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Spermatocyte chromosome analysis of the slug *Lehmannia melitensis* (Lessona and Pollonera, 1891) (Mollusca, Pulmonata) using conventional, NOR- and C-banding techniques

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SUMMARY — Diploid number $2n=40$ and haploid number $n=20$ for the slug *Lehmannia melitensis* have been determined. These chromosome values being considerably different from those reported for slugs belonging to other limacid genera support the notion that the genus *Lehmannia* is particular within the family Limacidae. Nucleolus organizer regions (NORs) and C-banding pattern of *L. melitensis* are described.

Key words: *Lehmannia melitensis*, Gastropoda, karyology, banding analysis.

INTRODUCTION

Limacid species are widely distributed in Europe, central Asia and north Africa. They usually inhabit forests or antropized and cultivated areas at variable altitude under stones and fallen tree trunks. Since slugs include morphologically similar forms, the taxonomy of the group has had a long history. In particular, *Lehmannia* Heynemann, 1862 was considered alternately as a synonim or as a subgenus of the genus *Limax* (Linnaeus, 1758) (PILSBRY 1940; KERNEY and CAMERON 1979).

With regard to the karyology, meiotic chromosomes of some slugs from England have been described (BEESON 1960) (Table 1). In particular, the haploid chromosome value $n=24$ has been found in *Lehmannia marginata* (Müller, 1774). This number differed considerably from the haploid values included in the range 30-33/34 of all other species of slugs there investigated.

In part to document whether a low chromosome number really occurs within the genus *Lehmannia* and partly to start a comparative chromosome analysis of slugs using the modern banding procedures, we have studied spermatocyte chromosomes of *L. melitensis* (Lessona and Pollonera 1891).

In this paper we describe conventionally, silver nitrate-stained, and C-banded chromosomes of this species from Sicily.

TABLE 1 - Chromosome numbers of 10 slugs belonging to the families Milacidae, Limacidae and Agriolimacidae.

Taxa	Chromosome number	References
Milacidae		
Milax		
gagates (Draparnaud, 1801)	$n=33$ or 34	BEESON 1960
nigrigans (Philippi, 1836)	$n=33$	VITTURI 1992
Tandonia		
sowerbyi (Ferussac, 1823)	$n=34$	BEESON 1960
Limacidae		
Limax		
cinereoniger (Wolf, 1803)	$n=31$	BEESON 1960
maximus (Linnaeus, 1758)	$n=31$	BEESON 1960
Lehmannia		
marginata (Müller, 1774)	$n=24$	BEESON 1960
melitensis (Lessona and Pollonera, 1891)	$n=20$	present paper
Agriolimacidae		
Deroceras		
agreste (Linnaeus, 1758)	$n=30$	BEESON 1960
reticulatum (Müller, 1774)	$n=30$	BEESON 1960

L. melitensis first identified by Issel (1868 sub *Limax* sp.) at Malta Island was described by LESSONA and POLLONERA (1891). Later, populations of this species have been found by GIUSTI (1973) in the Eolie Archipelago (Sicily).

MATERIALS AND METHODS

Sexually mature specimens of *Lehmannia melitensis* were collected in November 1990 from two geographical locations of Sicily. The first is Mussomeli (Caltanisetta) (10 individuals); the second is Mondello, a seaside resort near Palermo (6 individuals).

Specimens from Mussomeli were found under stones, beside intensively cultivated areas, while specimens from Mondello were collected by night along narrow paths of a public park.

Five voucher individuals were fixed in 70% ethanol solution and deposited at the Museum of the Institute of Zoology, University of Palermo, Italy.

Gathered specimens were classified following the guidelines of POLLONERA (1891), COLUCCI (1920) and GIUSTI (1973). They were identified as *L. melitensis* due to the presence in this species of a long flagellum with smooth outlines (Fig. 1) (GIUSTI *et al.* 1985).

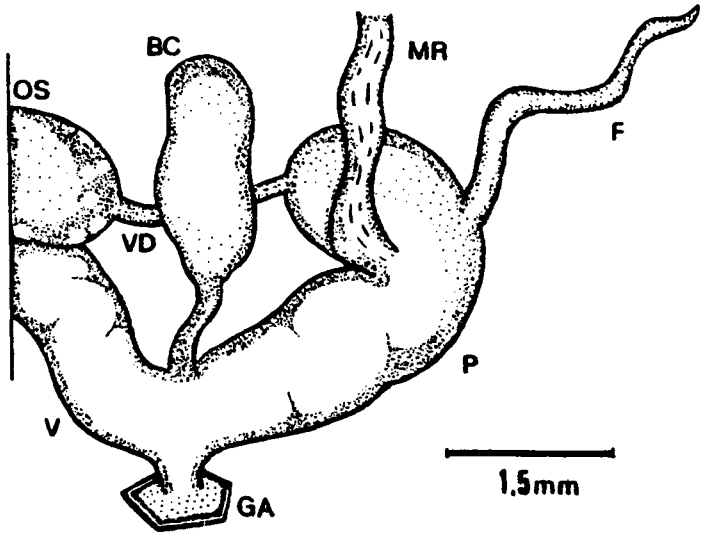


Fig. 1. — *Lehmannia melitensis* (Lessona and Pollonera, 1891). genital duct in a specimen collected at Mondello (Palermo). BC = bursa copulatrix; F = flagellum; GA = Genital atrium; OS = ovispermiduct; P = penis; PR = penial retractor muscle; V = vagina; VD = vas deferent.

Chromosome preparations were obtained from testes using the technique described by VITTURI *et al.* (1991). Mitotic metaphase chromosomes were prepared after a pretreatment with 0.025% colchicine in 0.075 M. KCl solution.

For the characterization of the NORs, slides of four specimens were stained with silver nitrate according to the method of HOWELL and BLACK (1980).

Constitutive heterochromatin was revelaed in slides of five spcimens according to the technique of SUMNER (1972).

Observation and photomicrographs were made with a «Jenaval 2» light microscope.

Chromosomes were classified on the basis of the arm length ratio using the criteria of LEVAN *et al.* (1964).

OBSERVATIONS

Acetic orcein stained chromosomes.

We have analysed a total of 48 spermatogonial metaphase plates where the chromosomes were spread well enough to be counted and karyotyped. Of these, 39 contained 40 chromosomes (Fig. 2a), while 9 had lower values; these latters were presumably due to technical artifacts.

TABLE 2 - Mean length and arm ratio of chromosomes of seven spermatogonial metaphase plates of *Lehmannia melitensis*.

Chromosome pairs	Mean length in microns	SD (±)	Arm ratio mean	Centromere position
1	3.3	0.26	1.5	M
2	3	0.07	1.5	M
3	2.75	0.10	1.4	M
4	2.40	0.26	5	ST
5	2.40	0.28	1.5	M
6	2.10	0.20	1.3	M
7	2	0.30	1.2	M
8	1.90	0.22	1.7	SM
9	1.80	0.17		A
10	1.80	0.17	1.2	M
11	1.75	0.16	1.9	SM
12	1.60	0.14	1.3	M
13	1.50	0.07	1.5	M
14	1.40	0.14	6	ST
15	1.30	0.05	3.3	ST
16	1.25	0.14	1.5	M
17	1.10	0.07	1	M
18	0.90	0.09	3.5	ST
19	0.90	0.09	2.5	SM
20	0.90	0.09	3.5	ST

Average karyotype (Table 2) was constructed from seven spreads arranging the chromosome pairs on the basis of decreasing size and centromere position (Fig. 2b, one spread is illustrated). Its analysis showed that *L. melitensis* possessed 20 pairs of autosomes, 6 of which being mono-armed (ST + A) and 14 bi-armed (M + SM).

Pachytene chromosomes (Fig. 3) were not homogenously stained because of light areas occurred along the chromosomal body. Counts of 124 diakinetic spreads (Fig. 4) gave the haploid number $n = 20$. Other 12 spreads displayed values lower than the mode. Due to the different locations of chiasmata, diakinetic bivalents had ring-, cross- and rod-morphologies.

Silver stained chromosomes.

After silver staining, variation of the NOR pattern occurred in both pachytene and diakinesis spreads. At pachytene, either NOR-negative spreads (Fig. 5a) or spreads with one (Fig. 5b) and two (Fig. 5c) silver positive areas which occasionally tended to fuse have been observed (Fig. 5d). When one nucleolus/nucleus could be visualized, it was variable in size (see Figs. 5b and e).

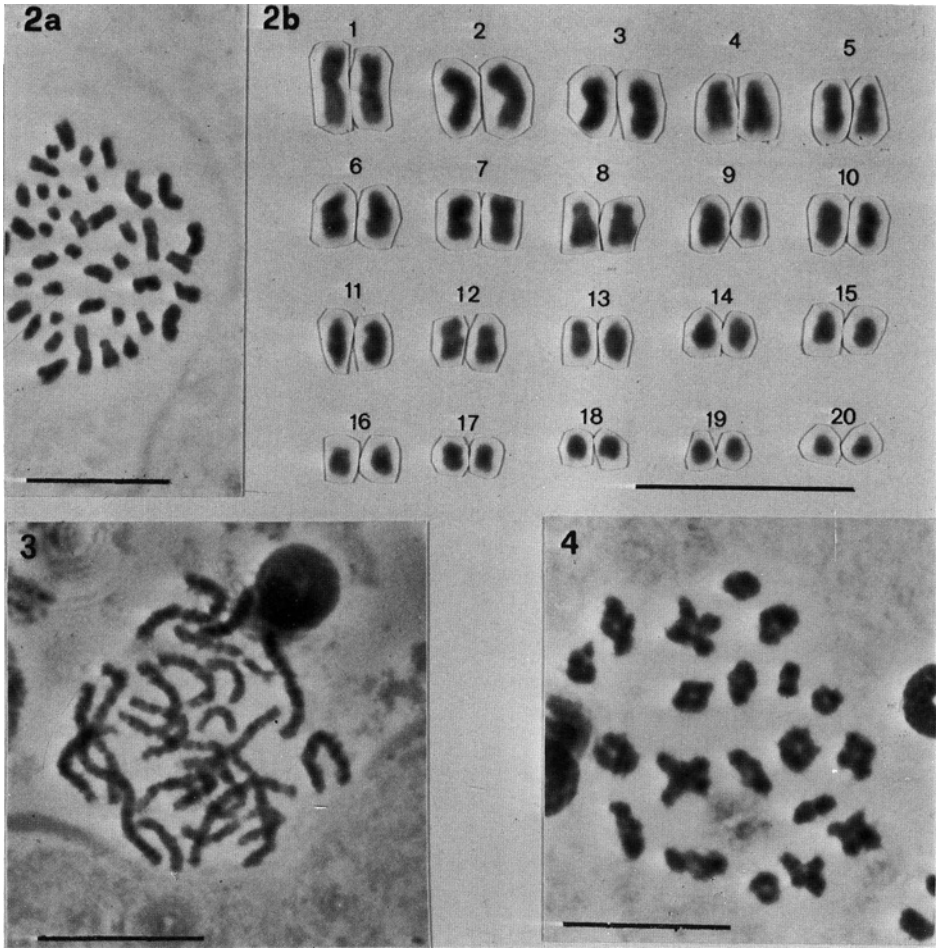


Fig. 2. — Acetic orcein stained mitotic metaphase of *L. melitensis*: a) spermatogonial metaphase; and b) karyotype.

Fig. 3. — Acetic orcein stained pachytene chromosomes from male gonads of *L. melitensis*.

Fig. 4. — Acetic orcein stained diakinetic bivalent, from male gonads of *L. melitensis*.

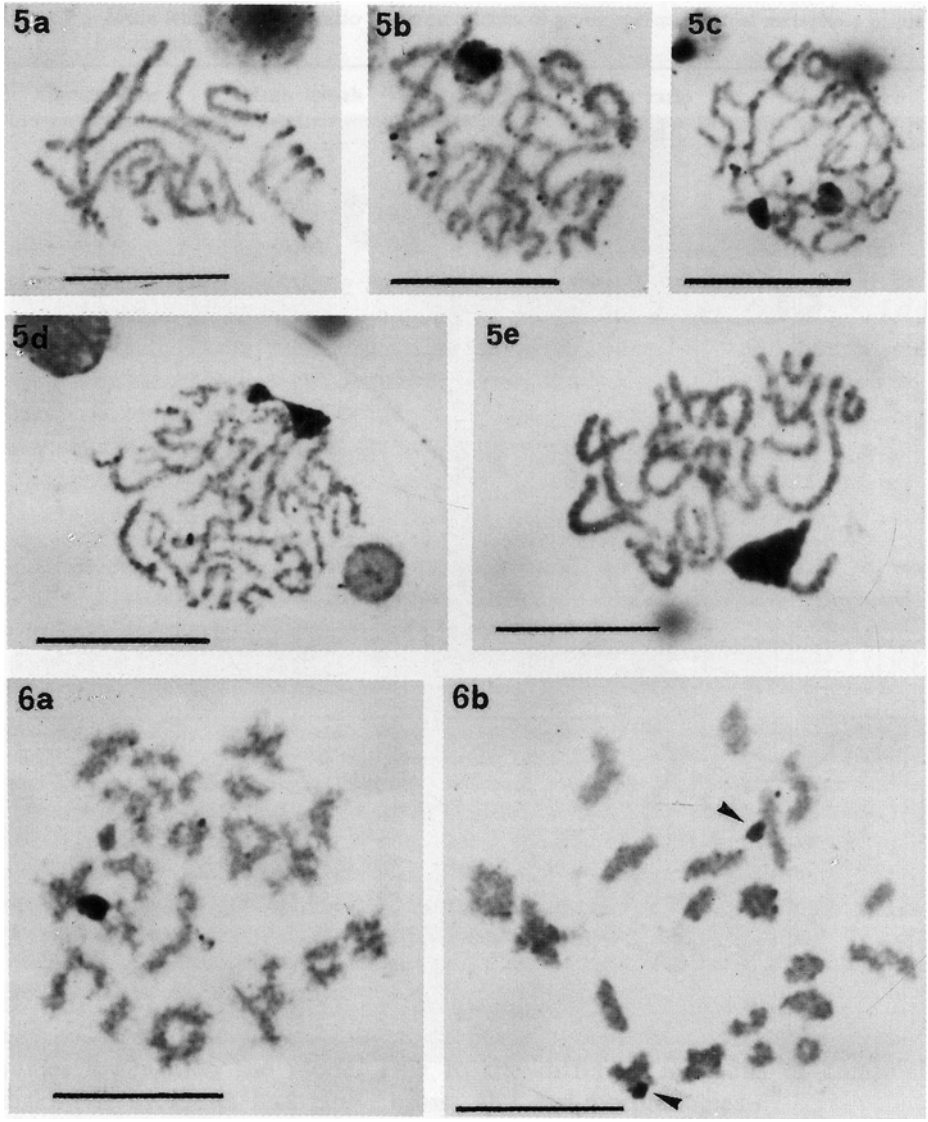
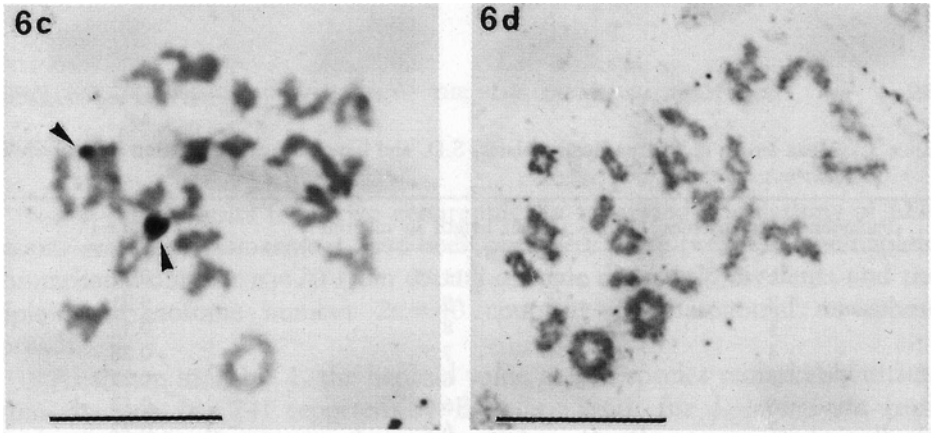
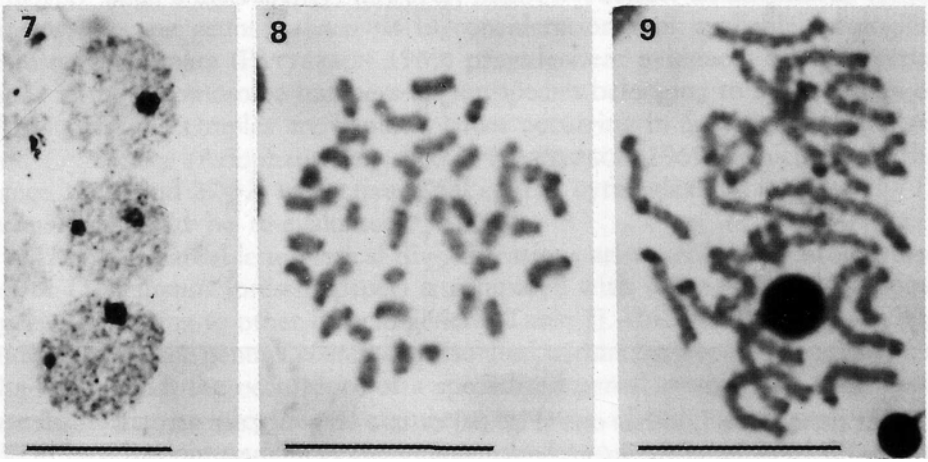


Fig. 5. — Silver stained pachytene chromosomes from male gonads of *L. melitensis*. a) NOR-negative; b) with one nucleolus; c); with two nucleoli; d) with two nucleoli which tend to fuse; and e) with one large nucleolus.

Fig. 6. — Silver stained diakinetic bivalents from male gonad of *L. melitensis*: a) with one silver positive area; b) with two silver positive areas similar in size (arrows); c) with two silver positive areas different in size (arrows); and d) NOR-negative.





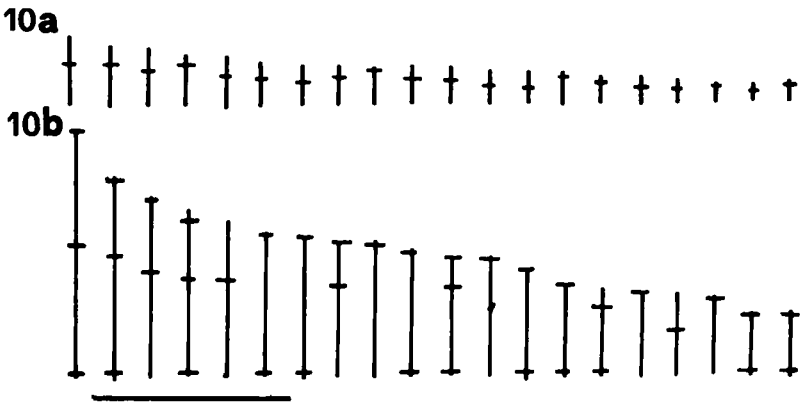


Fig. 7. — Silver stained nuclei with one nucleolus and two nucleoli.

Fig. 8. — C-banded spermatogonial metaphase.

Fig. 9. — C-banded pachytene spread from male gonads of *L. melitensis*.

Fig. 10. — a) Average karyotype obtained from seven spermatogonial metaphases and b) average idiogram obtained from five pachytene spreads (Bar = 10 μm).

TABLE 3 - Mean length of five pachytene plates, S.D. and heterochromatic location of *Lehmannia melitensis*.

Pachytene chromosomes	Mean length in microns	SD (±)
1	11.25	0.37
2	9	0.21
3	8	0.4
4	7.5	0.28
5	6.8	0.23
6	6.6	0.17
7	6.2	0.22
8	6	0.25
9	6	0.30
10	5.7	0.30
11	5.2	0.14
12	5.1	0.16
13	4.8	0.14
14	4.1	0.14
15	3.7	0.22
16	3.5	0.30
17	3.3	0.43
18	3.2	0.30
19	2.7	0.14
20	2.6	0.20

At metaphase-I, either one (Fig. 6a) or two (Fig. 6b, see arrows) bivalents per spread were involved in nucleolus organization in the proportion of 40% and 45%, respectively. When two bivalents showed nucleolus activity, the NORs were nearly similar (Fig. 6b) or different (Fig. 6c) in size. Moreover, 15% of the analysed spreads showed a NOR-negative appearance (Fig. 6d).

We have also observed 20 spermatogonial metaphases which consistently appeared to be NOR negative.

Analysis of 126 nuclei from one specimen stained by the silver method, revealed that 61 of them had one nucleolus and 65 two nucleoli (Fig. 7).

C-banded chromosomes.

At spermatogonial metaphase, the chromosomes looked like condensed rod-shaped bodies which occasionally showed small darkly stained terminal regions (Fig. 8).

Application of C-banding on pachytene bivalents allowed to better identify heterochromatic blocks because of these chromosomes were more relaxed (Fig. 9). These blocks are illustrated on an average ideogram constructed from five spreads (Table 3) (Fig. 10b). In seven chromosomes either interstitial or terminal bands were observed. In the other bivalents only terminal blocks occurred.

DISCUSSION

The same results from two geographically separated populations of *Lehmannia melitensis* (Limacidae) have been obtained: more precisely, the haploid chromosome number $n = 20$ from counts of male diakinetic bivalents and the diploid chromosome number $2n = 40$ counting spermatogonial metaphase spreads.

As shown in Table 1, the haploid value of this species remarkably differs from the one ($n = 24$) reported by BEESON (1960) for *L. marginata* from England. Such a discrepancy, however, might not reflect an anomalous condition within the genus *Lehmannia* in consideration that available cytological data on Pulmonata (PATTERSON 1969) provide clear evidences of wide variations in the chromosome numbers among species belonging to other genera of this subclass. Examples are haploid values occurring in *Succinea* (Succinidae) and *Cryptozona* (Ariophantidae) species (PATTERSON 1969), which lie in the range 17-22 and 27-32, respectively. Of course, cytological data concerning *L. marginata* should be re-evaluated.

More remarkable numerical diversifications arise, when the chromosome set of *L. melitensis* (present paper) is compared with those reported for some species belonging to other limacid genera (Table 1). Undoubtedly, this finding indicates that the genus *Lehmannia* is isolated within the family Limacidae. On the other hand, the occurrence of a specialized penial structure in *Lehmannia* species, seems to provide an argument in favor of this conclusion.

Since haploid numbers ranging from $n = 30$ to $n = 33$-34 have been ascertained in slugs of 5 genera and 3 families so far investigated (Table 1), we propose that one of these, or a value very close to these, may be considered as the basic chromosome number of slugs.

This leads to speculate that the genera *Limax*, *Limacus*, *Milax*, *Tandonia* and *Deroceras*, having $n = 33$-34, each is conservative for the chromosome number, or, at the most presents miniaml numerical changes during evolution. Conversely, *Lehmannia* is a derivate condition.

This notion is reliably supported by two considerations. The first is that, in *L. melitensis*, nearly all the largest chromosomes are bi-armed (metacentric and submetacentric) (Table 2), thus suggesting that the reduction in the chromosome number may be explained in terms of Robertsonian translocations. Secondly, although exceptions are certanly known (PATTERSON 1969; CURINI-GALLETTI 1988) specialization within either Mollusca (VITTURI and CATALANO 1989 and authors quoted by them) or other animal groups (MAYR 1970; COLOMBERA and LAZZARETTO-COLOMBERA 1978) is often accompained by a decrease in the chromosome number.

Application of banding techniques on chromosomes preparations of *L. melitensis* has allowed to describe the nucleolus activity during spermatogenesis as to detect the heterochromatin pattern of this species. As concerns the first

point, results of this study are in agreement with those previously reported for the land snail *Helicella virgata* (DA COSTA, 1778), family Helicidae (VITTURI *et al.* 1991) and the slug *Milax nigricans* (Philippi, 1836), family Milacidae (VITTURI 1992) as well as with results obtained for other marine gastropods such as *Buccinulum corneum* (VITTURI and CATALANO 1990) and *Pterotrachea hippocampus* (VITTURI *et al.* 1993). In particular, *L. melitensis* displays qualitative and quantitative intraindividual variations in the NOR pattern (see pachytenic and diakinetic spreads along with interphase nuclei), and, as has been reported for the slug *Milax nigricans* (VITTURI 1992) for this species as well, it seems that nucleolus activity at spermatogonial metaphase may be absent.

After C-banding satisfactory results have been obtained from analysis of pachytene chromosomes, because of these bivalents were more relaxed than the chromosomes at spermatogonial metaphase stage. Since, numerous bivalents dispalyed terminal and/or intestitial heterochromatic blocks, it is possible to conclude that a consistent heterochromatin amount occurs in the species investigated here. Moreover, comparing average karyotype (Fig. 10A) and average idiogram (Fig. 10B) it can be deduced that C-bands presumably correspond to the centromeric regions in numerous chromosomes (see chromosomes nos. 1, 2, 3, 4, 5, 8, 9, 11, 14, 15, 17 and 20).

However, due to the lack of comparative data in slugs, we cannot state what a role heterochromatin has played during evolution of this animal group. We can only affirm that a comparatively lower heterochromatin amount has been found in the slug *Milax nigricans* (VITTURI, 1992), while remarkable heterochromatin contents seem to occur in the mesogastropod *Pterotrachea hippocampus* (VITTURI *et al.* 1993) and in the ascidian *Clavelina lepadiformis* (VITTURI *et al.* 1991), both displaying specialized morphological characters.

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REFERENCES

BEESON G.E. , 1960. — *Chromosome numbers of slugs*. Nature, 186: 257-258.

COLOMBERA D. and LAZZARETTO-COLOMBERA I., 1978. — *Chromosome evolution in some marine invertebrates*. In «Marine organism» ed. by B. Battaglia and J. Beardmore, pp. 487-525, Plenum Press, New York.

COLOSI G., 1920. — *Limacidi e Arionidi conservati nel R. Museo Zoologico di Firenze*. Mon. Zool. Ital., 30: 57-63.

CURINI-GALLETTI M.C., 1988. — *Analyse du kayotype de Runcina coronata (Gastropoda, Cephalaspidea) (Gastropoda, Cephalaspidea)*. Cah. Biol. Mar., 29: 313-318.

GIUSTI F., 1973. — *Notulae Malacologicae, XVIII. I Molluschi terrestri e salmastri delle Isole Eolie*. Lavori Soc. Ital. Biogeografia, (N.S.), 3: 113-306.

GIUSTI F., CASTAGNOLO L. and MANGANELLI G., 1985. — *La fauna malacologica delle faggete italiane: brevi cenni di ecologia delle faggete italiane: brevi cenni di ecologia, elenco delle specie e chiavi per il riconoscimento dei generi e delle entità più comuni*. Boll. Malacol., 21: 69-144.

HOWELL W.M. and BLACK D.A., 1980. — *Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method.* Experientia, 36: 1014-1015.

HUBBELL H.R., 1985. — *Silver staining as an indicator of active ribosomal genes.* Stain Techn., 60: 285-294.

KERNEY M.P. and CAMERON R.A.D., 1979. — *A field guide to the land snails of Britain and North-West Europe.* pp. 288, Collins eds, London.

LESSONA M. and POLLONERA C., 1882. — *Monografia dei Limacidi italiani.* Mem. Acad. Sci. Torino, s. II, 35: 49-128.

LEVAN A., FREDGA K. and SANDBERG A.A., 1964. — *Nomenclature for centromeric position of chromosomes.* Hereditas, 52: 201-220.

MAYR E., 1970. — *L'evoluzione delle specie animali.* Vol. II, p. 485, Giulio Einaudi, Torino.

MILLER D.A., DEV U.G., TANTRAVAHI R. and MILLER O.J., 1976. — *Suppression of human nucleolus organizer activity in mouse-human somatic hybrid cells.* Exptl. Cell Res., 101: 235-243.

PATTERSON C.M., 1969. — *Chromosomes of Molluscs.* In: «Proceedings of 2nd Symposium on Mollusca». Ernakulam, Cochin, India. Vol. 2, pp. 635-689. Marine Biological Association of India, Ramanathapuram District, Madras State.

PILSBRY H.A., 1940. — *Land Mollusca of North America (north of Mexico)* Ac. Sci. Philadelphia, Monogr. No. 3, 1 (2): 575-994.

POLLONERA C., 1891. — *Appunti di Malacologia. VII. intorno ai Limacidi di Malta.* Boll. Mus. Zool. Anat. Comp. R. Università Torino, 6 (99), pp. 4.

SUMNER A.T., 1972. — *A simple technique for demonstrating centromeric heterochromatin.* Exptl. Cell Res., 75: 304-306.

VITTURI R., 1992. — *Conventionally stained chromosomes constitutive heterochromatin and nucleolus organizer regions in Milax nigricans (gastropoda, Pulmonata).* Chromatin, 1: 147-155.

VITTURI R. and CATALANO E., 1989. — *Spermatocyte chromosomes and nucleolus organizer regions (NORs) in Tricolia speciosa (Mühlfeld, 1824) (Prosobranchia, Archaeogastropoda).* Malacologia, 31: 211-216.

VITTURI R., CATALANO E., COLOMBERA D., AVILA A.L. and FUCÀ A.. 1993. — *Multiple sex-chromosome system and other karyological characterizations of Pterotrachea hippocampus (Mollusca, Mesogastropoda).* Marine Biol., 115: 581-585

VITTURI R., COLOMBERA D., CATALANO E. and AMICO F.P., 1991. — *Spermatocyte chromosome analysis of Helicella virgata (Pulmonata: Helicidae): Silver-stained and C-banded Chromosomes.* J. Hered., 82: 339-343.

VITTURI R., COLOMBERA D., CATALANO E. and TURON X., 1991. — *Ag-NOR and C-banding analysis of spermatocyte chromosomes of Clavelina lepadiformis (Ascidiacea, Aplousobranchiata).* Caryologia, 44: 343-347.

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