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Lodovico Galleni, Ileana Puccinelli

Karyometric analysis in Thysanozoon brocchii (Risso) (Turbellaria Polycladida)

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Zoologia. — *Karyometric analysis in* Thysanozoon brocchii (*Risso*) (*Turbellaria Polycladida*) (*). Nota di LODOVICO GALLENI (**) e ILEANA PUCCINELLI (**), presentata (***) dal Socio M. BENAZZI.

RIASSUNTO. — Gli Autori hanno compiuto, su piastre metafasiche ottenute da blastema rigenerativo caudale, una analisi cariometrica del patrimonio cromosomico diploide di *Thysanozoon brocchii*. Tale analisi ha permesso di ricostruire il cariogramma e l'idiogramma della specie.

T. brocchii possiede nove coppie di cromosomi omologhi, la cui lunghezza decresce gradatamente così che non è possibile dividerli in gruppi. Per quel che riguarda la posizione del centromero, i cromosomi nn. 3, 5 e 9, i cui indici centromerici sono rispettivamente 30.30, 27.37 e 31.33, sono submetacentrici. I cromosomi nn. 1, 2, 4, 6 e 7 sono metacentrici. I loro indici centromerici sono infatti: 41.19, 43.03, 47.42, 42.80 e 47.38 rispettivamente. Infine il cromosoma n. 8, il cui indice centromerico è 8.41, è chiaramente telocentrico.

In spite of its wide diffusion and the fact that this species is the best known among the Mediterranean Polyclads, so far our knowledge of the karyology of *Thysanozoon brocchii* (Risso) is not satisfactory.

A careful analysis of gametogenesis and of meiosis in male and female lines and of embryonic development including the definition of the haploid and diploid numbers was made by some Authors between the end of the 19th century and the first years of the present century [11, 12, 8, 9, 10]. A first karyotype description is furnished only in the paper of Bertani [1]: in fact the Author provided an analysis of chiasma frequency in the male line of two specimens of *T. brocchii* and a brief description of the chromosome morphology from somatic mitoses.

According to Bertani there is one pair of acrocentric chromosomes while the other eight pairs are metacentric or submetacentric. They are of different lengths, the shortest being one third of the longest one. The acrocentric pair is of medium length.

The present work accomplished during a research program on the karyology of marine Turbellaria [2, 3] allowed us to provide the karyogram of the species and its idiogram obtained through karyometric analysis of numerous metaphasic plates from regenerating blastema.

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(**) Istituto di Zoologia e Anatomia comparata dell'Università di Pisa.

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In 1974 some specimes of *T. brocchii* were collected along the shores near Pisa and Livorno. The karyotype has been analysed on mitoses from blastemata obtained three days after the transection of a large part of the caudal end of the animal. Specimens with regenerative blastema were put in 0.15 % colchicine Merck solution in sea water for a time varying from 3 to 4 hours. Then the portions with blastema were stained for 30 minutes with lactic acetic orcein and finally squashed.

The relative lengths (r.l.) and the centromeric indices (c.i.) were obtained by chromosome measurements of 6 plates from the same specimen chosen from 10 camera lucida drawings.

TABLE I

Chromosome measurements in 6 cells of Thysanozoon brocchii

Chromosome No.		1	2	3	4	5	6	Means
1	r.l.	15.41	15.43	14.75	13.45	14.64	15.16	14.81
	c.i.	38.67	38.96	42.86	41.15	40.11	45.41	41.19
2	r.l.	13.61	13.54	14.22	14.55	13.63	12.69	13.71
	c.i.	41.88	37.72	46.64	47.42	43.06	41.44	43.03
3	r.l.	13.12	13.32	12.43	12.90	13.01	12.94	12.95
	c.i.	34.03	30.00	29.18	25.03	28.04	35.52	30.30
4	r.l.	12.68	12.71	13.95	12.47	11.92	12.87	12.77
	c.i.	48.44	49.78	45.66	48.28	47.79	44.56	47.42
5	r.l.	11.22	11.26	11.80	11.14	10.78	12.04	11.37
	c.i.	29.97	27.27	27.67	26.05	22.51	30.74	27.37
6	r.l.	10.20	10.38	10.95	9.86	11.00	10.40	10.46
	c.i.	43.90	44.00	41.37	40.28	41.35	45.91	42.80
7	r.l.	8.83	8.71	7.74	10.41	8.98	8.34	8.84
	c.i.	48.00	43.93	48.37	46.36	48.69	48.91	47.38
8	r.l.	7.60	7.55	7.48	8.28	8.76	7.87	7.92
	c.i.	7.56	9.37	8.45	8.82	6.00	10.26	8.41
9	r.l.	7.33	7.10	6.68	6.94	7.28	7.69	7.17
	c.i.	29.53	29.68	36.20	26.66	31.44	34.48	31.33

r.l. = relative length: length of chromosome $\times 100$/total length of haploid genome.
c.i. = centrometric index: length of short arm $\times 100$/length of whole chromosome.

The diploid complement of the species is made up of 9 pairs of homologous chromosomes of different lengths (Plate I *a, b, c*); the karyometric analysis allowed us to ascertain that the longest chromosome is a little more than twice the size of the shortest one. In fact the relative lengths vary from 14.18 to 7.17. Moreover, the length of the chromosomes decreases so gradually that it is impossible to divide them into groups. In Table I the r.l. and the c.i. are reported. With regard to the centromeric position it is to be noted that chromosomes nos. 3, 5, 9 are submetacentric, according to the classification of Levan *et al.* [5]; their c.i. are, in fact, 30.30, 27.37, 31.33, respectively. Chromosomes nos. 1, 2, 4, 6, 7 are metacentric: their c.i. are, in fact, 41.19, 43.03, 47.42, 42.80, 47.38, respectively. Finally chromosome no. 8, whose c.i. is 8.41, is clearly telocentric. The idiogram of the species (Plate I *d*) has been based on the mean values presented in Table I. It may be observed that the order of the length of chromosomes is inverted in some plates. This fact is easy to explain, considering that the differences among chromosomes are so slight that they can be changed by slight variations in the level of spiralization. The chromosomes, in these cases, were arranged according to the position of the centromere.

Our data generally confirm the brief description of the karyotype given by Bertani [1], but being based on a karyometric analysis, allow us to furnish a more correct picture of the chromosome morphology. The telocentric chromosome must be arranged not among the medium sized chromosomes but in the shortest ones. Moreover, the differences among the chromosomes are not so great as those reported in Bertani's paper. In fact the longest pair of homologous chromosomes is only a little more than twice the shortest one.

With regard to the chromosome number, it is to be remembered that in the suborder Cotylea this varies from $n = 6$ to $n = 10$ [2, 4]; the number $n = 9$ is present also in *Stylostomum felinum* Marcus [7] and *Pseudoceros evelinae* Marcus [6]. It is to be pointed out that the latter species belongs to the Pseudoceridae family, the same as *T. brocchii*. However, neither any karyotype description nor any picture of *Pseudoceros evelinae* has been published to enable us to reach any conclusion as to the evolution of the karyotype of the family. Research is still in progress on a larger number of species to increase our knowledge of the karyology of the group.

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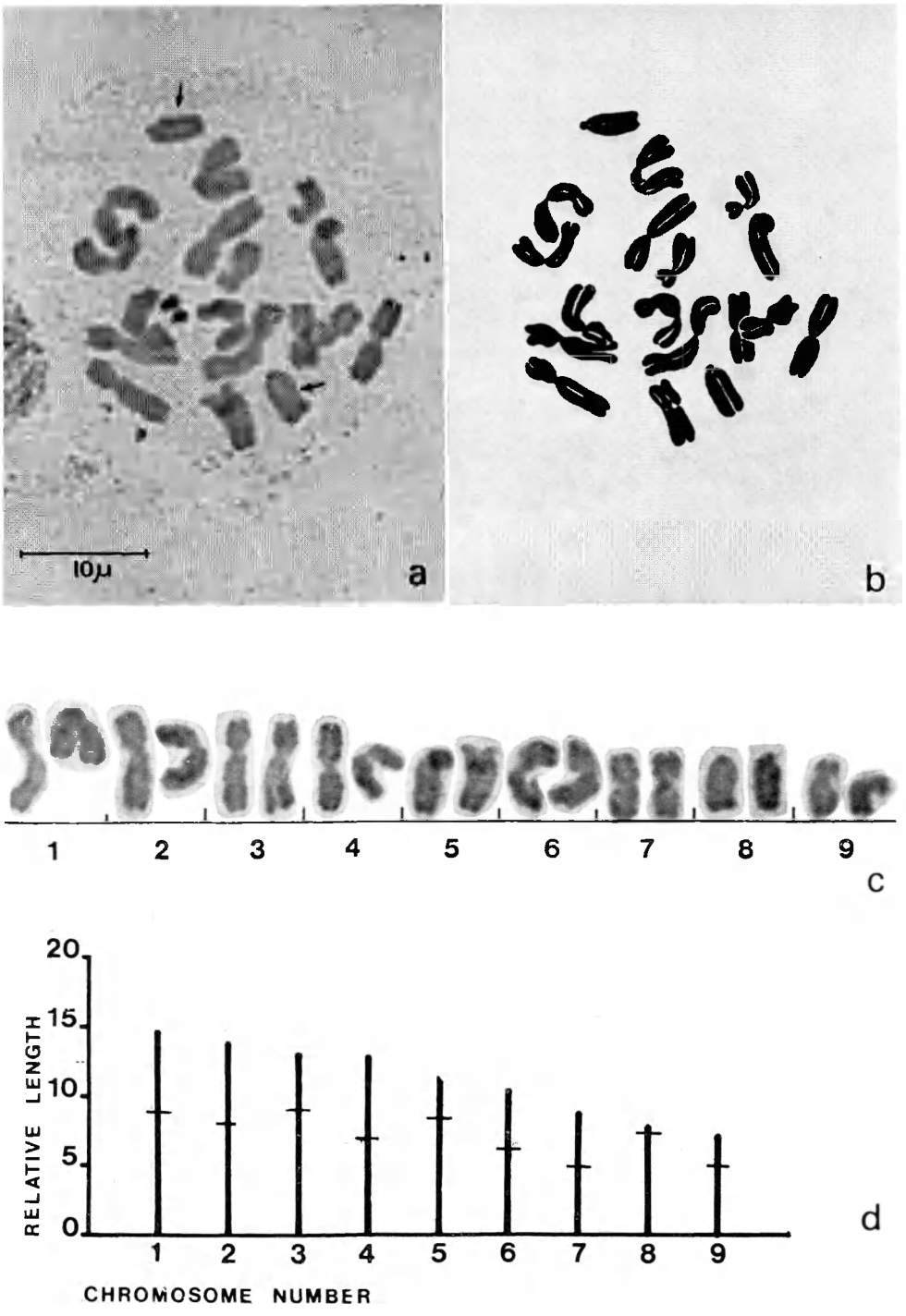

Plate I. – *a*) Metaphasic plate from neoblast of *Thysanozoon brocchii*. Arrows show the telocentric chromosomes; *b*) Camera lucida drawing of the same plate; *c*) Derived karyogram; *d*) Idiogram of the species based on the mean values reported in Table I.

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