Biology of long slender land planarians (Turbellaria) in Tokyo and environs

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Abstract

The common short-bodied species of *Bipalium* does not fragment, but individuals of two newly discovered long-bodied species -B nobile Kawakatsu & Makino, 1982, and *B. multilineatum* Makino & Shirasawa, 1983 - do regularly fission, usually behind the mouth or genital pore. Some experimental regenerates of these species form rings by adhesion of the anterior with the posterior cut surface. We found two other forms of *Bipalium*, perhaps representing a further two species, in Hino City, Tokyo, in 1983; and we have preliminarily arranged the forms of *Bipalium* known in the region into four groups distinguished on the basis of body coloring, position of the mouth, and structure of the copulatory organ.

Introduction

In contrast to the common *Bipalium fuscatum*, which we have found does not reproduce asexually by fragmentation, the newly discovered *B. nobile* Kawakatsu & Makino, 1982, (Fig. 1) and *B. multilineatum* propagate regularly in gardens (private garden of N.M.) and in the laboratory by fragmentation. Identification of species of *Bipalium* is difficult, however, and has been complicated especially by the discovery of several other forms of the genus, perhaps new species. We have devised methods for identification based on the natural coloration, relative position of the mouth and genital pore, and structure of the copulatory organ. Our preliminary work permits arrangement of the known forms into four groups.

Material and methods

Two long slender species were employed for the study of fragmentation: *Bipalium nobile* and *B*.

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multilineatum. Both were collected in the senior author's garden, and the latter was collected also in other areas of Tokyo. The worms were maintained at 18 °C in glass dishes in controlled-temperature chambers.

B. fuscatum, B. fuscolineatum, and B. hilgendorfi were collected in Hino City, Tokyo. Unnamed forms of Bipalium used include two worms with creamy body color and a strong medio-dorsal brown stripe and 10 worms having grey-green color with three dorsal stripes, both of which forms were collected in the neighborhood of the senior author's residence. An unidentified species of small worms having three dorsal stripes was obtained from the campus of Hirosaki University, Aomori Pref.

Specimens were anaesthetized by carbon dioxide (Makino & Seo, 1983) and fixed in Bouin's fluid. For histological study, Paraffin-embedded specimens were sectioned at $7-9 \ \mu m$ and stained with Mayer's haematoxylin and eosin.

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Results and discussion

Asexual and sexual reproduction in Bipalium nobile

Of the species of *Bipalium* we have raised in controlled-temperature chambers, *B. nobile* and *B. multilineatum* propagated asexually, while shortbodied species of the genus did not. Hyman (1951) reported that *B. kewense* Moseley propagated asexually; Morgan (1900) and Hauser *et al.* (1976) also studied regeneration in *Bipalium*.

The number of fragmentation pieces in *B. nobile* appeared to be directly related to body length. Individuals broke posterior to the mouth into two to four pieces of approximately equal size. If the initial fragment was small, as from a very small worm, no further divisions occured; when the fragment was large, it fragmented further in a single step into equal-sized secondary pieces (Fig. 5). After three or four weeks, each piece regenerated a complete worm. The process in *B. multilineatum* differed in that all pieces (5-8 in number) formed simultaneously (Fig. 5) rather than in two or three steps. Fragmentation in both species was restricted to the region behind the mouth and was possibly initiated by starvation. Well-fed individuals did not undergo fragmentation; and, indeed, a portion cut from such an individual simply flexed so that the anterior and posterior ends met, forming a ring, which died after a few weeks. Comparable results were obtained with the polyclad *Stylochus ijimai* (see Yamao & Makino, 1954).

The process of copulation in *B. nobile* was observed in the senior author's garden the evening of 28 June, 1984, over the period from 2120 to 2310 (Fig. 3). One of the individuals was brought into the laboratory for further observation. It weighed 7.31 g, and 18 days after copulation it laid a co-coon 8 by 7 mm weighing 280 mg (Fig. 4). Two off-



Fig. 1-4. (1) Bipalium nobile, a long slender yellowish-brown species with three dorsal longitudinal stripes; (2) Bipalium multilineatum, a long somber-green species with five dorsal and three ventral stripes, with a melanin ring around the mouth, and lacking a genital pore; (3) B. nobile, in copula; (4) Cocoon of B. nobile.

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Species	Specimen No.	Body Weight	A/L	(A + B)/L
B. nobile	No. 404	3.700 g	0.23	0.29
	No. 405	3.230	0.20	0.26
B. multilineatum	No. 410	0.252	0.43	-
	No. 533	0.457	0.44	-
B. sp. A*	No. 602	0.253	0.43	0.63
	No. 603	0.153	0.45	0.64
B. sp. B**	No. 613	0.251	0.47	0.59
	No. 649	0.313	0.47	0.59
B. hilgendorfi	No. 633	0.123	0.52	0.71
	No. 696	0.199	0.52	0.70
B. fuscatum	No. 474	1.431	0.52	0.62
	No. 475	1.677	0.52	0.67
B. fuscolineatum	No. 650	0.783	0.59	0.67
	No. 651	0.684	0.57	0.67

Table 1. Relative position of mouth and genital pore in species of Bipalium.

A - distance between head and mouth.

B - distance between mouth and genital pore.

L - total body length.

* milky colored with a brown stripe.

** grey-green with three black stripes.



Fig. 5-6. (5) Fragmentation in B. nobile and B. multilineatum; (6) The four types of copulatory organ in Bipalium. CB-copulatory bursa; OD-oviduct; P-penis; SD-seminal duct.

spring emerged from the cocoon on 22 August (38 d after oviposition). A second cocoon deposited on 14 August yielded one juvenile on 13 September (30 d after oviposition).

Taxonomic considerations

Species of *Bipalium* are cryptic and only a few specimens are usually collected at a time. We recommend the routine use of color photography to document distribution of pigment and shape of the head. The positions of the mouth and genital pore relative to total body length has proved to be significant in identifying species (Table 1).

Morphology of the copulatory organ appears especially important inasmuch as some individuals having similar external morphologies have different copulatory-organ structure. We have identified four general types of copulatory organ in our material (see also Kaburaki, 1922):

Type I. Penis lumen strongly plicate (Fig. 6, I) as in *B. kewense, B. nobile*, and perhaps *B. multilineatum*.

Type II. Penis globular as in *B. fuscatum* and *B. fuscolineatum* (Fig. 6, II).

Type III. Penis lumen broad and slightly folded as in *B. hilgendorfi* and in a cream-colored, unidentified species from the senior author's garden (Fig. 6, III).

Type IV. Penis lumen narrow (Fig. 6, IV) as in what appears to be a new species from Hirosaki (Aomori Pref.) and Hino City, Tokyo.

An illustrated guide to the Japanese species of *Bipalium* based on these types is in preparation (Makino & Shirasawa, in prep.).

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