A Molecular Cytogenetic Study on Some Icelandic Amphipods (Crustacea) by Fluorescence *In Situ* Hybridization (FISH)

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Abstract: A cytogenetical investigation was carried out on 8 species of amphipods from Iceland by using conventional staining, C-banding, fluorescence *in situ* hybridization (FISH) with rDNA and telomeric repeat probes, and flow cytometry genome size evaluation. Previously reported data on chromosome number were confirmed and the karyotype formula was determined for the first time in 3 species. The tendency of amphipods to be endowed by high symmetric karyotypes was once again pointed out. Heterochromatin distribution on chromosomes was revealed by C-banding and DAPI staining after FISH treatment. Heterochromatic bands are mainly centromeric and their total extension is directly proportional to genome sizes. The genome size (GS) in Icelandic species was relatively higher in comparison with the GS of related species from temperate climate, as a probable pre-adaptation to cold climate. Application of FISH with a rDNA probe evidenced a great variability among species concerning the number of autosome pairs carrying the nucleolar organizer regions (from 1 to 5). A probe containing the 'arthropod' type telomeric motif TTAGG was the only one to hybridize with the chromosomal termini in all the investigated amphipods. The molecular cytogenetical methods demonstrated to be a powerful tool to find differences in genome organization among related species of amphipods characterized by conservative karyotypes.

Keywords: Amphipoda, *in situ* hybridization, rDNA sequences, TTAGG telomeric motif, C-banding, Genome Size.

INTRODUCTION

With about eight thousands of ascribed species [1], Amphipoda is one of the most important orders among Crustacea. Amphipods inhabit all parts of the sea down to the depths of 9,100 meters, lakes, rivers, sand beaches, caves, and moist habitats on tropical islands, playing a fundamental role in the food chain of many environments and representing one of the most diversified groups in animal communities [2]. Despite the importance of Amphipods, cytogenetical knowledge on this taxon is still rudimentary, since only one hundred and twenty species have been studied and most of the families are still unexplored [3-7]. While haploid and/or diploid chromosome number are known for all the studied species, only a few papers reported the karyotype formulae [5 and reference therein, 6, 7]. Karyotypes of amphipods are generally characterized by high symmetry being composed almost exclusively by bi-armed chromosomes [5, 7]. For this fact, along with the presence of modal chromosome numbers, a low rate of karyotypical evolution was supposed in some amphipod taxa [4, 6, 8]. Fluorescence *in situ* hybridization (FISH) allows the localization of specific DNA sequences on chromosomes, which may be used as markers to identify peculiar patterns of karyotypical evolution, even in those taxa characterized by highly conservative karyotypes (for example see Gornung *et al.* [9] for animals and Fregonesi *et al.* [10] for plants). Concerning amphipods, only three papers have already reported the results of the FISH application [11-13]. In particular, Sahara *et al.* [11] demonstrated the presence of the pentameric repeat TTAGG in the telomeres of *Gammarus pulex* (Linnaeus, 1758), and Libertini *et al.* [12] and Krapp *et al.* [13] described the location of major and minor rDNA gene clusters in three species of Ischyroceridae.

The present study had the aim to localize the 45S rDNA and the telomeric repeats on chromosomes of 8 species belonging to 5 families from Iceland using FISH. Moreover, other karyological parameters (i.e. chromosome numbers, karyotype formulae, C-banding patterns and genome sizes) are going to be determined or confirmed.

For the herein studied species, the chromosome number, karyotype morphology and genome size of *Apohyale prevostii* (H. Milne Edwards, 1830) have already been reported in the literature [3, 7]. For *Calliopius laeviusculus* (Krøyer, 1838), *Echinogammarus finmarchicus* (E. Dahl, 1938), *E. obtusatus* (E. Dahl, 1938), and *Gammarus oceanicus* Segerstråle, 1947 only the chromosome numbers and some notes on the karyotype were already known [14, 15].

MATERIALS AND METHODOLOGY

Amphipod specimens were collected along the shores near the Sandgerði Marine Centre of the University of Iceland (SW Iceland: 64°02'20"N, 11°42'50"W), during low tide. Chromosome preparations were made using the hot-dry method applied to early embryos, or alternatively male

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gonads, as outlined by Libertini *et al*. [12]. The body of the female carrying the embryos or the remains of the dissected males were preserved in 70% ethanol for identification. Eight different species were identified: *Dexamine thea* Boeck, 1861 (family Dexaminidae); *Calliopius laeviusculus* (Krøyer, 1838) (Calliopiidae); *Apohyale prevostii* (H. Milne Edwards, 1830) (Hyalidae); *Caprella septentrionalis* Krøyer, 1838 (Caprellidae); and the Gammaridae *Echinogammarus finmarchicus* (E. Dahl, 1938), *Echinogammarus obtusatus* (E. Dahl, 1938), *Gammarus oceanicus* Segerstråle, 1947, and a *Gammarus* species morphologically-close to *Gammarus crinicornis* Stock, 1966, here reported as *Gammarus* sp. Species and genera names of Gammaridae follow Pinkster [16]. Voucher samples were deposited in the crustacean collection of the Civic Museum of Natural History in Verona (Italy), under the registration numbers : 30/417 (*A. p.*), 46/417 (*C. l.*), 51/417 (*C. s.*), 79/417 (*D. t.*) , 83/417 (*E. f.*), 102/417 (*E. o.*), 146/417 (*G.* sp), 164/417 (*G. o.*).

For conventional karyotyping, slides were stained for 20 minutes in 5% Giemsa solution in phosphate buffer (pH 6.8). At least 25 chromosome plates for each species were counted to determine the haploid and/or the diploid chromosome numbers. Karyotypes were arranged according to chromosome size and shape using digitized pictures of metaphase figures edited with Corel PhotoPaint. Chromosome classification was performed according to Levan *et al*. [17]; the centromeric index was evaluated following Naranjo *et al*. [18]. At least 5 karyotypes were examined for each species.

C-banding of chromosomes was revealed by treating the slides with barium hydroxide and staining with Giemsa, following the method used by Sumner [19], but reducing the treatment with alkaline solution to 30-60 seconds [12].

Mapping of the major (18S-5.8S-28S) rDNA genes and the telomeric repeats TTAGG and TTAGGG was performed by means of fluorescence in situ hybridization (FISH). A probe containing 18S-5.8S-28S genes plus the intergenic spacers of the fruit fly *D. melanogaster* (pDm238 [20]) was used for major rDNA FISH. The two probes for telomeric sequences were obtained by PCR in the absence of template [21] using $(TTAGG)_6$/$(CCTAA)_6$ or $(TTAGGG)_5$/$(CCCTAA)_5$ as primer pairs. Probes were labeled by nick translation with digoxigenin-11-dUTP or biotin-14-dATP (Roche Molecular Biochemicals or Invitrogen). Experimental conditions for performing FISH followed Krapp *et al*. [13]. Observations were made with a JenaMed 2-fluorescence microscope (Carl Zeiss Jena, Germany) equipped with the 410/450 and the 510/570 filter sets. Fluorescence images were taken with a Canon EOS 10D digital camera, processed and merged with Adobe Photoshop Elements 2.0.

Ag-NOR banding [22] was unsuccessfully attempted either on fresh preparations or on destained slides after FISH.

Genome size (GS) was evaluated using flow cytometry on amphipod cell suspensions of late embryos. Cell suspensions were prepared following Libertini *et al*. [7, 12]. A xenon-mercury lamp cytometer (BRYTE-HS, Bio-Rad Laboratories Inc., Hercules, California, USA) was used. Peripheral blood erythrocytes from chicken (2C GS=2.50 pg [23]) were added to the amphipod cells suspensions as an internal standard. The nuclei were stained with propidium iodide. For each sample at least 3,000 cells were examined and the DNA index (mean channel number of the G1/G0 peak of the amphipod cells over the mean channel number of the G1/G0 peak of the chicken cells) was evaluated after elaboration of the fluorescence data by means of the Modfit software (Verity Software House Inc., Topsham, Maine, USA). The average DNA indices of the samples, multiplied by half of the DNA content of the standard, gave the haploid value (C-value) assigned to each species (data are reported as mean ± standard deviation).

RESULTS

Family Dexaminidae: *Dexamine thea*

All the metaphase plates analyzed (N=55) from early embryos showed a diploid complement of 2n = 24 chromosomes. The karyotype (Fig. **1a**) is composed of 11

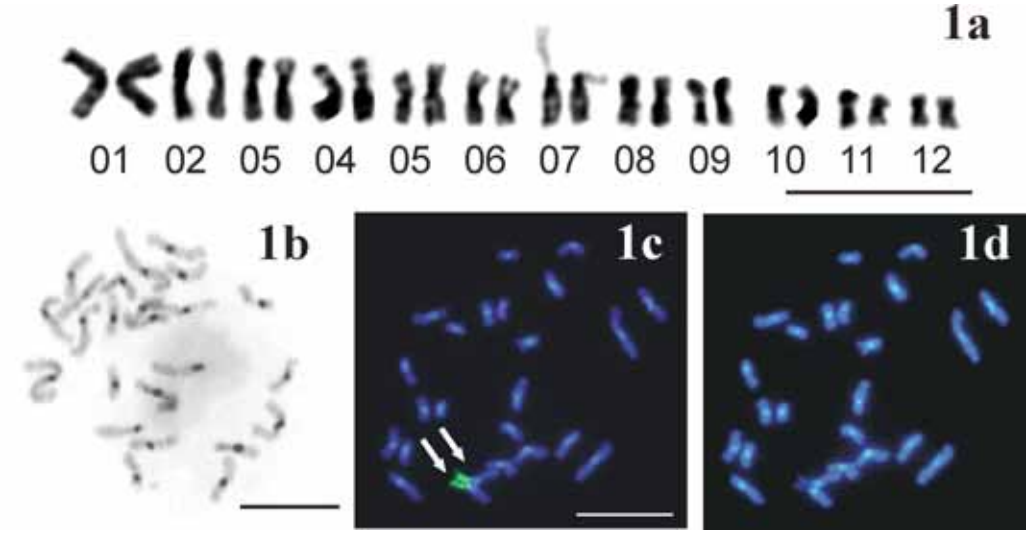

Fig. (1). *Dexamine thea*: **a.** Karyotype. **b.** Embryo metaphase chromosomes after C-banding. **c.** Embryo metaphase chromosomes after 45S rDNA FISH. **d.** The same previous metaphase stained with DAPI without the overlapping of positive FISH signals. Arrows indicate the hybridization signals. Scale bar = 10 μm.

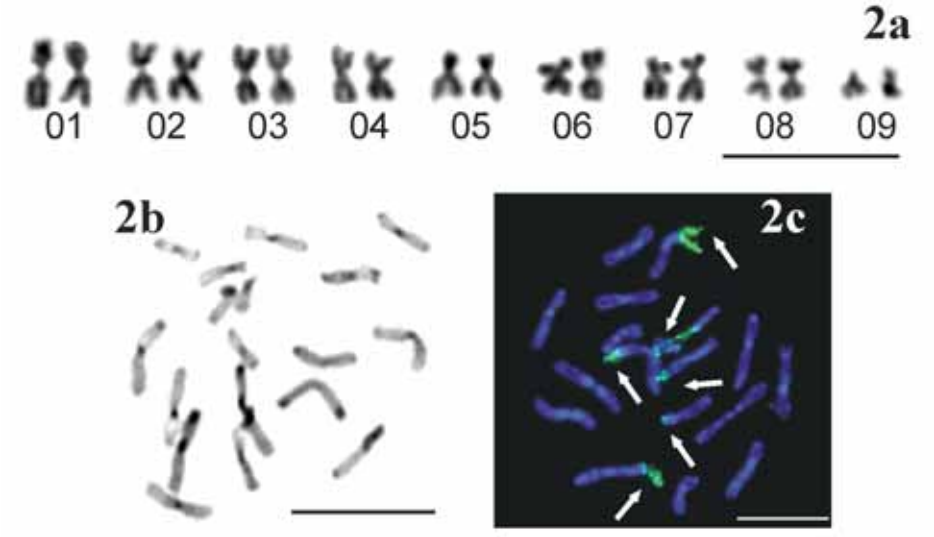

Fig. (2). *Calliopius laeviculus*: **a.** Karyotype **b.** Embryo metaphase chromosomes after C-banding **c.** Embryo metaphase chromosomes after 45S rDNA FISH. Arrows indicate the hybridization signals. Scale bar = 10 μm.

metacentric/submetacentric pairs and 1 subtelocentric pair, therefore the fundamental number of chromosome arms (FN) is 46. The short arm of the subtelocentric pair (Fig. **1a**, pair nr. 7) shows a secondary constriction with long and heteropycnotic chromatin treads. C-banding revealed small bands of heterochromatin in the centromeric regions of all chromosomes (Fig. **1b**).

In 35 analyzed plates, FISH with the 45S rDNA probe produced strong terminal signals in correspondence of the secondary constriction of the subtelocentric pair (compare Figs. **1c**, **d** and pair 7 in Fig. **1a**). After FISH treatment, DAPI stained intensively the pericentromeric regions of all chromosomes, coinciding with C-positive bands (Figs. **1c**, **d**).

Family Calliopiidae: *Calliopius laeviculus*

From the analysis of more than 250 early embryo dividing nuclei, the diploid chromosome number 2n = 18 was confirmed for this species [15].

The karyotype (Fig. **2a**) is composed of 8 medium-large sized metacentric/submetacentric pairs and 1 pair of small chromosomes that might be both submetacentric, both subtelocentric or one of each type (Fig. **2a**, pair 9). Therefore, depending on the morphology of the smallest pair, FN may range from 34 to 36.

C-banding revealed small bands of heterochromatin in most of the centromeres and in some telomeres (Fig. **2b**). A further proof of the location of heterocromatin either in the centromeric and the telomeric regions is given by the differential staining by DAPI after FISH treatments (Fig. **2c**). FISH with the 45S rDNA probe produced terminal signals in 4 (10 plates), 5 (11 plates) or 6 (33 plates) chromosomes (Fig. **2c**). The variable size of the hybridized regions did not allow to clearly ascertain the number of the NOR bearing homologous pairs (from 2 to 3 or more).

Haploid GS was evaluated through flow cytometric assay on late embryo cells and estimated as 2.84 ± 0.02 pg.

Family Hyalidae: *Apohyale prevostii*

Only the results of FISH experiments are described herein, since other karyological parameters have been recently reported elsewhere [3, 7].

Hybridization with the 45S rDNA probe produced intercalary signals on the long arm of a medium sized chromosome pair (Fig. **3a**).

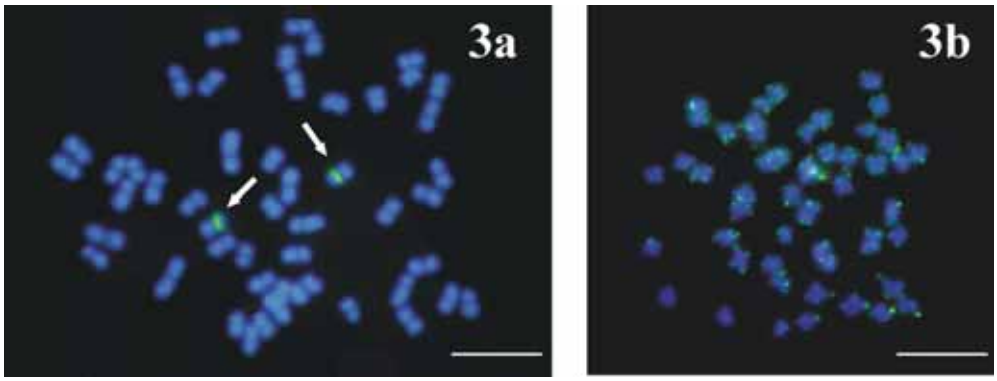

Fig. (3). *Apohyale prevostii*: **a.** Embryo metaphase chromosomes after 45S rDNA FISH. **b.** Embryo metaphase chromosomes after FISH with a $(TTAGG)_n$ probe. Arrows indicate the hybridization signals.Scale bar = 10 μm.

The telomeres of all chromosomes were hybridized by the probe containing the pentameric repeat $(TTAGG)_n$ (Fig. **3b**). The probe with the hexameric repeat $(TTAGGG)_n$ did not give any signals of hybridization.

Family Caprellidae: *Caprella septentrionalis*

From the analysis of 170 early embryo metaphase plates, a diploid complement of 2n = 24 chromosomes was determined. The karyotype (Fig. **4a**) is composed of 12 metacentric/submetacentric pairs, with a FN of 48.

C-banding revealed small centromeric bands of heterochromatin in all chromosomes (Fig. **4b**).

FISH with the 45S rDNA probe showed signals on the terminal regions of the short arms of 4 chromosomes belonging to a large size and a medium size metacentric pairs (73 plates observed, Fig. **4c**).

The probe with the pentameric repeat $(TTAGG)_n$ hybridized in the telomeric regions of all chromosomes, giving intense signals (Fig. **4d**).

By flow cytometric assay on late embryo cells, the haploid GS was estimated to be 1.52 ± 0.02 pg.

Family Gammaridae

Echinogammarus obtusatus

All the metaphase plates analyzed (N=25) from early embryos showed a diploid complement of 2n = 52 chromosomes, thus confirming the haploid chromosome number n = 26 previously found for this species [14]. The karyotype (Fig. **5a**) is composed of 24 metacentric/ submetacentric pairs and 2 subtelocentric pairs (pairs 5 and 6 in Fig. **5a**), therefore FN is 100. The 45S rDNA probe hybridized with the terminal regions of the long arms of two medium-large sized chromosomes (15 embryo plates observed, Fig. **5b**).

Echinogammarus finmarchicus

Chromosome counts in 47 spermatocytal plates (Figs. **5c**,**d**) and 2 spermatogonial mitotic metaphases (Fig. **5e**) gave a haploid and diploid value of n = 26 and 2n = 52, respectively, thus confirming the haploid chromosome number n = 26 previously found for this species [14].

C-banding revealed thick double bands of heterochromatin in all spermatocytal first metaphase bivalents (Fig. **5c**), these heterochromatin regions are probably located close to the centromeres, because they lay on opposite positions in each bivalent. A further proof of centromeric location of heterochromatin regions is shown by the differential staining by DAPI after FISH treatments (Figs. **5d,e**)

In 45S rDNA FISH experiments, two bivalents showed two opposite hybridization signals in spermatocyte plates (22 observations, Fig. **5d**), while 4 elements had a terminal signal on the short arm in spermatogonial metaphases (2 observations, Fig. **5e**).

Flow cytometric estimation of GS on late embryo cells gave a C-value of 7.00 ± 0.05 pg, the highest recorded among Gammaridae [24].

Gammarus oceanicus

Chromosome counts in 70 spermatocytal plates (Figs. **5f**,**g**) gave a haploid value of n = 27, the same number already reported for this species [15].

In spermatocytal metaphase I plates treated for 45S rDNA FISH, 8 signals were observed (26 plates): of which 6 were paired on the ends of 3 bivalents and 2 were heterozygously located in one end of other 2 bivalents (Fig. **5f**). Therefore, in *G. oceanicus* up to 5 chromosome pairs may carry major rDNA genes. In fact, 45S rDNA FISH in metaphase II plates showed 3, 4 or 5 chromosomes hybridized by the probe in the terminal regions of the short chromosomal arms (10 observations, Fig. **5g**).

The probe with the pentameric repeat $(TTAGG)_n$ hybridized in the telomeric regions of all the bivalent chromosomes, giving very intense signals (Fig. **5h**).

Gammarus sp.

Chromosome counts on 81 spermatocytal plates gave more frequently the haploid value n = 26 (Fig. **5i**), in some

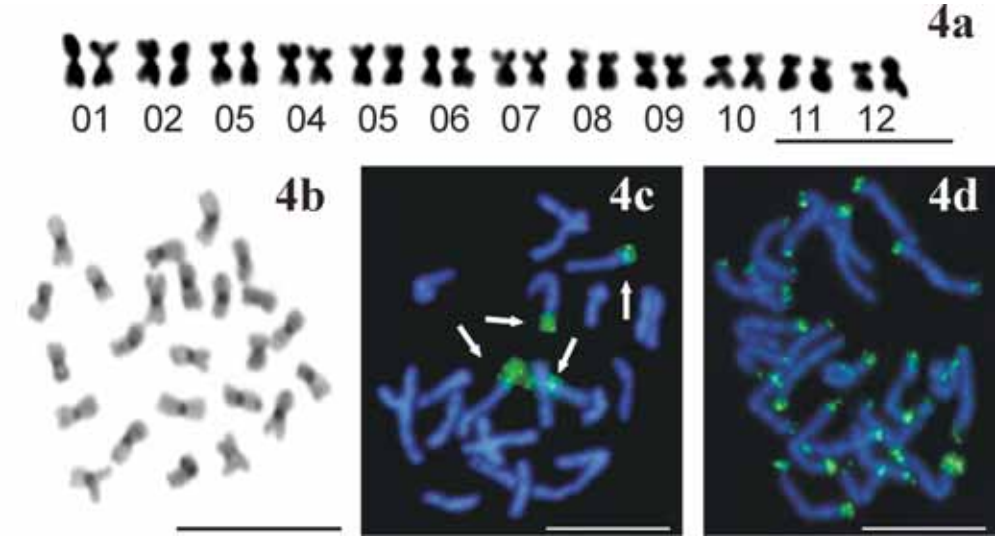

Fig. (4). *Caprella septentrionalis*: **a.** Karyotype. **b.** Embryo metaphase chromosomes after C-banding. **c.** Embryo metaphase chromosomes after 45S rDNA FISH. **d.** Embryo metaphase chromosomes after FISH with a $(TTAGG)_n$ probe. Arrows indicate the hybridization signals. Scale bar = 10 μm.

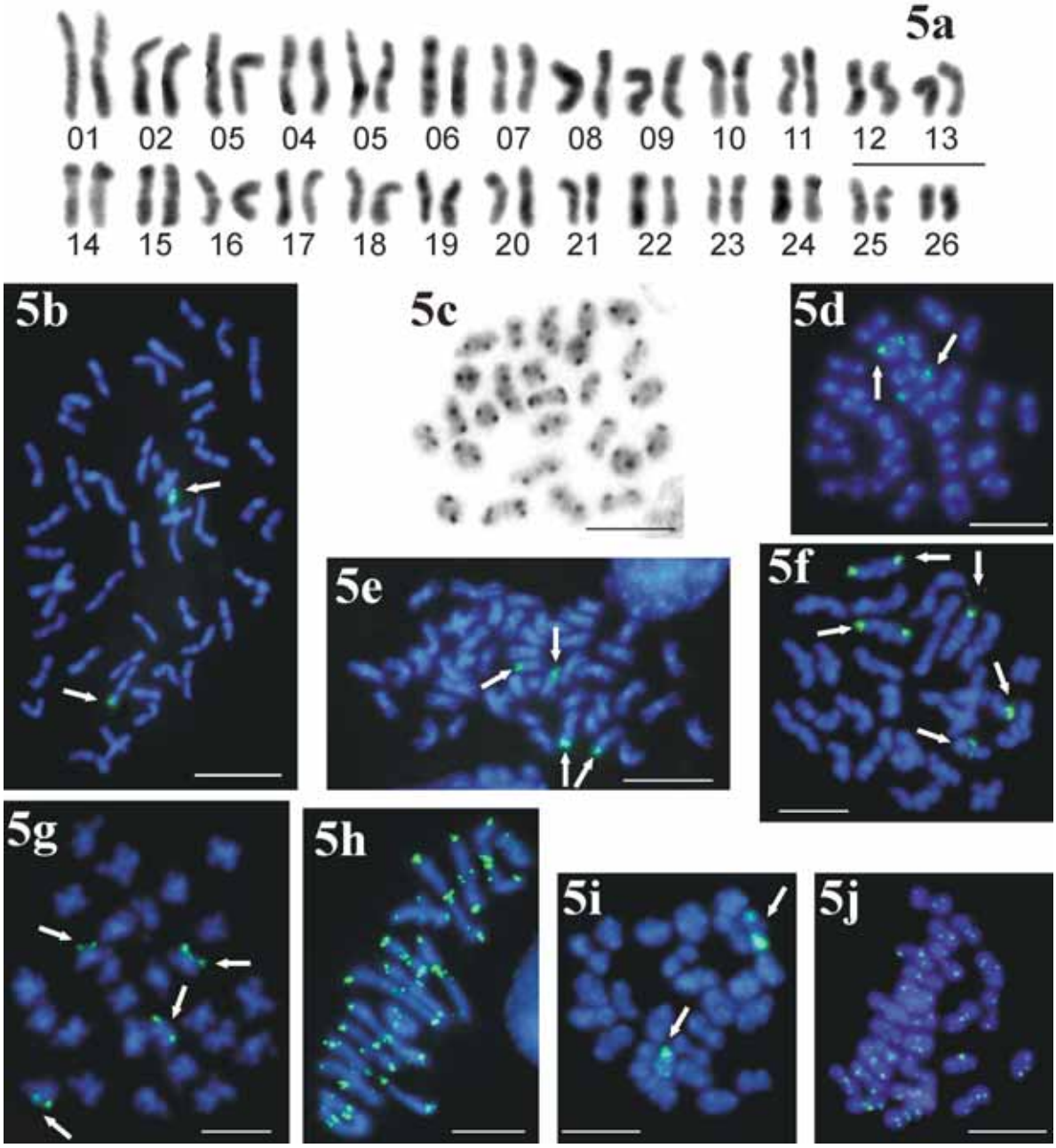

Fig. (5). Family Gammaridae: **a.** Karyotype of *Echinogammarus obtusatus*. **b.** *E. obtusatus* embryo metaphase chromosomes after 45S rDNA FISH. **c.** *Echinogammarus finmarchicus* spermatocyte bivalent chromosomes after C-banding. **d.** *E. finmarchicus* spermatocyte bivalents after 45S rDNA FISH. **e.** *E. finmarchicus* spermatogonial metaphase chromosomes after 45S rDNA FISH.: **f.** *Gammarus oceanicus* spermatocyte bivalents after 45S rDNA FISH. **g.** *G. oceanicus* spermatocyte metaphase II chromosomes after 45S rDNA FISH. **h.** *G. oceanicus* spermatocyte bivalents after FISH with a $(TTAGG)_n$ probe. **i.** *Gammarus* sp. spermatocyte bivalents after 45S rDNA FISH. **j.** *Gammarus* sp. spermatocyte bivalents after FISH with a $(TTAGG)_n$ probe. Arrows indicate the hybridization signals. Scale bar = 10 μm.

plates a supernumerary monovalent chromosome was also observed (B-chromosomes, figure not shown).

FISH with the 45S rDNA probe applied to first spematocytal metaphases (Fig. **5i**) showed a total of 4 signals, paired two-by-two in two bivalents (42 observations). Such a result attests the presence of two NOR bearing chromosome pairs in this species.

FISH with the probe containing the pentameric repeat $(TTAGG)_n$ on first spematocytal metaphases (Fig. **5j**) gave small hybridization signals in all the bivalents.

DISCUSSION

The chromosome numbers determined herein for the Icelandic populations of *C. laevisculus*, *E. finmarchicus*, *E. obtusatus*, and *G. oceanicus* are in accordance with those already reported for other conspecific populations from other sites of the North Atlantic [14, 15]. Data on chromosome numbers of *D. thea* and *C. septentrionalis* are new in the literature. The haploid chromosome number n=26 found in *G.* sp. is the most common value found in Gammaridae [3, 4]. Moreover the presence of B chromosomes detected in

this species is not a rare phenomenon within Amphipoda, especially among Gammaridae [25].

The karyotype formulae of *D. thea*, *C. septentrionalis* and *E. obtusatus* were determined for the first time. These karyotypes, along with that one of *C. laevisculus* [15, present paper], are characterized by a high symmetry due to the high frequency of bi-armed chromosome pairs and being mono-armed chromosomes limited to 0-2 pairs. *C. septentrionalis*, as well as all the *Caprella* species up-to-date analyzed [5, 6], are characterized by the same karyotype formula: 12 bi-armed chromosome pairs (FN = 48). For amphipods, mono-armed pairs may be a useful tool in karyotype comparison among phylogenetically close species that share similar chromosome numbers, as already reported for the genera *Gammarus* (Gammaridae) [5], *Apohyale* (Hyalidae) [7] and *Jassa* (Ischyroceridae) [13]. Table **1** summarizes the cytogenetical data available on the amphipod species analyzed up to now by FISH.

C-banding by alkaline treatment is the typical method to analyze constitutive heterochromatin on chromosomes [19] and was successfully applied to *C. laevisculus*, *D. thea*, *C. septentrionalis* and *E. finmarchicus*. Nevertheless, also DAPI staining after the application of the procedures for FISH may reveal heterochromatin, as already shown for *Gammarus pulex* by Sahara *et al.* [11] and furthermore herein in *C. laevisculus*, *D. thea* and *E. finmarchicus*. Probably, the steps of DNA denaturation and renaturation, characterizing the FISH procedure, allow a differential staining by DAPI in the more coiled fraction of genome (i.e. constitutive heterochromatin) that produces a C-banding like pattern. In the herein analyzed species heterochromatin was mainly distributed in the centromeric regions of all chromosomes as in most of the amphipods [7, 12], only in *C. laevisculus* heterochromatin was also found in telomeric positions.

Genome size was assessed for three species: *C. septentrionalis* (1.52 pg), *C. laevisculus* (2.84 pg) and *E. finmarchicus* (7.00 pg). In these species, the genome size and the heterochromatin distribution pattern seem to be directly related, since the species with a high GS also have large and widely distributed C-bands (see Table **1**). The direct correlation between total DNA content and C-heterochromatic DNA is a general rule for primate species [26] and was also shown for other amphipods (i.e. Talitridae [7]).

A general increase in the DNA amount in polar or sub-polar species in relation to those from temperate zones has been reported for amphipods [7, 27], as well as for other crustaceans (i.e. decapods [28]). The same trend is shown here by *C. septentrionalis* and *E. finmarchicus*, two typical

Table 1. Summary of the Cytogenetical Data Available on the Amphidod Species up to now Analyzed by FISH

	n	2n	Karyotype formula	FN	C-band	GS	NORs	$(TTAGG)_n$	References
Superfamily Dexaminoidea: Family Dexaminidae									
Dexamine thea		24	22m-sm + 2st	44	+		2(1)		pp
Superfamily Eusiroidea: Family Calliopiidae									
Calliopius laevisculus	9	18	16sm-st + 2 (sm or st)	34-36	++	2.84	4-6(2-3 or >3)		[15], pp
Superfamily Photoidea: Family Ischyroceridae									
Ischyrocerus anguipes		10	8m-sm + 2st	18			2(1)	positive	[13], unpub
Jassa marmorata	6	12	10m-sm + 2st	22	+	0.95	2(1)		[3], [12],[13]
Jassa cadetta		10	10m-sm	20			2(1)		[13]
Superfamily Talitroidea: Family Hyalidae									
Apohyale prevostii		50	50m-sm	100		1.89	2(1)	positive	[3], [7],pp
Superfamily Gammaroidea: Family Gammaridae									
Echinogammarus finmarchicus	26	52			+++	7.00	4(2)		[14],pp
Echinogammarus obtusatus	26	52	48m-sm + 4st	100			2(1)		[14],pp
Gammarus oceanicus	26-27						8(5)	positive	[15],pp
Gammarus pulex	26-27	52-54						positive	[14], [11], pp
Gammarus sp.	26 + B						4(2)	positive	pp
Superfamily Caprelloidea: Family Caprellidae									
Caprella septentrionalis		24	24m-sm	48	+	1.52	4(2)	positive	pp

n: haploid chromosome number; **2n**: diploid chromosome number; **FN**: fundamental number of chromosome arms; **C-band**: amount of heterochromatin after C-banding (+ = low; ++ = medium, +++=high); **GS**: Genome Size, C-value in picograms; **NORs**: number of chromosome bearing 18S-5.8S-28S rDNA genes after FISH, in brackets the forecast number of pertaining chromosome pairs; **$(TTAGG)_n$**: telomeric sequence assessed by FISH; **references**: references for the data (pp=present paper, unpub=Libertini and Rampin, unpublished).

species of the Icelandic rocky intertidal fauna [29], which have a genome size that is about the double of those in related species [24] inhabiting more temperate regions.

In the present paper, FISH allowed chromosomal mapping of the major rDNA gene clusters in all the 8 analyzed species, and the telomeric microsatellite $(TTAGG)_n$ in 4 species (Table **1**).

Since silver staining seems to be is ineffective in showing the nucleolar organizer regions of amphipod chromosomes [12, present paper], *in situ* hybridization with a rDNA probe is the only way to map the NORs.

Among amphipods, major rDNA genes are most frequently located in a single chromosome pair, although half of the described species have multiple NOR sites (Table **1**). A single pair of NOR-bearing chromosomes per karyotype is presumed to be the ancestral condition, both in vertebrates [30] and invertebrates including arthropods [31]. However, the presence of multiple sites for rDNA has been noted in various arthropod taxa: Copepoda [32], Decapoda [33] and Isopoda [34] among Crustacea, Diplopoda [35] and Insecta [31, 36-40].

Taking into account that a single NOR-bearing chromosome pair is the ancestral condition also for Amphipoda, all the Ischyroceridae, *D. thea* (Dexaminidae), *E. obtusatus* (Gammaridae)**,** and *A. prevostii* (Hyalidae) retained the ancestral trait, whereas an increase in the NOR number has occurred in the other Gammaridae, in *C. laevisculus* (Calliopiidae), and in *C. septentrionalis* (Caprellidae) (Table **1**).

Unfortunately, the ineffectiveness of Ag-NOR banding has not allowed to determine how many of the multiple rDNA loci are really active to organize the nucleolus.

Gammaridae give a clear example of the progressive increase in the number of NOR sites, with species having from 2 to 8 45S ribosomal cistrons located on 1 to 5 chromosome pairs, despite a relative constancy of the diploid chromosome number. Therefore, in this family the number of NOR sites represents a potential karyotype marker useful in phylogenetic reconstruction.

Transposition of ribosomal cistrons either by non-homologous exchange or by NOR associated transposons could be the most probable rearrangement [41] responsible for the multiplication and dispersion of ribosomal regions.

$(TTAGG)_n$ is considered as the 'arthropod' ancestral motif of telomeres [42] and, up to now, all the amphipod species investigated by FISH on the composition of the telomeric sequence revealed the presence of this pentanucleotide repeat (Table **1**). According to Vitkova *et al*. [42] the 'arthropod' ancestral motif TTAGG evolved from the ancestral sequence TTAGGG (the most common within bilaterian animals) at least 545 MYA. Among Peracaridan Crustaceans, telomeric sequences are known only for a few Isopoda and Amphipoda, while in the latter the 'arthropod' pentameric motif was the only one found, in the former species either the 'arthropod type' TTAGG and the 'bilaterian type' TTAGGG have been described [11, 33]. Further investigations are needed to confirm the constant presence of the $(TTAGG)_n$ motif within amphipods.

CONCLUSION

In addition to confirming previously observed trends of cytogenetic parameters this paper gives an insight on the use of molecular cytogenetical markers in amphipods. The great potential of this type of investigation concerns, in particular, ribosomal gene mapping. In fact, a high variability in NOR site numbers and their location was found among different species, suggesting that karyotypical evolution in Amphipoda involved replication and/or translocation of these gene tracts. Therefore, the number and/or the location of rDNA genes may represent in the future a potential karyotypical marker useful in phylogenetic analyses. Moreover, this demonstrates once again that when the cytogenetical analysis is not limited to conventional parameters (i.e. chromosome number and karyotype formula), markers such as the genome size, C-banding pattern and the location of repeated DNA sequences may point out clear differences among species, even in karyotypically conservative families like Gammaridae [this paper] and Talitridae [7].

These promising results encourage us to undertake further research on the application of FISH and other molecular cytogenetical techniques with the aim of getting a clearer picture of the patterns of karyotypical evolution in Amphipoda.

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