Cah. Biol. Mar. (1990), **31** : 463-472
Roscoff

Quantification of characters from live observations in meiobenthic Turbellaria-Macrostomida.

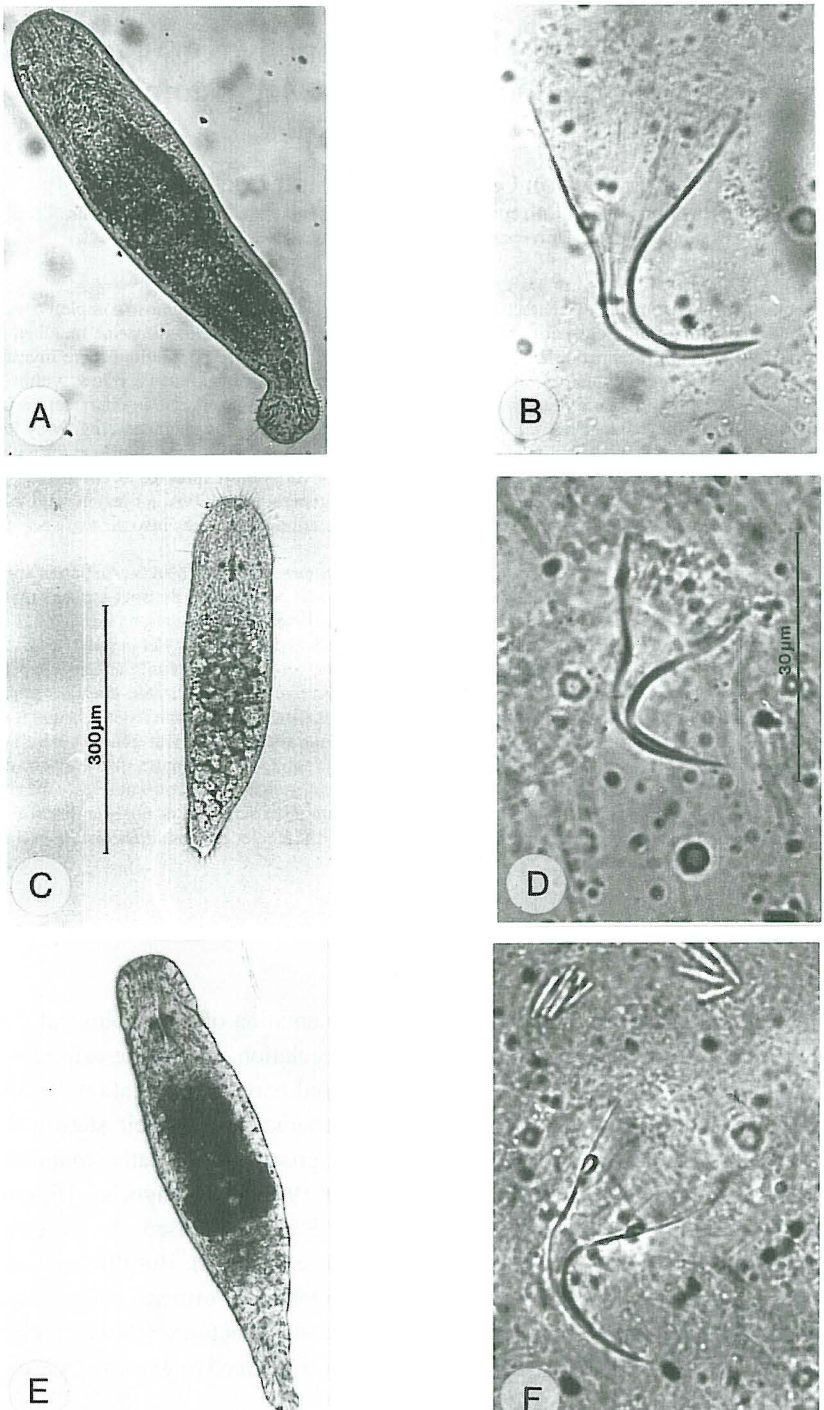
Marion Gelhen & Dr. Arnulf Lochs
Institut of Zoology - University of Innsbruck, Technikerstr. 25, 6020 Innsbruck
Computer-Center, University of Innsbruck, Technikerstr. 13, 6020 Innsbruck

Abstract : Quantitative estimates of character variability in Microurbellaria are almost completly wanting. This paper describes the within- and between population variability of three closly related marine meiobenthic species of the genus *Macrostomum* (Macrostomaida). Wild populations and laboratory populations were investigated. For sclerotic hard structures (eg. copulatory stylet), the present study corroborates the very low within population variability as suggested by one earlier study for the same order of Turbellaria. A rise in character variability was seen in laboratory cultures. Unexpected was rthe low variability of non-sclerotic characters (eg. body length, body width, position of female gonopore and eyes), which generally were thought to vary significantly within populations, but were shown in this study to have less or equal amounts of variation (expressed as coefficient of variability) as the same parameter of hard-bodied meiobenthic species. A general MANOVA, a t-test as well as a stepwise discriminant analysis show that such parameters allow a clear seperation of the three investigated species.

Résumé : Les estimations quantitatives de la variabilité des caractères chez les Mircoturbellariés sont pratiquement inexistantes. La présente étude décrit les variations au sein des populations de trois espèces marines meiobenthiques du genre *Macrostomum* (Macrostomida), étroitement parentes. Des populations sauvages et des populations élevées au laboratoire ont été étudiées. Pour les structures sclérosées dures (stylet copulateur, p. ex.), l'étude confirme la variabilité très faible à l'intérieur des populations, suggérée par une étude antérieure portant sur le même ordre de Turbellariés. Une variabilité plus grande des caractères a été constatée chez les populations de laboratoire. Une découverte inattendue était la faible variabilité des caractères non sclérosés tels que longueur, largeur des individus, position du gonopore femelle et des yeux, pour lesquels on avait généralement supposé une variation significative à l'intérieur des populations, alors que la présente étude montre que le degré de variation (exprimé par le coefficient de variabilité) est inférieur ou égal au même paramètre chez les espèces meiobenthiques à cuticule. Une analyse de variance à plusieurs variables, un test t ainsi qu'une analyse discriminatoire successive ont mis en évidence que de tels paramètres permettent de différencier nettement les trois espèces étudiées.

Introduction

In order to achieve more objectivity in the documentation of morphological data and to provide a better understanding of the processes of speciation, species descriptions in meiofauna should include numerical estimates of within and between population variability (see e.g. Westheide & Rieger 1987). Quantification of characters and their statistical analysis have been used increasingly in various meiobenthic groups (e.g. Gnathostomulida, Sterrer 1977 ; Gastrotricha, Hummon 1974, 1977, Ruppert 1977 ; Kinorhyncha, Higgins 1985 ; Nematoda, Geraert 1983 ; Tardigrada, Pollock 1970, Kristensen & Higgins 1984 ; Annelida, Westheide & Rieger 1987, Specht & Westheide 1988). But this approach is still rare in species identifications and descriptions of Turbellaria. Furthermore, most numerically estimated data of Turbellaria concern only sclerotic structures (former cuticular hard structure see Karling 1986 for terminology) of the reproductive system (see e.g. Rieger 1977, Heitkamp & Schrade-Mock 1977, Heitkamp 1982).


A
B
C
300µm
D
30µm
E
F

This study investigates whether, in addition to sclerotic structures, non-sclerotic parameters can be obtained reliably and accurately enough in living microturbellaria to characterise within and between population variability. The use of characters enumerated in this way for discrimination of closely related species is ascertained for marine species of the genus Macrostomum. A taxonomic revision of these forms, the Macrostomum hystricinum-species group (see Rieger 1977) will be published separately.

MATERIAL AND METHODS

Four populations of *M. hystricinum marinum* Rieger, 1977 (Fig. 1 a), *M.* cf. *beaufortensis* Ferguson, 1937 (Fig. 1 b) and *M. pusillum* Ax, 1951 (Fig. 1 c) have been compared. The samples of *M. hystricinum* marinum Rieger, 1977 and *M.* cf. *beaufortensis* Ferguson, 1937 have been taken from laboratory populations. These experimental populations have been cultured since 1984 without adding new individuals from the field (see Rieger *et al.*, 1988). In Summer 1988, samples of *M. pusillum* Ax, 1951 have been taken at Königshafen (Möwenbergwatt) and Rantum, Sylt (FRG). The two populations of Königshafen and Rantum are separated since 1927, through the construction of the Hindenburgdamm.

Observations were carried out on life animals after relaxing them in isotonic $MgCl_2$, using a Reichert Biovar equipped with phase contrast, a drawing tube and a semiautomatic microphotographic unit. All measurements except those of the sclerotic parts were taken without squeezing the specimens. The parameters chosen for the numerical description are shown in Fig. 2.

In order to estimate character variability, the coefficient of variation (CV ; standard deviation in % of mean) were calculated for each character and each population. The samples were tested for significance between population variability by general MANOVA and a t-test. All statistical treatments were performed at the Computer Center of the University of Innsbruck using the BMDP program.

RESULTS

The CVs of all variables studied (Tabl. I) range from 4.6 % (length of stylet, *M. pusillum* Möwenberg population) to 47.9 % (distance between eye spots, *M.* cf. *beaufortensis*).

Fig. 1 : Microphotographs of the whole animal (slightly squezzed) and the stylet (strongly squeezed) of the three species studies.
A) B) = *M. hystricinum marinum*,
A = a male mature specimen from the cultures
C) D) = *M.* cf. *beaufortensis*,
C = a subadult specimen from the cultures
E) F) = *M. pusillum*, wild population, Sylt.

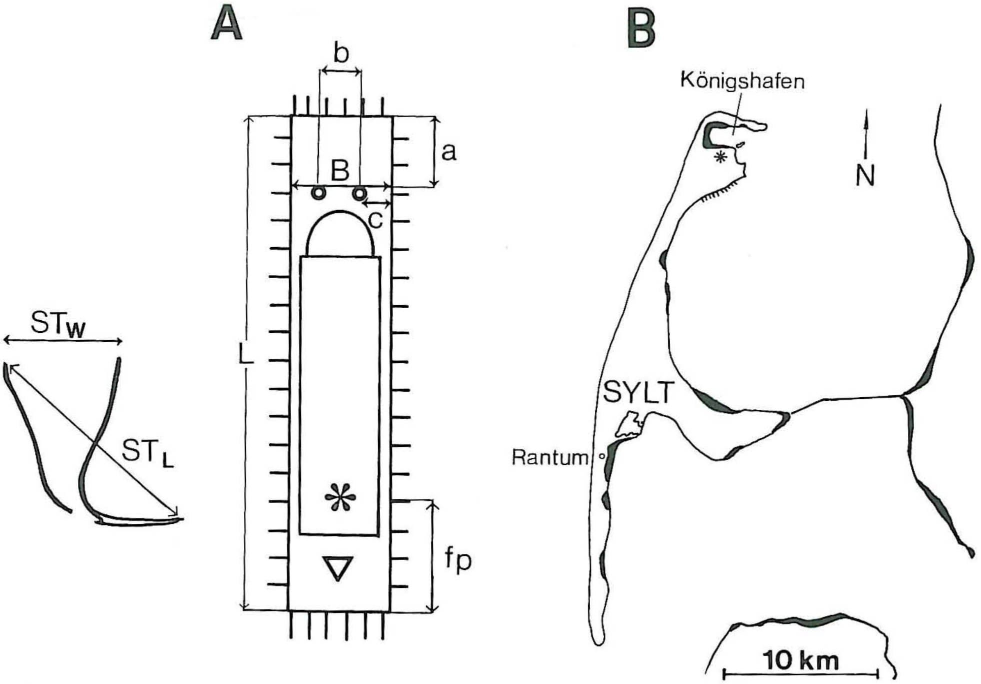


Fig. 2 : A. Position of the characters measured by life observations, illustrated in diagrammatic scheme of *M.* cf. *beaufortensis*.
L = total body length
B = body width
a, b, c = position
fp = female porus
St_l = stylet length
S_{tw} = stylet width
B. Location of the two populations of *M. pusillum* on Sylt.

Of the 8 investigated variables, those of sclerotic hard structures show the lowest range of coefficient of variation (CV 4.6 - 28.1 %, Tabl. I).

General MANOVA (Tabl. II) showed the populations to be significantly different. A t-test showed *M. hystricinum marinum* Rieger, 1977 to differ significantly in all variables studied ($p \leq 0.001$), whereas *M.* cf. *beaufortensis* Ferguson, 1937 differed from *M. pusillum* Ax, 1951 in body length, stylet dimension and the position of the female genital porus ($p \leq 0.001$). On the other hand, the two populations of *M. pusillum* Ax, 1951 were only slightly different in body length ($p \leq 0.01$).

A stepwise discriminant analysis was carried out and it gives a 100 % correct classification of the *M. hystricinum* marinum Rieger, 1977 and the *M.* cf. *beaufortensis* Ferguson, 1937 sample, whereas only 80.9 % for *M. pusillum* Rantum population and 81.8 % for *M. pusillum*, Möwenbergwatt population. The canonical variables were computed using L, c, St_L, St_W, and f_p (Fig. 2).

TABLE I

Basic data and coefficients of variation for *M. hystricinum marinum* Rieger, 1977 (1), *M. cf. beaufortensis* Ferguson, 1937 (2), *M. pusillum* AX, 1951, Möwenberg-population (3), *M. pusillum* AX, 1951, Rantum-population (4). n = number of cases ; min, max = minimum, maximum, avg = average, sd = standard deviation, CV = coefficient of variation. Abbreviations for characters see Fig. 2.

	Art	n	avg	sd	cv	min	max
L	1	20.0	924.195	159.917	17.303	741.700	1395.800
	2	23.0	381.700	58.491	15.324	262.800	497.100
	3	24.0	487.954	58.513	11.991	337.100	600.000
	4	23.0	556.304	61.415	11.040	446.700	666.700
B	1	21.0	159.976	28.723	17.954	116.400	232.700
	2	24.0	88.442	18.887	21.355	62.900	140.000
	2	22.0	88.441	9.008	10.186	66.700	100.000
	4	22.0	97.314	13.820	14.201	74.300	133.300
a	1	21.0	126.390	25.745	20.369	101.800	210.800
	2	24.0	69.692	12.863	18.458	45.700	109.100
	3	21.0	73.290	7.825	10.677	60.000	86.700
	4	22.0	79.359	8.286	10.441	65.700	93.300
b	1	21.0	32.562	9.654	29.648	21.800	50.900
	2	24.0	14.829	7.103	47.896	7.300	29.100
	3	21.0	11.414	2.747	24.070	6.700	13.300
	4	22.0	13.705	3.685	26.889	6.700	20.000
c	1	21.0	51.567	12.938	25.089	29.100	72.700
	2	24.0	32.558	12.319	37.836	20.000	74.300
	3	21.0	30.162	5.128	17.001	20.000	40.000
	4	22.0	32.218	6.309	19.583	26.700	46.700
ST_L	1	17.0	42.321	5.949	14.056	28.600	49.350
	2	18.0	27.397	2.217	8.093	23.600	31.450
	3	22.0	34.982	1.625	4.645	30.500	37.500
	4	21.0	32.838	2.140	6.516	28.500	36.800
ST_W	1	17.0	21.156	5.952	28.133	11.450	30.050
	2	18.0	12.744	2.090	16.399	8.550	16.450
	3	22.0	18.200	1.503	8.258	14.600	20.800
	4	21.0	16.957	2.182	12.868	12.500	20.100
f_p	1	10.0	297.700	44.771	15.039	218.200	370.800
	2	21.0	102.810	24.553	23.882	54.300	154.300
	3	21.0	137.371	24.886	18.116	80.000	206.700
	4	20.0	155.095	26.244	16.921	126.700	245.700

DISCUSSION

Variability of "soft" versus "hard" structures

Although the sclerotic structures show the lowest range of coefficient of variation (CV 4.6 - 28.1 %, Tabl. I), the maximal values observed are higher than those reported for sclerotic structures in Macrostomida by Rieger (CV 2.79 - 12.19 %, n = 10, Rieger 1977). The laboratory population of *M. hystricinum marinum* Rieger, 1977 and *M.* cf. *beaufortensis* Ferguson, 1937 are characterised by a higher CV compared to the field populations of *M. pusillum* Ax, 1951. If only the two wild populations are considered, the range for CV-values drops to 4.5 - 12.6 %. Of the two measurements of the sclerotic copulatory stylet, stylet width is much more prone to errors in taking measurements due to differences in squeezing conditions. By considering only stylet-length the range of coefficient of variation narrows to 4.5 - 6.4 %, falling within the range given for copulatory stylets in Macrostomida by Rieger (1977).

The data presented here further corroborate the low within-population variability of the sclerotic copulatory structures within the Macrostomida, a feature which is likely to be true for Turbellaria in general. But so far we are not aware of comparable data analysis of character variability of sclerotic structures in other Turbellarian groups. Heitkamp (1982) gives the mean values (n = 40) and the range for several parameters of the sclerotic copulatory organ in the freshwater dalyellioid *Gieysztoria rubra*. The data are apparently computed from several different populations, but no values of standard deviation are given and a comparison with the results of the present study is not possible.

Sterrer (1977) studied character variability of internal hard structures, the jaw apparatus in the soft-bodied Gnathostomulida. The author reported CV-values ranging from 2.3 - 12 % (n = 10). Only 2 of the 54 calculated CV exceeded 10 %. These data suggest an equally low within-population variability as demonstrated in this study for Turbellarian hard structures, although Gnathostomulid jaws - in opposition to the sclerotic penis stylet of Turbellaria - are apparently exclusively extracellular (see Lammert 1986).

The higher variability of non-sclerotic structures compared to sclerotic hard structures might be taken as evidence for the greater difficulty in enumerating such data. For the two wild populations of *M. pusillum* Ax, 1951, the range of CV observed for the parameters body length, body width, position of female pore, distance between eyes and rostral tip (CV : 10 - 17 %) fall well within the ranges of variability reported for similar measurements on hard-bodied meiofaunal groups. In the kinorhynchs *Echinoderes truncatus* and *Pycnophyes corrugatus* the CV-values of various parameters range from 3.6 - 31.3 % respectively (Higgins 1983). Similar for Tardigrada, *Styraconyx nanoqsunquak* : CV : 3.2-18.2 % and S. qivitoq : 6.0 - 24.1 % (Kristensen & Higgins 1984, 14 f, 19 f) and Gastrotricha, (e.g. *Turbanella ocellata* from smaller than 4% to up to 36 %, see Hummon 1974, and *Tetranchyroderma papii* from smaller than 5 % to up to 40 %, see Hummon 1977).

TABLE II

General Manova :

Variable V_1	... L	Groups	1 *M. hystricinum marinum*
V_2	... B		2 *M. cf. beaufortensis*
V_3	... a		*M. pusillum*
V_4	... b		3 Rantum population
V_5	... c		4 Möwenberg population
V_6	... St_l		
V_7	... St_w		
V_8	... f_p		

Variate	Statistic TSQ	F	DF		P
Grand Mean	12827.8	1458.39	8	77	0.0000
SS					
V_1	3.0605789E+7	3393.19	1	84	0.0000
V_2	1.0386403E+6	2915.61	1	84	0.0000
V_3	666891.135366	2839.59	1	84	0.0000
V_4	28831.464929	699.87	1	84	0.0000
V_5	59817.368521	1636.00	1	84	0.0000
V_6	104600.261320	8712.44	1	84	0.0000
V_7	58058.560700	1934.17	1	84	0.0000
V_8	2.3444867E+6	1923.26	1	84	0.0000

The above comparisons of some CV-values between soft and hard bodied meiofauna demonstrate that features as body length and width, position of eyes and the female gonopore in microturbellaria are as well suited for numerical analysis and estimation of within and between population variability as this is the case for hard bodied species. This finding was unexpected, because intuitively non-sclerotic or non-cuticular characters appear much more difficult to enumerate.

Species discrimination

Species characterisation and discrimination based on numerical data was possible between *M. hystricinum marinum* Rieger, 1977, *M.* cf. *beaufortensis* Ferguson, 1937 and *M. pusillum* Ax, 1951. Within the *M. pusillum* population, only a 80.9 % correct classification of the Rantum population and a 81.8 % correct classification of the Möwenbergwatt population was obtained. At the morphological level no significant changes occurred between the two *M. pusillum* populations during the past 62 years of reduced genetic exchange.

The *Macrostomum hystricinum*-species group comprises very similar marine and brackishwater species with a world-wide distribution (Rieger 1977). Whereas some of them are

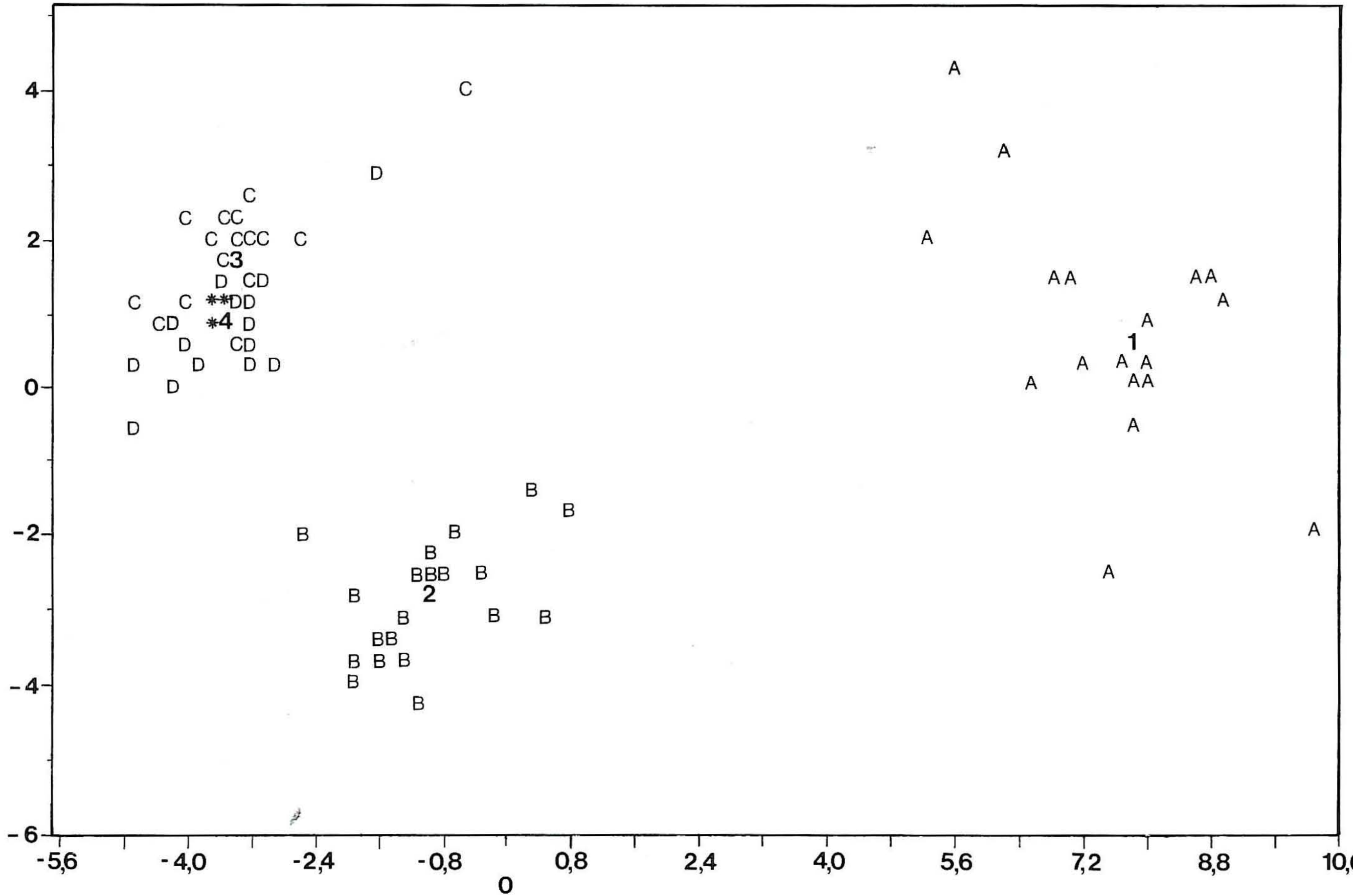


Fig. 3 : Analyses of discriminance ; groups : 1, A = *M. hystricinum marinum*, 2, B = *M.* cf. *beaufortensis*, 3, C = *M. pusillum* Rantum population., 4, D = *M. pusillum* Möwenberg population ; 1 to 4 indicate the centroid of each group ; overlap of different groups is indicated by*.

TABLE III

T-Test

Variable V_1 ... L
V_2 ... B
V_3 ... a
V_4 ... b
V_5 ... c
V_6 ... St_l
V_7 ... St_w
V_8 ... f_p

Groups
1 *M. hystricinum marinum* Rieger 1977
2 *M. nov. spec. I* *M. pusillum* AX 1951
3 Rantum population
4 Möwenberg population

Group	Level of significance	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
1 versus									
	2	***	***	***	***	***	***	***	***
	3	***	***	***	***	*	***	***	***
	4	***	***	***	***		***	***	***
2 versus									
	3	***		*		***	***	***	***
	4	***				***	***	***	***
3 versus									
	4	**							

Notation for Bonferroni significance levels
0.001 ***
0.01 **
0.05 *
not significant

well defined, the description does not allow clear identification for others. A numerical characterisation of those species including a thorough histological analysis would allow a better identification within this species group.

Numerical analysis of within- and between-population variability of characters is essential for our understanding of the processes of speciation in marine meiofauna. For example, Westheide & Rieger (1987) proposed a phylogenetic scheme of the *Microphthalmus-listensis*-species-group using a combined analysis of morphological, embryological, histological and behavioural characters. Similar studies using a standardised technique of character enumeration and evaluation (see e.g., Specht & Westheide 1988) are necessary for advancing our knowledge of species in Macrostomida and other groups of Turbellaria.

ACKNOWLEDGEMENT

This work was supported by FWF grant 6337 B (P. I. R.M. Rieger). The first author thanks Prof. Reise, Helgoland, Dr. Armonies, Sylt and her parents for their support of her trip to Sylt in order to collect *M. pusillum*.

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