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Genetic, Morphological, and Ecological Diversity of Spatially Separated Clones of *Meseres corlissi* Petz & Foissner, 1992 (Ciliophora, Spirotrichea)

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ABSTRACT. We investigated the intraspecific variation of the spirotrich freshwater ciliate *Meseres corlissi* at the level of genes (SSrDNA, ITS), morphology (14 characters), and ecophysiology (response to temperature and pH). Five of the eight clonal *M. corlissi* cultures isolated from five localities on four continents were studied at all levels. The null hypothesis was that geographic distance plays no role: *M. corlissi* lacks biogeography. The intraspecific variation was low at the genetic level (0%–4%), moderate at the morphological level (5%–15%), and high at the ecophysiological level (10%–100%). One clone, isolated from subtropical China, differed significantly at all levels from all other clones, suggesting limited dispersal and local adaptation among *M. corlissi*. However, other clones from distant areas, such as Australia and Austria, were genetically identical and differed only slightly in morphology and temperature response. We speculate that our findings may be typical for rare species; the chances may be equally high for both global dispersal of *most* and local adaptation of *some* populations in areas where dispersal has been permanently or temporarily reduced.

Key Words. Biogeography, ciliates, ecophysiology, morphology, phylogeny.

MESERES corlissi is a widely distributed but rare planktonic freshwater ciliate. Trophic cells seem to be restricted to ephemeric habitats and, therefore, short time periods (Gächter and Weisse 2006; Weisse et al. 2007). So far, the species has been recorded from eight localities on five continents (Fig. 1). Ecophysiological investigations of *M. corlissi* have shown significant clonal differences in temperature and pH preference and encystment pattern (Gächter and Weisse 2006; Müller, Foissner, and Weisse 2006; Weisse 2004; Weisse et al. 2007). Morphological studies described high similarity of trophic and cystic specimens from bromelia tanks in the Dominican Republic (DR) and from puddles in Austria (Foissner, Müller, and Weisse 2005; Petz and Foissner 1992). These results raise questions about the extent of genotypic and phenotypic divergence between different clones of *M. corlissi*, and how the variation measured at different levels is interrelated.

It is possible that *M. corlissi* comprises sibling species that account for the ecological differences, as we know from other ciliates, such as *Paramecium aurelia* and *Tetrahymena pyriformis* (Jerome and Lynn 1996; Jerome, Simon, and Lynn 1996; Stoeck et al. 2000). Ciliate identification by molecular methods does not always yield unequivocal results. While some *Tetrahymena* species share identical SSrDNA sequences (Sogin et al. 1986; Strüder-Kypke et al. 2001), the SSrDNA has been proven a suitable molecular marker for species identification in other ciliates. Furthermore, some studies have shown that the SSrDNA of spatially distant populations can be identical (Agatha, Strüder-Kypke, and Beran 2004; Strüder-Kypke et al. 2000), while other studies have found some genetic variation among populations of morphologically identical ciliates (Finlay et al. 2006; Katz et al. 2005; Schmidt, Ammerman, and Schlegel 2006). Foissner, Chao, and Katz (2008) suggest that future studies on those morphospecies will likely lead to the description of several distinct species to account for the observed genetic diversity.

Similar to the analysis of the SSrDNA, varying results were obtained with the ITS region to determine intraspecific variation of ciliate species. Snoeyenbos-West et al. (2002) have shown that there was only minimal clonal divergence within some oligotrich and choreotrich species. Coleman (2005), Barth et al. (2006), and Wright (1999) reported identical ITS sequences for *Paramecium* and *Isotricha* isolates from distant localities. In contrast, Miao et al. (2004) have found ITS sequences useful for establishing a phylogeography for *Carchesium polypinum* populations in China. Diggles and Adlard (1997) have also shown intraspecific variation in geographic isolates of the marine fish parasite *Cryptocaryon irritans*. With respect to the ongoing discussion about distribution and endemism of ciliate species (summarized in Foissner 2006; Foissner et al. 2008), our goal was a detailed analysis of the intraspecific variation in *M. corlissi* to determine patterns of evolutionary divergence and to infer and explain mechanisms of adaptation that may differ at the various levels of cellular organization (i.e. genes, ultrastructure, physiology, and behavior). We investigated whether clonal differences in molecular, morphological, and ecological features (1) increase with increasing geographical distance and (2) if those differences can be related to the break up of Pangaea into Gondwana and Laurasia. The null hypothesis was that geographic distance plays no role: *M. corlissi* lacks biogeography. Our study integrates, for the first time, molecular (SSrDNA, ITS) features with light microscopical (e.g. number of ciliary rows and of adoral membranelles, size of resting cysts), ultrastructural (e.g. number of cortical microtubules), and ecophysiological (temperature and pH response) characteristics to investigate intraspecific differences in a free-living ciliate.

MATERIALS AND METHODS

Collection of populations. Populations of *M. corlissi* Petz & Foissner, 1992 were collected from five sites representing four continents (Fig. 1). The origin, sampling date, collector/isolator, and GenBank accession number of the clones are summarized in Table 1. Clones AU1, AU2, AU3, and AU5 are from the type locality—soil and mud of an ephemeral meadow puddle in the City of Salzburg, Austria. For further details on sampling sites, see Gächter and Weisse (2006) and Weisse et al. (2007). The populations from soil were isolated with the non-flooded Petri dish method as described in Foissner, Agatha, and Berger (2002).

Cultivation. Clonal cultures for ecophysiological experiments were established and maintained as described by Weisse et al. (2007), with the exception of the DR population where cultures were established from several individuals. All cultures were maintained under controlled light and temperature conditions with the freshwater flagellate *Cryptomonas* sp. as food source. Specimens for the genetic investigations were harvested from exponentially

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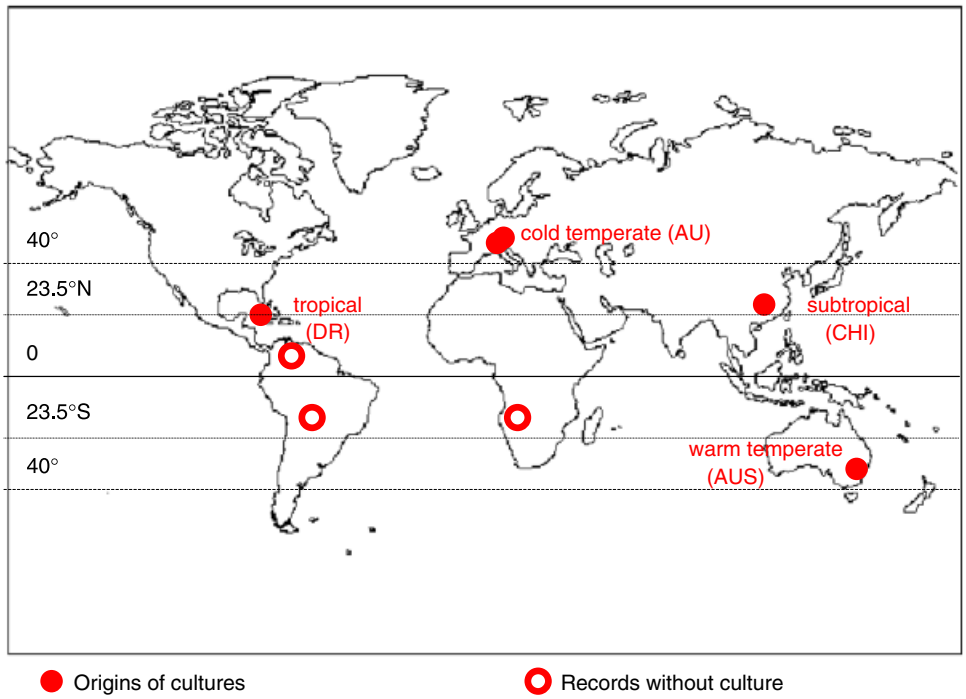

Fig. **1.** Map showing records of *Meseres corlissi* (open circles) and origin of the cultures with abbreviations of clones used in this study (closed circles).

growing cultures. Several hundred individual cells of the clones were fixed in 80% ethanol for DNA extraction.

Resting cysts were obtained from old cultures. When sufficient cysts had been formed, the medium was decanted and replaced by Eau de Volvic for 2 wk to be sure to investigate mature cysts. For the other morphological investigations, culturing was modified as described below (under subheading Light microscopy and morphometry).

DNA extraction and sequencing. DNA was extracted either following the protocol of Walsh, Metzger, and Higuchi (1991) as described by Strüder-Kypke and Lynn (2003) using 100 µl of 5% Chelex® 100 (Sigma, Oakville, ON, Canada) or using the MasterPure™ DNA Purification Kit (Epicentre, Madison, WI). Typically 4–10 µl of template were used in the subsequent PCR amplifications. PCR amplification of the rRNA genes was performed in a Perkin-Elmer GeneAmp 2400 thermocycler (PE Applied Biosystems, Mississauga, ON, Canada), following the procedure described in Wright, Dehority, and Lynn (1997) and using the universal forward primer A (5′-AACCTGGTTG ATCCTGCCAGT-3′; Medlin et al. 1988) and the reverse primer C (5′-TTGGTCCGTGTTTCAAGACG-3′; Jerome and Lynn 1996). PCR products were purified using the GeneClean kit (Qbiogen, Carlsbad, CA) and in some cases cloned with the TOPO TA Cloning Kit (Invitrogen, Burlington, ON, Canada). The cloned products were re-amplified and purified as described above. DNA was sequenced in both directions with a 3730 DNA Analyzer (Applied Biosystems Inc., Foster City, California), using ABI Prism BigDye Terminator (ver. 3.1) and Cycle Sequencing Ready Reaction kit. Usually, the SSrDNA sequence was obtained with three forward and three reverse internal universal SSrDNA primers (Elwood, Olsen, and Sogin 1985), while the ITS region was sequenced using the internal forward primer 1055F (5′-GGTGGTGCATGGCG-3′; Elwood et al. 1985) and reverse primer C.

Sequence availability and phylogenetic analyses. The nucleotide sequences used for our analyses are available from the GenBank/EMBL databases under the following Accession numbers:

Table **1.** Sampling data and GenBank Accession numbers for the studied clones of *Meseres corlissi*.

Clone	Sampling date	Origin	Collector/isolator	GenBank Accession number
Meseres corlissi				
DR	Spring 2002	Bromelia tank reservoir, fog rain forest, Santiago, Dominican Rep.	W. Till, W. Foissner/T. Weisse	EU399522
AU1	November 2002	Ephemeral meadow pond, Salzburg, Austria*	W. Foissner/H. Müller	EU399523
AU5	November 2002	Ephemeral meadow pond, Salzburg, Austria*	W. Foissner/H. Müller	EU399524
AU2	December 2003	Ephemeral meadow pond, Salzburg, Austria*	W. Foissner/E. Gächter	EU399525
AU3	December 2003	Ephemeral meadow pond, Salzburg, Austria*	W. Foissner/T. Weisse	EU399526
AU6	November 2004	Ephemeral meadow pond, Kefermarkt, Upper Austria	W. Foissner/T. Weisse	EU399527
AU7	November 2004	Ephemeral meadow pond, Kefermarkt, Upper Austria	W. Foissner/T. Weisse	—
CHI	October 2005	Zhu Jiang River, Guangzhou, China	W. Foissner/T. Weisse	EU399529
AUS	March 2006	Soil from the Murray River floodplain, Albury, Australia	W. Foissner/T. Weisse	EU399528

The asterisk marks the type locality of *M. corlissi*.

Engelmanniella mobilis AF164134, AF508757 (Hewitt et al. 2003), *Halteria grandinella* AF508759 (Hewitt et al. 2003), *Oxytricha granulifera* X53485 (Schlegel, Elwood, and Sogin 1991), and *Paraurostyla viridis* AF508766 (Hewitt et al. 2003). The sequences of the *M. corlissi* clones are reported in Table 1.

The sequence fragments were imported into Sequencher ver. 4.0.5 (Gene Codes Corp. Ann Arbor, MI), trimmed at the ends, assembled into contigs, and checked for sequencing errors. The SSrDNA sequences for the clones of *M. corlissi* were added to the existing Dedicated Comparative Sequence Editor (DCSE; De Rijk and De Wachter 1993) database and automatically aligned to the sequence of *H. grandinella* (Hewitt et al. 2003). We further refined the alignment by considering secondary structural features of the SSrRNA molecule.

For the phylogenetic analyses, MrModeltest (Nylander 2004) was employed to find the model of DNA substitution that best fits our data. The parameters were implemented into MrBayes ver. 3.1.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and two parallel runs of a Bayesian Inference (BI) analysis were performed over 1,000,000 generations, with every 50th tree sampled. The maximum posterior probability was determined out of the sampled trees, approximating it with the Markov Chain Monte Carlo (MCMC). The first 2,000 trees were discarded as burn-in. A maximum parsimony analysis with random sequence addition (MP) was performed with PAUP* ver. 4.0b10 (Swofford 2002). Genetic distances were calculated using DNADIST and the Kimura 2-parameter model (Kimura 1980) of the PHYLIP ver. 3.6a2 package (Felsenstein 2004) and Neighbor-Joining analysis (NJ; Saitou and Nei 1987) was performed to compute a tree. Both parsimony and distance data were bootstrap resampled 1,000 times.

Light microscopy and morphometry. Since the cells of the clonal cultures were too fragile for ordinary protargol impregnation, the cultures were maintained at room temperature with some crushed wheat grains and 1 ml of *Cryptomonas* sp. added as food source. After 2 wk cells stabilized and could be preserved with a fixative composed of 40-ml saturated mercuric chloride and 40-ml glutaraldehyde (25%, v/v). For fixation, 40 ml of an exponentially growing culture were poured into 40-ml fixative. Protargol impregnation was performed as described by Foissner (1991). For the morphological investigations, 21 well-prepared morphostatic specimens were selected from the permanent slides. The features investigated are shown in Fig. 2 and Table 5. The slides have been deposited in the Biologiezentrum of the Oberösterreichische Landesmuseum in Linz, Upper Austria.

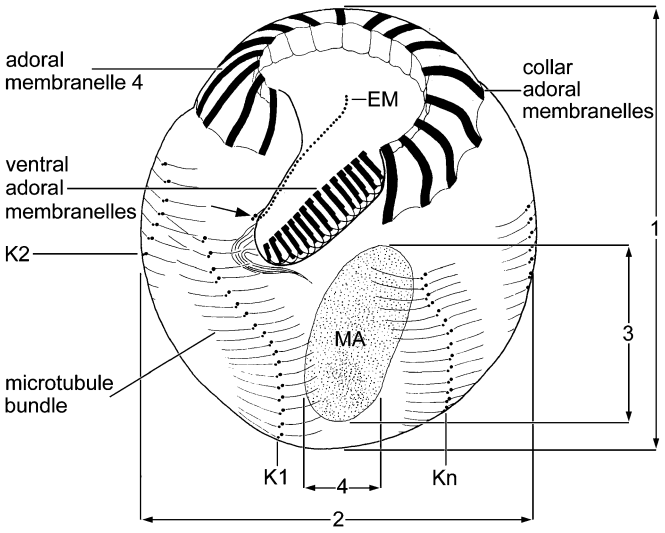

Fig. **2.** A slightly schematized ventral view of a protargol-impregnated specimen of *Meseres corlissi*, showing some of the features measured and counted (cf. Table 5). Arrow marks the minute paroral membrane. EM—endoral membrane; K1, K2—somatic kineties; Kn—last somatic kinety; MA—macronucleus; 1—body length; 2—body width; 3—macronucleus length; 4—macronucleus width.

Scanning electron microscopy (SEM). This was performed as described in Foissner (1991), but using 4% (w/v) osmium tetroxide. Further, specimens were mounted as described by Foissner and AL-Rasheid (2006).

Transmission electron microscopy (TEM). Exponentially growing cultures were fixed in a ''strong'' fixative composed of 10 ml of 25% (v/v) glutaraldehyde, 6 ml of 2% (w/v) aqueous osmium tetroxide, and 10 ml saturated mercuric chloride for 1 h at room temperature. Further manipulations were as described by Foissner (2005).

Since the investigations were very time consuming, only 7–12 specimens per population (actually twice as many, because transverse and longitudinal sections were needed) and only four populations were investigated (Table 6). To increase the accuracy of the values, we studied 6–12 sections from the middle third of each specimen. The data obtained were averaged and then analyzed statistically.

Ecophysiological experiments. Laboratory experiments on temperature and pH response were conducted with nine, and five *M. corlissi* clones, respectively, all harvested from cultures in exponential growth phase. Small culture flasks (50-ml vol.), 6-well (6-ml vol.), or 12-well (4-ml vol.) tissue plates, served as experimental containers. Ciliates were fed with *Cryptomonas* sp. at satiating levels during the experiments and acclimated step-wise, over several generations, to the respective experimental conditions. Ciliate cell size was measured with Lugol's fixed material using a semi-automatic image analysis system. Details of the experimental set up and the analyses have been reported by Gächter and Weisse (2006), Weisse (2004), and Weisse et al. (2007).

Statistical analyses. Morphological characters of the populations reported in Table 5 were compared by the non-parametric method according to Nemenyi (Sachs 1984). This analysis assumes k treatment groups (or populations, in our study) with equal sample sizes. We used $k = 7$ for the interphase specimen features, $k = 5$ for the resting cyst features, and $k = 4$ for the TEM features (Table 5). For each character, all ($n \times k$) observations were ranked from smallest to largest. In case of ties, we computed the average ranks. We then summed the ranks separately for each population and computed all possible absolute differences of these sums. If an observed difference between two treatments (populations) reached or exceeded a critical value D (Table 180 in Sachs 1984), the difference was significant. The critical value D is adjusted to the number of treatments (populations); thus, a Bonferroni adjustment is not needed. The results for 22 features are presented in the right part of Table 5. In order to discern geographical differences, we added the ''*n*umber of *n*ot *s*ignificantly *d*ifferent *c*haracters'' (NNSDC; Berger, Foissner, and Adam 1985) for each pair of populations at three significance levels. These values were converted to percentages, with 22 characters = 100%, which denotes ''total similarity'' (Table 6). The higher overall similarity at the higher level of significance (i.e. smaller P values) denotes that the number of significantly different characters decreases if P increases from 0.1 to 0.05 and 0.01. The populations AU5, AU6, and DR were not considered for the NNSDC method because resting cyst and/or TEM features were lacking.

We compared the morphological and ecophysiological diversity of five clones (AU2/AU3, AU5, AU6, AUS and CHI) for which all parameters were investigated. To render morphological

and ecophysiological measurements comparable, the raw data were converted to rank numbers for each clone and each parameter measured (i.e. the clone with the longest linear dimension, the highest number of kineties or the highest physiological rate received rank number 1). We used 13 of the 14 morphological characters of the interphase specimens listed in Table 5 for this statistical analysis; the number of collar adoral membranelles was discarded because it was constant among all clones. Similarly, we used the 14 features of the temperature (*T*) and pH response experiments listed in Table 7. The dataset was analyzed by ANOVA on ranks and pairwise post hoc tests (Student–Newman–Keuls method) to test for significant differences between the clones. Cluster analyses with unweighted pair-group average and nearest neighbor methods with Euclidean, squared Euclidean, and city block distance metrics were used to construct dendrograms (Statgraphics Plus 4.0, Manugistics Inc., Rockville, MD; Electronic Manual). We first calculated separate dendrograms for the morphological and the ecophysiological dataset, and then a dendrogram for the combined dataset. Since all cluster analysis methods yielded virtually identical results, we report results from the group average analysis and city block distance metric only. Statistical analyses were conducted using SigmaStat 2.03 and Statgraphics Plus 4.0.

RESULTS

Intraspecific genetic differences. We observed intraspecific conservation of length and GC content in all sequenced regions of the rDNA molecule of *M. corlissi*. The divergences among the clones of *M. corlissi* occurred mainly in the SSrDNA. The SSrDNA was generally 1,774 nucleotides long and had a GC content of 44%, and the 5.8S rDNA was conserved (153 nucleotides long, GC content 49%) in all studied clones. ITS 1 and ITS 2 were identical for all clones (125 and 199 nucleotides long, GC content 41% and 45%), except the Chinese one (CHI; 126 and 201 nucleotides long, GC content 40% and 45%), which showed eight differences to all other clones. Within the SSrDNA, we found differing nucleotide positions for clones DR (1), AU1 (4), AU5 (5), AU2 (2), and CHI (1) (Table 2).

The Austrian clone from Kefermarkt (AU6) was identical to clone AU3 from Salzburg and to the Australian clone (AUS). Sequences of *M. corlissi* clones AU2 and AU3 were inferred partly from the primary purified PCR product and partly from the reamplified clonal product. During sequence analysis with Sequencher ver 4.05, we discovered distinct nucleotide differences (A/G) in one (AU3) and two (AU2) positions of the SSrDNA between the primary and the clonal PCR product.

Table **3.** Similarities (in %) within the SSrDNA and ITS sequences of *Meseres corlissi* clones and *Halteria grandinella*.

	ITS1	5.8SrDNA	ITS2	All ITS	SSrDNA & ITS
CHI/other *M. corlissi*	96	100	98	98	99
Other *M. corlissi*/ *H. grandinella*	84	99	89	91	97
CHI/*H. grandinella*	82	99	89	90	97

CHI, Chinese *M. corlissi*.

The phylogenetic placement of *M. corlissi* was inferred from the SSrDNA sequence analysis of two clones of *M. corlissi* and *H. grandinella* (data not shown). Modeltest ver. 3 defined the Tamura-Nei model (TrN, Tamura and Nei 1993) with gamma distribution as the most suitable model for nucleotide substitution. A sister-group relationship of *Meseres* and *Halteria* is highly supported by ML (100%) and moderately supported by MP (54%) and NJ (64%). All clones of *M. corlissi* shared 99%–100% SSrDNA sequence similarity and showed only minimal evolutionary divergence (Table 3). The clones AU3, AU6, and AUS were identical. *Halteria grandinella* and *M. corlissi* shared over 98% similarity and the genetic divergence was 1.5%.

The phylogenetic analyses of the ITS1-5.8S-ITS2 region of the rDNA of *M. corlissi* included the ITS sequences of *H. grandinella*; three stichotrich species were used as out-group (data not shown). The ITS region was identical for the *Meseres* clones AU1, AU2, AU3, AU5, AUS, and DR: all clones formed a polychotomy. The Chinese clone (CHI) branched basally. Modeltest ver. 3 defined the model of HKY model (Hasegawa, Kishino, and Yano 1985) with gamma distribution as most suited for our ITS dataset. Based on the secondary structure model of the ITS2 (Coleman 2005), we constructed a model for the ITS2 of *Meseres* and *Halteria* (Fig. 3), illustrating the variable regions between the two genera. The substitutions are located in the loop of helix A, as well as in the subhelices and loops of helix B.

The third phylogenetic analysis was based on the combined data of both SSrDNA and ITS for *M. corlissi* and *H. grandinella* (Fig. 4). The stichotrichs *E. mobilis*, *Oxytricha granulifera,* and *Paraurostyla viridis* were used as out-group as these three species also have complete SSrDNA and ITS sequences. The tree topology is identical to the previous topologies: the Chinese clone (CHI) branched basally to the polychotomous clade of the remaining *M. corlissi* clones (Fig. 4). Table 3 lists the genetic divergences for the group of almost identical *M. corlissi* clones, the Chinese *M. corlissi* clones, and *H. grandinella*. The data show clearly that the ITS1 is the most diverse region: 4% divergence

Table **2.** Variable nucleotide positions among the clones of *Meseres corlissi* (SSrDNA).

Meseres corlissi	SSrDNA[a]													ITS1[a]					ITS2[a]		
Position/Clone[b]	118	146	206	273	318	499	511	687	736	1206	1359	1528	1694	1861	1893	1894	1895	1899	2179	2180	2253
DR	T	G	T	A	C	A	A	T	*C*	C	C	G	T	C	—	T	A	C	—	—	A
AU1	*C*	·	·	*G*	·	·	*G*	·	T	*T*	·	·	·	·	·	·	·	·	·	·	·
AU5	·	.	—	·	·	*G*	·	*C*	T	·	*G*	*A*	·	·	·	·	·	·	·	·	·
AU2	·	*C*	·	·	*T*	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·
AU3	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·
AU6	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·
AUS	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·
CHI	·	·	·	·	·	·	·	·	T	·	·	·	*C*	*T*	*A*	*C*	*C*	*A*	*G*	*C*	*T*

[a]Positions 118–1694 occur in the SSrDNA gene, positions 1861–1899 in the ITS1 region, and positions 2179–2253 in the ITS2 region.
[b]For designation of clones, see Table 1.
Positions are numbered with respect to the position in our SSrDNA alignment (Position 1).

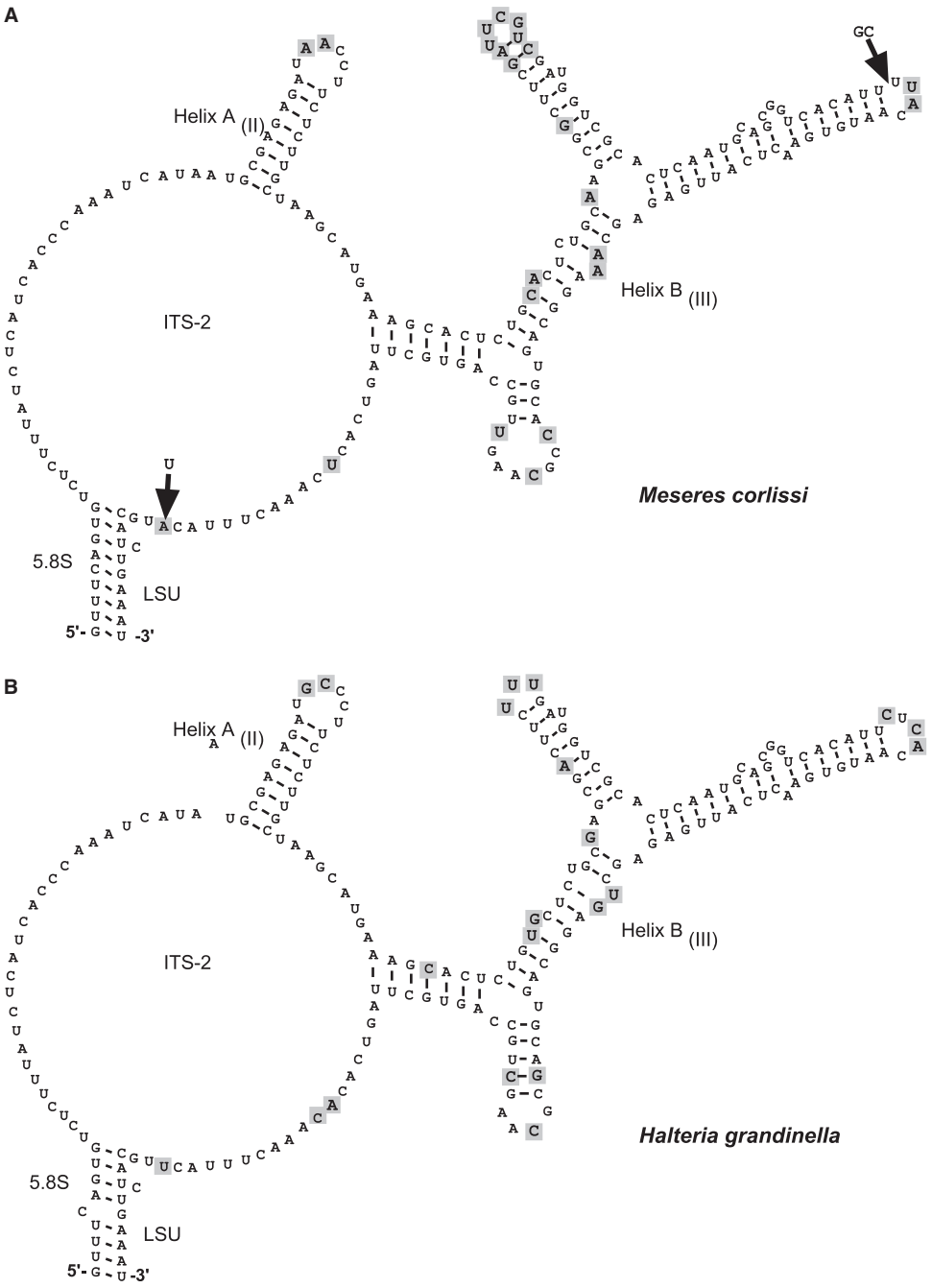

Fig. **3.** Models of the secondary structure of the ITS2 region of *Meseres corlissi* (**A**) and *Halteria grandinella* (**B**). Variable positions among *Meseres* and *Halteria* are highlighted by a grey background. The arrows in (**A**) mark the differences of the clone CHI to all other clones of *M. corlissi*.

within *M. corlissi* and 16%–18% divergence between *M. corlissi* and *H. grandinella*, while SSrDNA and 5.8S rDNA are more conserved. ITS2 shows mostly intergeneric variation (11% between *M. corlissi* and *H. grandinella*), but little intraspecific divergence (2% within *M. corlissi*).

Morphological variation. The most important morphological features investigated by light microscopy are shown schematically in Fig. 2; Table 4 summarizes the 14 morphological characters investigated in seven *M. corlissi* clones from different geographic regions. Ultrastructural details revealed by SEM and TEM are provided in the captions to Fig. 5–8.

The seven *M. corlissi* populations investigated showed conspicuous morphological differences (Tables 5 and 6). Even clones of the same population revealed significant differences; as an example, clones AU2 and AU5, both from the Austrian type locality, differed significantly in the number of ciliary rows: 8 and 9 on average respectively (Table 5). However, if only the presumably most important, (= presumably functional) morphological characters (i.e. number of ciliary rows, kinetids within a ciliary row, adoral membranelles, cortical microtubules) are considered, the populations are more similar. The variability of the main features was often so low that even minor differences between populations

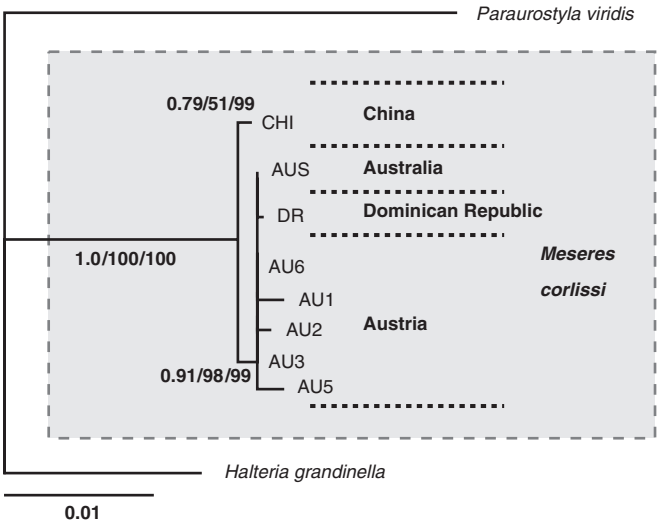

Fig. **4.** Neighbor joining tree of the combined small subunit rDNA and ITS sequences computed with NEIGHBOR and derived from the evolutionary distances computed by DNADIST (both PHYLIP ver. 3.6a2) using the Kimura-2-parameter model. Branch lengths represent evolutionary distance. The first numbers at the nodes represent the posterior probability values of the Bayesian analysis and the second and third numbers represent bootstrap values (percent out of 1,000 replicates) for maximum parsimony and neighbor joining, respectively. The scale bar represents one substitution per 100 nucleotide positions.

were significant; for instance, the number of ciliary rows was significantly different ($P \leq 0.01$, Table 5) between clones AU5 and AU7, although they differed by only 1 kinety on average. No significant differences were found in the fine structural (TEM) features selected (Table 5, Fig. 6–8).

Overall, there was a statistically significant difference between the populations (one-way ANOVA on ranks, $P < 0.001$). The Chinese clone was not different from the Austrian clones AU2 and AU5 from the type locality in Salzburg (pair-wise SNK posthoc tests); similarly, there was no statistical difference between AU2 and AU5 and between the Australian clone (AUS) and the Austrian clone from Kefermarkt (AU6). Pair-wise comparisons of all other clones yielded significant differences (data not shown). The differences obtained appear unrelated to geographical distance and historical break up of Pangaea because cluster analysis revealed that an Austrian population each (AU2, respectively AU6) was associated with the Chinese and Australian populations (Fig. 9(A)).

Ecophysiological variation. Significant differences between populations of *M. corlissi* have already been reported for the temperature response of nine clones (Gächter and Weisse 2006) and for the pH response of five clones (Weisse et al. 2007). Major results are summarized in Table 7 for the five *M. corlissi* clones that were investigated in both previous studies. If all 14 ecophysiological characters investigated were combined, all clones were different from each other with two exceptions: the Austrian clones AU3 and AU5 from Salzburg and the Austrian clone from Kefermarkt (AU6) vs. the Australian clone (AUS) did not differ (one-way ANOVA on ranks, pair-wise SNK posthoc tests). Cluster analysis confirmed and illustrated these results; the Salzburg clones formed one sister group, the Australian (AUS) and Chinese (CHI) clones another one, and the Austrian clone AU6 from Kefermarkt was more closely associated with the other Austrian than with the Asian/Australian clones (Fig. 9(B)).

Table **4.** Morphological features investigated in *Meseres corlissi*.

	Population						
Feature	AU2	AU5	AU6	AU7	AUS	CH1	DR
Interphase (Protargol)	x	x	x	x	x	x	x
Cyst (in vivo)	x			x	x	x	x
Interphase (TEM)	x			x	x	x	

For designation of populations, see Table 1.
DR, Dominican Republic; TEM, transmission electron microscopy.

Morphological and ecophysiological variation combined. If the datasets reported in Tables 5 and 7 are combined, a one-way ANOVA on ranks and pair-wise posthoc comparisons suggest that all clones but the clones from the type locality (AU2/AU3 vs. AU5) and the Australian clone (AUS) vs. the Austrian clone AU6 are significantly different from each other (data not shown). The Chinese clone differed from all other clones, while the Australian clone was identical with one Austrian clone. A biogeographic pattern is less obvious from cluster analysis (Fig. 9(C)) than with the ecological data alone (Fig. 9(B)).

DISCUSSION

Our study demonstrates that the level of detecting intraspecific differences was lowest in the conserved SSrDNA gene ($<0.5\%$ sequence divergence) and highest in ecological features, such as growth and production rates (up to $>100\%$ difference between maximum and minimum values). In the following, we will first address the question how to evaluate and rank variation of different characters at each level (i.e. genes, morphology, ecology) and whether biogeographic patterns emerge at each level. We will then synthesize the intraspecific variation across the three levels investigated with respect to our overall goal: does *M. corlissi* show biogeographic variability?

Genetic variation within *Meseres corlissi*. Among the eight clones of *M. corlissi*, we found 13 variable nucleotide positions in the SSrDNA sequence. The most divergent clones were AU1 and AU5 (0.47%)—both collected at the same locality and the same time. On the contrary, clones AU2 and AU3, also collected simultaneously at the type locality 1 yr later showed only 0.12% divergence. The clone from Kefermarkt (AU6) was identical to the Australian clone (AUS). The clone from the DR also showed little divergence to the Austrian clones—and less divergence to AU1 and AU5 (0.3%) than these two clones to each other. Snoeyenbos-West et al. (2002) have found 0.3%–0.9% intraspecific variation in other choreotrich and oligotrich clones, independent of sampling locality and sampling date. Katz et al. (2005) were able to distinguish three different clusters of *H. grandinella* sampled in locations on four continents. All clusters were separated by genetic divergences larger than 2% while the intraclade divergences were always smaller than 0.5%. Therefore, we conclude that all collected clones belong indeed to the same species, *M. corlissi*. The variations in the SSrDNA were all single nucleotide changes, occurring throughout the gene. A possible explanation is that these differences were introduced by the cloning procedure, since they only occurred in those strains that where cloned. The observed mismatches in the sequences of the primary purified PCR product and the clonal product in clones AU2 and AU3 (see Methods) do affirm this possibility. However, we do not have an explanation why the differences occur only in the SSrDNA but not in the ITS sequences. Previous studies have shown either identical sequences for both ITS and SSrDNA (Wright 1999) or evolutionary divergence to a similar degree in both regions or higher in ITS regions (Miao et al. 2004; Snoeyenbos-West et al. 2002).

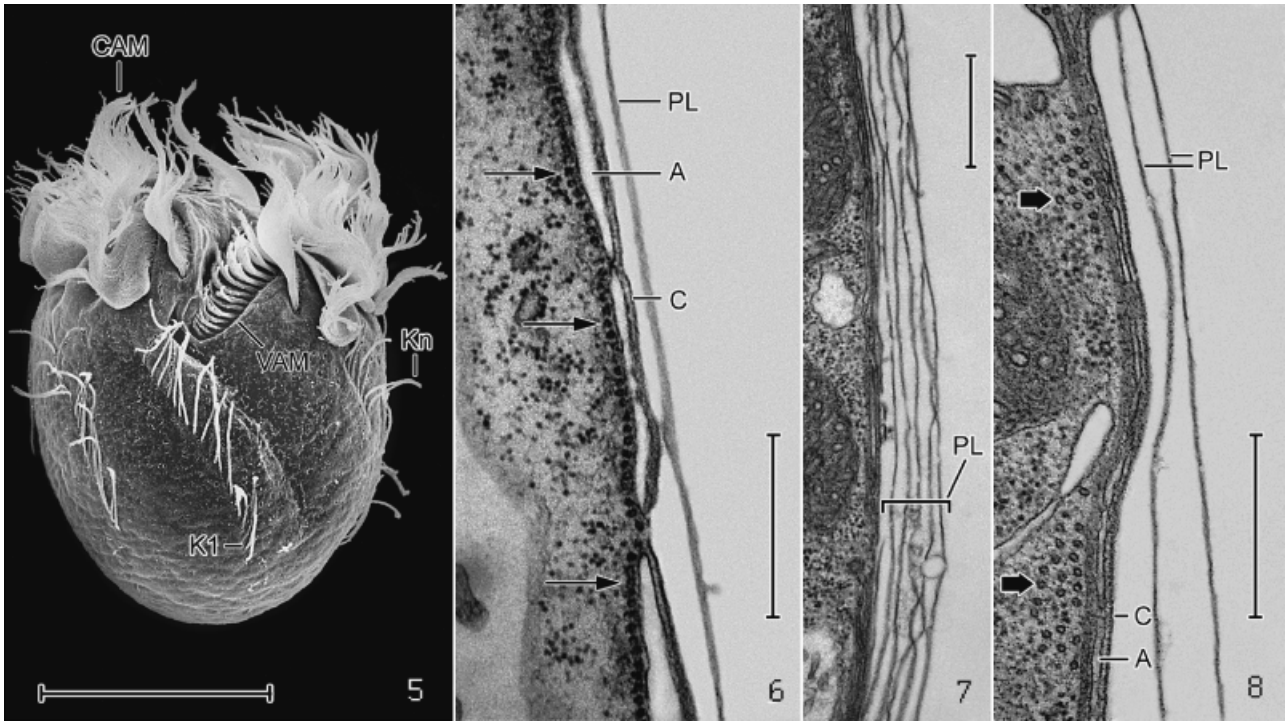

Fig. **5–8.** *Meseres corlissi*, Salzburg population in the scanning (5) and transmission (6–8) electron microscope, showing the general organization and most of the features investigated (cf. Table 5). **5.** Ventral view showing somatic kineties (K1, Kn) and collar (CAM) and ventral (VAM) adoral membranelles. **6.** Transverse section showing the cortical microtubules (arrows) whose number was counted in a length of 1 μm. **7.** Longitudinal section showing the perilemma sheets whose number is highly variable (cf. Fig. 6, 8). **8.** Longitudinal section showing two laterally extending microtubule bundles (arrows). The number of microtubules comprising the individual bundles was counted. Scale bars: 30 μm (Fig. 5) and 500 nm (Fig. 6–8). A—cortical alveoli, C—cell membrane, CAM—collar adoral membranelles, K1—somatic kinety 1, Kn—last somatic kinety, PL—perilemma sheets, VAM—ventral adoral membranelles.

The phylogenetic analyses of the ITS1-5.8S-ITS2 region of the rDNA revealed that the Chinese clone (CHI) differed by 2% from all other *M. corlissi* populations. Accordingly, the Chinese clone grouped separately and basally to all other clones. Since the Australian clone was identical in its ITS to the Austrian and the Central-American clones, the genetic data do not confirm our hypothesis that increasing geographical distance will show increased genetic divergence.

Intraspecific morphological variation. We found many morphological features that were significantly different within and between the *M. corlissi* populations. If all 13 variable characters reported were combined, the factor ''clone'' significantly affected the results (one-way ANOVA on ranks). However, seen with the taxonomist's eye, this variation is within the phenotypic range common in ciliates. For numeric characters, such as the number of ciliary rows and adoral membranelles, an intraspecific coefficient of variation (CV) of 5% is typical for most ciliates (Foissner 1984, 1993), and we found a CV of 4.5% for *M. corlissi*. Size-related distance measures, such as body length or macronucleus width, typically show a CV of 15% (Foissner 1984, 1993). *Meseres corlissi* adheres to this rule: if the first 4 morphometric characters reported in Table 5 are averaged, the CV of each population was 13.5%.

Similar to the genetic data reported above, we found significant differences in 10 out of 13 variable characters between the Austrian clones AU5 and AU6 and even significant differences between clones isolated from the same locality at the same time (AU6 vs. AU7), while the geographically distant clones from the DR and Australia (AUS) differed only little. The cluster analysis confirmed that the morphological results do not suggest increasing divergence with geographic distance.

Intraspecific ecological variation. The intraspecific differences that we obtained for the response to temperature and pH were relatively larger than the respective genetic and morphological differences. The maximum growth rate (μ_{max}), the average cell volume, and the maximum production rate measured in the temperature response experiments all varied by a factor of up to >2 between the clones. Note that some of the ecological features, such as the minimum pH tolerated, were measured at non-linear scales that cannot easily be compared with metric measures. Similarly, it is is difficult to compare relative thermal sensitivity to relative morphological variations because little information is available on inter- and intraspecific variation of the former.

Large intraspecific differences have been reported for growth, feeding, and production rates of several freshwater oligotrich and prostome ciliate species under experimental conditions comparable to those used in the present study (reviewed by Weisse 2006). While most of the previous investigations were conducted with clonal isolates obtained from different habitats or from the same locality at different years, Weisse and Rammer (2006) reported pronounced ecophysiological differences for sympatric clones of two freshwater ciliate species. These authors also discussed the ecological implications of presumably minor differences: 10% growth rate difference may significantly alter the clonal composition in the course of a ciliate peak, which typically lasts for 2–3 wk in temperate lakes (Weisse et al. 1990).

If the average μ_{max} of the temperature response experiments is calculated from the five clones investigated, the CV is 31.9%. In the pH response experiments, the CV of μ_{max} was somewhat lower

Table **5.** Morphometric characterization and comparison of seven populations of *Meseres corlissi*.

Characteristics[a]	Po	x	M	SD	SE	CV	Min	Max	N	Po	AU7	AU6	AU5	AU2	CH1	AUS
Interphase specimens																
Body, length	AU7	75.0	75.0	10.4	2.3	13.8	50.0	95.0	21	AU7						
	AU6	80.6	80.0	9.0	2.0	11.2	62.0	96.0	21	AU6	NS					
	AU5	62.4	62.0	12.4	2.7	19.9	43.0	90.0	21	AU5	*	**				
	AU2	69.9	70.0	9.7	2.1	13.9	56.0	88.0	21	AU2	NS	*	NS			
	CH1	66.7	67.0	8.5	1.9	12.7	55.0	83.0	21	CH1	NS	**	NS	NS		
	AUS	82.1	82.0	9.1	2.0	11.0	66.0	104.0	21	AUS	NS	NS	**	**	**	
	DR	76.4	80.0	9.7	2.1	12.7	55.0	95.0	21	DR	NS	NS	**	NS	x	NS
Body, width	AU7	64.6	68.0	9.2	2.0	14.3	42.0	78.0	21	AU7						
	AU6	69.2	67.0	8.4	1.8	12.2	55.0	87.0	21	AU6	NS					
	AU5	51.1	50.0	7.9	1.7	15.4	40.0	75.0	21	AU5	**	**				
	AU2	57.1	56.0	5.7	1.2	9.9	46.0	68.0	21	AU2	*	**	NS			
	CH1	55.6	56.0	4.1	0.9	7.5	49.0	62.0	21	CH1	**	**	NS	NS		
	AUS	68.3	69.0	4.0	0.9	5.8	62.0	76.0	21	AUS	NS	NS	**	**	**	
	DR	63.8	65.0	6.3	1.4	9.9	52.0	75.0	21	DR	NS	NS	**	NS	*	NS
Macronucleus, length	AU7	30.8	30.0	3.9	0.9	12.7	25.0	40.0	21	AU7						
	AU6	31.0	30.0	5.8	1.3	18.7	21.0	40.0	21	AU6	NS					
	AU5	32.1	32.0	7.2	1.6	22.3	22.0	50.0	21	AU5	NS	NS				
	AU2	31.9	32.0	4.4	1.0	13.9	25.0	39.0	21	AU2	NS	NS	NS			
	CH1	26.1	26.0	5.3	1.2	20.2	18.0	35.0	21	CH1	NS	NS	x	*		
	AUS	35.5	35.0	2.5	0.6	7.0	32.0	40.0	21	AUS	*	x	NS	NS	**	
	DR	31.5	32.0	5.3	1.2	16.7	23.0	40.0	21	DR	NS	NS	NS	NS	x	NS
Macronucleus, width	AU7	12.1	12.0	1.5	0.3	12.5	10.0	15.0	21	AU7						
	AU6	14.7	15.0	1.9	0.4	12.9	9.0	18.0	21	AU6	**					
	AU5	18.3	18.0	4.2	0.9	22.8	13.0	30.0	21	AU5	**	NS				
	AU2	14.1	14.0	2.0	0.4	14.5	10.0	17.0	21	AU2	x	NS	**			
	CH1	12.6	12.0	1.9	0.4	15.1	10.0	16.0	21	CH1	NS	*	**	NS		
	AUS	14.6	15.0	1.4	0.3	9.6	12.0	17.0	21	AUS	**	NS	NS	NS	*	
	DR	14.2	14.0	1.4	0.3	9.9	12.0	16.0	21	DR	*	NS	*	NS	NS	NS
Collar adoral membranelles, number	AU7	16.0	16.0	0.2	0.1	1.4	15.0	16.0	21	AU7						
	AU6	16.0	16.0	0.2	0.1	1.4	15.0	16.0	21	AU6	NS					
	AU5	16.0	16.0	0.5	0.1	2.8	15.0	17.0	21	AU5	NS	NS				
	AU2	16.1	16.0	1.0	0.2	6.5	13.0	17.0	21	AU2	NS	NS	NS			
	CH1	16.1	16.0	0.5	0.1	3.1	15.0	17.0	21	CH1	NS	NS	NS	NS		
	AUS	15.9	16.0	0.3	0.1	1.9	15.0	16.0	21	AUS	NS	NS	NS	NS	NS	
	DR	16.1	16.0	0.2	0.1	1.4	16.0	17.0	21	DR	NS	NS	NS	NS	NS	
Ventral adoral membranelles, number	AU7	15.1	15.0	0.9	0.2	6.1	14.0	17.0	21	AU7						
	AU6	14.5	14.0	1.0	0.2	7.1	13.0	16.0	21	AU6	NS					
	AU5	16.9	17.0	1.6	0.4	9.4	15.0	20.0	21	AU5	*	**				
	AU2	15.2	15.0	1.1	0.2	7.1	12.0	16.0	21	AU2	NS	NS	NS			
	CH1	14.5	14.0	0.8	0.2	5.2	13.0	16.0	21	CH1	NS	NS	**	NS		
	AUS	15.4	15.0	0.7	0.2	4.4	14.0	17.0	21	AUS	NS	NS	NS	NS	NS	
	DR	13.0	13.0	0.7	0.2	5.7	12.0	14.0	21	DR	**	*	**	**	*	**
Kinetids in kinety1, number	AU7	13.4	14.0	1.7	0.4	12.4	10.0	15.0	21	AU7						
	AU6	17.4	18.0	1.8	0.4	10.0	12.0	20.0	21	AU6	**					
	AU5	10.9	11.0	2.3	0.5	21.1	4.0	16.0	21	AU5	NS	**				
	AU2	12.9	13.0	2.3	0.5	17.5	8.0	16.0	21	AU2	NS	**	NS			
	CH1	16.5	16.0	1.7	0.4	10.3	14.0	20.0	21	CH1	**	NS	**	**		
	AUS	17.1	17.0	1.1	0.2	6.6	15.0	19.0	21	AUS	**	NS	**	**	NS	
	DR	14.2	15.0	1.9	0.4	13.3	9.0	16.0	21	DR	NS	**	*	NS	x	**
Kinetids in kinety 4, number	AU7	14.3	14.0	1.0	0.2	6.7	13.0	16.0	21	AU7						
	AU6	16.6	17.0	1.0	0.2	5.9	15.0	18.0	21	AU6	**					
	AU5	12.4	12.0	1.8	0.4	14.7	9.0	16.0	21	AU5	NS	**				
	AU2	14.5	14.0	1.9	0.4	13.4	12.0	19.0	21	AU2	NS	**	NS			
	CH1	15.0	15.0	0.9	0.2	5.8	14.0	17.0	21	CH1	NS	**	**	NS		
	AUS	15.5	15.0	0.8	0.2	5.3	14.0	17.0	21	AUS	x	NS	**	NS	NS	
	DR	15.4	16.0	1.3	0.3	8.4	13.0	18.0	21	DR	NS	NS	**	NS	NS	NS
Kinetids in kinety n, number	AU7	14.2	14.0	1.4	0.3	9.9	12.0	17.0	21	AU7						
	AU6	18.0	18.0	2.4	0.5	13.6	14.0	24.0	21	AU6	**					

Table **5.** (Continued).

Characteristics[a]	Po	x	M	SD	SE	CV	Min	Max	N	Po	AU7	AU6	AU5	AU2	CH1	AUS
	AU5	10.9	11.0	2.2	0.5	19.9	6.0	14.0	21	AU5	*	**				
	AU2	12.9	13.0	2.6	0.6	20.3	8.0	17.0	21	AU2	NS	**	NS			
	CH1	16.4	16.0	1.3	0.3	8.1	14.0	19.0	21	CH1	*	NS	**	**		
	AUS	15.6	16.0	1.6	0.3	10.1	11.0	18.0	21	AUS	NS	NS	**	*	NS	
	DR	14.8	14.0	3.3	0.7	22.1	12.0	28.0	21	DR	NS	**	*	NS	*	NS
Kinety 1, length	AU7	33.7	33.0	6.8	1.5	20.0	20.0	53.0	21	AU7						
	AU6	36.6	36.0	7.2	1.6	19.5	23.0	53.0	21	AU6	NS					
	AU5	24.5	23.0	6.8	1.5	27.8	12.0	40.0	21	AU5	*	**				
	AU2	31.8	33.0	5.4	1.2	16.8	24.0	42.0	21	AU2	NS	NS	NS			
	CH1	30.1	30.0	3.5	0.8	11.7	24.0	35.0	21	CH1	NS	*	NS	NS		
	AUS	43.0	42.0	5.8	1.3	13.4	33.0	57.0	21	AUS	**	NS	**	**	**	
	DR	39.1	40.0	10.7	2.3	27.4	20.0	68.0	21	DR	NS	NS	**	NS	*	NS
Kinety 4, length	AU7	26.4	26.0	3.2	0.7	12.2	20.0	32.0	21	AU7						
	AU6	29.8	30.0	3.1	0.7	10.5	22.0	37.0	21	AU6	x					
	AU5	25.6	25.0	6.3	1.4	24.4	16.0	41.0	21	AU5	NS	**				
	AU2	29.9	28.0	5.6	1.2	18.8	22.0	45.0	21	AU2	NS	NS	NS			
	CH1	23.5	25.0	2.4	0.5	10.2	18.0	27.0	21	CH1	NS	**	NS	**		
	AUS	29.0	29.0	2.9	0.6	9.9	25.0	35.0	21	AUS	NS	NS	x	NS	**	
	DR	27.4	28.0	2.7	0.6	10.0	22.0	30.0	21	DR	NS	NS	NS	NS	*	NS
Kinety n, length	AU7	27.8	28.0	4.2	0.9	15.3	18.0	35.0	21	AU7						
	AU6	34.6	34.0	5.8	1.3	16.7	22.0	43.0	21	AU6	*					
	AU5	22.1	22.0	5.7	1.3	26.0	11.0	32.0	21	AU5	x	**				
	AU2	28.6	28.0	7.3	1.6	25.4	20.0	45.0	21	AU2	NS	*	x			
	CH1	24.9	25.0	2.6	0.6	10.6	17.0	30.0	21	CH1	NS	**	NS	NS		
	AUS	31.7	32.0	3.1	0.7	9.9	24.0	36.0	21	AUS	NS	NS	**	NS	**	
	DR	31.1	32.0	6.2	1.4	19.9	15.0	42.0	21	DR	NS	NS	**	NS	**	NS
Adoral membranelle 4, length	AU7	13.2	13.0	1.0	0.2	7.9	11.0	15.0	21	AU7						
	AU6	15.1	15.0	0.5	0.1	3.3	14.0	16.0	21	AU6	**					
	AU5	13.6	14.0	0.8	0.2	5.9	12.0	15.0	21	AU5	NS	**				
	AU2	15.1	15.0	1.1	0.3	7.5	13.0	17.0	21	AU2	**	NS	**			
	CH1	13.4	13.0	1.4	0.3	10.2	10.0	15.0	21	CH1	NS	**	NS	**		
	AUS	14.1	14.0	0.7	0.2	5.1	13.0	15.0	21	AUS	NS	NS	NS	NS	NS	
	DR	12.3	13.0	0.9	0.2	7.4	11.0	14.0	21	DR	NS	**	x	**	NS	**
Somatic kineties, number	AU7	8.0	8.0	0.0	0.0	0.0	8.0	8.0	21	AU7						
	AU6	7.9	8.0	0.3	0.1	3.8	7.0	8.0	21	AU6	NS					
	AU5	9.3	9.0	0.9	0.2	9.2	8.0	11.0	21	AU5	**	**				
	AU2	7.8	8.0	0.6	0.1	8.1	6.0	9.0	21	AU2	NS	NS	**			
	CH1	8.0	8.0	0.2	0.1	2.7	7.0	8.0	21	CH1	NS	NS	**	NS		
	AUS	8.0	8.0	0.0	0.0	0.0	8.0	8.0	21	AUS	NS	NS	**	NS	NS	
	DR	7.9	8.0	0.4	0.1	4.6	7.0	8.0	21	DR	NS	NS	**	NS	NS	NS
Resting cysts																
Cyst, length in vivo	AU7	45.6	45.0	2.8	0.6	6.2	40.0	52.0	21	AU7						
	AU2	53.4	53.0	4.7	1.0	8.9	47.0	65.0	21	AU2	**					
	CH1	48.4	49.0	2.0	0.4	4.2	45.0	52.0	21	CH1	NS			**		
	AUS	50.1	50.0	3.5	0.8	7.0	42.0	55.0	21	AUS	**			NS	NS	
	DR	47.2	47.0	3.2	0.7	6.8	42.0	55.0	21	DR	NS			**	NS	x
Cyst, width in vivo	AU7	42.5	42.0	3.2	0.7	7.5	37.0	48.0	21	AU7						
	AU2	50.6	50.0	5.2	1.1	10.3	42.0	58.0	21	AU2	**					
	CH1	49.5	48.0	5.6	1.2	11.4	42.0	65.0	21	CH1	**			NS		
	AUS	47.3	48.0	4.3	0.9	9.1	40.0	55.0	21	AUS	**			NS	NS	
	DR	45.4	45.0	4.0	0.9	8.7	40.0	55.0	21	DR	NS			*	NS	NS
Cyst, length in vivo including lepidosomes	AU7	62.8	63.0	4.0	0.9	6.4	55.0	70.0	21	AU7						
	AU2	66.6	65.0	6.1	1.3	9.1	57.0	82.0	21	AU2	NS					
	CH1	59.1	60.0	5.1	1.1	8.7	48.0	65.0	21	CH1	NS			**		
	AUS	67.0	68.0	3.6	0.8	5.4	58.0	75.0	21	AUS	*			NS	**	
	DR	59.5	58.0	5.0	1.1	8.3	50.0	70.0	21	DR	NS			**	NS	**
Cyst, width in vivo including	AU7	60.0	60.0	4.0	0.9	6.7	50.0	66.0	21	AU7						

Table **5.** (Continued).

Characteristics[a]	Po	x	M	SD	SE	CV	Min	Max	N	Po	AU7	AU6	AU5	AU2	CH1	AUS
	AU2	63.6	62.0	7.0	1.5	11.0	50.0	75.0	21	AU2	NS					
lepidosomes	CH1	60.4	60.0	2.9	0.6	4.9	55.0	65.0	21	CH1	NS			NS		
	AUS	63.6	63.0	4.0	0.9	6.3	57.0	73.0	21	AUS	NS			NS	NS	
	DR	58.2	58.0	5.2	1.1	8.9	50.0	68.0	21	DR	NS			*	NS	**
Largest lepidosome, length in vivo	AU7	9.7	10.0	1.1	0.2	11.3	8.0	12.0	21	AU7						
	AU2	12.6	13.0	1.7	0.4	13.6	10.0	15.0	21	AU2	**					
	CH1	8.8	8.0	0.9	0.2	10.8	8.0	11.0	21	CH1	NS			**		
	AUS	11.4	11.0	1.1	0.2	9.8	10.0	13.0	21	AUS	*			NS	**	
	DR	9.1	9.0	1.7	0.4	19.0	6.0	14.0	21	DR	NS			**	NS	**
Transmission electron microscopy[b]																
TEM	AU7	1.7	1.7	0.6	0.3	36.0	1.0	2.7	6	AU7						
character 1	AU2	2.0	2.0	1.6	0.6	77.5	0.0	3.8	6	AU2	NS					
	CH1	1.7	1.6	0.7	0.3	43.3	0.7	2.8	6	CH1	NS			NS		
	AUS	1.1	1.0	0.3	0.1	31.1	0.8	1.7	6	AUS	NS			NS	NS	
TEM	AU7	11.3	10.9	1.8	0.7	15.6	9.3	13.4	6	AU7						
character 2	AU2	10.1	10.3	1.7	0.7	16.8	7.3	12.2	6	AU2	NS					
	CH1	10.9	12.0	3.3	1.4	30.3	5.3	14.0	6	CH1	NS			NS		
	AUS	14.1	12.9	3.6	1.5	25.3	10.4	20.0	6	AUS	NS			NS	NS	
TEM	AU7	18.8	19.5	5.4	2.2	28.5	9.0	24.7	6	AU7						
character 3	AU2	17.1	17.0	2.7	1.1	16.1	13.5	21.3	6	AU2	NS					
	CH1	15.9	15.9	2.6	1.1	16.3	13.0	19.3	6	CH1	NS			NS		
	AUS	17.8	16.8	3.5	1.4	19.8	14.8	24.5	6	AUS	NS			NS	NS	

[a]All measurements in μm.

[b]TEM character 1, number of perilemma sheets; TEM character 2, number of microtubules per bundle; TEM character 3, number of cortical microtubules per μ.

Multiple statistical comparison: NS = $P > 0.1$; x = $0.1 > P > 0.05$;

*$0.05 > P > 0.01$;

**$P < 0.01$; two-tailed.

CV, coefficient of variation (in %); M, median; Max, maximum value; Min, minimum value; n, sample size; Po, population, SD, standard deviation, SE, standard error of the arithmetic mean, *x*, arithmetic mean.

at 14.3%. In both cases, the variance between populations was distinctly higher than within populations. More importantly, the populations did not vary at random, but the difference increased with geographic distance. Gächter and Weisse (2006) demonstrated that the minimum temperature tolerated by clones of *M. corlissi* was positively related to the minimum temperature encountered in their natural habitats and that differences in the thermal sensitivity of the clones increased with increasing geographical distance and habitat difference. The clone-specific difference in temperature response did not result from phenotypic plasticity, since all strains used in this study had been kept under identical laboratory conditions for many generations prior to the beginning of the experiments. The effect of long-term acclimation of growth rates was studied with three Austrian clones of *M. corlissi* (Gächter and Weisse 2008). Although the acclimation effect was significant, its extent was minor relative to differences in the temperature response measured with the clones of *M. corlissi* originating from different climate zones (Gächter and Weisse 2006).

We conclude that, in contrast to the genetic and morphological characters, results from the ecophysiological laboratory experiments suggest a biogeographic pattern among populations of *M. corlissi*.

Comparing genetic with morphological and ecological clonal variation. How do we relate to each other the various levels of divergence within and between populations of *M. corlissi* shown by genes, morphology and ecophysiology? In other words, is there a correspondence between 1% genetic diversity, 10% morphological diversity, and 100% ecological diversity? The obvious answer is no, there is no direct correspondence. However, a more detailed comparison of our datasets suggests otherwise. The Chinese clone CHI differed by 1% in its combined SSrDNA and ITS sequence from all other *M. corlissi* clones. If this clone is compared morphologically with the Austrian clone AU6, it is obvious that they are different in all but one of the eight size-related characters: only the macronucleus length of clone CHI (26.1 ± 5.3 μm, SD) was not significantly smaller than that of AU6 (31.0 ± 5.8 μm, SD). Corresponding to these morphological results, the mean cell length and cell volume of CHI were significantly smaller than those of AU6 in both the temperature and pH response experiments. Similar size-related differences are apparent between CHI and the Australian clone (AUS), but less obvious between CHI and AU7, although the latter was isolated from the same locality as AU6. There were even fewer differences between CHI and AU5: only one out of the eight size-related features reported in Table 5 differed significantly between these clones. However, these clones differed significantly in all five numerical features investigated. Note that CHI did not differ in any of those numer-

Table **6.** Morphological similarity in percentages between four populations and at three significance levels (for details to the NNSDC method, see ''Material and Methods'' section).

Population	Significance level								
	x(0.10)			*(0.05)			**(0.01)		
	AU7	AU2	CH1	AU7	AU2	CH1	AU7	AU2	CH1
AU2	72.7	—	—	77.3	—	—	81.8	—	—
CHI	81.8	63.6	—	81.8	63.6	—	86.4	68.2	—
AUS	59.1	77.3	59.1	63.6	77.3	59.1	77.3	81.8	63.6

For designation of populations, see Table 1.

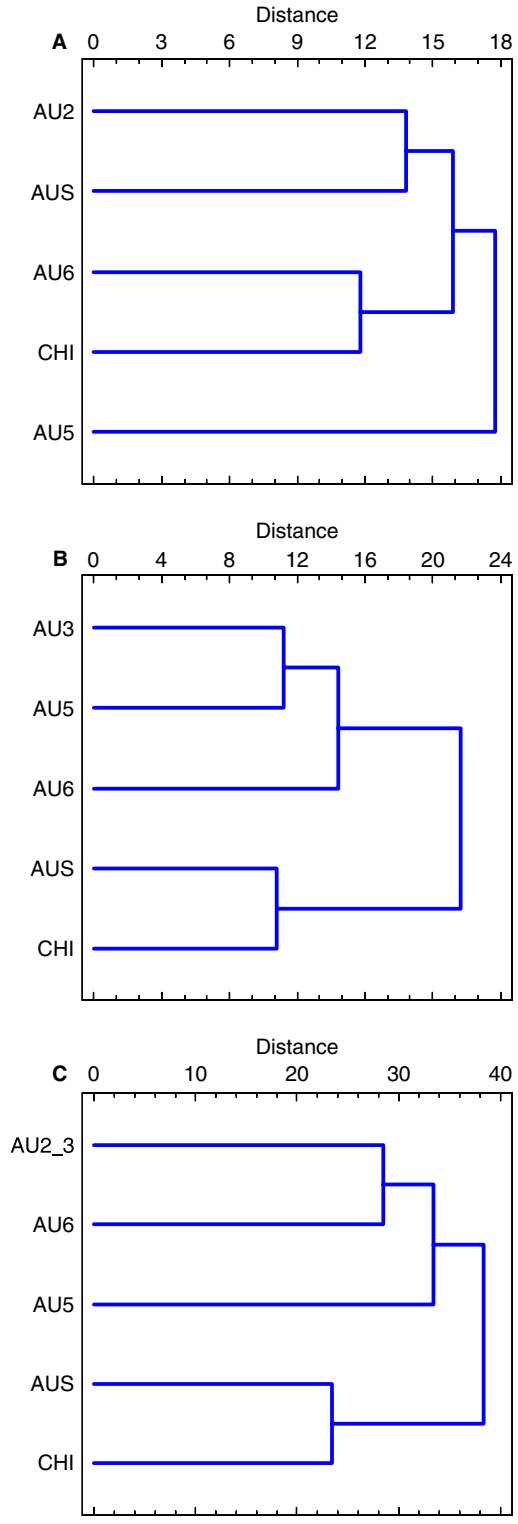

Fig. **9.** Cluster analysis of five clones of *Meseres corlissi* representing 13 morphological parameters (**A**), 14 ecophysiological parameters (**B**), and the combined 27 morphological and ecophysiological parameters (**C**). A biogeographic pattern is suggested by the ecophysiological parameters, but not obvious from the morphological parameters.

ical features from AUS and only in one numerical character from AU5. Accordingly, the Chinese clone was different from the Austrian clones AU5 and AU6 and the Australian clone in size-related ***or*** numerical characters. Ecologically, the Chinese clone was clearly separated from all other clones (our Results, details reported by Gächter and Weisse 2006; Weisse et al. 2007). The intraspecific ecological variation was as large as differences observed between ciliate species under comparable laboratory conditions as used in our study (Weisse 2006).

In conclusion, we demonstrated that small genetic intraspecific differences of 0%–4% in the SSrDNA and ITS sequences may correspond to a 5%–15%—approximately 10-fold larger—morphological variation in numerical and morphometric characters, and to an even larger ecophysiological variation (i.e. 10%–100% in pH and temperature response).

***Meseres corlissi*—a rare ciliate species with biogeography?** The late discovery (Petz and Foissner 1992) and the as yet only eight known records suggest that *M. corlissi* is a rare ciliate, likely preferring temporarily flooded habitats, such as small meadow ponds, floodplain soils, and tanks of bromeliads (WF., unpubl. data; Gächter and Weisse 2006; Petz and Foissner 1992; Weisse 2004). Accordingly, the possibilities for long-distance genetic exchange within metapopulations of this species may be limited. We never observed conjugation in non-clonal natural isolates or in the experimental clonal cultures. The ciliate swims continuously in the medium, and the ecological characteristics observed in our laboratory suggest that *M. corlissi* is a potentially highly competitive planktonic species (Weisse 2004). However, in the course of this study it became obvious that this ciliate is sensitive to particular environmental conditions and is difficult to cultivate in the trophic phase for an extended period of time (Müller et al. 2006; TW., unpubl. data). Furthermore, field studies showed that, for unknown reasons, it sometimes could not be reactivated from the cyst-containing mud and soil of the type locality (WF., unpubl. data). This is corroborated by laboratory observations (Müller 2007; Müller et al. 2006). Although cysts are readily formed and look ''healthy'', we were usually unable to initiate excystment: only when thousands of cysts were brought into fresh medium did some excyst. Similar observations have been made earlier with other ciliates (cited in Müller 2007), but the reason for the low percentage of successful excystment remained usually unknown. Foissner and Pichler (2006) suggested that the unusually complex structure of the cyst wall (five layers, each with a specific precursor) of *M. corlissi* might be responsible for this behavior. Since our methods of isolation and cultivation are all selective (Foissner 1999; Weisse 2006), *M. corlissi* may be much more common than indicated by the few records; likely, it needs highly specific conditions to excyst. If only some clones with certain predispositions excysted under our laboratory conditions, this could explain their high genetic similarity in spite of their wide distribution.

The peculiarities of the Chinese clone that we observed at all levels is consistent with local adaptation and, therefore, limited dispersal. Given our limited samples size, we cannot rule out that this clone is also present elsewhere. However, we found no clone that came close to the Chinese one at both Austrian localities. It remains an open question if dispersal of *M. corlissi* from other continents to the locality in China is rare or if local adaptation of the Chinese clone has progressed to an extent that immigration by other clones will be prevented, in spite of high dispersal rates. If the latter is the case and this situation persists, *M. corlissi* would undergo allopatric speciation (Weisse 2008). However, the fact that the Australian clone AUS and the Austrian clone AU6 were genetically identical in their SSrDNA and ITS sequences and did not differ significantly if all morphological and ecological characters were combined is consistent with cross-continental dispers-

Table 7. Overview of the major results obtained by ecophysiological laboratory experiments with five *Meseres corlissi* clones (Gächter and Weisse 2006; Weisse et al. 2007) that were used for statistical analysis in this study.

Clone	μ_{max} (d^{-1})	V_{avg} (μm^3)	P_{max} (μm^3/cil/d)	T at P_{max} (°C)	T_{min}	μ at 30 °C	T_{sens} (%)
Temperature response							
AU3	1.23[a] ± 0.25	71,290 ± 17,200	78,091 ± 38,295	20.0	7.65	0.6	5.45
AU5	1.25 ± 0.43	79,400 ± 20,840	88,832 ± 60,823	25.0	7.71	0.2	4.07
AU6	1.42 ± 0.41	87,250 ± 23,050	106,822 ± 35,986	30.0	7.67	1.4	8.11
AUS	2.40 ± 0.47	94,570 ± 11,170	162,078 ± 59,050	25.0	10.6	2.2	2.75
CHI	2.10 ± 0.16	48,220 ± 10,220	90,164 ± 36,016	30.8	14.3	2.0	5.42
For designation of populations, see Table 1.							
pH response				pH at P_{max}	pH_{min}	pH_{tol}	L_{avg} (μm)
AU3	2.56 ± 0.04	88,766 ± 15,362	256,000 ± 13,840	7.09 ± 0.02	5.9	2.7	64.44 ± 4.82
AU5	2.53 ± 0.13	97,412 ± 14,974	265,200 ± 19,740	7.26 ± 0.65	5.7	2.9	64.31 ± 4.89
AU6	2.76 ± 0.04	102,543 ± 15,063	283,900 ± 15,230	7.12 ± 0.34	5.5	3.1	66.46 ± 3.62
AUS	2.24 ± 0.19	86,110 ± 23,585	255,100 ± 20,690	5.82 ± 0.40	<4.4	>4.2	62.49 ± 6.12
CHI	1.88 ± 0.09	52,295 ± 12,018	112,100 ± 15,370	7.65 ± 0.39	5.1	3.6	53.57 ± 5.16

[a]Results reported are mean values ± 1 standard deviation.

μ_{max}, maximum growth rate; L_{avg}, average cell length; V_{avg}, average cell volume; P_{max}, maximum cellular production; T at P_{max}, temperature at maximum cellular production; T_{min}, minimum temperature tolerated [i.e., where $\mu \geq 0$]; μ at 30 °C, growth rate at 30 °C; T_{sens}, temperature sensitivity [i.e., % decrease in cell volume $°C^{-1}$]; pH at P_{max}, pH at maximum P, pH_{min}, minimum pH tolerated; pH_{tol}, width of pH tolerance [i.e., where $\mu \geq 0$; in pH units].

al of these clones. Given the large population sizes and high number of potentially suitable habitats of many ciliate species, we speculate that our findings may be typical for rare species; the chances may be equally high for both global dispersal of *most* and local adaptation of *some* populations in areas where dispersal has been permanently or temporarily reduced.

Methodological constraints and implications for future research. We have used selective methods of isolation, cultivation, and fixation of the ciliates that may have compromised our results. We can, for instance, not rule out that our isolation and cultivation procedures selected positively or negatively for certain *M. corlissi* clones. We did not succeed in isolating clones from encysted cells in each case; our attempts to isolate another *M. corlissi* clone from a soil sample in Brazil failed (reported in Weisse et al. 2007). Similarly, our analysis is limited with respect to the spatial and temporal dimensions of sampling and, accordingly, to the number of clones investigated. In addition to our comparatively small sample sizes, we are aware that each of the genetic, morphological, and ecological traits investigated has its own variability and so different sample sizes may be required to detect differences at the different levels (Martínez-Abraín 2007). Finally, ranking the results without weighing them may not be adequate; this is a principal problem with non-parametric statistics that cannot fully explore the information inherent in the datasets. It is, therefore, safe to conclude that our study provides a conservative estimate of the natural, intraspecific variation of *M. corlissi*.

In many cases the population variance estimated from sample means was larger than expected from the variability within the samples (= clones). Thus, we conclude that differences between the populations are real (i.e. exceed random effects). A corollary of this finding is that, rather than increasing the number of individuals/replicates studied, the number of different populations investigated should be increased to characterize adequately the morphological and ecophysiological variation within a species. This leads, however, to a principal dilemma with rare species; if a species can only be detected in <2% of all presumably suitable habitats, as it was the case with *M. corlissi* (Foissner et al. 2002), the efforts necessary for sampling and isolation easily exceed the means of most research projects.

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