ECOLOGY OF TESTATE AMOEBAE (PROTOZOA) IN TWO LAKE SUPERIOR COASTAL WETLANDS: IMPLICATIONS FOR PALEOECOLOGY AND ENVIRONMENTAL MONITORING

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Abstract: Testate amoebae are common inhabitants of moist soils, wetland, and lacustrine habitats. They produce a decay-resistant test, or shell, which can be identified to species in most cases and recovered from sediments in quantities sufficiently large to permit estimation of relative abundance. The objectives of this study were to assess the potential of testate amoeba assemblages as paleoenvironmental and environmental indicators in two Lake Superior coastal wetlands and to determine if morphological variation in four common taxa (*Arcella* spp., *Assulina* spp., *Centropyxis cassis* type, and *Nebela tincta-parvula-collaris* group) is related to microenvironment. Study localities included ridge-swale wetland systems adjacent to Grand Traverse Bay and Tahquamenon Bay in the Upper Peninsula of Michigan. Testate amoeba assemblages from 74 microsites were compared with percent moisture, depth to water table, pH, porosity, depth of living moss, and associated moss and vascular plant species. Morphometric analysis (e.g., test length and aperture diameter) was conducted on 25 individuals from at least 10 microsites for each of the four selected taxa. Gradient analysis indicated that testate amoeba assemblages are primarily controlled by substrate moisture and pH, consistent with results from other regions. Transfer functions for pH and substrate moisture were developed using 'jack-knifed' validation procedures. Little relationship was found between microenvironmental parameters and morphological variation in the investigated taxa, except for the *Nebela tincta-parvula-collaris* group, where test size was significantly correlated with pH ($r^2 = 0.68$). Results indicate that wetland testate amoeba assemblages in these coastal wetland systems are sensitive environmental and paleoenvironmental indicators that can be used to monitor and reconstruct water-level or pH changes.

Key Words: testate amoebae, paleohydrology, paleoecology, paleoenvironment, bioindicators, environmental indicators, Lake Superior wetlands, Great Lakes wetlands

INTRODUCTION

Testate amoebae (Protozoa: Rhizopoda) are common inhabitants of moist soils, wetlands, and lacustrine habitats (Tolonen 1986, Warner 1990). They produce a decay-resistant test, or shell, that protects the cell from desiccation. The shell may be proteinaceous, siliceous, or calcareous and may incorporate extraneous materials such as fungal hyphae, diatoms, and mineral grains (Ogden and Hedley 1980). The morphology of tests is usually unique, allowing species-level identification (Ogden and Hedley 1980, Tolonen 1986, Warner 1990). Fossil amoeba tests are especially abundant and well-preserved in Holocene *Sphagnum*-derived peats (Jung 1936, Schönborn 1963, Meisterfeld 1977, Tolonen 1986, Warner 1990).

The dominant control on testate amoeba distribution in peatlands is substrate moisture (Tolonen 1986, Warner 1987, 1990), although trophic status (Tolonen et al. 1992), pH (e.g., Charman and Warner 1992, Mitchell et al. 1999), and other aspects of water chem-

istry (Woodland et al. 1998, Mitchell et al. 2000a) are also important factors. The species-specific environmental tolerances of testate amoebae have supported qualitative environmental inferences from fossil data for many years (e.g., Grospietsch 1965, Aaby 1976, Tolonen et al. 1985, Beyens and Chardez 1987, Van der Molen and Hoekstra 1988). Recent investigations have focused on finding correlations among quantitative moisture parameters and modern testate amoeba assemblages (Charman and Warner 1992, 1997, Warner and Charman 1994, Charman 1997, Woodland et al. 1998, Mitchell et al. 1999). These relationships can be used to develop transfer functions for quantitative inference of past substrate moisture conditions (Charman 1997, Charman and Warner 1997, Woodland et al 1998, Charman et al. 1999, Mitchell et al. 2001). Many previous calibration studies have focused on ombrotrophic peatlands due to their sensitivity to climate change (Blackford 1993, Barber 1994). However, fossil testate amoebae may have more widespread ap-

plicability. Modern studies are needed from different geographic regions and peatland types to fully assess the potential of this proxy data source and to facilitate interpretation of fossil assemblages.

Due to their environmental sensitivity and rapid turnover rate, testate amoebae are also potentially sensitive bioindicators (Foissner 1987, 1999). Testate amoebae are excellent indicators of calcite precipitation (Casper and Schönborn 1985) and heavy metal pollutants (Patterson et al. 1996, Reinhardt et al. 1998) in lakes, and they may be used to assess rates of lake remediation (Patterson et al. 1996). Testate amoeba communities have shown significant compositional changes within 2 ½ years of peatland drainage (Warner and Chiemelski 1992). They have also been used to monitor the restoration and recovery of cutover bogs in Switzerland (Buttler et al. 1996). Additional knowledge of testate amoeba ecology would promote more widespread application in biomonitoring and wetland management.

In this study, I investigated the relationship between testate amoeba assemblages and microenvironmental parameters (pH, substrate percent moisture, water-table depth, porosity, evaporation potential, depth of living moss, associated plant species) in two Lake Superior coastal wetland systems. I compared assemblage variation between and within the wetlands and determined controls on testate amoeba distribution in these systems. Because morphological variation in some species has been found to occur in response to environmental conditions (Laminger 1978, Foissner 1987, Schönborn and Peschke 1988, Schönborn 1992, Wanner and Meisterfeld 1994, Bobrov et al. 1995, 1999, Wanner 1999), I compared morphological variation in four common species groups with microenvironmental conditions. I then developed transfer functions that can be used to infer microenvironmental parameters from fossil testate amoeba assemblages.

STUDY SITES

Two wetland systems along the southern shore of Lake Superior were investigated, one adjacent to Grand Traverse Bay and one adjacent to Tahquamenon Bay in the Upper Peninsula of Michigan, USA (Figure 1). Each of these systems is characterized by a series of alternating sand ridges and wetland depressions, which are oriented parallel to the lakeshore. Sand ridges were deposited during high stands of Lake Superior. The inter-ridge wetlands that subsequently developed are commonly referred to as swales. The ridges are progressively older inland from Lake Superior, and the timing of wetland development generally follows the same pattern (Johnston et. al 2000). These wetland systems are characterized by large inter-swale varia-
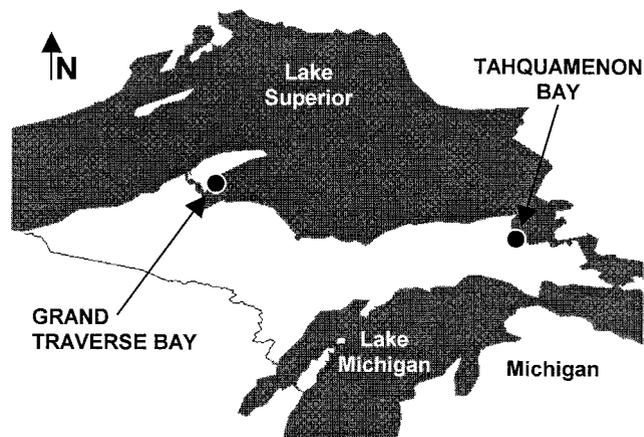

Figure 1. Location of the two wetland systems in the Upper Peninsula of Michigan, USA.

tion in vegetation and hydrology and thus provide a wide array of microenvironments for testate amoebae. Swales at both localities range from oligotrophic bogs to highly minerotrophic fens and marshes.

Vegetation on the younger ridges at Grand Traverse Bay is predominantly composed of shrubs (e.g., *Myrica gale* L., *Rosa palustris* Marsh., *Salix* spp., *Betula pumila* L.). Scattered *Pinus banksiana* Lamb. trees begin to appear by the 13th ridge inland, becoming more widespread and mixed with *Picea mariana* (Miller) BSP. by the 16th ridge. Mixed *Picea mariana* and *Pinus banksiana* stands continue to characterize the older ridges, with occasional *Betula papyrifera* Marsh. and *Pinus resinosa* Aiton. Vegetation in the swales closest to the lake is generally characterized by sedges (e.g., *Carex* spp., *Rhynchospora* spp., *Scirpus* spp.). *Sphagnum* moss is first found in abundance in the 16th swale inland from the lake and is abundant in most of the swales further inland. Other species that characterize the inland swales include *Chamaedaphne calyculata* (L.) Moench, *Kalmia polifolia* Wangenh., and *Ledum groenlandicum* Oeder. Standing water and aquatic vegetation (*Scirpus subterminalis* Torrey, *Nymphaea odorata* Aiton) also occur in several of the swales at Grand Traverse Bay.

The ridges at Tahquamenon Bay are dominated by *Pinus strobus* L. and *Picea mariana*. *Thuja occidentalis* L. and *Larix laricina* (DuRoi) K. Koch are abundant on several ridges and swales. The abundance of *Thuja occidentalis* and *Pinus strobus* is in contrast to Grand Traverse Bay, where these species are rare. Like Grand Traverse Bay, many of the swales are dominated by *Sphagnum* spp.; however, marshes dominated by *Typha latifolia* L. and *Carex lasiocarpa* Ehrh. also occur.

METHODS

Field sampling at both sites was conducted in a two-week period in late July 1999. The sampling procedure was modeled after previous investigations (Charman and Warner 1992, 1997, Warner and Charman 1994, Mitchell et al. 1999). Microsites within the swales were sampled in an attempt to include the natural heterogeneity of each wetland system. Specifically, sites were chosen to reflect the full range of surface moisture variability (e.g., hummocks, hollows, pools). I placed a 1-m² quadrat at each microsite and identified all plant species. A peat sample was collected from the middle of the quadrat. The peat sample was collected by pushing a can (7.5-cm diameter, 11-cm length) with one open end into the peat until it was level with the surface of the wetland. A knife was used to cut the peat around the can as the can was pushed into the peat. A small hole in the closed end of the can allowed air to escape and prevented peat compaction. The peat was held in the can with one hand and pulled up carefully. Peat sticking out of the bottom of the can was cut off, and the can was sealed. These peat samples were used to calculate percent moisture, porosity, and evaporation potential (amount of water that evaporates from sample in 12 days at ~26° C). From the edge of the hole left by the peat sample, a ~10-cm³ sample was collected from the brown *Sphagnum* directly below the green portion of the stems (~4 to 12 cm below surface). Samples from this location best represent what would be found in the fossil record (Warner 1987), and stratification of testate amoeba species has been observed along the chlorophyllous portion of *Sphagnum* stems (Heal 1962, Meisterfeld 1977). The green portion of *Sphagnum* around the hole was collected for species-level identification. At microsites lacking *Sphagnum*, a ~10-cm³ sample of peat or sediment was collected from the surface of the wetland. The depth to the water table and pH were measured at each sample locality.

Testate amoebae were isolated from peat using the method of Hendon and Charman (1997). Samples were boiled in distilled water for ten minutes and washed through 355-µm and 15-µm sieves. The material caught in the 15-µm sieve was stained with two drops of safranine and stored in glycerol. Slides were prepared, and all testate amoebae were identified and counted until a total of 200 was reached. Identifications were made using several available keys, atlases, and descriptions (Grospietsch 1958, Loeblich and Tappan 1964, Corbet 1973, Ogden and Hedley 1980, Warner 1987). The relative abundance of each taxon was calculated as a percent of the total number of testate amoebae counted. *Habrotrocha angusticollis*, a commonly fossilized rotifer (Warner 1988), was in-cluded in the analysis and count total. A total of 74 testate amoeba assemblages were analyzed from the two wetland systems. Fifty-three taxa were encountered, although three rare taxa (occurring in fewer than 4 samples) were excluded from analysis.

Morphometric analyses (e.g., test length and aperture diameter) were conducted on four species groups (*Arcella* spp., *Assulina* spp., *Centropyxis cassis* type, *Nebela collaris-parvula-tincta* group). These groups were chosen because they are common in many wetlands and/or are generally characterized by high levels of morphological variability (Medioli and Scott 1983, Foissner 1988). Twenty-five individuals from at least 10 microsites were measured for each of the four selected taxa (31 microsites for the *Nebela collaris-parvula-tincta* group) at 1000× magnification. Test length was measured in the *Assulina* species and the *Nebela collaris-parvula-tincta* group. Aperture diameter was measured for the *Arcella* spp., and aperture length and width were measured in *Centropyxis cassis* type.

Percent moisture, porosity, and evaporation potential were calculated using the cans of peat according to the following procedure (modified from Mitchell et al. 1999). Samples were weighed to obtain the fresh weight (FW). Each sample was then saturated with distilled water and weighed to obtain the saturated weight (SW). Samples were then allowed to stand open to the air for 12 days at room temperature (ca. 26°C) and weighed to obtain the evaporated weight (EW). The samples were then oven dried at 100°C for 48 hours and weighed to obtain the dry weight (DW). Percent moisture, evaporation potential, and porosity were calculated according to the following formulas:

Percent moisture (% of fresh weight)

$$= ((FW-DW)/FW)*100$$

Evaporation potential (% of saturated weight)

$$= ((SW-EW)/SW)*100$$

Porosity (% of saturated weight)

$$= ((SW-DW)/SW)*100$$

Gradient analysis was used to interpret the data using the software package PC-ORD (McCune and Mefford 1997). Detrended correspondence analysis (DCA), which only uses species data to constrain the ordination (indirect gradient analysis), was used to analyze species assemblage differences between the two sites and substrates (*Sphagnum* and non-*Sphagnum*). Canonical correspondence analysis (CCA), where the ordination is constrained by both environmental and species data (direct gradient analysis), was used to determine factors that affect testate amoeba community composition at the two sites.
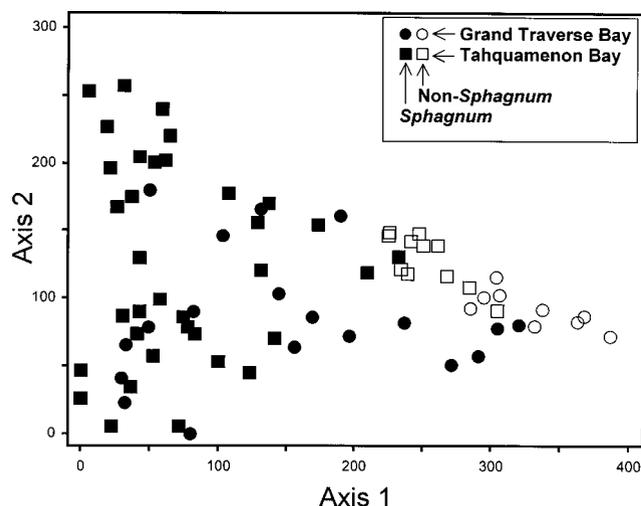
Figure 2. DCA ordination for testate amoeba assemblages characterizing *Sphagnum* and other substrates at the two sites.

Four models were used to develop transfer functions from the modern data set using the program CALIBRATE (Juggins 1998). These include weighted averaging (WA), tolerance downweighted averaging (WA-Tol), partial least squares (PLS), and weighted averaging partial least squares (WA-PLS) (Birks 1995). These models were chosen because they have been used successfully to develop transfer functions from testate amoeba data sets in Europe (Woodland et al. 1998), New Zealand (Charman 1997), and Canada (Charman and Warner 1997). 'Jack-knifing' validation was used to evaluate the transfer functions. This procedure allows the calculation of a model-predicted environmental value from each testate amoeba assemblage without including that assemblage in the data used to generate the model. Prediction errors (RMSEP), correlation coefficients (r^2), and 'maximum bias' can be used to assess the jack-knifed predictions. Prediction errors (RMSEP) are the differences between the observed and predicted environmental values. 'Maximum bias' is the largest mean bias of any particular section along the environmental gradient.

RESULTS

Site and Substrate Effects on Testate Amoeba Assemblages

DCA was performed on all samples to determine assemblage similarity between the two wetland systems and between *Sphagnum*-inhabiting and non-*Sphagnum* assemblages (Figure 2). Similarity exists between the Tahquamenon Bay and Grand Traverse Bay samples. However, *Sphagnum*-inhabiting assemblages are different from assemblages on other sub-

strates. Therefore, in addition to analysis on the entire data set, separate analysis was done on these two groups of samples.

Testate Amoebae–Microenvironment Relationships

Relationships between microenvironmental parameters and testate amoeba assemblages were investigated using CCA (Figure 3, Table 1). The presence of both standing water and plant species were included in these analyses, but they were kept passive in the procedure so they did not influence the ordination. The significance of species–microenvironment relationships was also tested using partial constrained analysis, where only one variable is included in the CCA (Table 2). Monte Carlo permutation tests were then used to test the statistical significance of each relationship.

For *Sphagnum* samples, water-table depth, substrate percent moisture, and pH were found to be dominant controls of testate amoeba species distribution (Figure 3a, Table 1). The relationships between each of these parameters and the species ordination were significant ($p < 0.05$), although the total variance explained by them individually was relatively low (Table 2). Testate amoeba species distribution on non-*Sphagnum* substrates is primarily related to pH (Figure 3b, Table 1). This was also the only statistically significant variable (Table 2), although the sample size was relatively low ($n = 20$). In fact, two species (*Lesquereusia epistomium*, *Lesquereusia modesta*) were restricted to sites with standing water. Analysis of the entire data set reveals that overall controls on distribution in these coastal wetland systems are pH, water-table depth, and percent moisture of the substrate (Figure 3c). All of these relationships were found to be significant, although partial constrained analysis indicates that the variance explained by each is relatively low (Table 2).

Associated plant species also are positioned in multivariate space according to their habitat preferences. In the *Sphagnum* sample CCA (Figure 3a), the location of *Sphagnum* species generally supports their known ecology. For example, *Sphagnum girgensohnii* and *Sphagnum centrale*, which were often found together in relatively dry, poor-fen environments, cluster on one side of the ordination. *Sphagnum majus*, which is commonly aquatic or grows near water level, is positioned on the opposite side of the ordination. In the non-*Sphagnum*-sample CCA diagram (Figure 3b), the positions of plant species generally correspond to the pH gradient. In the analysis of the entire data set (Figure 3c), plants characteristic of drier habitats, such as *Vaccinium myrtilloides* and *Cornus canadensis* are on one side of the ordination, whereas open water habitats and plants characteristic of wetter environments (e.g.,
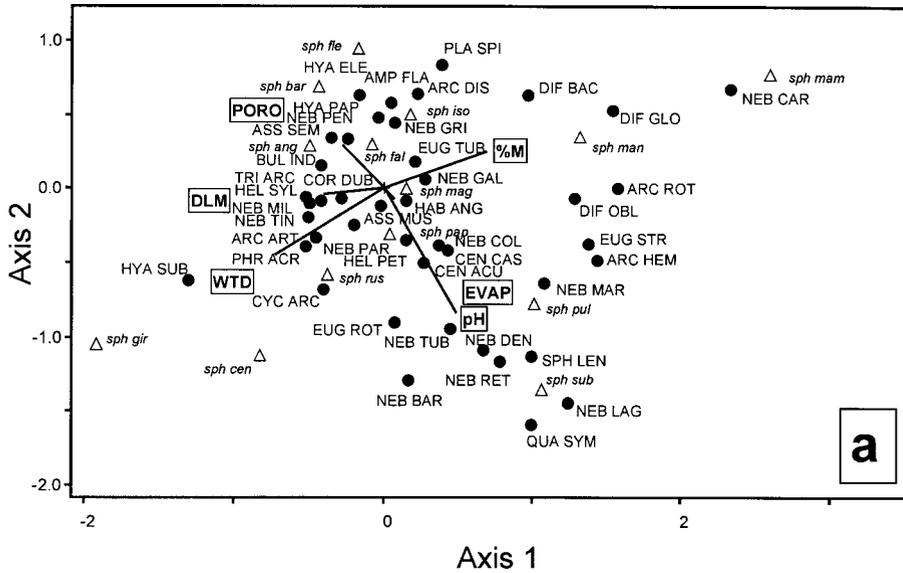
Abbreviations for testate amoebae:

AMP FLA	*Amphitrema flavum* (Archer)
ARC ART	*Arcella artocrea* Leidy
ARC CRE	*Arcella crenulata* Deflandre
ARC DIS	*Arcella discoides* Ehrenberg
ARC HEM	*Arcella hemisphaerica* Perty
ARC ROT	*Arcella rotundata* var. *aplanata* Deflandre
ASS MUS	*Assulina muscorum* Greeff
ASS SEM	*Assulina seminulum* (Ehrenberg)
BUL IND	*Bullinularia indica* Penard
CEN CAS	*Centropyxis cassis* type
CEN ACU	*Centropyxis aculeata* type
COR DUB	*Corythion dubium* type
CYC ARC	*Cyclopyxis arcelloides* Leidy
CYP AMP	*Cyphoderia ampulla* (Ehrenberg)
DIF BAC	*Difflugia bacillifera* Penard
DIF GLO	*Difflugia globulosa* Dujardin
DIF LEI	*Difflugia leidyi* type
DIF OBL	*Difflugia oblonga* Ehrenberg
DIF RUB	*Difflugia rubescens* Penard
EUG ROT	*Euglypha rotunda* type
EUG STR	*Euglypha strigosa* type
EUG TUB	*Euglypha tuberculata* type
HAB ANG	*Habrotrocha angusticollis* Murray
HEL PET	*Heleopera petricola/rosea*
HEL SPH	*Heleopera sphagni* (Leidy)
HEL SYL	*Heleopera sylvatica* Penard
HYA ELE	*Hyalosphenia elegans* Leidy
HYA PAP	*Hyalosphenia papilio* Leidy
HYA SUB	*Hyalosphenia subflava* Cash & Hopkinson
LES EPI	*Lesquereusia epistomium* Penard
LES MOD	*Lesquereusia modesta* Rhumbler
NEB BAR	*Nebela barbata* Leidy
NEB CAR	*Nebela carinata* (Archer)
NEB COL	*Nebela collaris* (Ehrenberg)
NEB DEN	*Nebela dentistoma* Penard
NEB GAL	*Nebela galeata* Penard
NEB GRI	*Nebela griseola* (Penard)
NEB LAG	*Nebela lageniformis* Penard
NEB LON	*Nebela retorta* type
NEB MAR	*Nebela marginata* Penard
NEB MIL	*Nebela militaris* Penard
NEB PAR	*Nebela parvula* Cash
NEB PEN	*Nebela penardiana* Deflandre
NEB TIN	*Nebela tincta* (Leidy)
NEB TUB	*Nebela tubulosa* Penard
PLA SPI	*Placocista spinosa* (Carter)
PHR ACR	*Phryganella acropodia* (Hertwig & Lesser)
QUA SYM	*Quadruella symmetrica* (Wallich)
SPH LEN	*Sphenoderia lenta* Schlumberger
TRI ARC	*Trigonopyxis arcula* (Leidy)
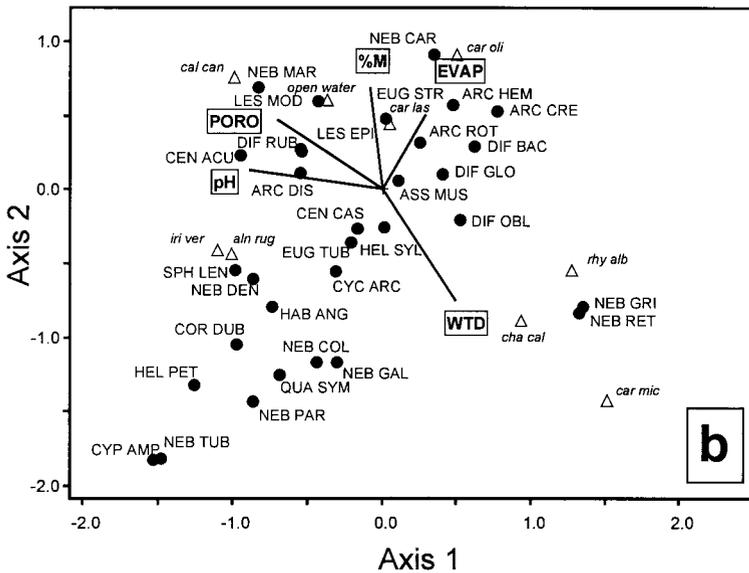
Abbreviations for plant taxa:

and gla	*Andromeda glaucophylla* Link
aro pru	*Aronia prunifolia* (Marsh.) Rehder
aln rug	*Alnus rugosa* (Duroi) Sprengel
cal can	*Calamagrostis canadensis* (Michaux) Beauv.
car str	*Carex stricta* Lam.
car tri	*Carex trisperma* Dewey
car las	*Carex lasiocarpa* Ehrh.
car oli	*Carex oligosperma* Michaux
car mic	*Carex michauxiana* Boeckl.
cha cal	*Chamaedaphne calyculata* (L.) Moench
cor can	*Cornus canadensis* L.
iri ver	*Iris versicolor* L.
kal pol	*Kalmia polifolia* Wangenh.
rhy alb	*Rhynchospora alba* (L.) Vahl.
smi tri	*Smilacina trifolia* (L.) Desf.
sph spp	*Sphagnum* spp.
sph ang	*Sphagnum angustifolium* (Russow) C. Jens.
sph bar	*Sphagnum bartlettianum* Warnst.
sph cen	*Sphagnum centrale* C. Jens.
sph fal	*Sphagnum fallax* (Klinggr.) Klinggr.
sph fle	*Sphagnum flexuosum* Dozy & Molk.
sph gir	*Sphagnum girgensohnii* Russow
sph iso	*Sphagnum isoviitae* Flatb.
sph mag	*Sphagnum magellanicum* Brid.
sph mam	*Sphagnum majus* (Russ.) C. Jens. ssp. *majus* Flatb.
sph man	*Sphagnum majus* (Russ.) C. Jens. ssp. *norvegicum* Flatb.
sph pap	*Sphagnum papillosum* Lindb.
sph pul	*Sphagnum pulchrum* (Braithw.) Warnst.
sph rus	*Sphagnum russowii* Warnst.
sph sub	*Sphagnum subsecundum* Nees in Sturm
vac myr	*Vaccinium myrtilloides* Michaux

Figure — ordination diagrams (a, b, c) plotting testate amoebae, plant taxa, and environmental variables (%M, EVAP, pH, WTD, PORO, DLM) along Axis 1 and Axis 2.
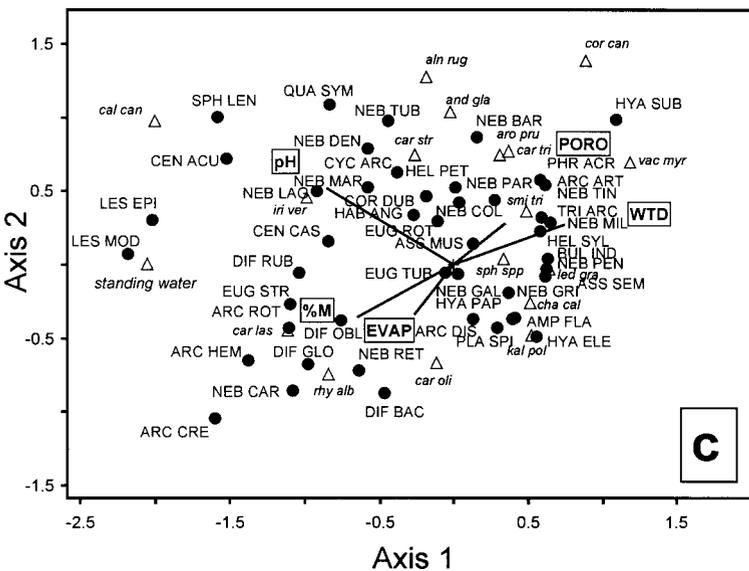
Table 1. Characteristics of CCA axes 1 and 2 and correlation coefficients.

Variable	*Sphagnum* Samples (n = 54)		Non-*Sphagnum* Samples (n = 20)		Entire Data Set (n = 74)	
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Axes characteristics						
Eigenvalue	0.280	0.185	0.472	0.348	0.408	0.180
% variance explained	9.7	6.4	14.5	10.6	9.6	4.2
Cumulative % explained	9.7	16.0	14.5	25.1	9.6	13.9
Correlation coefficients						
Water-table depth	−0.733	−0.454	0.406	−0.751	0.778	0.241
Percent moisture	0.688	0.246	−0.001	0.691	−0.676	−0.345
pH	0.490	−0.839	−0.892	0.040	−0.869	0.506
Porosity	−0.282	0.294	−0.673	0.405	0.296	0.289
Potential evaporation	0.048	−0.036	0.344	0.523	−0.278	−0.335
Depth of living *Sphagnum*	−0.409	−0.039	—	—	—	—

Calamagrostis canadensis) fall on the other side of this gradient.

Morphology–Microenvironmental Relationships

The relationship between morphological characteristics and microenvironmental variables was investigated using linear regression (Table 3). Little relationship was found between microenvironmental parameters and morphological variation in the investigated taxa, except for the *Nebela tincta-parvula-collaris* group (Figure 4), where test size was significantly correlated with pH ($r^2=0.68$). The strength of this relationship differed at the two sites, with a much stronger correlation characterizing the Grand Traverse Bay samples ($r^2=0.89$ vs $r^2=0.37$).

Development of Transfer Functions

WA-PLS consistently performed the best of the four models for water-table depth, percent moisture, and pH (Table 4). Assuming good modern analogues, all three of these parameters can be quantitatively inferred from fossil data sets (Figure 5). Water-table depth, percent moisture, and pH can be reconstructed from similar peatlands, with a mean error of ±7.5cm, ±4.2 percent, and ±0.41, respectively (Table 4).

Species Optima and Tolerances

The optima and tolerances of individual taxa were calculated using the program CALIBRATE (Figure 6). Taxa that are restricted to wet environments include *Lesquerusia* spp., *Nebela carinata*, *Difflugia* spp., and most of the *Arcella* species. Species characterizing drier substrates include *Hyalosphenia subflava*, *Nebela tincta*, *Heleopera sylvatica*, and *Trigonopyxis arcula*. Consistent with the bog-to-fen environmental gradient, many of the species characterizing wetter sites also have higher pH optima. However, exceptions exist, such as *Quadruella symmetrica*, which prefers high pH (>5.4) and only moderately wet to moderately dry substrates.

DISCUSSION

Assemblage Composition

The Grand Traverse Bay and Tahquamenon Bay sites were generally similar in testate amoeba assemblage composition. This is not surprising given that many species are cosmopolitan in distribution. Substrate moisture and pH are the dominant controls on testate amoeba species distribution in the *Sphagnum*-dominated swales at both sites, consistent with studies from other geographic regions (Charman and Warner 1992, 1997, Tolonen et al. 1992, Warner and Charman

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Figure 3. CCA ordinations for testate amoeba species and microenvironmental variables. a) *Sphagnum* samples, with associated *Sphagnum* species included as passive variables. b) Non- *Sphagnum* samples, with associated plant species included as passive variables. c) The entire data set, with associated plant species included as passive variables (species associated with 4 or more samples, *Sphagnum* species not included).

Table 2. Partial constrained analysis (CCA) on microenvironmental parameters, with p-values calculated from Monte Carlo permutation tests (1000 permutations). Asterisks denote significance.

Variable	*Sphagnum* Samples (n = 54)			Non-*Sphagnum* Samples (n = 20)			Entire Data Set (n = 74)		
	Eigen-value	% Variance Explained (r^2)	p-Value	Eigen-value	% Variance Explained (r^2)	p-Value	Eigen-value	% Variance Explained (r^2)	p-Value
Water-table depth	0.208	7.1	0.001*	0.166	8.0	0.084	0.321	8.9	0.001*
Percent moisture	0.164	5.6	0.032*	0.143	6.9	0.111	0.271	7.5	0.001*
pH	0.204	7.0	0.001*	0.322	15.4	0.004*	0.377	10.4	0.001*
Porosity	0.076	2.6	0.370	0.161	7.7	0.312	0.135	3.7	0.145
Potential evaporation	0.041	1.4	0.295	0.161	7.7	0.389	0.063	1.8	0.844
Depth of living *Sphagnum*	0.097	3.3	0.392	—	—	—	—	—	—

1994, Charman 1997, Woodland et al. 1998, Mitchell et al. 1999, 2000a). Although pH seems to be the most important factor in these non-*Sphagnum* wetlands, the sample size is low (n=20). More work is needed to better understand testate amoeba distribution patterns in non-*Sphagnum* wetlands before applications to paleoecology and management can be assessed.

The total variance explained by water-table depth, percent moisture, and pH, although relatively low (Table 1), is similar to that observed in other studies (e.g., Charman 1997, Woodland et al. 1998). The low r^2 observed may be a function of a scale differential between the environment as measured in these studies (i.e., coarse-grained) and the environment as perceived by testate amoebae (fine-grained). For example, there are microhabitat differences affecting testate amoeba species on the scale of centimeters or smaller within *Sphagnum* plants and carpets (Heal 1962, Corbet 1973, Mitchell et al. 2000b). In this study, environmental variables were only measured on the day of sample collection and do not take seasonal fluctuations into account. However, this has not been a problem in other studies (Warner 1987, Charman and Warner 1992, Tolonen et al. 1992, Charman 1997), apparently because species and environmental data have been collected during one field season and thus the measurements fall along relative environmental gradients.

Samples from different wetland types probably introduce additional noise into the environmental data. Seasonal water-level fluctuations probably differentially affect different wetland types, and testate amoeba species certainly respond to these differences in hydroperiod. A wide range of microsites (minerotrophic fens to oligotrophic bogs) was sampled in this study and may also contribute to the relatively low variance explained. However, paleoenvironmental reconstructions along a relative environmental gradient (e.g., wet to dry) are obtainable using instantaneous measurements of environmental variables (Figure 5). Improvements can be made using mean annual hydrologic data (Woodland et al. 1998), supporting the idea that testate amoebae are more sensitive than instantaneous environmental measurements indicate.

The use of the transfer functions presented in Table 4 and Figure 5 are only applicable when fossil and modern amoeba assemblages are similar in composition (i.e., good modern analogues exist for fossil assemblages). The fossil record suggests that, at least during the later part of the Holocene, communities of testate amoebae are structurally similar to those inhab-

Table 3. Results of linear regression between five microenvironmental variables and test morphological characteristics in four common species groups. Asterisks denote significance.

|	*Nebela* Group Mean Test Length		*Centropyxis cassis* Group Mean Aperture Length–Width Ratio		*Arcella* Group Mean Test Length–Aperture Ratio		*Assulina* Group Mean Test Length	
	r^2	p	r^2	p	r^2	p	r^2	p
Water-table depth	0.088	0.100	0.013	0.774	0.121	0.326	0.000	0.980
Percent moisture	0.004	0.736	0.143	0.316	0.080	0.801	0.199	0.196
pH	0.681	<0.001*	0.063	0.515	0.025	0.662	0.097	0.382
Porosity	0.034	0.315	0.012	0.776	0.043	0.563	0.140	0.287
Evaporation potential	0.001	0.852	0.085	0.448	0.166	0.243	0.225	0.166
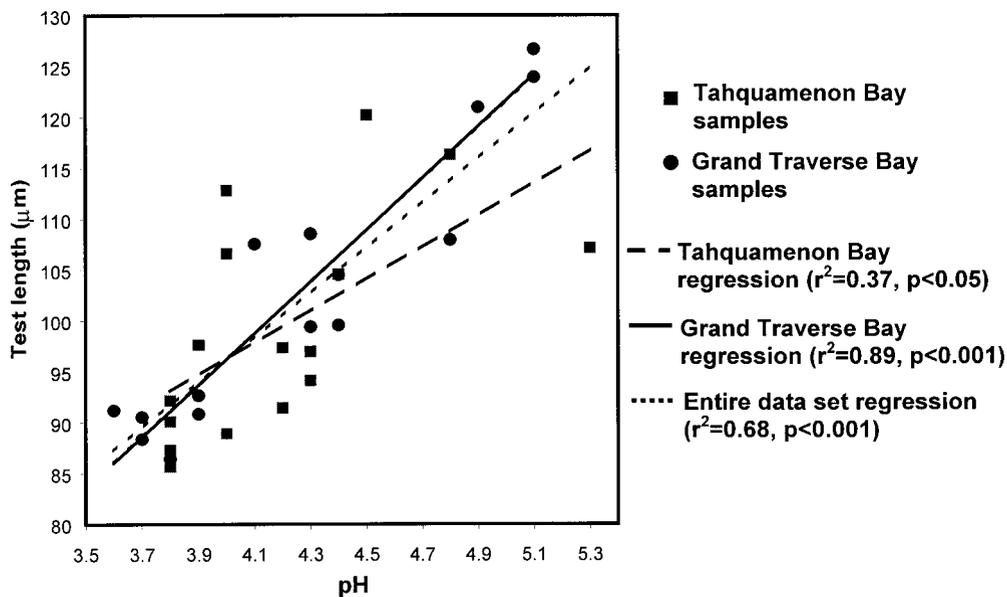
Figure 4. Relationship between test length and pH in the *Nebela tincta-parvula-collaris* group.

iting peatlands today (e.g., Beyens and Chardez 1987, Woodland et al. 1998, Hendon and Charmon 1999, Mitchell et al. 2001). However, in situations where no good modern analogues exist for fossil data, qualitative moisture and pH reconstructions are possible using the known tolerances of species (Figure 6), assuming that the entire environmental range of the species has been sampled.

It is difficult to compare quantitatively the optima and tolerances of individual species derived from this study to those from other regions because most studies have used instantaneous measurements of environmental factors. However, there is general agreement in the ranking of species along moisture gradients in this study with those from other areas (Figure 7), even though the actual values of the measured moisture parameters differ greatly from site to site. Many species, especially those with optima at the dry and wet ends

Table 4. Performance of the four transfer function models for the significant microenvironmental parameters assessed by jack-knifed scores (RMSEP), correlation coefficients (r^2), and maximum bias (M-bias).

| Variable/Model	Entire Data Set (n = 74)			*Sphagnum* Samples (n = 54)			Non-*Sphagnum* Samples (n = 20)		
	RMSEP	r^2	M-bias	RMSEP	r^2	M-bias	RMSEP	r^2	M-bias
Water-table depth									
WA	8.12	0.53	23.62	7.06	0.37	19.69			
WA-Tol	7.67	0.58	22.84	7.10	0.36	19.51			
PLS (1 component)	8.99	0.51	35.20	7.69	0.40	26.14			
WA-PLS (2 components)	7.48	0.61	18.99	6.53	0.47	16.58			
Percent moisture									
WA	4.39	0.46	10.66	4.58	0.34	9.74			
WA-Tol	4.38	0.47	10.68	4.87	0.28	9.70			
PLS (1 component)	4.43	0.47	10.40	4.81	0.32	9.66			
WA-PLS (2 components)	4.23	0.51	8.22	4.63	0.35	9.74			
pH									
WA	0.45	0.67	0.90	0.39	0.43	0.85	0.57	0.44	0.86
WA-Tol	0.49	0.61	1.05	0.39	0.42	0.83	0.47	0.60	0.52
PLS (1 component)	0.54	0.57	0.63				0.73	0.26	1.18
PLS (4 components)				0.43	0.44	0.75			
WA-PLS (1 component)							0.56	0.44	0.86
WA-PLS (2 components)	0.41	0.73	0.70	0.36	0.51	0.60			
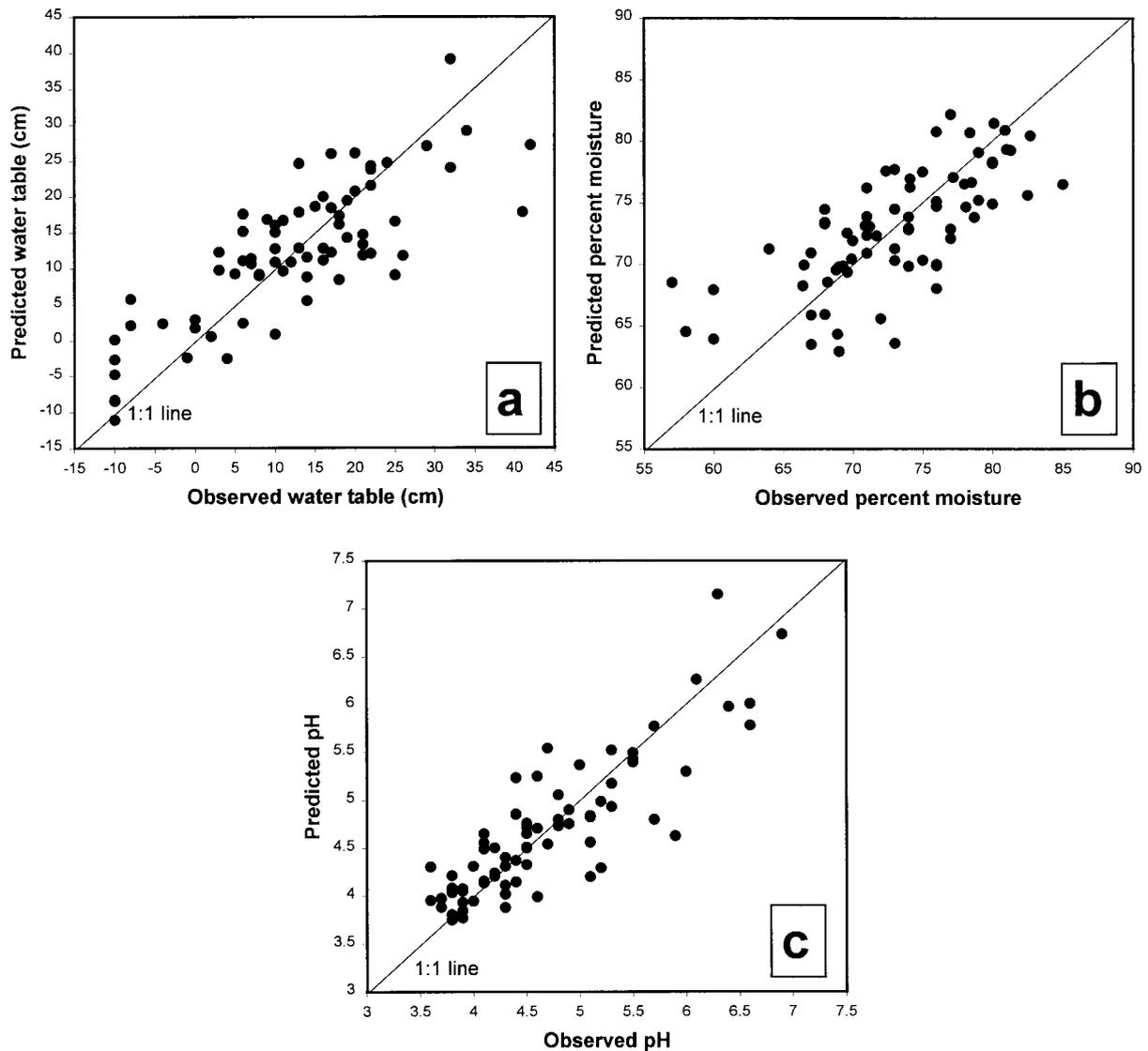
Figure 5.   Observed and predicted values for the samples based on jack-knifed estimates from a) WA-PLS for water-table depth, b) WA-PLS for percent moisture, and c) WA-PLS for pH. Relevant statistics are shown in Table 4.

of the gradient, have similar moisture preferences in North America, Europe, and New Zealand. Some species that are consistently more abundant in wetter habitats throughout the studied sites include *Amphitrema wrightianum* Archer, *Arcella discoides*, *Nebela carinata*, and *Difflugia bacillifera* (Figure 7). Species that are common in drier sites include *Trigonpyxis arcula*, *Bullinularia indica*, and *Hyalosphenia subflava*. Many of the amoebae with moisture optima in the mid-region of the moisture gradient show large variation in moisture preferences between sites. Different collection procedures, sampling times, wetland types, hydroperiods, and taxonomic uncertainties make it unclear how much of this variation is a result of real differences in microhabitat. However, it should be noted that some of the taxa have been reported as species groups in

one or more studies (e.g., *Centropyxis aculeata* type, *Cyclopyxis arcelloides* type), and the constituent species in these groups may have different environmental tolerances. Although more specific inferences are difficult due to the above-mentioned problems, many testate amoebae seem to conserve their hydrologic preferences across their distribution, especially those with moisture optima at the extremes of the wet-to-dry moisture gradient.

Morphological Variation

Morphological variation in the testate amoebae is to a large degree the result of environmental influences (Laminger 1978, Foissner 1987, Schönborn and Peschke 1988, Schönborn 1992, Wanner and Meisterfeld
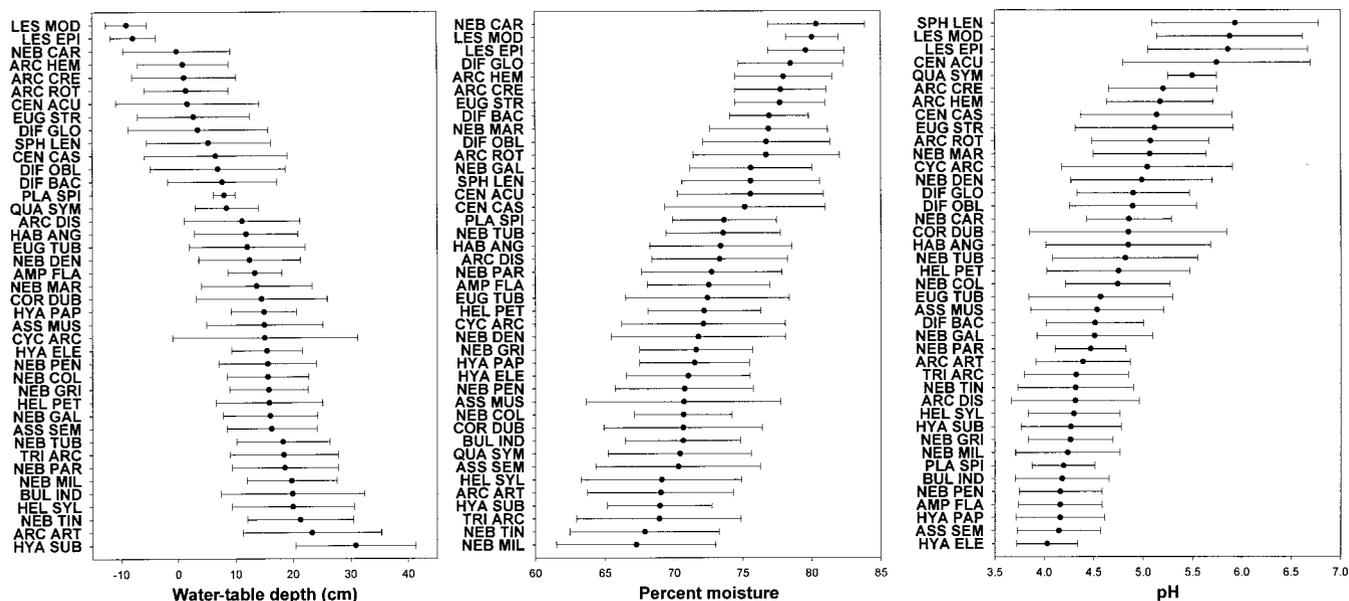
Figure 6. Testate amoeba taxa optima and tolerances for water-table depth developed using the Calibrate modelling program (see Figure 3 for species names).

1994, Bobrov et al. 1995, 1999, Wanner 1999). This morphological plasticity has led to confusion over the interpretation of biodiversity patterns in the fossil record (Charman 1999). However, from the perspective of paleoecology and bioindication, the variability in test morphology within taxa may be a valuable tool. This idea led Bobrov et al. (1999) to suggest that paleoecologists should attempt the lowest taxonomic divisions possible within environmentally sensitive groups. Although this is certainly possible in some taxa, in many species groups and genera, morphological variation appears to be intergradational, and distinctions between species become unclear (Medioli and Scott 1983). By measuring morphological characteristics of tests, I attempted an alternate approach not hindered by present taxonomic confusion. Although three of the four groups investigated show no significant relationship between measured morphological characteristics and measured environmental parameters, size in the *Nebela tincta-parvula-collaris* group was found to be related to pH (Figure 4).

The relationship between pH and test length in the *Nebela tincta-parvula-collaris* group is interesting in two respects. First, the relationship seems to be independent of substrate moisture levels (Table 3). Therefore, it may be a useful pH proxy for paleoecological studies, and it could be used in conjunction with modern analogue-based transfer functions (Figure 5c). Second, the relationship is much stronger in the Grand Traverse Bay wetlands than at the Tahquamenon Bay sites (Figure 4). Presumably, this is related to the relative importance of ground water and precipitation at

the two sites. Ground-water influence appears to be much greater at Tahquamenon Bay, and seasonal changes in water levels may alter pH more drastically at the wetland surface. Species differences between the two sites may also be attributable to differing hydrologic regimes. For example, *Trigonopyxis arcula*, a common and relatively xerophilous species, was extremely rare in the Grand Traverse wetlands. Similar microenvironments at Tahquamenon Bay contained abundant representatives of the species. The scarcity and abundance of *Trigonopyxis arcula* at the respective sites may be related to long-term hydrologic differences. Although significantly different hydrologic regimes at the two sites are supported by paleoecological data (R. Booth and S. Jackson, unpublished data), long-term hydrologic data are needed.

SUMMARY AND CONCLUSIONS

Testate amoebae have several strengths as environmental indicators relevant to both monitoring and paleoecological applications. Many species are cosmopolitan in distribution, and most species-environment relationships remain relatively consistent among locations (Charman 1997, Woodland et al. 1998, Mitchell et al. 2000a). They are more sensitive to minor differences in hydrology and water chemistry than macroscopic plants, and unlike most vascular plants, testate amoebae respond only to the conditions at the surface of a wetland (Mitchell et al. 2000a). Testate amoebae have a relatively short life cycle and therefore rapid response times to environmental changes.
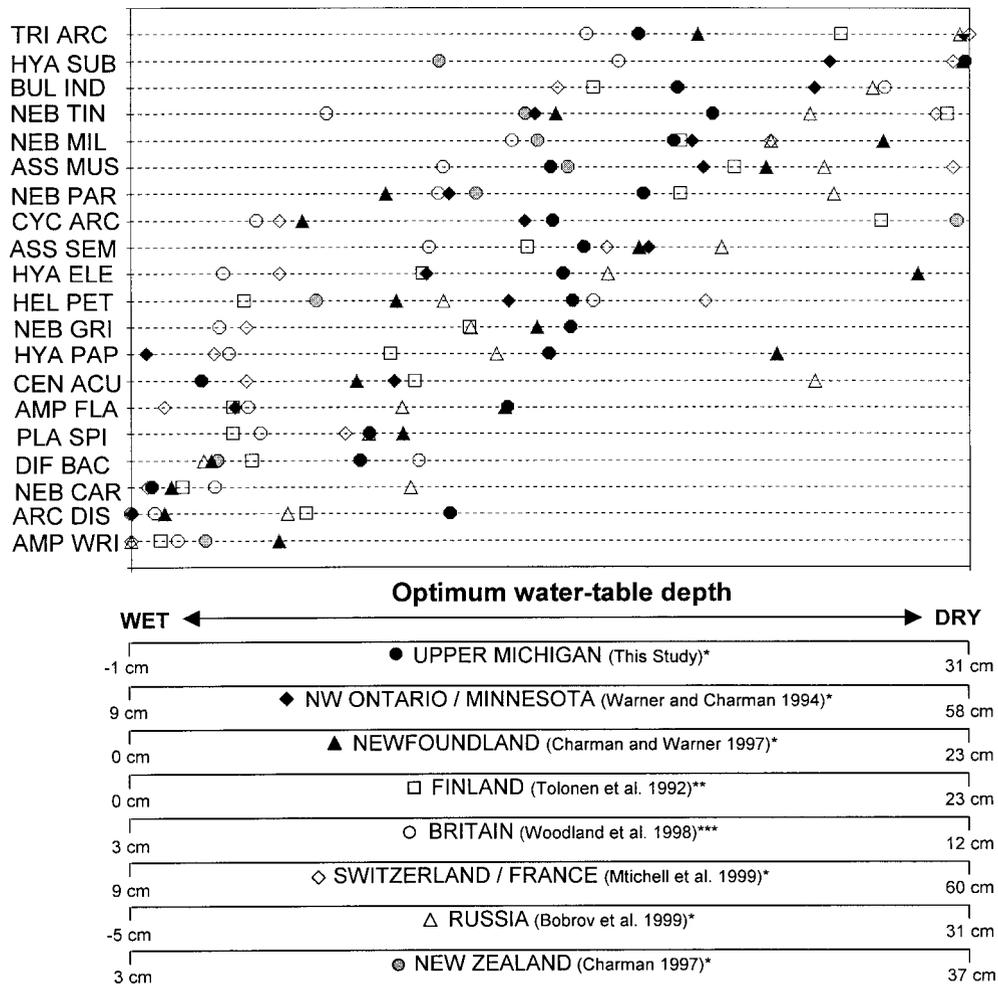
Figure 7. Comparison of the optimum water-table depth reported for 20 common species from geographically widespread sites. The endpoints of the x-axis were determined for each site depending on the wettest and driest values reported for these species. (*water-table measurements collected once during sampling, **water-table measurements a combination of annual data and one-time sampling, ***mean annual water-table-depth measurements)

These include hydrologic changes directly and indirectly associated with human disturbance, as well as those associated with long-term natural variability. A recent study of hydrologic proxy records from an ombrotrophic peatland suggests that the relative frequency of change in a fossil testate amoebae record is more similar to centennial-scale climatic trends (inferred from the wetness/dryness and mildness/severity indices of Lamb 1977) than plant macrofossil or humification records (Charman et al. 1999).

The results of this study indicate that testate amoeba species from *Sphagnum*-dominated Lake Superior coastal wetlands are potentially powerful tools in paleoecological and environmental monitoring contexts. More research is needed on testate amoeba environmental tolerances in substrates other than *Sphagnum*, although my results suggest that pH may be the most important factor influencing testate amoeba assem-

blages in these habitats. Assuming good modern analogues, fossil data can be used to accurately reconstruct water-table depth with a mean error of ±7.5 cm, percent moisture to within ±4.2 percent, and pH to within ±0.41. Test length in the *Nebela tincta-parvula-collaris* group may be an additional tool in the reconstruction of pH. Relationships between test morphology and environmental conditions may exist in other species and species groups, and more work should be done in this context. Research is still needed from different wetland types and geographic regions, increasing the number and diversity of modern analogue assemblages, and thereby improving interpretation of peatland fossil records.

ACKNOWLEDGMENTS

LITERATURE CITED

Aaby, B. 1976. Cyclic climatic variations in climate over the past 5,500 yr reflected in raised bogs. Nature 263:281–284.

Andrus, R. E. 1980. Sphagnaceae (peat moss family) of New York State. New York State Museum, Albany, NY, USA. Bulletin No. 442.

Andrus, R. E. 1986. Some aspects of *Sphagnum* ecology. Canadian Journal of Botany 64:416–426.

Barber, K. E. 1994. Deriving Holocene paleoclimates from peat stratigraphy: some misconceptions regarding the sensitivity and continuity of the record. Quaternary Newsletters 72:1–9.

Beyens, L. and D. Chardez. 1987. Evidence from testate amoebae for changes in some local hydrological conditions between c 5000 BP and c. 3800 BP on Edgeøya (Svalbard). Polar Research 5:165–169.

Birks, H. J. B. 1995. Quantitative paleoenvironmental reconstructions. p. 161–254. *In* D. Maddy and J. S. Brew (eds.) Statistical Modelling of Quaternary Science Data. Quaternary Research Association, London, UK.

Blackford, J. J. 1993. Peat bogs as sources of proxy climatic data: past approaches and future research. p. 47–56. *In* F. M. Chambers (ed.) Climate Change and Human Impact on the Landscape. Chapman and Hall, London, UK.

Bobrov, A. A., S. B. Yazvenko, and B. G. Warner. 1995. Taxonomic and ecological implications of shell morphology of three testaceans (Protozoa, Rhizopoda) in Russia and Canada. Archiv für Protistenkunde 145:119–126.

Bobrov, A. A., D. J. Charman, and B. G. Warner. 1999. Ecology of testate ameobae (Protozoa: Rhizopoda) on peatlands in western Russia with special attention to niche separation in closely related taxa. Protist 150:125–136.

Buttler, A., B. G. Warner, P. Grosvernier, and Y. Matthey. 1996. Vertical patterns of testate amoebae (Protozoa: Rhizopoda) and peat-forming vegetation on cutover bogs in the Jura, Switzerland. New Phytologist 134:371–382.

Casper, S. J. and W. Schönborn. 1985. *Difflugia limnetica* (Levander) Penard (Protozoa: Testacea) as indicator organism of calcite precipitation in Lake Stechlin, GDR. Archiv für Protistenkunde 130:305–311.

Charman, D. J. 1997. Modelling hydrological relationships of testate amoebae (Protozoa: Rhizopoda) on New Zealand peatlands. Journal of the Royal Society of New Zealand 27:465–483.

Charman, D. J. 1999. Testate amoebae and the fossil record: issues in biodiversity. Journal of Biogeography 26:89–96.

Charman, D. J. and B. G. Warner. 1992. Relationship between testate amoebae (Protozoa: Rhizopoda) and microenvironmental parameters on a forested peatland in northeastern Ontario. Canadian Journal of Zoology 70:2474–2482.

Charman, D. J. and B. G. Warner. 1997. The ecology of testate amoebae (Protozoa:Rhizopoda) in oceanic peatlands in Newfoundland, Canada: Modelling hydrological relationships for paleoenvironmental reconstruction. Ecoscience 4:555–562.

Charman, D. J., D. Hendon, and S. Packman. 1999. Multiproxy surface wetness records from replicate cores on an ombrotrophic

mire: implications for Holocene paleoeclimate records. Journal of Quaternary Science 14:451–463.

Corbet, S. A. 1973. An illustrated introduction to the testate rhizopods in sphagnum, with special reference to area around Malham Tarn, Yorkshire. Field Studies 3:801–838.

Foissner, W. 1987. Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. p. 69–212. *In* J. O. Corliss and D. J. Patterson (eds.) Progress in Protistology Vol 2. Biopress Ltd., Bristol, UK.

Foissner, W. 1988. Morphologic and biometric characterization of twenty-four soil testate amoebae (Protozoa, Rhizopoda). Archiv für Protistenkunde 136:153–189.

Foissner, W. 1999. Soil protozoa as bioindicators: Pros and cons, methods, diversity, representative examples. Agriculture, Ecosystems and Environment 74:95–112.

Grospietsch, T. 1958. Wechseltierchen (Rhizopoden). Kosmos, Stuttgart, Germany.

Grospietsch, T. 1965. Rhizopodenanalytische untersuchungen im Naturschutzgebiet Bernrieder Filz (Oberbayern). Archive für Hydrobiologie 61:100–115.

Heal, O. W. 1962. The abundance and micro-distribution of testate amoebae (Rhizopoda: Testacea) in *Sphagnum*. Oikos 13:35–47.

Hendon, D. and D. J. Charman. 1997. The preparation of testate amoebae (Protozoa:Rhizopoda) samples from peat. The Holocene 7:199–205.

Johnston, J. W., T. A. Thompson, and S. J. Baedke. 2000. Preliminary report of Late Holocene lake-level variation in southern Lake Superior. Indiana Geological Survey, Bloomington, IN, USA. Open File Study 99–18.

Juggins, S. 1998. CALIBRATE Version 0.82. University of Newcastle, Newcastle Upon Tyne, UK.

Jung, W. 1936. Thekamöben ursprünglicher lebender deutscher Hochmoore. Abhandlungen Landesmuseums Westfalens 7:1–87.

Lamb, H. H. 1977. Climate: Present, Past, and Future. Volume 2: Climatic History and the Future. Methuen, London, UK.

Laminger, H. 1978. The effects of soil moisture fluctuations on the testacean species *Trinema enchelys* (Ehrenberg) Leidy in a high mountain brown-earths-podsol and its feeding behaviour. Archiv für Protistenkunde 120:446–454.

Loeblich, A. R., Jr. and H. Tappan. 1964. Part C. Protista 2: Sarcodina chiefly ''thecamoebians'' and foraminiferida. p. C16-C54. *In* R. C. Moore (ed.) Treatise on Invertebrate Paleontology. University of Kansas Press and The Geological Society of America, Lawrence, KS, USA.

McCune, B. and M. J. Mefford. 1997. PCORD for windows: multivariate analysis of ecological data, version 3.0. MjM Software, Gleneden Beach, OR, USA.

Medioli, F. S. and D. B. Scott. 1983. Holocene Arcellacea (Thecamoebians) from Eastern Canada. Cushman Foundation for Foraminiferal Research, Special Publication 21:1–63.

Meisterfeld, R. 1977. Die horizontale und vertikale Verteilung der Testaceen (Rhizopoda, Testacea in *Sphagnum*). Archiv für Protistenkunde 79:319–356.

Mitchell, E. A. D., A. J. Buttler, and B. G. Warner. 1999. Ecology of testate amoebae (Protozoa: Rhizopoda) in *Sphagnum* peatlands in the Jura Mountains, Switzerland and France. Ecoscience 6:565–576.

Mitchell, E. A. D., A. Butler, P. Grosvernier, H. Rydin, C. Albinsson, A. L. Greenup, M. M. P. D. Heijmans, M. R. Hoosbeek, and T. Saarinen. 2000a. Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five *Sphagnum*-dominated peatlands in Europe. New Phytologist 146:95–106.

Mitchell, E. A. D., D. Borcard, A. J. Buttler, P. Grosvernier, D. Gilbert, and J. M. Gobat. 2000b. Horizontal distribution patterns of testate amoebae (Protozoa) in a *Sphagnum magellanicum* carpet. Microbial Ecology 39:290–300.

Mitchell, E. A. D., W. O. van der Knaap, J. F. N. van Leeuwen, A. Buttler, B. G. Warner, and J. M. Gobat. 2001. The palaeoecological history of the Praz-Rodet bog (Swiss Jura) based on pollen, plant macrofossils, and testate amoebae (Protozoa). The Holocene 11:65–80.

Ogden, C. G. and R. H. Hedley. 1980. An Atlas of Freshwater Tes-

tate Amoebae. British Museum of Natural History and Oxford University Press, London and Oxford, UK.

Patterson, R. T., T. Barker, and S. M. Burbridge. 1996. Arcellaceans (thecamoebians) as proxies of arsenic and mercury contamination in northeastern Ontario lakes. Journal of Foraminiferal Research 26:172–183.

Reinhardt, E. G., A. P. Dalby, A. Kumar, and R. T. Patterson. 1998. Arcellaceans as pollution indicators in mine tailing contaminated lakes near Cobalt, Ontario, Canada. Micropaleontology 44:131–148.

Schönborn, W. 1963. Die Stratigraphie lebender Testaceen im Sphagnetum der Hochmoore. Limnologica 1:315–321.

Schönborn, W. 1992. Adaptive polymorphism in soil-inhabiting testate amoebae (Rhizopoda): Its importance for delimitation and evolution of asexual species. Archiv für Protistenkunde 142:139–155.

Schönborn, W. and T. Peschke. 1988. Biometric studies on species, races, ecophenotypes and individual variations of soil-inhabiting Testacea (Protozoa, Rhizopoda), including *Trigonopyxis minuta* n. sp. and *Corythion asperulum* n. sp. Archiv für Protistenkunde 136: 345–363.

Tolonen, K., P. Huttunen, and H. Jungner. 1985. Regeneration of two coastal raised bogs in eastern North America. Annales Academiae Scientorum Fennicae Series A, Geologica-Geographica 139:5–51.

Tolonen, K. 1986. Rhizopod analysis. p. 645–666. *In* B. E. Berglund (ed.) Handbook of Holocene Palaeoecology and Palaeohydrology. John Wiley and Sons, New York, NY, USA.

Tolonen, K., B. G. Warner, and H. Vasander. 1992. Ecology of Testaceans (Protozoa: Rhizopoda) in mires in southern Finland: I. Autecology. Archiv für Protistenkunde 142:119–138.

Van der Molen, P. C. and S. P. Hoekstra. 1988. A palaeoecological study of the hummock-hollow complex from Engbertsdijksveen, in the Netherlands. Review of Palaeobotany and Palynology 56: 213–274.

Wanner, M. 1999. A review on the variability of testate amoebae: methodological approaches, environmental influences and taxonomic implications. Acta Protozoologia 38:15–29.

Wanner, M. and R. Meisterfeld. 1994. Effects of some environmental factors on the shell morphology of testate amoebae (Rhizopoda, Protozoa). European Journal of Protistology 30:191–195.

Warner, B. G. 1987. Abundance and diversity of testate amoebae (Rhizopoda, Testacea) in *Sphagnum* peatlands in southwestern Ontario, Canada. Archiv für Protistenkunde 133:173–189.

Warner, B. G. 1988. Holocene fossil *Habrotrocha angusticollis* (Bdelloidea: Rotifera) in North America. Journal of Paleolimnology 1:141–147.

Warner, B. G. 1990. Testate amoebae (Protozoa). p. 65–74. *In* B. G. Warner (ed.) Methods in Quaternary Ecology. Geological Association of Canada, St. John's, Newfoundland, Canada. Geoscience Canada Reprint Series 5.

Warner, B. G. and J. G. Chmielewski. 1992. Testate amoebae (Protozoa) as indicators of drainage in a forested mire, Northern Ontario, Canada. Archiv für Protistenkunde 141:179–183.

Warner, B. G. and D. J. Charman. 1994. Holocene changes on a peatland in northwestern Ontario interpreted from testate amoebae (Protozoa) analysis. Boreas 23:271–279.

Woodland, W. A., D. J. Charman, and P. C. Sims. 1998. Quantitative estimates of water tables and soil moisture in Holocene peatlands from testate amoebae. The Holocene 8:261–273.