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THE *GYRATRIX HERMAPHRODITUS* SPECIES-COMPLEX (PLATYHELMINTHES KALYPTORHYNCHIA) IN MARINE TROPICAL AREAS : FIRST DATA FROM THE CARIBBEAN

by

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SUMMARY

The finding of specimens belonging to the *Gyratrix hermaphroditus* species-complex (Platyhelminthes Kalyptorhynchia) in marine habitats of Puerto Rico and Bermuda is reported. They differ for karyotype and for details of the sclerotized organs, and are tentatively attributed to four sibling species.

INTRODUCTION

The *Gyratrix hermaphroditus* Ehrenberg, 1831 species-complex is represented in Western Europe by several sibling species, karyologically and ecologically distinct : species with $n=3$ are marine, whereas species with $n=2$ are found in fresh water. On the basis of outgroup comparisons, the haploid set formed by three isobrachial metacentric chromosomes and the marine habitat have been assumed to be plesiomorphic. Sets with $n=2$ have been interpreted as derived from a Robertsonian mechanism of fusion (Curini-Galletti and Puccinelli, 1989 ; Puccinelli and Curini-Galletti, 1987 ; Puccinelli *et al.*, 1990).

While the few data available from fresh water habitats in areas other than W. Europe (Russia (Birstein, 1991) ; N. Australia (Curini-Galletti and Puccinelli, 1990)) confirm the above picture, this clear cut scenario has been challenged by results on the composition of the species-complex in a tropical marine area (Darwin, Northern Territory, Australia) (Curini-Galletti and Puccinelli, 1990). There, both species groups with $n=2$ and $n=3$ have been found in intertidal habitats. However, the area around Darwin presents wide fluctuations of salinity (from 6 to 41 ‰), with coastal habitats being brackish for part of the year. Adaptations to local ecological conditions could result in the coexistence of the two species groups, as the high diversity of Platyhelminthes found in brackish waters has been shown to be the result of overlapping of marine and fresh-water species (Armonies, 1988a). More data from strictly marine tropical areas are therefore needed before any generalisation on the evolutionary biology of the group can be formulated.

Research on Platyhelminthes in Puerto Rico and Bermuda revealed few specimens of the *G. hermaphroditus* species-group ; although very limited, data are interesting, being the first contribution from tropical habitats without wide fluctuations of salinity.

MATERIAL AND METHODS

Samplings were performed in marine habitats off SW Puerto Rico (in the proximity of the Magueyes Marine Biological Station) in December 1988 and in Bermuda in August 1992. Specimens of the *Gyratrix hermaphroditus* species-group were found in two stations off Magueyes :

i) Corona de Pietra, $-4/-6$ m, poorly sorted silty medium sand (one specimen, Port 1),

ii) Turrumote Cay, exposed beach, intertidal, poorly sorted granule (two specimens, Port 2 ; Port 3).

In Bermuda, four stations yielded specimens of the group :

i) Flatts Inlet, intertidal, fine silty sand with vegetal debris (one specimen, Berm1) ;

ii) Flatts Inlet, lower intertidal, very poorly sorted medium sand (one specimen, Berm 2) ;

iii) off Non-Such Island, -10 m, fine sand (one specimen, Berm 3) ;

iv) Spanish Point, $-0.5/1$ m, poorly sorted medium sand with shell debris (one specimen, Berm 4).

Samples were collected by scooping the upper sediment layers ; extraction was done in laboratory using the $MgCl_2$ technique (Martens, 1984).

Measurements were taken for each specimen from camera lucida drawings of the two sclerotized pieces of the male copulatory apparatus (stylet and sheath) from semi-squashed mountings. For karyological purposes, specimens were placed in 0.2 % colchicine solution in sea water for 6-8 h, transferred into a 2 % acetic acid solution for about 1 min, stained with lactic acetic orcein for 3-5 min, and squashed. Relative lengths (r.l. = length of chromosome × 100/total length of haploid genome) and centromeric indices (c.i. = length of the short arm × 100/ length of entire chromosome) were obtained from measurements of camera lucida drawings of 5-10 spermatogonial mitoses for each specimen. Idiograms (Fig. 1) are based on mean values given in Table 1. Chromosome nomenclature is that of Levan *et al.*, 1964.

Data were subjected to a cluster analysis using NTSYS-pc, version 1.60 (Rohlf, 1990) (coeff. : average taxonomic distance ; method : UPGMA), based on the following parameters for each specimen : absolute length and centromeric index of each chromosome, length of stylet, and length of sheath.

TABLE 1

Karyometric and morphological data (means ± standard deviation) of the specimens of the *Gyratrix hemaphroditus* species-complex found in Puerto Rico and Bermuda. Nomenclature : m = metacentric ; st = subtelocentric.

Specimen		Chromosome			Haploid genome length (µm)	Stylet length (µm)	Sheath length (µm)
		1	2	3			
PORT 1	r.l.	37.69 ± 1.72	33.35 ± 1.64	28.96 ± 0.67	56.1 ± 3.5	186	179.5
	c.i.	46.70 ± 1.30	45.07 ± 2.41	44.29 ± 2.90			
	nom.	m	m	m			
PORT 2	r.l.	37.19 ± 1.30	33.17 ± 1.11	29.64 ± 1.82	15.4 ± 4.6	90	75.5
	c.i.	47.05 ± 1.21	43.54 ± 3.58	42.12 ± 2.33			
	nom.	m	m	m			
PORT 3	r.l.	58.73 ± 2.32	41.27 ± 2.32		16 ± 6.2	111.1	90
	c.i.	46.31 ± 1.56	22.78 ± 2.73				
	nom.	m	st				
BERM (4 specimens)	r.l.	37.51 ± 2.06	33.91 ± 2.35	28.57 ± 1.98	15.5 ± 3.9	121.5 ± 4.1	100.6 ± 4.5
	c.i.	46.37 ± 1.88	47.37 ± 2.64	46.85 ± 3.01			
	nom.	m	m	m			

RESULTS

Samples from both islands yielded few specimens of the *Gyratrix hermaphroditus* species-group (three in Puerto Rico and four in Bermuda). The three specimens from Puerto Rico differed markedly from each other either for chromosome numbers or for genome length and size of the sclerotized organs (Table 1 ; Figs 1, 2 A-F).

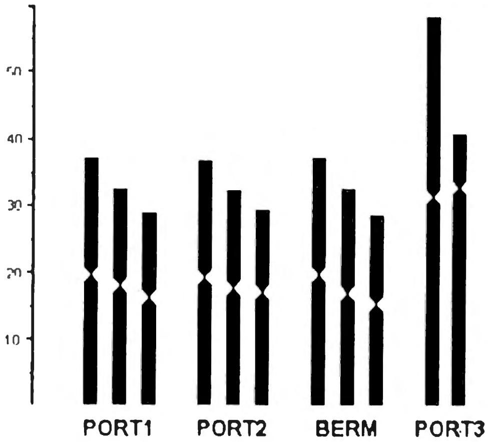

Fig. 1. — Idiograms based on the karyometric data given in Table 1.

Port 1 has three large metacentric pairs and large sclerotized organs.

Port 2, although similar to the former in the relative length and centromeric indices of the three chromosomes, has markedly smaller genome length (nearly one quarter) and sclerotized organs (nearly half the size).

Port 3 has n = 2, with one large metacentric and one smaller subtelocentric ; it has a small genome length and small copulatory organs.

The four specimens from Bermuda (Berm) do not differ appreciably among themselves as to the parameters above, and are grouped together in Table 1. They are similar to Port 2 for size and shape of chromosomes ; the sclerotized organs are, however, larger (Fig. 3 A-B).

In the cluster analysis performed on both cuticular and karyological data (Fig. 4), the specimens from Puerto Rico are markedly separated from each other, while the specimens from Bermuda are grouped together with Port 2 into a loose cluster.

Fig. 5 presents the result of a cluster analysis performed on the cuticular data of the specimens above plus four additional Bermudian specimens (K1-K4), whose biometric data are known (Karling, 1978). Though some differences are evident (most noticeably in the position of Port 3, isolated in Fig. 4 due to its unique

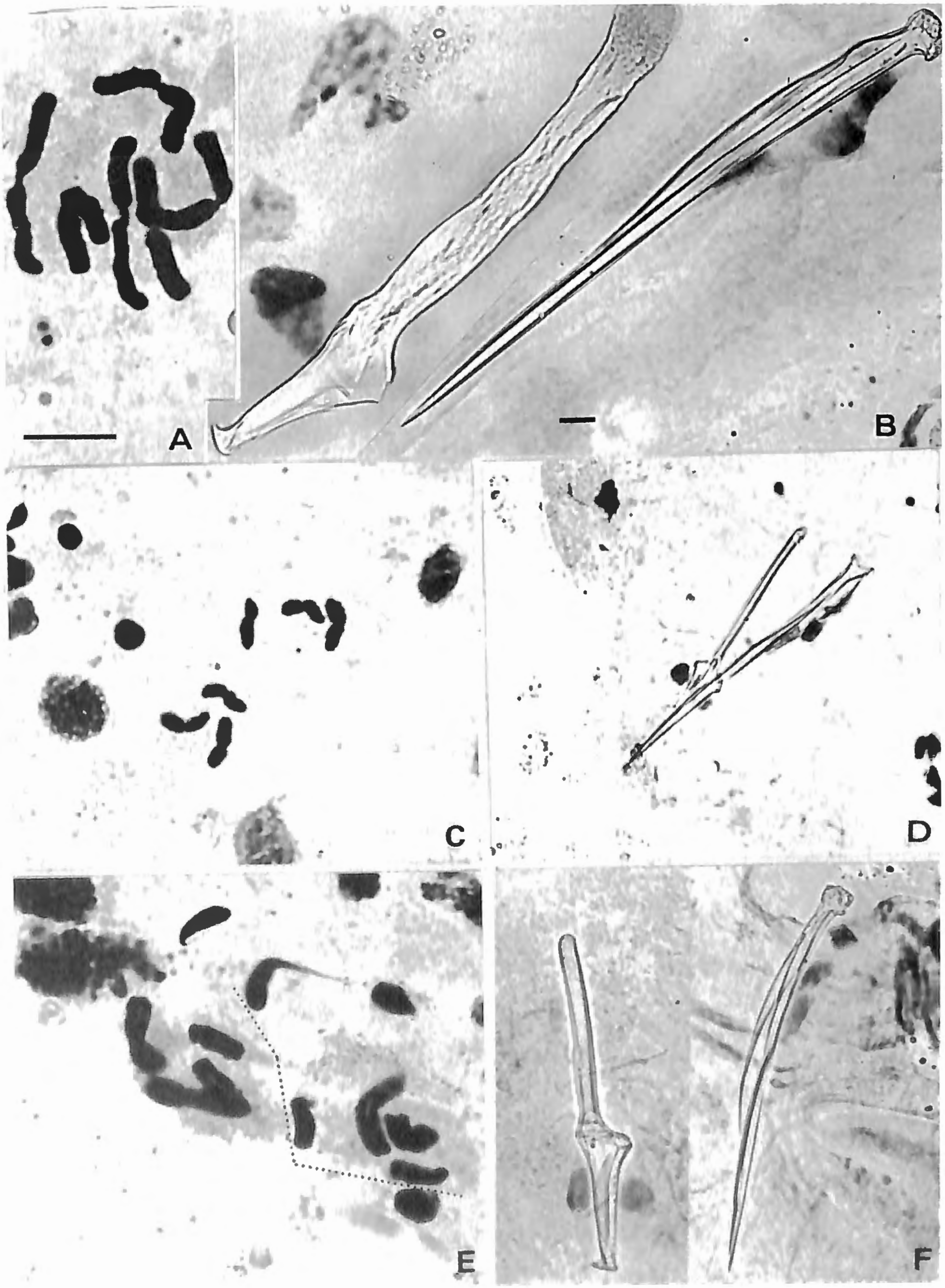

Fig. 2. — A-F, metaphase plates and male sclerotized organs of the three specimens of *Gyratrix hermaphroditus s.l.* from Puerto Rico. A-B, Port 1 ; C-D, Port 2 ; E-F, Port 3. Scale bars = 10 µm

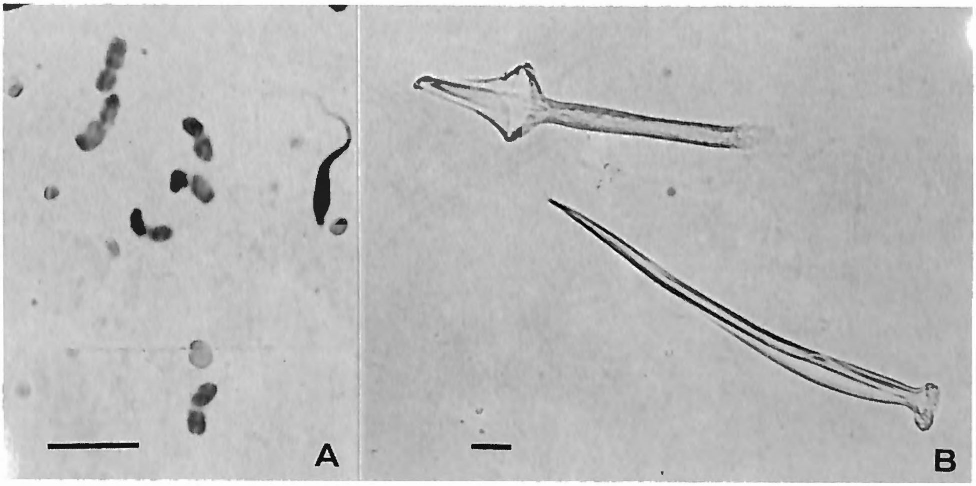

Fig. 3. — A-B, metaphase plate and male sclerotized organs of *G. hermaphroditus s.l.* from Bermuda. Scale bars = 10 µm.

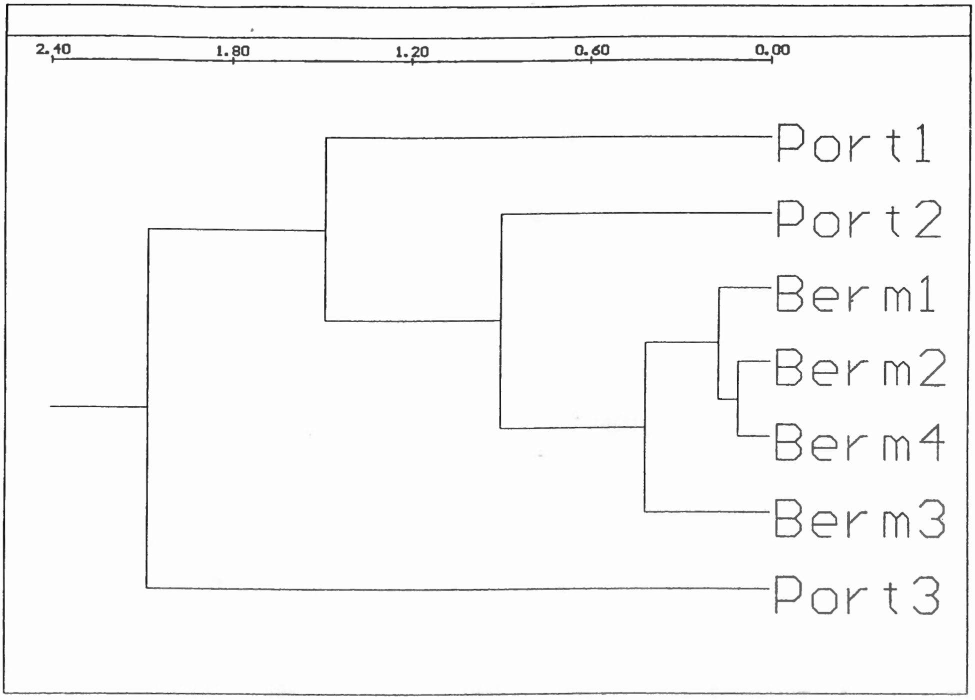

Fig. 4. — Cluster analysis performed on karyometrical and morphological data (see Table 1).

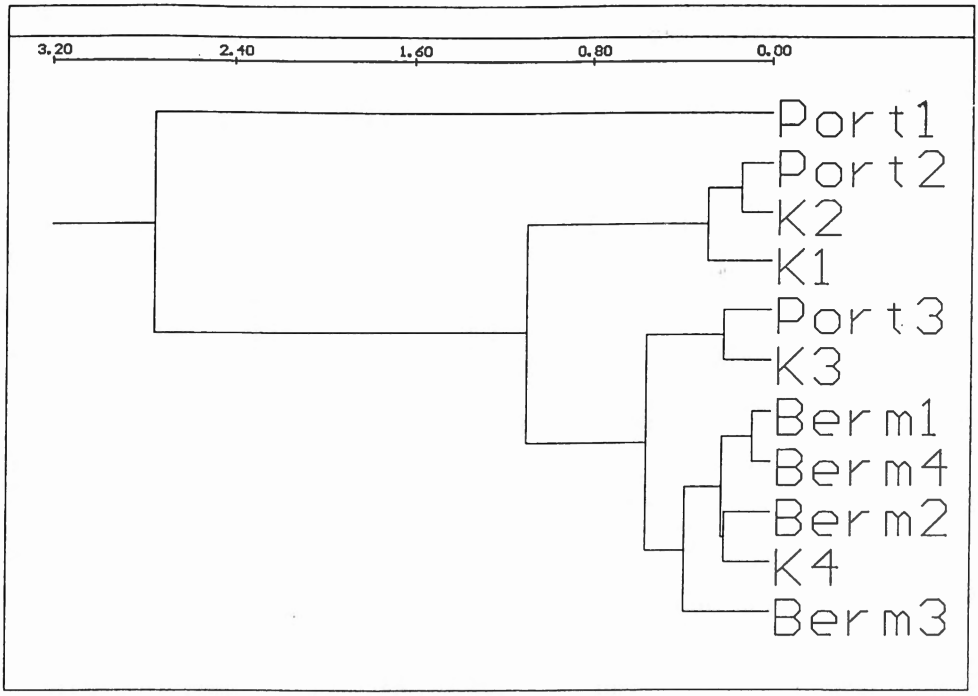

Fig. 5. — Cluster analysis performed on morphological data, including the specimens from Bermuda (K1-K4) described by KARLING (1978).

chromosome number), the number of clusters is comparable to the previous analysis.

DISCUSSION

Albeit the sample of the *Gyratrix hermaphroditus* species-complex from the Caribbean is limited and most certainly under-representative, it nonetheless allows some considerations :

Relationships among the n = 2 and n = 3 species-groups

Both in Puerto Rico and in Bermuda the stations sampled are not expected to withstand anything but negligible variations in salinity. The finding of one species with n = 2 in a truly marine environment in Puerto Rico confirms that in tropical areas the ecological separation between the two species groups observed in Europe is absent. The phenomenon is possibly related to the different level of species diversity in the areas considered. In Europe, in fact, marine species of the complex mostly occur singly, with one record only (Roscoff, Brittany) of two species occurring sympatrically (CURINI-GALLETTI and PUCCINELLI, 1989). On the contrary, samples from tropical areas have revealed a much higher number of sympatric species : eight in Darwin, at least three in Puerto Rico (where each specimen found belonged to a different species). Our sample from Bermuda is under-representative as well : KARLING (1978), sampling in different stations, possibly found two additional species (Fig. 5). A high number of sympatric species (at least seven) has also been found in the Northern Red Sea (CURINI-GALLETTI and PUCCINELLI, umpubl. data). All the sympatric species of the group known so far differ markedly either in size and shape of the sclerotized organs and/or in their karyotype (possible pre- and post-zygotic isolation mechanisms). Karyotype differentiation in the group usually proceeds through genome size growth and/or chromosome rearrangements, such as pericentric inversions and translocations (PUCCINELLI *et al.,* 1990). Chromosome fusion might be a further karyological evolution mechanism, evolved in areas of high selection for differentiated karyotype, *i.e.* areas with high species number, and thus a possibly homoplasous character. One or more lines in the group with n = 2 eventually managed to colonise fresh waters, spreading world-wide.

Relationships among the Caribbean species

Port 1 and Port 2 represent a striking example of differentiation between sibling species of the group. Port 1 has been found in mixed silty substrate, where most of the meiofauna was of the burrowing kind, thus comparatively large and stocky; Port 2 occurred in clean coarse sediments, and is a typical small interstitial organism. Their difference in body size is reflected by karyology : relative karyometric data are nearly identical, but the genome length is nearly four times larger in Port 1. Since these organisms are basically eutelic, an increase of genome and cell size is an effective means for attaining larger dimensions, and thus exploit different habitats. The sclerotized organs, whose definitive length — at least as far as the stylet is concerned — is attained within the cocoon, and is not related to the age of the animals (KARLING, 1963 ; CURINI-GALLETTI and PUCCINELLI, pers. obs.), are accordingly smaller in Port 2.

On the basis of data available, it is rather unclear whether or not Berm is a species distinct from Port 2 (see Fig. 4). They are karyologically nearly identical, though the difference in size of the sclerotized organ is remarkable. KARLING'S (1978) finding in Bermuda of specimens with sclerotized organs nearly identical to Port 2 (see Fig. 5) seems to suggest that Berm and Port 2 are indeed distinct and that the two species occur sympatrically in Bermuda. Furthermore, one of KARLING'S specimens is nearly identical to Port 3 — though the lack of karyological data does not allow further speculations.

As a rule, Bermuda is poorer in number of marine species than the rest of the Caribbean. This is reflected in the specific composition of another group of mesopsammic Platyhelminthes, the Proseriata (CURINI-GALLETTI, 1991), as well as by the marine fauna as a whole(STERRER, 1986), and is reasonably connected with the problems of colonisation of a relatively distant, minute oceanic island, enhanced, in the case of interstitial organisms, by dispersal limitation caused by direct development and low adult vagility. In the *G. hermaphroditus* species group, however, the number of species found in Bermuda and in Puerto Rico seems comparable. Since the sample of Bermuda is larger, results might be biased. However, *G. hermaphroditus* specimens are often found in the periphyton and are known to actively emerge into the water column (ARMONIES, 1988b). This can effectively result into larger distributions than strictly mesopsammic species.

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