

# Chromosomal evolution in marine triclads and polyclads (Turbellaria)

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## Abstract

From an analysis of chromosomal mutations in seven species among five genera of marine triclads and polyclads, three different types of mechanisms of such mutations are identified: 1) rearrangement involving the centromere such that its position is changed, 2) rearrangement of whole chromosome arms, and 3) Robertsonian mechanisms. These mechanisms are the same as those reported for freshwater turbellarians, but aneuploidy and polyploidy, which are common in freshwater species, were not detected in these marine turbellarians.

## Introduction

Since the publication of a revision of the karyology of Platyhelminthes by Benazzi & Benazzi Lentati (1976), several papers dedicated to karyology and cytotaxonomy of marine turbellarians have been published, mainly regarding benthic marine triclads and polyclads. Chromosome numbers of several species have been determined, but the most important gain in our knowledge is in karyometric analysis and idiograms, i.e. beta-level karyology, according to the classification of White (1978), as opposed to alpha-level karyology in which only numbers of chromosomes are reported.

This new material has allowed us to identify rearrangements of chromosomes that have occurred among related species in these marine groups and to propose a hypothesis on trends in the evolution of karyotypes in these species.

## Material and methods

Comparisons have been made using karyometric analyses of species in the genus *Procerodes*

(Tricladida) published by Galleni & Puccinelli (1979) and Galleni *et al.* (1984), of the polyclad species *Thysanozoon brocchii* and *Yungia aurantiaca* (see Galleni & Puccinelli, 1977, 1985), and of the polyclad genera *Notoplana* and *Leptoplana* (Galleni & Puccinelli 1975, 1981, 1984).

Relative length (r.l.) was calculated as mean total length of the two homologous chromosomes  $\times 100$  divided by the total length of the haploid genome; centromeric index (c.i.) was calculated as length of the short arm  $\times 100$  divided by total chromosome length.

Differences and similarities among the idiograms of these species have been evaluated with Student's test [see Benazzi & Puccinelli (1973) and Galleni & Puccinelli (1979) for application of the method to turbellarian karyology].

## Results

For marine triclads, we examined the relationships among idiograms of *Procerodes lobata*, *P. littoralis*, and *P. dohrni* (Fig. 1). The numerical data were published by Galleni & Puccinelli (1979) and

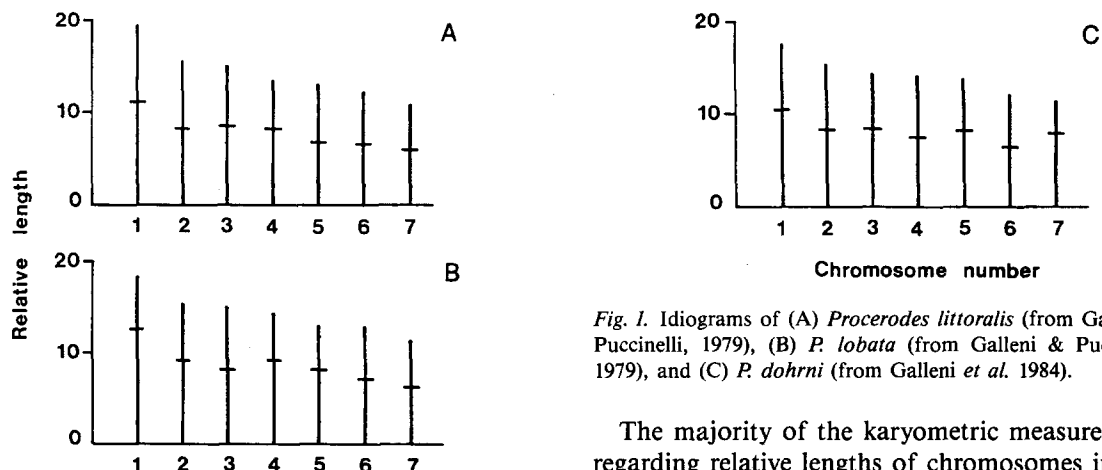


Fig. 1. Idiograms of (A) *Procerodes littoralis* (from Galleni & Puccinelli, 1979), (B) *P. lobata* (from Galleni & Puccinelli, 1979), and (C) *P. dohrni* (from Galleni *et al.* 1984).

Galleni *et al.* (1984). Numerical data reported by Ball (1976, 1979) on the same genus were not considered because he used different methods to order the chromosomes such that it was difficult to compare his data with ours.

The majority of the karyometric measurements regarding relative lengths of chromosomes in species of *Procerodes* are not significantly different, but measurements of centromeric indices are. This indicates that chromosomal rearrangements in this genus involve the centromere and thus changes in centromere position. Translocations, in contrast, seem to be rare (Table I, Fig. 1). The question of

Table I. Comparisons of relative length (r.l.) and centromeric index (c.i.) in three species of *Procerodes*. Values are reported as means  $\pm$  standard error of the mean. Numerical data from Galleni & Puccinelli (1979) and Galleni *et al.* (1984).

Chromosome number		<i>Procerodes littoralis</i> <i>Procerodes dohrni</i>	<i>Procerodes dohrni</i> <i>Procerodes lobata</i>	<i>Procerodes littoralis</i> <i>Procerodes lobata</i>
1	r.l.	1.85 $\pm$ 0.416 <sup>a</sup>	0.68 $\pm$ 0.462 <sup>d</sup>	1.17 $\pm$ 0.495 <sup>c</sup>
	c.i.	6.43 $\pm$ 0.703 <sup>a</sup>	7.61 $\pm$ 0.875 <sup>a</sup>	14.04 $\pm$ 0.890 <sup>a</sup>
2	r.l.	0.10 $\pm$ 0.349		
	c.i.	0.96 $\pm$ 0.875 <sup>d</sup>		
2/3*	r.l.		0.67 $\pm$ 0.386 <sup>d</sup>	0.77 $\pm$ 0.451 <sup>d</sup>
	c.i.		1.24 $\pm$ 0.738 <sup>d</sup>	0.28 $\pm$ 0.757
3/2*	r.l.		0.90 $\pm$ 0.437 <sup>d</sup>	0.46 $\pm$ 0.385 <sup>d</sup>
	c.i.		0.94 $\pm$ 1.178	3.19 $\pm$ 1.104 <sup>b</sup>
3	r.l.	0.44 $\pm$ 0.451		
	c.i.	2.25 $\pm$ 0.839 <sup>b</sup>		
4	r.l.	0.53 $\pm$ 0.236 <sup>c</sup>	0.10 $\pm$ 0.231	0.43 $\pm$ 0.293 <sup>d</sup>
	c.i.	8.01 $\pm$ 1.004 <sup>a</sup>	8.28 $\pm$ 1.335 <sup>a</sup>	0.23 $\pm$ 1.214
5	r.l.	0.86 $\pm$ 0.470 <sup>d</sup>	1.05 $\pm$ 0.498 <sup>d</sup>	0.19 $\pm$ 0.389
	c.i.	13.28 $\pm$ 0.789 <sup>a</sup>	3.73 $\pm$ 1.282 <sup>b</sup>	9.55 $\pm$ 1.045 <sup>a</sup>
6	r.l.	0.02 $\pm$ 0.331	0.68 $\pm$ 0.239 <sup>b</sup>	0.70 $\pm$ 0.282 <sup>c</sup>
	c.i.	0.94 $\pm$ 0.737 <sup>d</sup>	2.65 $\pm$ 0.967 <sup>b</sup>	1.17 $\pm$ 0.968 <sup>d</sup>
7	r.l.	0.76 $\pm$ 0.353 <sup>c</sup>	0.22 $\pm$ 0.558	0.54 $\pm$ 0.530 <sup>d</sup>
	c.i.	14.75 $\pm$ 1.460 <sup>a</sup>	16.79 $\pm$ 1.238 <sup>a</sup>	2.04 $\pm$ 1.622 <sup>d</sup>

<sup>a</sup> =  $p < 0.001$ ; <sup>b</sup> =  $0.01 < p < 0.02$ ; <sup>c</sup> =  $0.05 < p < 0.02$ ; <sup>d</sup> =  $p > 0.05$

\* Owing to small differences in r.l. in these cases, chromosomes with similar c.i. were compared.

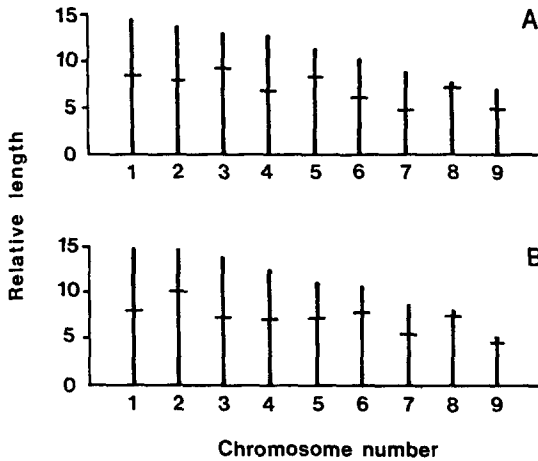


Fig. 2. Idiograms of (A) *Thysanozoon brocchii* (from Galleni & Puccinelli, 1979) and (B) *Yungia aurantiaca* (from Galleni & Puccinelli, 1985).

whether the rearrangements are due to pericentric inversions or to different levels of centromeric heterochromatin cannot be solved without the use of more detailed staining methods.

Karyometric comparisons between the polyclads *Thysanozoon brocchii* and *Yungia aurantiaca* reveal trends similar to those identified in *Procerodes* (see Galleni & Puccinelli, 1985). It is noteworthy that chromosome number 9 is submetacentric in *Thysanozoon brocchii* and acrocentric in *Yungia aurantiaca*. Since the long arm is of the same length in both species, the difference between the two must have arisen through rearrangement of the whole short arm (Fig. 2).

Comparisons of relative lengths and centromeric indices of *Notoplana igiliensis* and *Leptoplana tremellaris* (Table 2, Fig. 3) show that most measurements of relative lengths are not significantly different in the two species while those of centromeric index are.

Finally, the polyclad *Notoplana atomata* has a karyotype quite different from that of *Notoplana igiliensis* (see Galleni & Puccinelli, 1984). A chromosome number of  $2n=18$  in *Notoplana atomata* and of  $2n=20$  in *Notoplana igiliensis* and the lack of some pairs of heterobrachial chromosomes and the presence of a large metacentric chromosome in *Notoplana atomata* indicate that the difference in the karyotype of the two species could have arisen through a Robertsonian mechanism.

Table 2. Comparisons of relative length (r.l.) and centromeric index (c.i.) in *Notoplana igiliensis* and *Leptoplana tremellaris*. Values are reported as mean  $\pm$  standard error of the mean. Numerical data from Galleni & Puccinelli (1981).

Chromosome number		
1	r.l.	$0.48 \pm 0.546$
	c.i.	$26.99 \pm 2.678^a$
2	r.l.	$0.02 \pm 0.259$
	c.i.	$7.48 \pm 1.875^b$
3	r.l.	$0.11 \pm 0.413$
	c.i.	$25.09 \pm 1.044^a$
4	r.l.	$0.53 \pm 0.355^d$
	c.i.	$9.37 \pm 0.881^a$
5	r.l.	$0.37 \pm 0.310^d$
	c.i.	$26.59 \pm 1.346^a$
6	r.l.	$0.64 \pm 0.364^d$
	c.i.	$1.91 \pm 2.785$
7/8*	r.l.	$1.25 \pm 0.383^c$
	c.i.	$5.79 \pm 2.913^d$
8/7*	r.l.	$0.55 \pm 0.486^d$
	c.i.	$0.05 \pm 1.631$
9	r.l.	$0.19 \pm 0.540$
	c.i.	$0.27 \pm 1.040$
10	r.l.	$0.07 \pm 0.180$
	c.i.	$12.47 \pm 2.540^c$

<sup>a</sup> =  $p < 0.001$ ; <sup>b</sup> =  $0.001 < p < 0.01$ ; <sup>c</sup> =  $0.01 < p < 0.02$ ; <sup>d</sup> =  $p > 0.05$

\* = Owing to small differences in r.l., chromosomes with similar c.i. were compared.

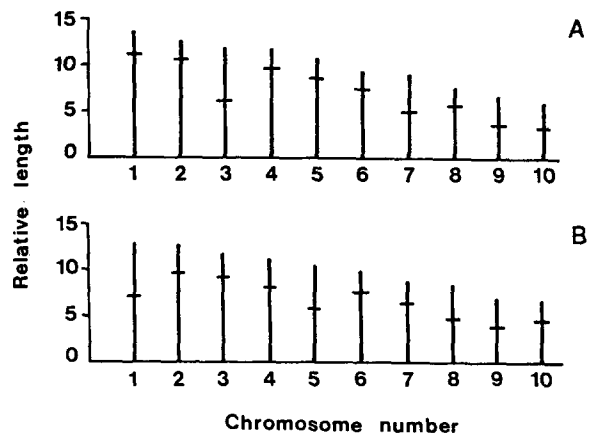


Fig. 3. Idiograms of (A) *Notoplana igiliensis* (from Galleni & Puccinelli, 1975) and (B) *Leptoplana tremellaris* (from Galleni & Puccinelli, 1981).

## Discussion

Three different types of chromosomal rearrangements are evident among these five genera of marine turbellarians: 1) rearrangements involving the centromere which is thus shifted in position, 2) rearrangements of whole chromosome arms, and 3) Robertsonian rearrangements.

According to Benazzi (1982) 'only minor chromosome mutations such as small pericentric inversions have accompanied speciation' at least in some freshwater triclads such as *Dugesia gonocephala* and the *Dugesia* sungenus *Girardia*. The same seems to obtain to the *Procerodes* species and to *Notoplana igiliensis* and *Leptoplana tremellaris*.

Major rearrangements involving a whole chromosome arm in the *Thysanozoon-brocchii*/*Yungia aurantiaca* example and a Robertsonian mechanism in *Notoplana atomata* are evident. A Robertsonian mechanism was also detected among biotypes E and F of the freshwater-triclad *Dugesia lugubris-polychroa* complex Benazzi & Puccinelli (1973). Thus chromosomal evolution among related species involves similar mechanisms whether they are marine or freshwater species. However, aneuploidy and polyploidy – which are commonly seen in freshwater turbellarians and are related to special forms of reproduction – both sexual (e.g. gynogenesis) and asexual – have not been detected in these marine polyclads and triclads.

It is not certain whether chromosomal rearrangements such as those we have found in these marine turbellarians are simply some of the many differences accumulated by differentiating populations or are the very cause of speciation itself as is hypothesized in the stasipatric model of speciation (White, 1978). Karyometric analysis by itself is not sufficient to determine how chromosomal differences among related species have arisen. Difficulties in comparing numerical data from different authors and in even conclusively identifying types

of chromosomal rearrangements show the need for applying chromosome-banding techniques to turbellarians, techniques such as that Galleni *et al.* (1985) perfected in a study of *Dugesia polychroa*.

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