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THE KARYOTYPE AND A NEW LOCALITY FOR
THE LAND NEMERTINE
GEONEMERTES PELAENSIS SEMPER, 1863

by

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INTRODUCTION

Chromosomal studies with detailed karyotypes are well known for Platyhelminthes, free living and parasitic. Even among land planarians a few reports are known (cf. BALL & DE VRIES, 1983; OKI, TAMURA, OGREN & KAWAKATSU, In press). On the other hand, in the phylum Nemertina (=Nemertea), only in a few species are chromosome numbers known, a fact illustrated by the following account from GONTCHAROFF (1961, pp. 838-839): "... quelques exceptions près (4 chromosomes chez *Tetrastemma vermiculus* LEBEDINSKY), le nombre diploïde des chromosomes est de 16: *Lineus ruber*, *Prostoma graecense*, *Malacobdella grossa*, ou de 32: *Cerebratulus marginatus*, *Emplectonema gracilis* et *Micrura caeca*."¹⁾

A literature search in BIOSIS 1969-1987 revealed only one recent report. ROE (1986), in a study of parthenogenesis in *Carcinonemertes* spp., haploid embryos contain 13 chromosomes per cell; the diploid number would be 26. He studied the species *C. epialti* and *C. errans* from the U.S. west coast.²⁾

The discovery of a population of land nemertines, on Chichijima Island of Japan, provided an opportunity to investigate Nemertine karyology according to methods now being used for land planarians (cf. KAWAKATSU, OKI, TAMURA, SEKIGUCHI & OGREN, 1987) and provide a new record.

The purpose of this paper is to present the chromosomal description of a small collection of land Nemertina from Chichijima (=Chichi Jima or Chichi-jima) Island (or Peel Island), the largest island in the Chichijima Islands (=Chichi Jima Rettô; also known as the Beechey Islands) belonging to the Ogasawara Islands (=Ogasawara Guntô; also called the Bonin Islands). Chichijima Island is located approximately 1,000 km SSE of Tôkyô, Japan. Living specimens of land nemertines collected by KITAGAWA from the Island were photographed and then preserved. Both live and preserved specimens with a color photograph of live specimens were sent to KAWAKATSU in 1986 and 1987. OKI and TAMURA, who received several live specimens from KAWAKATSU, succeeded in observing and determining the chromosome number of the animal. The species identification was made by OGREN, who received preserved specimens from KAWAKATSU at the occasion of the Fifth International Symposium on the Biology of Turbellaria held at Göttingen in the summer of 1987. Chromosomal analysis was made in the laboratory of OKI and TAMURA. Some part of the "Ecology Note" in the present paper is based upon KITAGAWA's observations made on the spot.

HABITATS, MATERIAL AND METHODS

The following samples were used.

1) KAWAKATSU's Specimen Lot No.1882. Locality: A roadside bush at Miyano-hama-michi, the NW of Chichijima Island; altitude, ca. 25 m. Collected by KITAGAWA on August 10, 1981. Five specimens were fixed with 70% ethanol.



Fig. 1. Live specimens of *Geonemertes pelaensis* SEMPER, 1863, from Chihijima Island.

1), 2). The taxonomic status and the complete scientific names of these 9 species are as follows:

Anopla

Heteronemertea

Lineidae

Cerebratulus marginatus PENIER, 1804

Lineus ruber MÜLLER, 1774

Micrura caeca VERRILL, 1892

Enopla

Hoplonemertea

Monostylifera

Carcinonemertidae

Carcinonemertes epialti COE, 1902

Carcinonemertes errans WICKHAM, 1978

Emplectonemertidae

Emplectonema gracilis (JOHNSTON, 1837)

Tetrastemmatidae

Prostoma graecence (BÖHMIG, 1892)

Tetrastemma vermiculus (QUATREFAGES, 1846)

Bdellonemertea

Malacobdellidae

Malacobdella grossa (MÜLLER, 1776)

2) KAWAKATSU's Specimen Lot No. 1899. Locality: Under a flowerpot placed in a garden at Nishi-machi, near the first locality of Chichijima Island; altitude, ca. 10 m. Collected by KITAGAWA on July 18, 1987. Seven specimens were fixed with 70% ethanol (2 of them were studied by OGREN).

3) Nine live specimens collected by KITAGAWA from the first locality on June 20, 1987, were received in Sapporo in a good condition; 5 of them were fixed with Bouin's fluid in KAWAKATSU's laboratory (Specimen Lot No. 1900). The remaining 4 live specimens were sent to the laboratory of OKI and TAMURA in Ōsaka; 2 of them were studied cytologically by them.

KAWAKATSU prepared a whole mount of an unstained, well-preserved specimen (No. 1899 -a). Serial transverse sections of worms (Specimen Lot No. 1899) for histological examination prepared in OGREN's laboratory were stained with hematoxylin and eosin. For the chromosomal analysis, regenerating somatic cells were observed according to the squash method for land planarians (cf. KAWAKATSU, OKI, TAMURA, SEKIGUCHI & OGREN, 1987, p. 23, fig. 5).

TAXONOMIC ACCOUNT

Class ENOPLA

Order HOPLONEMERTEA

Suborder MONOSTYLIFERA

Family PROSORHOCHMIDAE

Genus *GEONEMERTES* SEMPER, 1863

Geonemertes pelaensis SEMPER, 1863

External Appearance. The general appearance in life of several specimens, reproduced from a color photograph, is shown in Figure 1. Photographs of a whole mounted specimen are shown in Figure 2 (A-F). The following observations by KAWAKATSU and OGREN are based upon living and preserved worms. The largest living specimen measured approximately 60 mm in length in the creeping state, and 2 mm in diameter. Smaller specimens were 45 to 50 mm long.

The body is cylindrical in shape, but ventral surface may be slightly flattened. Elongated body has a blunt anterior head portion bearing 2 pairs of eyes; the anterior pair are large cup-shaped, the posterior pair are small and less pigmented. In one specimen with extruded proboscis, the body slightly narrows just behind the head; body tapers at the rear to a distinct point (Figs. 1 and 2 A-F).

In life the body is semitranslucent, unpigmented, and whitish, or slightly pale brown, in coloration. There is a single, narrow, brown pigmented, middorsal stripe which extends from just behind the level of the eyes almost to the posterior end of the body (Figs. 1, 2 A and D). The pigment of the stripe is primarily in the epithelium not the underlying tissue. Examination of animals with high-power of the stereomicroscope proved that many, small, blackish pigment spots of an irregular size are scattered alongside of the middorsal stripe; the concentration of blackish pigments is also present at the tail region (Fig. 2 A, D and F). The ventral side of the body lacks pigment patterns (Fig. 2 B).

The proboscis was extruded from the anterior end in some preserved specimens (Fig. 2 C and E). One middle-sized preserved worm, 44 mm long and 2 mm diameter, examined by OGREN, had

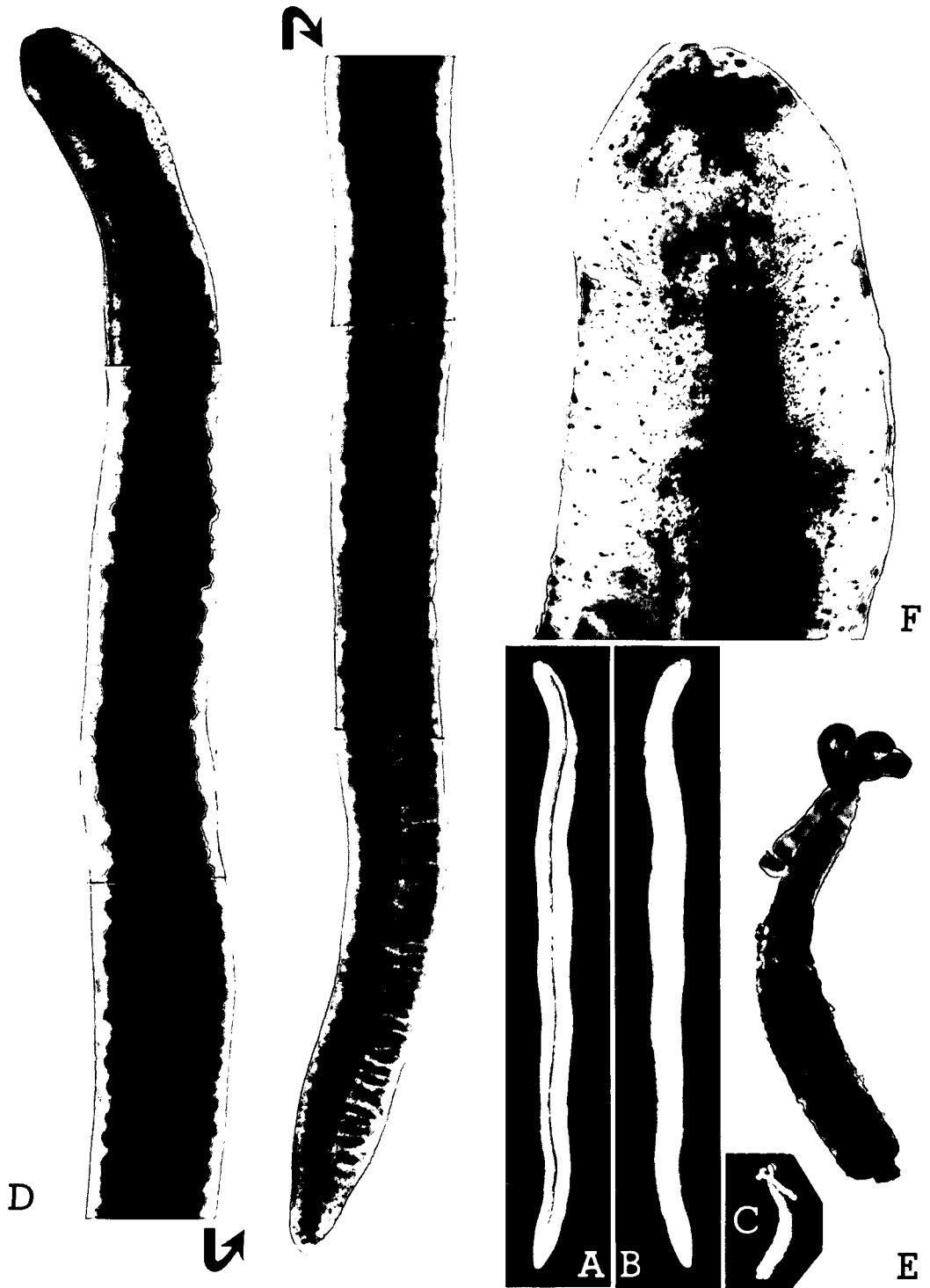


Fig. 2. *Geonemertes pelaensis* SEMPER. A-F: Photographs (A, B and C) and photomicrographs (D, E and F) of a preserved specimen (Specimen Lot No. 1899-a). A, dorsal view; B, ventral view; C, proboscis; D and E, enlarged body and proboscis (whole mount); F, the enlarged head region showing eyes and pigment spots on the dorsal side of the body (whole mount).

an extruded proboscis, 12 mm long and 1 mm diameter.

Important Histological Features. The cross section of the cylindrical body shows hoplonemertine features: Tall epidermis of ciliated and glandular columnar cells underlain by a dense layer of connective tissue; thick body musculature in two layers underlain by a loose connective tissue containing flame cells; rhynchocoel enclosed by muscular proboscis sheath of outer circular and inner longitudinal layers; conspicuous lateral nerve cords. The dorsal blood vessel is just below the proboscis sheath in the tissue; lateral blood vessels are present.

Specialized features of *Geonemertes* and the "*pelaensis* group" include: 1) Body musculature and proboscis sheath show distinct ring of circular fibers whereas the longitudinal fibers are arranged into thick bundles. 2) The flame cells, abundant in the parenchymal tissue, are binucleate and the tubule is strengthened by transverse as well as longitudinal structures, resulting in a ladder appearance. 3) A small accessory nerve is present beside the lateral nerve. Taken together these features provide strong confirmation that these specimens are *Geonemertes pelaensis*.

KARYOLOGICAL OBSERVATIONS

The result of the cytological study of 2 specimens of *Geonemertes pelaensis* is shown in Table 1. Photomicrographs of the chromosomes are shown in Figure 3 (A and B); the provisional idiogram is also shown in Figure 4.

The chromosomes are small and similar in appearance. Examination of mitotic figures reveals a diploid chromosome number of 32. Four meiotic figures were also observed, but their chromosome numbers could not be determined. A detailed karyological analysis awaits further study.

Table 1. Result of cytological observation of *Geonemertes pelaensis* from Chichijima Island.

No. of specimens examined cytologically			Chromosome nos. & the no. of cells studied in parentheses		Karyotype
Total	Sexual	Asexual	Meiosis	Mitosis	
2	2	—	(4)	32 (7).	2x=32

ECOLOGY NOTE

Chichijima Island is volcanic in nature of the Tertiary Period (lat. 27°2' to 27°7' N. and long. 142°11' to 142°14' E.). Topographically, it is a hilly island surrounded by eroded sea cliffs (ca. 23.99 km²; the highest peak, Mt. Chû'ô-san, is 319 m in altitude). The island is composed of andestic and basaltic lavas and pyroclastic sediments. Sedimentary rocks such as sandstone and limestone are distributed locally in the southwestern area (cf. IWASAKI & AOSHIMA, 1975). Yearly average tempera-

Fig. 3. Photomicrographs of the chromosomes of *Geonemertes pelaensis* SEMPER. Both light-toned (A) and thick-toned (B) figures were printed from the same film. Notice the position of the centromere in each chromosome in Figure A. Two club-shaped bodies in Figure B are bacteria.



A



B

Fig. 3. For explanation see page 71.

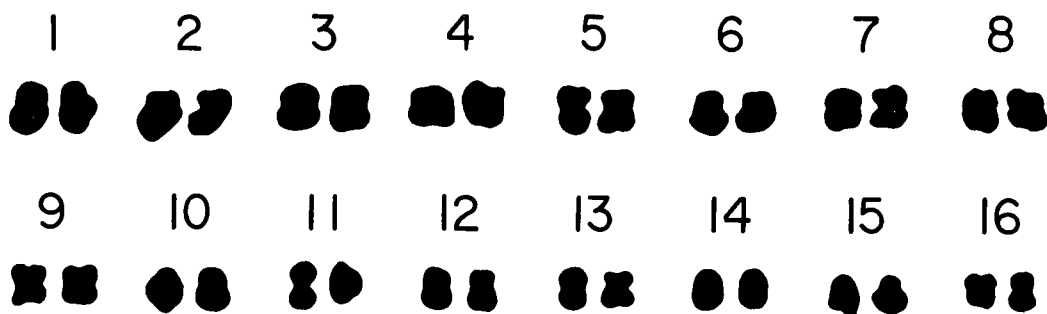


Fig. 4. Idiograms of *Geonemertes pelaensis* SEMPER.

ture is 22.7°C (19°C in winter and 27°C in summer). Yearly average humidity is 78%. Annual rainfall attains 1211.7 mm (cited from the publication of the Ogasawara General Office issued in 1986).

Most of the island is covered with slightly dried, subtropical forests. The following plant communities are conspicuous in the Chichijima Island (cf. YAMAZAKI, 1975).

1) Seaside vegetation. *Vitex rotundifolia*-*Sporoborus virginicus* glassland; *Calophyllum inophyllum*-*Terminalia catappa* forest; *Scaevola sericea* scrub.

2) Mountain vegetations. Forest: *Schima mertensiana*-*Ardisia sieboldii* association; *Ardisia sieboldii*-*Diplazium virescens* association. Scrub: *Distylium lepidotum*-*Pouteria obovata* var. *dubia* association; *Dendrocacalia crepidifolia* association.

Chichijima Island is also characterized by rich flora of lichens (cf. INOUE, 1975).

As far as KITAGAWA's observations go, *Geonemertes pelaensis* is a rather common animal found beneath flowerpots inhabiting garden soil and the leaf mold layer in roadside bushes of the northern and central parts of Chichijima Island (Fig. 5). There is no knowledge of what plants have been introduced into the garden from locations where the worm occurs in the Pacific. Animals are sometimes found beneath stones and fallen trees in subtropical forests of the island. They are also found beneath artificial rubbish, such as blocks, vinyl bags, pieces of old carton boxes, etc.

TAXONOMY AND DISTRIBUTION OF *GEONEMERTES PELAENSIS* SEMPER, 1863

A great deal has already been written concerning taxonomy and distribution of land nemertines, hence only key articles need to be mentioned (PANTIN, 1961, 1969; GIBSON, 1972, 1982; MOORE & GIBSON, 1981, 1985; MOORE, 1985). Following the critique of PANTIN (1969), a fundamental review of *Geonemertes* by MOORE & GIBSON (1981), resulted in formation of several new genera with separations based on musculature and flame cell structure. The amended genus *Geonemertes* now contains only *G. pelaensis* SEMPER, 1863, and *G. rodericana* (GULLIVER, 1879), separable on the basis of color and external appearance. Specimens studied for this present account display major features of *G. pelaensis*, which has become one of the best known land nemertines (cf. SCHRÖDER, 1918; PANTIN, 1961, 1969; MOORE & GIBSON, 1981, 1985).

Fig. 5. Map showing the localities of *Geonemertes pelaensis* SEMPER in Chichijima Island. Solid circles mark localities where specimens were found and collected. Solid squares mark, those localities where animals were observed by KITAGAWA.

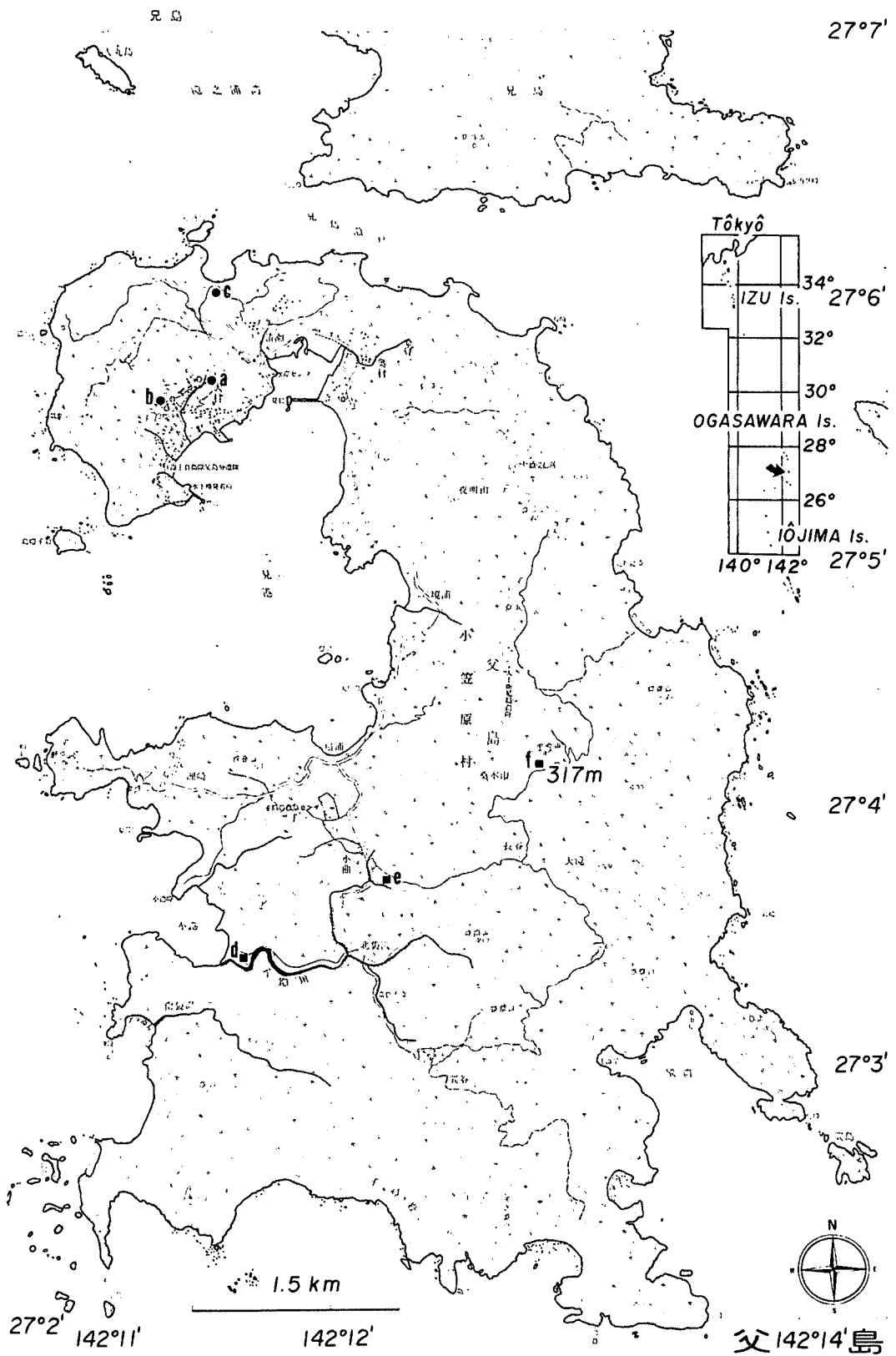


Fig. 5. For explanation see page 73.

The distribution of *Geonemertes palaensis* is well documented (cf. COE, 1940; HETT, 1927; MOORE, 1975, 1985; MOORE & GIBSON, 1986; MOORE & MOORE, 1982; PANTIN, 1961, 1969; PUNNETT, 1907; SCHRÖDER, 1918). The majority of habitats are island localities as follows: Indopacific islands of Micronesia (Carolina Is., Palau or Pelew Is.); Polynesia (Upholo Is. in Samoa); Philippines Is.; Hawaiian Is.; Sri Lanka, Celebes, Papua New Guinea, and Samaria Is.; Kei Is. in Moluccas Group; Mahé Is. in Seychelles Group and Mauritius Is. The species is also reported from the Caribbean region (cf. MOORE & MOORE, 1982; MOORE & GIBSON, 1986: West Indies, Dominica and Jamaica). It is also reported from Florida, U. S. A. (cf. MOORE & GIBSON, 1981). The present report from Chichijima Island represents a new locality from the North Pacific Island area.

MOORE (1985) discusses adaptations of these worms favoring distribution and considers the following important: Ability to form mucus coatings that resist desiccation and the hermaphroditic sexual condition. Human activities may be responsible for passive distribution of this species with soil and plants, in some cases with coconuts.

SUMMARY

This is the initial report on the karyotype and the first record of the occurrence in the Territories of Japan of the land nemertine *Geonemertes palaensis* SEMPER, 1863. The diploid chromosome number of the species is $2x=32$. The species is rather common in leaf mold layers of subtropical forests on Chichijima Island belonging to the Pacific Ogasawara Islands south-southeast of Tōkyō, Japan.

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Note. Original spelling of the specific epithet was "*pelaensis*" for Pelew Island now called Palau Is., hence, in some cases writers have introduced an alternate spelling of "*palaensis*".

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