Vol. 10: 193–200, 2010
doi: 10.3354/ab00278

AQUATIC BIOLOGY
Aquat Biol

Published online August 31

Cytogenetics of *Hediste diversicolor* (Annelida: Polychaeta) and comparative karyological analysis within Nereididae

Alexandra Leitão[1,*], Susana Carvalho[1], Radhouan Ben-Hamadou[2], Miguel B. Gaspar[1]

[1]Instituto Nacional de Recursos Biológicos (INRB, I.P.)/IPIMAR, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal
[2]Centro de Ciências do Mar (CCMAR), EcoReach Research Group, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

ABSTRACT: The karyotype of the common ragworm *Hediste diversicolor* (OF Müller 1776) (Polychaeta, Nereididae) was determined for the first time; it comprised 8 metacentric, 2 submetacentric and 4 subtelocentric chromosome pairs. In a few metaphases, we observed an easily distinguishable undersized supernumerary chromosome. Recent taxonomic revisions of the family Nereididae have highlighted the need for further studies in several genera. To understand the taxonomic relationships in this family, we carried out a comparative karyological analysis through multivariate analyses (principal component analysis and hierarchical cluster analysis), using morphological and structural features of karyotypes, between *H. diversicolor* and 10 other Nereididae species. This analysis showed the distinctiveness of *H. limnicola*, *H. diversicolor* and *Nereis oligohalina*, which could be explained by the presence of 3 or 4 morphological categories of chromosomes in these species. The remaining species presented more symmetrical karyotypes with only 2 morphological categories and were grouped in 2 main clusters, mainly due to different percentages of metacentric chromosomal pairs. This study highlights the importance of cytogenetics in taxonomic studies of polychaetes.

KEY WORDS: Chromosomes · *Hediste diversicolor* · Karyotype · Nereididae · Polychaeta · Taxonomy

INTRODUCTION

The common ragworm *Hediste diversicolor* (OF Müller 1776), a member of the family Nereididae, order Phyllodocida, is a common species inhabiting both sandy and muddy estuarine and coastal lagoon sediments, and is among the few polychaete species of commercial interest as fish bait (see Scaps 2002 for references). Due to its bioturbation activity, *H. diversicolor* plays a relevant role in several biogeochemical processes at the sediment and sediment–water interface (Banta & Andersen 2003, Carvalho et al. 2007). Moreover, it is also an important food resource for many estuarine fish and wading birds (Masero et al. 1999, Cabral 2000). Furthermore, as a robust sentinel species in European beaches and estuaries, this polychaete has been recommended as a toxicity test organism for the assessment of heavy metal pollution (Ozoh 1992, Solé et al. 2009).

Nevertheless, there is little consensus regarding its generic name. While most ecologists still refer to the genus *Nereis* for this species since the publication of Fong & Garthwaite (1994), population geneticists are using the genus *Hediste* (Scaps 2002). As with many other polychaetes, several groups of nereidids consist of morphologically indistinct species (Wilson & Glasby 1993). The polychaete family Nereididae comprises over 500 nominal species, constituting a diverse group of animals that occur both in marine, brackish water and freshwater habitats (Rouse & Pleijel 2001). Several attempts have been made to establish taxonomic relationships within the Nereididae at the subfamily level (Fitzhugh 1987, Glasby 1999). However, these were mainly based on overall morphological similarities,

*Email: aleitao@ipimar.pt

highlighting the need for further studies of Nereididae relationships using a multidisciplinary approach.

In this family, the karyotype determination has been performed in only a few species. In the genus *Hediste*, the species *H. japonica* (Izuka 1908), *H. diadroma* (Sato & Nakashima 2003) and *H. atoka* (Sato & Nakashima 2003) presented a very similar karyotype, with a chromosome number of 2n = 28. Sato & Ikeda (1992) and Tosuji et al. (2004) also suggested the presence of an XX-XY (male heterogametic) sex chromosome system for these species. In contrast, the closely related congener *H. limnicola* (Johnson 1903) presented a diploid number of 2n = 26 and absence of sex chromosomes (Tosuji et al. 2010). In *Nereis oligohalina* (Rioja 1946) a 2n = 28 diploid number was observed with a karyotype composed of 7 metacentric, 1 submetacentric, 3 subtelocentric and 3 telocentric chromosome pairs (Ipucha et al. 2007). Within the genus *Perinereis*, *P. macropus* (Claparède 1870) also presented the diploid chromosome number of 2n = 28 with a karyotype consisting of 6 metacentric, 5 submetacentric and 4 subtelocentric chromosome pairs (Lipari et al. 1994). On the other hand, *P. ponteni* Kinberg 1866, *P. vancaurica* (Ehlers 1868) and *P. anderssoni* Kinberg 1886 all presented the same diploid number of 2n = 28, but with karyotypes mostly composed of metacentric and submetacentric chromosomes (Ipucha et al. 2007). The same main cytogenetic features were also observed by the same authors in *Pseudonereis palpata* (Treadwell 1923) and *Platynereis dumerilii* (Audouin & Milne Edwards 1833). In the latter, the karyotype comprised 10 metacentric pairs and 4 submetacentric pairs. However, Jha et al. (1995) observed a different karyotypic formula in the same species, consisting of 7 metacentric and 7 submetacentric chromosomal pairs. The karyological variations observed could be due either to the effect of different researchers or methodology applied, although a different location of the chromosomal Ag-nucleolar organizer regions (NORs) was also observed between both studies. On the other hand, the material examined had different origins. While Ipucha et al. (2007) analysed specimens from the southeastern Atlantic, specimens used by Jha et al. (1995) were from the North Atlantic. *P. dumerilli* was included in the group of cosmopolitan polychaete species recognised by Fauchald (1977) as an alleged species complex (Jha et al. 1995), and more recently, Ipucha et al. (2007) suggested that the species is polytypic. In another nereidid, *Neanthes arenaceodentata* (Moore, 1903), a different diploid chromosome number was observed in 2 populations of this species (Pesch et al. 1988, Weinberg et al. 1990). While a diploid chromosome number of 18 was observed in a Californian population (Pesch & Pesch 1980), 24 chromosomes were reported in a Connecticut population of *N. arenaceodentata* (Pesch et al. 1988). Moreover, a different chromosomal morphology was also observed between the 2 populations, which led the authors to suggest, from the karyotype analyses, that those populations represented 2 different species. The results of Pesch et al. (1988) emphasise the importance of further taxonomic studies in the Nereididae (with multidisciplinary approaches) and the role of cytogenetics.

Here we describe for the first time the karyotype of *Hediste diversicolor*. This information was used to perform a comparative karyological analysis within the family Nereididae, using multivariate analysis methods, in order to shed light on the taxonomic relationships within this family.

MATERIALS AND METHODS

Biological material. *Hediste diversicolor* used in the present experiment were hatchery-reared in the Fish Culture Experimental Station of IPIMAR at Olhão. They were randomly selected from culture tanks and transferred to the Molluscan Aquaculture Experimental Station of IPIMAR (Tavira). Before processing, specimens were acclimated for 1 wk. Worms were maintained in tanks with seawater and a thin sediment bottom layer (ca. 1 to 2 cm), constant aeration and under a natural photoperiod. *H. diversicolor* specimens were fed ad libitum with dry fish feed.

Chromosome preparation. For chromosome preparation, we generally followed the regenerated tail methodology (e.g. Tosuji et al. 2004, Ipucha et al. 2007). After 1 wk, the posterior end of each worm (ca. 5 mm long) was cut with a razor blade. The worms were kept in the same laboratory conditions for approximately 30 d to allow the development of a regenerated tail. After this period, whole individuals were incubated for 70 min in a 0.05% solution of colchicine in seawater at 18°C. Afterwards, the regenerated tail tissue (ranging from 1 to 6 setigers) was removed with a razor blade, and treated with 4 successive solutions of sodium citrate and seawater (2:1, 1:1, 1:2 and 1:3, 10 min each). The material was then fixed in a freshly prepared mixture of absolute alcohol and acetic acid (3:1) with 3 baths of 20 min each. Fixed pieces of the regenerated tail tissue from each individual were dissociated in 50% acetic acid with distilled water solution. Slides were prepared following an air-drying technique (Thiriot-Quiéveux & Ayraud 1982).

Karyotyping. Slides were stained with Giemsa (4%, pH 6.8) for 10 min. Images of Giemsa-stained metaphases were acquired with a digital camera (Nikon DSFi 1) coupled to a light microscope (Nikon Eclipse 80i). Digital images were processed with Adobe Photoshop (edition 5.0) using functions affecting only the whole image. After karyotyping of the 42 metaphases, the 10 best karyotypes were selected for chromosome

measurements using ImageJ (version 1.32j). Relative length was expressed as 100 times the absolute chromosome length (µm) divided by the total length of the haploid complement. The centromeric index was calculated by dividing 100 times the length of the short arm by the total chromosome length. The arm ratio was also determined (length of short arm divided by length of long arm). Both centromeric index and arm ratio are given because both express centromere position, allowing the comparison with other karyological studies. Terminology relating to centromere position followed that of Levan et al. (1964).

Comparative karyological analysis. Comparative karyological analysis within the family Nereididae was performed with average chromosomal measurements of *Hediste diversicolor* and the 10 other species for which those measurements had already been published: *Hediste* (*H. atoka*, *H. diadroma*, *H. japonica*, *H. limnicola*), *Nereis* (*N. oligohalina*), *Perinereis* (*P. anderssoni*, *P. ponteni*, *P. vancaurica*), *Platynereis* (*P. dumerilii*) and *Pseudonereis* (*P. palpata*). However, as the karyotype of *P. dumerilii* was determined by 2 different authors, we decided to analyse each karyotype separately. Although identified as the same species, data were analysed separately; therefore, comparative karyological analysis will hereafter be considered as being performed with 12 species. It is worth noting that in the World Register of Marine Species (www.marinespecies.org/index.php) *N. oligohalina* and *P. ponteni* are presented as invalid taxa. The former is given as *Neanthes oligohalina* Rioja, 1946 and is considered a synonymised taxon of *Nereis pelagica occidentalis* Hartman, 1945, and the latter is presented as a heterotypic synonym of *P. anderssoni*. Since we did not identify the specimens, we decided to maintain the original names presented by the authors. Moreover, while studying the genus *Nereis* from the northeast Brazilian coasts, Santos & Lana (2003) found different morphological characters to those described for *N. occidentalis* and decided to maintain the name of the species as *N. oligohalina*.

For this comparative karyological analysis, a data set was constructed aiming to summarise structural and morphological features of the species' karyotypes. For each of the studied species, we considered the number of chromosomes, number of morphological categories, proportion of each of the latter (respectively metacentric, submetacentric, subtelocentric and telocentric), and the Shannon diversity index, calculated using the standardised relative length of chromosomes (*HL*) as:

$$HL_i = -\sum_{ch=1}^{N} \mathrm{RL}_{ch} \log \mathrm{RL}_{ch} \quad (1)$$

where N is the total number of chromosome pairs of species i, ch is the chromosome number and RL_{ch} is the relative length of chromosome ch.

We also calculated an index of symmetrical level of the karyotype (SK), calculated for each species as:

$$\mathrm{SK} = \frac{m + sm}{(m + sm) - (st + t)} \quad (2)$$

where m, sm, st and t are, respectively, the number of metacentric, submetacentric, subtelocentric and telocentric chromosomes within the species karyotype.

For each karyotype, the RL measurements were divided by the maximum value observed within the chromosomal pairs. For each species, the standardised values of RL obtained ranged from 1 (from the largest chromosomal pair) to a minimum value obtained by dividing the smallest by the largest RL measured in each karyotype. Also, to avoid statistical bias due to experimental discrepancy between studies and to attain multinormality, which is a requisite for further multivariate analysis, raw values of each column (descriptor) were standardised. The normalised value of a descriptor for a species was calculated as the ratio of the difference between the raw value and the average of the 12 species and divided by the SD:

$$Xs_{ij} = \frac{X_{ij} - \overline{X_{.j}}}{STD(X_{.j})} \quad (3)$$

where Xs_{ij} is the normalised value of the descriptor j for species i. X_{ij} is the raw value; $\overline{X_{.j}}$ and $STD(X_{.j})$ are, respectively, the mean and SD values of the descriptor j across the 12 species.

The obtained data matrix was used for principal component analysis (PCA) coupled to a hierarchical clustering analysis (HCA; single linkage) using the Manhattan distance (Legendre & Legendre 1998). The clustering validation was carried out using the Silhouette criteria (Rousseeuw 1987). Information on the clusters obtained was used to produce confidence ellipses on the PCA graphic depicting cluster splitting. The confidence ellipse for the group mean was based on Hotelling's T^2 statistic and was defined for the 99% confidence level. These analyses were carried out using Matlab 6.5 (MathWorks).

RESULTS

Analysis of 42 metaphases from 8 specimens of *Hediste diversicolor* confirmed the diploid chromosome number of 2n = 28 scored by Christensen (1980). The mitotic index (number of observed metaphases) was higher in the individuals presenting more regenerated setigers. A detail of the anterior and posterior ends of an individual of this species and a mitotic metaphase of regenerated tail tissue of *H. diversicolor* are presented in Fig. 1. The karyotype

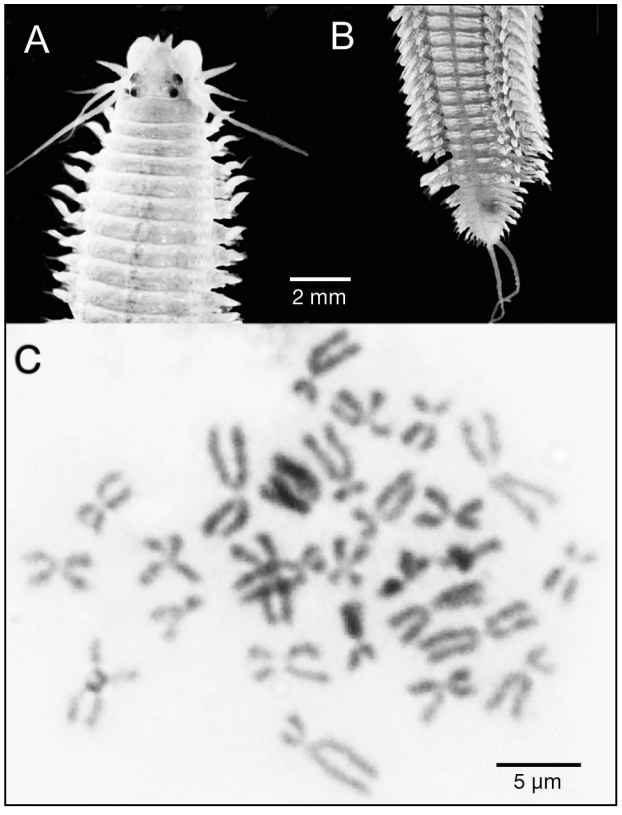

Fig. 1. *Hediste diversicolor.* Dorsal views of the (A) anterior and (B) posterior ends; (C) mitotic metaphase of the regenerated tissue

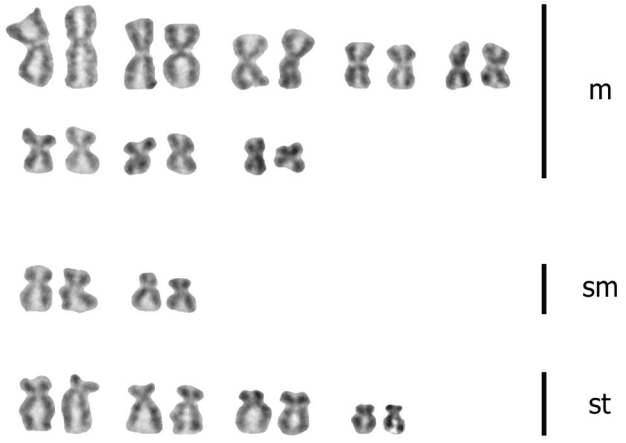

Fig. 2. *Hediste diversicolor*. Karyotype. Chromosomes are arranged in order of decreasing size within each morphological class. m: metacentric, sm: submetacentric, st: subtelocentric

(Fig. 2, Table 1) consisted of 8 metacentric, 2 submetacentric and 4 subtelocentric chromosome pairs (Fig. 2).

We did not observe any heteromorphisms between homologous chromosomes that would suggest the existence of sex chromosomes. However, after removal of the regenerated tail tissue, all individuals were sacrificed and analysed for sex determination, by the presence of female or male gametes in the coelom, and all were revealed to be females. A supernumerary chromosome, smaller than the ones of the standard diploid complement, was observed in 3 metaphases of 1 individual (Fig. 3).

Results of the comparative karyological analysis of the features presented in Table 2 are illustrated in Fig. 4 for the PCA and the HCA. Using the Silhouette validation criteria in the clustering analysis, 5 different clusters were obtained: (1) *Perinereis ponteni*, *P. vancaurica*, *Hediste atoka*, *H. diadroma* and *H. japonica*; (2) *Platynereis dumerilii*, *P. dumerilii* 2, *Perinereis anderssoni* and *Pseudonereis palpata*; (3) *H. diversicolor*; (4) *Nereis oligohalina*; (5) *H. limnicola*.

Hediste diversicolor, *Nereis oligohalina* and *H. limnicola* presented individual dissimilarities with the remaining species, forming separate individual clusters. It is worth noting that *Perinereis anderssoni* was grouped with species of another genus instead of being grouped with the 2 remaining *Perinereis* species. The 2 karyotypes available for *P. dumerilii* were grouped together, although PCA and HCA showed relative dissimilarities between them (Fig. 4A,B).

DISCUSSION

A diploid chromosome number of 2n = 28 was observed in *Hediste diversicolor*, which is the most commonly observed number within the family Nereididae (e.g. Christensen 1980, Jha et al. 1995, Tosuji et al. 2004). The relative constancy of the diploid number within the family might suggest that structural rearrangements in chromosome complements (without producing changes in chromosome number) may be the origin of the interspecific differences observed.

The karyotype of *Hediste diversicolor* from the southern Portuguese populations determined in this study comprised 8 metacentric, 2 submetacentric and 4 subtelocentric chromosome pairs. However, it is worth noting that allozyme evidence of genetic differentiation between *H. diversicolor* populations was already observed in different European regions, namely among western Mediterranean populations (Abbiati & Maltagliati 1996), between North Sea and Baltic Sea populations (Röhner et al. 1997), and between the Weser estuary and the Jadebusen in Germany (Fong & Garthwaite 1994). More recently, a comprehensive phylogenetic analysis of several European populations was undertaken by Virgilio et al. (2009), confirming the existence of 3 main lineages: (1) from northeast Atlantic coasts (from Germany to Morocco) and from

Table 1. *Hediste diversicolor*. Mean chromosome measurements (± SD) and classification in 10 metaphases. m: metacentric, sm: submetacentric, st: subtelocentric. See 'Materials and Methods' for the calculation of the centromeric index and arm ratio

Chromosome pair no.	Relative length (%)	Centromeric index	Arm ratio	Chromosome type
1	11.63 ± 0.15	45.56 ± 0.20	0.86 ± 0.08	m
2	10.18 ± 0.07	41.28 ± 0.42	0.70 ± 0.11	m
3	8.68 ± 0.09	44.37 ± 0.33	0.82 ± 0.20	m
4	7.76 ± 0.13	45.45 ± 0.23	0.84 ± 0.06	m
5	6.93 ± 0.24	39.24 ± 0.30	0.65 ± 0.09	m
6	5.89 ± 0.03	43.41 ± 0.86	0.76 ± 0.07	m
7	5.20 ± 0.17	45.26 ± 0.43	0.85 ± 0.06	m
8	4.87 ± 0.24	43.58 ± 0.50	0.77 ± 0.09	m
9	5.48 ± 0.06	33.17 ± 0.31	0.51 ± 0.08	sm
10	5.04 ± 0.15	34.57 ± 0.12	0.53 ± 0.05	sm
11	9.40 ± 0.04	18.42 ± 0.09	0.20 ± 0.16	st
12	8.24 ± 0.22	22.38 ± 0.25	0.29 ± 0.15	st
13	6.24 ± 0.30	23.17 ± 0.08	0.37 ± 0.07	st
14	4.46 ± 0.44	19.42 ± 0.12	0.24 ± 0.20	st

part of the western Mediterranean; (2) from the Mediterranean Sea; and (3) from the Black and Caspian Seas. The reduced gene flow observed among populations of this species may result from its limited dispersal capacity (Fong & Garthwaite 1994, Abbiati & Maltagliati 1996, Röhner et al. 1997), or from strong short-term selection (Fong & Garthwaite 1994), contributing to intraspecific morphological and genetic differences (Scaps 2002). Therefore, our results may represent one of the sibling species of *H. diversicolor* (possibly of the northeast Atlantic lineage following Virgilio et al. 2009).

In the present study, we observed a small supernumerary chromosome in 3 metaphases. To our knowledge, this is the first record of supernumerary chromosomes in nereidids. Supernumerary chromosomes, also called accessory or B-chromosomes in order to distinguish them from the standard A-chromosomes, were firstly detected in 1906 by Wilson (1909). Generally, B-chromosomes differ in size, form and DNA composition from the normal A-chromosome complement (Fregonezi et al. 2004). B-chromosomes can originate intraspecifically from the standard A complement or interspecifically as the result of interspecies mating (Camacho et al. 2000). Investigations of B-chromosomes in other populations of *Hediste diversicolor* and other species of Nereididae could provide new information on the genetics and evolutionary relationships within this family.

Because of the design of the present study, we cannot draw conclusions on the existence of sex chromosomes in this species, as all individuals analysed were females. It is known that the sex ratio in natural populations of *Hediste diversicolor* is female-biased (e.g. Olive & Garwood 1981, Mettam et al. 1982, Abrantes et al. 1999). For example, in populations from the Thames estuary (England) the percentage of males was estimated to be less than 10% (Dales 1950). To date, of all Nereididae species that have been studied cytogenetically, the presence of sex chromosomes was only suggested for *H. japonica* (Tosuji et al. 2004), *H. diadroma* and *H. atoka* (Sato & Ikeda 1992, Tosuji et al. 2004). However, in those studies, the presence of an XX-XY (male heterogametic) sex chromosome system was proposed based only on the subjective pairing of homologous chromosomes from Giemsa standard staining metaphases.

The comparative karyological analysis of the Nereididae species performed in this study highlights the uniqueness of *Hediste limnicola*, *H. diversicolor* and *Nereis oligohalina*, which might be explained by the presence of 3 (*H. diversicolor*) or 4 (*H. limnicola* and *N. oligohalina*) morphological categories in these species, while the remaining presented more symmetrical karyotypes with only 2 morphological categories. Species with more symmetrical karyotypes could be considered plesiomorphic. Indeed, karyotypes with a higher proportion of metacentric pairs are probably primitive and present a relative chromosomal stability (White 1978). The cytogenetic isolation of *H. limnicola* might also be corroborated by the different diploid

Fig. 3. *Hediste diversicolor*. Karyotype of a hyperdiploid metaphase (2n = 29), with a supernumerary B-chromosome

Table 2. Number of chromosomes (NC), number of morphological categories (MC), proportion of metacentric (m), submetacentric (sm), subtelocentric (st) and telocentric (t) chromosomes, Shannon diversity index calculated using the standardised relative length of chromosomes (*HL*) and index of symmetrical level of thekaryotype (SK) for each species considered in multivariate analysis

Species	NC	MC	m (%)	sm (%)	st (%)	t (%)	*HL*	SK	Source
Hediste diversicolor	28	3	57.0	14.0	29.0	0.0	2.5259	1.69	Present study
Hediste atoka	30	2	67.0	33.0	0.0	0.0	2.5447	1.00	Tosuji et al. (2004)
Hediste diadroma	30	2	87.0	13.0	0.0	0.0	2.5541	1.00	Tosuji et al. (2004)
Hediste japonica	30	2	87.0	13.0	0.0	0.0	2.5483	1.00	Tosuji et al. (2004)
Hediste limnicola	26	3	85.0	7.5	0.0	7.5	2.5239	1.09	Tosuji et al. (2010)
Nereis oligohalina	28	4	50.0	7.0	21.5	21.5	2.5191	4.07	Ipucha et al. (2007)
Perinereis anderssoni	28	2	71.0	29.0	0.0	0.0	2.5140	1.00	Ipucha et al. (2007)
Perinereis ponteni	28	2	93.0	0.0	0.0	7.0	2.4938	1.08	Ipucha et al. (2007)
Perinereis vancaurica	28	2	93.0	7.0	0.0	0.0	2.5447	1.00	Ipucha et al. (2007)
Platynereis dumerilii	28	2	79.0	21.0	0.0	0.0	2.5027	1.00	Ipucha et al. (2007)
Platynereis dumerilii 2	28	2	50.0	50.0	0.0	0.0	2.5230	1.00	Jha et al. (1995)
Pseudonereis palpata	28	2	79.0	21.0	0.0	0.0	2.4676	1.00	Ipucha et al. (2007)

chromosome number of this species. From all species analysed in the present study, *H. limnicola* is the only species able to colonise freshwater habitats. *H. limnicola* also stands apart from the remaining species due to its reproductive features, as it is the only hermaphroditic viviparous species (Smith 1950). Moreover, *Neanthes arenaceodentata* presents diploid numbers of 2n = 18 and 2n = 24 instead of the 2n = 28 commonly observed within the family (e.g. Pesch et al. 1988). Although not unique in Nereididae, this species broods its offspring in the tube (Jha et al. 1995) instead of releasing planktonic larvae. The relationship between the existence of different diploid numbers and unusual reproductive behaviour, as well as the constancy of the diploid number of the other species of the family, could suggest that interspecific changes in chromosome number within the Nereididae are related to differences in reproductive behaviour (Jha et al. 1995). However, 2 of the species analysed in the present study (*H. diversicolor* and *H. atoka*) also present similar reproductive behaviour to *N. arenaceodentata*, despite having the most common diploid number of 2n = 28. This suggests that other factors, besides diploid chromosome number, must have a role in the determination of reproductive behaviour.

The remaining species were grouped together in 2 main clusters (Fig. 4A). The first group comprised *Hediste diadroma*, *H. japonica*, *Perinereis vancaurica* and *H. atoka*, which were closely related. Although presenting a lower similarity to the above species, *P.*

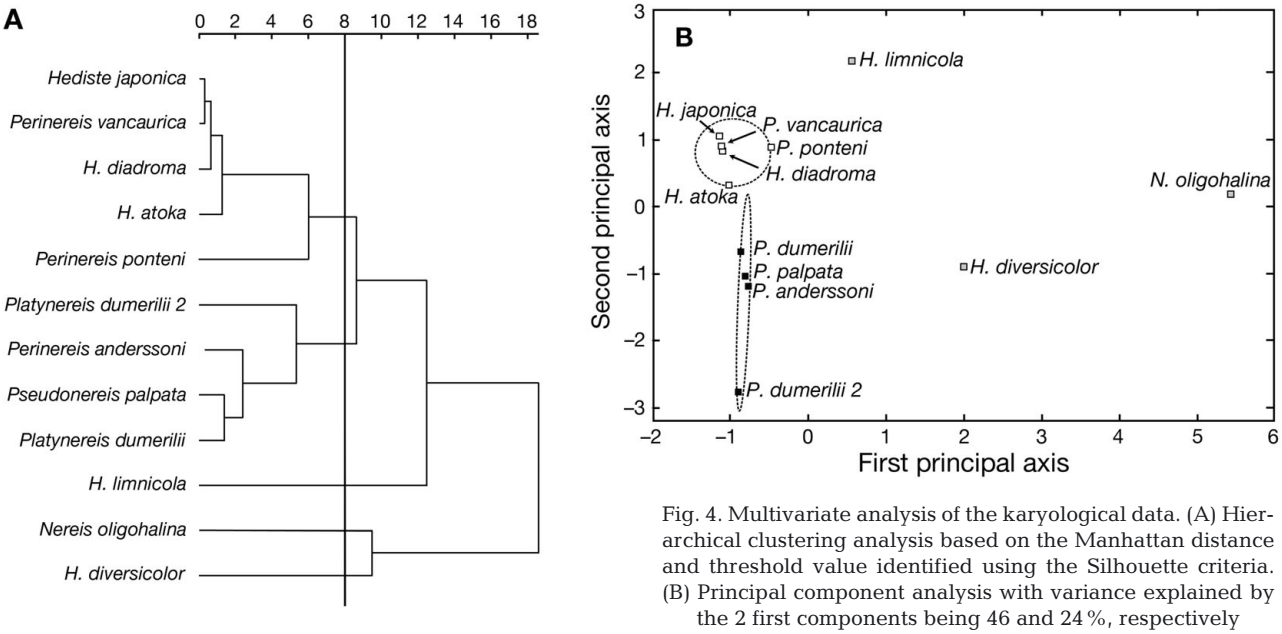

Fig. 4. Multivariate analysis of the karyological data. (A) Hierarchical clustering analysis based on the Manhattan distance and threshold value identified using the Silhouette criteria. (B) Principal component analysis with variance explained by the 2 first components being 46 and 24 %, respectively

ponteni also belonged to this major cluster. The first 4 species presented highly symmetrical karyotypes with only metacentric and submetacentric chromosomes, whilst the karyotype of *P. ponteni* was composed of metacentric and telocentric chromosomal pairs. However, all 5 species share the common feature of presenting an extremely high percentage of metacentric chromosomal pairs, higher than 90% for *H. diadroma*, *H. japonica*, *P. vancaurica* and *P. ponteni*. The 3 *Hediste* species included in this group were previously regarded as belonging to a single species, *H. japonica* (Sato & Nakashima 2003). Although they all grouped together in a main cluster, *H. japonica* and *H. diadroma* were found to be more closely related to *P. vancaurica* than to *H. atoka*. This separation, based on cytogenetic data, is also observed in reproductive behaviour. While *H. diadroma* and *H. japonica* produce planktonic larvae, development in *H. atoka* occurs without a planktonic stage (Sato & Nakashima 2003). Moreover, viable hybrid offspring have already been produced from cross-insemination experiments between *H. japonica* and *H. diadroma* (Tosuji & Sato 2006).

Although the species comprising the second cluster also presented 2n = 28 and karyotypes with only 2 morphologic categories (in this case, metacentric and submetacentric chromosomal pairs), the percentage of metacentric chromosomal pairs was lower than in the first group. *Pseudonereis palpata*, *Perinereis anderssoni* and *Platynereis dumerilii* (Ipucha et al. 2007) presented very similar proportions of the 2 chromosomal morphological categories. *P. dumerilii* 2, which corresponds to the karyotype measurements published by Jha et al. (1995), presented a lower similarity to the 3 other species due to a karyotype with a different proportion of the 2 chromosomal categories (50% metacentric and 50% submetacentric). The differences observed between the 2 studies performed in this species might be due to several causes, as stated in the 'Introduction'.

One of the most striking results of our karyological comparative analysis was the placement of 2 *Perinereis* species in different clusters. It was surprising that *P. ponteni* and *P. anderssoni* were not closely related, as the former is considered an invalid species and a synonym of the latter. Assuming that no misidentification of 1 of the species occurred, our study suggests that these are different species. A phylogenetic study of the Nereididae with pharyngeal paragnaths (Nereidinae) did not support monophyly of the genus *Perinereis* (Bakken & Wilson 2005). Moreover, the need for further taxonomic studies on groups such as *Nereis* (sensu lato), *Perinereis* and *Platynereis* was also expressed by Rouse & Pleijel (2001). Our study highlights the potential contribution of cytogenetics to taxonomic studies of polychaetes.

Acknowledgements. We thank P. Vasconcelos for collaborating in the laboratorial procedures, J. Cúrdia for the photos of *Hediste diversicolor* and P. Pousão-Ferreira and J. Guerra for supplying live specimens of *H. diversicolor*. Thanks also to the editor and 4 anonymous referees for valuable comments that greatly improved the manuscript. This study was partially supported by a post-doctoral grant to S.C. (SFRH/BPD/26986/2006), the project-PTDC/MAR/72163/2006 and the 'Programa Ciência 2007' from the Fundação para a Ciência e Tecnologia (FCT).

LITERATURE CITED

Abbiati M, Maltagliati F (1996) Allozyme evidence of genetic differentiation between populations of *Hediste diversicolor* (Polychaeta: Nereididae) from the Western Mediterranean. J Mar Biol Assoc UK 76:637–647

Abrantes A, Pinto F, Moreira MH (1999) Ecology of the polychaete *Nereis diversicolor* in the Canal de Mira (Ria de Aveiro, Portugal): population dynamics, production and oogenic cycle. Acta Oecol 20:267–283

Bakken T, Wilson RS (2005) Phylogeny of nereidids (Polychaeta, Nereididae) with paragnaths. Zool Scr 34:507–547

Banta GT, Andersen O (2003) Bioturbation and the fate of sediment pollutants. Experimental case studies of selected infauna species. Vie Milieu 53:233–248

Cabral HN (2000) Comparative feeding ecology of sympatric *Solea solea* and *S. senegalensis*, within the nursery areas of the Tagus estuary, Portugal. J Fish Biol 57:1550–1562

Camacho JPM, Sharbel TF, Beukeboom LW (2000). B chromosome evolution. Philos Trans R Soc Lond B Biol Sci 355: 163–178

Carvalho S, Barata M, Gaspar MB, Pousão-Ferreira P, Cancela da Fonseca L (2007) Enrichment of aquaculture earthen ponds with *Hediste diversicolor*: consequences for benthic and natural productivity. Aquaculture 262: 227–236

Christensen B (1980) Annelida. In: John B (ed) Animal cytogenetics, Vol 2. Gebrüder Borntraeger, Stuttgart, p 1–81

Dales RP (1950) The reproduction and larval development of *Nereis diversicolor* O.F. Müller. J Mar Biol Assoc UK 29: 321–360

Fauchald K (1977) Family revision and cladistic analysis of the Nereididae (Polychatea: Phyllodocida). Invertebr Taxon 7: 1551–1573

Fitzhugh K (1987) Phylogenetic relationships within the Nereididae (Polychaeta): implications at the subfamily level. Bull Biol Soc Wash 7:174–183

Fong PP, Garthwaite RL (1994) Allozyme electrophoretic analysis of the *Hediste limnicola* – *Hediste diversicolor* – *Hediste japonica* species complex (Polychaeta:Nereididae). Mar Biol 118:463–470

Fregonezi JN, Rocha C, Torezan JMD, Vanzela ALL (2004) The occurrence of different Bs in *Cestrum intermedium* and *C. stringilatum* (Solanaceae) evidenced by chromosome banding. Cytogenet Genome Res 106:184–188

Glasby CJ (1999) The Namanereidinae (Polychaeta: Nereididae). Part I. Taxonomy and phylogeny. Rec Aust Mus Suppl 25:1–129

Ipucha MC, Santos CG, Lana PC, Sbalqueiro IV (2007) Cytogenetic characterization of seven south American species of Nereididae (Annelida:Polychaeta): implications for the karyotypic evolution. J Basic Appl Genet 18: 15–22

Jha NA, Hutchinson TH, Mackay JM, Elliot BM, Pascoe PL, Dixon DR (1995) The chromosomes of *Platynereis dumerilii* (Polychatea: Nereididae). J Mar Biol Assoc UK 75:

551–562

Legendre P, Legendre L (1998) Numerical ecology. Developments in environmental modelling. Elsevier Science B.V., Amsterdam

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromere position in chromosomes. Hereditas 52: 201–220

Lipari R, Vitturi R, Badalamenti F (1994) Cariotipo e organizzatori nucleolari (NORs) in *Perinereis macropus* (Claparede, 1870) (Annelida, Polychatea). Biol Mar Mediterr 1:361–362

Masero JA, Perez-Gonzalez M, Basadre M, Otero-Saavedra M (1999) Food supply for waders (Aves: Charadrii) in an estuarine area in the Bay of Cadiz (SW Iberian Peninsula). Acta Oecol 20:429–434

Mettam C, Santhanam V, Havard MSC (1982) The oogenic cycle of *Nereis diversicolor* under natural conditions. J Mar Biol Assoc UK 62:637–645

Olive PJ, Garwood PR (1981) Gametogenic cycle and population structure of *Nereis diversicolor* and *Nereis pelagica* from north-east England. J Mar Biol Assoc UK 61:193–213

Ozoh PTE (1992) The importance of adult *Hediste* (*Nereis*) *diversicolor* in managing heavy metal pollution in shores and estuaries. Environ Monit Assess 21:165–171

Pesch GG, Pesch CE (1980) Chromosome complement of the marine worm *Neanthes arenaceodentata* (Polychaeta: Annelida). Can J Fish Aquat Sci 37:286–288

Pesch GG, Pesch CE, Mueller C (1988) Chromosome complements from two populations of the marine worm *Neanthes arenaceodentata* (Annelida: Polychaeta). Ophelia 28: 163–167

Röhner M, Bastrop R, Jürss K (1997) Genetic differentiation in *Hediste diversicolor* (Polychaeta: Nereididae) for the North Sea and the Baltic Sea. Mar Biol 130:171–180

Rouse GW, Pleijel F (2001) Polychaetes. Oxford Univeristy Press, Oxford

Rousseeuw P (1987) Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. J Comput Appl Math 20:53–65

Santos CSG, Lana PC (2003) Nereididae (Polychaeta) da costa nordeste do Brasil. III. Géneros *Ceratonereis* e *Nereis*. Iheringia. Ser Zool 93:5–22

Sato M, Ikeda M (1992) Chromosomal complements of two forms of *Neanthes japonica* (Polychaetea, Nereididae) with evidence of male-heterogametic sex chromosomes. Mar Biol 112:299–307

Sato M, Nakashima A (2003) A review of Asian *Hediste* species complex (Nereididae: Polychaeta) with descriptions of two new species and redescription of *Hediste japonica* (Izuka, 1908). Zool J Linn Soc 137:403–445

Scaps P (2002) A review of the biology, ecology and potential use of the common ragworm *Hediste diversicolor* (O.F. Müller) (Annelida: Polychaeta). Hydrobiologia 470: 203–218

Smith RI (1950) Embryonic development in the viviparous nereid polychaete, *Neanthes lighti* Hartman. J Morphol 87:417–465

Solé M, Kopecka-Pilarczyk J, Blasco J (2009) Biomarkers in two estuarine invertebrates, *Nereis diversicolor* and *Scrobicularia plana*, from a marsh ecosystem in SW Spain. Environ Int 35:523–531

Thiriot-Quiéveux C, Ayraud N (1982) Les caryotypes de quelques espèces de bivalves et gastéropodes marins. Mar Biol 70:165–175

Tosuji H, Sato M (2006) Salinity favourable for early development and gamete compatibility in two sympatric estuarine species of the genus *Hediste* (Polychaeta: Nereididae) in the Ariake Sea, Japan. Mar Biol 148:529–539

Tosuji H, Miyamoto J, Hayata Y, Sato M (2004) Karyotyping of female and male *Hediste japonica* (Polychaeta, Annelida) in comparison with those of two closely related species, *H. diadroma* and *H. atoka*. Zool Sci 21:147–152

Tosuji H, Togami K, Miyamoto J (2010) Karyotypic analysis of the hermaphroditic viviparous polychaete, *Hediste limnicola* (Polychaeta: Nereididae): possibility of sex chromosome degeneration. J Mar Biol Assoc UK 90:613–616

Virgilio M, Fauvelot C, Costantini F, Abbiati M, Backeljau T (2009) Phylogeography of the common ragworm *Hediste diversicolor* (Polychaeta: Nereididae) reveals cryptic diversity and multiple colonization events across its distribution. Mol Ecol 18:1980–1994

Weinberg JR, Starczak VR, Mueller C, Pesch GC, Lindsay SM (1990) Divergence between populations of a monogamous polychaete with male parental care: premating isolation and chromosome variation. Mar Biol 107:205–213

White MJD (1978) Modes of speciation. W.H. Freeman, San Francisco, CA

Wilson EB (1909) Studies on chromosomes. V. The chromosomes of *Metapodius*. A contribution to the hypothesis of the genetic continuity of chromosomes. J Exp Zool 6: 147–205

Wilson RS, Glasby CJ (1993) A revision of the *Perinereis nuntia* species group (Polychaeta: Nereididae). Rec Aust Mus 45:253–277

Editorial responsibility: Nikolaos Schizas, Mayagüez, Puerto Rico, USA

Submitted: January 15, 2010; Accepted: July 22, 2010
Proofs received from author(s): August 30, 2010