Microbial Ecology (2020) 79:443–458
https://doi.org/10.1007/s00248-019-01429-0

METHODS

Behind the Organic Veil: Assessing the Impact of Chemical Deflocculation on Organic Content Reduction and Lacustrine Arcellinida (Testate Amoebae) Analysis

Nawaf A. Nasser[1] · Braden R. B. Gregory[1] · Riley E. Steele[1] · R. Timothy Patterson[1] · Jennifer M. Galloway[2,3]

Received: 17 May 2019 / Accepted: 13 August 2019 / Published online: 20 August 2019

Abstract

Arcellinida (testate lobose amoebae) are widely used as bio-indicators of lacustrine environmental change. Too much obscuring organic material in a gridded wet Petri dish preparation makes it difficult to observe all specimens present and slows quantification as the organic material has to be carefully worked through with a dissection probe. Chemical deflocculation using soda ash ($Na_2CO_3 \cdot H_2O$), potassium hydroxide (KOH), or sodium hexametaphosphate ($(NaPO_3)_6$) has previously been shown to disaggregate and reduce organic content in lake sediments, but to date, no attempt has been made to comparatively evaluate the efficiency of these deflocculants in disaggregating organic content and their impact on Arcellinida analysis in lacustrine sediments. Here, we assess the effectiveness of soda ash, potassium hydroxide, and sodium hexametaphosphate treatments on removing organic content and the impact of those digestions on Arcellinida preservation in 126 sample aliquots subdivided from three sediment samples (YK-20, YK-25, and YK-57) collected from three lakes near Yellowknife, Northwest Territories, Canada. Following treatment, cluster analysis and Bray-Curtis dissimilarity matrix (BCDM) were utilized to determine whether treatments resulted in dissolution-driven changes in Arcellinida assemblage composition. Observed Arcellinida tests in aliquots increased drastically after treatment of organic-rich samples (47.5–452.7% in organic-rich aliquots and by 14.8% in aliquots with less organic matter). The BCDM results revealed that treatment with 5% KOH resulted in the highest reduction in observed organic content without significantly affecting Arcellinida assemblage structure, while soda ash and sodium hexametaphosphate treatments resulted in marginal organic matter reduction and caused severe damage to the arcellinidan tests.

Keywords Arcellinida · Lake sediments · Organic content · Chemical treatment · Sample preparation methods · Arcellinidan analysis

Introduction

Arcellinida (testate lobose amoebae) are a cosmopolitan group of benthic-shelled microorganisms (15–200 μm) that are important bio-indicators of environmental change in fresh and brackish water aquatic systems [1, 2]. The group is characterized by tests (i.e., shells) that are either secreted by the organism or constructed by binding particles from the surrounding environment (e.g., sediment particles and/or diatom frustules), with the latter type being of greater use in limnological and paleolimnological studies [2]. Arcellinida analysis of samples derived from lake sediments generally involves identifying and quantifying tests in wet preparations in a Petri dish under a dissecting microscope. Prior to analysis, it is desirable to remove as much organic and minerogenic material as possible as this extraneous matter obscures specimens. In the early

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-019-01429-0) contains supplementary material, which is available to authorized users.

✉ Nawaf A. Nasser
Nawaf.nasser@carleton.ca

[1] Ottawa-Carleton Geoscience Centre and Department of Earth Sciences and, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

[2] Geological Survey of Canada/Commission géologique du Canada, Calgary, Alberta T2L 2A7, Canada

[3] Aarhus Institute of Advanced Studies, Aarhus University, 8000 Aarhus, Denmark

1980s, Arcellinida researchers adapted the approach used in analysis of foraminifera in organic-rich salt marsh samples (e.g., [3–5]) to lacustrine environments. That sample preparation protocol involves gently washing sediment samples through coarse (500–1000 μm) and fine (44–63 μm) sieves to remove organic and minerogenic particles that are larger and smaller than arcellinidan tests. Scott and Hermlin [6] complemented sieving with using an improved version of the Elmgren [7] wet splitter, which allowed for dividing sieved samples into smaller and more manageable aliquots.

While sieving and splitting still form the standard arcellinidan sample preparation protocol, variations in the mesh-size used for coarse (1000–100 μm) and fine sieving (63–15 μm) and types of wet splitter (six vs. eight chambers) have been employed over the past 30 years (Table 1). A potential byproduct of these variations in preparation protocols is the introduction of various types of uncertainties during and after sample preparation that can negatively impact the quality of quantitative Arcellinida data. A few recent studies have investigated the potential statistical impact of preparation protocol variation focusing on the loss of arcellinidan tests during sample preparation [8, 9], the impact of using different filters on the density and species richness [10], and the effects of refrigerated sample storage on assemblage composition [11]. A potential source of uncertainty that has been overlooked is the impact of residual organic content on the quality of quantitative arcellinidan analysis.

Significant amounts of residual organic debris often survive the standard preparation processes and may mask or trap arcellinidan tests, which consequently impedes their analysis. Sample agitation, using either a stirring rod or laboratory benchtop wrist shaker, or repeating the process of sieving and splitting have been suggested as means to further loosen the organic colloidal content (e.g., [4, 5]). However, these methods do not substantially reduce the total amount of organic content and may increase the potential of Arcellinida test loss during sample processing (e.g., process of decanting, sieving, and splitting; [12]). In order to better assess the impact of the organic content on arcellinidan analysis, the development of a more robust sample preparation technique that can maximize the reduction of the organic content is required.

Numerous studies on lacustrine (e.g., chironomids; [13, 14]) and marine bio-indicators (e.g., foraminifera; [15, 16]) have utilized chemical deflocculation to disaggregate organic and minerogenic colloidal content prior to sample preparation. Chemical sample preparation methods often involve soaking sediments in a known volume and concentration of a selected chemical deflocculant for a specific duration prior to sieving and splitting the sample. To the best of our knowledge, only a limited number of lacustrine arcellinidan studies employed chemical deflocculants including sodium hexametaphosphate ($(NaPO_3)_6$), sodium carbonate monohydrate (soda ash; $Na_2CO_3{\cdot}H_20$), or potassium hydroxide (KOH), to disaggregate and reduce amorphous organic content in lake sediment samples. The lack of enthusiasm among Arcellinida researchers to employ chemical deflocculation is attributed to a perception that the harsh nature of these methods may cause severe damage to arcellinidan tests [2]. Hendon and Charman [17] have compared the effects of using several sample preparation methods, including KOH treatment, on peatland testate amoebae density, species richness, and test retention. While their study revealed that KOH treatment produced superior results in terms reduction of organic content and retention of arcellinidan tests; it also showed that treatment damaged a significant number of tests in the process thus impacting the statistical quality of the resultant quantitative arcellinidan analysis. The use of soda ash and sodium hexametaphosphate have also been reported to reduce organic content in lake sediments while causing little to no damage to arcellinidan tests [18–20]. However, no attempt has previously been made to compare the efficacy of organic content reduction using these various chemical deflocculants and to assess their impact on arcellinidan test preservation and associated assemblage structure in lake sediment samples.

In this study, we assess the impact of three chemical deflocculant treatment methods; soda ash ($Na_2CO_3{\cdot}H_2O$), potassium hydroxide (KOH), and sodium hexametaphosphate ($(NaPO_3)_6$), on organic content reduction and arcellinidan test preservation in 126 subsample aliquots derived from three sediment-water interface samples (YK-20, YK-25, and YK-57) from three lakes near the City of Yellowknife, Northwest Territories, Canada. The study aims to (1) determine the optimal deflocculant method and deflocculant chemical concentration for reducing organic content in arcellinidan bearing lake sediment preparations, (2) assess the level of arcellinidan dissolution caused by the various chemical treatment methods, and (3) assess the impact of chemical treatment and residual organic content on arcellinidan analysis and faunal composition.

Study Area

The samples, used in this study, were collected from three lakes located within a radius of ~ 30 km around the City of Yellowknife, Northwest Territories, Canada (Fig. 1). The elevation of the study area ranges from 157 m above mean sea level (AMSL) close to Great Slave Lake to 350–400 m AMSL to the north of Thistlethwaite Lake [21]. The Yellowknife River, the main drainage system in the study area, flows southward into Yellowknife Bay of the Great Slave Lake. The study area is characterized by a subarctic, continental climate where

Table 1 Sample preparation techniques employed in 44 Arcellinida studies between 1983 and 2018

Author (s)	Year	Coarse sieving (μ)	Fine sieving (μ)	Splitting (Scott and Hermlin, 1993)	Chemical treatment
Scott and Medioli	1983	500	63	No	
Patterson et al	1985	500	63	No	
Ellison and Ogden	1987	100	44	No	6% Calgon
Medioli and Scott	1988	500	63	No	
Collins et al	1990	Not mentioned	63	No	
Asioli et al	1996	Not mentioned	45	No	
Patterson et al	1996	1000	63	Yes	
Bubridge et al.	1998	Not mentioned	44	No	Soda ash
Reinhardt et al	1998	1000	55	Yes	
Kliza and Shroeder-Adams	1999	Not mentioned	63	No	
Dalby et al.	2000	1000	43	Yes	
Dallimore et al	2000	Not mentioned	44	Yes (eight chambers)	
Kumar and Patterson	2000	1000	55	Yes	
Patterson and Kumar	2000	1000	55	Yes	
Dalby et al.	2002	1000	43	Yes	Soda ash
Patterson and Kumar	2002a	1000	40–55	Yes	
Patterson and Kumar	2002b	454	43	Yes	
Dallimore	2004	Not mentioned	44	Yes	
Boudreau et al.	2005	500	44	Yes	
Escobar et al	2005	Not mentioned	63	No	
Reinhardt et al	2005	150	45	Yes	
Kaupilla et al	2006	1000	56	Yes (eight chambers)	
Roe and Patterson	2006	250	37	Yes	
Escobar et al	2008	707	53	Yes	
Kihlman and Kauppila	2009	1000	56	Yes	
Lorencova	2009	1000	36	Yes	
Sonnenburg and Reinhardt	2009	250	45	Yes	
Kihlman and Kauppila	2010	500	56	Yes	
Roe et al	2010	Not mentioned	37	Yes (not specified)	
Neville et al	2010	500	45, 63	No	
Wall et al	2010	250	25, 47, 63	No	
Farooqui et al	2012	105	20	No	5% KOH
Kihlman and Kauppila	2012	500	56	Yes	
McCarthy et al	2012	Not mentioned	63	No	10% Calgon (HMP)
Patterson et al	2012	250	37	Yes	
Warchorn et al	2012	Not mentioned	43	No	
Neville et al	2013	250	37	Yes	
Patterson et al	2013	Not mentioned	37	Yes	
Qin et al	2013	300	35	No	
Sonnenburg et al	2013	1000	250	Yes (eight chambers)	
Patterson et al	2015	297	37	Yes	
Nasser et al	2016	297	37	Yes	
Qin et al	2016	500	35	No	
Segala et al	2016	Not mentioned	Not mentioned	No	
Steel et al	2018	297	37	Yes	

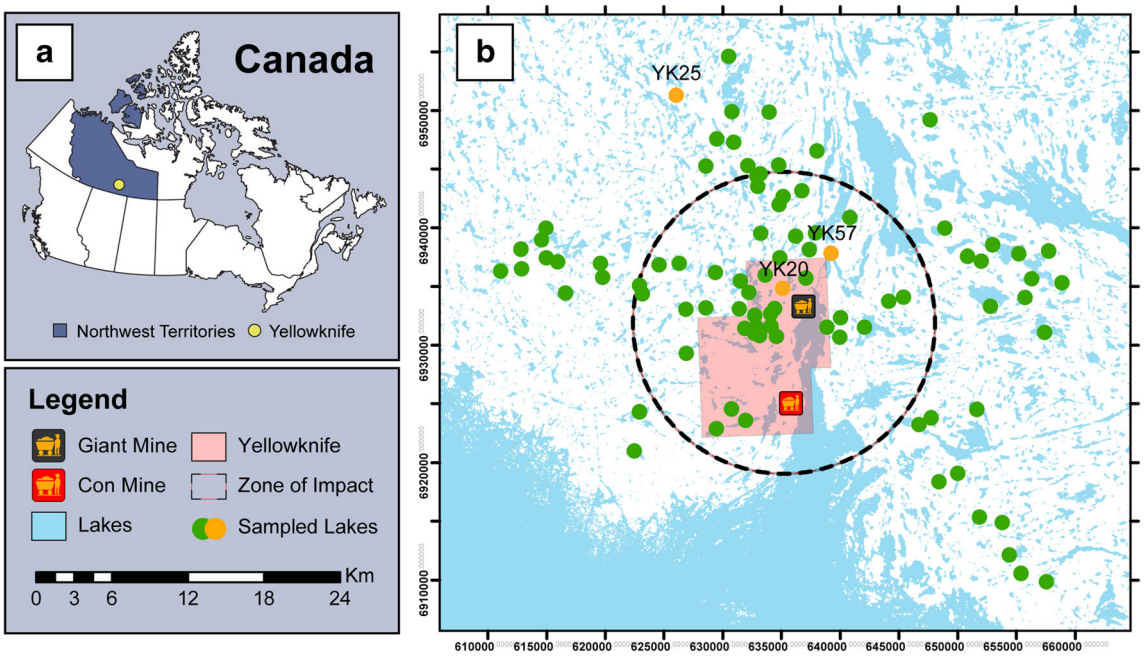

Fig. 1 **a** Map of Canada showing the study location in the Northwest Territories, Canada (dark blue) as a yellow circle. **b** A map showing sampling sites (green and yellow circles) within 30-km radius around the City of Yellowknife. Lakes sampled in this study ($n = 3$) are represented as yellow circles

summers are short, dry, and cool with a mean annual temperature of − 4.3 °C and a relatively low mean annual precipitation of 170.7 mm [22].

Rocks underlying the investigated lakes belong to the Yellowknife Supergroup of the southern Slave structural province of the Canadian Shield. These include Archean metavolcanic and metasedimentary rocks intruded by younger granitoids and diabase dykes. Surficial sediments in the Yellowknife area are dominated by fine clastic lacustrine sediments deposited in Glacial Lake McConnell and a relatively thick (< 2 m) and discontinuous layer of glacigenic sediments [21]. Holocene-aged peat is also common in the study region and can be greater than 1 m thick in bogs and wetlands [21].

Methodology

Sample Selection

The 126 aliquots analyzed here were subsampled from three samples (YK20, YK25, and YK57) collected in June 2014 using an Ekman grab (Fig. 1). More than 100 lakes from this area are being investigated as part of an inter-lake spatial assessment of the impact of legacy gold mine-induced arsenic contamination on the arcellinidan distribution within a radius of 30 km around the City of Yellowknife ([23, 24). The three lakes selected for this study were chosen to include samples with both high and low total organic carbon (TOC) from that large data set based on observed organic concentration using Rock-Eval® Analysis 6, as well as visual inspection of the density of amorphous organic matter under a dissecting microscope. The highest organic content density and TOC (26.38%) was observed in YK20. Surprisingly, YK57 is also characterized by high observed organic content density even though the sample's TOC is the lowest among the selected samples (7.03%). In contrast, a low organic matter density was observed in YK25 despite the relatively higher TOC of the sample (26.08%) (Table 2).

Arcellinida Taxonomy

The arcellinidan species and strains were identified using several well-illustrated papers that employed the strain taxonomic concept (e.g., [25–28] [29–31]; [23]). As lacustrine arcellinidan species can display a significant amount of

Table 2 Total organic matter (TOC) and particle size analysis results for YK20, YK25, and YK57

Site ID	Rock Eval	Particle size analysis		
	TOC (%)	Sand (%)	Silt (%)	Clay (%)
YK20	26.38	5.87	76.82	17.31
YK25	26.08	13.61	71.19	15.2
YK57	7.03	10.13	77.43	12.44

environmentally controlled morphological variability (e.g., [32, 34]), the accepted practice has been to designate informal infrasubspecific "strain" names for these ecophenotypes ([2, 33]). Ic under the International Zoological Code of Nomenclature (art. 45.5; 4th edition, [61]). However, they have been extensively used in the literature for delineating environmentally significant populations within lacustrine environments ([2, 26, 35]; [36, 37]).

Chemical Treatment Methods

To determine the most efficient combination of treatment method, chemical concentration, and treatment exposure time for reducing the organic content in arcellinidan samples, the experimental component of the study was divided into three testing stages. Figure 2 provides an overview of the standard arcellinidan sample preparation method (Fig. 2a) and the steps

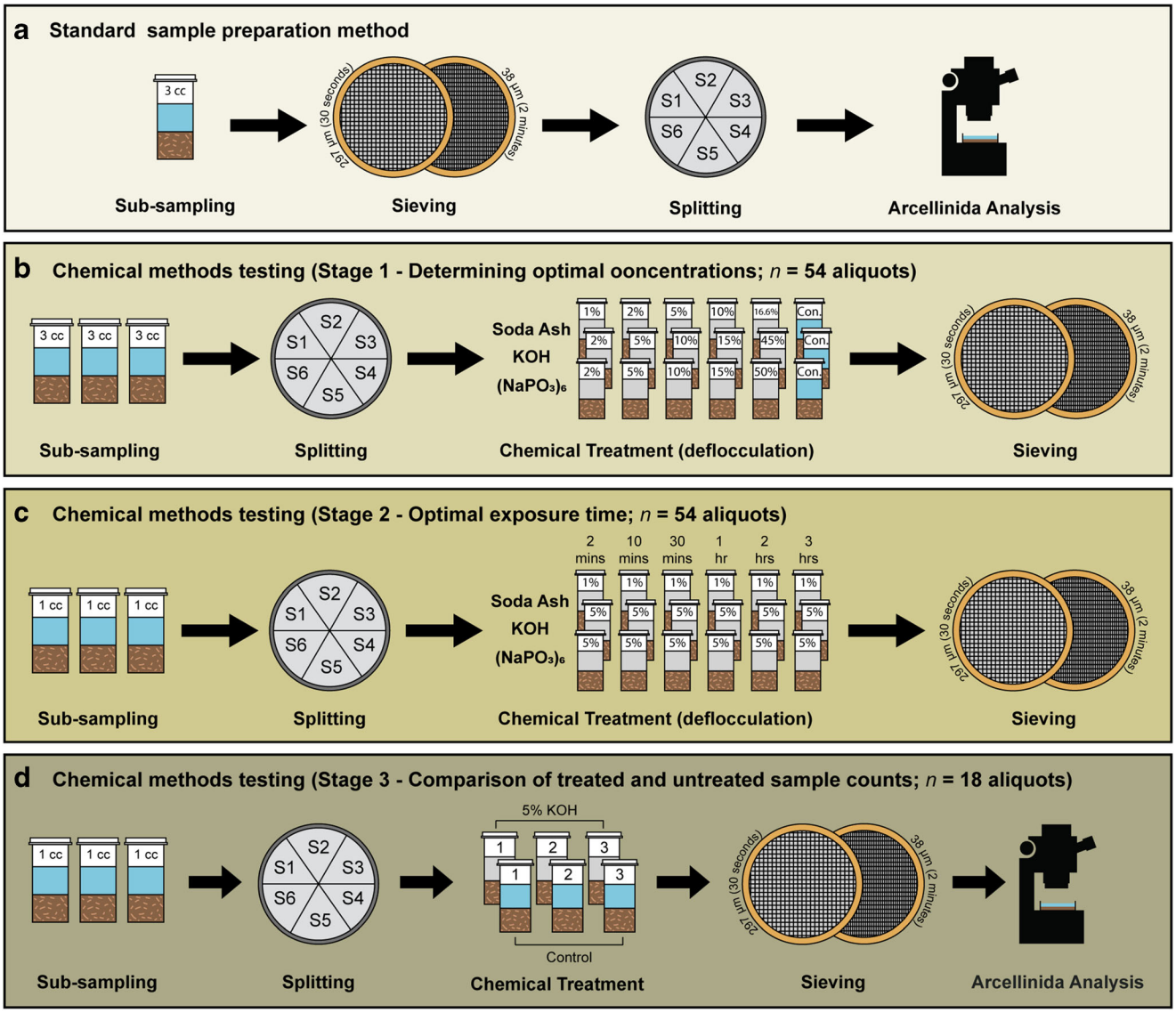

Fig. 2 Schematic view of sample preparation methods used in this study. **a** Standard Arcellinida sample preparation protocol. A sediment sample (often 3 cm^3) is sieved through coarse (297 µm; 30 s) and fine (38 µm; 2 min) sieves and split prior to arcellinidan analysis. **b** First chemical methods testing stage, in which the impact of chemical treatment on organic content reduction was assessed. Three sediment subsamples (3 cm^3 each), from each YK sample, were split into six aliquots (n = 54). Five aliquots were treated with different concentrations of a chemical deflocculant for 3 h before sieving. A single aliquot was processed following the standard sample preparation protocol (control subsample). **c** Second chemical methods testing stage, in which optimal treatment duration was determined and impact on Arcellinida tests was assessed. Three sediment subsamples (1 cm^3 each), from each YK sample, were split into six aliquots (n = 54). Aliquots were treated with one selected concentration of each chemical deflocculant for different treatment durations prior to sieving. **d** Third chemical methods testing stage. Three sediment subsamples (1 cm^3 subsample per YK sample) were split into six aliquots (n = 18). Three aliquots were treated with the optimal chemical method and concentration for 3 h, and three were processed following the standard sample preparation protocol. Treated and control sub-samples were sieved prior to arcellinidan analysis

followed during the three chemical testing stages (Fig. 2b, c, d). Nine subsamples (3 cm^3 each) were collected from the YK20, YK25, and YK57 lake sediment grab samples (three subsamples per YK sample) to carry out the first testing stage. Each of the nine subsamples was divided into six aliquots using a wet splitter, resulting in a total of 54 aliquots [6]. The aliquots were then centrifuged at 4000 rpm for 4 min and excess water was carefully decanted to mitigate diluting the chemical concentrations that were to be added to each sub-sample. A single aliquot from each sample served as a control sub-sample and was prepared following the standard arcellinidan sample preparation protocol described above (Fig. 2a), while the remaining aliquots were treated using 20 ml of five different concentrations of soda ash, KOH, and sodium hexametaphosphate for 3 h (Fig. 2b). After 3 h, all treated aliquots were gently sieved using 297 μm (30 s) and 37 μm sieves (2 min). The aliquots were then placed in a gridded Petri dish and the proportion of residual organic content was visually inspected under an Olympus SZH dissecting binocular microscope. Photomicrographs of random grids for treated and control aliquots were taken to determine the difference between the amount of organic content reduced by the chemical treatment methods and the standard sample preparation protocol (Figs. 3, 4, and 5). The chemical concentration of each chemical deflocculant responsible for reducing the highest proportion of organic content was selected for further testing.

During the second stage of the experiment, three 1-cm^3 subsamples from each YK sample was split into six aliquots (total number of aliquots = 54) and treated with the selected

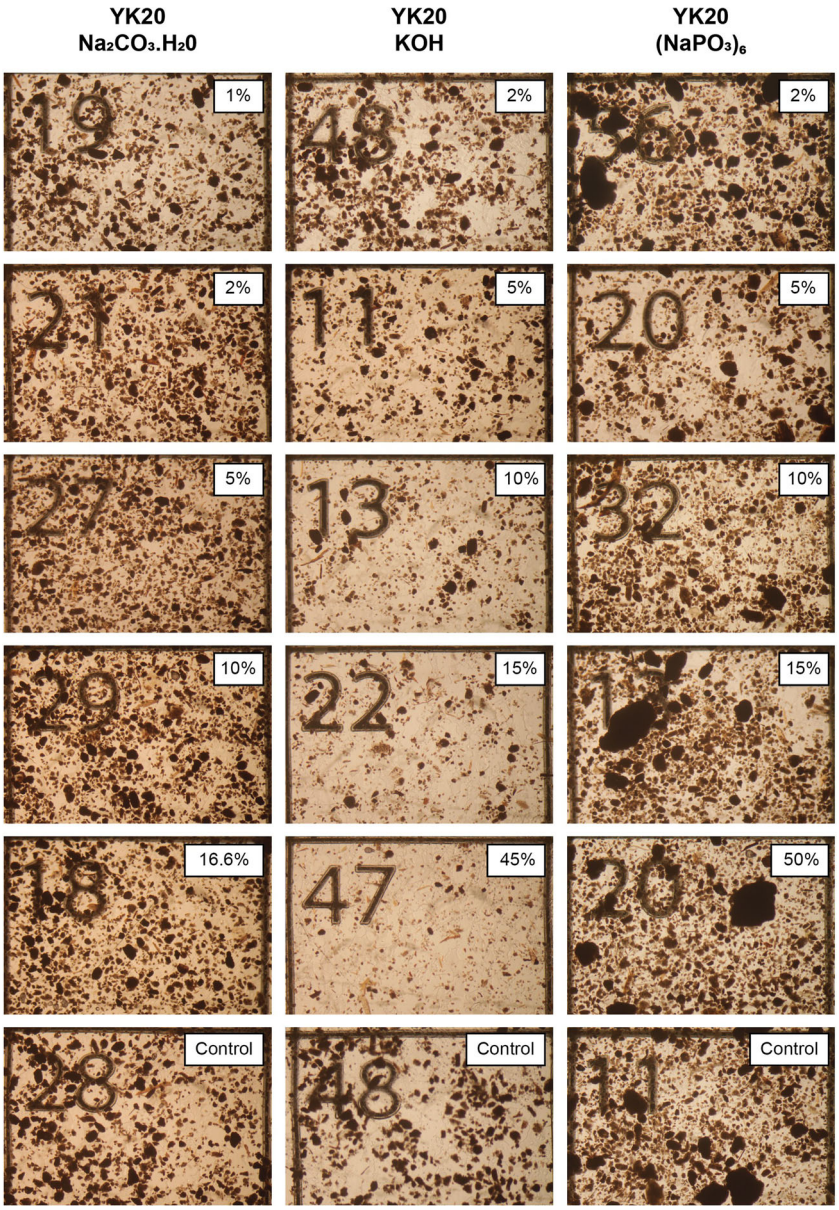

Fig. 3 Photomicrographs showing the level of organic content reduction in chemically treated, with different concentrations of soda ash, KOH, and sodium hexametaphosphate, and control YK20 sub-samples

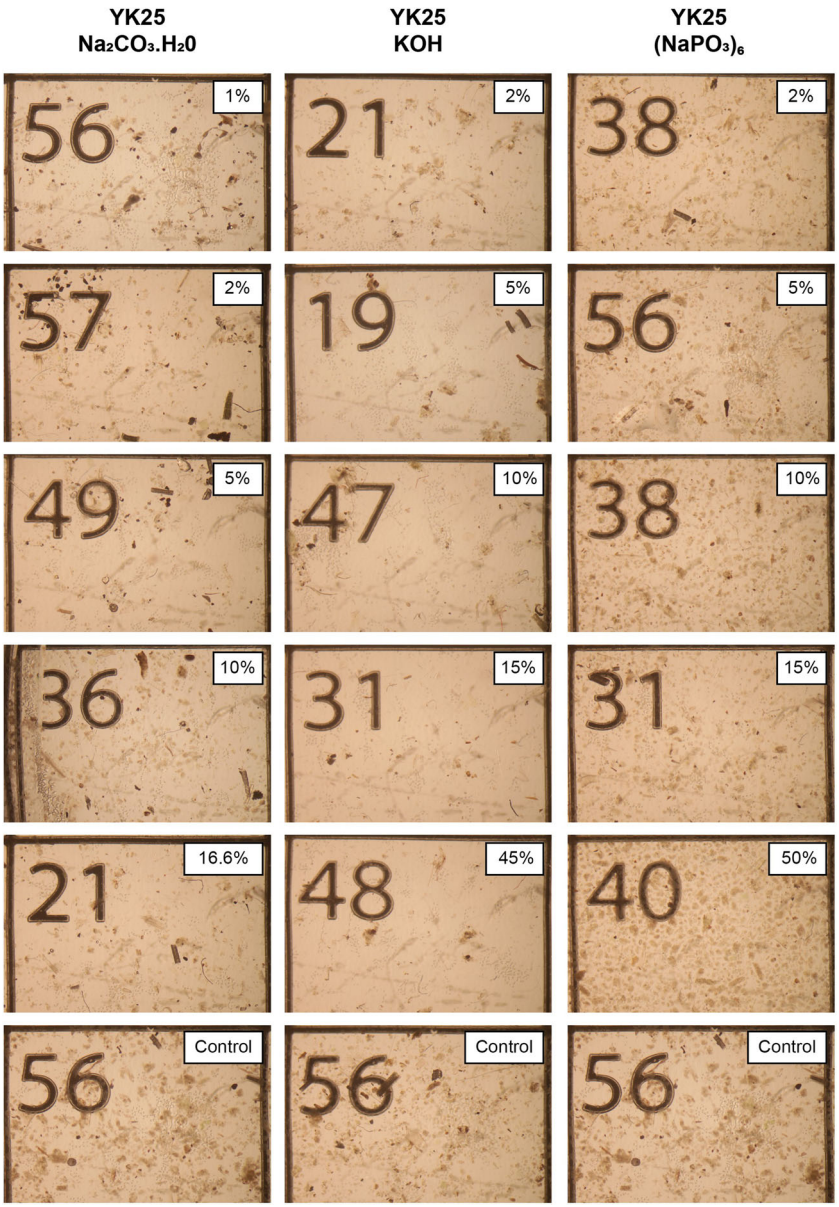

Fig. 4 Photomicrographs showing the level of organic content reduction in chemically treated, with different concentrations of soda ash, KOH, and sodium hexametaphosphate, and control YK25 sub-samples

chemical concentrations for different time intervals prior to sieving (i.e., 2 min, 10 min, 30 min, 1 h, 2 h, and 3 h; Fig. 2c). This stage was essential for determining the optimal duration for the treatment and assessing the impact of the chemical treatment on the integrity of arcellinidan tests. The chemical method causing the least damage to Arcellinida tests was selected for further testing in stage 3.

The final testing stage involved splitting an additional three 1 cm^3 from each YK sample into six aliquots (total number of aliquots = 18), treating three aliquots with the selected chemical, and preparing the remaining three aliquots following the standard sample preparation protocol (Fig. 2a, d). Arcellinida in all aliquots were identified and quantified in totality to determine whether treating the subsample with the selected chemical treatment method will allow for identifying more arcellinidan tests, which would otherwise be concealed or trapped within the organic cover. Results from this stage were important for assessing the impact of residual organic content on the arcellinidan test retention as well as the impact of the selected chemical treatment on arcellinidan tests and population composition.

Statistical Methods

Arcellinidan data generated in the third testing stage (Table 3) was analyzed using RStudio statistical software (version 0.98.1028; [52]). Q-mode cluster analysis was performed on the Arcellinida fractional abundance data using the Ward Minimum Variance clustering method [53] to identify any variations in the composition of faunal assemblages resulting

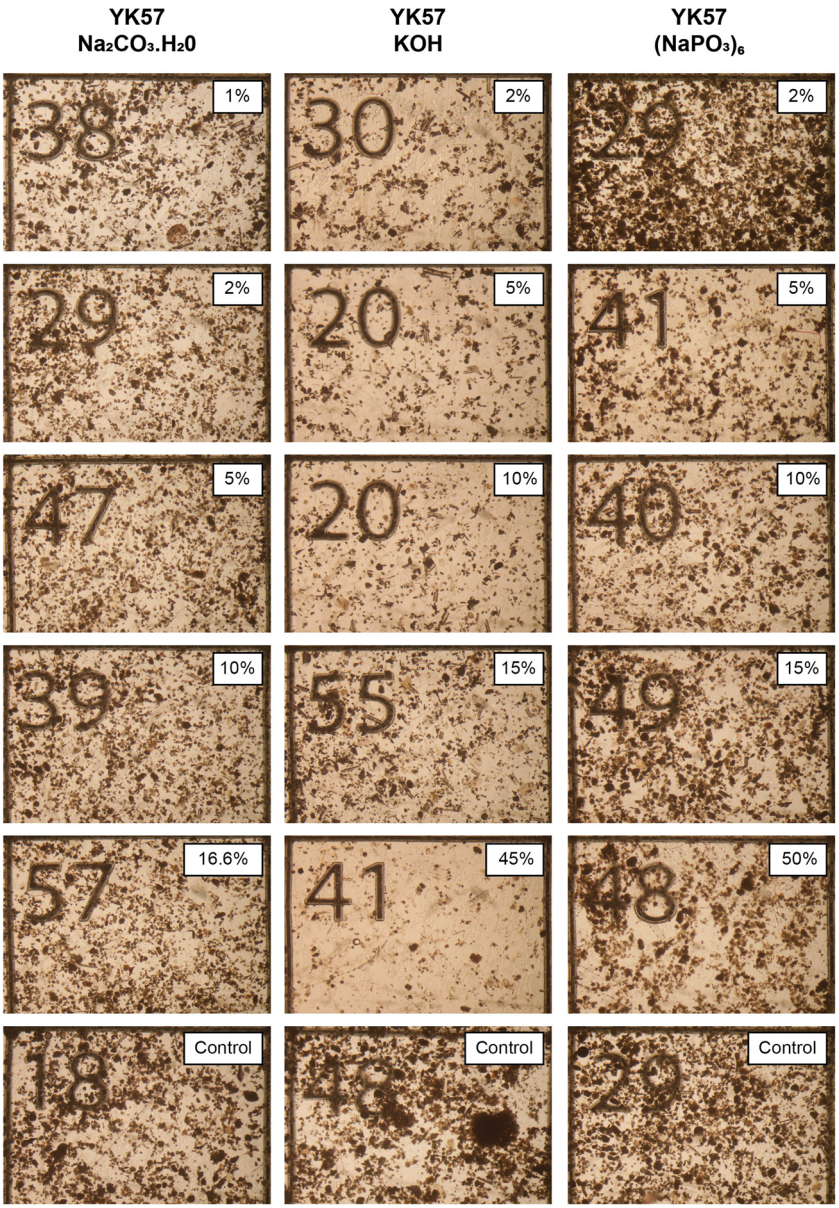

Fig. 5 Photomicrographs showing the level of organic content reduction in chemically treated, with different concentrations of soda ash, KOH, and sodium hexametaphosphate, and control YK57 sub-samples

from the chemical treatments. The statistical significance of each identified assemblage was determined using the PVClust package (Fig. 6). The Bray-Curtis dissimilarity matrix (BCDM; [55]) of the Arcellinida count data was generated to assess the level of similarity between the samples comprising each assemblage (Supplementary Table 1), and thus detect any subtle changes in faunal composition after chemical treatment. Standard error (Sxi) was calculated for each taxon in the treated and control sub-sample using the following equation:

$$Sxi = 1.96\sqrt{\frac{F1(1-F1)}{Ni}}$$

where $F1$ is the fractional abundance of species and Ni is the total number of counts. Determining the standard error was important for assessing whether a reduction in organic content via chemical deflocculation is associated with lower standard errors of arcellinidan total counts of dominant, common, and minor taxa ([54]; Supplementary Table 2).

Results

Chemical Methods Testing

Stage 1—Determining Optimal Concentrations

Of the three chemicals used in the experiment, treatment with KOH showed the greatest reduction in organic content in comparison to the control sub-samples ($n = 3$). Higher

Table 3 Raw Arcellinida counts for the control and 5% KOH-treated subsamples of YK20, YK25, and YK57

Sample	YK20						YK25						YK57					
Preparation method	Control			Treated			Control			Treated			Control			Treated		
Sub-sample	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Arcella vulgaris Ehrenberg, 1830	0	0	1	2	0	0	0	2	0	1	2	0	2	2	1	20	15	19
Centropyxis aculeata Ehrenberge, 1983 "aculeata" Centropyxis aculeata Ehrenberge, 1983 "aculeata" Centropyxis aculeata Ehrenberge, 1983 "aculeata"	33	60	55	88	95	87	20	19	15	24	14	21	72	81	98	459	559	534
Centropyxis aculeata Ehrenberge, 1983 "discoides" Centropyxis aculeata Ehrenberge, 1983 "discoides" Centropyxis aculeata Ehrenberge, 1983 "discoides"	29	34	33	49	66	97	2	0	1	3	1	6	4	9	4	128	109	141
Centropyxis constricta [38] "aerophila" Centropyxis constricta [38] "aerophila" Centropyxis constricta [38] "aerophila"	23	22	33	46	52	46	1	1	4	3	1	3	1	3	1	43	17	13
Centropyxis constricta [38] "constricta" Centropyxis constricta [38] "constricta"Centropyxis constricta [38] "constricta"	15	2	12	26	12	17	11	4	9	4	4	3	5	6	12	62	10	12
Centropyxis constricta [38] "spinosa"Centropyxis constricta [38] "spinosa"Centropyxis constricta [38] "spinosa"	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	2
Cucurbitella tricuspis [39]	5	5	3	13	6	2	2	5	3	0	5	1	16	20	15	19	15	21
Conicocassis pontigulasiformis [40] [41]	16	20	22	35	41	45	0	0	0	0	1	0	0	0	0	0	0	0
Difflugia globulosa Dujardin, [42]	23	19	19	15	24	18	3	1	0	0	2	1	0	8	5	111	119	137
Difflugia amphora Leidy, [43]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Difflugia glans *[44]* "glans"Difflugia glans *[44]* "glans" Difflugia glans *[44]* "glans"	10	9	17	16	13	7	0	0	1	0	1	3	1	1	1	4	4	2
Difflugia glans *[44]* "distenda"Difflugia glans *[44]* "distenda"Difflugia glans *[44]* "distenda"	8	15	19	17	3	14	0	1	0	0	0	0	0	1	0	0	3	0
Difflugia spe.	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Difflugia lenceolata [45]	0	0	0	0	0	0	0	0	0	0	0	0	5	4	9	4	2	1
Difflugia linearis [45] Gauthier-Lièvre and Thomas, [46]	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia oblonga Ehrenberg, 1932 "oblonga"Difflugia oblonga Ehrenberg, [47] "oblonga"	32	33	31	23	29	38	0	1	1	0	1	0	5	8	2	1	1	5
Difflugia oblonga Ehrenberg, 1932 "spinosa"Difflugia oblonga Ehrenberg, 1932 "spinosa"	0	1	0	1	3	0	0	0	0	0	0	0	0	0	1	0	0	0
Difflugia oblonga Ehrenberg, 1932 "tenuis"Difflugia oblonga Ehrenberg, 1932 "tenuis"	6	9	5	2	9	4	1	0	1	1	1	1	0	0	0	0	1	0
Difflugia protaeiformis [49]	0	0	2	2	0	0	7	12	8	3	9	2	13	5	8	5	11	11
Difflugia acuminata Ehrenberg, [48]	3	1	1	1	3	4	0	0	0	0	1	0	0	6	4	1	0	2
Difflugia claviformis Penard, [50]	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0
Difflugia urceolata [51] "urceolata"Difflugia urceolata [51] "urceolata"	0	0	0	4	12	5	0	0	0	1	0	0	0	0	0	3	7	4
Difflugia urceolata [51] "elongata"Difflugia urceolata [51] "elongata"	0	1	0	0	2	4	0	0	0	0	0	0	0	0	1	6	3	3

Table 3 (continued)

Sample	YK20						YK25						YK57					
Preparation method	Control			Treated			Control			Treated			Control			Treated		
Difflugia urens [4]	0	0	0	9	6	16	0	0	0	0	4	0	0	2	1	2	3	3
Difflugia curvicaulis Penard, 1899	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Difflugia elegans [45]	107	95	101	133	121	116	29	25	31	45	37	40	9	8	9	43	27	23
Difflugia bicornis [45]	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Lagenodifflugia vas (Leidy, 1874)	10	8	5	6	8	11	1	1	0	0	0	1	0	0	0	0	0	0
Lesquereusia spiralis (Ehrenberg, 1840)	4	8	5	3	3	3	0	0	0	0	0	0	0	0	0	0	0	0
Pontigulasia compressa [51]	1	2	3	3	3	3	0	0	0	1	0	0	0	1	0	0	0	0
Total	327	345	369	495	512	538	77	72	74	87	85	84	134	165	174	912	908	934
Median	347			512			74			85			165			912		
Shannon diversity index (SDI)	2.28	2.3	2.34	2.35	2.35	2.34	1.69	1.75	1.7	1.44	1.94	1.63	1.64	1.91	1.73	1.67	1.39	1.45
Number of species	17	19	19	22	21	20	10	11	10	11	16	12	12	16	18	17	18	18
Total number of species	21			23			14			20			21			18		
Percentage of increase (total test count)	NA			47.55%			NA			14.86%			NA			452.70%		

concentrations of KOH provided greater reduction in visible organic content, with 45% concentration providing the greatest reduction, and 2% still producing a notable decrease in visible organic content. Because the 5%, 10%, and 15% KOH concentrations provided a similar reduction in visible organic debris as 45% KOH exposures, a 5% KOH concentration was selected for further testing.

Treatment of aliquots using different concentrations of soda ash (1%, 2%, 5%, 10%, and 16.6%; $n = 15$) showed negligible reduction in visible organic debris except for a meager reduction observed in sub-samples treated with 1% soda ash (Figs. 3, 4, and 5). Therefore, the 1% soda ash concentration was selected for the next testing stage.

A minor reduction of visible organic content occurred after treatment with sodium hexametaphosphate in comparison to standard sieving treatments ($n = 15$; Figs. 3, 4, and 5). The 5% sodium hexametaphosphate concentration was selected for the second testing stage as it reduced slightly higher proportions of organic content compared to the rest of the sodium hexametaphosphate concentrations (2%, 10%, 15%, and 50%).

Stage 2—Determining Optimal Exposure Times

Treatment of organic-rich samples with KOH provided a significant reduction in organic content for all exposure times (2 min, 10 min, 30 min, 1 h, 2 h, and 3 h; Fig. 2c). Samples treated for longer durations (1 h, 2 h, and 3 h) exhibited greater reduction in visible organic content, with the 3-h treatment exhibiting the highest reductions in organic content. Exposure to the chemical for > 24 h did not further reduce organic matter substantially. The removal of organic content when sodium hexametaphosphate and soda ash were used did not differ substantially over time and remained similar in both the treated and control sub-samples when visually inspected under a dissecting microscope.

In terms of damage/dissolution to arcellinidan tests, treatment with sodium hexametaphosphate caused severe damage to Arcellinida tests, most of which were agglutinated, with signs of damage (e.g., test breakage) manifesting after only 2 h of low concentrations treatment (2% and 5%) and after 45 min when higher concentrations were used (10–50%). Samples treated with soda ash exhibited no discernable signs of damage after 24 h of treatment in lower concentrations (1–2%) and after 3 h in higher concentrations (5–16.6%). However, a peculiar side effect of the treatment was that tests, particularly those of pyriform and globular morphologies, became more elastic the longer the treatment lasted. These tests eventually became easier to break. This change in test robustness was only detected when the test condition was carefully examined using a microscope probe. Severe arcellinidan test damage caused by KOH treatment was observed after 1 day for the 2% concentration and after 3 h for the 5%

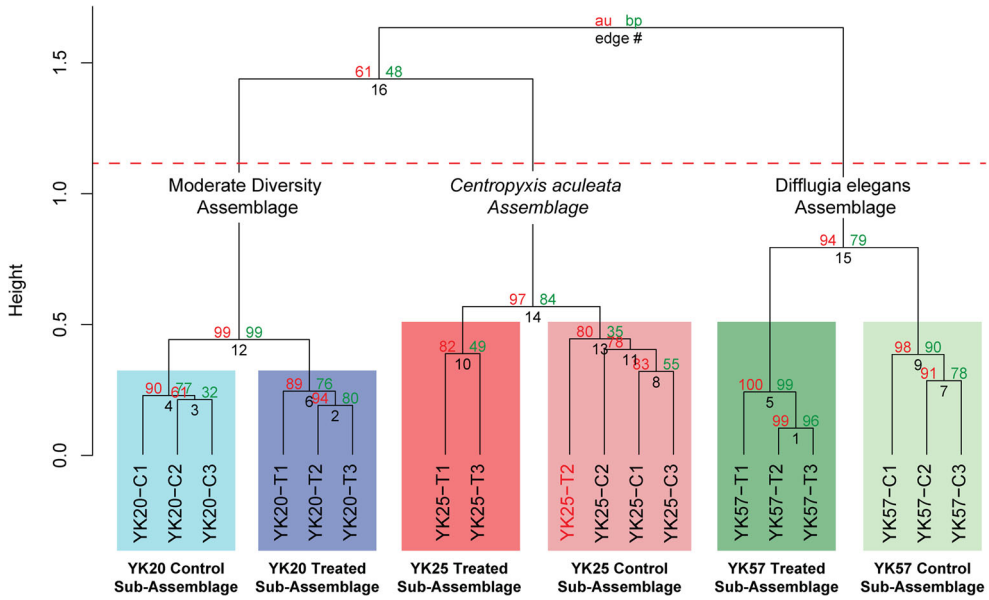

Fig. 6 Q-mode cluster analysis dendrogram showing three identified Arcellinida assemblages: (1) *moderate diversity assemblage* (MDA), (2) *Centropyxis aculeata* assemblage (CPA), and (3) *Difflugia elegans* assemblage (DEA). Six faunal sub-assemblages are also indicated with the colored rectangles. The statistical significance of each assemblage and sub-assemblage was indicated by the results of PVClust (red numbers; p value < 0.05)

concentration. However, the tests were damaged after 1 h when higher concentrations are used (10–45%). Damage caused by lower concentrations did not impede the identification of Arcellinida tests. Therefore, treatment with 5% KOH for 3 h was selected as the most efficient chemical treatment method for reducing organic content and was further tested in the third stage.

Stage 3—Comparison of Treated and Untreated Sample Counts

The control subsamples of YK20 have a median total arcellinidan count of 347 (range = 327–369) and a total identified species of 21 (Table 3; Fig. 7). It was possible in the treated subsamples to increase the total counted arcellinidan

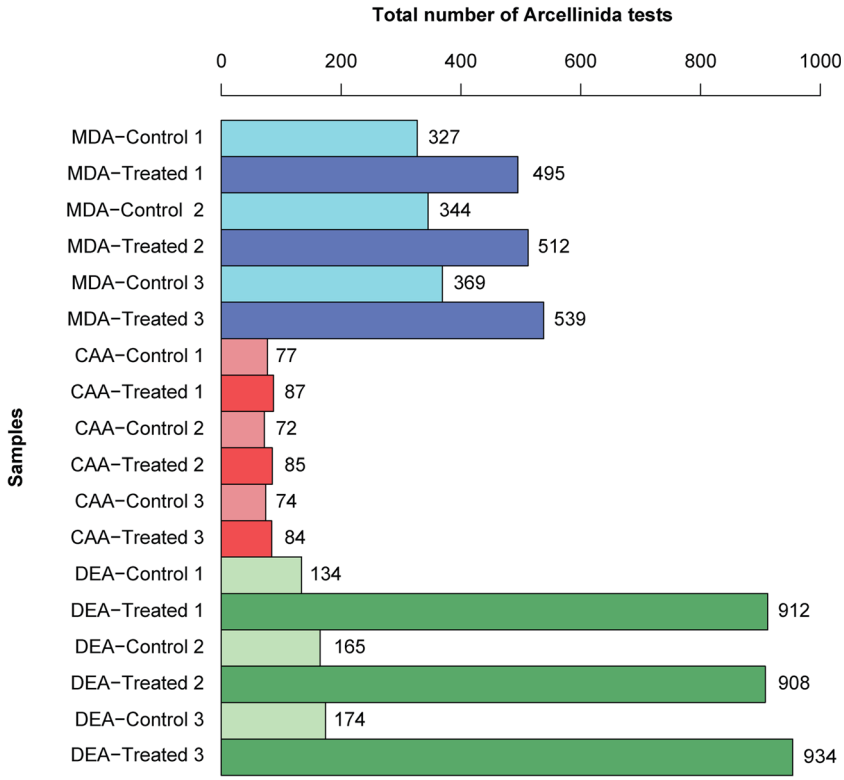

Fig. 7 A histogram plot showing the total number of Arcellinida tests (x-axis) retained in all 5% KOH treated and control subsamples (y-axis). The different colors reflect the identified assemblages and sub-assemblages. MDA—moderate diversity assemblage; CAA—*Centropyxis aculeata* assemblage; DEA—*Difflugia elegans* assemblage

tests to a median of 512 tests and a range of 495–538 (Table 3; Fig. 7). The total arcellinidan test counts in the treated and control splits of sample YK25 only differed slightly with a median of 74 and 85, and a range of 72–77, and 84–87, respectively. However, the number of identified species differed notably, with 14 identified in the control splits, and 20 in the treated splits (Table 3; Fig. 7). Subsamples of YK57 showed a significant (p value < 0.0001; $n = 6$) disparity in the total tests counted for control and treated subsamples. Control aliquots yielded a median total test count of 165 (range = 134–174), while treated aliquots yielded a significantly higher median of 908 (range = 908–912) (Table 3; Fig. 7). Surprisingly, the number of species identified in the treated splits ($n = 19$) was slightly less than that identified in the control splits ($n = 21$). This small decline in species richness in the treated aliquots likely attributes low abundance of the missing taxa, even in the control aliquots, rather than damage caused by the chemical treatment because damaged remains of the missing species were not observed in the treated aliquots.

Identified Arcellinida Assemblages

Interpretation of the Q-mode cluster analysis, supplemented with PVClust results, led to the identification of three distinct arcellinidan assemblages that corresponded well with the three analyzed surface sediment samples: (1) moderate diversity assemblage (MD; statistical significance = 99%; p value < 0.05), (2) *Difflugia elegans* assemblage (DE; statistical significance = 97%; p value < 0.05), and (3) *Centropyxis aculeata* assemblage (CA; statistical significance = 94%; p value < 0.05) (Fig. 6). Based on the PVClust and Bray-Curtis results, each assemblage was further divided into two sub-assemblages: (1) moderate diversity control sub-assemblage (MDC; statistical significance = 90%; p value < 0.05), (2) moderate diversity treated sub-assemblage (MST; statistical significance = 89%; p value < 0.05), (3) *Difflugia elegans* control sub-assemblage (DEC; statistical significance = 80%; p value < 0.05), (4) *Difflugia elegans* treated sub-assemblage (DET; statistical significance = 82%; p value < 0.05), (5) *Centropyxis aculeata* control sub-assemblage (CAC; statistical significance = 98%; p value < 0.05), and (6) *Centropyxis aculeata* treated sub-assemblage (CAT; statistical significance = 100%; p value < 0.05) (Fig. 6). A single outlier (YK25–treated 3) is observed plotting with the samples of the DEC sub-assemblage, when it should be plotting with DET.

Moderate Diversity Assemblage (MD)

The MD assemblage was found only in the YK20 aliquots ($n = 6$) (Fig. 6). The faunal composition of the assemblage is co-dominated by *Difflugia elegans* [45] (median proportion = 27.1%) and *Centropyxis aculeata* [56] strain "aculeata" (median proportion = 16.8%) (Table 3). Other taxa like *C. aculeata* strain "discoides" (median proportion = 9.9%; $n = 6$), *C. constricta* [38] strain "aerophila" (median proportion = 8.7%; $n = 6$), *D. oblonga* Ehrenberg, 1932 strain "oblonga" (median proportion = 7.7%; $n = 6$), *Conicocassis pontigulasiformis* [40] [41] (median proportion = 6.5%; $n = 6$), and *D. globulosa* Dujardin, 1837 (median proportion = 4.6%; $n = 6$) are commonly present in all aliquots (Table 3). The sediment composition of MD samples is dominated by the silt fraction (76.82%; Table 2). Faunal diversity of MD is moderate with the calculated Shannon Diversity Index (SDI) values falling in the range of 2.28–2.35 [57] (Table 1).

The MDC and MDT sub-assemblages share a relatively similar faunal structure, with *D. elegans* being the dominant taxa (median proportion = 27.5% and 23.6% in MDC and MDT, respectively). However, the sub-assemblages differed in the proportions of *D. oblonga* "oblonga: (MDC median proportions = 9.6%; MDT median proportions = 7.7%) and *C. constricta* "aerophila" (MDC median proportions = 7%; MDT median proportions = 9.6%). Additionally, the total number of species identified increased slightly in MDT ($n = 23$) compared to MDC ($n = 21$) (Table 3). A single species, *Difflugia curvicaulis* Penard, 1899, was only present in MDC, while *D. urceolata* [51] stain "urceolata", *D. urens* [4], and *D. bicornis* [45] were only present in MDT. Total arcellinidan counts in treated samples were associated with lower median standard error values with a percentage of reduction ranging between 0.54 and 50.10% ($n = 3$) (Supplementary Table 2). Only one taxon (*D. acuminata*) exhibited a notable increase in median standard error (median Sxi increase % = 16.5; $n = 3$).

Results of the BCDM showed that the faunal structure of the MD assemblage differed notably from that of the DE (median dissimilarity coefficient = 0.43) and CA assemblages (median dissimilarity coefficient = 0.59). The results also showed that while the dissimilarity coefficients for the two MD sub-assemblages are notably low (median = 0.144; $n = 36$), MDT subsamples were characterized by a slightly higher median (0.12) when compared to samples of MDC (median dissimilarity coefficient = 0.09).

Difflugia Elegans Assemblage (DE)

The DE assemblage was found exclusively in YK25 subsamples (Fig. 6). Similar to the MD assemblage, both *D. elegans* (median proportion = 42.7%; $n = 6$) and *C. aculeata* "aculeata" (median proportion = 25.5%; $n = 6$) dominated the arcellinidan population in the DE assemblage. Notable numbers of *D. protaeiformis* [49] strain "protaeiformis" (median proportion = 7%; $n = 6$) and *C. constricta* "constricta" (median proportion = 5.1%; $n = 6$) are also present in most DE samples. Samples of DE were collected from a silt-dominated substrate (71.19%; Table 2). The SDI range calculated for the assemblage (1.44–1.94) was reflective of relatively poor faunal diversity [57].

The faunal composition of the DEC and DET sub-assemblages was relatively similar, except for small but notable differences in the abundance of the dominating taxa, with DEC samples having higher numbers of *C. aculeata* "aculeata" (median proportion = 25.5%; $n = 3$) compared to DET (median proportion = 22%; $n = 3$), and DET samples having slightly higher proportions of *D. elegans* (median proportion = 47.6%; $n = 3$) in comparison to DEC (median proportion = 39.8%; $n = 3$). The number of species identified notably increased from 14 in the DEC samples to 20 in the DET samples. Additionally, *D. glans* [44] strain "distenda" were only present in the control samples, while *C. pontigulasiformis*, *D. acuminata* Ehrenberg, 1838, *D. urceolata* "urceolata", *D. urens*, and *Pontigulasia compressa* [51] were only present in the treated aliquots. Similar to the MDA, counted arcellinidan taxa were associated with relatively low median standard error values (median reduction % range = 2.24–48.3%; $n = 3$) (Supplementary Table 2). Two taxa, *C. constricta* "aerophila" (median Sxi increase % = 41.8; $n = 3$) and *C. aculeata* "discoides" (median Sxi increase % = 45.7; $n = 3$) exhibited a notable increase in median standard error.

The BCDM results showed that samples of the DE assemblage differed from that of MD (median dissimilarity coefficient = 0.43) and CA assemblages (median dissimilarity coefficient = 0.55). The median dissimilarity coefficient for the DEC and DET (0.21) was low, indicating a high level of similarity between the faunal assemblage seen in the DE sub-assemblages.

Centropyxis aculeata Assemblage (CA)

The CA assemblage was found in YK57 subsamples. Unlike the previous two assemblages, the faunal composition of the CA assemblage was dominated by *C. aculeata* "aculeata" (median proportion = 57%; $n = 6$). Species like *C. aculeata* (Eherenberg, 1838) strain "discoides" (median proportion = 14%; $n = 6$) and *D. globulosa* (median proportion = 13.1%; $n = 6$) are also present in elevated numbers. Similar to MD and DE, sample of CA samples was collected from a silty substrate (77.43%; Table 2). The SDI values for the assemblages are between 1.39 and 1.91 and are reflective of relatively poor faunal diversity of the assemblage [57].

While *C. aculeata* "aculeata" dominated the faunal structure of the CAC (median proportion = 57.3%; $n = 3$) and CAT (median proportion = 53.7%; $n = 3$). However, higher numbers of *Cucurbitella tricuspis* (Carter, 1856) (median proportion = 11.9%; $n = 3$) and *D. elegans* (median proportion = 5.2%; $n = 3$) characterized samples of CAC, while *C. aculeata* "discoides" (median proportion = 14%; $n = 3$) and *D. globulosa* (median proportion = 13.1%; $n = 3$) were more abundant in the CAT samples. A small decline in the number of identified species was observed in the CAT samples ($n = 18$) compared to the CAC samples ($n = 21$). Four arcellinidan taxa, namely *D. amphora* [44], Difflugia sp., *D. oblonga* Ehrenberg 1838 strain "spinosa", and *D. curvicaulis* are only present in the CAC samples, while *D. oblonga* "oblong" was only present in the CAT samples. Total counts of Arcellinida taxa were associated with notably reduced median standard error values in the treated aliquots (median reduction % range = 0.379–93.2%; $n = 3$) (Supplementary Table 2).

Similar to the previous assemblages, the BCDM results revealed that the faunal structure of CAC differed notably from that of MD (median dissimilarity coefficient = 0.59) and DE (median dissimilarity coefficient = 0.55). While the dissimilarity coefficients for the CAC and CAT sub-assemblages are notably low (median = 0.21; $n = 36$), CAT sub-samples are characterized by notably lower median dissimilarity coefficient (0.06) when compared to the CAC sub-samples (median = 0.29).

Discussion

Reduction of Organic Content Using Chemical Treatment

Previous lake arcellinidan and peatland testate amoebae studies have reported a reduction in the organic content in samples treated with soda ash [19], sodium hexametaphosphate [18, 20], or KOH [17, 58, 59]. Our results show that the three tested chemical treatment methods removed varying proportions of organic content depending on the type of chemical used, its concentration, and the duration of treatment. Organic-content removal was highest in subsamples treated with KOH, varying to inconsequentially small amounts in sodium hexametaphosphate and soda ash-treated subsamples (Figs. 3, 4, and 5). The substantial reduction of organic content in the KOH-treated subsamples is consistent with the findings of other testate amoebae and Arcellinida studies [17, 58, 59]. Our results also reveal an increase in organic content reduction with increase in KOH concentration. In contrast, the meager reduction of organic content in soda ash- and sodium hexametaphosphate-treated sub-samples is surprising, especially since multiple studies have reported notable reductions in organic content in sample treated with sodium hexametaphosphate (e.g., [18, 20]) and soda ash (e.g., [19]). The sodium hexametaphosphate- and soda ash treatments are less effective than KOH in deflocculating the organic colloidal particles, and in turn reducing the organic content in lake sediments.

Damage to Arcellinida Tests

While various chemical treatment methods have typically been used in the preparation of palynological and microfaunal samples, such methods have been infrequently used in

peatland testate amoebae and, to an even lesser extent, lake arcellinidan studies due to the potential for destruction of tests. Our results show that all three tested chemical treatment protocols resulted in damage to arcellinidan tests, but the degree of damage depended on the type of chemical, the concentration used, and the duration of exposure to the treatment. Test damage caused by the treatment methods was not exclusive to a certain test type (i.e., secreted vs. agglutinated), taxon, or a set of species and strains of Arcellinida.

The rapid severe damage to tests caused by sodium hexametaphosphate treatment was surprising since previous studies that have employed different concentrations of sodium hexametaphosphate (6–10%; [18, 20]) reported insignificant, or no damage to arcellinidan test even after treating samples for long periods of time (e.g., 12 h; [18]). More surprising, and concerning, is the nature of damage induced by the soda ash treatment method. The peculiar side effect of the treatment described above—more elastic tests resulting from longer chemical exposure—may only be detected through examining test robustness with a microscope probe. This form of test damage has not previously been reported (e.g., 30-min treatment; [19]) or even after several weeks of continuous soda ash treatment [60].

Treatment using KOH led to varying degrees of test damage. Hendon and Charman [17] found that treating peatland samples with 10% KOH led to the identification of more Arcellinidan tests but caused enough damage to hinder statistical analysis of arcellinidans in some samples. Conversely, Charman et al. [58] reported notably enhanced arcellinidan analysis and significantly lower test damage when samples were treated with 5% KOH instead. Our results corroborated the findings of Charman et al. [58] and showed that test damage caused by the 2% and 5% KOH concentrations was not severe enough to hamper test identification and enumeration. However, Farooqui et al. [59] reported notable test damage in samples treated with 5% KOH, even though the arcellinidan population in treated samples was found to be relatively abundant and diverse. The samples were initially treated in 5% KOH and later aceytolyzed with acetic acid. In their study, to test different testate amoebae sample preparation methods, Hendon and Charman [17] found that acetylation was the most damaging of all evaluated methods including treatment using KOH. This suggests that test loss reported by Farooqui et al. [59] may be attributed to the adverse effects of acetylation rather than treatment with KOH.

Arcellinidan Test Retention and Faunal Composition

Testing of the selected chemical treatments on the three samples demonstrates that chemical treatment is beneficial in samples with high organic content. Comparing treated and untreated sample aliquots demonstrated that treatment liberated a significant proportion of tests from organic colloidal content. These tests would otherwise be undetected when the samples were prepared with the standard methods.

The results of the third testing stage revealed that 5% KOH treatment enhanced arcellinidan test identification and enumeration by reducing significant amounts of organic debris without causing severe damage to arcellinidan tests. This improvement in analysis quality was reflected in a notable increase in the total arcellinidan tests identified in the treated subsamples, with an elevated increase in YK20 subsamples (median increase percentage = 47.5%) and a more drastic increase in YK57 subsamples (median increase percentage = 452.7%; Fig. 7, Table 3). Similar findings have been reported in studies that observed notable reductions of organic content and elevated number of tests identified in KOH-treated samples (e.g., [17, 58, 59]). The disparity between the total test counts in control and KOH-treated subsamples confirmed that a considerable number of tests may be trapped or concealed by residual organic content when the standard sample preparation method is employed. Interestingly, the total number of tests identified in the treated subsamples of YK25 increased slightly (median increase percentage = 14.8%; Fig. 7, Table 3). This small increase in test retention is expected and is attributed to the thin coverage of organic detritus in the YK25 subsamples (Figs. 3, 4, and 5). These results suggest that chemical treatment may only be necessary for samples characterized by dense organic content (e.g., YK20 and YK57).

The three identified assemblages represented the three analyzed samples and were characterized by a relatively consistent faunal structure (Fig. 6, Table 3, Supplementary Table 1). Similarly, sub-assemblages identified in this study shared a relatively consistent arcellinidan composition, differing only in the concentrations of the dominant arcellinidan taxa, and uncommon taxa (< 5% relative abundance). These results are consistent with the findings of Charman et al. [58] who observed relatively consistent faunal structure for the arcellinidan population identified in 5% KOH-treated samples. This consistency in the faunal compositions of the identified assemblages and sub-assemblages suggests that the chemical treatment is not differentially impacting the faunal structure of arcellinidan assemblage in general, or certain arcellinidan taxa in particular. Additionally, the notable reduction in the median standard error (Sxi) for most total arcellinidan taxa counts in the treated samples suggest an enhanced accuracy of the arcellinidan analysis (Supplementary Table 2).

Conclusions

We assessed the impact of three chemical deflocculation methods (soda ash ($Na_2CO_3 \cdot H_2O$), potassium hydroxide (KOH), or sodium hexametaphosphate ($(NaPO_3)_6$) on organic content reduction and destruction of arcellinidan tests in three

lacustrine sediment samples. Of the three tested chemical methods, treatment with 5% KOH was the most effective in reducing considerable proportions of organic content, which led to the identification and enumeration of much higher numbers of arcellinidan tests.

However, improvement of the total number of arcellinidan tests retained was more subtle in samples characterized by reduced organic concentrations to start with, indicating that chemical treatment is generally only necessary in samples where both TOC is elevated (e.g., TOC > 20%) and the observed organic content density is high when visually assessed under a dissecting microscope. The 5% KOH treatment caused acceptable levels of damage that was neither exclusive to certain arcellinidan taxa nor severe enough to hamper arcellinidan analysis. The arcellinidan assemblages and subassemblages identified in 5% KOH-treated and control subsamples were characterized by relatively consistent faunal compositions, indicating that chemical treatment is not severely impacting the faunal composition of the identified assemblages. However, the aggressive nature of KOH treatment, even at lower concentrations, must be taken into consideration when samples characterized by fragile or damaged tests are analyzed.

Acknowledgments Funding for this research project was provided by a NSERC Discovery Grant to RTP as well as a NSERC Strategic Project Grant, a Department of Aboriginal and Northern Affairs Cumulative Impact Monitoring Program grant, and a Polar Knowledge grant awarded to JMG and RTP. This project was also supported by the Geological Survey of Canada, Environmental Geoscience Program (JMG). Additional direct and in-kind funding was provided by the Northwest Territories Geological Survey and Natural Resources Canada Polar Continental Shelf Program. Funding was also provided by Loeblich and Tappan Student Research Award (NAN). This contribution represents NRCan Contribution Number 20190265.

References

1. Charman DJ (2001) Biostratigraphic and palaeoenvironmental applications of testate amoebae. Quat. Sci. Rev. 20(16–17):1753–1764
2. Patterson RT, Kumar A (2002) A review of current testate rhizopod (thecamoebian) research in Canada. Palaeogeogr. Palaeoclimatol. Palaeoecol. 180(1–3):225–251. https://doi.org/10.1016/S0031-0182(01)00430-8
3. Scott DB, Medioli FS (1983) Testate rhizopods in Lake Erie: modern distribution and stratigraphic implications. J. Paleontol. 57:809–820
4. Patterson RT, MacKinnon KD, Scott DB, Medioli FS (1985) Arcellaceans (Thecamoebians) in small lakes of New Brunswick and Nova Scotia: modern distribution and Holocene stratigraphic changes. J Foramin Res 15:114–137
5. Medioli FS, Scott DB (1988) Lacustrine thecamoebians (mainly arcellaceans) as potential tools for palaeolimnological interpretations. Palaeogeogr. Palaeoclimatol. Palaeoecol. 62(1–4):361–386. https://doi.org/10.1016/0031-0182(88)90062-4
6. Scott DB, Hermelin JOR (1993) A device for precision splitting of micropaleontological samples in liquid suspension. J. Paleontol. 67:151–154
7. Elmgren R (1973) Method of sampling sublittoral soft bottom meiofauna. Oikos, Supple 15:112–120
8. Payne R (2009) The standard preparation method for testate amoebae leads to selective loss of the smallest shells. QNewsl 119:16–20
9. Avel E, Pensa M (2013) Preparation of testate amoebae samples affects water table depth reconstructions in peatland palaeoecological studies. Estonian J. Earth Sci. 62:113–119
10. Wall AAJ, Gilbert D, Magny M, Mitchell EAD (2010) Testate amoeba analysis of lake sediments: impact of filter size and total count on estimates of density, species richness and assemblage structure. J. Paleolimnol. 43(4):689–704. https://doi.org/10.1007/s10933-009-9360-5
11. Mazei Y, Chernyshov V, Tsyganov AN, Payne RJ (2015) Testing the effect of refrigerated storage on testate Amoeba samples. Microb. Ecol. 70(4):861–864. https://doi.org/10.1007/s00248-015-0628-1
12. Collins ES, McCarthy FM, Medioli FS, Scott DB, Honig CA (1990) Biogeographic distribution of modern thecamoebians in a transect along the eastern north American coast. In: Hemleben C, Kaminski MA, Kuhnt W, Scott DB (eds) Paleoecology, biostratigraphy, paleoceanography and taxonomy of agglutinated foraminifera. NATOAdvanced Study Institute Series, Series C, Mathematical and Physical Sciences, vol 327, pp 783–791
13. Peter K, Bent B, Ole VO, John N (2001) Chironomid stratigraphy in the shallow and eutrophic Lake Søbygaard, Denmark: chironomid–macrophyte co-occurrence. Freshw Biol:253–267
14. Brooks SJ (2000) Late-glacial fossil midge stratigraphies (Insecta: Diptera: Chironomidae) from the Swiss Alps. Palaeogeogr. Palaeoclimatol. Palaeoecol. 159(3–4):261–279. https://doi.org/10.1016/S0031-0182(00)00089-4
15. Barker S, Greaves M, Elderfield H (2003) A study of cleaning procedures used for foraminiferal mg/ca pale- othermometry. Geochem. Geophys. Geosyst. 4(9):8407. https://doi.org/10.1029/2003GC000559
16. Feldmeijer W, Metcalfe B, Scussolini P, Arthur K (2013) The effect of chemical pretreatment of sediment upon foraminiferal-based proxies. Geochem. Geophys. Geosyst. 14(10):3996–4014. https://doi.org/10.1002/ggge.20233
17. Hendon D, Charman DJ (1997) The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. Holocene 7(2):199–205. https://doi.org/10.1177/095968369700700207
18. Ellison RL, Ogden CG (1987) A guide to the study and identification of fossil testate amoebae in quaternary Lake sediments. Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie 72(5):639–652. https://doi.org/10.1002/iroh.19870720509
19. Burbidge SM, Schröder-Adams CJ (1998) Thecamoebians in Lake Winnipeg: a tool for holocene paleolimnology. J. Paleolimnol. 19(3):309–328. https://doi.org/10.1023/A:1007942301638
20. McCarthy F, Tiffin S, Sarvis A, McAndrews J, Blasco S (2012) Early Holocene brackish closed basin conditions in Georgian bay, Ontario, Canada: microfossil (thecamoebian and pollen) evidence. J. Paleolimnol. 47(3):429–445
21. Kerr DE, Wilson P (2000) Preliminary surficial geology studies and mineral exploration considerations in the Yellowknife area, Northwest Territories. Geol Sur Can Curr. Res. C3:8. https://doi.org/10.4095/211101
22. Environment Canada (2019) National Climate Data and Information Archive, Canadian Climate Normals or Averages 1981–2010, Yellowknife A, Northwest Territories; Environment

Canada, <http://www.climate.weatheroffice.gc.ca> [accessed March 10, 2019]
23. Nasser NA, Patterson RT, Galloway JM, Roe HM, Neville LA, Macumber AL, Sanei H, Patterson RT, Palmer MJ, Spence C (2016) Lacustrine Arcellinina (testate amoebae) as bioindicators of arsenic contamination. Microb. Ecol. 72(1):130–149. https://doi.org/10.1007/s00248-016-0752-6
24. Nasser NA, Cullen J, Patterson CW, Patterson RT, Roe HM, Galloway JM (2019) Inter-annual Arcellinida (testate lobose amoebae) assemblage dynamics within lacustrine environments. Limnologica 76:60–71
25. Kumar A, Dalby PB (1998) Identification key for Holocene lacustrine arcellacean (thecamoebian) taxa. Palaeontol. Electron. 1(1):34
26. Reinhardt EG, Dalby A, Kumar A, Patterson RT (1998) Utility of arcellacean morphotypic variants as pollution indicators in mine tailing contaminated lakes near cobalt, Ontario, Canada. Micropaleontol 44:131–148. https://doi.org/10.2307/1486066
27. Roe HM, Patterson RT, Swindles GT (2010) Controls on the contemporary distribution of lake thecamoebians (testate amoebae) within the greater Toronto area and their potential as water quality indicators. J. Paleolimnol. 43:955–975
28. Patterson RT, Roe HM, Swindles GT (2012) Development of an Arcellacea (testate lobose amoebae) based transfer function for sedimentary phosphorous in lakes. Palaeogeogr Palaeocl Palaeoecol. 348-349:32–44. https://doi.org/10.1016/j.palaeo.2012.05.028
29. Patterson RT, Lamoureux EDR, Neville LA, Macumber AL (2013) Arcellaceans (testate lobose amoebae) as pH indicators in a pyrite mine acidified lake, northeastern Ontario, Canada. Microb. Ecol. 65(3):541–554. https://doi.org/10.1007/s00248-012-0108-9
30. Macumber AL, Patterson RT, Roe HM, Neville LA, Swindles GT (2014) Autoecological approaches to resolve subjective taxonomic divisions within Arcellacea. Protist. 165:305–316. https://doi.org/10.1016/j.protis.2014.03.004
31. Neville LA, Patterson RT, Gammon P, Macumber AL (2014) Relationship between ecological indicators (Arcellacea), total mercury concentrations and grain size in lakes within the Athabaska Oil Sands region, Alberta. Environ. Earth Sci. 72:577–588. https://doi.org/10.1007/s12665-013-2979-6
32. Medioli FS, Scott DB (1983) Holocene Arcellacea (Thecamoebians) from eastern Canada. Cushman Foundation For Foraminiferal Research special Publication 21:63
33. Asioli A, Medioli FS, Patterson RT (1996) Thecamoebians as a tool for reconstruction of paleoenvironments in some Italian lakes in the foothills of the southern Alps (Orta, Varese and Candia). J Foraminifer Res 26(3):248–263
34. Medioli FS, Scott DB, Abbott BH (1987) A case study of protozoan intraclonal variability; taxonomic implications. J Foraminifer Res 17(1):28–47
35. Patterson RT, Kumar A (2000) Assessment of arcellacean (thecamoebian) assemblages, species, and strains as contaminant indicators in James Lake, northeastern Ontario, Canada. J Foramin Res 30:310–320
36. Escobar J, Brenner M, Whitmore TJ, Kenney WF, Curtis JH (2008) Ecology of testate amoebae (thecamoebians) in subtropical Florida lakes. J. Paleolimnol. 40:715–731
37. Kihlman S, Kaupila T (2009) Mine water-induced gradients in sediment metals and arcellacean assemblages in a boreal freshwater bay (Petkellajti, Finland). J. Paleolimnol. 42:533–550
38. Ehrenberg CG (1843) Verbreitung und Einfluss des mikroskopischen Lebens in Su¨d-und Nord Amerika: Ko¨nigliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen 1841:291-446
39. Carter HJ (1856) Notes on the fresh water Infusoria of the island of Bombay. Ann Mag Nat Hist 18(105):221–249
40. Beyens L, Chardez D, De Bock P (1986) Some new and rare testate amoebae from the Arctic. Acta Protozool. 25:81–91
41. Nasser NA, Patterson RT (2015) *Conicocassis*, a new genus of Arcellinina (testate lobose amoebae). Palaeontol. Electron. 18:1–11. https://doi.org/10.26879/538
42. Dujardin F (1837) Sur une nouvelle esp~ce de Gromia et sur les Difflugies. Ann Sci Nat 2(8):310–313
43. Leidy J (1874) Notice of some fresh-water and terrestrial rhizopods. Proc Acad Natl Sci Phila 26:86–88
44. Penard E (1902) Faune Rhizopodique du Bassin du Lèman. Henry Kündig, Libraire de L'institut, Genéve pp 712
45. Penard E (1890) Etudes sur les rhizopodes d'eau douce. Mém Soc Phys Hist Nat Genève 31:1–230
46. Gauthier-Lièvre L, Thomas R (1958) Les genres Difflugia, Pentagonia, Maghrebia et Hoogenraadia (Rhizopodes testaces) en Afrique. Arch Protistenkd 103:241–370
47. Ehrenberg CG (1832) Über die Entwicklung und Lebensdauer der Infusionsthiere, nebst ferneren Beitra¨gen zu einer Vergleichung ihrer organischen Systeme. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen 1931, 1-154
48. Ehrenberg CG (1838) Die Infusionsthierchen als vollkommene Organismen. Leipzig, Leopold Voss. p. 547
49. Lamarck JB (1816) Histoire Naturelle des Animaux sans Vèrtèbres. Verdièie, Paris, tome 2:1–568
50. Penard ME (1899) Les Rhizopodes de faune profonde dans le lac Léman. Rev Suisse Zool 7:1–142
51. Carter HJ (1864) On freshwater Rhizopoda of England and India. Ann Mag Nat Hist ser 3(13):18–39
52. Core Team R (2014) R: a language and environment for statistical computing. Available at: http://www.r-project.org (accessed 10th, October, 2018)
53. Ward JH (1963) Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc. 58:236–244
54. Patterson RT, Fishbein E (1989) Re-examination of the statistical methods used to determine the number of point counts needed for micropaleontological quantitative research. J Paleontol 63(2):245–248
55. Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27:325–349. https://doi.org/10.2307/1942268
56. Ehrenberg CG (1832) Über die Entwicklung und Lebensdauer der Infusionsthiere, nebst ferneren Beitra¨gen zu einer Vergleichung ihrer organischen Systeme. Königliche Akademie der Wissenschaften zu Berlin Physikalische Abhandlungen 1931:1–154
57. Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton, 185 pp
58. Charman DJ, Gehrels WR, Manning C, Sharma C (2010) Reconstruction of recent sea-level change using testate amoebae. Quat. Res. 73(02):208–219. https://doi.org/10.1016/j.yqres.2009.11.009
59. Farooqui A, Kumar A, Swindles GT (2012) Thecamoebian communities as proxies of seasonality in Lake Sadatal in the ganga-Yamuna Plains of North India. Palaeontol. Electron. 15(1):1–19
60. Dalby AP (2002) Application of Multivariate Statistical and Analytical Techniques in the Examination of Lacustrine Arcellacean Data From Southwestern New Brunswick. Ph.D.Thesis, Carleton University, Ottawa, Ontario, Canada
61. International Commission on Zoological Nomenclature: International Code of Zoological Nomenclature (1999) 4th edition. The International Trust for Zoological Nomenclature, London