# Karyology of four land-planarian species of the genus *Bipalium* from Japan

Iwashiro Oki<sup>1</sup>, Sachiko Tamura<sup>2</sup>, Robert E. Ogren<sup>3</sup> & Masaharu Kawakatsu<sup>4,\*</sup>

<sup>1</sup>Ôsaka Environmental Project Authority MFC 7F, Uchihon-machi 1-2-15, Chû'ô-ku, Ôsaka 540, Japan
<sup>2</sup>Ôsaka Prefectural Institute of Public Health, Nakamichi-1-chôme 3-69, Higashinari-ku, Ôsaka 537, Japan;
<sup>3</sup>Wilkes University, Wilkes-Barre, Pennsylvania 18766, USA; <sup>4</sup>Biological Laboratory, Fuji Women's College, Kita-16, Nishi-2, Kita-ku, Sapporo (Hokkaidô) 001, Japan (\* author for correspondence)

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### Abstract

We have empolyed a new scale for characterizing chromosomal forms in the karyotypes of four species of *Bipalium* from five localities in Japan. Specimens of *Bipalium nobile* Kawakatsu et Makino, 1982, from Yokohama had a diploid chromosome number of 2x = 10 (2m + 2sm + 2sm + st & sm + 2sm); specimens of the same species from Toyonaka had this number as well but with slightly different chromosomal form (2m + 2sm + sm & st + 2st + m & sm). An undescribed species from Sanjô, *Bipalium* sp. 2, with two dorsal stripes and a yellow head crescent, had 2x = 10 (2m + 2sm + 2sm + 2sm + 2sm + 2m); and another undescribed species from Chichijima Island, *Bipalium* sp. 3, with five dorsal stripes, had 2x = 10 (2m + 2sm + 2sm + 2sm + 2m); are more than the same species from Chichijima Island, *Bipalium* sp. 3, with five dorsal stripes, had 2x = 10 (2m + 2sm + 2sm + 2sm + 2m); and another species from Chichijima Island had 2x = 18 (2m + 2m + 2sm + 2sm + 2sm + 2sm + 2sm + 2sm + 2sm).

## Introduction

Karyotypes reported for species of the genus *Bipalium* Stimpson (Bipaliidae) show some variety both among and within its recognized species. Seo *et al.* (1988) found that *Bipalium nobile* and *Bipalium multilineatum* Makino et Shirasawa, 1983, from Japan both have a diploid chromosome number of 2x = 10 while *Bipalium kewense* from Japanhad2x = 18. Other specimensof *B. kewense* from Australia were found by Winsor (1981, 1983) to have a diploid chromosome number of 2x = 16. We have found three additional, undescribed species of *Bipalium* from Sanjô, Japan, and Chichijima Island, and our karyological studies of two of these show a diploid chromosome

number of 2x = 10. As a continuation of our karyological studies of land planarians (Oki *et al.*, 1988), we report here on their karyotypes and of other Japanese specimens of *B. kewense* (?) and of *B. nobile*, and we describe a new method for measuring and determining the form of chromosomes.

## Materials and methods

*B. nobile* was collected from two localities. From the campus of Yokohama National University, Tokiwadai, Hodogaya-ku, Yokohama, near Tôkyô, Japan, a single sexually mature specimen was collected by Dr J.-I. Aoki on July 15, 1988. It was 300 mm long and 5-7 mm wide in its creeping form and had the coloration and longitudinal stripes on both dorsal and ventral surfaces of the body typical of the species. The live specimen was photographed by Kawakatsu and sent to the laboratory of Oki and Tamura for karyological analysis. From Toyonaka-chô, Toyonaka, near Ôsaka, Japan, a single non-sexual specimen that was similar to B. nobile in general appearance and 60 mm long and 5 mm wide was collected and forwarded from the Health Center of Toyonaka to the Ôsaka Prefect. Inst. of Public Health for identification, in August, 1988. The animal had separated into four pieces when received. A regenerated head piece was kept for taxonomic purposes by Kawakatsu (Specimen Lot No. 1931).

Of three undescribed species of Bipalium we have found (see Kawakatsu et al., 1990, for photographs), two were subjected to karyological analysis. (Not yet completed is analysis of Bipalium sp. 1, collected from Sanjô, near Nîgata, Japan; it has three stripes and is similar in external morphology to Bipalium pennsylvanicum Ogren, 1987.) Bipalium sp. 2, with two dorsal stripes and a yellow head crescent, was collected from a garden of Mr Z. Kozakai's residence, Sanjô, near Nîgata, Japan, by Mr Kozakai, July 11, 1987. Two sexually mature specimens examined were 70-75 mm long and 7-8 mm wide, dark gray above with two indistinct, wide, longitudinal, lateral stripes; the lunate head plate had a crescent of yellowish brown color. Taxonomic samples collected in June and July were retained by Kawakatsu (Specimen Lot Nos. 1901, 1903) and Ogren. This unidentified species has a superficial resemblance to Bipalium floweri von Graff. 1899, from Sri Lanka, and Bipalium univittatum Grube, 1866, from India.

*Bipalium* sp. 3, with five dorsal stripes, was collected from Chichijima Island, the Ogasawara (or Bonin) Islands, approximately 1000 km SSE of Tôkyô, Japan, by Mr K. Totani; his laboratory stock, hatched on August 25, 1988, originated from animals collected in October, 1987. Two sexually mature specimens examined were 30–35 mm long and 3–4 mm wide; they were yellow-

ish brown above with five dark-colored longitudinal stripes; the median stripe extended into an elongate, black, oval mark on the head, and the marginal stripes were rather wide and curved onto the neck; on the ventral surface were four longitudinal stripes. Taxonomic samples of this species collected later were retained by Kawakatsu (Specimen Lot Nos. 1910, 1936, 1937, 1951). (Note: A photograph of a live specimen was published by Ogren & Kawakatsu [1988].)

A single, non-sexual specimen, tentatively identified as *Bipalium kewense* on the basis of external features (*see* Kawakatsu, 1985), was collected from Chichijima Island (the same as that of *Bipalium* sp. 3) by Mr K. Totani in 1988. It was 80 mm long and 4 mm wide. Specimens were retained by Kawakatsu for taxonomic purposes (Specimen Lot Nos. 1909, 1938, 1939).

Preparations of chromosomes were made using a squash method (Oki et al., 1988; see also



Fig. 1. A scale for classifying the form of chromosomes and demonstration of its use. See Materials and Methods for explanation.

Kawakatsu *et al.*, 1987), and photomicrographs of chromosome sets were enlarged to a size suitable for measurement. To classify chromosomal form, we used a scale newly devised by one of us (Oki). This scale (Fig. 1A) was drawn on mmruled graph paper. On the y-axis, reference points defined according to the Denver report (1960) were established: c (0.0, the standard reference point, location of centromere), s (1.0, terminal point of short arm), m (1.7, maximum for arm ratio of a metacentric chromosome), sm (3.0, maximum for arm ratio of a submetacentric chromosome), st (7.0, maximum for arm ratio of a subtelocentric chromosome). On the x-axis, 0 was set at 5 units from the y-axis, and straight lines connecting this 0-point to each of the reference points on the y-axis were drawn. For each photographic image of a chromosome, a flexible leader (thread, silkworm gut, or a fine wire; k in Fig. 1B) was aligned with the image and marked for the position of the centromere (c', Fig. 1B) and terminal points of both arms (s' for short arm; l' for long arm). The marked leader was then made straight and placed on the scale parallel to the y-axis, with the c' mark on the 0-c line and the s' mark intersecting the 0-s line (Fig. 1A). The form of the chromosome was finally read from the y-axis according to which segment of the axis was intersected by the l' mark (in Fig. 1A, the example is a submetacentric chromosome).



Fig. 2. Photomicrographs of the chromosomes of three bipaliid species from Japan. A), B) Bipalium nobile (2x = 10) from Yokohama population (A) and Toyonaka population (B). C) Bipalium sp. 2 (with two dorsal stripes and yellow head crescent) from Sanjô (2x = 10). D) Bipalium sp. 3 (with five dorsal stripes) from Chichijima Island (2x = 10). E) Bipalium kewense (?) from Chichijima Island (2x = 18).

# Results

Both the Yokohama and Toyonaka populations of *B. nobile* had a chromosome number of 2x = 10in diploid cells. While we have not yet definitively characterized the karyotypes (see discussion), we tentatively identify them as 2m + 2sm + 2sm + st& sm + 2sm in the Yokohama population and 2m + 2sm + sm & st + 2st + m & sm in the Toyonaka population (Figs 2A, 2B, 3).

The chromosome number of diploid cells in *Bipalium* sp. 2 (Figs 2C, 3) was 2x = 10, with a karyotype of 2m + 2sm + 2sm + 2sm + 2m. In *Bipalium* sp. 3 (Figs 2D, 3) the chromosome number was 2x = 10 and the karyotype 2m + 2sm + 2sm + 2sm + 2sm + 2m. *Bipalium kewense* (?) had a chromosome number of 2x = 18, 2m + 2m + 2m + 2sm +

# Discussion

Our study of B. nobile confirms the report of Seo et al. (1988) that the diploid chromosome number for this species is 2x = 10. Their data, based upon material collected from Hino in Tôkyô, show a somewhat different karyotype of 2sm + 2m +2sm + 2st + 2sm. They present four idiograms of enlarged photomicrographs of chromosomes. Three sets show aberrations such as translocation or breakage. We also found variations, or chromosomal aberrations, in our samples of B. nobile: those from the Yokohama population showed differences in the lengths of homologous chromosomes of the 4th set (Fig. 3), and those of the Toyonaka population had variations in the 3rd and the 5th sets (Fig. 3). Such differences and variability indicate that a definitive karvotype for this species will be difficult to obtain.

Similarities in karyotypes of *B.nobile* and *B. multilineatum*, however, may be useful in showing the close linkage between these two species which are similar in external morphology. Both have the 2x = 10 diploid number, and there are overlapping similarities in their idiograms (Seo *et al.*, 1988). Similarly, *Bipalium* sp. 3 from



Fig. 3. Idiograms of four bipaliid species from Japan.

Chichijima Island, with five dorsal stripes, is similar to B. nobile. Our finding an identical chromosome number and similarities in the idiograms of these two species (together with similarities observed in taxonomic study) strongly suggest a close relationship between the two species.

The diploid number in our specimens of B. kewense (?) is 2x = 18, the same as that reported by Seo et al. (1988) for specimens from Tôkyô and by Dr E. M. Froehlich of Universidade de São Paulo, Brazil (personal communication) on Brazilian specimens. Data of Seo et al. (1988) can be summarized as 2x = 18 (2m + 2m + 2m + 2sm + 2s2st + 2sm + 2st + 2st + 2sm); they did not believe their idiogram was the definitive representation of this karyotype because of the striking resemblances of the middle-sized and small chromosomes (nos. 4 to 9 in their Fig. 4). This characterization is substantially the same as what we found except for the classification of the 7th and the 8th sets of chromosomes. In contrast, the first report on the karyotype of B. kewense, that by Winsor (1981) using material from Townsville, Australia, described a chromosome number of 2x = 16. Winsor (1983: 78-79, Fig. 8) later published an idiogram quite different from that of the animals from Japan. His data can be summarized as 2x = 16 (2m + 2m + 2m + 2m + 2st + 2sm + 2sm + 2sm). Additional studies correlating taxonomy and karyology on a broad geographic basis are clearly needed to learn how widespread these two diploid numbers are.

For *Bipalium* sp. 2 from Sanjô, we found a diploid number of 2x = 10 and an idiogram with such similarities and differences from that of *B. nobile* that diagnosis is difficult. These two species are so different in external morphology that they are readily recognized as separate taxa. The karyotypes of the two *Bipalium* species to which *Bipalium* sp. 2 appears most similar in external morphology, *viz.*, *B. floweri* and *B. univittatum*, are unknown.

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