified by TESHIRGI & ITAGAKI (1965) who studied the karyotypes of the Japanese species from four different localities: Otaru in the south-central part of Hokkaidō, Hirosaki in the northern part of Honshū, Kyūto in the central part of Honshū, and Matsuyama in Shikoku. Their observation was made in the mitotic cells of neoblasts in regenerating pieces (see also BENAZZI & BENAZZI-LENTATTI, 1976, pp. 99 - 100).

Recently, the chromosome observation of *Dugesia japonica* from several localities of Central Japan has been studied by SUGINO and his co-researchers (SUGINO, HIROSE & KATÔ, 1973; HIROSE,

1) Preliminary reports of some of this work were presented at the 46th Annual Meeting of the Zoological Society of Japan, Kyōto, October 1975 (KAWAKATSU, 1975), and at the 47th Annual Meeting of the Zoological Society of Japan, Hiroshima, October 1976 (ONI, TAMURA, KAWAKATSU & SUGINO, 1976; KAWAKATSU, ONI, TAMURA & SUGINO, 1976).
Katō & Sućino, 1974) and Oki & Tamura (1974, 1975a, b). Although the animals from almost all of the populations investigated by them have a chromosome number of $2n = 16$, it has been found out that the variation of chromosome numbers of animals occurs in some populations. According to them, the chromosome number of the specimens from a stream at Kurama in the northern part of Kyōto is 25 (cf. Sućino, Hirose & Katō, 1973). Moreover, it was found out that the specimens from three localities near Ōsaka (the Kinokawa River at Kokawa in Wakayama Pref., a brook at Nozaki-kan'non, the northern part of the Ikoma Mountains, Daitō City, and the Mino'ō River at Segawa, Mino'ō City, in Ōsaka Pref.) have heterogeneous cells with two different numbers of chromosomes in one body. Namely, the tissue of many asexual specimens examined from these localities consists of two types of somatic cells intermingled: $2n = 16$ and $3n = 24$ (cf. Hirose, Katō & Sućino, 1974; Oki & Tamura, 1974, 1975a). Oki & Tamura (1975b) also reported the occurrence of a number of sexual specimens in the Mino'ō locality ($n = 8$ and $2n = 16$; somatic cells, $2n = 16$ and $3n = 24$).

The recent chromosomal findings of Dugesia japonica mentioned above led the authors to give a reconsideration to the taxonomy of this species based upon the morphological, anatomical and karyological data. According to this line of the authors' cooperative investigations, Kawakatsu reexamined the slides of Dugesia japonica from various localities including the type-series which were preserved in his laboratory; the chromosomal observation of the specimens from Japan (Mino'ō in Ōsaka Pref. and Urasoe in Okinawa Pref.) and Korea (Wooyi-dong and Mt. Kangnac in Seoul) for comparison with the karyotype of the specimens recollected in the type-locality was investigated by Oki, Tamura and Sućino. From these studies the authors have come to the conclusion that Dugesia japonica should be subdivided into two subspecies, one nominative and the other new one.

In the present paper, the results of the morphological, anatomical and karyological observations with materials of Dugesia japonica from several localities including the type-locality, as well as a redescription of the nominative subspecies and an original description of the new subspecies, are described, together with some remarks about the taxonomy and karyotype of this widely distributed, polymorphic species.

II. LIST OF LOCALITIES

The animals collected from the following seven localities in Japan and two localities in Korea were used in the present study. The Specimen Lot Number given for each group is the number registered in Kawakatsu's fixing notebook according to his permanent recording system.


Additional sexual specimens from the type-locality used in the present study were collected by Mr. F. Nīmura several times from March to May, 1976. Both histological (Specimen Lot No. 1385 group) and chromosomal examinations were made.

No. 2. Specimen Lot No. 1358 group. A spring-fed stream near the First Sōenbashi Bridge of the Mino'ō River at Segawa, Mino'ō City, Ōsaka Pref., Honshū, Japan (alt. 43 m). A considerable number of sexual and asexual specimens (mostly asexual) were collected and cultured by Oki and Tamura since 1963. Water temperature, 16~21°C. Cf. Oki & Tamura (1972, 1974, 1975a, b; Tamura (=Yoshida) & Oki, 1969). Both histological and chromosomal examinations were made.
No. 3. Specimen Lot No. 68 group. A spring-fed stream near Masumizu-hara Hütte, the western foot of Mt. Daisen (Masumizu slope), Tottori Pref., Honshū, Japan (alt. 720 m). A considerable number of specimens, both sexual and asexual, were collected by KAWAKATSU on April 27, 1958. Water temperature, 9.3°C. This locality was discovered by KAWAKATSU and Mr. Y. TARUI on July 4, 1954 (cf. KAWAKATSU, 1955, p. 46, Fig. 1, st. 18; KAWAKATSU & ŌGAWARA 1969). Only histological examination was made.

No. 4. Specimen Lot Nos. 1379, 1386, 1387, and 1388 groups. A stream at Nakamayama-gawara, Urasoe City (about 5 km northeast of Naha), Okinawa Island, Okinawa Pref., Japan (alt. 20 m). A considerable number of specimens, both sexual and asexual, were collected by Mr. I. Tanaka several times from April to May, 1976. Water temperature, 18 - 20°C. Both histological and chromosomal examinations were made. This is the type-locality of the new subspecies of Dugesia japonica which will be described in the present paper.

No. 5. A stream at Wooyi-dong, the northeastern part of Seoul, Korea. Twelve live specimens (including only one sexual specimen) collected by Mr. W. K. Lee late in April, 1976, were received. Water temperature, 6 - 8°C. Only chromosomal examination was made. The ecological nature and the taxonomic description of the specimens from this locality were found in Kim (1964) and KAWAKATSU & Kim (1966).

No. 6. A stream near the gate of the New Campus of Seoul National University, the southern part of Seoul (between the Han River and Mt. Kangnac), Korea. Nine asexual specimens collected by Dr. S. W. Kang early in May, 1976, were received (the animals injured when they were arrived). Water temperature, 10 - 15°C. Only chromosomal examination was made. Although the sexual specimens were not obtained from this locality, the genital anatomy of the animals from several populations in the vicinity if this locality is found in KAWAKATSU & Kim (1966).

No. 7. Specimen Lot Nos. 384 and 391 groups. A stream at Hangchow, Che-Kiang, China. A considerable number of specimens were obtained by courtesy of Dr. Wang Yu-Lan and Dr. Chiang Shi-Ming. Cf. Ichikawa & KAWAKATSU (1967). Only histological examination was made.

III. METHODS

For general observation of animals, both live specimens and whole mounts (animals killed by Sugino's method were fixed in Nozawa's fluid and were stained in borax-carmin as a whole mount) have been used. Animals fixed in Bouin's fluid were used for histological examination. Serial sagittal sections of several orientations (7 - 8 micra in thickness) were stained with Delafield's haematoxylin and eosin (sometimes erythrosin was used). Some of the sections were stained with Mallory's triple stain.

For chromosomal studies, the animals were acclimatized to the laboratory culture condition. They were kept in culture pans with tap water (boiled and then aerated after cooling) and were fed with chicken liver. Then the healthy animals were starved for more than 4 days before cutting. The procedures of the samples used for chromosomal studies are as follows:

1) The animals cut transversely at the posterior level of the mouth were kept in the culture pans (water temp., 16 ± 2°C); the sexual animals were cut at the middle level between the mouth and the genital pore. 2) After 2 to 4 days of culture, the anterior portions of the regenerated pieces were cut transversely and then longitudinally into 2 small pieces (each piece measures less than 1 mm² in volume). 3) The pieces were immersed in 5ml of 10⁻⁶M colchicine (Merck) for 1 to 3 hours (16 ± 2°C). 4) The treated pieces were retransferred to 5ml of 0.1% potassium chloride for about
30 minutes (16±2°C). 5) Then each piece transferred onto a slide glass was stained in aceto-orcein (ca. 1%) for 10 to 30 minutes. 6) The stained pieces were squashed under a cover glass and were sealed by paraffine-balsam.

A more detailed technique of chromosomal studies employed will be found in the authors' Japanese article (Oki, Tamura & Kawakatsu, 1976).

IV. PRESENTATION OF RESULTS

Order TRICLADIDA
Suborder PALUDICOLA or PROBURSALIA
Family Planariidae
Genus Dugesia Girard, 1850
Dugesia japonica ICHIKAWA et KAWAKATSU, 1964
[Japanese name: Nami-uzumushi ナミウズムシ]
Dugesia japonica japonica ICHIKAWA et KAWAKATSU, 1964


Dugesia japonica should be subdivided into two subspecies (see the original description of the new subspecies which will be described in the present paper) and the nominative form then becomes Dugesia japonica japonica.


External characters. The general appearance of the sexual specimens from the Matsumoto population was given by ICHIKAWA & KAWAKATSU (1964). The animals are moderately large in size and dark brown in color with many light colored splotches of varying size on the dorsal side; non-splotchy animals are also common. Photographs and sketches showing the typical appearance of the animal are found in previous articles (cf. ICHIKAWA & KAWAKATSU, 1964, p. 188, Fig. 1 A-C; see also ICHIKAWA & KAWAKATSU, 1961, Pl. I, Fig. 1; KAWAKATSU, 1968 b, p. 42, Pl. Fig. 1-a, 1969 b, p. 89, Pl. VII, Fig. 1-a, 1973, Pl. Fig. 1-a, 1974, p. 295, Fig. 2 a-1).

The photographs of living specimens are shown in Figure 1 (A-D).

Internal characters. The distinctive histological and anatomical characteristics of the Matsumoto specimens have already been described by ICHIKAWA & KAWAKATSU (1964). The authors wish to add here only some additional observations concerning the genital anatomy of the animals based upon the slides of the type-series and of the newly prepared ones.

The photomicrographs of the sagittal and horizontal sections of the copulatory apparatus are shown in Figures 1 (E) and 2 (A-C). The sagittal view of the copulatory apparatus, reconstructed from several sets of fully sexually mature animals including the holotype (Specimen No. 103 c), is also shown in Figure 3 (see also ICHIKAWA & KAWAKATSU, 1964, p. 190, Fig. 3, p. 191, Fig. 4 A).

The penis of the Matsumoto specimens has a large, semiovoidal bulb and a large conical papilla of a highly asymmetrical form. It is strongly muscular in nature. A wide bulbular cavity (seminal vesi- cule) with a more or less irregular outline is lined by a highly glandular, nucleate epithelium. The two sperm ducts open into the bulbular cavity separately. The dorsal lip of the papilla is larger than the ventral one. The outer wall of the papilla is lined with a nucleate cubical epithelium (it is thick on the

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2) According to Mr. F. Nishida's recent data in the Matsumoto locality (August, 1976), the rate of occurrence of splotchy animals was 38.1%.
dorsal lip). Below the epithelium there are two layers of muscle fibres, one thick circular and the other thin longitudinal. A rather wide ejaculatory duct, which is separated from the bulbar cavity by a well-developed valve or diaphragm, opens into the male genital antrum on the ventral side of the papilla. Its lining glandular epithelium consists of cubical cells and contains numerous eosinophilous granules.

The genital antrum of the present form is divided into two cavities, i.e., a wide male antrum and a rather narrow common antrum which connects with the terminal part of the bursal canal or the vagina. The former is lined by a nucleate, very thick epithelium. There are two layers of the subepithelial muscle fibres, the inner thick circular and the outer thin longitudinal. Usually, a glandular fold or a slit accompanying eosinophilous glands is conspicuous on the floor of the male antrum near the genital pore.

The present form has a large copulatory bursa and a wide, rather long bursa stalk, of which the cavity (bursal canal) opens into the common genital antrum. The bursa stalk is divided into two parts: a wide anterior section which connects with the copulatory bursa and a posterior slightly narrow but thick-walled section (i.e., the vagina). After a close reexamination of the newly prepared serial sections of the Matsumoto specimens, it has been found out that the bursa stalk has a thick glandular epithelium of nucleate type (cf. Ichikawa & Kawakatsu, 1964, p. 192). The muscle coat surrounding the bursa stalk consists of three layers of fibres, i.e., a thin layer of longitudinal, a wide layer of circular, and a thin layer of longitudinal. In the posterior two-third section, the middle circular and the outer longitudinal layers of the muscle fibres become thicker than those of the anterior one-third section and slightly intermingled at the regions of the vagina and of the posterior wall of the common genital antrum. As shown in the photomicrograph (Fig. 2B), the vagina is surrounded by a very wide halo-like structure. Histologically, it consists of mesenchymal tissue traversed by several coarse rows of longitudinal muscles and less-developed radial ones. The wall of the vagina is pierced by numerous ducts of strongly eosinophilic glands and the cyanophilic gland ducts which open into the cavity. The two ovovitelline ducts accompanying strongly eosinophilic glands open into the vagina. Weakly eosinophilic cement glands open into the common antrum near the genital pore.

Cytological observation. As already mentioned, Dahm (1963) studied the karyotype of Dugesia species, "Matsumoto stock", which was later described under Dugesia japonica Ichikawa et Kawakatsu. In two sexually mature specimens examined cytologically 100 mitoses (2n=16) and 22 meioses (n=8) were observed (Fig. 15A and B). The karyotype consists of 8 pairs of metacentric chromosomes in descending order of size. The chromosome labelled 4 seemed to be slightly submetacentric (Fig. 18-a A). The present examination of the chromosomes of Dugesia japonica japonica from the type-locality is coincident with Dahm's description (1963, pp. 60-62, Figs. 19 and 20; see also Teshirogi & Itagaki, 1965). See also Table 1.

Differential diagnoses of Dugesia japonica and Dugesia japonica japonica. The differential diagnosis of Dugesia japonica (cf. Ichikawa & Kawakatsu, 1964, p.193) should be revised as follows: living animals moderate to large; head triangular with a rather short, bluntly pointed but definite auri- cles; eyes two (supernumerary eyes may occur); colored but highly variable, sometimes with spots on the dorsal surface; external muscle layers of the pharynx consist of outer longitudinal and inner circular fibres (additional inner longitudinal fibres may occur); testes numerous and dorsal, extending in two lateral rows throughout the body; penis bulb moderate to large and strongly muscular with a wide retort-shaped bulbar cavity into which sperm ducts enter without previous union (spermiducal vesicles well-developed); highly asymmetric muscular penis papilla large and conical; separation of the bulbar cavity and ejaculatory duct by a well-developed diaphragm and external opening of the
Table 1 Variation of the chromosome numbers and karyotypes of *Dugesia japonica* ( *Dugesia japonica japonica* and *Dugesia japonica ryukyuensis* subspec. nov.) from 5 localities in Japan and Korea.

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<tr>
<th>Two subspecies &amp; their localities</th>
<th>No. of specimens examined cytologically</th>
<th>Chromosome nos. &amp; the no. of cells studied in parentheses</th>
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<td>Total</td>
<td>Sexual specimens</td>
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<td><strong>Matsumoto, Japan</strong></td>
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<td><strong>Urasoe, Okinawa, Japan</strong></td>
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* The type-locality of *Dugesia japonica*.
** The sexual condition of the animals was determined according to the presence of the genital pore and/or sperms.

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Fig. 1. *Dugesia japonica japonica* [ICHIRAWA et KAWAKATSU, 1964], from the Matsumoto locality (the type-locality). A-C: Three living sexually mature specimens. D: Head of a living sexually mature specimen. No. 1385 group. E: Photomicrograph of the parts of the copulatory apparatus (horizontal section, No. 1385 j).

bc, bulbar cavity; cb, copulatory bursa; od, oovitelline duct; pp, penis papilla; sv, spermiducal vesicle.
Fig. 1
Fig. 2. (A and B). Photomicrographs of the copulatory apparatus of *Dugesia japonica japonica* from the Matsumoto locality. A: Penis of the holotype (sagittal section, No. 102c). B: Vagina of the holotype (sagittal section, No. 102c). Arrow indicates the opening of the ovovitelline duct. See Ichikawa & Kawakatsu (1964, p. 191, Fig. 4 A and D).

**bc**, bulbar cavity; **ca**, common antrum; **ed**, ejaculatory duct; **gp**, genital pore; **ma**, male antrum; **od**, ovovitelline duct; **pb**, penis bulb; **pp**, penis papilla; **v**, vagina.
Fig. 2 (C). Photomicrograph of the copulatory apparatus of *Dugesia japonica japonica* from the Matsumoto locality (sagittal section, No. 1385 g). Abbreviations, see Fig. 2 (A and B).

Fig. 3. Diagram showing the sagittal view of the copulatory apparatus of *Dugesia japonica japonica* from the Matsumoto locality (redrawn from the slides of the holotype, No. 102 c).

bc, bulbar cavity; bs, bursa stalk; ca, common antrum; cb, copulatory bursa; cg, cement gland; ed, ejaculatory duct; gp, genital pore; ma, male antrum; od, ovovitelline duct; pb, penis bulb; pp, penis papilla; sd, sperm duct; sv, spermiducal vesicle; v, vagina.
ejaculatory duct on the under side of the penis; copulatory bursa large, folded, with a wide bursal canal which opens into the common antrum near the genital pore; vagina well-developed (typically, it is surrounded by a wide halo-like structure) into which ovovitelline ducts enter separately; cocoon spherical in shape and stalked.

The nominative subspecies, *Dugesia japonica japonica*, is characterized in having a penis papilla without a well-developed valve surrounding its basal part and also in having a well-developed vagina. This subspecies has a chromosome number of \( n = 8 \) and \( 2n = 16 \). The chromosome number of \( 24 \) (\( 3n \)) is also found in the asexual specimens from certain localities.

*Type-series.* Holotype — One set of serial sagittal sections (Specimen No. 102c, 3 slides) will be deposited in the Department of Zoology, National Science Museum, Tokyō. Two paratypes (Nos. 102 e and 286 f) and one set of additional serial sagittal sections and one whole mount of the animals from the type-locality (No. 1385E, K) will be deposited in the same Museum. The remaining slides including the paratypes and several whole mounts are retained in Kawakatsu’s collection (Kawakatsu’s laboratory, Fuji Women's College, Sapporo). Cf. Ichikawa & Kawakatsu (1964, p. 193); Kawakatsu (1969 b, p. 50).


The distribution of *Dugesia japonica japonica* will follow in the section "Taxonomic and Zoogeographical Remarks".

2. Specimens from the Mino’o Population, Honshū, Japan.

*External characters.* The sexually mature specimens were rare in the Mino’o population. The specimens may attain about 25 mm in length and 4 mm in width. The animals are of a light brownish color (cf. Oki & Tamura, 1975 a).

*Internal characteristics.* Only one sexual specimen was available for histological examination. The musculature of the pharynx shows the typical arrangement of *Dugesia japonica* (cf. Ichikawa & Kawakatsu, 1964, p. 189).

The testes, ovaries and yolk glands have been observed in the slides examined. It is, however, very interesting that the ovaries are extraordinary large in size and occupy almost all the dorso-ventral space at the usual anterior level of the body.3

The photomicrographs of the copulatory apparatus are shown in Figure 4 (A and B); see also Figure 5. The Mino’o specimen shows the typical genital anatomy of *Dugesia japonica japonica*. But the halo-like structure surrounding the vagina in the Mino’o specimen shows a slightly low degree of differentiation compared with that of the animals from the type locality.

*Cytological observation.* The karyotype of the animals belonging to this population was reported in a preliminary form by Oki & Tamura (1974, 1975 a, b). In the present study the result of the cytological examination of additional specimens, both sexual and asexual, are included.

The authors have examined 10 sexual specimens. One hundred and twenty-six mitoses in 3 of them revealed a chromosome number of \( 2n = 16 \). During meiosis 8 bivalents were found. No apparent differences in the morphology of the karyotype could be observed between the sexual specimens from the Mino’o population and the Matsumoto population. From the study of the somatic cells of the sexual

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3. Sugino (1969, p. 297, Fig. 26; see also Sugino, 1971, p. 12, Fig. 26) described an extraordinary large ovaries induced in the body of the asexual form of *Dugesia japonica* (Kyōsōyū stock) when the anterior piece of the asexual form and the posterior piece of the sexual form (Mt. Shigisan stock) had been united by a transplantation technique.
Fig. 4. Photomicrographs of the copulatory apparatus of *Dugesia japonica japonica* from the Mino'o locality. A: Penis (sagittal section, No. 1358 a). B: Vagina (sagittal section, No. 1358 a).

- **bc**: bulbar cavity
- **buc**: bursal canal
- **ca**: common antrum
- **cb**: copulatory bursa
- **gp**: genital pore
- **ma**: male antrum
- **ph**: pharynx
- **pp**: penis papilla
- **v**: vagina
specimens the occurrence of two different types of cells intermingled in one animal was observed. The first type of cells had 16 chromosomes, while the second type had 24 (Fig. 15 C and D). In one of them, 8 bivalents during meiosis were found.

Three kinds of the asexual specimens having different karyotypes were found in the Mino'o population. They were: the diploid specimens (2n = 16), the specimens with the chromosome number of 16 and 24 in one animal (2n = 16 and 3n = 24), and the triploid specimen (3n = 24). See also Figure 18-a (B and C) and Table 1.

**Material.** One set of serial sagittal sections (Specimen No. 1358 a) is retained in KAWAKATSU's laboratory, Fuji Women's College, Sapporo.

**Locality.** See "List of Localities".


**External characters.** The sexually mature specimens of this population are very large in size. The animals may attain over 35 mm in length and 5 to 7 mm in width. They are of a brownish color. Photographs of the preserved specimens including bicephalous animal taken from a lot of regeneration experiments are shown in Figure 6 (A-C).

**Internal characters.** In histological sections, it was observed that the arrangement of the pharynx musculature of the Mt. Daisen specimens is different from the typical arrangement of *Dugesia japonica*. Namely, its outer musculature consists of three layers, i.e., the outer thin longitudinal, the middle thick circular, and the inner thin longitudinal; the inner musculature shows the typical arrangement (Fig. 6 D).

There are no histological peculiarities about the testes, ovaries and yolk glands.

The photomicrographs of the copulatory apparatus are shown in Figure 7 (A and B). The sagittal view of the copulatory apparatus, reconstructed from several sets of sections, is shown in Figure 8. In the penial anatomy, the Mt. Daisen specimens of *Dugesia japonica japonica* have a small valve at the basal part of the dorsal lip of the penis papilla. This character is found in all of the five specimens examined. The halo-like structure surrounding the vagina is well-developed.
Fig. 6. *Dugesia japonica japonica* from the Mt. Daisen locality. A: Preserved sexually mature specimen. B: Ventral view of the specimen A. C: Preserved bicephalic specimen. No. 68 group. D: Photomicrograph of the sagittal section of the part of the pharynx (No. 68 a).

- **ecm**: external circular muscle layer
- **eilm**: external irregular longitudinal muscle layer
- **elm**: external longitudinal muscle layer
- **icm**: internal circular muscle layer
- **ilm**: internal longitudinal muscle layer

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Fig. 7. Photomicrographs of the copulatory apparatus of *Dugesia japonica japonica* from the Mt. Daisen locality. A: Penis (sagittal section, No. 68 c.). B: Vagina (sagittal section, No. 68 c.).

*buc*, bursal canal; *gp*, genital pore; *pb*, penis bulb; *pp*, penis papilla; *sd*, sperm duct; *v*, vagina.
Fig. 8. Diagram showing the sagittal view of the copulatory apparatus of *Dugesia japonica japonica* from the Mt. Daisen locality (No. 68 c).

**Material.** Five sets of serial sagittal sections (Specimen No. 68 a-e) are retained in KAWAKATSU’s laboratory, Fuji Women’s College, Sapporo.

**Locality.** See “List of Localities”.


**External characters.** The sexual specimen of this population may attain about 15 mm in length and 2 mm in width. The animal is of a dark brownish color with an indistinct light brownish longitudinal band along the median line of the body. The anterior margin of the body is mottled with indistinct sensory spots. Photographs of the preserved specimens from the Wooyi-dong population are found in KIM (1964, p. 261, Fig. 1 A and B) and KAWAKATSU & KIM (1966, p. 104, Fig. 1 A).

**Internal characters.** The description of the genital anatomy of the specimens from the Wooyi-dong population was described in the previous paper (cf. KAWAKATSU & KIM, 1966, p. 105, Fig. 3 B). After reexamination of the slides (Specimen No. 403 group), it must be said that the histological details and the anatomical characters of the copulatory apparatus of the Korean specimens are coincident with those of *Dugesia japonica japonica* from the type-locality. The halo-like structure surrounding the vagina in the Wooyi-dong specimens shows a slightly low degree of differentiation.

**Cytological observation.** One sexual and 5 asexual specimens were examined cytologically. In the sexual specimen the chromosome number is \(2n = 16\) in 32 mitoses. The genome consisted of 8 metacentric chromosomes, of which no. 4 seemed to be slightly submetacentric. In meiosis 8 bivalents were formed (Fig. 16 A). The karyotype of the present material is very similar to that of the Matsu-moto specimens.
In the asexual specimens, two kinds of karyotypes were found. The three specimens had the cells with 16 chromosomes and 24 chromosomes in one animal \( (2n = 16 \text{ and } 3n = 24) \) (Fig. 16 A). The remaining 2 specimens showed a peculiar karyotype. Namely, they had two types of cells with the chromosome number of 25 and 26 in one animal. It was observed that the cells with the chromosome number of 25 contained one acrocentric chromosome in addition to the usual karyotype of the triploid specimens (Fig. 16 B). The cells with 26 chromosomes were fundamentally triploid except no. 2 which consisted of two chromosomes. In addition to these chromosomes, extra three chromosomes were found. They were acrocentric or telocentric (Fig. 16 C). See also Figure 18-a (D-G) and Table 1.

Material. Only used for karyological examination. The serial sagittal sections of the Wooyi-dong specimens used in the previous study (cf. \textit{Kawakatsu \\& Kim}, 1966; Specimen Nos. 403 a and 404 a) are retained in \textit{Kawakatsu}'s laboratory, Fuji Women's College, Sapporo.

Locality. See "List of Localities".


External characters. The general appearance of the asexual specimens from the Seoul population is very similar to that of the specimens from the Wooyi-dong population. The animals are about 10 mm in length and 1.5 mm in width.

Cytological examination. Six asexual specimens were examined cytologically. The three specimens had the cells with 16 chromosomes and 24 chromosomes in one animal \( (2n = 16 \text{ and } 3n = 24) \) (Fig. 16 D). The remaining 3 specimens had only triploid cells \( (3n = 24) \). No diploid specimens were found in this population. See also Figure 18-a (H and 1) and Table 1.

Material. Only used for karyological examination.

Locality. See "List of Localities".

\textit{Dugesia japonica ryukyuensis} \textit{Kawakatsu} subspec. nov.,


The original description of \textit{Dugesia japonica ryukyuensis} subspec. nov. will be given below based upon the material from the Urasoe population in Okinawa.

1. Specimens from the Urasoe Population, Okinawa, Japan.

External characters. The general appearance of both live and preserved specimens from the Urasoe locality is shown in photographs in Figure 9 (A-H). Externally, the animals are indistinguishable from the nominative subspecies. The sexually mature specimens attain 10 to 15 mm in length.
Fig. 9. *Dugesia japonica ryukyuensis* subspec. nov. from the Urasoe locality (the type-locality).

and 1.5 to 2 mm in width. The two eyes (irregularities may occur) with usual rounded clear areas are situated on the dorsal side of the head. The pharynx is inserted somewhat behind the middle of the body. The genital pore opens at the slightly anterior level of the halfway between the mouth and the posterior end of the body.

The general color of the live specimens is light brown to grayish brown on the dorsal surface with numerous dark brown to blackish brown pigments or small granules. The body margin and the areas above the pharynx and copulatory apparatus are of a lighter hue. The auricular sense organ which is a small, elongated leaf shape is indistinctly visible on each side of the head. In the Urasoe specimens examined, no sensory spots were observed at the anterior margin of the head.

**Internal characters.** In large specimens the anterior intestinal trunk bears 8 to 12 lateral branches; each posterior trunk has 12 to 15 or more short lateral branches. It was observed in the histological sections that the outer musculature of the pharynx consists of two layers, the outer thin longitudinal and the inner thick circular muscle fibres.

The ovaries, dorsal testes and yolk glands are conspicuous in every sexually mature specimen examined. A pair of well-developed seminal ducts is seen in the usual position.

The photomicrographs of the copulatory apparatus are shown in Figures 10 (A and B) and 11 (A-F). The sagittal view of the copulatory apparatus of the well-extended specimen (Specimen No. 1379 a; holotype) is shown in Figure 12.

The penis bulb is large and hemispherical in shape and is strongly muscular in nature. It contains a rather wide retort-shaped cavity which is lined by a thick, nucleate epithelium. The two sperm ducts open into the bulbular cavity separately. The bulb is pierced by numerous ducts of penis glands. The secretion of these ducts consists of pale eosinophilous granules. The penis papilla having a strong constriction at its basal part is rather long, conical and highly asymmetrical in shape. It contains a narrow long ejaculatory duct which opens into the male genital antrum on the ventral side of the papilla.

A well-developed diaphragm is present in the penis lumen at the beginning of the ejaculatory duct. The outer wall of the papilla (and the valves of the papilla) is lined with a nucleate epithelium. Below the epithelium there are two layers of muscle fibres, one circular and the other longitudinal.

The male genital antrum is lined by a glandular nucleate epithelium. The subepithelial muscle fibres show the same arrangement as those of the papilla. A small glandular fold or a slit is differentiated on the floor of the male genital antrum near the genital pore.

The constriction or the valve surrounding the basal part of the penis papilla is the most important taxonomic character which separates the present new subspecies from the nominate form. As will be seen in Figures 10 (A and B) and 12, the dorsal lip of the valve is larger than the ventral one. The valves (especially the dorsal valve) contain numerous ducts of eosinophilic glands. The secretion of these ducts consists of heavily stained granules with erythrocin.

The copulatory bursa is moderate to large in size. The bursa stalk which is a rather wide and long duct opens into the narrow common genital antrum near the genital pore (common antrum is usually narrow in the present new subspecies). The bursal canal has a glandular nucleate epithelium. At the anterior two-thirds of the bursa stalk, there are two layers of muscle fibres, the inner thick circular and the outer thin longitudinal. The posterior one-third of the stalk become thick and to form the less-developed vagina. In this part the subepithelial muscle fibres consist of three layers: the

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4) In many specimens from several localities of the Ryūkyū Islands, the anterior margin of the head is mottled with indistinct white stipples (cf. Ichikawa & Kawakatsu, 1967, pp. 179-180, Fig. 2 H and I: Kawakatsu & Tanaka 1971, p. 49, 1975, p. 74, Fig. 1 B-D)
Fig. 10. Photomicrographs of the copulatory apparatus of *Dugesia japonica ryukyuensis* subspec. nov. from the Urasoe locality. 

A: Penis of the holotype (sagittal section, No. 1379 a ).

B: Vagina of the holotype (sagittal section, No. 1379 a ). Arrow indicates the opening of the ovovitelline duct.

bc, bulbar cavity; dlp, dorsal lip of the penis papilla; gp, genital pore; ma, male antrum; od, ovovitelline duct; pb, penis bulb; pp, penis papilla; vilp, ventral lip of the penis papilla.

- 99 -
Fig. 11. Photomicrographs showing the parts of the copulatory apparatus of *Dugesia japonica ryukyuensis* subsp. nov. from the Urasoe locality. A: Sagittal section of a part of the prepharyngeal region (No. 1379 a). Notice the arrangement of the dorsal testes. B: Sagittal section of a part of the postpharyngeal region (No. 1379 a). C: Near mid-sagittal section of the copulatory apparatus (No. 1379 a). D: Sagittal section through the middle part of the bursal canal (No. 1379 a). E: Penis (sagittal section, No. 1379 b). F: Penis (sagittal section, No. 1379 f). G: Penis (horizontal section, No. 1379 h).

bc, bulbar cavity; buc, bursal canal; cb, copulatory bursa; ed, ejaculatory duct; ma, male antrum; pb, penis bulb; pc, pharyngeal chamber; pp, penis papilla; sv, spermiducal vesicle.
Fig. 12. Diagram showing the sagittal view of the copulatory apparatus of *Dugesia japonica ryukyuensis* subspec. nov. from the Urasoe locality (holotype, No. 1379 a).

*bc*, bulbary cavity; *bs*, bursa stalk; *ca*, common antrum; *cb*, copulatory bursa; *cg*, cement gland; *dip*, dorsal lip of the penis papilla; *ed*, ejaculatory duct; *gp*, genital pore; *m*, mouth; *ma*, male antrum; *od*, ovovitelline duct; *pb*, penis bulb; *ph*, pharynx; *pp*, penis papilla; *sd*, sperm duct; *sv*, spermiducal vesicle; *v*, vagina; *vlp*, ventral lip of the penis papilla.
inner thin longitudinal, the middle thick circular, and the several coarse rows of the outer longitudinal. It is observed that the second and the third muscle layers are slightly intermingled with each other. The wall of the vagina is pierced by numerous ducts of eosinophilic and cyanophilic gland ducts. Two ovovitelline ducts open into the terminal part of the vagina. Weakly eosinophilous cement glands open into the common genital antrum near the genital pore.

The cocoon is spherical in shape and stalked.

*Cytophysical examination*. Four sexual and 8 asexual specimens were examined cytotopically. One-hundred and seventeen mitoses in 2 sexual specimens revealed a chromosome number of $2n = 14$. During meiosis 7 bivalents were found (Fig. 17 A). The genom consisted of 6 metacentric chromosomes and one acrocentric chromosome (Fig. 17 B). The third sexual specimen had the somatic with same mitotic plate described above. It was however observed in the fourth sexual specimen that a small excess chromosome existed in addition to the diploid components ($2n + 1 = 14 + 1$) (Fig. 17 C). Although the sperms were found in this specimen, no mitotic plate was observed in the germ cells.

In the asexual specimen examined, two kinds of karyotypes were found. The two specimens had the cells with 6 pairs of metacentric chromosomes and one pair of acrocentric chromosomes ($2n = 14$). The remaining 6 asexual specimens were clearly the triploid forms. However, some variations were observed in their chromosome numbers and the karyotypes. Namely, their mitotic plates showed the chromosome number of $3n = 21$, $3n + 1 = 21 + 1$, $3n + 2 = 21 + 2$, and $3n + 3 = 21 + 3$, respectively. The excess chromosomes (1 to 3 in numbers) were small in size and morphologically similar to that of the fourth sexual specimen described above (Fig. 17 D). See also Figure 18-b (J-L) and Table 1.

*Differential diagnosis of Dugesia japonica ryukyuensis*. The present new subspecies, *Dugesia japonica ryukyuensis*, differs from the nominate subspecies in the following characters of the genital anatomy: penis papilla with a well-developed valve surrounding its basal part; vagina less-developed. The specimens from the type-locality have a chromosome number of $n = 7$ and $2n = 14$. The chromosome number of 21 (3n) is also found in the asexual specimens from the type-locality.

**Type-series.** Holotype —— One set of serial sections (Specimen No. 1379a, 3 slides) will be deposited in the Department of Zoology, National Science Museum, Tōkyō. Two paratypes (No. 1379 g, h) and two whole mounts (No. 1386 a, b) will also be deposited in the same Museum. The remaining slides including the paratypes and several whole mounts are retained in KAWAKATSU's collection (KAWAKATSU's laboratory, Fuji Women's College, Sapporo).

**Type-locality.** A stream at Nakamayama-gawara, Urasoe City, Okinawa, Japan. Altitude, about 20 m. Collected by Mr. I. TANAKA on April 20, 1976 (holotype).

The distribution of *Dugesia japonica ryukyuensis* will follow in the section "Taxonomic and Zoogeographical Notes."

2. Specimens from the Hangchow Population, Che-Kiang, China.

Although the description of the specimens from the Hangchow populations was given in a previous paper (cf. ICHIKAWA & KAWAKATSU, 1967), the result of the reexamination of them will be given bellow.

**External characters.** See ICHIKAWA & KAWAKATSU (1967, pp. 178 - 179, Fig. 2 J, pp. 184 - 185).

**Internal characters.** Serial sagittal sections of the only one fully sexually mature specimen (Specimen No. 391 a) was available for this study. The outer musculature of the pharynx consists of two layers.

The testes, ovaries and yolk glands have been observed in the slides examined. The photograph of the copulatory apparatus is shown in Figure 13. The sagittal view of the copulatory
Fig. 13. Photomicrograph of the copulatory apparatus of *Dugesia japonica ryukyuensis* subspec. nov. from the Hangchow locality, China (No. 391 a). After Ichikawa & Kawakatsu (1967, p. 183, Fig. 4 H). 

*bc*, bulbar cavity; *dlp*, dorsal lip of the penis papilla; *ed*, ejaculatory duct; *ma*, male antrum; *pb*, penis bulb; *pp*, penis papilla; *sd*, sperm duct; *vlp*, ventral lip of the penis papilla.

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Fig. 14. Diagram showing the sagittal view of the copulatory apparatus of *Dugesia japonica ryukyuensis* subspec. nov. from the Hangchow locality, China (redrawn from the slides of the specimen No. 391 a). See Ichikawa & Kawakatsu (1967, p. 182, Fig. 3 C).
Fig. 15. Photomicrographs of the chromosomes of Dugesia japonica japonica from Japan. Ca. \( \times 1000 \). A and B: Matsumoto (the type-locality). A, Meiosis (\( n = 8 \)). B, Mitosis (\( 2n = 16 \)). Notice the sperm. C and D: Mino' o. C, Meiosis (\( n = 8 \)). D, Mitoses (\( 2n = 16 \) and \( 3n = 24 \)). Notice the sperms.
Fig. 17. Photomicrographs of the chromosomes of *Dugesia japonica* ryukyuensis subspec. nov. from Okinawa, Japan. Ca. ×1000. A-D: Urasoe (the type-locality). A, Meiosis (n = 7). B, Mitosis (2n = 14). C, Mitosis (2n + 1 = 14 + 1). Notice the sperms. D, Mitosis (3n + 1 = 21 + 1).

Fig. 18-b (on page 108). Idiogram analysis of the metaphase plates of *Dugesia japonica ryukyuensis* subspec. nov. from Okinawa, Japan. J-L: Urasoe (the type-locality). J, 2n = 14. K, 2n + 1 = 14 + 1. L, 3n + 1 = 21 + 1.
apparatus redrawn from the slides ( Specimen No. 391 a ) is also shown in Figure 14 ( cf. Ichikawa & Kawakatsu, 1967, pp. 182 - 183, Fig. 3C and 4H ). The Hangchow specimen show the typical genital anatomy of Dugesia japonica ryukyuensis. The epideria of the penis papilla, the male genital antrum and the bursal canal are the nucule type. The constriction or the valve surrounding the basal part of the papilla is conspicuous ( the ventral lip of the valve is very small ). The histological structure of the vagina is similar to that of the specimens from the type-locality of Dugesia japonica ryukyuensis.

**Material.** Ten sets of serial sagittal sections ( Specimen No. 391 a - j ) including one sexually mature specimen are retained in Kawakatsu's laboratory, Fuji Women's College, Sapporo.

**Locality.** See "List of Localities".

V. TAXONOMIC AND ZOOGEOGRAPHICAL NOTES

As regards the taxonomy and distribution of Dugesia japonica, a frequent mention was made in the previous articles published by Kawakatsu and his co-researchers ( see "Introduction" ). This commonest and a wide-distributed species in the Far East including the Japanese Islands shows a considerable degree of variation in its morphology and anatomy. Physiologically, the species can be classified into two main groups or so-called physiological races according to the differences of the mode of reproduction : the sexual and the asexual races ( cf. Kawakatsu, 1965, 1967, 1971, 1974; Okugawa, 1955, 1957; Okugawa & Kawakatsu, 1954 - 1958; Sugino, 1969, 1960, 1962, 1969, 1971 ) also demonstrated that Dugesia japonica may consist of many biotypes if classified based upon the physiological and ecological natures. The animals from different localities have shown the various degrees of physiological endurance for the physico-chemical conditions of cultures ( see also Kawakatsu's note including in the paper by Sugino, 1969, pp. 302 - 303; Kawakatsu, 1971 ).

In the comparative genital anatomy of Dugesia japonica from various localities in Japan and its neighbouring countries, the most noticeable variation has been found in the shape of the penis papilla and the degree of development of the vagina. The animals from the type-locality in Middle Japan have a large and highly asymmetrical penis papilla and a well-developed vagina surrounded by thick musculatures. These anatomical and histological characters are more or less conspicuous in the specimens from every known localities in Japan, Korea and Taiwan. On the other hand, a well-developed constriction or the valve surrounding the basal part of the penis papilla was found in the specimens from the Ryūkyū ( Looc choo ) Islands in Southwest Japan and Hangchow in Middle China. It is usually observed that their vagina is somewhat poorly developed compared with that of the specimens from the other geographical areas ( cf. Ichikawa & Kawakatsu, 1967; Kawakatsu, 1971; Kawakatsu & Tanaka, 1971, 1976 ). When Dugesia japonica was described as a new species, the animals from Okinawa and China were also identified as the same species after some hesitation ( cf. Ichikawa & Kawakatsu, 1964, pp. 186 - 187, footnote 2 ). The only root reason of this identification was that the sharp separation of the species into two forms based upon a little difference in the penial anatomy seemed to be unjustifiable because a transitional form was found in Kagoshima, the southernmost part of Kyūshū, in Japan. Namely, a considerably large valve was found at the basal part of the dorsal lip of the penis papilla in the animals from the Kagoshima population ( cf. Kawakatsu & Iwaki, 1967 b, pp. 182 - 184, Fig. 3 ).

Ball ( 1970 ), who studied the taxonomy of several Dugesia species from the Oriental Region, discussed the taxonomic status of Dugesia japonica in comparison of the "atrial fold" ( cf. Kawakatsu, 1972 b, p. 342 ) found in the Dugesia japonica—Dugesia bauiensis complex ( see also Fig. 7 on page 283 of his paper ). Conclusively, he expressed the opinion as follows: "On the available data it seems
desirable that this form of *D. japonica* with the atrial fold should be given separate status, at least at the level of the subspecies" (op. cit., pp. 283–284). Dr. Ball’s proposal was not accepted by Kawakatsu, for which the reason was mentioned above (cf. Kawakatsu, 1971; Kawakatsu, Horikoshi & Akama, 1972, p. 125; Kawakatsu & Mack-Firá, 1975, p. 81; Kawakatsu & Tanaka, 1971, p. 49).

The discovery of the occurrence of the different karyotypes in the animals of the *Dugesia japonica* populations in the Far East might support a possibility of subdivision of this polymorphic species into two subspecies based upon the morphological and anatomical characters. From the result of the comparative reexamination of the Kawakatsu’s slides of *Dugesia japonica* from various localities, it has been found out that the degree of development of the valve surrounding at the basal part of the penis papilla shows a wide variation. A rather wide variation is also found in the anatomy of the vagina.

The distribution of the animals of *Dugesia japonica* in the Far East has been plotted in Figure 19 which is based on the anatomical data of the valve of the penis papilla. This structure in the penial anatomy is classified into 8 degrees as follows: 1) without any valve; 2) with a slightly developed valve at the dorsal lip; 3) with a slightly developed valve at the ventral lip; 4) with slightly developed valves at both the dorsal and ventral lips; 5) with a moderately developed valve at the dorsal lip; 6) with moderately developed valves at both the dorsal and ventral lips; 7) with a well-developed valve surrounding the basal part of the papilla but less-developed at the ventral lip; 8) with a well-developed valve surrounding the basal part of the papilla (usually the valve of the ventral lip is smaller than that of the dorsal lip).

As will be seen in the map of Figure 19, the animals without any valve at the basal part of the penis papilla are distributed in North China (Judged from the figures of the copulatory apparatus in Hsiao, 1934, Tu, 1934 and Kato, 1944), Taiwan (including Kawakatsu’s unpublished data), Korea including Quelpart (Cheju) Island, and Honshu, Shikoku and Hokkaido in the Japanese Islands. The animals with a small valve at the basal part of the penis papilla (located dorsally and/or ventrally of the papilla) are distributed in Hong Kong, Taiwan (including Kawakatsu’s unpublished data), Korea, and the southwestern and the central areas of the Japanese Islands (i.e., the Tsushima Islands, Kyushu, and the Chugoku, the Kinki, the Hokuriku, and the Kantō Regions in Honshu; including Kawakatsu’s unpublished data). A well-developed or a rather well-developed vagina is differentiated in the animals from these geographical areas.

It must be said from the penial anatomy of *Dugesia japonica* that a well-developed valve surrounding the part of the penis papilla like a collar is a general character in the penial anatomy of the animals from the Ryūkyū Islands—the genital anatomy has been studied in the animals from Okinawa Island, Miyako-jima Island, and Ishigaki-jima Island, the Mt. Arisan district in the central part of Taiwan (Kawakatsu’s unpublished data), and Hangchow in Middle China (cf. Ichikawa & Kawakatsu, 1967; Kawakatsu & Tanaka, 1971, 1976). In some Asiatic species of *Dugesia* with a valve surrounding the penis papilla, a narrow space between the under surface of the dorsal valve and the upper surface of the dorsal lip of the penis papilla forms a glandular cavity or a hollow adenodactyl-like structure (cf. Kawakatsu, 1973a, p. 100). This character is not clear in the animals of *Dugesia japonica* from the Ryūkyū Islands, Taiwan and Middle China. Although the function of the valve of the penis papilla is not known, it is supposed that the valve may carry out the elastic movement of the penis when the animals are copulated. A less-developed vagina is found commonly in them.

Among the Asiatic *Dugesia* species, *Dugesia japonica* is an only species investigated karyologically. According to the information of cytologists of Panjab University, the chromosome number of *Dugesia lindbergi* from North India (cf. Kawakatsu & Ogawara, 1974) is 2n =16, but their study has not yet
Fig. 19. Map of the Far East, showing the geographical distribution range of *Dugesia japonica* (*Dugesia japonica japonica* and *Dugesia japonica ryukyuensis* subspec. nov.). Symbols represent the degree of development of the valve at the basal part of the penis papilla.

- : Without any valve;  : With a slightly developed valve at the dorsal lip;  : With a slightly developed valve at the ventral lip;  : With slightly developed valves at both the dorsal and ventral lips;  : With a moderately developed valve at the dorsal lip;  : With moderately developed valves at both the dorsal and ventral lips;  : With a well-developed valve surrounding the basal part of the papilla but less-developed at the ventral lip;  : With a well-developed valve surrounding the basal part of the papilla.
been completed (in litt.). It is true that the karyological data for Dugesia japonica are very limited at present and we need to know the karyotypes of this species from various populations of Japan, especially, the populations of Southwest Japan including the Ryūkyū Islands, Taiwan, Hong Kong, China, and Korea. But the following speculation concerning the chromosomal variations of Dugesia japonica may be interesting for the future studies.

Typically, the chromosome number of the diploid specimens of Dugesia japonica japonica is 2n = 16. On the contrary, the diploid specimens of Dugesia japonica ryukyuensis from the Urasoe population in Okinawa have the chromosome number of 2n = 14. Their karyotype consists of 6 pairs of metacentric chromosomes and one pair of acrocentric chromosomes. Moreover, a number of small excess chromosomes (supernumerary chromosome or microchromosome) are found in some of both diploid and triploid specimens examined. For making a comparison of the idiograms between the Urasoe specimens (2n = 14) and the Matsumoto specimens (2n = 16), the authors assume that a pair of acrocentric chromosomes in the former probably correspond with the fourth pair of chromosomes in the latter; one pair of chromosomes which will be correspond with the sixth pair of chromosomes in the specimens having 2n = 16 chromosomes seems to be missing in the Urasoe specimens (Fig. 21, Table 4).

The interesting karyotypes are found in two triploid specimens from the Wooyi-dong population in Korea. They have cells with the chromosome number of 25 and 26 in one animal. In cells with 25 chromosomes, one additional acrocentric chromosome is found. In cells with 26 chromosomes, the extra three chromosomes are found. The authors speculate that two of these chromosomes, when they are united, seem to be classified as a component of the second set of chromosomes. In other words, one component of the second set may have been divided into two extra chromosomes (Fig. 20 a and β), while the remaining one may correspond to extra acrocentric chromosome represented in the preceding figure (Fig. 20 γ).

What is the origin and the karyological significance of the acrocentric chromosomes found in the animals of Dugesia japonica from certain localities in Okinawa and Korea? At present the authors have no sufficient data for discussing the problem, only to emphasize that the occurrence of these excess chromosomes in some specimens of Dugesia japonica japonica from Korea and Dugesia japonica ryukyuensis from Okinawa may indicates their intraspecific near relationship in a widely distributed, polymorphic species Dugesia japonica.

Considerations about the geographical distribution of Dugesia japonica based upon the topographical and the geological data around the East China Sea area may also be interesting for the analysis of the subspeciation of this species. Both of the Yellow Sea (less than 50 meters in depth) and the East China Sea (less than 200 meters in depth) are characterized as the continental shelves of the Asiatic Continent. The Southwest Islands of Japan, which consist of many small islands, can be classified into three topographical groups. They are: 1) the Ōsumi Islands (Tanegashima Island, Yaku-shima Island, etc.) and the Tokara Islands; 2) the Amami Islands (Amami-Ōshima Island, etc.) and the Okinawa Islands (Oki-no-erabu-jima Island, Okinawa Island, etc.); 3) the Sakishima Islands (Miyako-jima Island, Ishigaki-jima Island, Iriomote-jima Island, Yonaguni Island, etc.). Topographically, the Okinawa Islands and the Sakishima Islands are called the Ryūkyū Islands.

The depth of the straits of the East China Sea and its neighbouring areas is as follows: about 140 meters at the Korean Straits (between the Korean Peninsula and the Tsushima Islands); about 120 meters at the Tsushima Straits (between the Tsushima Islands and Kyūshū); about 200 meters at the Ōsumi Straits (between Kyūshū and Tanegashima-Yakushima Islands); about 1000 meters at the Amami Straits (between the Tokara Islands and Amami-Ōshima Island); about 1000 meters at the Okinawa Straits (between Okinawa Island and Miyako-jima Island), about 1000 meters at the straits between
Fig. 20. Explanatory idiograms of the Korean *Dugesia japonica japonica* from the Wooyi-dong locality. F: Chromosome nos. 25 (see Fig. 18-a, F). G': Chromosome nos. 26 (see Fig. 18-a, G). Explanation in the text.

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Fig. 21. Explanatory idiogram of *Dugesia japonica ryukyuensis* subspec. nov. with the chromosomes of 2n = 14 (J') in comparison with the idiogram of *Dugesia japonica japonica* from the type-locality. (A, 2n = 16; the Matsumoto specimen). The numbers in parentheses of the bottom row correspond with the numbers shown in Fig. 18-b. Explanation in the text.

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(5) (6)
Yonaguni Island and Taiwan; about 35 meters at the Taiwan (Formosan) Straits (between Taiwan and Fukien in China). The Bashi Channel is about 1850 meters in the deepest part (between the South Cape of Taiwan and Luzon Island of the Philippines.

According to the previous studies by geologists, geographers and palaeo-climatologists, the Japanese Islands constituted a part of the northeastern edge of the Asiatic Continent until the middle of the Oligocene in the Palaeogene Tertiary (ca. 25 m. y. ago). The climate of the Palaeogene Period in that latitude seemed to be tropical or subtropical and humid. In the Miocene of the Neogene Tertiary, the Old Sea of Japan was depressed and the greater part of Japan had sunk beneath the waves except for the Southwest Japan and the central parts of Middle and North Japan (ca. 19-9 m. y. ago). During this age the Old Japanese Islands seemed to be under a benign climate. In the Pliocene of the Neogene Tertiary, or perhaps, even in the earliest Pleistocene of the Quaternary Period, the landmass of the Old Japanese Islands were mostly united with the northeastern edge of the Continent except for the areas around the Old Sea of Japan. The Yellow Sea and the East China Sea did not exist in that age (ca. 6-2 m. y. ago). From the late Miocene and the middle Pliocene, the climate may have become rather cold; in the late Pliocene it might become warmer. The separation of the Old Southwest Islands into several groups of islands by the openings of straits might begin in the late Pliocene or the early Pleistocene (ca. 2-0.8 m. y. ago). The formation of the Old East China Sea might also be in progress. But the openings of the Taiwan Straits, the Korean Straits and the Tsushima Straits seemed to occur later — probably not until the late Pleistocene (ca. 0.15 m. y. ago). The Yellow Sea might appear gradually in that Epoch. In the Quaternary Period, the Northern Hemisphere had been attacked by the glaciations — Günz (ca. 0.6 - 0.5 m. y. ago), Mindel (ca. 0.48 - 0.38 m. y. ago), Riss (ca. 0.25 - 0.15 m. y. ago), and Würm W I - W IV (ca. 0.07 - 0.01 m. y. ago), and the eustatic movement had often been repeated. The climate of the second interglacial epoch (i.e., the Mindel-Riss interglacial epoch) seems to have been very temperate. The climate of the subinterglacial epochs in the Würm Glaciation seems to have been rather colder than that of the present. The present shoreline of the Japanese Islands which consist of four main islands (Kyūshū, Shikoku, Honshū, and Hokkaidō) and many small islands belonging to them may have been completed in the early Holocene (ca. 0.15 - 0.01 m. y. ago). The final opening of the Tsugaru Straits (the BLAKISTON'S Line) between Honshū and Hokkaidō seems to have been about 0.18 million years ago (cf. MINATO et al., 1973; and others). The local flora and fauna of the Japanese Islands were effected by the repeated submerision of the ocean, the volcanic activity, and the change of climates during the Cainozoic Era.

KAWAKATSU (1965, pp. 375 - 377; see also 1967) considered from the geological history and zoogeography of the trilobed turbellarians that the Old Japanese Islands were invaded by the proto-species of Dugesia japonica which came through the southern transmigrating route. And then, the species may have extended its distribution from south to north during the Pliocene or the Pleistocene. He supposed the transmigrating route of the species as follows: From South China to Taiwan (Formosa) and the Loocchoo Islands and Kyūshū in South Japan; and from Middle China to Korea and Kyūshū (cf. KAWAKATSU, 1974, pp. 301 - 307). KAWAKATSU'S hypothesis mentioned above is quite tenable due to the occurrence of Dugesia japonica in Hong kong (cf. KAWAKATSU & WONG, 1975) and several islands in the Ryūkyū Islands (cf. KAWAKATSU & TANAKA, 1971, 1976).

Dugesia japonica is a eurythermic species adapted to the warm waters and is distributed widely in the Far East. Judging from the evidences of the geological history and the palaeoclimatological data of the Far East, there is a high possibility that the arrival of the old proto-species of Dugesia japonica to the Old Japanese Islands is earlier than KAWAKATSU'S previous supposition cited above. At its earliest, it can be retroacted to the Palaeocene in the Palaeogene Tertiary (ca. 65 - 45 m. y. ago). At that
age the present North Japan was under a tropical or subtropical climate (a fossil freshwater mud-snail, *Cipangopaludina ishibariensis* and, a bivalve, *Lanceolaria pisciformis*, were common in the strata of this age in Hokkaidō). Some of the animals of the old proto-species could survive free of the waves in the Miocene and glacial periods in the Quaternary Period even in Middle and North Japan. The invaded animals of the proto-species which had invaded into the Old Southwest Islands through the southern route were gradually isolated in that area since the early Pleistocene. On the other hand, the transmigrations of the animals of the proto-species of the other groups were possible from the Continent to Taiwan, North China, Korea, and the Old Japanese Islands across the land of the Old Yellow Sea. It seems to be that the complete separation of the faunal elements between Korea and North Kyūshū in Japan (and between South Kyūshū and the Ōsumi Islands) may have not occurred before the early Holocene.

Considerations about the zoogeography and the comparative genital anatomy of the Asiatic species of the genus *Dugesia* will be discussed here. Figure 22 shows the world-wide distribution of the species of the “Dugesia gonoccephala group”; the possible transmigrating routes of them are also illustrated in Figure 23 (for details, see Kawakatsu & Ōgawara, 1974, pp. 89 - 93; see also Ball, 1975). All of the Asiatic *Dugesia* species belong to this group. And they can be divided into four groups by the characters of the penial anatomy. Namely:


Only *Dugesia izuensis* lacks a diaphragm in the penis lumen.

2). A group having an asymmetrical penis papilla; with a diaphragm in the penis lumen; without a valve at the basal part of the penis papilla; without adenodactyl: *Dugesia indica* Kawakatsu, 1969, from Middle India; *Dugesia nannophallus* BALL, 1970, from Sri Lanka (= Ceylon; cf. Kawakatsu & Ōgawara, 1974); *Dugesia andamanensis* (Kaburaki, 1925) from the Andaman Islands; *Dugesia burmaensis* (Kaburaki, 1918) from Burma; *Dugesia borneana* Kawakatsu, 1972, from North Borneo (cf. Kawakatsu & Ōgawara, 1974); *Dugesia novaguineana* Kawakatsu, 1976, from New Guinea.

*Dugesia annandalei* (Kaburaki, 1918) from Burma has a symmetrical penis papilla and lacks a diaphragm in the penis lumen. But reexamination of this uncertain species should be necessary for the taxonomic discussion.

3). A group having an asymmetrical penis papilla; with a diaphragm in the penis lumen; with a valve at the basal part of the penis papilla; without adenodactyl: *Dugesia lindbergi* De Beauchamp 1959, from Afghanistan, Pakistan (cf. Kawakatsu, 1973) and North India (cf. Kawakatsu & Ōgawara, 1974); *Dugesia krishnasuwamyi* Kawakatsu, 1975, from South India; *Dugesia batuensis* Ball, 1970, from Malaya (cf. Kawakatsu, 1972a, b); *Dugesia indonesiana* Kawakatsu, 1973, from Indonesia (Sumatra and Java); *Dugesia hymanae* (Šivicke, 1928) from the Philippines (cf. Kawakatsu, 1972).

4) A group having an asymmetrical penis papilla; with a diaphragm in the penis lumen; with or without a valve at the basal part of the penis papilla (a wide variation is found in this character); without adenodactyl: *Dugesia japonica* Ichikawa et Kawakatsu, 1964, from the Far East including the Japanese Islands (for literature, see foregoing sections).

Of these four groups of the Asiatic *Dugesia* species, the phylogenetical relation of the first groups seems to be rather distant. But there may be a deep relationship among the second, the third and the fourth groups. Generally the genital anatomy of each species of the second group is very
Fig. 22. The distribution of the species of the *Dugesia gonocephala* group. The geographical distribution ranges of *Dugesia lindbergi* and *Dugesia japonica* are also shown. Symbols represent the anatomical characters of the male couplatory organs.

○: Penis papilla symmetrical, without adenodactyl (s); ○: Penis papilla symmetrical, with adenodactyl (s); ○: Penis papilla asymmetrical, with adenodactyl (s).

The other symbols represent the species having an asymmetrical penis papilla and without adenodactyl (s). They are classified into ten groups according to the degree of development of the valve at the basal part of the penis papilla.

□: Without any valve; □: With a slightly developed valve at the dorsal lip; □: With a slightly developed valve at the ventral lip; □: With slightly developed valves at both the dorsal and ventral lips; □: With a moderately developed valve at the dorsal lip; □: With a well-developed valve at the dorsal lip; □: With moderately developed valves at both the dorsal and ventral lips; □: With a well-developed valve surrounding the basal part of the papilla but less-developed at the ventral lip; □: With a well-developed valve surrounding the basal part of the papilla but moderately developed at the ventral lip; □: With a well-developed valve surrounding the basal part of the papilla.
Fig. 23. The distribution and dispersal of the species of the Dugesia gonocephala group. The solid lines represent the proto-Dugesia group without the valve at the basal part of the penis papilla. The broken lines represent the proto-Dugesia group with the valve at the basal part of the penis papilla. See Fig. 22.

Fig. 24. Phylogenetic diagram of the known Asiatic Dugesia species. 1: A group having a symmetrical penis papilla; with adenodactyl (s). 2: A group having an asymmetrical penis papilla; with a diaphragm in the penis lumen; without a valve at the basal part of the penis papilla; without adenodactyl (s). 3: A group having an asymmetrical penis papilla; with a diaphragm in the penis lumen; with a valve at the basal part of the penis papilla; without adenodactyl (s). 4: A group having an asymmetrical penis papilla; with a diaphragm in the penis lumen; with or without a valve at the basal part of the penis papilla; without adenodactyl (s). Explanation in the text.
Proto-Dugesia of Asia
(Dugesia gonocephla group)

Proto-Dugesia

Fig. 24
similar to that of *Dugesia japonica* from many populations in Taiwan, North China, Korea, and Japan (Shikoku, Honshū including the type-locality and Hokkaidō). Among the species of the third group, the valve at the basal part of the dorsal lip of the penis papilla is only found in *Dugesia lindbergi*. This character is also found in *Dugesia japonica* from some localities, such as Hong Kong, Taiwan, Korea, and Japan (Kyūshū and the south and central areas of Honshū). In the other species of this group, i.e., *Dugesia batuensis*, *Dugesia indonesiana*, *Dugesia hymanae*, and especially *Dugesia kryshnaswamyi*, a well-developed valve surrounding the basal part of the penis papilla like a collar is differentiated. The same character in the penial anatomy is also found in *Dugesia japonica* from Middle China, Taiwan, and the Ryūkyū Islands in Southwest Japan.

Judging from the similarity in the morphological and anatomical characters of the Asiatic *Dugesia* species, the following speculation can be possible for the understanding of the speciation and subspeciation of *Dugesia japonica* as a polymorphic species. We may first suggest that the old land-mass of Japan was invaded by some of the earlier proto-species of the second group (without valve at the basal part of the penis papilla). At that age the Old Ryūkyū Islands was not existent. We must admit the possibility that there were slow and repeated northward movements of the proto-species through the Southeast Asia when various sea barriers were removed even in the early Pleistocene according to the repeated eustacy. At that age the Malay Peninsula, Indonesia, Borneo, the Philippines, and Taiwan were connected with each other. In the late Miocene or the early Pliocene, it seem to be that the proto-species of the *Dugesia lindbergi* type in the third group (with a valve only at the basal part of the dorsal lip of the penis papilla) may also have arrived at the Old Ryūkyū Islands and the south and the central areas of the Old Japanese Islands through the southern and south-eastern transmigrating routes. This proto-species may have adapted themselves to a rather cold-water habitat in the third group.

In the middle Pliocene, some of the proto-species of the third group (with a well-developed valve surrounding the basal part of the penis papilla) may have arrived at Taiwan, Middle China and the Old Ryūkyū Islands through the southern migration route as the new comers. They might be the warm-water inhabitants. Although their farthest northern outpost seems to have been the land of the Old Yellow Sea (which is now the continental shelf) and the western area of the Korean Peninsula, their rapid north-eastward migration may have been obstructed by the cold climate of the middle Pliocene and the isolation of the Old Ryūkyū Islands in the late Pliocene or the early Pleistocene.

In conclusion, the authors suppose from the foregoing considerations that the proto-species of *Dugesia japonica* must have been originated from the ancestral forms of the second and the third groups of the Asiatic *Dugesia* species. The crossing of the animals of the proto-species of the second group and of the *Dugesia lindbergi* type in the third group may have accelerated the subspeciation of *Dugesia japonica*. The subspeciation of *Dugesia japonica ryukyuensis* may have been accelerated by the crossing of the animals of the proto-species of the second and the third groups including the new comers which might be differentiated in Southeast Asia.

The above discussed schema is illustrated in Figures 24 and 25. More light could be thrown on the problem by the further study of the probursalan fauna of the Himalaya-Tibet-China area.

From the foregoing considerations, the geographical distribution ranges of two subspecies of *Dugesia japonica* will be defined as follows:

*Dugesia japonica japonica* Ichikawa et Kawakatsu : Hong Kong, Taiwan (probably entire region), North China, Korea, and Kyūshū (including the Satsunan Islands), Shikoku, Honshū, Hokkaidō, and many small islands in the Japanese Islands.

*Dugesia japonica ryukyuensis* Kawakatsu subspec. nov.: Taiwan (probably limited in the
Fig. 25. The distribution and dispersal of *Dugesia japonica*. The solid lines (2') represent the proto-*Dugesia japonica* without the valve at the basal part of the penis papilla. The broken lines (3') represent the proto-*Dugesia japonica* with the valve at the basal part of the dorsal lip of the penis papilla. The chain lines (3'') represent the proto-*Dugesia japonica* with the valve surrounding the basal part of the penis papilla. See also Fig. 19. Explanation in the text.
central mountainous area), Middle China (only Hangchow is known at present), and the Ryūkyū Island and the Amami Islands in Southwest Japan.

VI. SUMMARY

The taxonomic revision of a freshwater planarian species *Dugesia japonica* Ichikawa et Kawakatsu, 1964, based upon the morphological, anatomical and karyological data, is described in the present paper. A redescriptions of the nominative subspecies, *Dugesia japonica japonica* Ichikawa et Kawakatsu, and an original description of a new subspecies, *Dugesia japonica ryukyuensis* Kawakatsu, subsp. nov., are also given. The former is characterized in having a penis papilla without a well-developed valve surrounding its basal part and in having a well-developed vagina (chromosome no.: n = 8, 2n = 16, 3n = 24; distribution: Hong Kong, Taiwan, North China, Korea, and Japan except for the Ryūkyū Islands). The latter, new subspecies, is characterized in having a penis papilla with a well-developed valve surrounding its basal part and in having a less-developed vagina (chromosome no.: n = 7, 2n = 14, 3n = 21; distribution: Taiwan, Middle China, and the Ryūkyū Islands and the Amami Islands in Southwest Japan).

The problems concerning the possible transmigrating routes of the proto-species of *Dugesia japonica* and their speciation and subspeciation are discussed from the taxonomic, karyological and the zoogeographical viewpoints. The conclusion is illustrated in Figures 24 and 25.

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補遺．川勝正治．日本列島を含む東北地域に広く分布しているナミウズムシは，形態的・生理的な形質の他，染色体構成についても変異の認められる多型種である。分類学的再検討の結果，本論文で，本種を2亜種に分冊した。本種は実験動物として著明なものであり，その分類に関しては国際を含む目的で試験を付した。

ナミウズムシ Dugesia japonica Ichikawa et Kawakatsu, 1964. 極東地域に分布し，下記の2亜種を含む。

Dugesia japonica japonica Ichikawa et Kawakatsu, 1964. 冠名亜種で，香港・台湾（ほぼ全地域）・中国北部・韓国；日本列島のうち，九州（薩南諸島を含む）・四国・本州・北海道，及び小島嶼に分布。

Dugesia japonica ryukyuensis Kawakatsu, 1976. 本論文で原記載した新亜種で，台湾（主に中央山地）・中国中部；琉球諸島・奄美諸島に分布。

上記の2亜種に対して，適当な和名を与えるべきかどうかについても検討した。結論として，下等動物の亜種に和名を与えることは独立種と誤解される恐れもあるので，ナミウズムシという和名を適意で用いることにした。この件に関し，御意見をうかがった市川純彦博士と川合順次博士（樋崎目の分類）に感謝する。

沖村・田村・川勝・杉野（1976）及び，川勝・沖村・村村・杉野（1976）の記事（動物学雑誌，85巻，507-508頁）は本論文の和文摘要の性格を持ち，本種のさまざまな問題に関する解説的記事は改めて別報する。

なお，非専門家の方々の記事中に，日本産のナミウズムシに対して Dugesia gonocephala の学名をあてているものを散見するが ヨーロッパナミウズムシ Dugesia (= Planaria ; = Euplanaria) gonocephala (Ducès, 1830) は，ヨーロッパとアフリカにだけ分布する別種であって，東北地域には産しない（市川・川勝，1964，動植，73巻，305頁；川勝，1965，遺伝，19巻，10号，31-37頁，他），学名の誤用をさけるとともに，非分類学的論文についても実験材料の採取地を明記することが望ましいと考えられる。

December 25, 1976.

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