Acta Protozool. (2001) 40: 83 - 97

ACTA
PROTOZOOLOGICA

Biogeographical Differences in a Common Soil Ciliate, *Gonostomum affine* (Stein), as Revealed by Morphological and RAPD-Fingerprint Analysis

Wilhelm FOISSNER[1], Thorsten STOECK[2], Helmut SCHMIDT[2] and Helmut BERGER[3]

[1]Universität Salzburg, Institut für Zoologie, Salzburg, Austria; [2]Universität Kaiserslautern, Abteilung Ökologie, Kaiserslautern, Germany; [3]Technisches Büro für Ökologie, Salzburg, Austria

Summary. *Gonostomum affine* is a common, hypotrichous soil ciliate showing a wide variety of more or less distinct morphologies which, depending on the view, can be considered as taxonomic entities or variations of a single morphotype. Thus, we chose it as a model to investigate some main questions in ciliate alpha-taxonomy and biodiversity, viz., (i) the power of morphological methods and RAPD-fingerprints to separate two distinct morphospecies of *Gonostomum*; (ii) whether morphology and RAPD agree in separating the most dissimilar morphotypes; and (iii) whether *G. affine* has a biogeographical population structure. Accordingly, we investigated one *G. strenuum* population from Australia and compared it with six *G. affine* populations from Europe, Africa, and South America. Data were analysed with classical similarity trees and two new methods combining morphological features and RAPD-fingerprints in single similarity trees. The main results of this study were: (i) Morphology could separate the two morphospecies very clearly, while RAPD could not, irrespective of the indices and clustering algorithms used; (ii) Morphotrees did not agree with RAPD-trees and not show a distinct biogeographical pattern. However, a rather distinct biogeographical population structure became recognisable when morphological features and RAPD-fingerprints were combined in a single similarity tree, indicating (iii) a certain degree of geographical uniqueness of various genotypes. Generally, however, separation was rather weak and highly dependent on the indices and clustering algorithms used, indicating that, at the present state of knowledge, most *Gonostomum affine*-like morphotypes fall into the range of natural variability of a single species.

Key words: biodiversity, biogeography, Ciliophora, Protozoa, similarity trees, soil ciliates.

INTRODUCTION

Gonostomum affine is a very common ciliate in limnetic and terrestrial habitats, usually preferring the latter, where it may gain high abundances. Apparently, *G. affine* has a cosmopolitan distribution, although many more or less distinct morphotypes exist, several of which have Linnean names (for reviews, see Foissner 1998 and Berger 1999). Most of the variability concerns body size (length 50-160 μm), and number (6-22) and pattern of the fronto-ventral-transverse cirri. Recent authors tend to interpret these differences as part of the natural variability of this species and thus synonymise most of the named populations (Buitkamp 1977, Foissner 1982, Berger 1999), although different opinions exist (Maeda and Carey 1984) and gene sequence data and ecological specialisations indicate that, in general, mor-

Address for correspondence: Wilhelm Foissner, Universität Salzburg, Institut für Zoologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria (Europe); Fax +43 (0) 662 8044-5698

phologists heavily under-split ciliates (Nanney *et al.* 1998, Dini and Nyberg 1999).

It has not yet been investigated whether variability follows a biogeographical pattern in *G. affine*. Generally, such studies are rare in ciliates. The best studied examples are the sibling species of *Tetrahymena pyriformis*, *Paramecium aurelia*, and *Stylonychia mytilus*, some of which are very likely restricted to certain geographic regions (Nanney and McCoy 1976, Ammermann 1985, Wichtermann 1986, Nanney *et al.* 1998). On the other hand, several reproductively isolated *Euplotes* strains obviously lack a geographic pattern (Dini and Gianni 1985) and a morphological analysis of four *Mesanophrys* species, all described from different hosts, strongly suggests that all belong to a single, facultatively histophagous morphospecies (Wiąckowski *et al.* 1999).

RAPD-fingerprinting and riboprinting are new tools for investigating population structures and have been successfully used also in ciliates (Kusch and Heckmann 1996; Kusch 1998; Stoeck and Schmidt 1998; Stoeck *et al.* 1998, 2000a). And even three common soil ciliate species of the genus *Colpoda* have been investigated (Bowers and Pratt 1995, Xu *et al.* 1997, Bowers *et al.* 1998). The riboprints and some main morphometrics did not show a biogeographical population structure in these species. However, the conclusion is not definitive because the rRNA gene region used was probably not informative enough (Bowers *et al.* 1998). Furthermore, molecular and morphological data were not combined in the similarity trees, so a common failure that often makes it difficult to know the extent results are influenced by incomplete data analysis. Others abandon morphology altogether, ascribing an unfounded power to molecular methods (Malpartida *et al*. 1995, Martin *et al.* 1997).

Our study is designed to overcome some of these shortcomings and specifically addresses the following matters: (i) to develop methods combining morphological data with RAPD-fingerprints in a single similarity (dissimilarity) tree; (ii) to compare the power of morphological methods and RAPD-fingerprints in separating two morphospecies of *Gonostomum*; (iii) to investigate whether morphology and RAPD agree in separating the most dissimilar morphotypes; and (iv) to investigate whether *G. affine* has a biogeographical population structure. Such questions address biodiversity and conservation matters, both very controversially discussed in ciliates (Finlay and Fenchel 1999, Foissner 2000a).

MATERIALS AND METHODS

Material. We investigated seven *Gonostomum* populations belonging to two morphospecies, viz., *Gonostomum affine* (Stein, 1859) Sterki, 1878 and *G. strenuum* (Engelmann, 1862) Sterki, 1878. Identification followed the review by Berger (1999). Although *G. affine* is a very common ciliate in soils worldwide (Foissner 1998), we could establish pure cultures (on Eau de Volvic enriched with some crashed wheat grains or baker's yeast) only from the *G. affine* population of Namibian site (31) and from the *G. strenuum* population of Australia. Raw cultures were set up from air-dried soil with the non-flooded Petri dish method, as described in Foissner (1987).

Population 1: *Gonostomum affine* from a beech forest brown earth soil in the surroundings of Salzburg, Austria, Europe (E 13°40' N 47°47').

Population 2: *Gonostomum affine* from a very sandy pasture soil near the village of Alqasab, about 130 km northeast of Riyadh, Saudi Arabia, Africa (E 47°30' N 25°).

Population 3: *Gonostomum affine* from Namibian site (26), that is, from organic debris and sand collected in the Sossus Vlei of the Namib Desert, Southwest Africa (E 15°20' S 24°40').

Population 4: *Gonostomum affine* from Namibian site (31), that is, from very sandy soil around a *Welwitschia* plant near Swakopmund, Namibia, Southwest Africa (E 15°30' S 22°40').

Population 5: *Gonostomum affine* from a cloud rain forest in the Henry Pittier National Park, Venezuela, South America (W 77° N 12°).

Population 6: *Gonostomum affine* from soil of the gallery forest at the bank of the Rio Negro (Amazon floodplain), Manaus, Brazil, South America (W 60° S 3°).

Population 7: *Gonostomum strenuum* from soil of the River Murray floodplain near Albury, Australia (E 147° S 37°).

Morphological analysis. Specimens from the raw or pure cultures were protargol-impregnated following the protocol A as described in Foissner (1991). This method provides permanent slides and shows the structures measured or counted very clearly (Fig. 4). Measurements and counts were performed at a magnification of X1000 (oil immersion), where a measuring unit is 1 µm in the eye piece. Scanning electron microscopy was performed as described in Foissner (1991), using concentrated aqueous mercuric chloride and osmium tetroxide (10:1) as fixative. The features investigated and the terminology used are shown in Fig. 1.

RAPD-fingerprinting. Three to five specimens each were used for the fingerprints, either directly from the (raw) non-flooded Petri dish cultures or the pure cultures. Specimens from the raw cultures were identified in vivo at a magnification of X250 and in protargol preparations (Figs. 7-9, 11, 12). Pre-treatment of specimens (three washes in SMB medium etc.) and fingerprinting were performed as described by Stoeck and Schmidt (1998), using primer Ro 360-04, with the sequence: 5' CCCTCATCAC (Roth, Karlsruhe, Germany). Furthermore, a control without any DNA-template was run with each of the populations.

Data analysis. The morphological data (Table 1) were compared with the Coefficient of Racial Likeness (CRL) described by Pearson (1926). The coefficient is a scale-independent distance function, which considers both the mean and variance of the variables (Sneath and Sokal 1973). Invariable features (number of dorsal kineties,

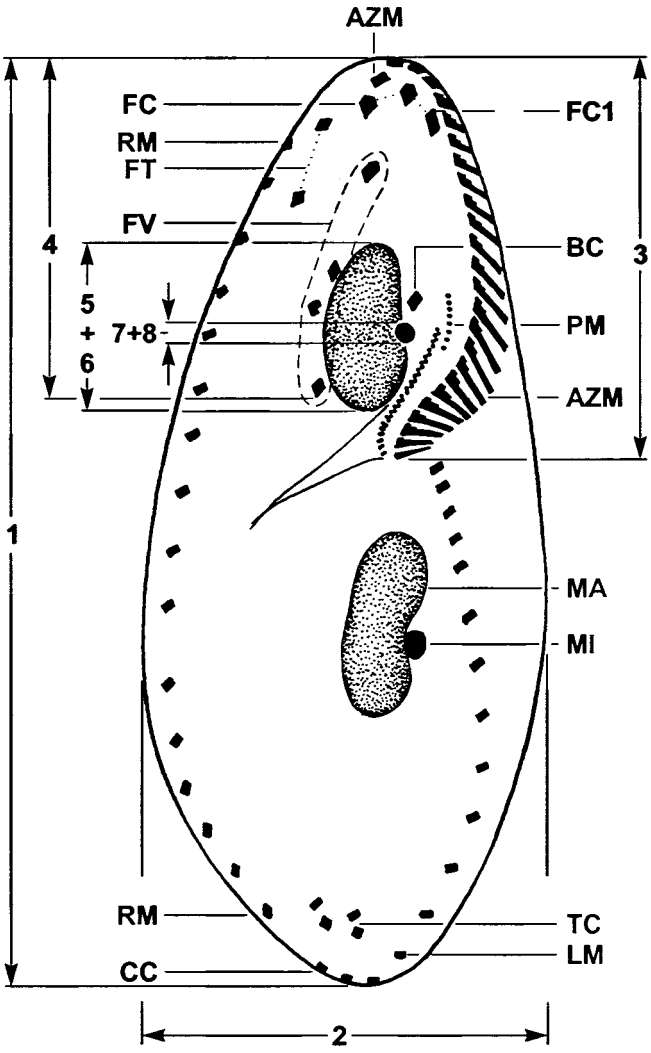


Fig. 1. Terminology used and features measured (numbers) or counted (labels) in *Gonostomum* populations. 1 - length of body; 2 - maximum width of body; 3 - length of adoral zone of membranelles, that is, distance between anterior body end and last adoral membranelle; 4 - distance between anterior body end and last frontoventral cirrus; 5 + 6 - length and width of anterior macronuclear nodule; 7 + 8 - length and width of anterior micronucleus; AZM - number of adoral membranelles; BC - number of buccal cirri; CC - number of caudal cirri; FC - number of frontal cirri; FC1 - first frontal cirrus; FT - number of frontoterminal cirri; FV - number of remaining frontoventral cirri; LM - number of cirri in left marginal row; MA - number of macronuclear nodules; MI - number of micronuclei; PM - number of cilia composing paroral membrane; RM - number of cirri in right marginal row; TC - number of pretransverse and transverse cirri

macronuclear nodules, frontal cirri and buccal cirri) have been omitted. Furthermore, features 7 and 8 were excluded because they are difficult to measure. Thus 16 characteristics remained (Table 1).

The fingerprints were aligned by eye (Fig. 16) and analysed with the association (band sharing) index of Simpson using the biodiversity program of Baev and Penev (1995). This index divides the number of species (= DNA bands) in common (= a) by (a) plus the number of species present in the shorter list only (b). The data were also analysed with the computer program HENNIG 86 (Farris 1988). In one trial, the Australian population (= *G. strenuum*) was defined as outgroup, in another trial we used an artificial outgroup in which all characteristics (= DNA bands) studied are absent (Lorenzen and Sieg 1991).

To analyse morphological and fingerprint data together two methods were developed. The first is a modification of the NNSDC (= Number of Not Significantly Different Characters) method proposed by Berger *et al.* (1985). The seven populations were compared by the nonparametric a posteriori testing procedure of Nemenyi, as described in Sachs (1984): There are k treatment groups (= populations) with equal sample size n; in the present study, k = 7 and n = 21 for each feature (Table 1). Rank all (k x n) observations from smallest to largest when pooled together into a single sample. In case of ties, compute the average ranks. Sum the ranks separately for each population and make all 21 possible absolute differences of these sums. If an observed difference between two treatments is as great as a critical value D (Table 180 in Sachs 1984) then a (real) difference exists. Only the 5% significance level was used. The fingerprint data of Table 2 were compared in pairs as follows: +/+ and -/- became - (= no difference); +/- and -/+ became + (= difference). Now both the 36 fingerprint bands and the 20 morphological features (Table 1; exclusive micronucleus measurements) are arranged in a table. Then the number of the not significantly different characteristics for each pair of the seven populations is added. These values are converted to percentages with 56 (36 + 20) equaling 100%, that is, "total similarity", and UPGMA (unweighted pair-group method using arithmetic averages) clustered.

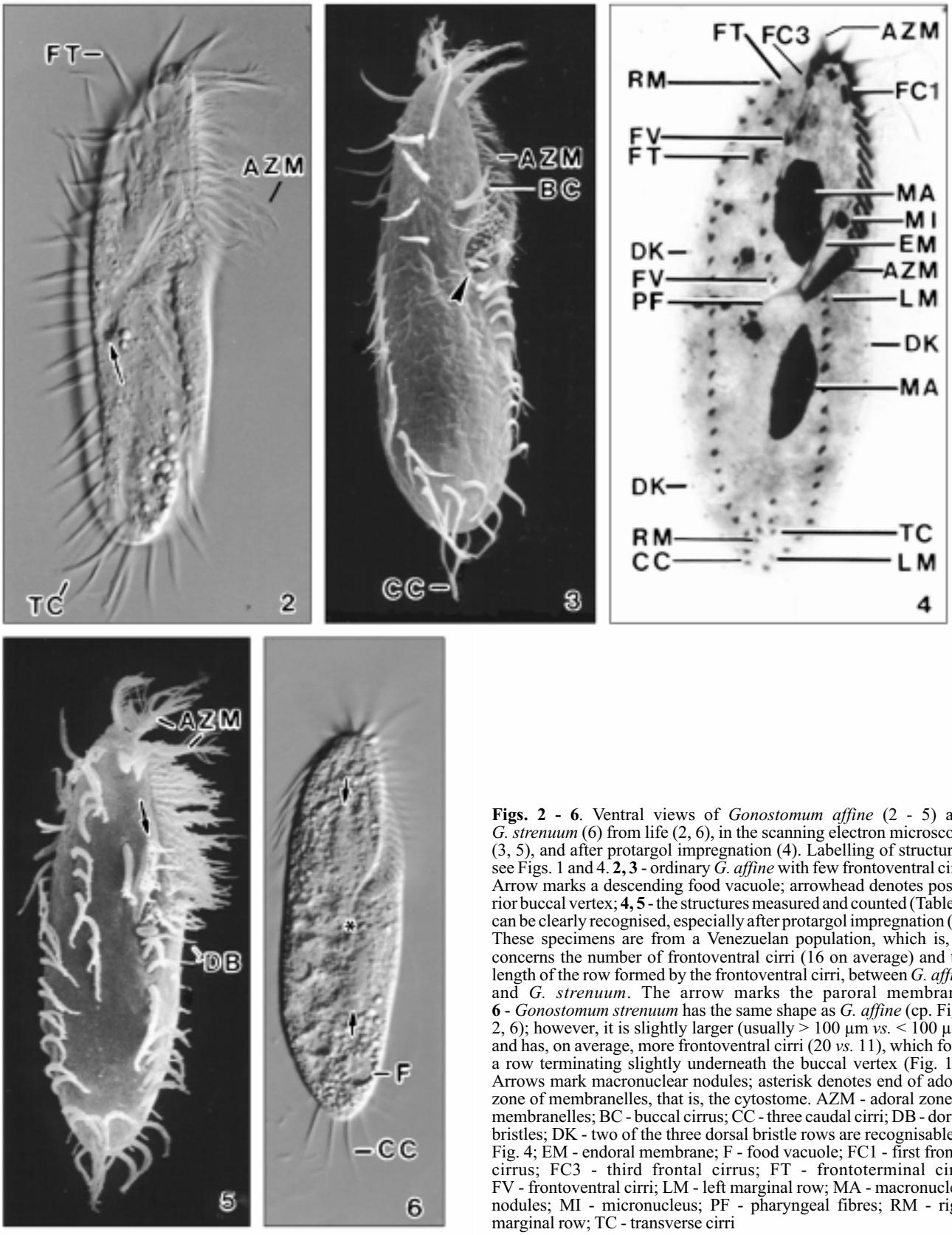
The program of Baev and Penev (1995) was used for the second method to analyse RAPD-fingerprints and morphological data together. For this, the morphological values shown in Table 1 were transformed to simple presence/absence data, applying the following rule: if the arithmetic mean over all populations (n 7 x 21 = 147) was smaller than the mean of a single population (n = 21), then a minus was assigned and vice versa. For equal means a plus was assigned. We suggest naming the method "presence/absence transformation". Several indices were tried. Best results were obtained with the index of Sørensen (1948).

Dendrograms were constructed using various algorithms (Sneath and Sokal 1973, Pielou 1984, Baev and Penev 1995). The graphs presented were constructed by the UPGMA (unweighted pair-group method using arithmetic averages) and the complete linkage (farthest neighbour) method, which provided the most meaningful results. Furthermore, all methods used do not weigh individual features, that is, are unbiased, but the load of the fingerprints is greater in the combined trees, where 36 fingerprint bands (= features) are opposed to only 20 morphological features.

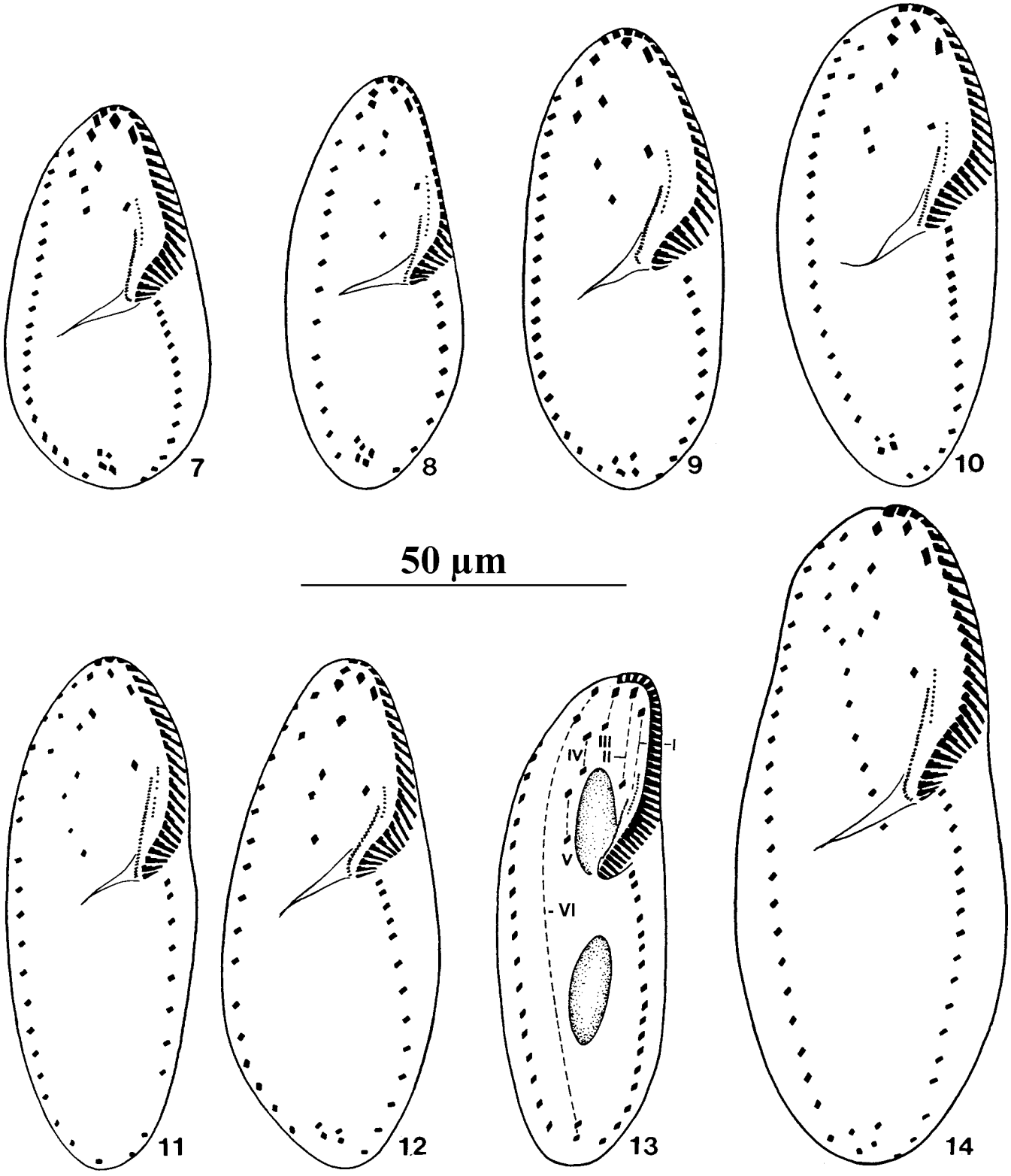
RESULTS

Brief description of the species investigated

Both species investigated were recently reviewed by Berger (1999). Thus, we provide only a brief description



Figs. 2 - 6. Ventral views of *Gonostomum affine* (2 - 5) and *G. strenuum* (6) from life (2, 6), in the scanning electron microscope (3, 5), and after protargol impregnation (4). Labelling of structures, see Figs. 1 and 4. **2, 3** - ordinary *G. affine* with few frontoventral cirri. Arrow marks a descending food vacuole; arrowhead denotes posterior buccal vertex; **4, 5** - the structures measured and counted (Table 1) can be clearly recognised, especially after protargol impregnation (4). These specimens are from a Venezuelan population, which is, as concerns the number of frontoventral cirri (16 on average) and the length of the row formed by the frontoventral cirri, between *G. affine* and *G. strenuum*. The arrow marks the paroral membrane; **6** - *Gonostomum strenuum* has the same shape as *G. affine* (cp. Figs. 2, 6); however, it is slightly larger (usually > 100 µm *vs.* < 100 µm) and has, on average, more frontoventral cirri (20 *vs.* 11), which form a row terminating slightly underneath the buccal vertex (Fig. 14). Arrows mark macronuclear nodules; asterisk denotes end of adoral zone of membranelles, that is, the cytostome. AZM - adoral zone of membranelles; BC - buccal cirrus; CC - three caudal cirri; DB - dorsal bristles; DK - two of the three dorsal bristle rows are recognisable in Fig. 4; EM - endoral membrane; F - food vacuole; FC1 - first frontal cirrus; FC3 - third frontal cirrus; FT - frontoterminal cirri; FV - frontoventral cirri; LM - left marginal row; MA - macronuclear nodules; MI - micronucleus; PF - pharyngeal fibres; RM - right marginal row; TC - transverse cirri



Figs. 7 - 14. Ventral infraciliature (~ cirral pattern) of the *Gonostomum affine* (7 - 12) and *G. strenuum* (14) populations investigated. All protargol-impregnated and drawn to scale. For labelling of structures, see Figs. 1 and 4. **7** - specimen from Namibian site 26, length 60 µm; **8** - specimen from Austria, length 65 µm; **9** - specimen from Brazil, length 71 µm; **10** - specimen from Namibian site 31, length 75 µm; **11** - specimen from Saudi Arabia, length 78 µm; **12** - specimen from Venezuela, length 79 µm; **13** - schema showing origin of the fronto-ventral-transverse cirri (from Berger 1999, modified). Cirri originating from the same anlage (Roman numbers) are connected by broken lines; **14** - specimen from Australia, length 93 µm

Table 1. Morphometric data on six populations of *Gonostomum affine* (Austria, Saudi Arabia, Namibian site 26, Namibian site 31, Venezuela, Brazil) and a population of *G. strenuum* from Australia. All - all *G. affine* populations combined. Numbers refer to Fig. 1

Characteristics[a]	Number	Populations	x	M	SD	SE	CV	Min	Max	n
Body, length	1	Austria	79.0	80.0	11.6	2.5	14.7	63	100	21
		Saudi Arabia	68.8	68.0	9.4	2.1	13.7	50	85	21
		Namibia 26	60.5	62.0	7.3	1.6	12.1	50	75	21
		Namibia 31	75.8	74.0	7.2	1.6	9.5	67	97	21
		Venezuela	72.5	71.0	6.0	1.3	8.3	60	82	21
		Brazil	81.0	82.0	8.7	1.9	10.8	70	93	21
		Australia	93.9	94.0	10.6	2.3	11.2	75	112	21
		All	72.9	71.0	10.9	1.0	14.9	50	100	126
Body, maximum width	2	Austria	34.7	33.0	5.3	1.2	15.4	28	44	21
		Saudi Arabia	28.6	30.0	4.1	0.9	14.2	21	35	21
		Namibia 26	29.2	29.0	4.5	1.0	15.6	21	37	21
		Namibia 31	31.8	31.0	3.8	0.8	12.0	26	40	21
		Venezuela	29.9	32.0	5.4	1.2	18.0	20	40	21
		Brazil	32.8	33.0	5.1	1.1	15.5	24	41	21
		Australia	38.3	37.0	4.8	1.0	12.4	32	48	21
		All	31.2	31.0	5.1	0.5	16.5	20	44	126
Anterior body end, distance to posterior end of adoral zone of membranelles (~ length of adoral zone)	3	Austria	41.9	43.0	7.6	1.7	18.0	29	55	21
		Saudi Arabia	33.2	34.0	4.1	0.9	12.4	26	40	21
		Namibia 26	29.2	29.0	3.7	0.8	12.5	25	42	21
		Namibia 31	36.6	37.0	1.5	0.3	4.1	34	40	21
		Venezuela	33.2	33.0	2.9	0.6	8.7	29	38	21
		Brazil	39.2	39.0	1.9	0.4	4.9	36	44	21
		Australia	44.5	44.0	2.7	0.6	6.0	40	50	21
		All	35.5	35.0	5.8	0.5	16.4	25	55	126
Anterior body end, distance to last ventral cirrus	4	Austria	33.6	33.0	7.3	1.6	21.6	22	49	21
		Saudi Arabia	25.9	26.0	3.2	0.7	12.3	21	32	21
		Namibia 26	17.6	17.0	3.3	0.7	18.6	13	28	21
		Namibia 31	21.7	22.0	2.1	0.5	9.7	18	25	21
		Venezuela	26.5	26.0	2.6	0.6	9.9	21	30	21
		Brazil	26.2	26.0	2.8	0.6	10.8	19	32	21
		Australia	49.5	49.0	6.3	1.4	12.7	41	65	21
		All	25.2	25.0	6.2	0.6	24.7	13	49	126
Macronucleus, length anterior nodule	5	Austria	17.1	18.0	3.3	0.7	19.2	11	22	21
		Saudi Arabia	14.0	13.0	3.2	0.7	23.1	10	20	21
		Namibia 26	11.4	12.0	1.7	0.4	14.5	9	15	21
		Namibia 31	14.1	14.0	2.3	0.5	16.0	10	18	21
		Venezuela	13.1	13.0	2.2	0.5	16.5	9	17	21
		Brazil	14.0	14.0	2.2	0.5	15.3	11	19	21
		Australia	16.7	17.0	2.9	0.6	17.5	13	25	21
		All	14.0	13.5	3.0	0.3	21.5	9	22	126
Macronucleus, width anterior nodule	6	Austria	7.9	8.0	0.9	0.2	11.2	6	10	21
		Saudi Arabia	7.1	7.0	1.1	0.2	14.9	5	9	21
		Namibia 26	6.6	6.0	0.7	0.1	10.3	6	8	21
		Namibia 31	6.8	7.0	0.9	0.2	13.1	5	9	21
		Venezuela	7.9	8.0	0.9	0.2	10.9	6	10	21
		Brazil	7.7	8.0	1.2	0.3	16.2	6	12	21
		Australia	6.9	7.0	0.9	0.2	12.9	5	9	21
		All	7.3	7.0	1.1	0.1	14.6	5	12	126
Micronucleus, length	7	Austria	3.1	3.0	0.8	0.2	25.4	2	4	21
		Saudi Arabia	2.3	2.5	0.3	0.1	15.0	2	3	21
		Namibia 26	2.2	2.0	0.5	0.1	20.9	2	3	21

Table 1 (contd.)

		Namibia 31	not measured							
		Venezuela	2.1	2.0	0.3	0.1	13.0	1	3	21
		Brazil	2.8	3.0	0.4	0.1	14.3	2	4	21
		Australia	3.0	3.0	0.3	0.1	10.5	3	4	21
		All	2.5	2.5	0.6	0.1	24.9	1	4	105
Micronucleus, width	8	Austria	2.3	2.5	0.5	0.1	21.9	2	3	21
		Saudi Arabia	1.9	2.0	0.2	0.1	12.4	2	3	21
		Namibia 26	1.4	1.5	0.3	0.1	21.6	1	2	21
		Namibia 31	not measured							
		Venezuela	1.9	2.0	0.2	0.1	12.7	1	2	21
		Brazil	2.0	2.0	0.2	0.0	11.2	2	3	21
		Australia	2.3	2.0	0.3	0.1	13.5	2	3	21
		All	1.9	2.0	0.4	0.0	22.5	1	3	105
Adoral membranelles, number	9	Austria	28.8	30.0	4.2	0.9	14.6	23	35	21
		Saudi Arabia	21.5	22.0	2.2	0.5	10.4	17	24	21
		Namibia 26	22.0	22.0	2.0	0.4	9.2	18	27	21
		Namibia 31	25.9	26.0	1.0	0.2	3.8	24	28	21
		Venezuela	24.7	25.0	1.6	0.4	6.6	21	27	21
		Brazil	27.1	27.0	0.9	0.2	3.3	25	29	21
		Australia	27.9	28.0	1.3	0.3	4.7	25	30	21
		All	25.0	25.0	3.4	0.3	13.7	17	35	126
Dorsal kineties, number	10	Austria	3.0	3.0	0.0	0.0	0.0	3	3	21
		Saudi Arabia	3.0	3.0	0.0	0.0	0.0	3	3	21
		Namibia 26	3.0	3.0	–	–	–	2	3	21
		Namibia 31	3.0	3.0	0.0	0.0	0.0	3	3	21
		Venezuela	3.0	3.0	0.0	0.0	0.0	3	3	21
		Brazil	3.0	3.0	0.0	0.0	0.0	3	3	21
		Australia	3.0	3.0	0.0	0.0	0.0	3	3	21
		All	3.0	3.0	–	–	–	2	3	126
Macronuclear nodules, number	11	Austria	2.0	2.0	0.0	0.0	0.0	2	2	21
		Saudi Arabia	2.0	2.0	0.0	0.0	0.0	2	2	21
		Namibia 26	2.0	2.0	0.0	0.0	0.0	2	2	21
		Namibia 31	2.1	2.0	–	–	–	2	3	21
		Venezuela	2.0	2.0	0.0	0.0	0.0	2	2	21
		Brazil	2.0	2.0	0.0	0.0	0.0	2	2	21
		Australia	2.0	2.0	0.0	0.0	0.0	2	2	21
		All	2.0	2.0	–	–	–	2	3	126
Micronuclei, number	12	Austria	1.9	2.0	0.6	0.1	30.9	0	3	21
		Saudi Arabia	2.3	2.0	1.0	0.2	44.1	0	4	21
		Namibia 26	1.9	2.0	0.4	0.1	22.9	1	3	21
		Namibia 31	0.0	0.0	0.0	0.0	0.0	0	0	21
		Venezuela	1.9	2.0	1.0	0.2	51.9	0	4	21
		Brazil	1.7	2.0	0.6	0.1	32.7	0	2	21
		Australia	3.1	3.0	1.3	0.3	40.6	0	6	21
		All	1.6	2.0	1.0	0.1	62.2	0	4	126
Right marginal row, number of cirri	13	Austria	16.6	17.0	2.9	0.6	17.2	10	21	21
		Saudi Arabia	17.8	18.0	2.9	0.6	16.0	12	23	21
		Namibia 26	16.5	16.0	2.3	0.5	13.7	11	20	21
		Namibia 31	19.5	19.0	2.3	0.5	12.0	16	26	21
		Venezuela	17.7	17.0	2.5	0.5	14.2	13	23	21
		Brazil	18.8	18.0	1.8	0.4	9.5	15	22	21
		Australia	25.4	24.0	4.2	0.9	16.5	21	33	21
		All	17.8	18.0	2.6	0.2	14.8	10	26	126

Table 1 (contd.)

Left marginal row, number of cirri	14	Austria	11.0	11.0	1.9	0.4	17.5	7	15	21
		Saudi Arabia	11.8	12.0	2.4	0.5	20.3	6	16	21
		Namibia 26	12.3	12.0	2.9	0.6	23.3	8	22	21
		Namibia 31	14.8	15.0	1.3	0.3	8.5	12	17	21
		Venezuela	12.6	12.0	1.6	0.3	12.6	10	16	21
		Brazil	12.3	12.0	1.1	0.2	8.6	11	15	21
		Australia	17.3	18.0	2.6	0.6	15.1	13	21	21
		All	12.4	12.0	2.2	0.2	18.0	6	22	126
Frontal cirri, number	15	Austria	3.0	3.0	0.0	0.0	0.0	3	3	21
		Saudi Arabia	3.0	3.0	0.0	0.0	0.0	3	3	21
		Namibia 26	3.0	3.0	0.0	0.0	0.0	3	3	21
		Namibia 31	3.0	3.0	0.0	0.0	0.0	3	3	21
		Venezuela	3.0	3.0	0.0	0.0	0.0	3	3	21
		Brazil	3.0	3.0	0.0	0.0	0.0	3	3	21
		Australia	3.0	3.0	0.0	0.0	0.0	3	3	21
		All	3.0	3.0	0.0	0.0	0.0	3	3	126
Frontoterminal cirri, number	16	Austria	2.0	2.0	0.0	0.0	0.0	2	2	21
		Saudi Arabia	2.9	3.0	–	–	–	2	3	21
		Namibia 26	2.0	2.0	–	–	–	2	3	21
		Namibia 31	2.0	2.0	0.0	0.0	0.0	2	2	21
		Venezuela	1.9	2.0	–	–	–	1	2	21
		Brazil	1.9	2.0	0.4	0.1	22.9	0	2	21
		Australia	4.1	4.0	0.6	0.1	13.8	3	6	21
		All	2.1	2.0	0.4	0.0	20.3	0	3	126
Remaining frontoventral ventral cirri, number	17	Austria	5.5	5.0	0.9	0.2	15.8	5	7	21
		Saudi Arabia	5.9	6.0	0.8	0.2	13.0	4	7	21
		Namibia 26	3.8	3.0	1.1	0.2	28.3	3	6	21
		Namibia 31	4.8	5.0	0.8	0.2	17.4	3	6	21
		Venezuela	5.0	5.0	0.4	0.1	7.8	4	6	21
		Brazil	3.1	3.0	0.5	0.1	15.2	3	5	21
		Australia	11.7	11.0	1.3	0.3	10.8	10	15	21
		All	4.7	5.0	1.2	0.1	26.0	3	7	126
Buccal cirri, number	18	Austria	1.0	1.0	0.0	0.0	0.0	1	1	21
		Saudi Arabia	1.0	1.0	0.0	0.0	0.0	1	1	21
		Namibia 26	1.0	1.0	0.0	0.0	0.0	1	1	21
		Namibia 31	1.0	1.0	0.0	0.0	0.0	1	1	21
		Venezuela	1.0	1.0	0.0	0.0	0.0	1	1	21
		Brazil	1.0	1.0	–	–	–	0	1	21
		Australia	1.0	1.0	0.0	0.0	0.0	1	1	21
		All	1.0	1.0	–	–	–	0	1	126
Transverse cirri, number	19	Austria	4.7	4.0	1.1	0.2	22.8	3	6	21
		Saudi Arabia	1.0	1.0	–	–	–	0	1	21
		Namibia 26	2.6	4.0	1.7	0.4	68.0	0	4	21
		Namibia 31	4.0	4.0	–	–	–	3	4	21
		Venezuela	4.0	4.0	0.7	0.1	16.9	3	6	21
		Brazil	3.9	4.0	0.5	0.1	12.4	2	4	21
		Australia	4.0	4.0	0.0	0.0	0.0	4	4	21
		All	3.3	4.0	1.5	0.1	45.8	0	6	126
Caudal cirri, number	20	Austria	3.0	3.0	0.0	0.0	0.0	3	3	21
		Saudi Arabia	3.0	3.0	–	–	–	2	3	21
		Namibia 26	2.8	3.0	0.7	0.2	25.4	0	3	21
		Namibia 31	2.5	3.0	0.7	0.2	29.7	1	3	21
		Venezuela	3.0	3.0	0.0	0.0	0.0	3	3	21
		Brazil	3.0	3.0	0.0	0.0	0.0	3	3	21

Table 1 (contd.)

		Australia	3.0	3.0	0.3	0.1	10.5	2	4	21
		All	2.9	3.0	0.5	0.0	15.9	0	3	126
Paroral, number of cilia	21	Austria	13.6	14.0	3.1	0.7	22.8	8	19	21
		Saudi Arabia	10.6	11.0	2.1	0.5	19.6	5	14	21
		Namibia 26	8.6	8.0	1.8	0.4	20.6	6	12	21
		Namibia 31	10.2	10.0	2.1	0.5	20.5	6	14	21
		Venezuela	9.0	9.0	1.7	0.4	19.3	6	11	21
		Brazil	12.5	13.0	1.9	0.4	14.9	6	16	21
		Australia	13.4	13.0	1.8	0.4	13.2	11	17	21
		All	10.8	11.0	2.8	0.2	25.7	5	19	126
Fronto-ventral-transverse cirri, total number	22	Austria	16.1	17.0	1.2	0.3	7.7	14	18	21
		Saudi Arabia	13.7	14.0	1.1	0.2	8.0	11	15	21
		Namibia 26	12.3	13.0	1.2	0.3	10.0	10	15	21
		Namibia 31	14.7	15.0	0.8	0.2	5.7	13	16	21
		Venezuela	14.8	15.0	0.7	0.2	4.7	14	17	21
		Brazil	12.9	13.0	0.9	0.2	7.1	10	15	21
		Australia	23.8	23.0	1.2	0.3	5.2	22	26	21
		All	14.1	14.0	1.6	0.1	11.6	10	18	126

[a]Data based on protargol-impregnated (Foissner 1991 method), mounted, randomly selected, morphostatic specimens. All measurements in µm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, SE - standard error of arithmetic mean, x - arithmetic mean

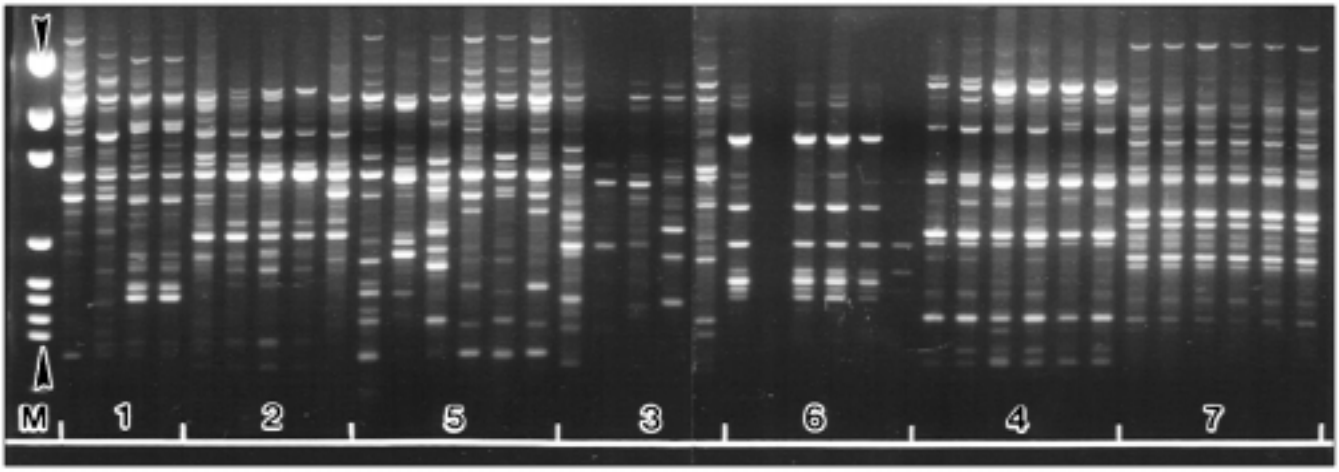


Fig. 15. RAPD-fingerprints from six *Gonostomum affine* populations (numbers 1- 6) and one *G. strenuum* population (number 7). M - pGEM-marker, arrowheads mark 222 and 1,605 bp

focusing on features important for the present paper. Furthermore, all relevant structures are shown in Figs. 1-6.

Gonostomum affine (Stein, 1859) Sterki, 1878 is a colourless, ellipsoidal, and laterally more or less distinctly flattened, oxytrichid ciliate having a size of about 90 x 30 µm, with length ranging from about 50 to 160 µm. As it is a hypotrich, the cilia are grouped into compound organelles, the cirri, which form a specific pattern. Usually, there are two ellipsoidal macronuclear nodules, each with a single micronucleus attached. The oral apparatus extends along the left margin of the anterior body half. It is composed of a conspicuous adoral zone of membranelles at the left margin of the oral field and two short, inconspicuous undulating membranes at the right. One of the undulating membranes, the paroral, is composed of few, comparatively widely spaced cilia, a specific feature of gonostomoid ciliates. The buccal cavity is small and inconspicuous. Only the right (ventral) side bears cirri, the dorsal surface is bare, except

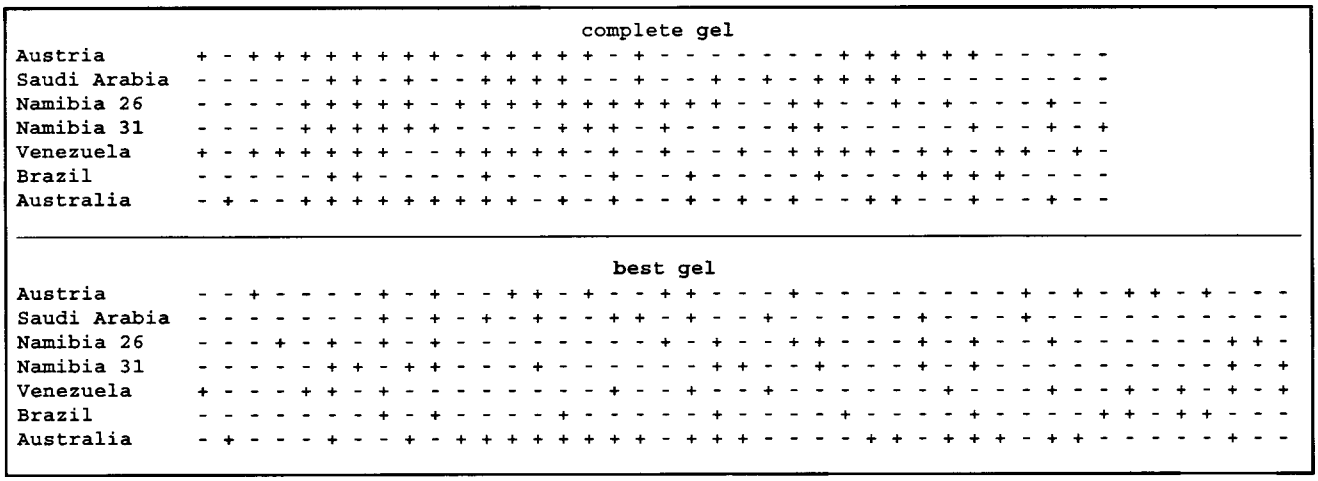
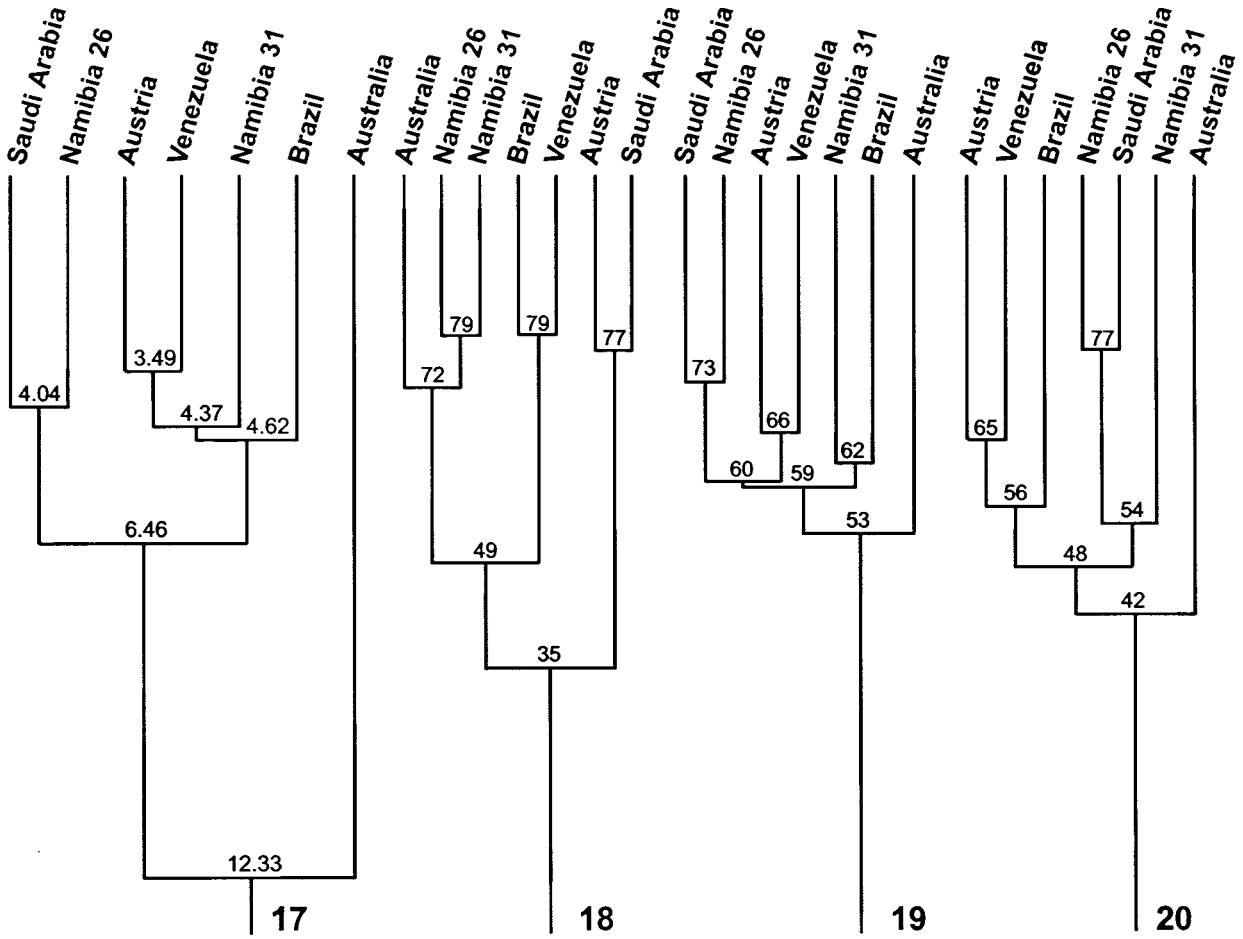


Fig. 16. Band pattern shown in Fig. 15 transformed to a presence/absence (+/-) matrix used for calculating band sharing indices. In the upper lane, all usable fingerprints were evaluated, in the lower lane only the "best looking" fingerprint from each population was used



Figs. 17 - 20. Similarity trees. **17** - morphological tree with the coefficient of racial likeness UPGMA clustered; **18** - RAPD-fingerprint tree with the association (band sharing) index of Simpson complete-linkage clustered; **19** - combined morphological and RAPD-fingerprint tree based on the "number of not significantly different characters" UPGMA clustered; **20** - combined morphological and RAPD-fingerprint tree based on the "presence/absence transformation" data clustered with the complete-linkage method. For details on methods, see "data analysis" in the "Materials and Methods" section

for three rows of minute bristles associated with three caudal cirri at the posterior body end. The cirri form a row each at the right and left margin of the cell and several groups on the ventral surface, as shown in Figs.1and 4. Briefly, there are a highly variable number of fronto-ventral cirri (6-15) in the area between the anterior body end and the oral vertex, and an also highly variable number of transverse cirri (0-7) near the posterior body end. The fronto-ventral cirri are classified in frontal cirri (usually three at anterior body margin), buccal cirri (usually one near paroral membrane), frontoterminal cirri (usually two at right anterior body margin), and ventral cirri (all other cirri in the area mentioned above) forming a more or less distinct row never surpassing the oral vertex. *Gonostomum affine* usually feeds on bacteria, prefers terrestrial habitats, and has been recorded from all main biogeographical regions (Foissner 1998, Berger 1999).

Gonostomum strenuum (Engelmann, 1862) Sterki, 1878 (Figs. 6, 14) is rather similar to *G. affine*, but a distinct morphospecies, although Foissner (2000b) very recently described a population from Venezuela, which is intermediate in all main features (Figs. 4, 5). Usually, *G. strenuum* is slightly larger than *G. affine* (110 x 40 *vs.* 90 x 30 μm) and has more frontoterminal (4 *vs.* 2) and fronto-ventral cirri (20 *vs.* 11 on average). Thus, the row formed by the ventral cirri is much more conspicuous than in *G. affine* and extends beyond the level of the oral vertex. *Gonostomum strenuum* feeds mainly on bacteria and is a rare species preferring limnetic habitats (Berger 1999). Previously, it was known only from Eurasia, our record from the Murray River floodplain is the first from Australia.

Morphological investigations (Figs. 7-14, 17; Table 1)

The six populations of *G. affine* investigated are well within the range recorded in the literature (Berger 1999). Thus, identification is beyond reasonable doubt. Likewise, the Australian *G. strenuum* matches the literature data.

The smallest *G. affine* specimens are those from the most extreme environment, that is, Namibian site 26, the Namib Desert. This population is smaller also in all other features, such as the length of the adoral zone of membranelles, the width and length of the macronuclear nodules, the number of adoral membranelles, and the number of marginal and fronto-ventral-transverse cirri. The Saudi Arabian specimens are unique in having three frontoterminal cirri, thus bridging the gap between *G. affine* (usually 2) and *G. strenuum* (usually 4). Furthermore, they usually have only a single transverse cirrus, while all other populations have four on average (median). The Austrian specimens are unique in having most adoral membranelles, paroral cilia, and fronto-ventral-transverse cirri. Furthermore, this population has the highest average coefficient of variability. The average variation coefficient of the features 1-6, 9, 13, 14, 17, 21, 22 in Table 1 is distinctly higher in the Austrian (16.3%), Namibian site 26 (15.7%), and Saudi Arabian (14.8%) populations than in the Namibian site 31 (11%), Brazilian (11%), and Venezuelan (11.4%) populations.

The similarity tree (Fig. 17) shows two conspicuous attributes: (i) the two species, *G. affine* and *G. strenuum*, are very distinctly separated, while (ii) the *G. affine* populations do not show a consistent biogeographical pattern, although the resolution capacity of the coefficient is excellent, as shown by the two distinct *G. affine* subclusters. However, at least the South American populations are in the same subcluster. The Namibian site 26/Saudi Arabian subcluster matches the peculiarities mentioned above, but excludes the Austrian population although it is rather peculiar, too.

RAPD - fingerprints (Figs. 15, 16, 18)

Each of the seven populations has a distinct pattern (Fig. 15). Thus, the overall fingerprint similarity is only 64%. Within the parallel probes, those from populations 4 and 7 (both pure cultures started with one or two specimens), but also those from population 6, a raw culture, are very similar, indicating that each consists of only a single genotype. In contrast, the parallel probes from populations 1 and 3 each are very different, indicating that most belong to distinct genotypes. This matches the high average morphological variability coefficients of these populations (see above).

The similarity trees from the RAPD-fingerprints are all rather chaotic, irrespective of the indices and cluster methods used. None separates the two morphospecies as distinctly as the morphological trees do (Figs. 17, 18). However, at least the South American and Namibian populations each form a distinct subcluster. To improve the tree, we tried not only various indices and cluster methods, but also confined the analysis to the "best looking" fingerprint from each population (Fig. 16). Furthermore, we tried a matrix based only on the more conspicuous bands. However, trees could not be improved, that is, the two morphospecies were never clearly separated and the three African populations never in a single subcluster.

Combined similarity trees

Combining the morphological and fingerprint data should increase resolution because of the increased data set. This is indeed the case (Figs. 19, 20). Both methods applied can at least distinctly separate the two morphospecies, and the complete-linkage tree even provides a meaningful geographical population structure, that is, distributes the African and South American *G. affine* populations in distinct subclusters (Fig. 20). However, we emphasise that other indices and/or cluster algorithms (for instance, UPGM, average-linkage) provide much less "beautiful" trees, indicating that the differences are subtle.

DISCUSSION

Methodological problems

Methodological problems and constraints are often underestimated in investigations of the kind we performed. However, they greatly influence data interpretation and conclusions, and thus they are discussed in advance.

Correlated features: Basically, the quality of similarity trees increases with the number of **independent** features analysed. This is why we tried to combine morphological traits and RAPD-fingerprints in a single tree. However, not only the quantity but also the quality of the features influences the outcome. Features which depend on each other, that is, are correlated can produce artificial groupings simply by their numerical dominance (Remane 1952, Mayr 1975). In hypotrichous genera, for instance, body length is often correlated with body width and the number of adoral membranelles is usually correlated with the length of the adoral zone. Actually, two of the four features are redundant. Unfortunately, recognition of correlated features is impossible in fingerprints and also rather difficult for morphological traits. Thus, "uncleared" data were used. In combined trees, numerical character equivalence is an additional source of errors. In our trees, 20 morphological traits are somewhat outrivaled by 36 RAPD-bands.

Band-alignment: Fig.16 shows that RAPD-bands are subject to different interpretations because the intensity of the bands varies in parallel probes and some bands are so faint that it depends on the classifier whether they are used or not. Furthermore, if many probes are analysed they become distributed over several gels, and even the molecular marker cannot guarantee that different gels are entirely correctly aligned.

Probably, some variation of DNA band patterns is due to the problems with reproducibility, which are typical for RAPD patterns (Skroch and Nienhuis 1995, Woodburn *et al.* 1995). It is known, that the intensities of several amplified bands can vary between experiments with the template DNA (Stoeck and Schmidt 1998). Thus, it might be possible that DNA bands, which are relatively weak in one sample, could be missing in another sample from the same clone. Therefore, it is necessary to study different cells from a clone in several experiments to obtain a reliable and specific pattern. Differences in the patterns of populations 1 and 3 contribute to a large extent to the relatively low overall similarity of 64%; thus, this value is assumed to be an underestimation.

Indices and algorithms: it is well-known and confirmed by our experience that both the coefficient used for estimating similarity and the algorithm applied to the similarity matrix highly influence the similarity analysis. The coefficient of racial likeness, the Simpson index (and variations), and the Sørensen index have simple structures and are widely used. Thus, they are perhaps unproblematic. The two methods for combining morphological and fingerprint data are empirical and need a detailed mathematical investigation, which is beyond our possibilities. We developed them because no appropriate methods were available from the literature. In any case, both methods are simple and provide binary data, which can be clustered.

There is no "best" cluster algorithm because all have some disadvantages and the structure of the data influences clustering. Specialists recommend using several methods on the same data set (Pielou 1984, Birks 1987). If similar results emerge, one can have more confidence that the groups have some reality and are not simply artifacts of any one method. However, it is widely accepted that UPGM clustering is one of the best methods because it represents the original data matrix better than other algorithms (Sokal and Rohlf 1981). Thus, our farthest-neighbour clusters are more problematical than our UPGM clusters because the "representative point" is always "extreme" rather than "typical" of the cluster it represents (Pielou 1984).

Resolution power of RAPD-fingerprints and morphology

It is known that RAPD patterns are sometimes difficult to interpret when applied for taxonomical questions (Stoeck *et al.* 2000b). Nothing is known about the

DNA sequences amplified in RAPD fingerprints (Tingey and Tufo 1993); specific DNA bands cannot be associated with particular loci in the genome (Lynch 1991); and difficulties arise concerning the mathematical analysis of RAPD fingerprints (Backeljau *et al.* 1995, Skroch and Nienhuis 1995, Landry and Lapointe 1997). Consequently, RAPD data on their own are not sufficient to unambiguously answer taxonomical questions. However, Stoeck *et al.* (2000b) were successful in combining two molecular biological methods, RAPD and amplified ribosomal DNA restriction analyses (ARDRA), also known as riboprinting, to reject the hypothesis of sibling species in *Paramecium caudatum*.

As concerns the present study, we have three indicators that the investigations are technically correct: (i) two of the three *G. affine* populations with the highest average variability coefficients also have highly variable fingerprints; (ii) the combined similarity trees give a higher resolution than the individual trees; (iii) pattern variability within the pure cultures (sites/gels 4, 7 in Fig. 15) is much smaller than within the raw cultures, where it is likely that several genotypes occur (sites/gels 1, 2, 3, 5, 6 in Fig. 15). Thus, the failure of the RAPD method to distinguish between the two morphospecies of *Gonostomum* is very likely not a technical artifact but indicates high genotype variability in *G. affine* and/or a lower resolution power than morphology. Furthermore, we must not forget that other primers might have produced clearer results.

Are the morphotypes of *Gonostomum affine* distinct taxonomic entities?

Kusch and Heckmann (1996) investigated a hypotrichous ciliate genus with RAPD-fingerprints. They found that different *Euplotes* species shared 38-46% of their fingerprint bands, while sexually recombined strains of *E. octocarinatus* and *E. aediculatus* shared 60-70%. Within-population variability of *E. daidalos* was only 3% when originating from the same pond, but 40% in populations from different ponds. Similar results were obtained by Stoeck *et al.* (2000a) in *Paramecium novaurelia*, a sibling species of the *P. aurelia* complex: 14 strains showed 85.7-97.1%, on average 92.3% band pattern similarity. Several strains of *P. pentaurelia* showed no variability at all. Kusch (1998) investigated 721 individuals of *Stentor coeruleus* from seven sites up to 400 km apart. He observed four distinct genotypes, while about 15 genotypes were recognisable in the *G. affine* populations from the six sites and three continents. Comparing these similarity values with the 64% overall band sharing in the six *G. affine* populations, it becomes obvious that they are in the range of populations, and thus should not get Linnean names. This is emphasised by the overlapping morphologies (Table 1) and the possibility that the 64% overall band similarity is an underestimation caused by technical problems (see above).

Biogeographical patterns

Euplotes spp. (Kusch and Heckmann 1996), *Colpoda* spp. (Bowers *et al.* 1998), *Stentor coeruleus* (Kusch 1998), *Paramecium novaurelia* (Stoeck *et al.* 2000a), and *Gonostomum affine* (this paper) agree in having several distinct genotypes apparently lacking a geographic pattern. However, a rather distinct pattern becomes recognisable in *G. affine* when morphological and molecular data are combined in single similarity trees (Figs. 19, 20). Likewise, RAPD-fingerprints and strain crossing show a restricted geographical distribution of certain genotypes of *Paramecium sexaurelia*, another species of the *P. aurelia* complex (Stoeck *et al.* 1998). Unfortunately, strain crossing was impossible in *G. affine* because only one of the six populations could be grown in pure culture.

To sum up, it seems possible that further investigations will reveal a biogeographical population structure in *G. affine* and other ciliates, especially when several methods (morphology, gene sequences, mating reaction) are combined in **single** similarity trees. Such method not only increases the number of features considered, but possibly also reduces the bias caused by technical problems. This suggestion is supported by the molecular biological data which almost invariably show that morphological traits are much more conservative than DNA molecules (Nanney *et al.* 1998). The morphostasis of protists is still unexplained, but recognisable in the fossil record (Schönborn *et al.* 1999).

Acknowledgements. Supported by the Austrian Science Foundation (FWF project P 12367-BIO) and the Deutsche Forschungsgemeinschaft (DFG project IIB3 Schm 544/7-1). The technical assistance of Dr. B. Moser, Dr. E. Herzog and Mag. E. Strobl is gratefully acknowledged.

REFERENCES

Ammermann D. (1985) Species characterization and speciation in the *Stylonychia/Oxytricha* group (Ciliata, Hypotrichida, Oxytrichidae). *Memorie Soc. tosc. Sci. nat., Serie B* **92:** 15-27

Backeljau T., de Bruyn L., de Wolf H., Jordaens K., van Dongen S., Verhagen R., Winnepenninckx B. (1995) Random amplified poly-

morphic DNA (RAPD) and parsimony methods. *Cladistics* **11:** 119-130
Baev P. V., Penev L. D. (1995) BIODIV program for calculating biological diversity parameters, similarity, niche overlap, and cluster analysis. Version 5.1. Pensoft, Sofia, Moscow. ISBN: 0-925031-23-2
Berger H. (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichia). Kluwer, Dordrecht
Berger H., Foissner W., Adam H. (1985) Morphological variation and comparative analysis of morphogenesis in *Parakahliella macrostoma* (Foissner, 1982) nov. gen. and *Histriculus muscorum* (Kahl, 1932), (Ciliophora, Hypotrichida). *Protistologica* **21:** 295-311
Birks H. J. B. (1987) Recent methodological developments in quantitative descriptive biogeography. *Ann. Zool. Fennici* **24:** 165-178
Bowers N. J., Pratt J. R. (1995) Estimation of genetic variation among soil isolates of *Colpoda inflata* (Stokes) (Protozoa: Ciliophora) using the polymerase chain reaction and restriction fragment length polymorphism analysis. *Arch. Protistenkd.* **145:** 29-36
Bowers N., Kroll T. T., Pratt J. R. (1998) Diversity and geographic distribution of riboprints from three cosmopolitan species of *Colpoda* Müller (Ciliophora: Colpodea). *Europ. J. Protistol.* **34:** 341-347
Buitkamp U. (1977) Über die Ciliatenfauna zweier mitteleuropäischer Bodenstandorte (Protozoa; Ciliata). *Decheniana* **130:** 114-126
Dini F., Gianni A. (1985) Breeding systems in the *Euplotes vannus-crassus-minuta* group. *Memorie Soc. tosc. Sci. nat., Serie B* **92:** 75-93
Dini F., Nyberg D. (1999) Growth rates of marine ciliates on diverse organisms reveal ecological specializations within morphospecies. *Microb. Ecol.* **37:** 13-22
Engelmann T. W. (1862) Zur Naturgeschichte der Infusionsthiere. *Z. wiss. Zool.* **11:** 347-393
Farris, J. S. (1988) Hennig 86, version 1.5. Port Jefferson Station, New York
Finlay B. J., Fenchel T. (1999) Divergent perspectives on protist species richness. *Protist* **150:** 229-233
Foissner W. (1982) Ökologie und Taxonomie der Hypotrichida (Protozoa: Ciliophora) einiger österreichischer Böden. *Arch. Protistenkd.* **126:** 19-143
Foissner W. (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. *Progr. Protistol.* **2:** 69-212
Foissner W. (1991) Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Europ. J. Protistol.* **27:** 313-330
Foissner W. (1998) An updated compilation of world soil ciliates (Protozoa, Ciliophora), with ecological notes, new records, and description of new species. *Europ. J. Protistol.* **34:** 195-235
Foissner W. (2000a) Protist diversity: estimates of the near-imponderable. *Protist* **150** (year 1999): 363-368
Foissner W. (2000b) Notes on ciliates (Protozoa, Ciliophora) from *Espeletia* trees and *Espeletia* soils of the Andean Páramo, with descriptions of *Sikorops espeletiae* nov. spec. and *Fragmocirrus espeletiae* nov. gen., nov. spec. *Stud. neotrop. Fauna Environm.* **35:** 52-79
Kusch J. (1998) Local and temporal distribution of different genotypes of pond-dwelling *Stentor coeruleus*. *Protist* **149:** 147-154
Kusch J., Heckmann K. (1996) Population structure of *Euplotes* ciliates revealed by RAPD fingerprinting. *Écoscience* **3:** 378-384
Landry P. A., Lapointe F. J. (1997) RAPD problems in phylogenetics. *Zool. Scr.* **25:** 283-290
Lorenzen S., Sieg J. (1991) Phylip, Paup, and Hennig 86 - how reliable are computer parsimony programs used in systematics? *Z. zool. Syst. Evolut.-forsch.* **29:** 466-472
Lynch M. (1991) Analysis of population genetic structure by DNA fingerprinting. In: DNA Fingerprinting: Approaches and Applications, (Eds. T. Burke, G. Dolf, A. J. Jeffreys and R. Wolffs). Birkhäuser, Basel, 113-126
Maeda M., Carey P. G. (1984) A revision of the genera *Trachelostyla* and *Gonostomum* (Ciliophora, Hypotrichida), including redescriptions of *T. pediculiformis* (Cohn, 1866) Kahl, 1932 and *T. caudata* Kahl, 1932. *Bull. Br. Mus. nat. Hist. (Zool.)* **47:** 1-17
Malpartida J. M., Martin-González A., Gutiérrez J. C. (1995) Comparison of PCR fingerprintings (RAPD method) and phylogenetic analysis of European, African and American colpodid species. *Second Europ. Congr. Protistol., Clérmont-Ferrand*, Abstract 155
Martín A., Palacios G., Olmo A., Martín-González A., Ruiz-Perez L. M., Gutiérrez J. C. (1997) Karyotypic variability in ribosomal DNA subchromosome size among colpodid ciliates, a possible tool to differentiate colpodid species. *Appl. Environ. Microbiol.* **63:** 1602-1605
Mayr E. (1975) Grundlagen der zoologischen Systematik. Parey, Hamburg
Nanney D. L., McCoy J. W. (1976) Characterization of the species of the *Tetrahymena pyriformis* complex. *Trans. Am. microsc. Soc.* **95:** 664-682
Nanney D. L., Park C., Preparata R., Simon E. M. (1998) Comparison of sequence differences in a variable 23S rRNA domain among sets of cryptic species of ciliated protozoa. *J. Euk. Microbiol.* **45:** 91-100
Pearson K. (1926) On the coefficient of racial likeness. *Biometrika* **18:** 105-117
Pielou E. C. (1984) The Interpretation of Ecological Data. Wiley & Sons, New York
Remane A. (1952) Die Grundlagen des natürlichen Systems, der vergleichenden Anatomie und der Phylogenetik. Akademische Verlagsgesellschaft, Leipzig
Sachs L. (1984) Angewandte Statistik. 6 ed. Springer, Berlin
Schönborn W., Dörfelt H., Foissner W., Krienitz L., Schäfer U. (1999) A fossilized microcenosis in triassic amber. *J. Euk. Microbiol.* **46:** 571-584
Skroch P., Nienhuis J. (1995) Impact of scoring error and reproducibility of RAPD data on RABD based estimates of genetic distance. *Theor. appl. Genet.* **91:** 1086-1091
Sneath P. H. A., Sokal R. R. (1973) Numerical Taxonomy. The Principles and Practice of Numerical Taxonomy. Freeman, San Francisco
Sørensen T. (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. *K. danske Vidensk. Selsk. Skr.* **5:** 1-35
Sokal R. R., Rohlf F. L. (1981) Biometry. 2 ed. Freeman, San Francisco
Stein F. (1859) Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reihenfolge bearbeitet. I. Abtheilung. Allgemeiner Theil und Naturgeschichte der hypotrichen Infusionsthiere. Engelmann, Leipzig
Sterki V. (1878) Beiträge zur Morphologie der Oxytrichinen. *Z. wiss. Zool.* **31:** 29-58
Stoeck T., Schmidt H. J. (1998) Fast and accurate identification of European species of the *Paramecium aurelia* complex by RAPD-fingerprints. *Microb. Ecol.* **35:** 311-317
Stoeck T., Przyboś E., Schmidt H. J. (1998) A combination of genetics with inter- and intra-strain crosses and RAPD-fingerprints reveals different population structures within the *Paramecium aurelia* species complex. *Europ. J. Protistol.* **34:** 348-355
Stoeck T., Przyboś E., Kusch J., Schmidt H. J. (2000a) Intra-species differentiation and level of inbreeding of different sibling species of the *Paramecium aurelia* complex. *Acta Protozool.* **39:** 15-22
Stoeck T., Welter H., Seitz-Bender D., Kusch J., Schmidt H. J. (2000b) ARDRA and RAPD-fingerprinting reject the sibling species concept for the ciliate *Paramecium caudatum* (Ciliophora, Protoctista). *Zool. Scr.* **29:** 75-82
Tingey S. V., Tufo J. P. (1993) Genetic analyses with random amplified polymorphic DNA markers. *Pl. Physiol.* **101:** 349-352
Wiąckowski K., Hryniewiecka-Szyfter Z., Babula A. (1999) How many species are in the genus *Mesanophrys* (Protista, Ciliophora,

facultative parasites of marine crustaceans)? *Europ. J. Protistol.* **35:** 379-389

Wichterman R. (1986) The Biology of *Paramecium*. 2 ed. Plenum Press, New York

Woodburn M. A., Youston A. A., Hilu K. H. (1995) Random amplified polymorphic DNA fingerprinting of mosquito-pathogenic and nonpathogenic strains of *Bacillus sphaericus*. *Int. J. Syst. Bact.* **45:** 212-217

Xu Z., Bowers N., Pratt J. R. (1997) Variation in morphology, ecology, and toxicological responses of *Colpoda inflata* (Stokes) collected from five biogeographic realms. *Europ. J. Protistol.* **33:** 136-144

Received on 25th August, 2000; accepted on 7th February, 2001