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Karyological observations on Turbellaria Proseriata: karyometric analysis of *Monocelis fusca, M. lineata* (Monocelididae) and *Parotoplana macrostyla* (Otoplanidae)

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ABSTRACT: A karyometric analysis of the chromosome set of the marine turbellarians *Monocelis fusca, M. lineata* and *Parotoplana macrostyla* has been carried out. The karyotype of the two *Monocelis* species investigated (2n = 6) is formed by three pairs of small and similarly sized chromosomes: In *M. fusca*, chromosome 1 is metacentric, chromosome 2 acrocentric and chromosome 3 is subtelocentric. *M. lineata* also presents one pair of metacentric chromosomes (chromosome 2), while chromosomes 1 and 3 are submetacentric. *P. macrostyla* (2n = 12) reveals two pairs of large metacentric and four pairs of small chromosomes, three of which are metacentric, whereas the last is subtelocentric.

INTRODUCTION

At present, the knowledge on the karyology of marine Turbellaria is fairly satisfactory only for polyclads and marine triclads (Galleni & Puccinelli, 1981, 1982). Within the other marine groups, Proseriata are of special interest, mainly due to their phylogenetic position and relation to triclads (Ehlers, 1984; Sopott-Ehlers, 1984). Nevertheless, while much morphological research work has recently been carried out on Proseriata, resulting in numerous new taxa, karyological investigations are almost entirely lacking. The chromosome number is known of only two species, *Monocelis fusca* Oersted (Monocelididae) and *Bothrioplana semperi* M. Braun (Bothrioplanidae, a freshwater group whose systematic position still needs to be clarified); however, no karyotype reconstructions were reported (see Benazzi & Benazzi Lentati, 1976).

The present paper provides the first contribution to the karyology of Proseriata based on karyometric analysis and reconstruction of idiograms. Among the species considered, *Parotoplana macrostyla* Lanfranchi belongs to the family Otoplanidae, while *Monocelis fusca* and *M. lineata* (Müller) belong to the Monocelididae. Owing to the close relationship existing between the latter two species, a comparative analysis of their karyotypes is worthy of interest in order to clarify the possible evolutionary mechanisms in related species.

MATERIALS AND METHODS

Specimens of *Monocelis lineata* were collected from under stones in the Bay of Argostoli (Ionian island of Cephalonia, Greece) in August 1981, using the bait method reported by Wilhelmi (1909) for marine triclads. Other specimens of the same species were found in August 1983 among mussels on the Danish coasts of the Øresund between Helsingør and Hornebaeck.

Specimens of *Monocelis fusca* were found in samplings of coarse sand collected intertidally in the harbour of Portoferraio (Island of Elba, Italy) in October 1983. Recently, Martens (pers. comm.) expressed the opinion that the specimens from Portoferraio could be referred to as a new undescribed species closely related to *M. fusca*.

Specimens of *Parotoplana macrostyla* were collected from a sandy beach on the shore of the Ligurian sea near Leghorn (Tuscany, Italy) during Spring, 1982. Whole worms were maintained from 3 to 4 h in 0.3 % colchicine Sigma solution in sea-water; thereafter, the animals were transferred into a 2 % acetic acid solution for about 1 to 2 min, stained with lactic-aceto-orcein for 5 min and finally squashed between slide and coverglass. Moreover some specimens of *Monocelis lineata* were transferred, after colchicine treatment, to a 1 % sodium citrate solution for 20 min, then into Carnoy solution for 30 min. Finally, they were dissociated on a slide in a drop of 60 % acetic acid. After drying and rapid rinsing with Carnoy solution, they were stained for 25 min with a 2.5 % Giemsa solution buffered with a 7.0 phosphate buffer. This method allowed to obtain metaphasic chromosomes presenting a lower degree of coiling, which is more suitable for karyometric analysis. Results of measurements obtained by use of both methods are similar.

The diploid chromosome numbers were determined from spermatogonial mitoses, the haploid number from metaphasic primary and secondary spermatocytes. The karyometric analysis was carried out on C-mitoses from the male germinal line: the mitotic figures most suitable for karyometric analysis in each species were drawn with the aid of a camera lucida, and the chromosome lengths measured. In the same plate, the homologous chromosomes were paired according to their length and to the position of their centromere.

RESULTS

The diploid complement of both *Monocelis lineata* and *M. fusca* is formed by three pairs of homologous chromosomes (2n = 6) of approximately the same length. The first two pairs almost have the same relative length (r. l.); the smallest is more easily recognizable, being approximately 5/7 the length of the longest pair in *M. fusca* and 6/7 the length in *M. lineata*. The two karyotypes, anyway, show good similarities as far as r. l. are concerned (Table 1, Fig. 1, Fig. 2 A–D).

According to the position of the centromere, chromosomes 1 and 3 of *M. lineata*, whose centromeric indices (c. i.) are 35.54 and 36.57 respectively, are submetacentric (sm according to Levan et al. 1964), while chromosome 2 (c. i. = 43.85) is clearly

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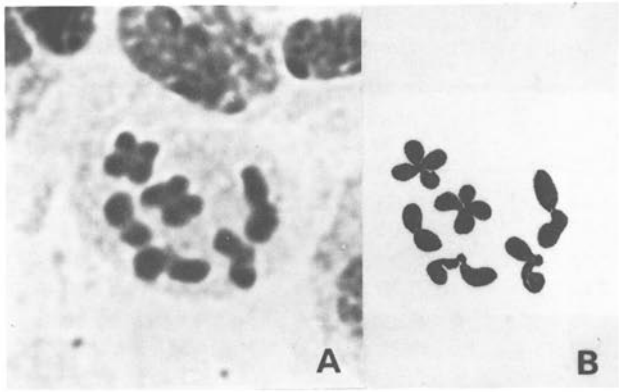
Fig. 1. (A) metaphasic plate from spermatogonium of *Monocelis fusca* (lactic-aceto-orcein); (B) camera lucida drawing; (C) derived karyogram; (D) idiogram based on the values reported in Table 1; (E) metaphasic plate from spermatogonium of *Monocelis lineata* (Giemsa stained); (F) derived karyogram; (G) idiogram based on values reported in Table 1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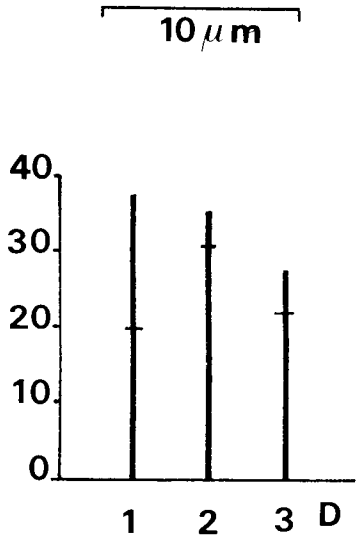
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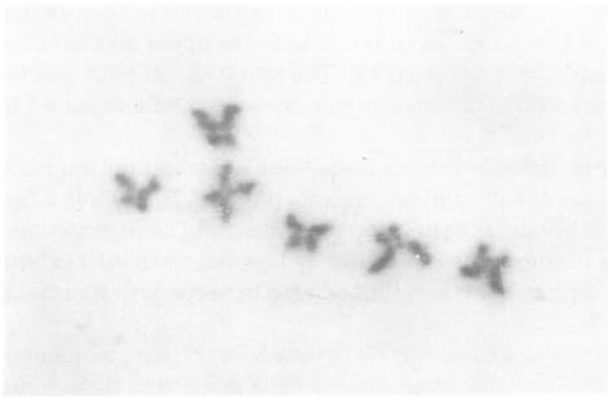
E


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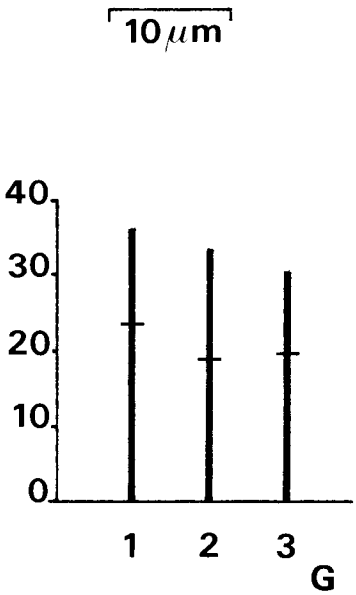
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Table 1. Data on chromosome measurements based on 8 cells for each turbellarian species. r. l. = relative length: length of chromosome × 100/total length of haploid genoma. c. i. = centromeric index: length of short arm × 100/length of entire chromosome. ± standard deviation

Chromosome	*Monocelis lineata*	*Monocelis fusca*	*Parotoplana macrostyla*
1 r. l.	35.91 ± 1.48	37.85 ± 2.91	30.12 ± 3.96
c. i.	35.54 ± 1.92	48.05 ± 1.93	46.76 ± 1.78
2 r. l.	33.38 ± 1.55	35.03 ± 2.57	28.19 ± 2.26
c. i.	43.85 ± 1.88	11.78 ± 4.97	41.06 ± 1.77
3 r. l.	30.72 ± 1.78	27.12 ± 1.03	12.67 ± 2.19
c. i.	36.57 ± 2.73	18.70 ± 4.21	39.09 ± 2.53
4 r. l.			11.21 ± 1.61
c. i.			43.65 ± 2.67
5 r. l.			8.97 ± 1.39
c. i.			42.38 ± 3.62
6 r. l.			8.64 ± 1.35
c. i.			18.15 ± 5.24

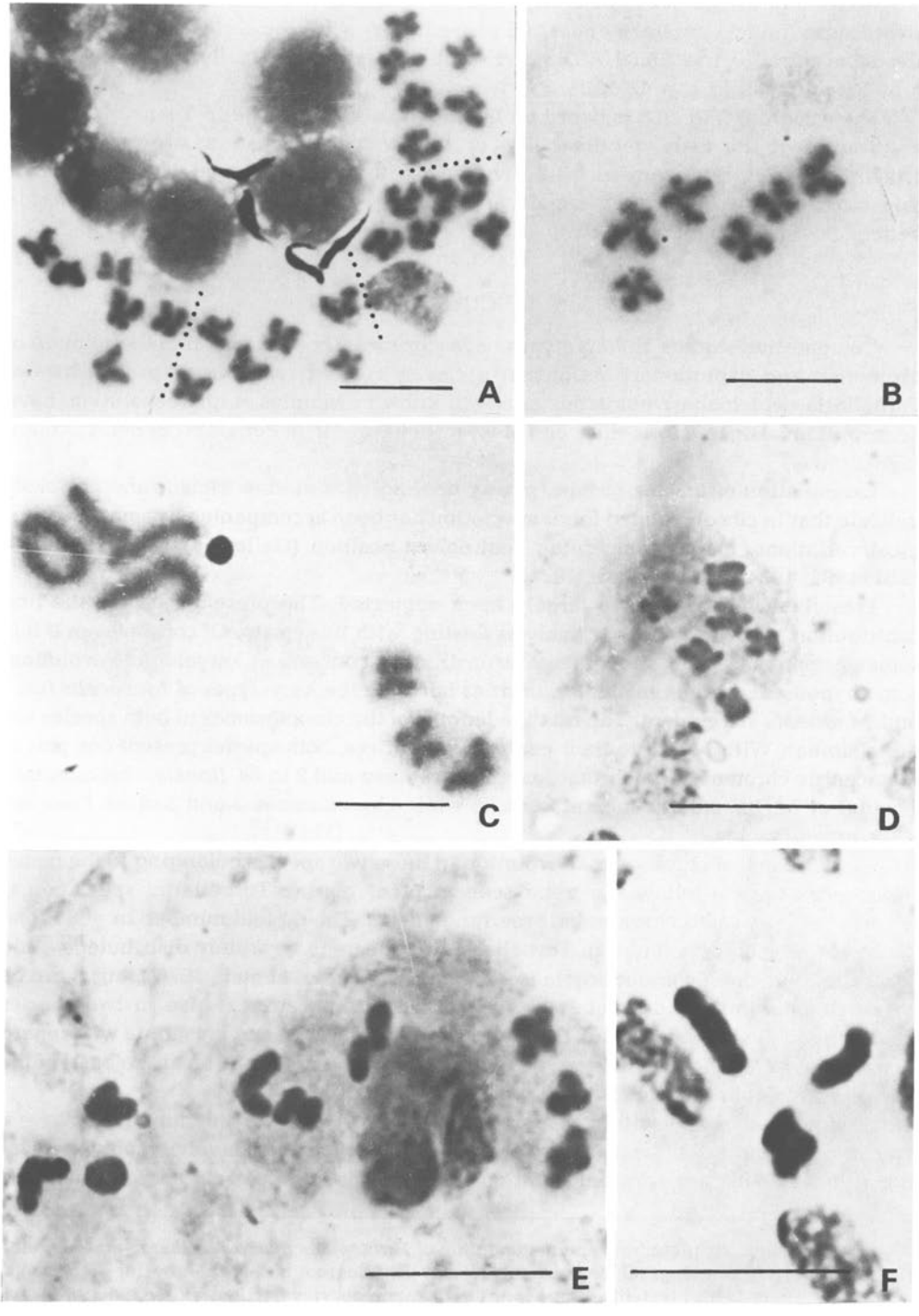
metacentric (m). However, it must be pointed out that the measurement of chromosome 3 showed some degrees of variability; it was at the highest limit for submetacentric class, and found to be metacentric in some plates. Mediterranean and Øresund populations of *M. lineata* do not show any relevant discrepancy, both in c. i. and r. l.

On the contrary, the first pair of *M. fusca* is clearly metacentric (m) (c. i. = 48.05), while the second is acrocentric (t) (c. i. = 11.78) even if just near the upper border of the class; finally the third is subtelocentric (st) (c. i. = 18.70). The idiograms of both species (Fig. 1D, G) are based on the mean values of chromosome measurements reported in Table 1.

In both *M. fusca* and *M. lineata,* three bivalents have been observed during male meiosis, mostly with a single, terminal or subterminal chiasma (Fig. 2C, E, F). Only a few plates with one or more ring-shaped bivalents exhibiting two chiasmata have sometimes been observed. The mean chiasma frequency is consequently low, being about 3 in both species. The haploid number (n = 3) has been ascertained also in secondary spermatocytes (Fig. 2C).

The diploid complement of *Parotoplana macrostyla* is formed by 6 pairs of homologous chromosomes of diverse length. The longest chromosome is more than three times the length of the shortest one, their relative lengths being 30.12 and 8.64 respectively (Fig. 3; Table 1). In its karyotype it is possible to distinguish two pairs of large chromosomes (chromosomes 1 and 2) and a group of four pairs of small chromosomes (Nos 3, 4, 5, 6), presenting only slight length differences.

Fig. 2. (A–E) *Monocelis lineata.* (A–B) spermatogonial metaphasic plates (Giemsa); (C) primary spermatocyte with one ring-shaped bivalent and two with one chiasma, and secondary spermatocyte (Giemsa); (D) spermatogonial metaphasic plate (lactic-aceto-orcein); (E) three primary spermatocytes (lactic-aceto-orcein); (F) bivalents from primary spermatocyte of *Monocelis fusca* (lactic-aceto-orcein). Bars = 10 μm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According to the position of the centromere, the two larger chromosomes are metacentric (m) (c. i. = 46.76 and 41.06 respectively). Also the chromosomes 3, 4 and 5 are metacentric (c. i. = 39.09, 43.65 and 42.38 respectively). Finally, chromosome 6 is subtelocentric (st) (c. i. = 18.15).

The idiogram (Fig. 3D) is based on the mean values reported in Table 1.

Studies of the male germinal line of *P. macrostyla* revealed large numbers of primary spermatocytes formed by 6 bivalents, with a single terminal or subterminal chiasma or with two or more chiasmata, approximately in equal numbers. Mean chiasma frequencies were about 9 (Fig. 3E).

DISCUSSION

Comparative studies on karyotypes have significantly contributed to the solution of taxonomic and evolutionary problems in closely related species groups. Freshwater Turbellaria, which show numerous and well known examples of microevolution, have been well investigated from this point of view (Benazzi, 1982; Benazzi & Benazzi Lentati, 1976).

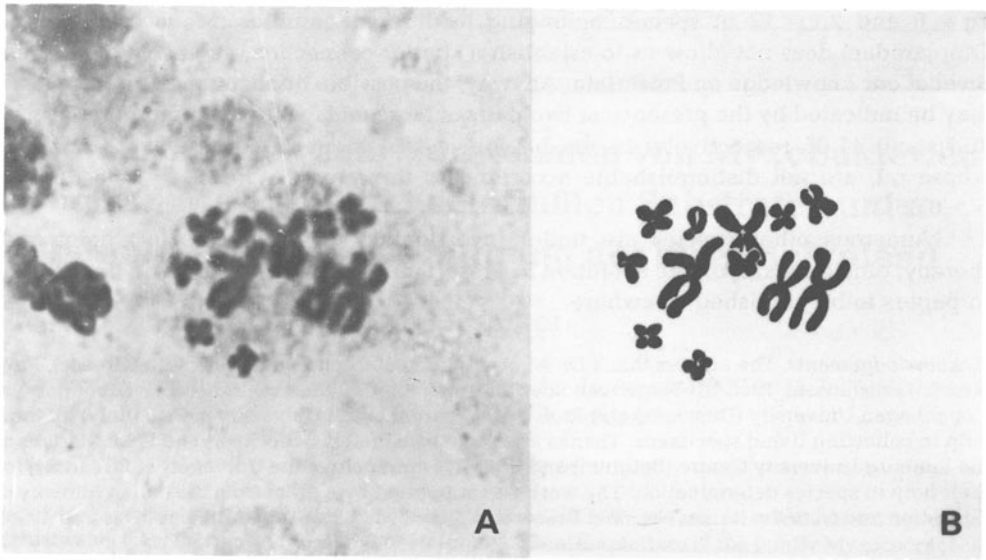
Examination of marine groups, mostly dealing with marine triclads and polyclads indicate that in closely related forms speciation has been accompanied by small karyological variations, mainly concerning centromere position (Galleni & Puccinelli, 1979, 1981, 1982, 1984; Galleni et al., 1984).

Proseriata, however, have largely been neglected. The present data are the first contribution to the karyometric analysis dealing with this group. Of course, even if it is somewhat premature to attempt any reconstruction concerning karyological evolution, some hypotheses can be made. Similarities between the karyotypes of *Monocelis fusca* and *M. lineata* are evident. The relative lengths of the chromosomes in both species are quite similar. With regard to their centromeric indices, both species present one pair of metacentric chromosomes: chromosome 1 in *M. fusca* and 2 in *M. lineata;* chromosomes 1 and 3 of *M. lineata* are submetacentric, while chromosomes 2 and 3 of *M. fusca* are more heterobrachial.

Mechanisms of chromosomal evolution in these two species belonging to the genus *Monocelis* seem to follow the trend seen in other marine Turbellaria: speciation is accompanied by small chromosomic rearrangements. The diploid number 2n = 6 is one of the lowest numbers found in Turbellaria; it appears to be widely distributed within Macrostomida and Neorhabdocoela (see Benazzi & Benazzi Lentati, 1976); thus it can be considered as the basic number of such orders. Its occurrence also in two species belonging to the Monocelididae, the most primitive family among Proseriata with regard to many morphological characters, suggests that this number may be at the basis of the karyological evolution of Proseriata too.

Congeneric species within Macrostomida showing chromosome numbers of 2n = 6 and 2n = 12 suggested the existence of an evolutive mechanism involving polyploidization (White, 1978). In the present situation, however, the finding of chromosome numbers

Fig. 3. (A) metaphasic plate from spermatogonium of *Parotoplana macrostyla* (lactic-aceto-orcein); (B) camera lucida drawing; (C) derived karyogram; (D) idiogram based on the values reported in Table 1; (E) bivalents from two primary spermatocytes (lactic-aceto-orcein)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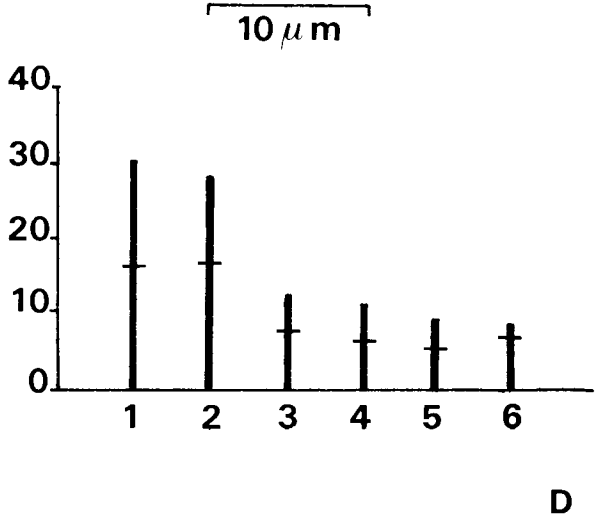
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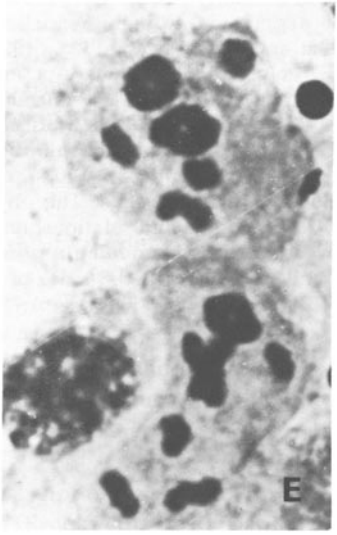
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2n = 6 and 2n = 12 in species belonging to different families (Monocelididae and Otoplanidae) does not allow us to establish a similar connection, at least at the present level of our knowledge on Proseriata. Anyway, the possible duplication of the karyotype may be indicated by the presence of two pairs of large metacentric chromosomes (c. i. = 46.76 and 41.06 respectively) in the haploid complement of *Parotoplana macrostyla* whose r. l. are not distinguishable according to the t test ($\Delta \pm$ E.S. = 1.93 ± 1.52; $p > 0.05$).

Numerous other species are under investigation to clarify this last point and, thereby, elucidate karyologic evolution in Proseriata. Respective results will be reported in papers to be published elsewhere.

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