The Polytene Chromosomes of *Cnesia dissimilis* (Edwards) and Three Species of *Gigantodax* Enderlein (Diptera: Simuliidae) from Lanin National Park (Argentina)

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Cytological studies were made on larvae of Gigantodax marginalis, G. chilensis, G. fulvescens *and* Cnesia dissimilis *from four creeks in Lanin National Park, Neuquen province, Argentina. Chromosome maps and idiograms of these species are presented. The following inversions were observed:* G. marginalis*: IL-1 (X-linked inversion), IL-2 (Y-linked inversion), IIS-1.2, IIL-1, IIIL-4,5;* G. chilensis*: IL-4 (X-linked inversion), IIS-1.2, IIIL-4,5;* G. fulvescens*:IL-1 (X-linked inversion), IL-3 (Y-linked inversion), IIS-1.2, IIL-1, IIIL-4,5;* C. dissimilis: *IL-1, IL-5, IIIL-1. Karyological information was used to construct a cladogram and* Cnesia *sp. Was found to show close resemblance to the three* Gigantodax *spp.*

Key words: Argentina - Neotropics - Simuliidae - blackflies - polytene chromosomes - cytotaxonomy

Studies of larval salivary gland chromosomes have been of major importance in the taxonomy of a number of Diptera worldwide. The differences observed in chromosome banding patterns allow the recognition of biologically distinct sibling species which is useful to establish species identity and phylogenetic relationships. In the neotropical region, blackfly chromosome studies are very scarce. Of about 350 known species (Crosskey & Howard 1997) in this area only a few have been studied cytogenetically (Hirai & Uemoto 1984, Shelley et al. 1986, Hirai 1987 a,b, Conn 1988, 1990, Conn et al. 1989, Millest 1992, Charalambous et al. 1993 a,b, 1996, Hirai et al. 1994, Muñoz de Hoyos 1995).

Gigantodax is a Prosimuliini genus (*sensu* Crosskey & Howard, 1997), composed of 71 species distributed along the Andean range from Mexico to Tierra del Fuego (Argentina). Females are not anthropophilic and can be found as well as in mountain creeks from sea level to 4,700 m of altitude (Wygodzinsky & Coscarón 1989). *Gigantodax* is a peculiar genus, showing the greatest diversity among the Prosimuliini genera, with synapomorphies in imago and preimaginal stages that help to differentiate species in this genus from other genera. The unusual morphology of the respiratory filaments in the pupal stage is useful in differentiating species.

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Cnesia, another Prosimuliini genus, is sympatric with southern *Gigantodax.* Both genera breed on both sides of the Andean range in Chile and Argentina in the subantarctic, central Chile and Patagonia realms. In this area these species are sympatric with *Simulium (Pternaspatha)* in the fast streams (Coscarón & Coscarón Arias 1995).

The object of this study is to provide cytological descriptions of three species of *Gigantodax* and one species of *Cnesia*, to supply basic chromosome maps for future comparisons and to achieve a more complete resolution of their phylogenetic relationship. This objective could explain possible relationships among Simuliidae genera and to ascertain if there is agreement with Wygodzinsky and Coscarón´s (1989) group species division using exosomatic characters. One *Cnesia* species and three of *Gigantodax* were analyzed cytologically. A cladistic study using the cytological characters analyzed by the HENNIG86 program was done to evaluate the relationships between the species in these two genera.

MATERIALS AND METHODS

The larval collections available for this analysis are part of a study on the ecology of blackflies from the Lanin National Park (Neuquen Province, Argentina) (Coscarón 1989). The *Gigantodax* species studied here are *G. marginalis* (Edwards), *G. fulvescens* (Blanchard) and *G. chilensis* (Philippi). The first species is distributed on both sides of the southern Andes area, from Valparaiso to Llanquihue (Chile) and from the center of Neuquén to Chubut (Argentina). *G. fulvescens* has a similar distribution but it extends from Coquimbo to Chiloe

in Chile. *G. chilensis* has a larger range in the south from Coquimbo to Magallanes in Chile and from Neuquén to Tierra del Fuego in Argentina (Wygodzinsky & Coscarón 1989, Coscarón 1991). *C. dissimilis* is sympatric with the *Gigantodax* species studied, ranging from Valparaiso to Magallanes in Chile and from Neuquén to Chubut on the eastern flank of the Andes (Wygodzinsky & Coscarón 1973, Coscarón 1991).

Samples of *G. marginalis* were from Chapelco Grande, Yuco and Quitrahue brooks, while *G. chilensis* was collected in the Yuco, Quitrahue and Telesilla brooks. *G. fulvescens* was collected in Yuco and Telesilla brooks and *C. dissimilis* in Chapelco Grande, Yuco and Quitrahue brooks. All collections were made from 1980 to 1983 (Fig. 1, Tables I-IV). Larval instars were identified using Wygodzinsky and Coscaron (1989). The sampling and cytological methods follow standard procedures (Rothfels & Dunbar 1953, Rothfels et al. 1978).

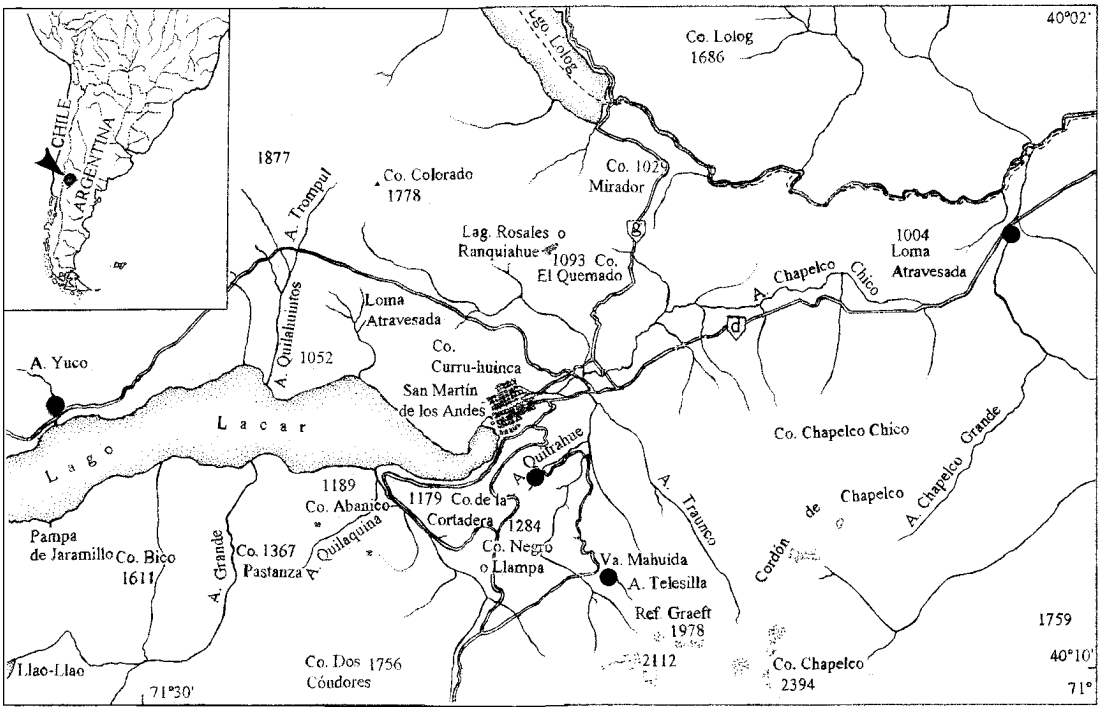

Fig. 1: map with breeding sites.

TABLE I

Seasonal distribution of *Gigantodax marginalis, G. chilensis* and *Cnesia dissimilis* in Quitrahue brook

Date	*Gigantodax marginalis*		*Gigantodax chilensis*		*Cnesia dissimilis*	
	Females	Males	Females	Males	Females	Males
10/05/80	8	3	1	3		
12/06/80	0	1			2	1
15/07/80					0	1
8/08/80	1	0			3	2
11/09/80			0	2	1	1
14/10/80	4	6			12	7
11/11/80	4	7	4	6	26	16
5/03/81	7		8	4		
9/05/81			1	1		
5/08/81			1	1		
19/10/81	5	3	0	1	8	13
16/11/81	2	5	4	0	6	4
12/12/81	10	7	15	9	1	0

TABLE II

Seasonal distribution of *Gigantodax marginalis* and C*nesia dissimilis* in Chapelco Grande brook

Date	*Gigantodax marginalis*		*Cnesia dissimilis*	
	Females	Males	Females	Males
10/05/80	0	1		
12/06/80	1	1		
15/07/80			12	8
8/08/80	1	1	10	6
11/09/80			3	7
14/10/80			2	1
11/11/80			0	1
5/04/81	12	18	1	1
9/06/81	3	1	4	2
5/08/81	1	2	0	1
21/09/81			9	3
19/10/81			14	10
16/11/81			7	4
12/12/81	5	5	2	3

Chromosomal nomenclature and mapping conventions are those in general use (Gordon 1984). Briefly, the three chromosomes are numbered in descending order of length using roman numerals, S (short) or L (long) to denote the arm. Inversions are numbered in order of their discovery. The landmarks: ring of Balbiani, parabalbiani, nucleolar organizer, grey band, shield, frazzle, blister are designated as RB, pB, NO, gB, S, F and B respectively.

Prosimulium mixtum and *P. fuscum* (IIIL) were used as the standard pattern because the genera *Gigantodax* and *Cnesia* belong to the same tribe (Crosskey & Howard 1997). The standard banding sequence has been previously reported Basrur (1959) and the major chromosomal landmarks are summarized in Fig. 2A. Briefly, chromosome I is characterized by the presence of the NO in IL. IS has the "3 heavy group" at the base near an expanded region. In IIS the RB is located near the centromere while IIL has two distinctive landmarks, the "group of 5", and the pB. Chromosome III is characterized by not having an expanded region. IIIS begins with the F. The B can be observed with two associated dark bands. The S and triad are located on IIIL.

Phylogenetic systematics, developed by Hennig (1966), was used. Characters analyzed were derived from the karyological maps. The chromosomal changes in relation to the standard are considered as a plesiomorphic state, which is placed as an apomoporphic character. Character polarity was determined by outgroup comparison (Nixon & Carpenter 1993) using *Prosimulium* for comparison. Data were analyzed using HENNIG86 version 1.5 (Farris 1988); the ie* (implicit enumera-

TABLE IV

Seasonal distribution of *Gigantodax chilensis* and *G. fulvescens* in Telesilla brook

Date	*Gigantodax chilensis*		*Gigantodax fulvescens*	
	Females	Males	Females	Males
12/04/82			12	20
17/08/82	16	11		
15/08/83	9	7	5	8
21/09/83	5	7	9	6
14/10/83	6	6	8	6
11/11/83	0	2	10	15

TABLE III

Seasonal distribution of *Gigantodax marginalis*, *G. chilensis*, *G. fulvescens* and C*nesia dissimilis* in Yuco brook

Date	*Gigantodax marginalis*		*Gigantodax chilensis*		*Gigantodax fulvescens*		*Cnesia dissimilis*	
	Female	Male	Female	Male	Female	Male	Female	Male
3/03/82			20	18	1	4		
12/04/82			12	16	6	4		
8/05/82	3	1	8	8	0	4		
19/06/82			1	2			2	1
15/07/82							1	0
17/08/82			24	19			0	1
22/03/83	3	5	2	2	4	2		
16/04/83			0	1	5	8		
18/05/83			0	3	3	5		
20/06/83			5	2	1	4		
22/07/83			4	0	2	1	3	6
21/09/83	26	30					8	4
14/10/83	12	11	1	0	1	0	5	2
11/11/83	5	1	2	2	0	3	2	1
14/12/83	1	1	17	11	2	5	1	1

TABLE V

Data matrix for *Cnesia* sp. and *Gigantodax* spp. using *Prosimulium* sp. as outgroup

	Character							
	1	2	3	4	5	6	7	8
Prosimulium	0	0	0	0	0	0	0	0
C. dissimilis	0	0	0	0	0	0	1	1
G. marginalis	1	1	1	1	1	1	1	2
G. chilensis	1	1	1	3	1	1	1	2
G. fulvescens	1	1	0	2	1	1	1	2

TABLE VI

Characters and character stats used in cladistic analysis of *Cnesia* sp. and *Gigantodax* spp.

0: Plesiomorph	1: apomorph
1: IS with three heavy group,	
0: present	1: absent
2: NO position,	
0: NO in IL	1: NO in IS
3: determination of males,	
0: absent	1: IL-2
4: determination of females,	
0: absent	1: IL-1 2: IL-3 3: IL-4
5: RB position,	
0: proximal to the centromere	1: distal to the centromere
6: included inversion in IS (IIS-1.2)	
0: absent	1: present
7: pB position	
0: distal to the centromer	1: proximal to the centromere
8: inversion in IIIL	
0: absent	1: IIIL-1 2: IIIL-4,5

tion) option was used for calculating trees. Similar weight was given to all the characters. Table V contains the data matrix used for analysis and Table VI the characters selected. Multistate characters are considerate not additive.

RESULTS

Due to the difficulty in identifying the centromere in all the species analyzed its position was determined by comparison with the banding pattern of the standard species.

Gigantodax marginalis (Figs 2-4)

The centromeres were diffuse but still observed in all chromosomes.

Chromosome I - IS is identical to banding pattern in the standard, however, the NO is present in sections 18-19 rather than in IL (Figs 3B, 4). IL is the sex determining arm, with inversion IL-2 Y-linked in section 38, and inversion IL-1 in sections 30-34 X-linked (Figs 3B, 4).

Chromosome II - IIS has inversion IIS-1 in sections 50-57 which includes the RB; an included inversion IIS-1.2 is also recognized in the same region (sections 50-52) (Figs 2, 5A). The pB, a characteristic landmark of the IIL, is in section 66. This section, along with sections 65 and 67, has been translocated between section 59 and 60 (Fig. 5B). A fixed inversion IIL-1 is present in section 68 (Fig. 5B).

Chromosome III - The banding pattern of IIIS is identical to the standard pattern (Fig. 5C). However, the IIIL arm is characterized by possessing two fixed inversions: IIIL-4 and IIIL-5 in sections 88-89 and 93-96, respectively (Fig. 5D).

Gigantodax chilensis (Figs 2D, 6-8)

Chromosome I - Like *G. marginalis*, IS has the same banding pattern as the standard except that the NO is in section 18/19 (Figs 6A, 7A). The long arm has a sex-linked inversion (IL-4) which involves sections 29-37 and is observed only in females (Fig. 7B). No Y-linked inversions were present (Fig. 6B).

Chromosome II - The short arm of the chromosome II has the same included inversion (IIS-1.2) as *G. marginalis* (Fig. 6C).

G. chilensis also possesses the translocation of

the segment 65-66 between sections 59-60 as in *G. marginalis* (Fig. 6D).

Chromosome III - The short arm of this chromosome is identical to the IIIS of the standard. The IIIL has the same inversion (IIIL-4) as *G. marginalis* in sections 88-89 (Fig. 8A). *G. chilensis* also has inversion IIIL-5 (sections 93-96) (Fig. 8B).

Gigantodax fulvescens (Figs 2E, 9-11)

Chromosome I - *G. fulvescens* can be homologized with the standard banding pattern, with the exception that the NO is in section 18-19, very near the centromere (Figs 9B, 10C) as in *G. marginalis* and *G. chilensis*. IL is the sex determining arm as in *G. marginalis*, i.e., inversion IL-2 in section 38 is exclusive to males (Y-linked) (Fig. 9C). The females have an inversion in sections 29-39 (X-linked) which is denoted as IL-3 (Fig. 10C).

Chromosome II - IIS shares the included inversion IIS-1.2 with *G. marginalis* and *G. chilensis* (Fig. 11A). There is a translocation of the segment involving sections 65 and 66 in the long arm of this chromosome which is rearranged between segments 59 and 60. There is an intraspecific inversion between section 69-70 (IIL-1) (Fig. 11B).

Chromosome III - The IIIS has the same banding pattern as the standard (Fig. 11C). In the long arm (IIIL) two interspecific inversions are observed: IIIL-4 and IIIL-5 (Fig. 11D), as in the other two species of *Gigantodax* described here.

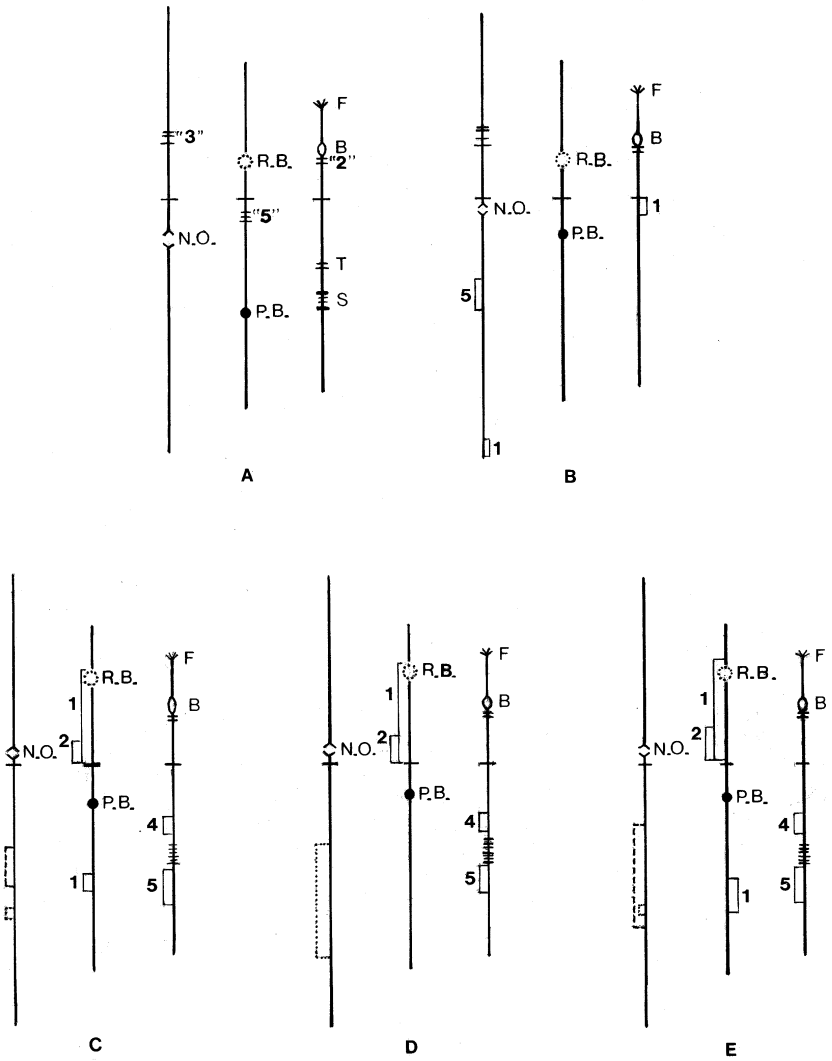

Fig. 2: idiograms of *Prosimulium mixtum* and *P. fuscum* III L (A), *Cnesia dissimilis* (B), *Gigantodax marginalis* (C), *G. chilensis* (D), and *G. fulvescens* (E). Interspecific inversion shown by brackets to the left of chromosome arms, floating inversions by brackets to the right, X inversions by broken line, and Y inversions by dotted lines. For full explanations of chromosomes landmarks see text.

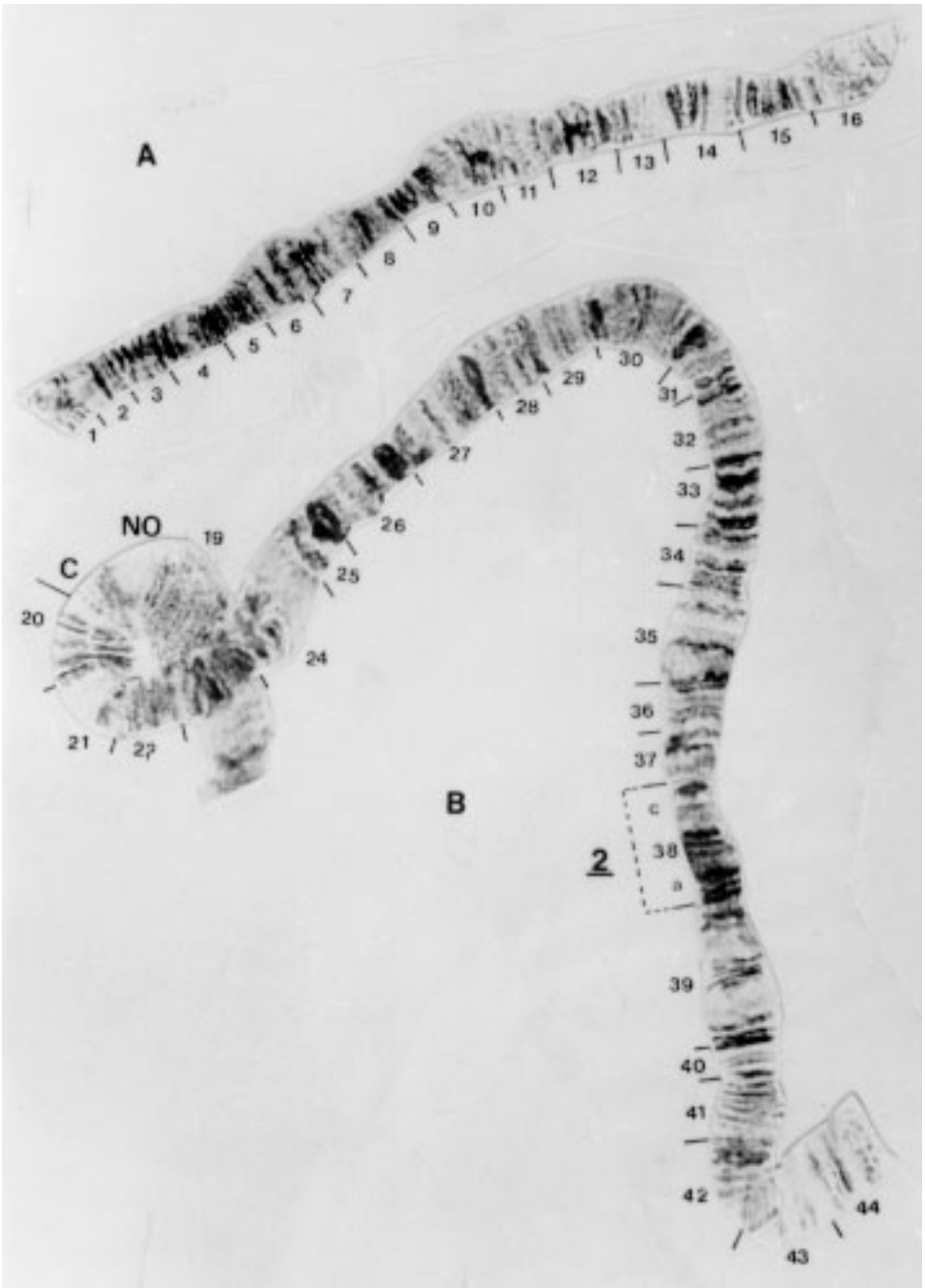

Fig. 3: *Gigantodax marginalis* (male) - A: chromosome IS; B: chromosome IL.

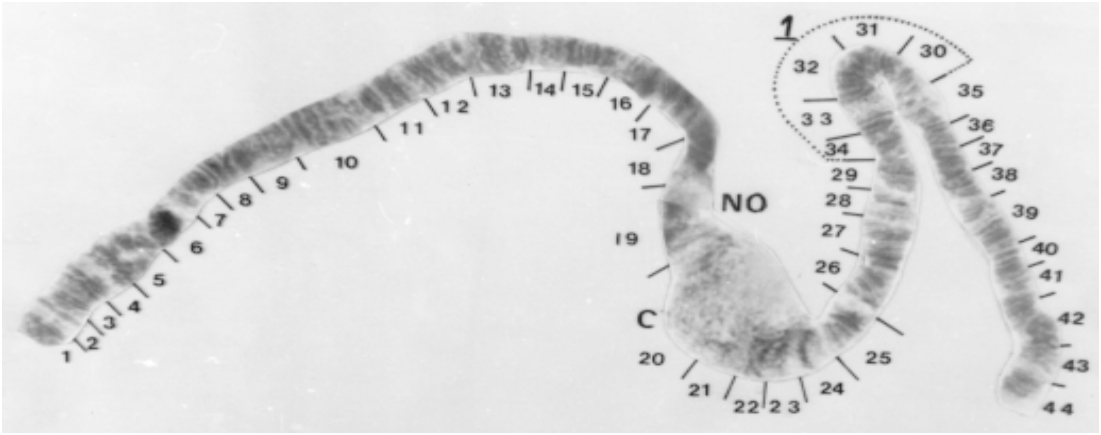

Fig 4: *Gigantodax marginalis* (female) chromosome I.

Cnesia dissimilis (Figs 2B, 12)

No cytological characteristic could differenciate the sexes.

Chromosome I - The short arm has the same banding pattern as the standard (Fig. 12A). The NO is in IL (Fig. 12 B) as in *P. mixtum*. Two inversions were found in IL. IL-5 is an interspecific inversion which includes sections 28-32 and there is an intraspecific inversion IL-1 in sections 43-44 (Fig. 12B).

Chromosome II - Chromosome II of *C. dissimilis* is identical with the standard banding pattern except that in IIL the pB (66) is translocated between 59/60 as in *G. fulvescens* and *G. chilensis* (Fig. 12C).

Chromosome III - The short arm is similar to the standard species but the subterminal blister is not well developed (Fig. 12D). In IIIL an intraspecific inversion (IIIL-1) is observed (83-84).

Phylogeny - The cladistic analysis gave only one cladogram (Fig. 13) with 11 steps, a consistency index of 1 and retention index of 1. The first clade shows that *C. dissimilis* has a relationship with *Gigantodax* species, supported by one synapomorphy, but *Cnesia* is the outermost taxon of them. A clade includes the three *Gigantodax* species and is supported by five synapomorphies. Among them *G. marginalis* and *G. chilensis* show a relationship supported by one synapomorphy.

DISCUSSION

Comparing these three species of the genus *Gigantodax* from Argentina with the five species analyzed by Hirai (1987c) from Ecuador where only the gross features are described and there is no standard species mentioned, we can say that the five species from Ecuador and the three species from Argentina share the following characters: the frazzle end in IIIS; the ring of Balbiani in IIS is inverted; the NO in species *Gigantodax 1*, *2* and *4* of Hirai are in the short arm of chromosome Y and the sex determining factor of this group may be located in chromosome I.

Muñoz de Hoyos (1995) states that *G. osornorum*, *G. ortizi*, *G. fulvescens*, *G. marginalis* and *G. chilensis* are homologous for chromosome I and that the species from Colombia differ from the species analyzed here in the position of the NO. This author suggests an indepth analysis of chromosome I is required. We also suggest that it may also be important to examine IIIL because *G. osornorum* has an inversion in sections 88-90 and in *G. fulvescens*, *G. marginalis* and *G. chilensis* the inversion comprises sections 88-89.

From the genus *Cnesia* only one species was analyzed. The *C. dissimilis* banding pattern is most similar to that of the members of the complex *P. mixtum*, *P. fuscum* IIIL. This would suggest that among the taxa here analyzed, this species is nearest to the standard. Therefore, it could be considered a primitive species.

Comparing *C. dissimilis* with the *Gigantodax* spp. from the Lanin National Park: there are similarities between *Gigantodax* spp. and *Cnesia* sp. (translocation and location of the ParaBalbiani, frazzled end in IIIS, chromosome III has the same banding pattern as the standard and IS). These characteristics show the proximity of these two genera emphasizing what was established morphologically by Wygodzinsky and Coscarón (1973) and Py Daniel (1994).

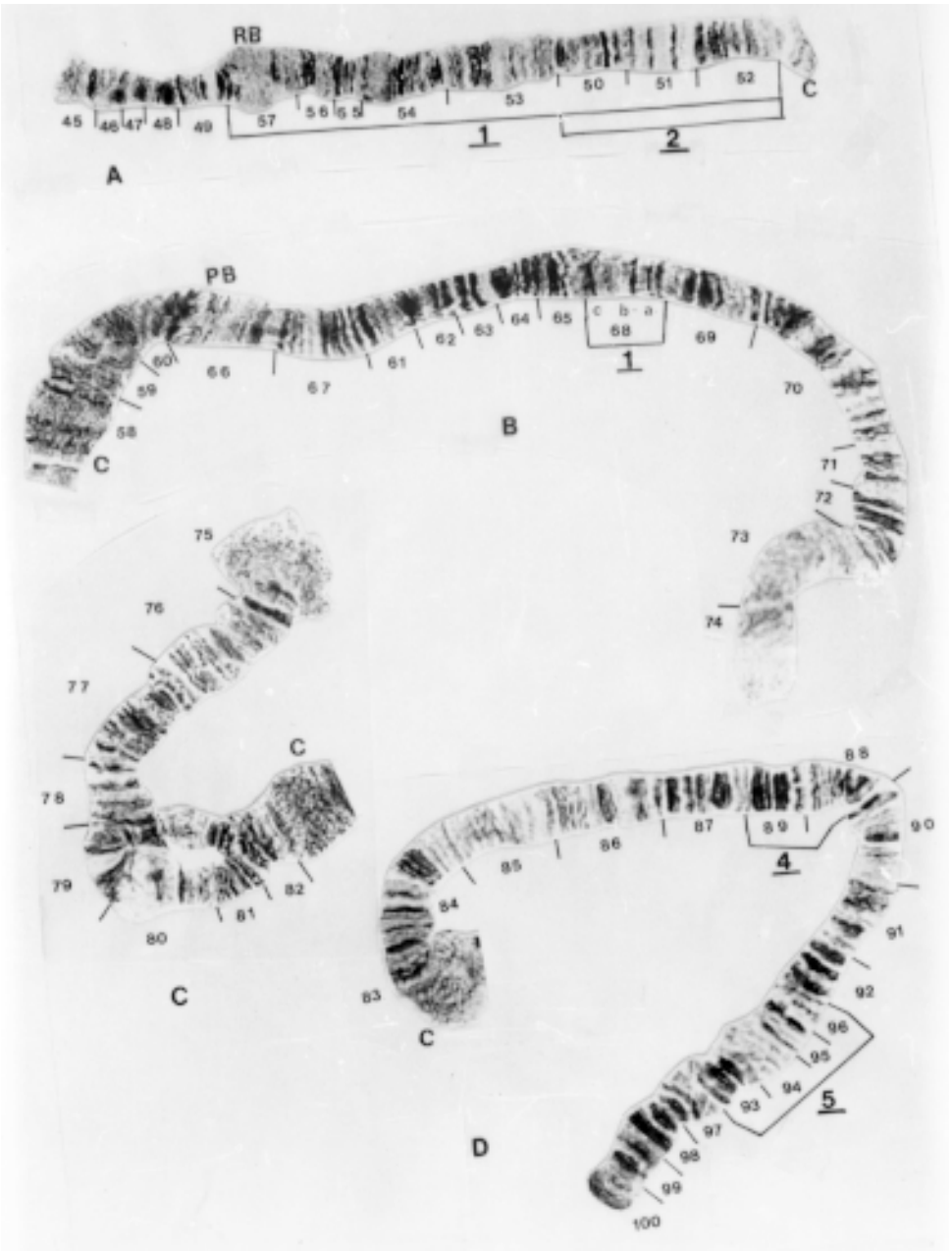

Fig. 5: *Gigantodax marginalis* - A: chromosome IIS; B: chromosome IIL; C: chromosome IIIS; D: chromosome IIIL.

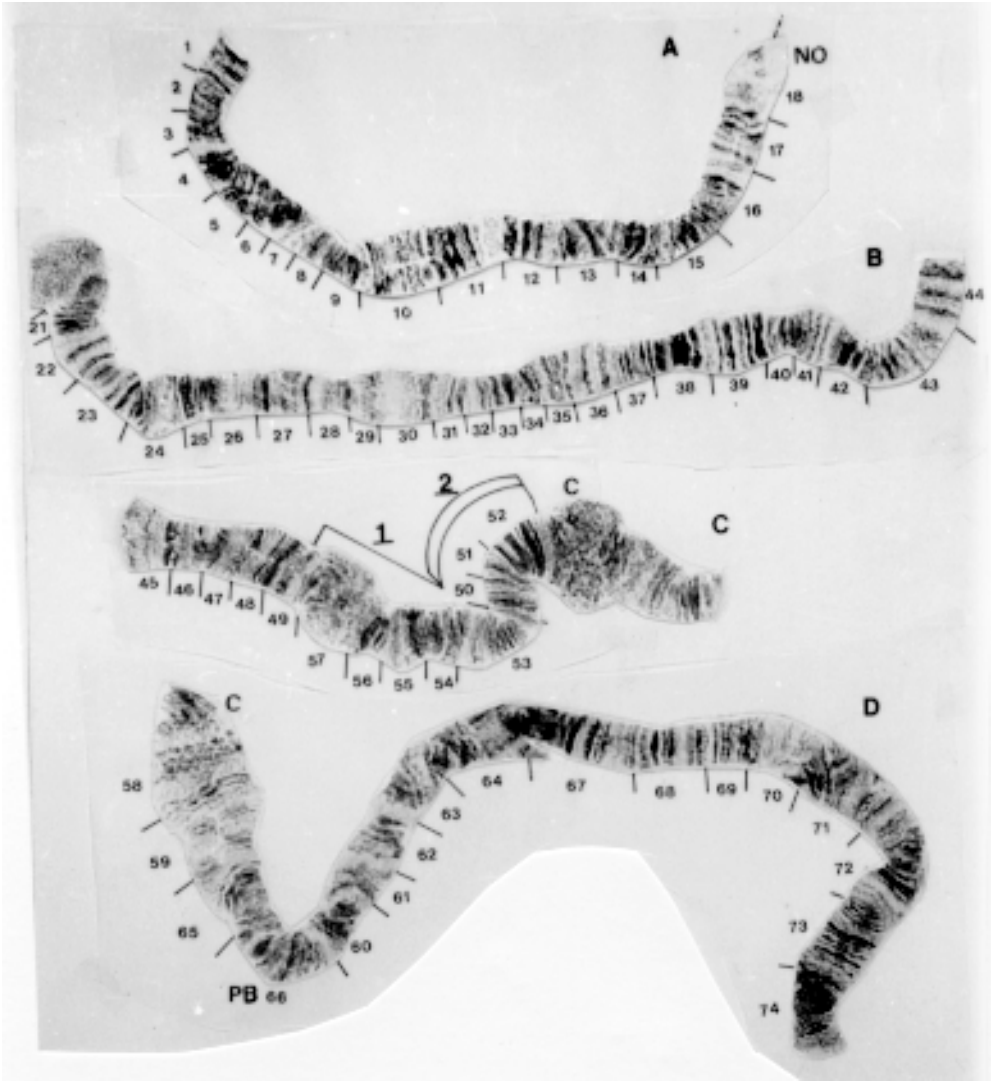

Fig. 6: *Gigantodax chilensis* - A: chromosome IS (male); B: chromosome IL (male); C: chromosome IIS; D: chromosome IIL.

The cladogram shows that *C. dissimilis* is closer to the standard than the species from the genus *Gigantodax*. Also, *Gigantodax* has a well supported monophyly, and *G. marginalis* and *G. chilensis* are the most closely related as they share a translocation of 65-66 (where the paraBalbiani is) to 59-60. In fact they only differ in their sex determination and the inversion IIL-1. This relationship is congruent with the placement of these species in the *brophyi* group and *fulvescens* in *cilicinus* group (Wygodzinsky & Coscarón 1989).

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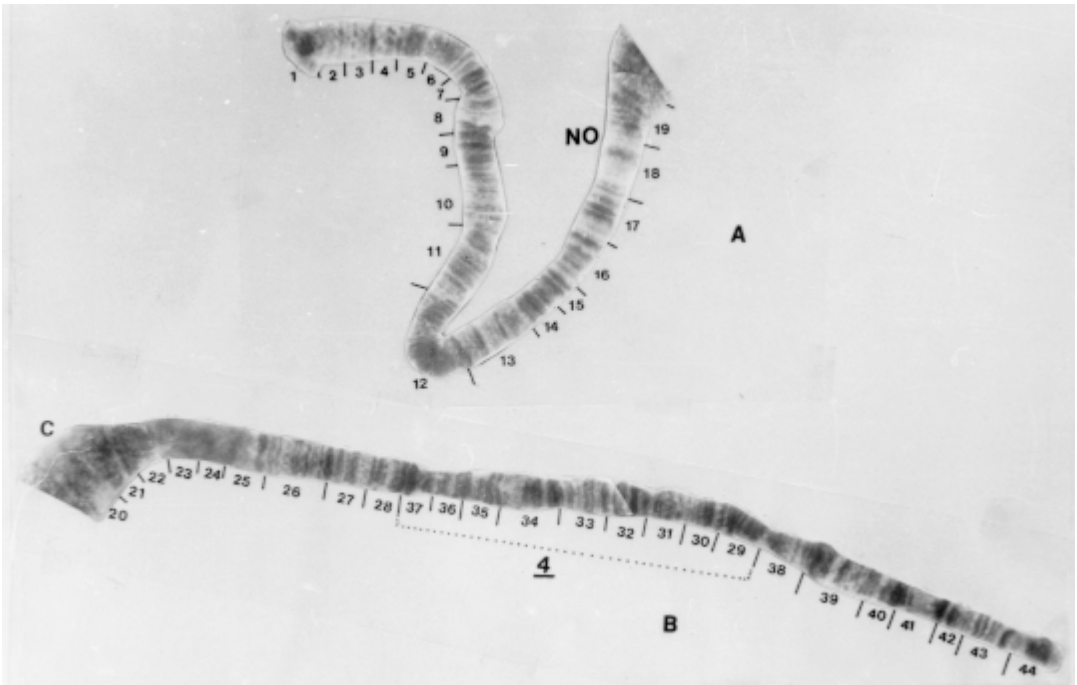

Fig. 7: *Gigantodax chilensis* (female) - A: chromosome IS; B: chromosome IL.

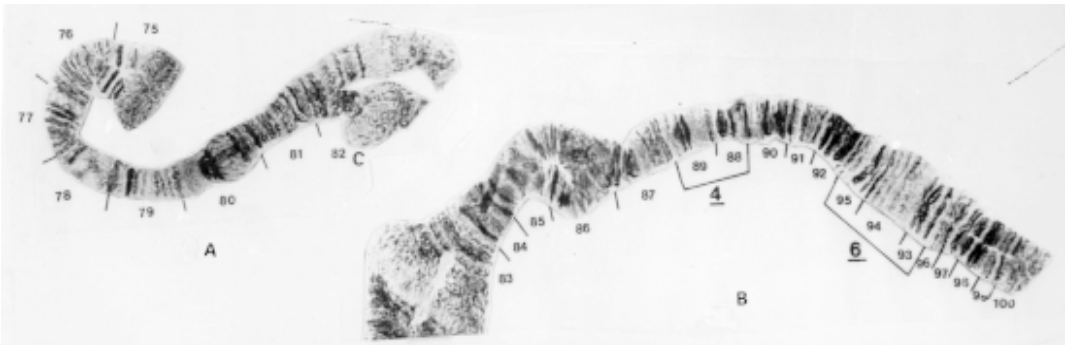

Fig. 8: *Gigantodax chilensis* - A: chromosome IIIS; B: chromosome IIIL.

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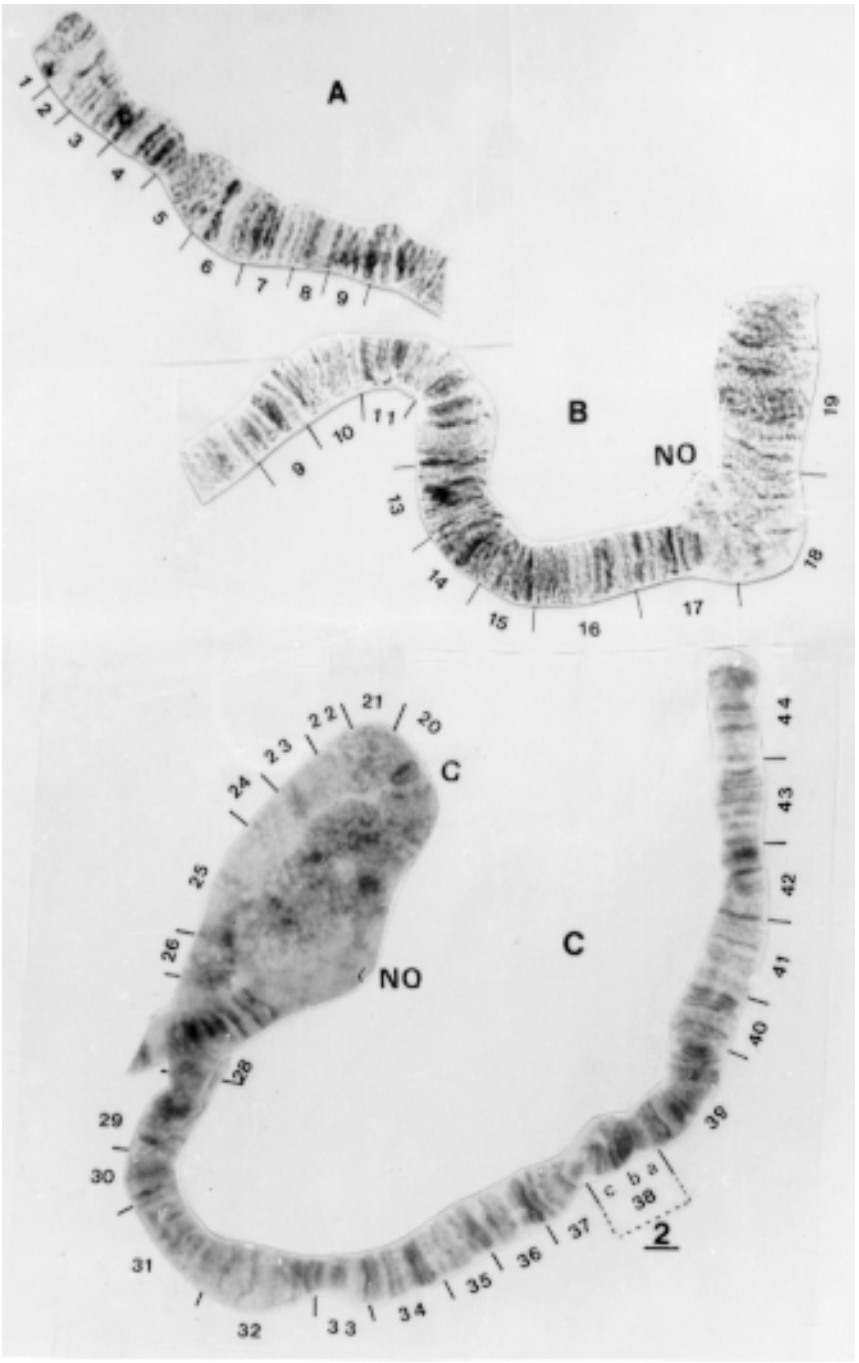

Fig. 9: *Gigantodax fulvescens* (male) - A, B: chromosome IS; C: chromosome IL.

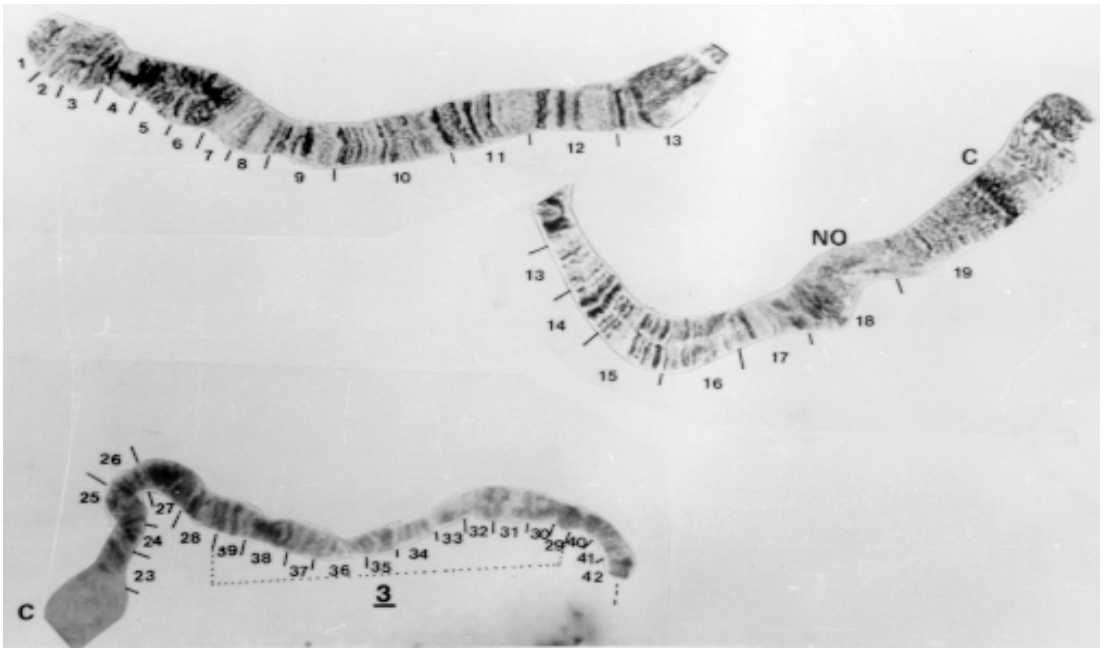

Fig. 10: *Gigantodax fulvescens* (male) - A, B: chromosome IS: C: chromosome IL.

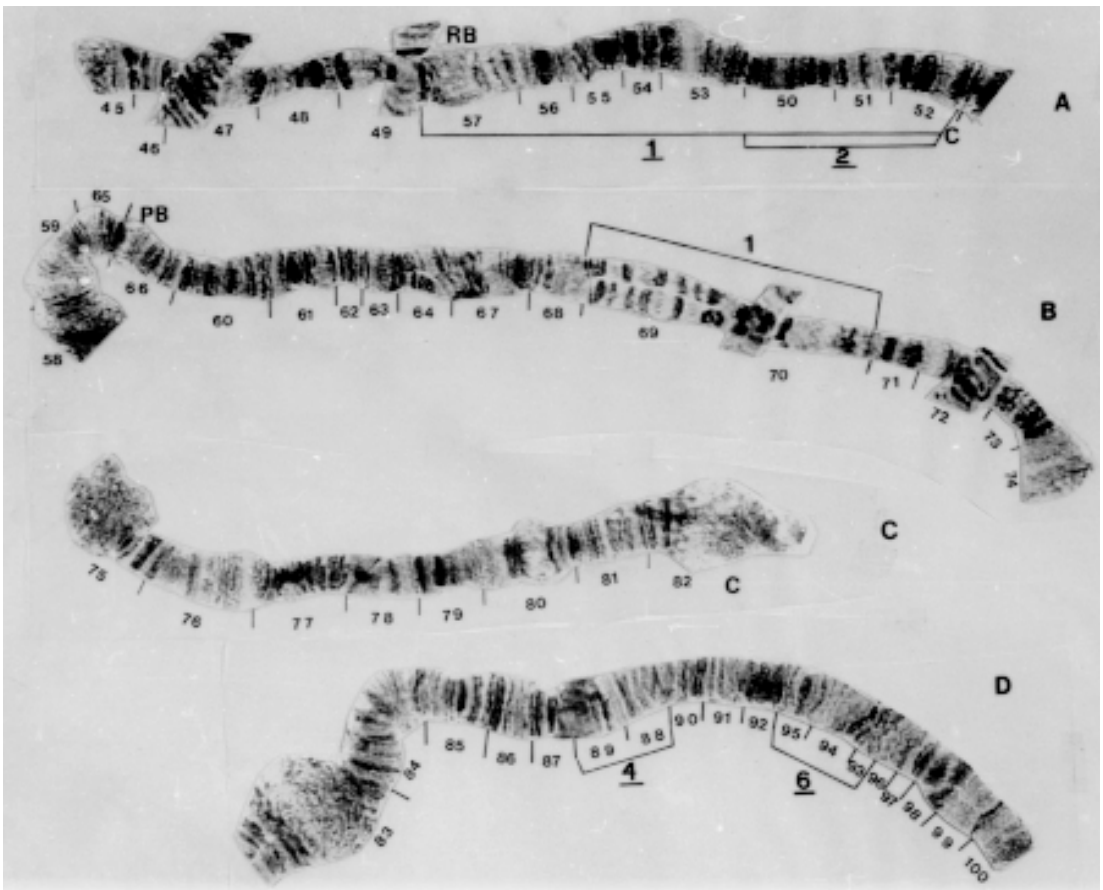

Fig. 11: *Gigantodax fulvescens* - A: chromosome IIS (part); B: chromosome IIL; C: chromosome IIIS; D: chromosome IIIL.

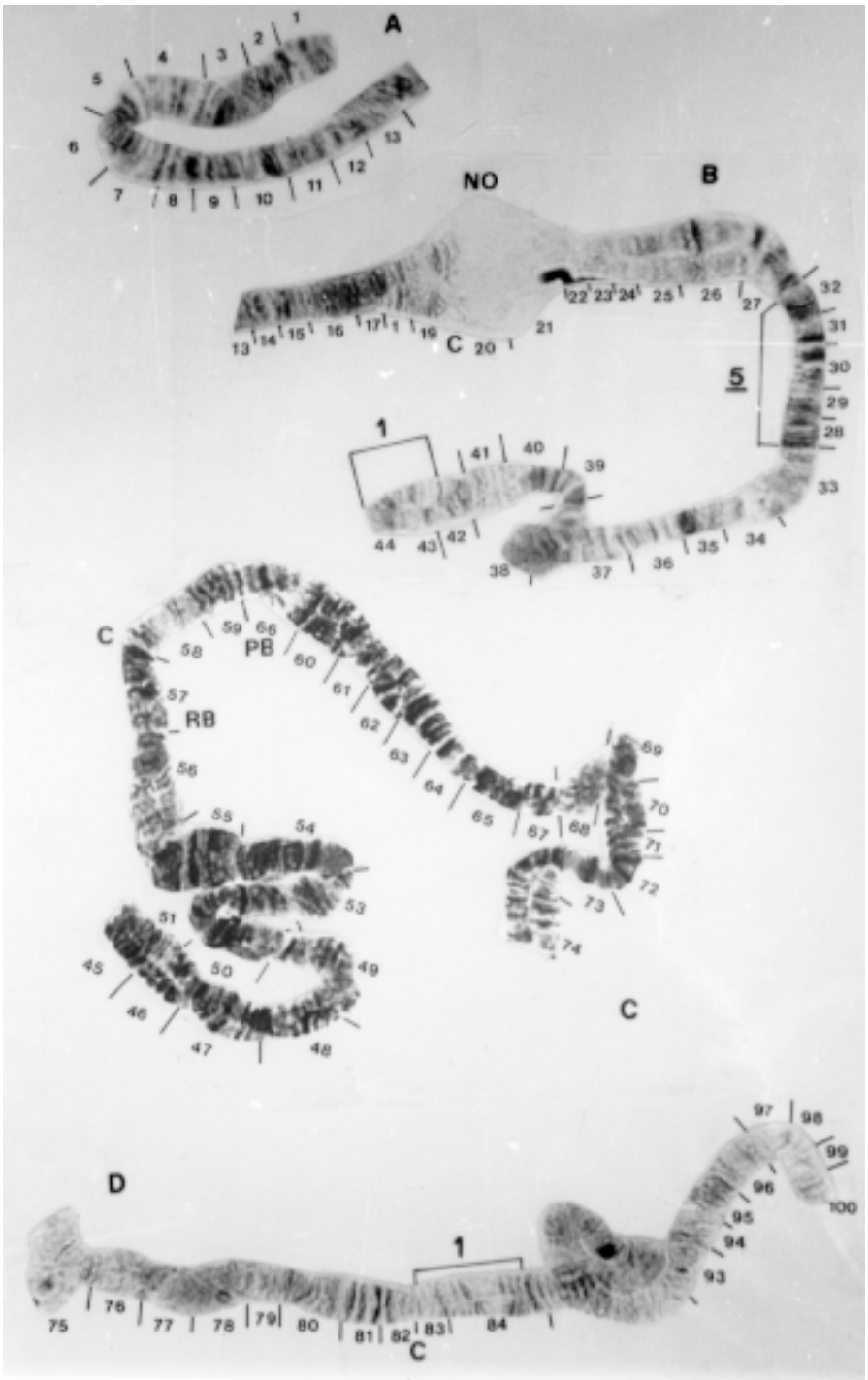

Fig. 12: *Cnesia dissimilis* - A: chromosome IS (part); B: chromosomes IS (part) and IL; C: chromosome IIL; D: chromosome III.

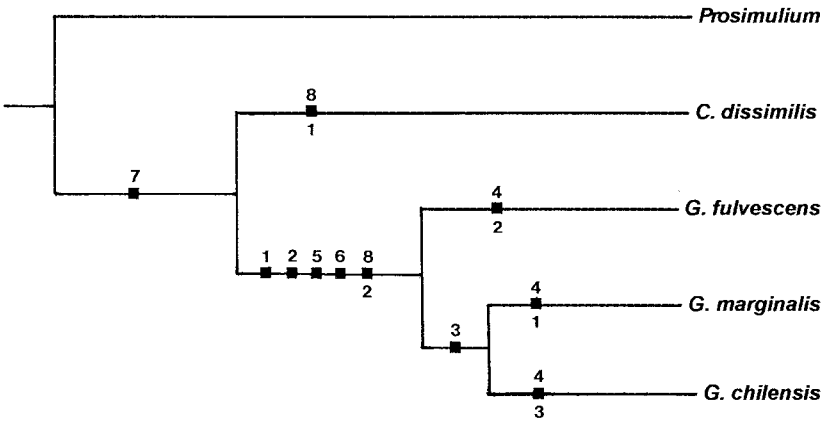

Fig. 13: cladogram. Length: 14 steps. Consistency Index: 1.0. Retention Index:1.0.

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