Cytogenetic studies on marine myodocopid Ostracoda: the karyotypes of some species of halocyprids

ALICIA MOGUILEVSKY

Institute of Earth Studies, University of Wales, Aberystwyth SY23 3DB, UK.

ABSTRACT - Nincteen species of halocyprid ostracods were dissected and analysed for karyotype studies. Results were obtained for only six species, 5 belonging to *Conchoecia* and 1 to *Halocypris*. The uniformity in the chromosome morphology of these species (metacentric/submetacentric) is similar to that found for the cypridinids. The size range of their chromosomes falls within the lower end of the studied cypridinid species. The chromosome counts, in some preparations, were somewhat impaired by uncertain cell boundaries and widely scattered chromosomes. In these cases, the morphology of the identifiable chromosomes and their size ranges were noted and wherever possible a provisional karyotype was suggested. J. Micropalaeontol. **14**(2): 81–95, October 1995.

INTRODUCTION

The present paper extends the previous work by the author on the cytotaxonomy of the cypridinid Myodocopida (Moguilevsky, 1985, 1990; Moguilevsky & Whatley, 1988). An attempt is made to establish the karyotypes for some halocyprid species in order to make comparisons within the group and with other Ostracoda.

All marine pelagic Ostracoda belong to the Myodocopida and the majority are found within the Halocypridacea. Planktonic ostracods are still poorly known, despite the fact that many species are widely distributed in the World Ocean and are surprisingly abundant. At all depths in the tropics and below 100 m at high latitudes, they are often the second most abundant group of macroplankton after copepods (Angel, 1981).

The taxonomic criteria used to distinguish species in this group are based on carapace size, shape, sculpturing and the position of the asymmetrical glands as well as the setation of the appendages. As Angel (1981) points out, some of these criteria are often trivial. He further remarks that a certain amount of doubt must be felt as to whether a good number of the present species are valid or should instead be considered as sub-species or perhaps races of a central species, and that similarly, some of the ubiquitous species may, on more careful taxonomic examination, prove to be species complexes. Several species complexes, in which different sibling species occur in each oceanic gyre system, have already been identified (Deevey, 1978; Gooday, 1976, 1981; Angel, 1981).

Many of the problems found in the taxonomy of the halocyprids are further compounded by the fact that the type material is difficult to locate and that some of the original drawings and descriptions are not detailed enough or just incomplete.

The majority of the still unresolved systematic problems concern the subdivision of the large, and perhaps 'umbrella', genus *Conchoecia*. Angel (1982) has helped by solving some of the confusion within the genus *Halocypris*. He suggested that the influence of interspecific competition between closely related species may result in character displacement as seems to be the case in the sibling species of *Halocypris pelagica* Claus, 1890 and *H. inflata* (Dana, 1849), which he redescribes.

Poulsen (1973) attempted to clarify some of the taxonomic intricacies which make the systematics of the halocyprids so unsatisfactory and confusing, concentrating on the genus Conchoecia which he subdivided into a number of new genera. Unfortunately, as Angel (1983) indicates, the result of Poulsen having reversed Müller's (1906a) synonymization of Claus's many genera into a single large genus Conchoecia was far from a clarification of the problem. Summarizing Angel's remarks: 'As Martens (1979) pointed out, neither Claus's nor Poulsen's new genera had designated type species, nor were there any adequate generic descriptions. It is clear from Poulsen's key that he considered characteristics of the mandibles important criteria in separating the genera. Unfortunately, Poulsen's use of these minute characters without explanatory diagrams makes his key totally unusable'. 'Martens (1979) and a number of other authors have continued to follow Poulsen's lead in recognising Claus's various genera. While recognising that certain species groups are readily identifiable within the main Conchoecia complex, I have felt that Poulsen's assignment of species to some of the groupings is arbitrary'. Angel considered that 'without a substantial clarification of the whole of the Conchoecia complex, I prefer to take the pragmatic if cautious approach of following Müller (1912) in treating it as a single, if overlarge, genus'. Poulsen left large heterogeneous groups of species undivided and did not explain the taxonomic relationships between the species.

Angel (1983) suggested that one way of helping to resolve some of the taxonomic problems in the genus *Conchoecia* would be an SEM study of the mandibular structures used by Poulsen to designate his genera.

Taking all this into account, the present author decided to take a different route and approach some of the problems of the halocyprids by analysing the karyotypes of a number of species. The aim of this work was to establish at least the

















Fig. 1. Conchoecia curta Lubbock, 1860. 1–3. C-metaphase chromosomes obtained from testis tissue squash (spermatogonia). Note the scattering of chromosomes and uncertainty of the cell boundaries; 1a-3a. interpretation and diagrams of 1–3. 1b-3b. suggested '2n' and 'n' karyotypes; 4–6. metaphase chromosomes of similar morphology to those in 1–3 (different cells, same specimen).



Fig. 1. (Continued.)

number and morphology of the chromosomes for each of the species collected and, where ever possible, concentrating on species which are members of 'species groups'.

Specimens of some 19 species were recovered from a number of RRS Discovery cruises between 1981 and 1985. Males, females and juveniles of all those species were subjected to colchicine culturing processing although some were fixed immediately in 1/3 acetic alcohol and preserved accordingly. As many specimens as possible of each species were dissected and analysed. Since none of the females of the available species retain their embryos in a brood chamber, eggs, ovaries and testes tissue squash were used instead.

The species studied are listed below:

Halocyprididae

Conchoecinae Elegans Group: Conchoecia elegans; Curta Group: C. curta *; Magna Group: C. magna, C. macrocheira, C. lophura, C. hyalophyllum; Spinifera Group: C. spinifera; Loricata Group: C. loricata, C. ctenophora; Daphnoides Group: C. daphnoides; Imbricata Group: C. ametra*, C. imbricata*;

Mollis Group: C. kampta*, C. rynchena *;

Bispinosa Group: C. haddoni, C. incisa;

Halocypris pelagica*, H. inflata, Halocypria globosa.

Over 150 slides were prepared and analysed; only some of those obtained from the species, which are marked with an * in the list above, yielded dividing cells from which a karvotype could be ascertained. These species and the results obtained, are discussed below.

THE SPECIES Curta Group Müller, 1906a

C. curta, C. echinulata, C. acuticostata and C. stigmatica are four small species (0.75–1.25 mm in length) grouped by Müller (1906a) in the Curta Group. These species are small, compact and rounded. The shell is short and high with a strongly curved ventral margin and strikingly sculptured. The first two species are less than 1 mm long and the remaining two, over 1 mm (Deevey, 1968). This group is considered by Skogsberg (1920) to be a natural one. Of the 4 species of the Curta Group, only specimens of C. curta were available for this study.

Conchoecia curta, Lubbock, 1860 (Fig. 1). The males and females of this species are of slightly different shape: the males have a straight rostrum while that of the female curves downwards. In both, the shell is strikingly sculptured and reticulated with striations paralleling the shell margins cut across by finer cross-striations, although they are less noticeable in the female (Deevey, 1968).

C. curta is epipelagic to mesopelagic with the females extending down to mesopelagic depths, and is one of the most abundant species in tropical and subtropical waters. Moguilevsky & Angel (1975) recorded it as one of the more frequent species of halocyprids occurring in the neuston of the Atlantic. It is widely distributed between 48°N and 37°S in the Atlantic and also in the Indian and Pacific Oceans and the Mediterranean. At latitudes higher than 40° its occurrence is seasonal (Angel, 1981). It is also recognized by Angel (1979) as a 'principally epipelagic species associated with North Atlantic Central Waters', and one of the small species capable of migrating vertically for considerable distances. C. curta is one of the species observed by Angel (pers. comm.) capable of displaying controlled sinking behaviour.

Halocyprinae

Merrett & Roe (1974) and Foxton & Roe (1974) have reported this species as one of the main food items in the diet of the hatchet fish *Argyropelecus aculeatus* as well as being eaten by some decapods.

Results. The specimens used in this study were recovered from surface waters (Neuston Net) at RRS *Discovery* St. 11266#2, at approximately 35°N 21°W, during night sampling. The material was cultured in 0.1% colchicine for 24 hours and placed in a hypotonic solution of 2/3 sea water/fresh water for 5 minutes prior to fixation in 1/3 acetic alcohol.

A number of preparations were obtained in the usual manner from gut, ovaries and testes tissue squash. The results were disappointing since only some of the slides made from testes tissues rendered some visible chromosomes. These appear scattered and all cell boundaries indistinguishable. This type of pattern can often be a result of overculturing. Further experiments, altering the time of culturing and/or a change in the concentration of the colchicine solution, are needed before a conclusive explanation could be offered.

The photomicrographs in Fig. 1, figs 1–3 show a scattering of c-metaphase chromosomes, with uncertain cell boundaries, obtained from testis tissue squash (spermatogonia). Figure 1, 1a–3a, shows interpretative diagrams of 1–3, showing the uniform morphology of the identifiable chromosomes (all metacentric or very slightly submetacentric) and their size range $(7.3-3.46 \,\mu\text{m})$ (1a), 5.76–3.07 μm (2a), 5.38–3.07 μm (3a)).

An attempt was made to arrange these chromosomes according to morphology and size and suggested '2n' and 'n' karyotypes are offered in Fig. 1, 1b–3b. The morphology of the identifiable chromosomes in 4-6 (same specimen as in 1-3), is similar to that shown in the suggested karyotypes.

Although the material available for this study was not adequate for the study of chromosome numbers, the results obtained show that the morphology of these chromosomes, as well as their size range, is consistent with that obtained for other myodocopid species.

Imbricata Group Müller

Müller (1906a) included in this group 5 species characterized by their large size and strikingly sculptured shell. The largest of these species is *Conchoecia plinthina* (4.8–5.9 mm) and in decreasing order of size: *C. symmetrica* and *C. ametra* (3.7-4.2 mm), *C. squamosa* (3.75-4.2 mm) and the smallest *C. imbricata* (2.5–3.5 mm). As Deevey (1968) points out, a sixth species *C. prosadene*, was thought by Müller (1906a) also to be related to this group although it differs from the other 5 species in its general shape and lack of shell sculpture. Skogsberg (1920) considers that 'this group, comprising 5 species, is certainly quite natural' and that *C. prosadene* does not seem to belong to it.

In all 5 species, the postero-dorsal corner of the shell is produced into a definite point on both valves. The postero-ventral corner of the right valve, or of both valves, shows a process which bears either the openings of the asymmetrical and lateral corner glands, or only of the latter. A characteristic shared with species of the *Mollis* Group, is the presence of a row of lamellae in the principal seta of the male. In some species, this seta may show instead a row of large teeth which stand at right angles to the seta.

Of this group, only *C. ametra* and *C. imbricata* were available for karyotype analysis. Although these two species share a number of similarities in the morphology of their limbs, they are clearly different in other aspects such as size range and overall shape of the carapace as well as distribution, vertical migration behaviour and luminescent patterns.

Conchoecia ametra Müller, 1906 (Figs 2, 3 and 4). Deevey (1968) describes this highly sculptured species as follows: 'the rostrum is rounded, but both sides come to a sharp point, the left point definitely longer than the right. The opening of the left lateral corner gland clearly projects beyond the shell margin at the postero-ventral corner, but the projection in the same location on the right shell is larger and also includes the opening of the right asymmetrical gland'.

C. ametra has a widespread distribution throughout all oceans. Angel (1979) defines it as a medium to high latitude, deep mesopelagic to bathypelagic species. Poulsen (1973) recorded its occurrence in Dana samples taken from 63°N-36°S. Deevey (1974) extends the southerly range to 44°S. Angel & Fasham (1975) found the species to be less common south of 30°N in the Northeast Atlantic, with its vertical range becoming shallower to the north. Angel (1979) records maximum numbers caught between 600-800 m at 30°N 23°W, with the adults remaining most abundant at 600-700 m and the juveniles at 700-800 m, close to the influence of the Mediterranean Water. He also reports that at 32°N 64°W (area of the Ocean Acre programme), the females of C. ametra were most abundant at 900-1250 m and males at 900-1000 m with little diel change, although the juveniles showed a small amount of sinking during ontogeny.

At 53°N 20°W Angel & Fasham (1975) encountered the main bulk of the population between 400-600 m in waters of $8^{\circ}-9^{\circ}$ C. Further north (60°N 20°W) the zone of abundance was found in deeper, cooler waters (900–1250 m, 4° –5°C). Angel (1979), concludes that at those higher latitudes there seems to be indications that C. ametra undergoes a seasonal migration, whereas in the central gyre water of the Atlantic, at least during spring, it becomes a deep mesopelagic species. Gooday & Moguilevsky (1975) found that although this species, which only migrates modest vertical distances (about 100 m) and did not sink readily unless disturbed, when induced to do so was able to stop sinking and remain neutrally buoyant for up to 6.5 minutes. The passive sinking velocity of C. ametra was recorded as 1.00-1.20 cm/sec which is slightly faster than the slowest species (< 1.00 cm/sec) but much slower than the fastest of the species studied by Gooday & Moguilevsky (1975) which registered at 1.80-2.00 cm/sec.

Results. The material used in this study was recovered from samples taken in the Northeast Atlantic at St 11272#1 (approximately 41°N 14°W) by RRS *Discovery*, Cruise 156. The specimens were collected by a RMT1 multiple net from a depth of 800–620 m. These were



Fig. 2. Conchoecia ametra Müller, 1906. Spermatogonia in testis tissue squash. 1, 2. Metaphase chromosomes (17) in different stages of contraction; 1a, 2a. interpretation and diagrams of 1 & 2; 1b, 2b. karyotypes from 1 & 2 showing the uniformity of these chromosomes in terms of morphology (metacentric to submetacentric), and gradual variation in size between the largest $(7.14-5.71 \,\mu\text{m})$ and the smallest $(4.28-3.14 \,\mu\text{m})$.





10 µm

Fig. 3. Conchoecia ametra Müller, 1906. Spermatogonia in testis tissue squash (same specimen as in Fig. 2); 1. metaphase chromosomes (16) showing some degree of overlap; 1a, interpretation and diagram of 1; 1b. the 16 identified chromosomes arranged according to size. Note the similar morphology and size variation to those shown in Fig. 2.

cultured in a 0.1% colchicine solution for 15 hours and subsequently fixed and preserved in 1/3 acetic alcohol.

The photomicrographs in Fig. 2, figs 1, 2 show 17 metaphase chromosomes (spermatogonia in testis tissue squash), in different stages of contraction. Figure 2, figs 1b, 2b represent the karyotypes from Figs 1 and 2 and show the uniformity of these chromosomes in terms of morphology (metacentric to submetacentric), and gradual variation in size between the largest $(7.14-5.71 \,\mu\text{m})$ and the smallest $(4.28-3.14 \,\mu\text{m})$. Although only 16 chromosomes were identified in the photomicrograph in Fig. 3 (fig. 1), these show similar morphology and size variation as those seen in the previous plate.

Figure 4 represents a preparation obtained using the same specimen as in Figs 2 and 3. The interpretative diagram of the chromosomes shown in Fig. 4, fig. 1 reveals that their

morphology and size variation, between the largest $(7.14 \,\mu\text{m})$ and the smallest $(3.71 \,\mu\text{m})$, are similar to those seen before, although in this case the number of chromosomes identified is 21.

It is difficult, at this stage, to be able to explain the variation in the number of chromosomes observed in this species or even to propose, with any certainty, a firm karyotype without first analysing many more specimens.

Conchoecia imbricata Brady, 1880 (Fig. 5). This species is distinguished from the others of the group by its smaller size, shell shape and the armature of the male principal seta. The postero-dorsal corners of the shell are extended into sharp points, that of the left valve is considerably longer than that of the right. The right asymmetrical gland and the lateral corner glands open into large processes at the postero-ventral corners. In ventral view, the rostrum







Fig. 4. Conchoecia ametra Müller, 1906. Spermatogonia in testis tissue squash (same specimen as in Fig. 2). 1. Metaphase chromosomes (21) showing some degree of overlap; 1a. interpretation and diagram of 1; 1b. the 21 identified chromosomes arranged according to size. Note the similar morphology and size variation to those shown in Figs 2 & 3.

appears sharply pointed (Deevey, 1968). As Angel (1979) describes it, *C. imbricata* 'is one of the most easily recognised halocyprid species with its clear carapace sculpturing and long curved rostral processes, posterior dorsal spines and posterior ventral tubercles'.

Poulsen (1973) records its occurrence in the Dana material, from 5°N in the Atlantic to 45°S in the Pacific. Deevey (1968) gives its distribution as 60°N and 55°S in the Atlantic and also occurring in the Pacific and Indian Oceans. On the basis of the geographical range and vertical distribution of this species, Angel & Fasham (1975) and Angel (1979) describe it as a widespread, shallow mesopelagic species. They also include in this group such species as *C. rhynchena*, *C. intermis*, *C. hyalophyllum* and *C. spinifera*.

Although *C. imbricata* is considered to be a vertically migrating species, as with other mesopelagic species which are nearly ubiquitous, it changes from being strongly migrant towards the centre to being non-migrant or weakly migrant at the extremities of their ranges of distribution (Angel & Fasham, 1975).

Angel (1968) in his study of bioluminescence in halocyprid ostracods, groups *C. imbricata* with those species in which the luminescence is retained within the glands. This pattern of display is usually associated with certain morphological modifications of the shell, which in the case of this species refers to the tips of the postero-ventral processes of the valves, where the luminescence is observed. The tips of the rostral processes and the postero-dorsal spines also carry luminous organs (Angel, 1981).

Gooday & Moguilevsky (1975) studied the sinking velocities of some 9 species of halocyprids. They found that 'the frequency and duration of sinking in an undisturbed ostracod may be related to the magnitude of its diurnal vertical migration. Thus, natural sinking was least common in *C. haddoni* a species for which Angel (1969) found no evidence of vertical migration, and most frequent in *C. imbricata* which according to Angel (1969) migrates vertically as much as 500 m.' Angel (1970) also points out that the large vertical migrations undertaken by some ostracods must involve active downward swimming as well as passive sinking. This particularly applies to *C. imbricata* which has a slow sinking velocity (1 cm/sec) despite undertaking extensive vertical migration.

This species seems to be able to control the velocity at which it sinks, slowing down and speeding up repeatedly. Although other species could also swim up and sink down in a controlled manner, *C. imbricata* seems more able to do this since it could also stop sinking and remain neutrally buoyant for up to 6.5 minutes (Gooday & Moguilevsky, 1975).

Results. The material used in this study was recovered from samples taken by RRS Discovery at Station 11261#34 (31°N 25°W) in a 600–0 m haul. The very few specimens available (only males) were cultured in colchicine for 24 hours and subsequently fixed and preserved in acetic alcohol.

The photomicrographs in Fig. 5 (figs 1-4) represent c-metaphase in spermatogonia obtained from testis tissue

squash. Unfortunately, in all of these the cell boundaries are uncertain and therefore, the chromosome count is rendered impossible. Nevertheless, as shown in the interpretative diagrams of Fig. 5, figs 1a-4a, a certain amount of information can be drawn regarding the morphology and size of the chromosomes involved. These are metacentric and submetacentric, and they vary in size between $5.35 \,\mu$ m, for the largest, and 2.8 μ m for the smallest. The reason for the breakage of the cell boundaries and the scattered appearance of the chromosomes, is unclear at this stage although similar configurations have been observed and obtained as a result of over culturing or because of specimens having been exposed to too high concentrations of colchicine solution and/or excessive hypotonic treatment prior to fixation.

Obviously, many more specimens need to be analysed, experimenting with different colchicine concentrations and culturing times, before a chromosome complement could be ascertained.

Mollis Group Müller

Summarizing from Deevey (1968). Müller (1906a) included in this group eleven large species of about 3 mm long, with sculptured shells. Only one of these species, *C. distans*, is slightly smaller (c.2.15 mm in length) and has no visible sculpturing. The postero-dorsal and ventral corners of the shell are rounded. The asymmetrical glands are in the usual place. Single lateral corner glands are present in both valves, opening on or near the margin. Medial dorsal glands occur in both sexes and sometimes are displaced from the postero-dorsal corner.

Four species of this group (C. mollis, C. amblypostha, C. kampta and C. acanthopora) are very similar, particularly their females, which are difficult to distinguish. The armature of the proximal secondary seta is a useful character to separate the males.

The following three species, which are also included in this group, are less closely related: *C. tyloda*, *C. borealis* (var. maxima and var. antipoda), and *C. distans*. Müller also included *C. dichotoma*, *C. plactolycos* and *C. rhynchena*. *C.* sibogae which has been described by Müller (1906b) from the Siboga Expedition material, is believed to be closely related to *C. rhynchena*. Deevey (1982) describes a new species, *C. eltaninae*, which she includes within this group as been closely related to *C. rhynchena* and *C. sibogae*.

Only two representatives of this group (*C. rhynchena* and *C. kampta*) were available for this study.

Conchoecia rhynchena Müller, 1906 (Fig. 6). This species was first recorded by Müller (1906a) from samples taken between $32^{\circ}N$ and $36^{\circ}S$ in the Atlantic and Indian Ocean. Since then Poulsen (1969) has found it in the Gulf of Guinea and in 1973 extended its distribution in all oceans from $46^{\circ}N$ to $46^{\circ}S$. Deevey (1982) gives the distribution of this species as $60^{\circ}N-46^{\circ}S$ in the Atlantic, to $49^{\circ}S$ in the Pacific and in the equatorial Indian Ocean and Indonesian seas.

Angel & Fasham (1975) regarded C. rhynchena as a mesopelagic species with its maximum abundance at $30^{\circ}N$ and $40^{\circ}N$ and, although becoming rare to the south, it was



Fig. 5. Conchoecia imbricata Brady, 1880. C-metaphase in spermatogonia obtained from testis tissue squash. 1-4. The uncertainty of the cell boundaries shown in these preparations have rendered the chromosome count impossible. However, the interpretation and diagrams of 1a-4a reveal information regarding the morphology and size of the chromosomes involved. These are metacentric and submetacentric and vary in size between 5.35 μ m for the largest, and 2.8 μ m for the smallest.



Fig. 5. (Continued.)

still one of the common species up to 60°N 20°W in waters of around 8°C. Very high numbers were also found by Angel (1977a) just south of the Bay of Biscay (44°N 13°W).

Angel (1979) in his study of vertical distribution of the halocyprids along a transect from Africa to Bermuda, found *C. rhynchena* to be one of the three most abundant species at 30°N 23°W. Although the migration of *C. rhynchena* is much more limited than in other species such as *C. imbricata*, Angel describes the following pattern: by day, the majority of the adult population was found between depths of 500-600 m with the females extending the range up to

400 m and the males down to 800 m; the juveniles were most abundant at 400–600 m. At night the pattern changed with the upper range of the females and juveniles becoming shallower (up to 200 m) and the total main adult concentration rising to 600-500 m, a zone in which the temperature range was $11.6-12.8^{\circ}$ C.

Angel also found that *C. rhynchena*, like many other northern mesopelagic species along the 32°N transect, was most abundant in the eastern region with numbers decreasing notably west of 44°W. The few specimens caught off Bermuda occurred much deeper, possibly as a response



Fig. 6. Conchoecia rhynchena Müller, 1906. C-metaphase in spermatogonia obtained from testis tissue squash. 1. Shows 19 chromosomes with considerable degree of uniformity in terms of morphology (these are all metacentric or very slightly submetacentric) and size; 1a. interpretation and diagram of 1; 1b. karyotype from 1; the gradual decrease in size between the largest chromosome (c. $10 \,\mu$ m) and the smallest (c. $4.8 \,\mu$ m) renders them individually unidentifiable except for the first pair which exhibit a secondary constriction.

to the 14°C isotherm being identified there at nearly 600 m.

C. rhynchena migrates vertically only moderate distances of 100-150 m. Gooday & Moguilevsky (1975) found that although this species would not readily sink unless disturbed, when forced to do so, it would show a passive sinking velocity of 1.00-1.20 cm/sec. This is similar to that of C. ametra, another species with moderate vertical migration. These two species together with C. inermis and C. imbricata exhibited controlled sinking behaviour and were found to be able to stop sinking and remain neutrally buoyant for up to 6.5 minutes.

Results. The specimens available for this study were recovered from samples taken at RRS *Discovery* St. 11272#2 from 300–100 m depth and cultured in 0.1% colchicine for 24 hours. Only the preparations obtained from testes tissue squash yielded some dividing cells. The photomicrographs in Fig. 6, fig. 1 shows 19 chromosomes in c-metaphase spermatogonia. These chromosomes (see fig. 1b) exhibit a considerable uniformity in terms of morphology and size. All chromosomes are metacentric or very slightly submetacentric. Their similarity in size, with a gradual decrease in size between the largest ($c. 10 \,\mu$ m) and the shortest ($c. 4.8 \,\mu$ m), renders them individually unidentifiable except for the first pair (the longest) which exhibit a secondary constriction.

It is, therefore, suggested here that the chromosome complement for the male is composed of 18 autosomes and a single unpaired 'X' (19 = 18A + XO), and that of the female a possible 20 chromosomes (18A + XX).

Conchoecia kampta Müller, 1906 (Fig. 7). Angel (1979) identified, in *Discovery* samples from Atlantic waters, 3 species belonging to Müller's *Mollis* Group; these were *C. tyloda*, *C. mollis* and *C. kampta*. He could easily identify *C. tyloda* on the basis of its distinctive carapace sculpture. The identification of the other two species offered him the same difficulties encountered by Poulsen (1973) who made the decision to take these 3 species, plus another 3 from Müller's *Mollis* Group, and erect the new genus *Mollicia*.

Although the morphological distinction between *C. mollis* and *C. kampta* relies only on characters of the setation of the first and second antenna of the male and slight differences in carapace size, these two species are also found at different levels in the water column.

Angel & Fasham (1975) identified *C. kampta* as a species that, in the NE Atlantic, is associated with the NACW (North Atlantic Central Water). Although both *C. kampta* and *C. mollis* co-occurred at 18°N 25°W, *C mollis* displaces *C. kampta* at the lower end of its vertical range. At 30°N 23°W, only *C. kampta* is recorded with the adult population predominating both by day and night in the 800–1000 m



10 um 🔒

1b

1a







Fig. 7. Conchoecia kampta Müller, 1906. C-metaphase in spermatogonia obtained from testis tissue squash. 1. Note the presence of 19 chromosomes, highly uniform morphologically (all metacentric) and of similar size; 1a. interpretation and diagram of 1. Dotted lines indicate the uncertainty of the outline as a result of some overlapping; 1b. karyotype of 1. Note the gradual decrease in size between the largest (6.28 μ m) and the smallest (3.24 μ m); 2, 3. although these photomicrographs exhibit a higher degree of overlapping than that in 1, the identifiable chromosomes are of similar morphology and size as those described in 1.

zone (within temperature and salinity ranges of $8.1-9.5^{\circ}$ C and $35.37-35.41_{\infty}$, respectively). At this latitude Angel (1979) recognized this species to be positioned around the top of the influence of Mediterranean Water, and that at 44° N 13°W it was found at 700-1000 m in waters of below 10°C and salinity which represents the salinity maximum of Mediterranean Water. He also records that at Ocean Acre (1° square centred at 32°N 64°W) *C. kampta* is of rather uncommon occurrence; the few adults caught there were recovered by day from the 800-1500 m samples, and the juveniles from the 800-900 m. By night, the adults were found deeper in the 1000-1500 m hauls were the temperature range was $4.4-7.0^{\circ}$ C and salinity 35.06-35.14‰.

Results. The specimens used in this study were recovered from samples taken during RRS *Discovery* Cruise 176, at Station 11272#1 in a 620-800 m haul. All specimens were cultured in colchicine for 24 hours and treated in a hypotonic solution prior to fixation. Both males and females were dissected and preparations made from testes and ovaries. Only those slides obtained from testis tissue squash yielded a number of dividing cells.

Figure 7, fig. 1. shows a photomicrograph of c-metaphase in spermatogonia in which 19 chromosomes can be distinguished. Figure 7, fig. 1a represents an interpretative diagram of these chromosomes with dotted lines indicating the uncertainty of the outline as a result of some overlapping. 1b shows that, in terms of centromere location (metacentric) and very slight variation in size between the chromosomes, this karyotype exhibits considerable uniformity. The largest of the chromosomes is 6.28 μ m in length and the shortest pair, 3.42 μ m.

The additional photomicrographs on Fig. 7, figs 2, 3, although exhibiting a higher degree of overlapping than that of fig. 1, show that the identifiable chromosomes are of similar morphology and size to those described above. It is, therefore, suggested that the chromosome complement for the male of *C. kampta* is composed of 19 (18A + XO) chromosomes which are metacentric and exhibiting a gradual variation in size between the longest and the shortest, and that possibly for the female the complement would be composed of 20 (18A + XX) chromosomes.

Halocyprinae

Halocypris pelagica Claus, 1890 (Fig. 8). Since originally described by Claus (1890) this species, like others in this genus and for that matter in this subfamily, has been the object of misidentification, synonymization, obscurity and finally re-identification or rather re-validation by Angel (1982).

Confusion originated as a result of a combination of factors, such as specimens exhibiting considerable size variation with the clear occurrence of both large and small forms, and differences in the mesh size of the nets used by various expeditions. This led a number of authors (Dana, 1849, 1852; Claus, 1874; Vavra, 1906; Müller, 1906a; Skogsberg, 1920, etc.) to mistakenly assign males of one species to females of another, and to split or lump together valid and distinct species within this group. After re-examining a large number of specimens recovered from samples taken at different times, latitudes and seasons during various *Discovery* cruises, Angel (1968, 1969, 1979) was able to re-assess the complicated situation. Finally in 1982 he produced an important and clarifying study of this species. He proposes that the large form should be distinguished as *Halocypris inflata* (=*brevirostris* =*concha sensu* Claus and *sensu* Vavra, 1906) and that the small form should be recognized as *H. pelagica* Claus, 1890 and which he re-describes as a valid species.

Angel (1977b, 1979) found that size is a useful taxonomic character in halocyprids since, with few exceptions, they have very restricted size ranges. The different size groupings which he observed in *H. pelagica* and in *H. inflata* juveniles were more noticeable when the two species co-occur. He further suggests that these two sibling species possibly show character displacement and that this may help to minimize the extent of spatial overlap by reducing the possibility of interspecific competition 'assuming the size of food taken is related to the organism's size'.

H. pelagica and *H. inflata* are vertical diel migrants. Angel (1982) summarizes the pattern of migration at different latitudes: 'off Bermuda *H. pelagica* migrates from 100-300 m to 10-100 m and, whereas this species seems to be associated with North Atlantic Central Water, *H. inflata* is associated with South Atlantic Central Water. Where the two species co-occur on the Equator the hydrographic conditions are complicated by the equatorial current and counter-current'. From the only day samples available from the Equator (0°23'W), he concludes that the adults were almost restricted to 50-150 m zone. The juveniles of both species seem to show less segregation than the adults since these were found together in the 50-100 m zone. Deevey (1974) indicates a distribution in the SW Atlantic between $0^{\circ}-32^{\circ}S$.

When this study was planned, one of the aims was to analyse and compare the karyotypes of both *H. pelagica* and *H. inflata* with the view to test to what extent the number and morphology of their chromosomes reflected Angel's (1982) findings regarding character displacement and speciation. Of all the specimens analysed, including males and females of both species, only the preparations obtained from the males of *H. pelagica* yielded some useful material. No dividing cells were found in any of the slides obtained from the others.

Adults of *H. pelagica* measure 1.00-1.40 mm (female) and 1.0-1.26 mm (male). Those of *H. inflata* 1.48-1.82 mm (female) and 1.32-1.60 mm (male). Their small size, combined with the fact that females of this group do not retain the embryos in a brood chamber, made matters more difficult. In previous work, embryos have proved to be the most valuable tissue for the study of the karyotypes and whereas testes tissue does normally provide some good meiotic and also mitotic plates, ovaries were never found to yield useful preparations.

The material used herein was collected from surface waters at approximately 31°N 25°W, Station 11261 of *Discovery* cruise 156 in July 1985. The specimens were cultured in colchicine for 24 hours and treated in a







2b

Fig. 8. *Halocypris pelagica* Claus, 1890. Spermatogonia in testis tissue squash. 1. Shows 19 c-metaphase chromosomes (all metacentric or very slightly submetacentric); 1a. interpretation and diagram of 1 in which the dotted lines indicate some uncertainty in the outline of the overlapping chromosomes; 1b. karyotype of 1, showing the chromosomes arranged according to size. There is a gradual decrease in size between that in position 1 and that in position 18 (6.06–2.72 μ m) The 19th chromosome is much smaller, measuring 1.51 μ m; 2, 3. show chromosomes of similar morphology and size to those in 1 but with varying degree of contraction and overlapping; 2a, 3a. interpretation and diagram of 2, 3; 2b, 3b. karyotypes of 2, 3 arranged according to size.

hypotonic solution of 2/3 sea water prior to fixation. The results of the photomicrographs taken of the testes tissues squash preparations can be seen in Fig. 8. Figure 8, fig. 1 shows c-metaphase in spermatogonia with 19 identifiable chromosomes. 1a represents an interpretative diagram of

Fig. 1 in which the dotted lines indicate some uncertainty in the outline of the overlapping chromosomes. The karyotype in fig. 1b shows the chromosomes arranged according to morphology and size. All chromosomes are metacentric or very slightly submetacentric. There is a gradual decrease in



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Fig. 8. (Continued.)

size between that in position 1 and that in position 18 $(6.06-2.72 \ \mu m)$. The 19th chromosome is much smaller, measuring $1.51 \ \mu m$. A similar pattern is shown in figs 2, 2a-b and 3, 3a-b.

All the preparations analysed exhibited chromosomes in a rather contracted stage and with a considerable degree of overlapping. Some of these preparations also show widely scattered chromosomes indicating rupture of the cells boundaries. The uncertainty which resulted from chromosomes overlapping and disruption of cell boundaries rendered it impossible to count the chromosomes in as many slides as desired. The reasons for both the excessive overlapping and scattering of the chromosomes may be related to a combination of factors such as colchicine.

Many more specimens of both males and females of this species need to be examined to confirm the suggested karyotype of 19 chromosomes (18A + XO), for the male, and a possible 20 chromosomes (18A + XX) for the female, as well as for the study the behaviour of the chromosomes at meiosis.

SUMMARY OF CONCLUSIONS

Of the nineteen species studied data were obtained for only six, 5 belonging to the *Conchoecia* and 1 to *Halocypris*. The type of information gathered varied according to a number of factors which are summarized below:

(i) No dividing cells were obtained from any of the female tissues analysed.

(ii) Embryo tissue was not available since none of these species retain embryos in a brood chamber.

(iii) Although testes tissue yielded some mitotic plates (spermatogonia), no meiotic division was found.

(iv) The chromosome counts, in some preparations, were somewhat impaired by uncertain cell boundaries and widely scattered chromosomes. In these cases, the morphology of the identifiable chromosomes and their size ranges were noted and wherever possible a provisional karyotype was suggested.

(v) Possible causes of the excessive disruption of cell boundaries and the scattering of the chromosomes may be (a) culturing in a colchicine solution of extreme concentration (each species seems to have an optimum concentration); (b) prolonged exposure to a hypotonic solution.

A synopsis of the results obtained in this study is given below and in Table 1:

Curta Group

Conchoecia curta typical neuston dweller, epi-mesopelagic. Preparations: mitosis in spermatogonia, uncertain cell boundaries, scattered chromosomes, some overlapping. Chromosome morphology: metacentric/slightly submetacentric.

Size range: $3.2-6.14 \mu m$.

Suggested number of chromosomes per cell (2n): 12?, 14?.

Imbricata Group

Conchoecia imbricata ((400–600 m) – 100 m, vertical migrant).

Preparations: mitosis in spermatogonia, uncertain cell boundaries, scattered chromosomes, some overlapping.

Chromosome morphology: metacentric to submetacentric. Size range: $2.8-5.35 \ \mu$ m.

Suggested number of chromosomes per cell: uncertain.

Conchoecia ametra (600-1000 m, non migrant).

Preparations: mitosis in spermatogonia, cell boundaries moderately undisturbed although, chromosome numbers per cell not constant (polymorphic?), some overlapping observed.

Chromosome morphology: metacentric/very slightly submetacentric.

Size range: 4.09–6.71 µm.

Suggested number of chromosomes per cell: 17 (16A + XO), although 16 (one missing?) and 21 (polymorphic?) also identified.

These two species are easily distinguished from one another on the basis of their morphology and depth distribution. In terms of their karyotypes, although it was not possible to determine the chromosome number with any certainty, the points to note are: the similarity in the morphology of their chromosomes, and the slightly larger size of the chromosomes in *C. ametra*.

Mollis Group

Conchoecia rynchena (500–800 m (900 m), moderate vertical migrant (100 m)).

Preparations: mitosis in spermatogonia, some chromosomes overlapping.

Chromosome morphology: metacentric, 1st pair exhibits secondary constriction.

Size range: 4.8–10 μ m.

Suggested karyotype: male, 19 (18A + XO); female 20 (18A + XX).

Conchoecia kampta (800-1500 m, vertical migrant).

Preparations: mitosis in spermatogonia, some chromosomes overlapping.

Chromosome morphology: metacentric.

Size range: $3.42-6.28 \ \mu m$.

Suggested karyotype: male, 19 (18A + XO); female 20 (18A + XX).

These two species occupy different levels in the water column and exhibit different migrational patterns. Although the morphology and chromosome numbers are similar in both species, their karyotypes differ in that the 1st pair of chromosomes in *C. rhynchena* exhibit a secondary constriction and, on average, its chromosomes are larger than in *C. kampta*.

Halocyprinae

Halocypris pelagica (epipelagic).

Preparations: mitosis in spermatogonia, some uncertainty of cell boundaries and scattering of chromosomes.

Chromosome morphology: metacentric/very slightly submetacentric.

Size range: $(1.60 \ \mu m)$, 2.57–6.30 μm . Suggested karyotype: 19 (18A + XO).

Points to note

From the data presented above, although inconclusive in part, the following points may be noted:

(1) The uniformity in the chromosome morphology of all the halocyprid species studied herein (metacentric/ submetacentric) which, in this sense, is similar to that found for the cypridinids (see Moguilevsky, 1985, 1990; Moguilevsky & Whatley, 1988).

(2) The size range of their chromosomes falls within the lower end of the size range of studied cypridinid species $(2.2-10.2 \ \mu m)$, for the species of *Vargula*).

(3) The chromosome number found for these halocyprid species, although provisional in same cases, are also within the numerical range of the cypridinids (16–18).

(4) Within the halocyprids studied, there is as much variation in the number and morphology of the *Conchoecia* species as to include the single species of *Halocypris*. The minimum size range of *H. pelagica*, however, is lower than that of the *Conchoecia* species, although its maximum is within the range of the latter species.

Species	Chromosomes		
	2n	Size-range	Morphology
Conchoecia curta	12? 14?	3.2–6.14 μm	m/sm
Conchoecia imbricata	uncertain	$2.8-5.35 \mu m$	m/sm
Conchoecia ametra	17? 21?	4.09–6.71 μm	m
Conchoecia rhynchena	19(m); 20(f)	4.8–10 µm	m
Conchoecia kampta	19(m); 20(f)	$3.2-6.28 \mu m$	m
Halocypris pelagica	19(m); 20(f)	1.60–6.3 μm	m/sm

Table 1. Results.

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