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CHROMOSOME DIVERSITY IN MEDITERRANEAN AND ANTARCTIC EUPHAUSIID SPECIES (EUPHAUSIACEA)

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ABSTRACT

Chromosome number and morphology were studied in gonadal tissue from eight euphausiid species, using an air-drying technique and Giemsa staining. Among Mediterranean species, haploid chromosome numbers were: n = 19 in *Euphausia hemigibba, Euphausia brevis,* and *Nematoscelis megalops,* n = 18 in *Euphausia krohni,* and n = 11 in *Nyctiphanes couchi.* Among Antarctic species, haploid chromosome numbers were: n = 17 in *Euphausia superba,* n = 20 in *Thysanoessa macrura,* and n = 13 in *Thysanoessa vicina.* Chromosome morphology was further studied for three species after chromosome measurements: *Euphausia hemigibba* karyotype showed 17 metacentric and 2 submetacentric chromosome pairs, *Euphausia krohni* 17 metacentric and 1 submetacentric chromosome pairs, and *Nematoscelis megalops* 17 metacentric and 2 submetacentric chromosome pairs. Three species sharing the same chromosome number of n = 19, i.e., *Euphausia hemigibba, Nematoscelis megalops,* and *Meganyctiphanes norvegica* were distinguished by a different slope of the decrease in chromosome size and by the presence/absence of metacentric and submetacentric chromosome pairs differing in number and position. Chromosome morphology of other species was derived from the observation of meiotic metaphases II. A majority of metacentric chromosomes was also displayed. Cytotaxonomic differences in haploid chromosome number allow the discrimination of morphologically neighboring sympatric species (e.g., *Meganyctiphanes norvegica* and *Nyctiphanes couchi*; *Thysanoessa vicina* and *T. macrura*). Euphausiids share common chromosomal features which clearly separate them from other eucarids, i.e., a narrow range of haploid chromosome number, a large chromosome size, and a majority of metacentric chromosomes.

Euphausiids are important components of the pelagic communities. They are distributed in all the world oceans. Species such as *Euphausia superba* Dana in the Antarctic Ocean, or *Meganyctiphanes norvegica* M. Sars in northern Atlantic and adjacent seas, may reach high densities and biomass. Known as krill, they contribute to a major part of the diet of large marine vertebrates, from fishes and birds to whales (Mauchline and Fisher, 1969).

Eighty-six species of Euphausiacea are now identified (Baker *et al.,* 1990). They share common morphological traits which distinguish them easily from other shrimplike crustaceans, such as mysids (Peracarida) and eucarid decapods. However, among euphausiids, neighboring species are sometimes difficult to recognize when they belong to the same community, especially in larval and juvenile stages, which do not exhibit the adult sexual characteristics (petasmata in males, thelycum in females). This is the case of *Meganyctiphanes norvegica* and *Nyctiphanes couchi* Bell in the Ligurian Sea (northwestern Mediterranean), and *Thysanoessa vicina* Hansen and *T. macrura* G. O. Sars in the Southern Ocean.

A better knowledge of the genetic structure of euphausiids may help to clarify their taxonomy and systematic position between Peracarida and Eucarida Decapoda, as well as to recognize the ecological importance of the different species belonging to various regions of the pelagic ecosystem.

Few karyological studies have been carried out on euphausiids. The number of chromosomes is known for *Euphausia pacifica* Hansen (n = 16; Yabu and Kawamura, 1981) and for *E. superba* where different counts have been given: n = 16 (Yabu and Kawamura, 1984) and n = 17 (Phan *et al.,* 1989).

In the course of a comparative investigation on euphausiid chromosomes, a previous karyological study of *Meganyctiphanes norvegica* (see Thiriot-Quiévreux and Cuzin-Roudy, 1995) gave the haploid chromosome number of n = 19, with a karyotype showing only metacentric, large chromosome pairs of gradually decreasing size, and no heteromorphic sexual chromosomes. In the present paper, we report the results of a study on chromosome number and morphology of five Mediterranean and three Antarctic species, using *Meganyctiphanes norvegica* as a basis for comparison.

Table 1. Summary of karyological results for the nine euphausiid species studied.

Euphausiacea	Number of animals	Number of mitoses	2n	Number of meioses diakinesis—metaphase II	n	Number of karyotypes	Chromosome morphology
Mediterranean species							
Meganyctiphanes norvegica (1)	9	23	38	8–38	19	10	19m*
Euphausia hemigibba	12	27	38	28–2	19	14	17m/2sm*
Euphausia krohni	10	8	36	11–12	18	6	17m/1sm*
Nematoscelis megalops	21	49	38	31–6	19	24	17m/2sm*
Euphausia brevis	3	—	—	7–4	19	—	18m/1sm
Nyctiphanes couchi	2	1	22	6–1	11	—	11m
Antarctic species							
Euphausia superba	6	2	34	5–21	17	1	17m
Thysanoessa macrura	8	—	—	35–12	20	—	18m/2sm
Thysanoessa vicina	3	2	26	5–13	13	—	12m/1sm

2n = diploid chromosome number, n = haploid chromosome number.
(1) after Thiriot-Quiévreux and Cuzin-Roudy (1995).
* after chromosome measurements, m = metacentric, sm = submetacentric.

Materials and Methods

Samples of Mediterranean euphausiid species: *Euphausia hemigibba* Hansen, 1910, *Euphausia krohni* (Brandt, 1851), *Nematoscelis megalops* G.O. Sars, 1883, *Euphausia brevis* Hansen, 1905, and *Nyctiphanes couchi* (Bell, 1853) were collected in an area situated from 4–10 nautical miles out of the Bay of Villefranche-sur-Mer (Ligurian sea), using an ORI (Ocean Research Institute) net, towed at night in the upper 100 m of the water column, in order to recover the animals with as little stress as possible.

Live euphausiids were kept in cool (13°C) sea water and treated the next morning. Specimens were injected in the pericardial region with 200 µl of 0.01% colchicine in sea water and kept for 4 h in aerated sea water. The gonads were then dissected out, incubated for 45 min in 0.9% sodium citrate and fixed in a freshly prepared mixture of absolute ethanol and acetic acid (3:1) with three changes of 20 min each. Slide preparations were made from each individual gonad, using an air-drying technique (Salemaa, 1979; Thiriot-Quiévreux and Ayraud, 1982), then stained for 10 min with Giemsa (pH 6.8). Photomicrographs of meiotic stages and mitotic metaphases were taken with a Zeiss III photomicroscope.

Samples of Antarctic euphausiids, *Euphausia superba* Dana, 1850, *Thysanoessa macrura* G.O. Sars, 1883, and *Thysanoessa vicina* Hansen, 1911, were collected in the same way, in the South Indian sector of the Antarctic Ocean, on a N.-S. transect situated at 62°E, west of Kerguelen Islands, from 48°59′S to 58°50′S. Live krill were treated on board with colchicine, the dorsal part of the cephalothorax containing the gonad was cut out, then treated and fixed in toto following the same procedure as above. Specimens were kept refrigerated in the fixative until they could be processed in the laboratory. Dissection of the gonads, slide preparations, and staining were made 2 months later.

The most suitable material was obtained from young adults sampled during the period of early development of the gonad. In euphausiids that are reproducing, the germinal cells are not conspicuous among the sexual products, especially in females, as described for *Meganyctiphanes norvegica* and *Euphausia superba* (Cuzin-Roudy, 1993). All results of the present study were obtained from males.

The number of chromosomes was scored on meiotic stages (diakinesis and metaphase II) and on spermatogonial mitotic metaphases.

The morphology of chromosomes was determined on meiotic metaphase II and on mitotic metaphases. Karyotypes were constructed for 3 Mediterranean species where a sufficient number of mitotic metaphases was available. Morphometric measurements of chromosomes were made according to the method described in Thiriot-Quiévreux and Cuzin-Roudy (1995). Terminology relating to centromere position followed that of Levan *et al.* (1964).

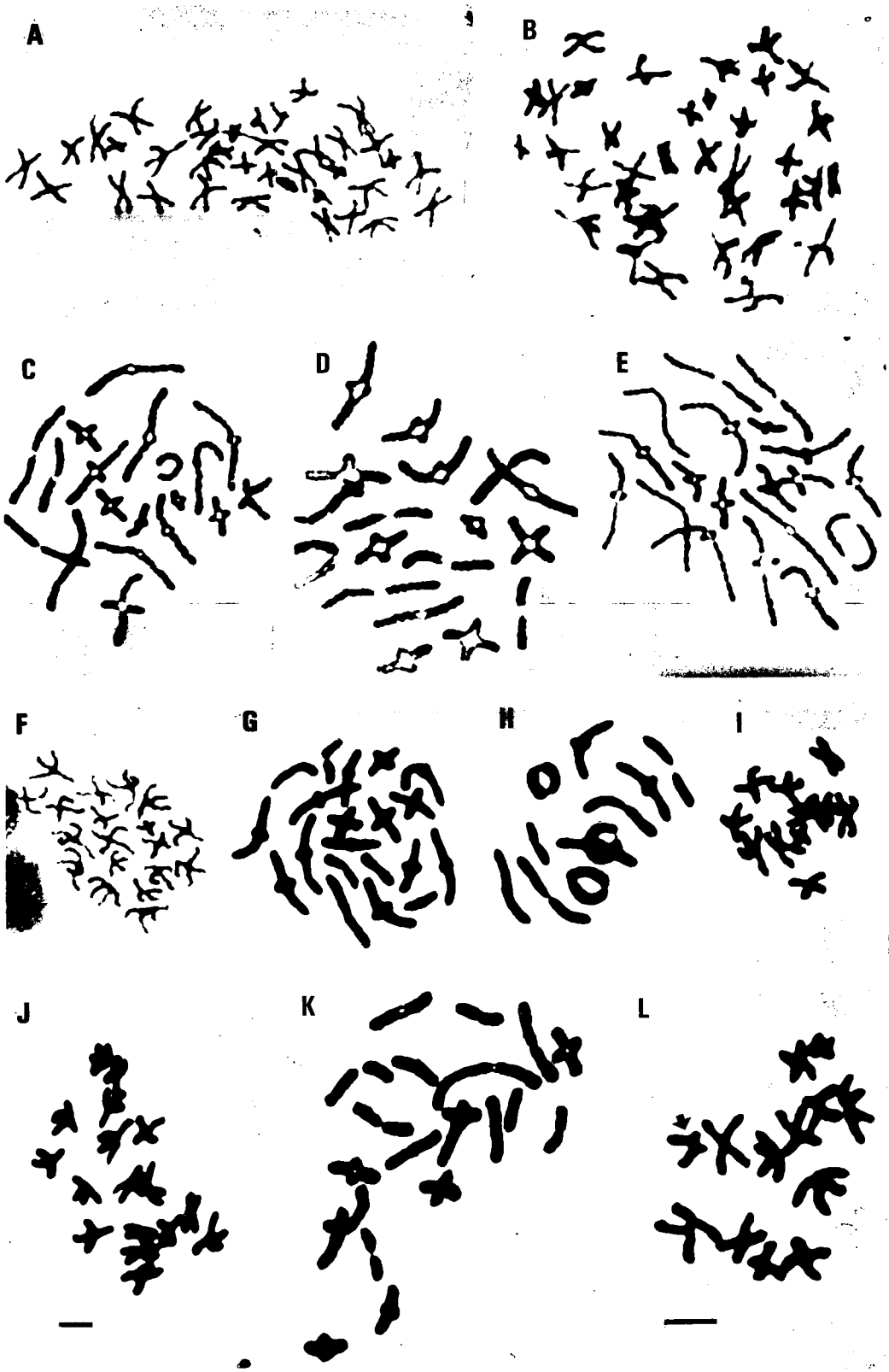
Results

The results obtained for each of the species studied are summarized in Table 1.

Mediterranean Species

Euphausia hemigibba.—Spermatogonial metaphases showed a diploid chromosome number of 2n = 38 (Fig. 1A) and meiotic stages a haploid chromosome number of n = 19 (Fig. 1C). Chromosome measurements of 10 mitotic metaphases were performed. The karyotype (Fig. 2A) consisted of 17 metacentric and two submetacentric chromosome pairs. The chromosome pairs numbers 14–19 displayed a sharp decrease in size, the last pair being four times smaller than the first pair. Heteromorphic sexual chromosomes were not observed.

Euphausia krohni.—Spermatogonial metaphases had 2n = 36 (Fig. 1B) and meiotic stages n = 18 (Fig. 1D). Chromosomes of six mitotic metaphases were measured. The karyotype (Fig. 2B) included 17 metacentric and one submetacentric chromosome pairs. The two last chromosome pairs were significantly smaller than the others. Heteromorphic sexual chromosomes were not observed.



Nematoscelis megalops.—Spermatogonial metaphases showed 2n = 38 and meiotic stages n = 19 (Fig. 1E, F). Chromosome measurements were performed on 10 mitotic metaphases. The karyotype (Fig. 2C) consisted of 17 metacentric and two submetacentric chromosome pairs of gradually decreasing size. Heteromorphic sexual chromosomes were not observed.

Euphausia brevis.—Meiotic stages displayed n = 19 (Fig. 1G). Chromosome morphology was observed in metaphases II. All chromosomes appeared metacentric, except one small submetacentric chromosome.

Nyctiphanes couchi.—One spermatogonial metaphase showed 2n = 22 and meiotic stages n = 11 (Fig. 1H). All chromosomes appeared metacentric (Fig. 1I).

Antarctic Species

Euphausia superba.—Two spermatogonial metaphases had 2n = 34 and meiotic stages n = 17 (Fig. 1J). All chromosomes appeared metacentric.

Thysanoessa macrura.—Meiotic stages showed n = 20 (Fig. 1K). Metaphases II displayed 18 metacentric and two submetacentric chromosomes.

Thysanoessa vicina.—Two spermatogonial metaphases had 2n = 26 and meiotic stages n = 13. In metaphases II, 12 chromosomes appeared metacentric and one clearly submetacentric (Fig. 1L).

Discussion

Based on morphological characteristics, Euphausiacea occupy a transitional systematic position in Malacostraca, between Mysidacea (Peracarida) and Decapoda Dendrobranchiata (Abele and Felgenhauer, 1986). They share with the latter group important developmental characteristics: eggs are released freely in the water and larval development is complex, with a nauplius followed by numerous larval stages.

A study of the phylogenetic relationships among three species of the genus *Euphausia* from the Southern Ocean and *Meganyctiphanes norvegica* has been made using sequencing of the 16S ribosomal mitochondrial gene (Patarnello *et al.*, 1996). The results revealed a large genetic divergence between the Antarctic and sub-Antarctic species.

Considering the haploid chromosome number of the species studied here, the range of n = 11–20 observed was narrower than the range given in the literature, i.e., n = 5–56 in peracarids (Salemaa, 1986; Salemaa and Kamaltynov, 1994; Thiriot-Quiévreux, 1994), n = 32–188 in decapods (Mauchline, 1980; Hedgecock *et al.*, 1982; Nakamura *et al.*, 1988; Ahmed and Nayak, 1991). It is also smaller than the chromosome numbers known for Decapoda Dendrobranchiata, i.e., n = 44–45 given for four species of the genus *Penaeus* (Penaeidae) (Chow *et al.*, 1990), and n = 41 observed in Mediterranean specimens of *Sergestes arcticus* (Sergestidae) (unpublished data).

Among the nine euphausiid species compared here, the range was similar for the Mediterranean and the Antarctic species, with n = 11–19 and n = 13–20, respectively. Nevertheless, neighboring species can be differentiated by their chromosome number, i.e., *Euphausia hemigibba* and *E. krohni* (n = 19 and n = 18, respectively), *Meganyctiphanes norvegica* and *Nyctiphanes couchi* (n = 19 and n = 11, respectively), *Thysanoessa macrura* and *T. vicina* (n = 20 and n = 13, respectively).

In the Antarctic krill *Euphausia superba* the haploid chromosome number of n = 17 was given by Phan *et al.* (1989) for specimens from the Admiralty Bay (King George Island) population, but a different chromosome number of n = 16 was found by Yabu and Kawamura (1984) for specimens from Wilkes Coast (East Antarctica). Such a difference in

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Fig. 1. A, Spermatogonial metaphase of *Euphausia hemigibba* (2n = 38); B, spermatogonial metaphase of *Euphausia krohni* (2n = 36); C, meiotic diakinesis of *Euphausia hemigibba* (n = 19); D, meiotic diakinesis of *Euphausia krohni* (n = 18); E, meiotic diakinesis of *Nematoscelis megalops* (n = 19); F, meiotic metaphase II of *Nematoscelis megalops* (n = 19); G, meiotic diakinesis of *Euphausia brevis* (n = 19); H, meiotic diakinesis of *Nyctiphanes couchi* (n = 13); I, meiotic metaphase II of *Nyctiphanes couchi* (n = 13) (see all metacentric chromosomes); J, meiotic metaphase II of *Euphausia superba* (n = 17) (see all metacentric chromosomes); K, meiotic diakinesis of *Thysanoessa macrura* (n = 20); L, meiotic metaphase II of *Thysanoessa vicina* (n = 13) (see all metacentric chromosomes except one submetacentric, arrow). Scale bars = 10 μm, A–J and K,L.

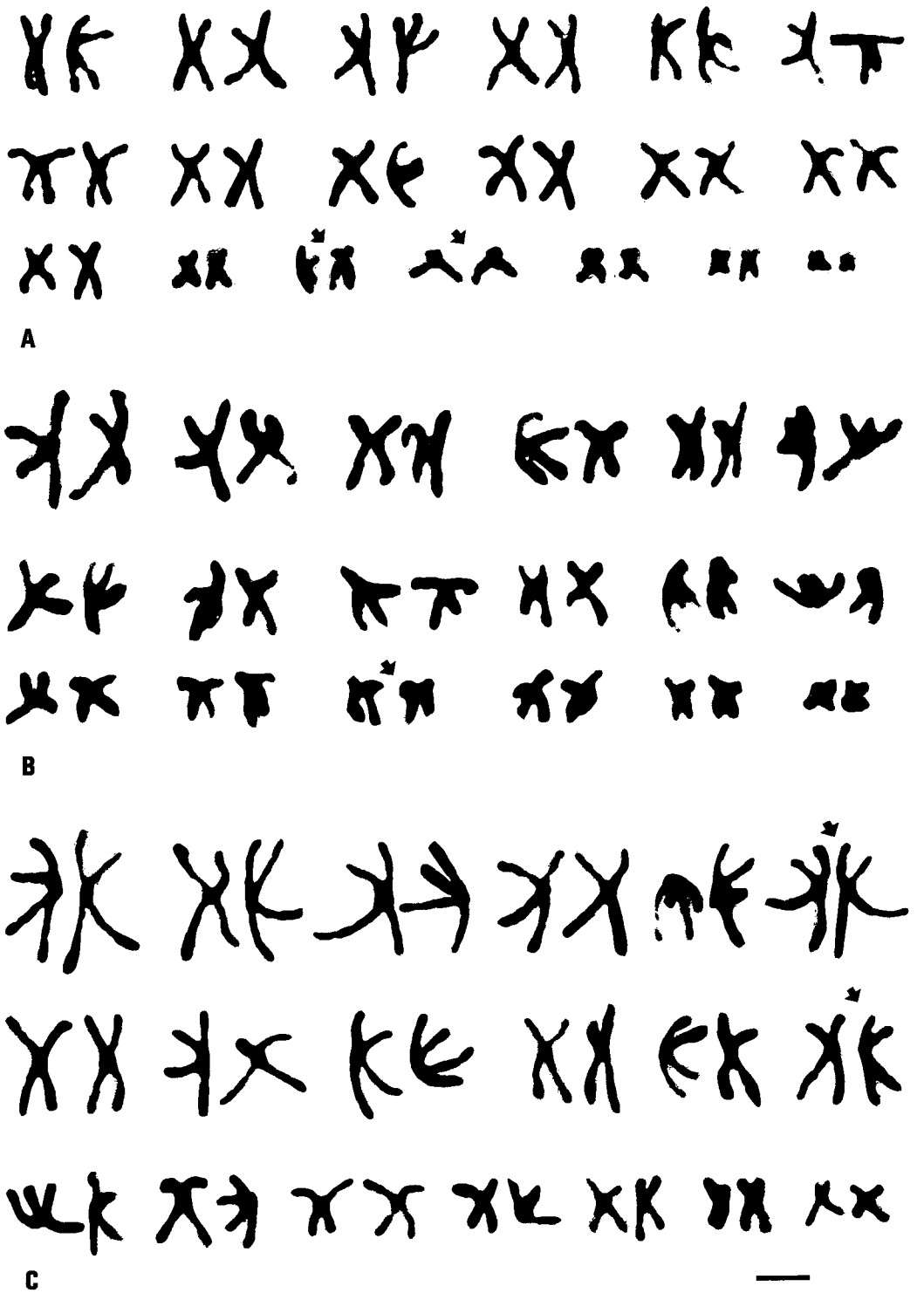

Fig. 2. A, karyotype of *Euphausia hemigibba* (2n = 38); B, karyotype of *Euphausia krohni* (2n = 36); C, karyotype of *Nematoscelis megalops* (2n = 38). Arrows = submetacentric chromosome pairs. Scale bar = 10 μm.

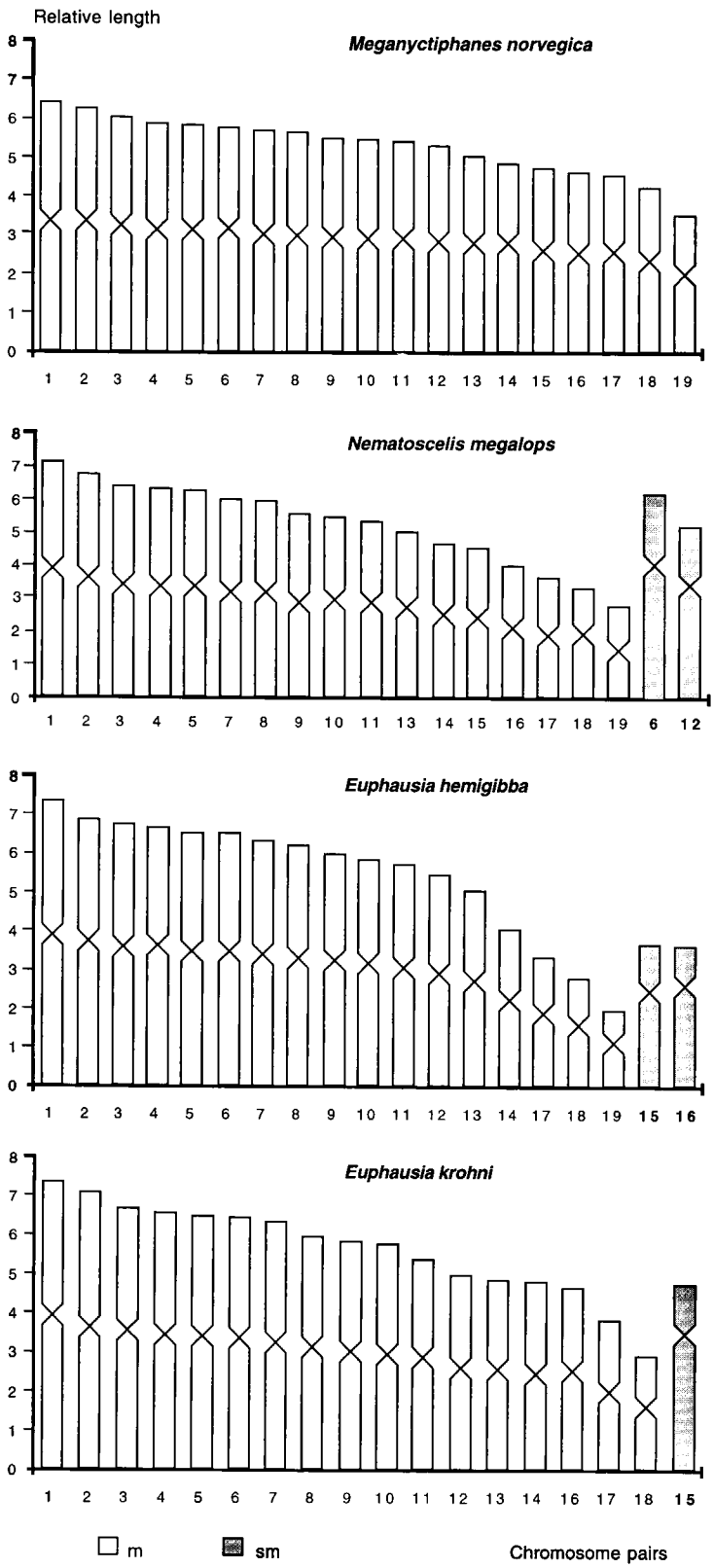

Fig. 3. Comparative ideograms constructed from relative length and centromeric index values in four euphausiid species. Chromosome pairs are numbered according to their size and ordered according to their morphology. m = metacentric, sm = submetacentric.

chromosome counts may correspond to genetically different populations of *E. superba,* as suggested by Phan *et al.* (1989). This assumption is not confirmed by the number obtained here for the population sampled in a region which occupies an intermediate geographical situation.

Chromosome size in euphausiids, at least in the species studied here, appeared uncommonly large when compared to size reported in the literature for other karyotyped malacostracans (e.g., Di Castro *et al.,* 1989; Chavez-Justo *et al.,* 1991; Trentini *et al.,* 1992; Gomes *et al.,* 1993; Libertini and Lazzaretto, 1993). The large chromosome size highlighted by Thiriot-Quiévreux and Cuzin-Roudy (1995) for *Meganyctiphanes norvegica* was corroborated here: sizes about 10 μm were observed for the largest chromosomes of all the species studied (see Figs. 1, 2).

Among euphausiids, details of chromosome morphology have been known only for *Meganyctiphanes norvegica.* The karyotype of this species was characterized by 19 metacentric chromosome pairs, slightly and regularly decreasing in size (Thiriot-Quiévreux and Cuzin-Roudy, 1995). The ideograms constructed from chromosome measurements of three Mediterranean species, *Euphausia hemigibba, E. krohni,* and *Nematoscelis megalops* and *Meganyctiphanes norvegica* (Table 1; Thiriot-Quiévreux and Cuzin-Roudy, 1995) are shown in Fig. 3. The four species are characterized by a different slope of the decrease in chromosome size, and by the presence/absence of metacentric and submetacentric chromosome pairs differing in number and position. Species sharing the same chromosome number of 2n = 38 are then distinguishable, i.e., *Meganyctiphanes norvegica* with only metacentric chromosome pairs, while *Euphausia hemigibba* and *Nematoscelis megalops* have two submetacentrics located at different positions in the karyotypes.

When chromosome morphology was derived only from observation of a limited number of mitotic metaphases and meiotic metaphases II, as in the other species studied here, a majority of metacentric chromosomes was also displayed, as in the fully karyotyped species. Therefore, symmetry appeared as a general characteristic of euphausiid chromosomes.

In conclusion, euphausiids from various genera and species share common traits in chromosome characteristics that clearly separate them from the other eucarids: a narrow range of haploid chromosome number, large chromosome size, and a majority of metacentric chromosomes. Compared to peracarids, euphausiids may be placed near isopods and amphipods, rather than near mysids. Isopods and amphipods show a modal chromosome number of n = 28 and n = 26, respectively, and a relatively large chromosome size, while mysids have haploid chromosome numbers varying from 5 to 56, without modal number, and small-sized chromosomes (Salemaa, 1986; Salemaa and Kamaltynov, 1994; Thiriot-Quiévreux, 1994). Within euphausiids, cytotaxonomic differences are sufficiently well expressed to allow the discrimination of neighboring species.

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