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Individual and micro-geographical variability of the marine nematode *Metachromadora vivipara* de Man 1907, (Chromadoria: Desmodorida) in the White Sea

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Summary. Males of *Metachromadora vivipara* from eight localities in the vicinity of White Sea Biological station of Moscow State University differed in the number of preanal supplements (NPS) present and in their karyotype. Karyotypes of *M. vivipara* from populations with a mean NPS greater than 20 had optional B-chromosomes. Conversely, populations of *M. vivipara* with a mean NPS less than 20 did not contain B-chromosomes. This correlation is considered evidence of a genetic basis for the polymorphism. These two features were used to distinguish the populations examined into three groups. Differences in mean NPS were stable during several years of study. Populations with similar characters had close geographical affinity and distances of only a few kilometers were sufficient for the populations to be distinct.
Key words: *Metachromadora vivipara*, karyotype, B-chromosomes, variability, White Sea.

Metachromadora vivipara de Man 1907, a morphologically highly variable species (Tchesunov & Krasnova, 1985), is one of several dominant species in the nematode community of the middle-grain sands of the White Sea intertidal zone in the vicinity of the White Sea Biological Station of Moscow State University. Specimens from eight sampling locations in the vicinity of the type locality were used to examine inter-population variation of the number of male preanal supplements (NPS). Also, karyological data were obtained to determine if differences in NPS were determined by phenotype or genetic factors. Some data are available of the chromosome numbers in marine free-living nematodes (Cobb, 1928; Timm, 1953; Malakhov & Cherdantsev, 1974; Pegova, 1995). However, in the study reported here cytogenetic data is applied to an inter-populational study to examine morphological variation in relation to differences in nematode karyotype.

MATERIALS AND METHODS

Metachromadora vivipara nematodes were collected from 8 sampling locations (Fig. 1) in the vicinity of the White Sea Biological Station of Moscow State University: 1) Kislaya Inlet; 2) inlet at the Biological Station settlement; 3) bay near the Poyakonda moorage; 4) southern shore of Velikij Island (Kandalaksha State Nature reserve); 5) Vonutchaya Inlet; 6) Biofiltry Bay; and 7 & 8) small beaches on the northern and southern shores, respectively, near Zeleny Cape. Locality 1 was sampled by E.A. Tchusova during 1978-79 and by the authors during 1988-89 and 1993-94. The other localities were sampled by the authors: locality 2 in 1988-89 and in 1993-94; locality 3 in summer 1994; localities 4 & 5 in summer 1993 and localities 6 to 8 in summer 1995. A total of 1464 male specimens recovered from all of the localities were fixed in formalin and mounted in glycerin.

The difference and similarity of the frequency distributions were analyzed using chi-squared, Student's t-test of weighted means' differences, Chekanovskii index and the Fisher's F-test of variances. The grouping cluster analysis by average distances method was used for the populations. The analyses were done using the computer programs STATGRAPHICS and SYSTAT.

A total of 15 female *M. vivipara* from locality 1, 18 from locality 2 and 12 from locality 3, all collected during 1994-95 were used for cytogenetic examination. Embryos were removed from females and kept in 0.01% colchicine (Sigma) in sea water for 2 to 4 hr and the medium subsequently replaced with 0.6%

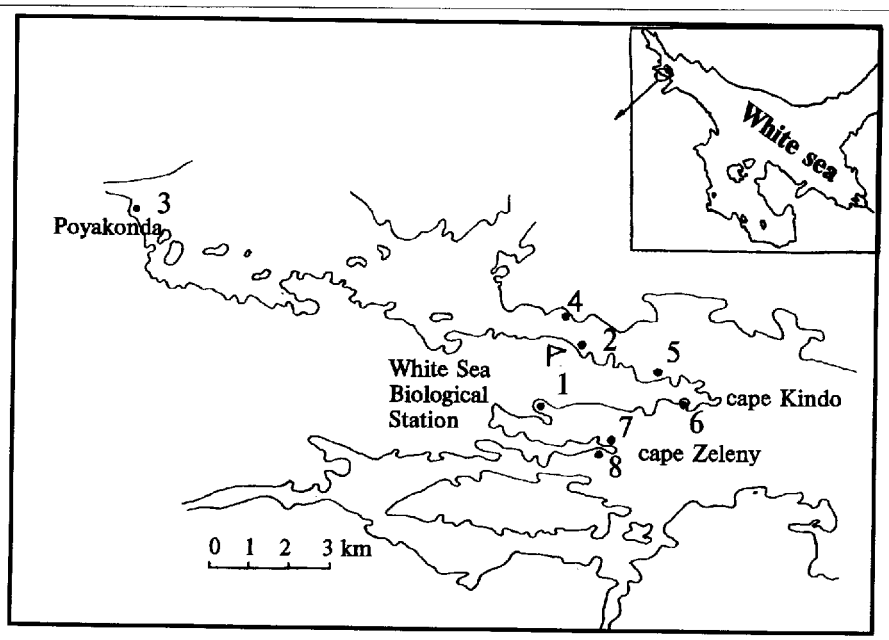

Fig. 1. Eight sampling locations for *Metachromadora vivipara* in the vicinity of the White Sea Biological Station of Moscow State University.

sodium citrate. Hypotonic treatment was performed for 4-8 min followed by fixation in cold, freshly made Carnoy's solution (3 parts ethanol: 1 part glacial acetic acid). Slide preparation was done using the standard procedure described by Kligerman & Bloom (1977). Following dissociation of the embryos with 60% acetic acid, nuclei spreads were obtained by placing drops of the cell suspension on to slides heated on a hotplate and subsequently the dried slides were stained for 15-30 min with 2% Giemsa solution.

RESULTS

The mean NPS for all examined *M. vivipara* was 20.0±0.1, the range being 12-32, with an almost normal frequency distribution. The longest temporal study and most data were obtained for localities 1 and 2 and consequently these two sites were examined in most detail. The frequency distributions of the NPS for males from localities 1 and 2 are presented in Figure 2, and both have a near normal distribution but are displaced from one another. Differences between frequency distributions and mean values were statistically significant at a level of $P<0.05$.

Long-term variations for the mean NPS for males from locations 1 and 2 are presented in Table 2 and Figure 3. The confidence intervals of the mean NPS calculated for location 1 were 18.1-20.1 and for location 2 were 19.2-22.8. The intervals for the mean values did not overlap, an exception being for specimens from location 2 collected in 1988. *M. vivipara* produce two generations per year, summer and winter (Tchesunov & Krasnova, 1985) and seasonal difference in NPS between the summer (May to September) and winter (January to March) generations were not significant at a level of $P<0.05$. Almost normal frequency distributions were evident for the NPS for locations from 1 to 8 and the mean NPS for these localities are given in Table 1.

The various statistical analyses used produced similar results and revealed the presence of three groups of populations. Group I is represented only by the population from location 1 which had a mean NPS less than 20.0, and the lowest intra-population variation. Populations from locations 3, 7 and 8 comprised Group II with the mean NPS being approximately 20.5. Group III is comprised of populations from locations 2, 4, 5 and 6, with their mean NPS exceeding 21 and these populations had the the largest NPS variation (Fig. 4).

The karyotype of *M. vivipara* was found to contain 14 chromosomes (n=7; 2n=14). The first and second pairs of chromosomes were most different from the others due to their large size. The III-V chromosome pairs were of almost identical size which makes their individual identification difficult. The relative sizes of chromosomes (ratio of the length of each chromosome to the sum of the lengths of the haploid as a percentage) were: I - 22%; II - 15%; III, IV, V - 14%; VI - 12%; VII - 9%. The karyotype of *M. vivipara* from locality 1 was as described above, with the diploid number of 14 chromosomes being constant. The karyotypes of some *M. vivipara* from localities 2 and 3 were the same as that obtained for specimens from localitiy 1, however, several specimens from these two localities contained one or two additional B-chromosomes. The relative size of the

Table 1. The mean number of preanal supplements (NPS) in *Metachromadora vivipara* males from eight locations.

Location	NPS	Total number of males
1	19.3±0.1	543
2	21.0±0.2	314
3	20.3±0.5	73
4	21.3±0.5	88
5	21.5±0.3	46
6	21.5±0.2	292
7	20.2±0.3	100
8	20.0±0.4	79

Table 2. Temporal variation in the mean number of preanal supplements (NPS) in *Metachromadora vivipara* males from locations 1 and 2.

Year	Location 1		Location 2	
	NPS	Total number of males	NPS	Total number of males
1978	18.6±0.5	48	–	–
1979	18.8±0.3	118	–	–
1988	19.6±0.5	109	20.6±1.4	24
1989	19.5±0.4	89	22.2±0.6	62
1993	19.1±0.6	47	21.1±0.7	50
1994	19.4±0.3	132	20.7±0.3	178

supernumerary chromosomes was about 3.0%. The karyotypes with and without the B-chromosomes are shown on Figure 5.

DISCUSSION

Most putative species belonging to the *Sabatieria pulchra* - group are considered different ecophenotypes (Vincx, 1986) as they inhabit different habitats in the same geographic area and have only minor differences in NPS. Most *Metachromadora* species vary in their NPS (Gerlach, 1951) and such variation may be correlated with geographical location. Blome (1974) reported difference in NPS between males of *M. quadribulba* Gerlach 1956. Specimens from the French Atlantic described by Gerlach had 23 preanal supplements whereas specimens from Sylt Island in the North Sea had 26-27.

As a result of the natural variation in the NPS of *Neochromadora paraminuta* (Boucher, 1976) and *N. minuta* Lorenzen, 1972, Vincx (1986) synonymized the two species. The NPS differs in these two morphotypes with males in some localities having 9 supplements, in others 12, and in several localities examples of both types were reported. Polymorphism in the NPS was present in populations of *M. vivipara* examined in the present study, with the NPS being relatively constant with time and season. Each population in the study could be distinguished by the frequency distribution, mean value and variation in the NPS. Also, variation in the NPS of *M. vivipara* was correlated with differences in the karyotypes. B-chromosomes were absent in the population of *M. vivipara* with a mean NPS < 20 but were present in the group of populations with a mean NPS > 20.

The variation in NPS could be used to separate the populations of *M. vivipara* into three groups. The first group, represented only by population 1, had a mean NPS of less then 20 and chronological changes in frequency distribution, mean value and variance range of the NPS were considerably less in this than in other populations. In the other seven populations the average NPS was greater than 20. Group 2 was comprised of three populations (3, 7, and 8) with a mean NPS of 20.5, whereas Group 3 consisted of four populations (2, 4, 5, and 6) with a mean NPS of 21 or greater. The three groups of populations corresponded closely to their geographical location. Population 1 was from Kislaya Inlet which is located rather distantly from all the other populations and this population had the largest differences e.g. absence of B-chromosomes, relatively smaller NPS. Populations 7 and 8, which were similar to one another, came from locations on different sides of one cape, divided by a ridge. Populations 2, 5 and 6 were from locations along one coast of a wide strait and population 4 came from a locality situated on the opposite side of this strait. These four populations were similar. Population 3 came from a geographically more distant site with the nearest populations, from which it differed by its wide variability, being 7 and 8 . Thus, a distance of only a few kilometres was

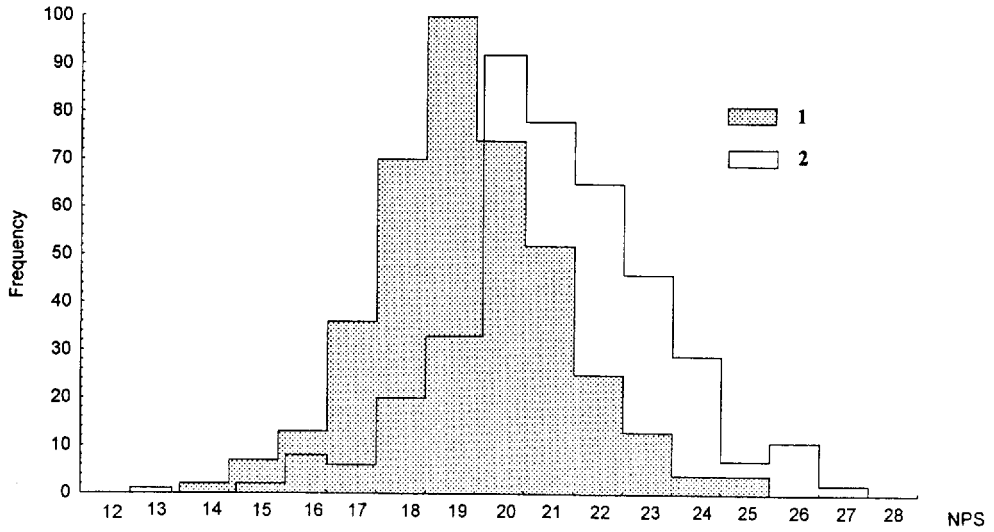

Fig. 2. Frequency distributions of the number of preanal supplements (NPS) in *Metachromadora vivipara* males from locations 1 (1) and 2 (2); (Y-axis, frequency; X-axis, NPS).

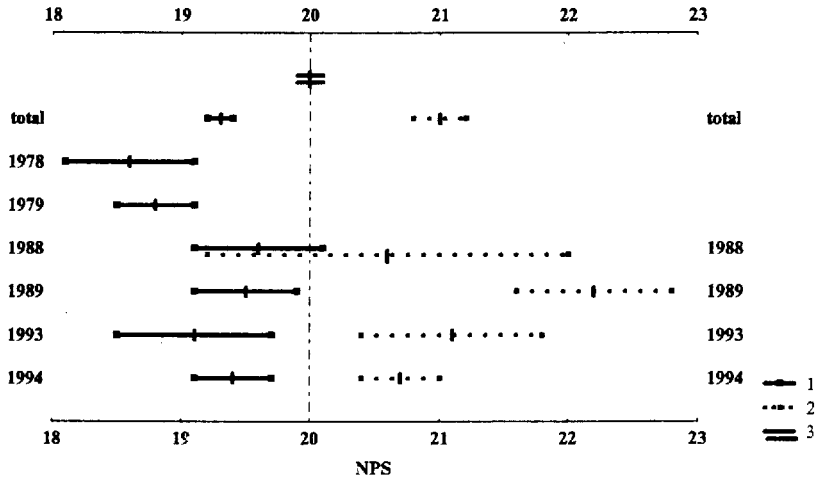

Fig. 3. Confidence intervals of the mean number of preanal supplements (NPS) in *Metachromadora vivipara* males from locations 1 (1) and 2 (2) collected in different years and (3) for the total number of male specimens recovered from eight locations examined in the study. Hatched line indicates 20 preanal supplements.

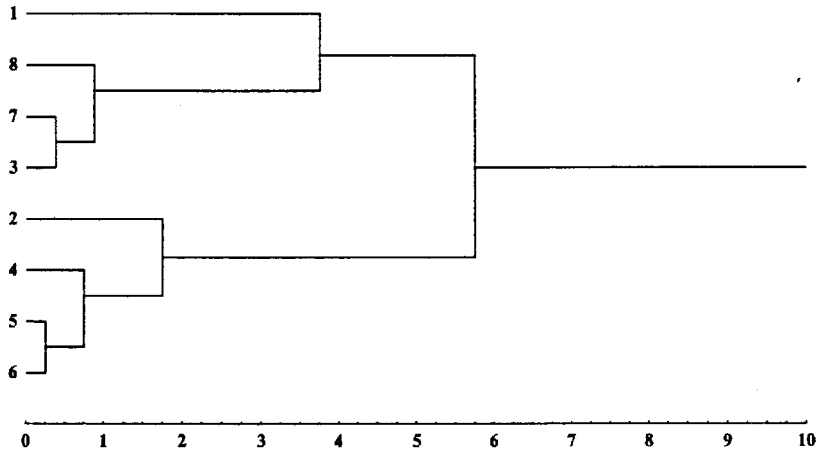

Fig. 4. Clustering of *Metachromadora vivipara* populations from 8 locations using the Student's t-test values. (X axis, Student's t-test values).

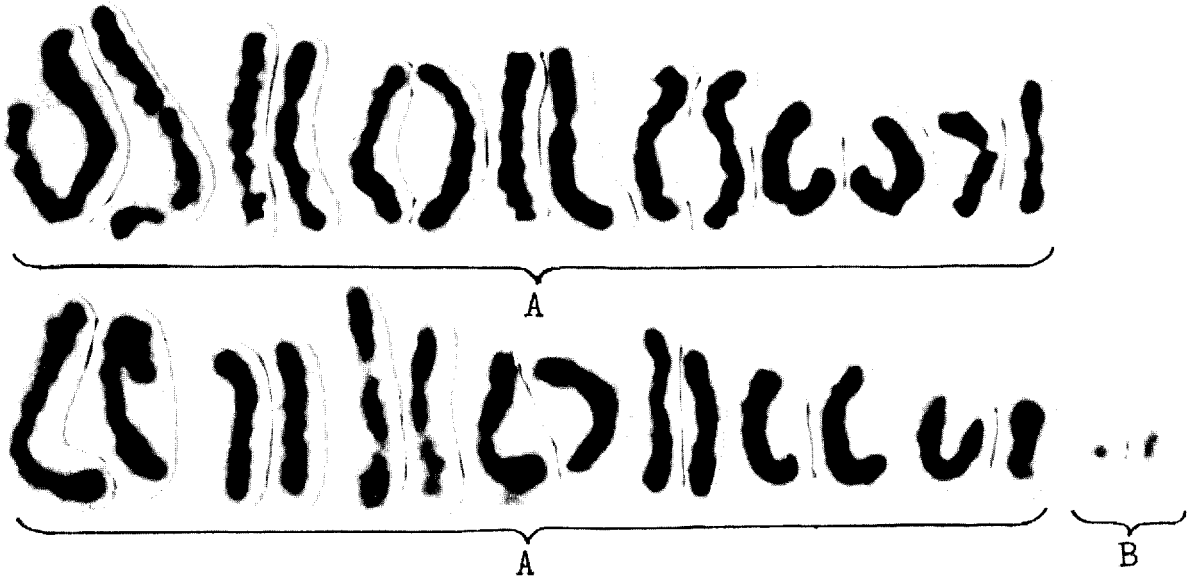

Fig. 5. Karyotypes of *Metachromadora vivipara*: 1) location 1; 2) locations 2 and 3. A: A-elements; B: Supernumerary B-chromosomes.

apparently sufficient for two morphotypes to evolve independently.

Males use their preanal supplements during copulation and it appears possible that differences in the NPS can influence this behaviour, resulting in the development of an isolation mechanism for the nematodes.

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Краснова Е. Д., Пегова А. Н. Индивидуальная и микрогеографическая изменчивость морской нематоды *Metachromadora vivipara* de Man, 1907 (Chromadorida: Desmodorida) в Белом море.
Резюме. Показано, что самцы *Metachromadora vivipara,* собранные в восьми пунктах вокруг Беломорской биологической станции Московского Государственного Университета, различаются по числу преанальных супплементов (ЧПС) и по кариотипу. Кариотипы *M. vivipara* со средним значением ЧПС более 20 часто характеризуются присутствием В-хромосомы. Напротив, популяции *M. vivipara* с средним ЧПС менее 20 никогда не имеют В-хромосому. Это соответствие рассматривается как генетическая основа наблюдаемого полиморфизма. Два данных признака были использованы для дифференциации исследованных популяций, которые оказались принадлежащими к трем основным группам. Различия в среднем значении ЧПС оказались достаточно стабильными на протяжении нескольких лет исследований. Популяции со сходными особенностями оказались наиболее близкими друг к другу географически, и расстояния в несколько километров было достаточно для существования различных популяций.