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Testate Amoebae as Proxy for Water Level Changes in a Brackish Tidal Marsh

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Abstract. Few studies have examined testate amoebae assemblages of estuarine tidal marshes. This study investigates the possibility of using soil testate amoebae assemblages of a brackish tidal marsh (Scheldt estuary, Belgium) as a proxy for water level changes. On the marsh surface an elevation gradient is sampled to be analyzed for testate amoebae assemblages and sediment characteristics. Further, vegetation, flooding frequency and soil conductivity have been taken into account to explain the testate amoebae species variation. The data reveal that testate amoebae are not able to establish assemblages at the brackish tidal marsh part with flooding frequencies equal to or higher than 36.5%. Further, two separate testate amoebae zones are distinguished based on cluster analysis. The lower zone's testate amoebae species composition is influenced by the flooding frequency (~ elevation) and particle size, while the species variability in the higher zone is related to the organic content of the soil and particle size. These observations suggest that the ecological meaning of elevation shifts over its range on the brackish tidal marsh. Testate amoeba assemblages in such a brackish habitat show thus a vertical zonation (RMSEP: 0.19 m) that is comparable to the vertical zonation of testate amoebae and other protists on freshwater tidal marshes and salt marshes.

Key words: Testate amoebae, elevation, sea-level change, water-level change, estuary.

Abbreviations: PSU – practical salinity unit; MHWL – mean high water level; ~ MHWL – relative to mean high water level; WA-PLS – weighted averaging-partial least squares regression; RMSEP – root mean square error of prediction; DCCA – detrended canonical correspondence analysis; SD – standard deviations; LOI – loss on ignition; RDA – redundancy analysis.

INTRODUCTION

In the last two decades more and more research is done on protists from salt marshes all over the world (Zong and Horton 1999, Sawai *et al*. 2002, Charman *et al*. 2002, Horton *et al*. 2006, Woodroffe 2009, Gabriel *et al*. 2009). The studies are mainly focusing on the potential of using soil protists (mainly diatoms, foraminifera and testate amoeba) as proxies for sea level, with the goal of reconstructing Holocene sea level changes from sediment cores in which protists are preserved. The reconstructed sea level changes can help to understand the ongoing present and future sea level changes resulting from global warming, as the reconstructions can be used to test sea level change models. These reconstructions are established by the use of a transfer function, in which the relationship between modern protist

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(assemblages) and an environmental variable (e.g. elevation) is used to infer past environmental variables from Palaeo protist assemblages preserved in sediment profiles. The accuracy of the sea level reconstruction is dependent on the protists used. The highest precision is found in a study on testate amoeba giving an accuracy that might be up to 4 cm (Gehrels *et al.* 2006). Further, compared to foraminifera, testate amoebae show only limited infaunal distribution (3 cm deep) (Roe *et al.* 2002), and can be quicker to investigate than diatoms (Gehrels *et al.* 2001).

The effects of recent sea level rise may be amplified towards the more inland part of estuaries due to human modifications of the estuarine morphology. As a consequence of land reclamation, the reduced water storage capacity of the estuary may result in considerable enlargement of the tidal range. This means that areas lower than 10 m above present Mean Sea Level are vulnerable for future inundations (McGranahan *et al.* 2007). Among these areas are large coastal cities like e.g. New York, Miami, Shanghai and Mumbai. The fact that inland water levels are highly influenced by the sea level rise, makes it interesting to reconstruct estuarine water level changes. This might help to reach a better understanding of the relation between sea level rise and estuarine tidal water level changes. For example, sea level rise at the Belgian coast was about 3 mm/yr during the past century, while mean high water level rise was up to 15 mm/yr in the inland part of the Scheldt estuary (Temmerman *et al.* 2004). Though, there is only one study that investigated the use of protists (testate amoeba) as proxy for water level changes in the inland part of an estuary (Ooms *et al.* 2011). While that study was conducted at a freshwater marsh, present study investigates the modern testate amoebae assemblages of a brackish tidal marsh, expecting different species assemblages.

Brackish tidal marshes have a unique character by their occurrence at the transition from the freshwater to marine parts of an estuary. The average salinity (5–18 PSU) of these brackish areas is intermediate between that of the freshwater and marine parts and is highly variable during the year. The major causes of salinity variations in estuaries are freshwater discharge from the upstream and tributary rivers and the mixing of fresh and sea water through wind and tidal action (Peterson 2007). These salinity variations occur over large distances within the estuary. The salinity instability, in combination with tidal currents and high sediment load, makes it more difficult for species to settle or adapt in the brackish part of an estuary in comparison to the more stable salinity conditions in the marine or freshwater zones (Little 2000). Therefore, brackish environments are typically species poorer than marine and freshwater ecosystems (Remane and Schlieper 1971). Yet the study of Więski (2010) discusses that plant diversity, primary production and nutrient recycling within brackish marshes is equal to freshwater tidal marshes and exceeds those of salt marshes. Furthermore, species densities and biomass might be bigger in brackish marshes. Species living in this environment are mostly marine or freshwater species that have adapted to this habitat, while species that only occur in the brackish zone are rare (Little 2000).

Here, we focus on the protozoan group of testate amoebae. Testate amoebae are shelled amoebae with an average size between 20–200 μm (Hendon and Charman 1997). They can be found in a variety of moist to wet environments. Until now, around 2000 species have been described (Mitchell *et al.* 2008). Testate amoebae have been studied in marine salt marshes in the UK and North America as proxies for Holocene sea level changes (Charman *et al.* 1998, Gehrels *et al.* 2001, Charman *et al.* 2002, Gehrels *et al.* 2006, Riveiros *et al.* 2007, Charman *et al.* 2010). So far, only one study is known of a freshwater tidal marsh (Scheldt estuary, Belgium) (Ooms *et al.* 2011). To our knowledge, no studies have been reported on modern testate amoebae assemblages of brackish marshes yet.

The objectives of this study are to investigate the modern testate amoebae species composition of a brackish tidal marsh, in relation to environmental variables (elevation, flooding frequency, particle size, vegetation). Further, a transfer function will be developed to assess if the relationship between testate amoebae and elevation is comparable to that of other marsh types (salt and freshwater tidal marsh).

MATERIAL AND METHODS

Study area

The Scheldt river is a rain-fed lowland river that has its source in France (St. Quentin) and streams through Belgium and The Netherlands to flow into the North Sea at Vlissingen. The tidal part of the Scheldt river is 160 km long, stretching between Ghent (Belgium) and the North Sea (Fig. 1). The Scheldt estuary has a semidiurnal meso- to macrotidal regime and is characterized by a full salinity gradient with a freshwater, brackish and marine part (Fig. 1).

The tidal marshes and mudflats of Groot Buitenschoor are located in the mesohaline, brackisch part (5–18 PSU) of the Scheldt estuary at the border of Belgium and The Netherlands (Meire *et al.* 2005). The tidal difference between mean high water and mean low water is here 4.98 m (Taveniers and Mostaert 2009).

The intertidal area of Groot Buitenschoor is 226 ha (± 2.3 km long and max. 1200 m broad). Groot Buitenschoor consists of a vegetated marsh with a mud flat in front. The marsh sediments consist of fine grained particles ranging from clay to fine sands. The vegetation zones on this marsh are, from high to low elevation, willows (*Salix* sp.), herbaceous vegetation (*Urtica dioca*, *Elytrigia atherica*), reed (*Phragmites australis)* and pioneer vegetation (*Scirpus maritimus)*. At some parts of the marsh, the marsh edge is eroding, creating a cliff with a height up to about 1 m between the high marsh vegetation and the pioneer vegetation (*Scirpus maritimus*) on the mudflat (Fig. 1).

Sampling method

Samples were taken at the Dutch part of Groot Buitenschoor along the biggest altitudinal gradient (–1.2–2.31 m ~ MHWL) (Fig. 1). The sampling campaign took place in April of 2010. The samples were collected along two altitudinal gradients, one running from the highest point, with *Salix* vegetation, to the transition between *Phragmites australis* and *Scirpus maritimus*, and the second, at a location with a smoothly sloping elevation gradient, from the transition zone between *Phragmites australis* and *Scirpus maritimus* to the lowest reach of the *Scirpus maritimus* vegetation on the mudflat. In this way, the whole elevation gradient, spanning all vegetation communities, was sampled. The sampling started at the highest point and for every elevation decrease of 5 cm, determined with a laser level, a sampling location was marked. In this way, 80 sampling locations were indicated along the elevation gradi-

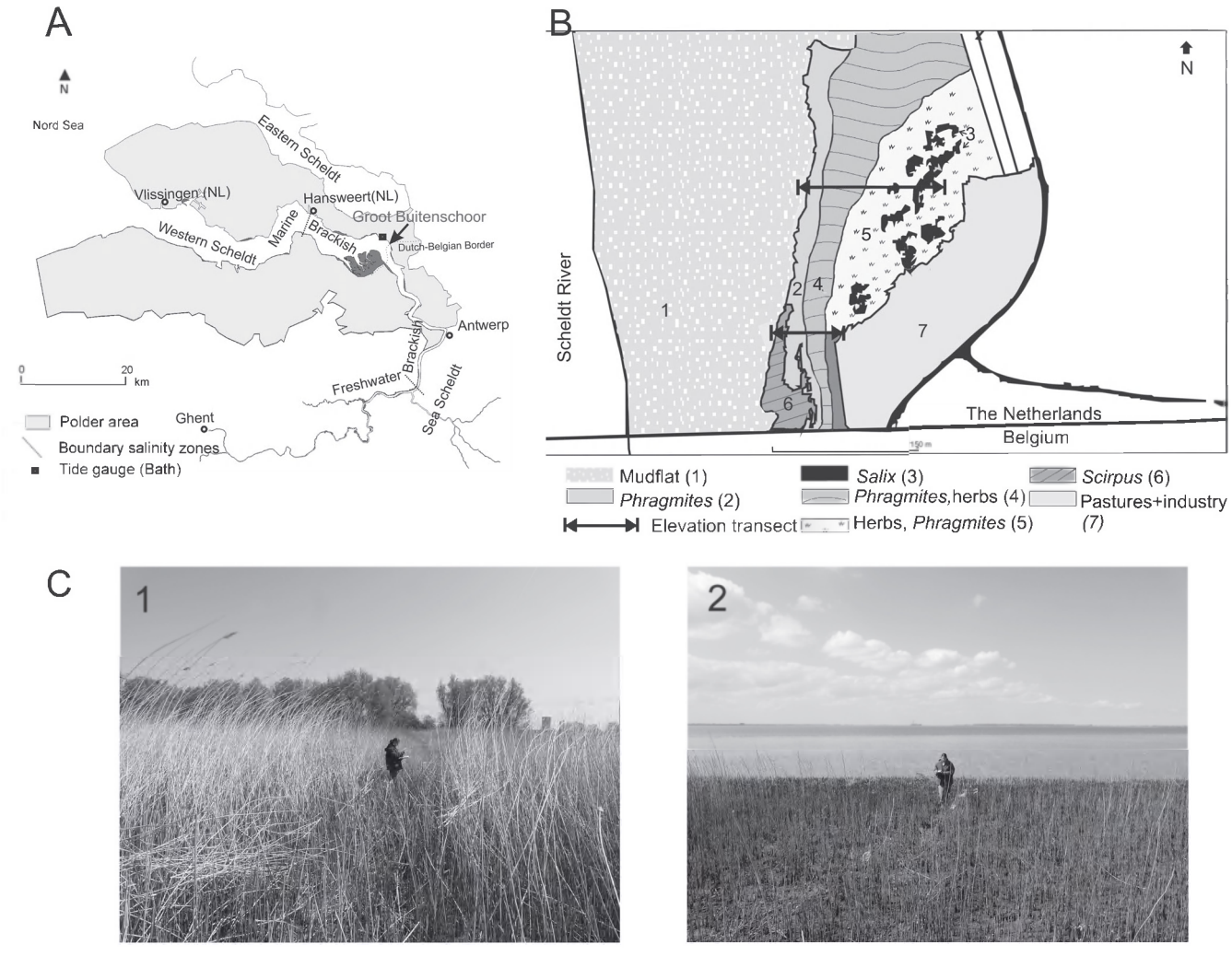

Fig. 1. A – map of the Scheldt estuary with location of Groot Buitenschoor; **B** – map of the brackish tidal marsh Groot Buitenschoor with indication of vegetation zones and the elevation transects that are sampled; **C** – photos of the two sampled transects. Photo 1 – from *Salix* to outer edge of *Phragmites australis* vegetation; Photo 2 – from *Phragmites australis* to outer edge of *Scirpus maritimus*.

ents. The accurate elevation of each sample location was measured with a Total Station Sokkia set $5X_{10}$ (vertical error of ± 2 mm). The elevation measurements were relative to a benchmark (ALTI-Hd34) from the NGI (National Geography Institute). Further, two sediment samples (testate amoebae analysis + sediment properties) were collected from each sample location.

The first sediment sample was a mixed sample for testate amoebae analysis, taken by the pooling method. For this method, a grid of 20 × 20 cm was laid down on the ground and in each corner and in the middle of the grid, a sediment sample of 2 cm deep was taken with a small corer (diameter = 0.9 cm). Pooling of the 5 cores smoothed out local variation of species assemblages. Considering a local marsh sedimentation rate of about 2 cm per year (Temmerman *et al.* 2003a, b), the soil sampling of 2 cm deep comprised the testate amoebae assemblage over about a one-year period.

The second sediment sample was taken by pushing a metal ring of fixed volume (84.5 cm^2) into the ground within the pooling grid. The sediment sample was used for analyzing sediment properties (bulk density, loss on ignition, particle size and soil conductivity).

Preparation method for testate amoebae

Testate amoebae samples were fixated with alcohol (95%) directly after field sampling.

Samples were dried in the oven at 30°C for 36 hours. Only 2.5 g of sediment was used for testate amoebae preparation. Before preparation, *Lycopodium* spores (Stockmarr 1971) were added to each sample for estimating testate amoebae concentrations. The preparation method was based on Hendon and Charman (1997), but samples were stained with Rose Bengal instead of Safranine. Analyses of testate amoebae assemblages were done on an OLYMPUS BX50 microscope with Nomarski optics. If the first slide of a sample contained at least 10 testate amoebae, the sample was further counted until 150 tests were found. a number of 150 testate amoebae gave enough precision for this study following Patterson and Fishbein (1989) (Error calculations are in appendix). Samples with less than 10 testate amoebae per slide were omitted from further analysis, because of low testate amoebae concentrations. Differentiation between death, empty, tests and living testate amoebae, tests with amoebae inside, was made during counting, but total assemblages were used in the analyses.

Measuring and calculating of environmental data

Bulk density and loss on ignition were measured using a standard analysis method (Last and Smol 2001). Fresh sediment material was oxidized with H_2O_2 before particle size analysis by the laser diffraction technique (Malvern 1000). Conductivity was measured using the standard protocol of Tucker and Beatty (1974), by mixing of 5 g of soil with 25 ml of distilled water for 60 min. and settling for 30 min. before measuring electrical conductivity. The measured elevations were transferred to elevations relative to mean high water level by subtracting the mean high water level (MHWL).

The MHWL was calculated from nearby tide gauge data of Bath from the period of the 1st of January 2010 to the 1st of November 2010. These data were downloaded from the *on-line* database "waterbase" from the Dutch Rijkswaterstaat. The dataset contained measurements of the water level in 10 min. intervals. The MHWL was determined by calculating the average of all the highest water levels for each tide. Flooding frequency was calculated by ranking the highest water levels for each tide from highest to lowest. Every highest water level was cumulatively numbered according to the number of times the flooding height was reached. The highest water level got value 1 and the lowest water level got the value corresponding to total water level values. Based on this ranking, flooding frequency was computed by dividing the number of ranking by total water level values.

The normalized elevation was calculated using the formula of Gehrels *et al.* (2001) to be able to compare the performance of our transfer function with those published for different locations:

Normalized Elevation = (Absolute Elevation – Mean Tidal Level)/(Mean High Water of Springtides – Mean Tidal Level)

Data analysis

Relative abundances of testate amoebae species were used in all analyses. Furthermore, all species that never reached 2% of relative abundance in one of the samples were deleted from the analyses. Patterson and Fishbein (1989) stated that for 150 specimens counted, only rare species of at least 2% relative abundance could be used to help distinguish environments that differ by 2% abundance. The species values were square root transformed for cluster and ordination analyses, to give more weight to less dominant species.

Cluster analysis was performed to distinguish different testate amoebae zones. The analysis was done with the dissimilarity coefficient Edwards and Cavalli-Sforza's chord distance in the Tilia program CONISS (Grimm 2004). Ordination analyses were carried out, in the program CANOCO 4.5 (ter Braak and Šmilauer 1998), to study the relationship between testate amoebae species and environmental variables. First, an unconstrained Detrended Canonical Correspondance Analysis (DCCA) was run to find out if a linear or unimodal ordination method was appropriate. A DCCA gradient length smaller than 2 Standard Deviations (gradient length = 1.448 SD) indicated the use of a linear ordination model. Therefore, direct linear gradient analysis was performed, called Redundancy Analysis (RDA). This type of analysis uses the environmental variables to explain the species data. The RDA calculates fitted values by performing a multiple regression for each species on the environmental variables (ter Braak and Šmilauer 1998). To limit the number of variables, a forward selection procedure was carried out. The selection method ranks environmental variables following their importance for determining the species data (ter Braak and Šmilauer 1998). Here, only significant ($p < 0.05$) environmental variables were used in the redundancy analysis. The partial variance was determined in Total, with all significant variables, and separately for Elevation and the subset of other significant variables using the Monte Carlo Permutation Test (999 permutations).

Transfer functions were made for elevation and normalized elevation, using multiple regression models (partial least squares, weighted averaging, tolerance weighted averaging and weighted averaging-partial least squares (WA-PLS)). More information about the regression models can be found in ter Braak (1987a, b) and ter Braak and Juggins (1993). The regression models were calculated in C2 (Juggins 2007). The outcome of the regression models was tested by the jack-knifing technique. This technique is related to the bootstrapping technique, but leaves one sample out of the dataset with every cycle. The dataset is run for multiple cycles and is every time divided in a calibration and validation set (Birks *et al.* 1990, ter

Braak and Juggins 1993). The resulted r^2 and root mean square error of prediction (RMSEP) values can be used to test the robustness of the models. The r^2 value indicated the strength of the correlation between the observed modern data and the inferred data, and RMSEP is a measure of the prediction error (Horton *et al.* 2006). After determination of the best suited regression model, analysis of outliers was performed on the full transfer function model by checking for residual values that were higher than the standard deviation of the environmental variable (Edwards *et al.* 2004).

RESULTS

Environmental variables

The environmental variables that were taken into account in this study are shown in Fig. 2. The horizontal lines showed the bio-zonation based on cluster analyses of the testate amoebae assemblages (Fig. 3). Two major bio-zones (A and B) were separated, which were each splitted into two smaller bio-zones (A1, A2, B1, B2). The A-zones were separated from the B-zones by the very low or lacking flooding frequency. The characteristic differences for dividing zones A1 and A2 are related to the high amount of organic matter of zone A2 and the low amount of clay in zone A1. Zone B is divided into the low zone B2 with highest flooding frequency and zone B1 with highest amount of sand and highest bulk density. The particle size distribution follows the changes in flooding frequency really well. For elevations with a tidal flooding frequency of more than 10%, the grain size distribution is characterized by a high silt content (60–75%), moderately high clay content (16–22%), and low sand content (< 10%), which is typical for low-energy tidal deposition in tidal marshes in the Scheldt estuary (e.g. Temmerman *et al.* 2004, Ooms *et al.* 2011). For higher elevations with less than 10% flooding frequency, the silt and clay contents gradually decrease while the sand content increases, which can be explained because the higher elevations are only rarely flooded by very shallow water depths, so that few or no tidal deposition occurs anymore and a transition towards a terrestrial soil takes place.

Testate amoebae species composition

The whole vegetated marsh elevation range was sampled to investigate the testate amoebae boundary of occurrence. Following the elevation range, 50 highest samples were investigated on testate amoebae content. Forty, mostly elevated, samples were fully counted (150 testate amoebae individuals/sample) and used for analyses. The other 10, mostly lower samples, did not bear enough testate amoebae to count the whole sample (< 10 testate amoebae/slide) and were only used for calculating testate amoebae concentrations (see appendix).

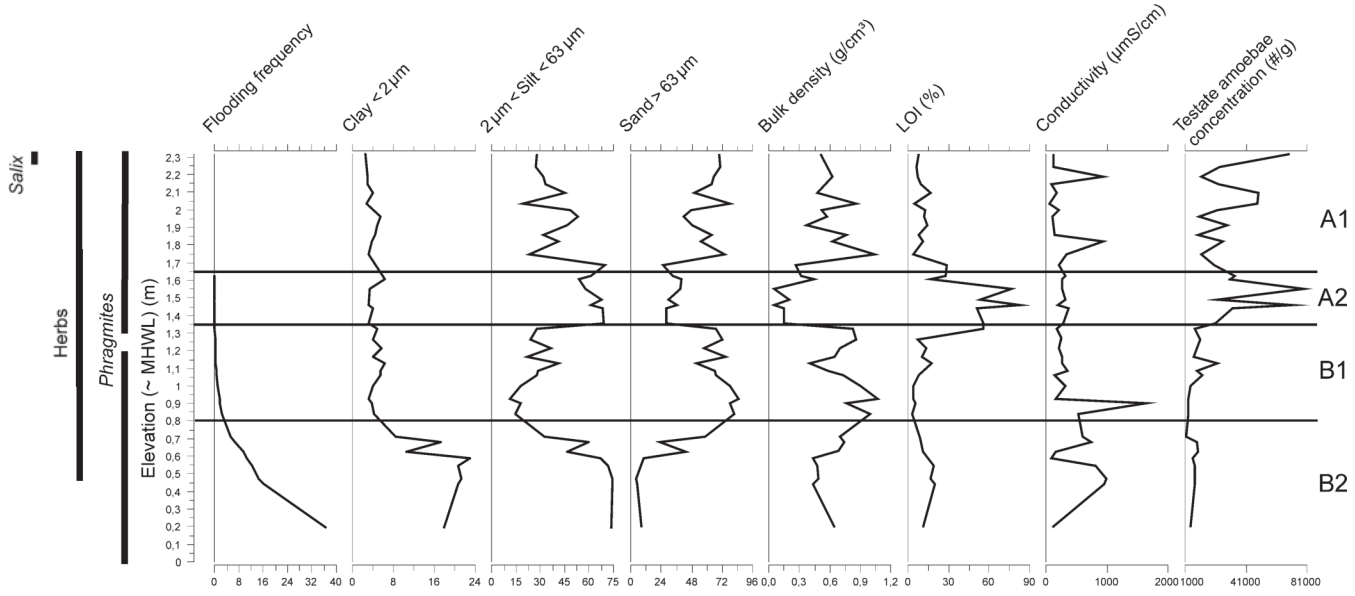

Fig. 2. Environmental variables (flooding frequency, particle sizes, organic content, bulk density, soil conductivity, vegetation) and testate amoebae concentrations over the elevation gradient (m ~ MHWL) of the brackish tidal marsh. The four indicated zones are based on cluster analyses of testate amoebae assemblages (see Fig. 3).

This lack of testate amoebae in lower samples and the knowledge of decreasing testate amoebae concentrations with decreasing elevation on the marsh (Charman *et al.* 2002), gave reason to expect insufficient testate amoebae numbers in the remaining 30 samples, which is why they were not counted. The lower boundary of testate amoebae assemblage occurrence was set at an elevation of ± 20 cm above MHWL. In total, 43 testate amoebae species were found within 40 samples. The most abundant species were *Tracheleuglypha dentata*, *Trinema enchelys* and *Difflugia globulus*. They were found along with *Trinema lineare* and *Euglypha rotunda* in all analyzed samples. *Difflugia elegans var. parva* was found in high relative abundances in the analyzed samples with insufficient testate amoebae numbers (pioneer vegetation). Most of the found species were known from freshwater biotopes, but there were also some marine interstitial species (e.g. *Cyphoderia littoralis*, *Pseudocorythion acutum*, *P. wailesi*) present (Golemansky 1971, Golemansky and Todorov 2004).

The testate amoebae species belonged to 19 taxa. The genus *Trinema* represented more than 30% of the found testate amoebae. The most abundant species, however, was *Tracheleuglypha dentata*, which accounted for more than 20% of all counted testate amoebae. The genera of *Campascus*, *Centropyxiella* and *Paulinella* were only found once. On Fig. 4, for us unknown testate amoebae species/type was found that is called for this study *Pseudohyalosphenia* sp. 1. This species had the appearance of *Hyalosphenia*, but with a broad collar. The found specimens had a length of ± 50 µm, a width of ± 40 µm and a pseudostome of ± 20 µm. The body was round, only the pseudostome was flattened.

Testate amoebae assemblages

After deleting species with low relative abundance (< 2%), 27 testate amoebae species were kept for further analyses. They are all shown in Fig. 3. The cluster analyses shown in the same figure made a division in two distinctive testate amoebae zones (A and B), which could both be subdivided in two separate zones (A1, A2, B1, B2). The two cluster zones a and B followed the environmental separation of intertidal and supratidal zone. Since these two zones represented different environmental conditions (tidal flooding or not), they were treated separately in the Redundancy Analysis.

The species variation of intertidal zone B was partly explained by the environmental variables Elevation, Flooding, Clay and Silt (47.7%; $p = 0.0010$) (Fig. 5).

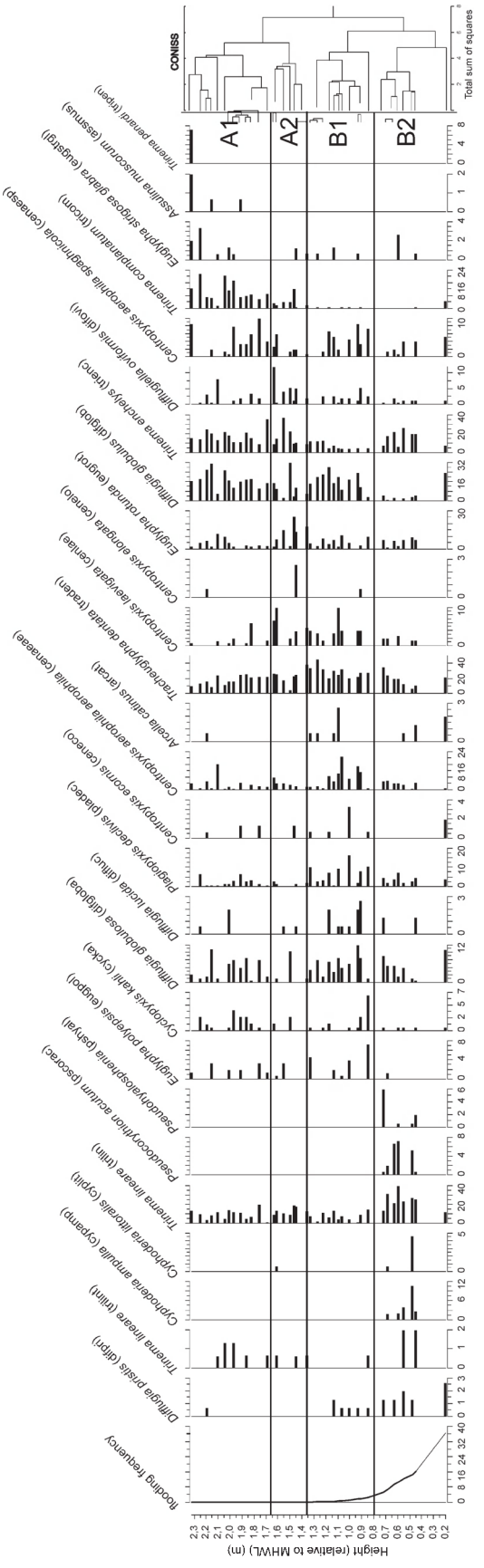

Fig. 3. Relative abundances of testate amoebae over the elevation range (m ~ MHWL) and division of testate amoebae in four different bio-zones based on cluster analysis (right side of graph). The species' names are followed by the abbreviation used in the RDA analysis.

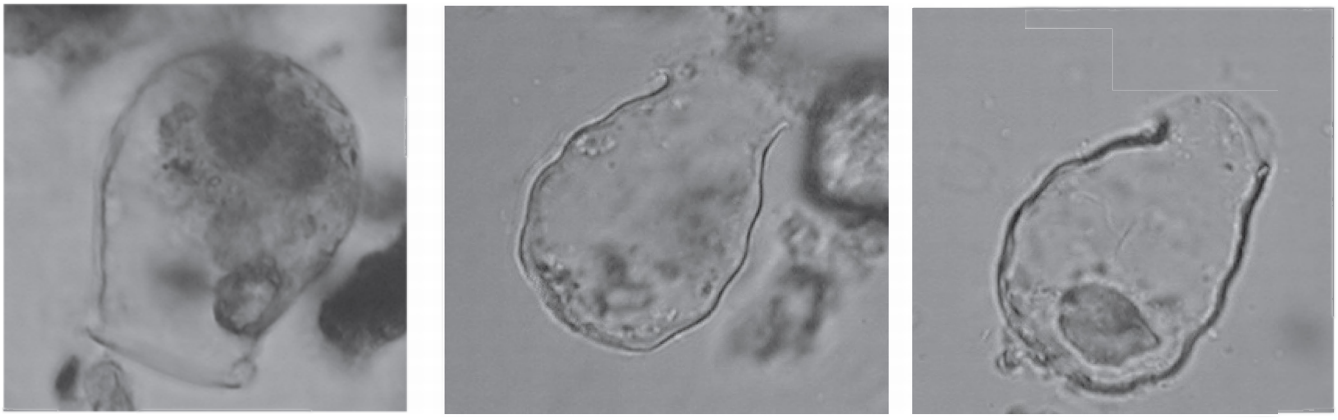

Fig. 4. Photographs of unidentified species, called here *Pseudohyalosphenia* sp. 1.

Elevation is not a real ecological measure, but rather an anthropogenic one. This means that Elevation does not explain much species variation by itself. They are the real environmental variables (vegetation, flooding, conductivity, LOI, particle size, …) that are linked to and change with elevation that explain the species variation. Partial RDA was calculated (Fig. 6) to understand the relationship between the environmental variables and Elevation. It showed that the biggest part of the species variation caused by elevation could be assigned to the relation with flooding and particle size (clay, silt). Flooding and particle size are only loosely linked to Elevation, because 30.5% of the species variation was explained by Flooding, Clay and Silt.

The RDA of the supratidal zone a was executed without the variables Flooding, Herbs, Reed and Scirpus, as they were not of interest. The result of the RDA (Fig. 5) showed that Elevation, Loss on Ignition and Clay explained a significant part of the species variation of zone a (26.1%; $p = 0.0150$). Partial RDA (Fig. 6) showed that the biggest part of species variation due to Elevation could be explained by LOI and amount of clay (6.7% in common). Apart from the interaction with Elevation, LOI and Clay explain together another 15.7% of the species variation.

The lowest zone (zone B2) (0.2–0.8 m ~ MHWL) was characterized by the presence of *Pseudohyalosphenia* sp. 1, *Pseudocorythion acutum*, *Cyphoderia ampulla*, *Cyphoderia littoralis* and highest abundances of *Difflugia pristis*, *Trinema lineare* and *Trinema lineare* var. *truncatum*. The species variation of this zone was mainly determined by the particle size (Fig. 5). However, *Difflugia pristis* seemed to be more influenced by the amount of flooding.

Zone B1 (0.8–1.35 m ~ MHWL) was represented by a higher number of species, namely *Euglypha polyepsis*, *Cyclopyxis kahli*, *Difflugia lucida*, *Plagiopyxis declivis*, *Centropyxis ecornis*, *Centropyxis aerophila* var. *aerophila*, *Arcella catinus*, *Tracheleuglypha dentata* and *Centropyxis laevigata*. Most of these species can be found in the left lower corner of the RDA (Fig. 5). These clustered species were not influenced by Flooding or Elevation, but were negatively correlated with Silt and Clay. *Tracheuglypha dentata* showed a good relation with Elevation.

The highest middle zone A2 (1.35–1.65 m ~ MHWL) had high abundances of *Centropyxis elongata*, *Euglypha rotunda* and *Difflugiella oviformis*, which were positively correlated with the amount of organic matter in the ground (LOI) (Fig. 5). *Centropyxis laevigata* was very abundant in the highest part of this zone. This was related to a small rise in clay. Zone A1 of the marsh (1.65–2.3 m ~ MHWL) was determined by the presence of *Trinema complanatum*, *Euglypha strigosa* var. *glabra*, *Assulina muscorum* and *Trinema penardi*, which were all correlated with Elevation (Fig. 5).

Transfer function

The best regression method for Elevation and Normalized elevation was WA-PLS (component 2) with both highest r^2 and lowest RMSEP (Table 1; Fig. 7). The analysis on outliers within the residual$_{jack}$ values (standard deviation environmental variable > residual$_{jack}$ value), resulted in the omission of three samples (S07,

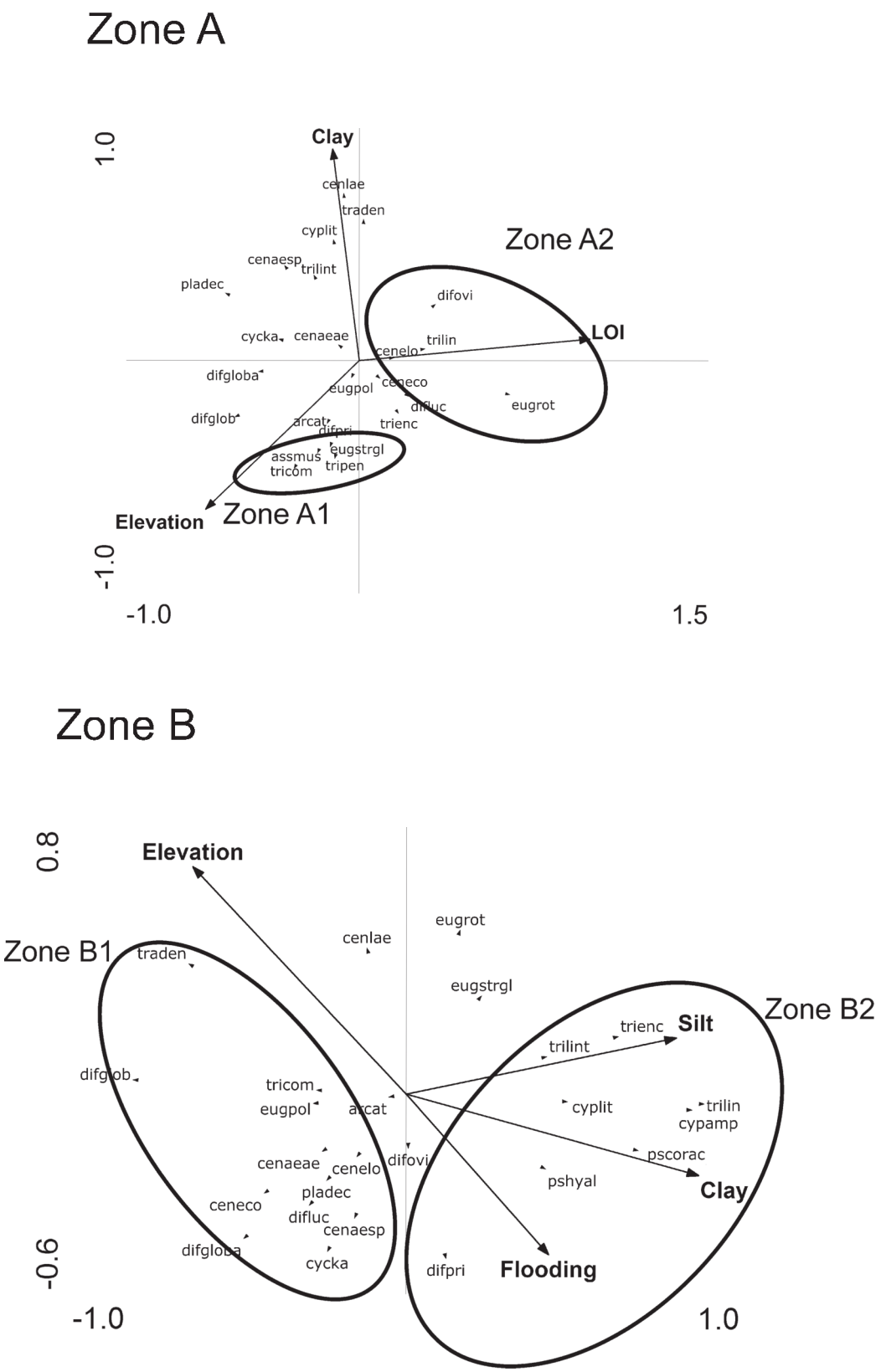

Fig. 5. Result of the RDA both on the intertidal zone and the supratidal bio-zone. Sub-zones were indicated by circles. Species abbreviations were used, full names can be found in Fig. 3.

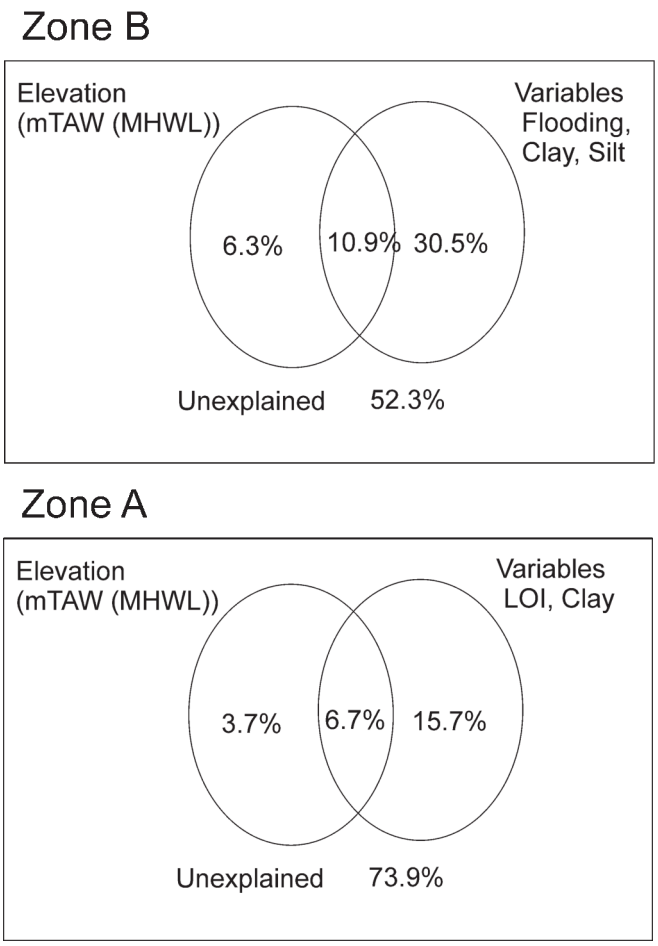

Fig. 6. Results of the partial RDA for both intertidal (zone B) and supratidal (zone A) bio-zones. The values in the intersection of the circle are the common variation explained by the two variables.

S11, S46; see appendix). These samples had deviated species composition, probably related to the high flooding frequency (S46) or (antropogenic) disturbance of the environment (S07, S11). After removing the outliers, the model was highly improved (Fig. 7). This resulted in an increase of 0.14 for the r^2 value and decrease of the prediction error (RMSEP) with ± 0.08 m (Table 1).

Based on cleaned transfer function, a partial transfer function was built using only the intertidal data (zone B) (Table 1, Fig. 7). The results showed lowest RMSEP values ($RMSEP_{Normalized\ Elevation} = 0.14$), while the r^2 value was comparable to that of the complete data set transfer function.

The partial transfer function underestimated the highest marsh levels of the intertidal zone (Fig. 7).

Table 1. Jack Knifed Results of the WA-PLS method for the environmental variables Elevation and Normalized Elevation. The table is split in three parts: the WA-PLS model with complete data set (N = number of samples), after removing outliers and the partial transfer function for the intertidal zone.

	WA-PLS (component 2)	
Complete data set (N = 40)	r^2_{jack}	$RMSEP_{jack}$
Elevation	0.66	0.23 m ~ MHWL
Normalized Elevation	0.66	0.27 m
After removing outliers (N = 37)		
Elevation	0.8	0.24 m ~ MHWL
Normalized Elevation	0.8	0.19 m
Partial Transfer function (zone B) (N = 19)		
Elevation	0.67	0.17 m ~ MHWL
Normalized Elevation	0.67	0.14 m

DISCUSSION

Two testate amoebae species boundaries have been discovered on this brackish marsh. Firstly, the boundary separating the testate amoebae assemblage zones from the testate amoebae poor zone. Secondly, the border between intertidal (B1 and B2) and supratidal testate amoebae assemblages (A1 and A2). The different zones divided by these boundaries will be discussed below, starting with the lowest, the testate amoebae poor zone.

Testate amoebae poor zone (lower than 0.2 m ~ MHWL)

This testate amoebae poor zone contains the whole pioneer vegetated *Scirpus maritimus* zone and also the lowest part of the *Phragmites australis* zone.

All the investigated samples of the *Scirpus maritimus* zone have average testate amoebae concentrations of approximately 450 tests g^{-1}. The *Phragmites australis* vegetation close to the marsh edge has slightly higher testate amoebae concentrations (average ± 970 tests g^{-1}). These very low testate amoebae numbers together with the fact that only dead testate amoebae are found, might indicate that the few found tests are possibly allochtonous. This might also imply that the environment in the pioneer vegetation is too harsh for testate amoebae to survive or settle. The tidal inunda-

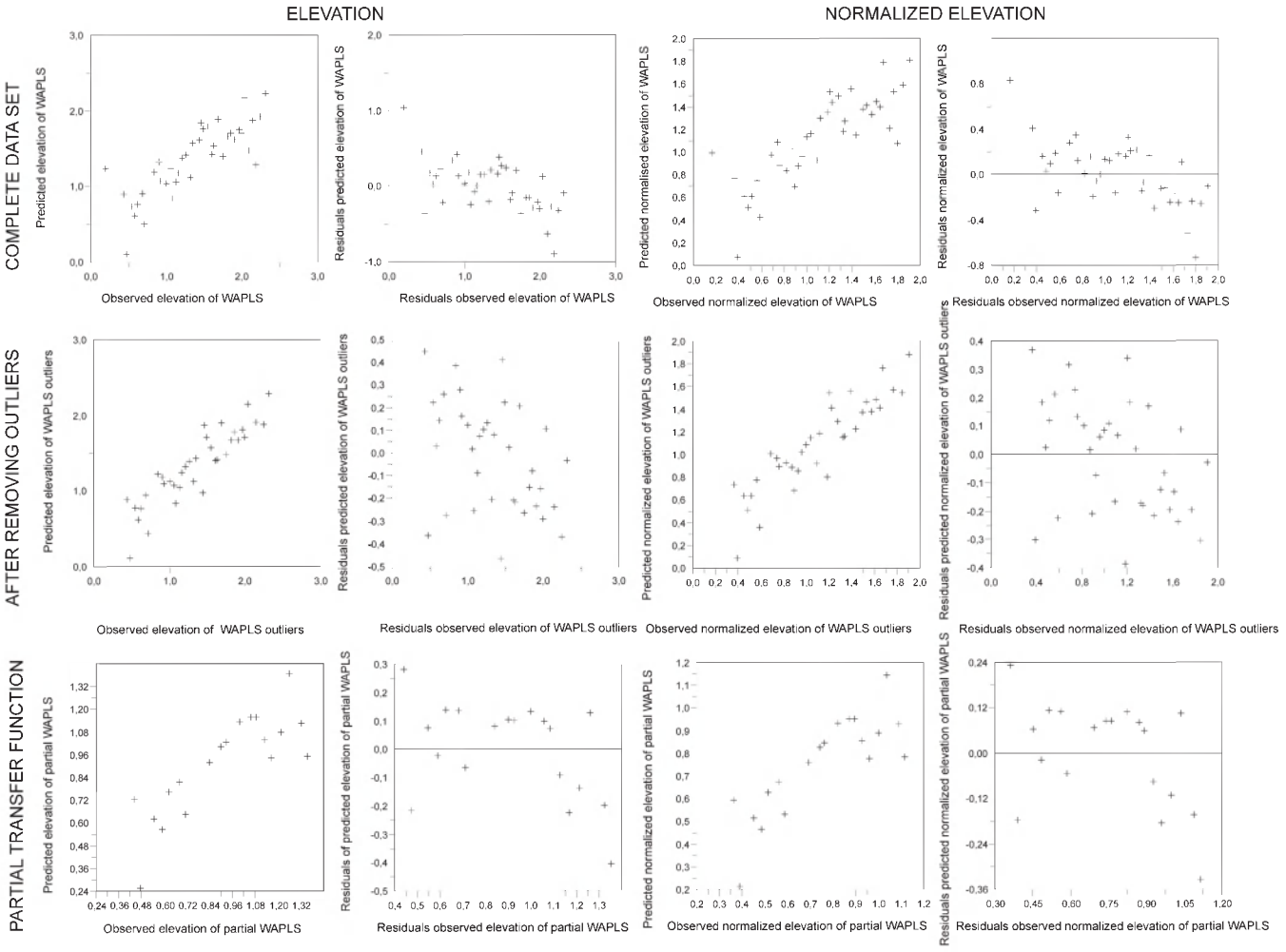

Fig. 7. Graphs of observed versus estimated Elevation and Normalized elevation, predicted by the transfer function based on Jack-knifed WA-PLS (component 2) for the complete dataset, after the removing of outliers and for the partial dataset.

tion and salinity stress are possibly preventing testate amoebae to live within this zone. The boundary between poor testate amoebae densities and the presence of testate amoebae assemblages is set at a flooding frequency of 36.5%, which corresponds with the appearance of countable testate amoebae concentrations (± 6560 tests g^{-1} (Table 2)).

The intertidal testate amoebae zone (Zone B)

This zone (0.2–1.35 m ~ MHWL) reaches from 36.5 to 1% of flooding frequency. The testate amoebae inhabitants of this zone are mainly related to the particle size of the soil and the flooding frequency (~ elevation) (Figs 5 and 6). Since the two subzones (B1 and B2) have different testate amoebae assemblages and dominant environmental variables (Figs 2 and 3), they will be discussed separately.

The lowest zone B2 (0.2–0.8 m ~ MHWL) starts with the highest flooding frequency (36.5–3.5%), at which testate amoebae could form an assemblage. The high flooding frequency with brackish water makes it possible for marine interstitial species to establish. These testate amoebae species (*Pseudocorythion spp.*, *Cyphoderia littoralis*) (Fig. 3) are almost exclusively related to this zone. Apart from the fact that their appearance is associated with high flooding frequency,

Table 2. Mean and total mean species numbers and testate amoebae concentrations over the different zones.

	Mean species number	Concentration
Zone A1	14.53846	27 867.03
Zone A2	13	41 231.93
Zone B1	14.36364	8 264.44
Zone B2	17.375	6 560.11
Total mean	14.79487	20 169.13

they seem to be more related to the interstitial space made by the particle size of the soil (Fig. 5). The interstitial space of this zone is rather small by the high concentration of clay and silt in the soil. This results in a testate amoebae assemblage of small species (e.g. *Difflugia pristis*).

Looking at Table 2 with mean species numbers for each zone, zone B2 shows the highest species number, but also bears the lowest testate amoebae concentrations. The fact that there are many species (almost all had living representatives) but low concentrations might be explained in multiple ways. A couple of the possible explanations are:

Firstly, the high species number might be explained by the tidal inundation. Every flooding gives the opportunity for allochtonous tests, carried through the river, to come ashore. The high flooding frequency in this zone facilitates the immigration. However, this would also mean that testate amoebae can be picked up in this zone and carried away with the tide. This might be the reason for low testate amoebae concentrations.

A second explanation might be that the salty environmental conditions in this zone are very stressful for the organisms. Testate amoebae should osmoregulate to maintain or restore their cell volume. These high energy costs might slow down growth and therefore extend the generation time of the species.

It is suggested in the study of Mbugua (2008) on marine Gymnamoebae that these amoebae have optimum growth at lower salinity levels because of the saving on energetic costs involved in osmoregulation. Following this hypothesis, a number of species may colonize the site, but the stress prevents them from reproducing rapidly and reaching large populations. The low concentration of testate amoebae implies low competition between species, facilitating a diverse species assemblage to establish.

Further *Cyphoderia ampulla* was only found within this brackish marsh in the *Phragmites australis* vegetated zone.

This finding is consistent with the exclusive presence of *Cyphoderia ampulla* within the reed zone of salt and freshwater marshes (Charman 2001, Ooms *et al.* 2011). Possibly, it is more related to the environmental conditions in which *Phragmites australis* occurs, than to the presence of *Phragmites australis*, as *Cyphoderia ampulla* is also found within the deeper parts of lakes (e.g. Schönborn 1962) and in moss (Todorov *et al.* 2009).

The second zone (zone B1) (0.8–1.35 m ~ MHWL) has a very low flooding frequency (between 3.5 and 1%) compared to the B1 zone. It also differs from the zone B2 by its high amount of Sand, which also results in high bulk density values. This sandy soil facilitates the occurrence of bigger species, like *Centropyxis* species (Charman *et al.* 2002), since interstitial space is bigger. Therefore, it is not surprising that this zone has the highest percentage over the four zones of *Centropyxis* (15%) and *Difflugia* (26%). Probably due to competition with bigger species, the concentrations of *Trinema* species are halved in this zone (Fig. 3). Most of the dominant species of this zone are not influenced by the flooding frequency at all (Fig. 5). For this zone, the tidal inundation (~ elevation) has no determining effect on the species assemblage.

The supratidal zone (zone A)

Flooding frequency has a negligible effect on this zone (1.35–2.30 m ~ MHWL), as there is only one occurrence known of flooding in this zone for the past five years (highest water level: 1.49 m ~ MHWL). The environmental variables that influence the species composition of the testate amoebae assemblages in this supratidal zone are the amount of organic matter in the soil (LOI), particle size (Clay) and also the Elevation.

This zone will also be separated in two sub-zones A1 and A2 for discussion.

Zone A2 has a very high level of organic matter content of the soil. The highest concentrations of testate amoebae are also found within this zone. The testate amoebae concentration curve and the LOI curve (Fig. 2) follow the same pattern in this zone, indicating that they might be linked. The testate amoebae concentration peaks when organic content is highest. Although the testate amoebae concentration is highest in this layer, the number of species (Table 2) is lowest.

This low number of testate amoebae species might be explained by (selective) predation, competition be-

tween testate amoebae and/or between the different groups of protozoa. It is also possible that the yearly accumulating plant litter creates an uncolonized new habitat. Hence it could mean that only pioneer species have had the chance to colonize this new habitat.

Following Fig. 5, the highest zone (A1) is highly influenced by the elevation on the marsh. Here, species like *Assulina muscorum* and *Trinema penardi* which like dry areas appear (Decloitre 1981, Charman *et al.* 2000).

This zone has lower species concentrations than the zone below. This is probably related to the fact that this zone might be too dry in comparison with the third zone. The species number rises slightly compared to the lower zone.

Comparison between intertidal and supratidal zone

Although there is a clear boundary between the intertidal and the supratidal zone based on the cluster analysis (Fig. 3), there are a lot of species that occur in both zones. The exceptions are the marine interstitial species for the intertidal zone and *Trinema complanatum*, *Assulina muscorum* and *Trinema penardi* for the supratidal zone. These species inhabit mainly the lowest (B2) and highest zone (A1). This might be explained by the fact that these two outer zones are more stable. Zones B2 and A1 are either regularly flooded or not flooded at all. The intermediate zones B1 and A2 undergo a more irregular or occasional flooding and suffer more from the salinity variations throughout the year, as they appear as a sudden event. Therefore, the specific species assemblage inhabiting this zone is adapted to this (extreme) environment, which might explain the lack of high species variations in these two zones.

The anthropogenic measure Elevation has a different ecological meaning for the intertidal and supratidal zones (Fig. 6). The variable Elevation has no direct ecological meaning, since it pools together a number of environmental variables. For the intertidal and supratidal zone it can clearly be pointed out that the ecological meaning of the variable Elevation can change over the elevation gradient. The Elevation of the intertidal zone of the marsh (zone B) is mainly linked with Flooding and particle size, while in the supratidal zone (zone A) the Elevation greatly covers the differences in amount of organic matter and particle size.

Transfer function

The transfer function on the full dataset show large prediction errors (RMSEP ± 0.30 m (~ MHWL)) for Elevation and Normalized Elevation compared to published transfer functions of freshwater tidal marshes and salt marshes (Table 3) and also compared to transfer functions based on other protists. After removing outliers, the regression model has a comparable r^2 and RMSEP value as to other published studies. Still, two things need to be kept in mind.

Firstly, the high accuracy of this transfer function should be treated with caution, as the sample number of the transfer function model is rather low (37 samples). However, it has been shown that transfer functions with around 40 samples can give good results (Ooms *et al.* 2011, Patterson *et al.* 2012). The partial intertidal transfer function has good results, but is in need of extra samples to become useful for actual Palaeo water level reconstructions.

Secondly, as pointed out above, the ecological meaning of Elevation for testate amoebae assemblages has changed over the elevation gradient, as the intertidal zone is linked to the flooding frequency and supratidal zone to soil organic matter content. This raises the question whether the variable Elevation is useful for the reconstruction of water level changes if samples from higher on the marsh are included, which is often the case in testate amoebae studies.

For now, we can say that the results of the transfer function give us the indication that the vertical testate amoebae assemblages of a brackish marsh have a range comparable to other protist species vertical marsh assemblages.

CONCLUSION

There are multiple environmental variables that influence the soil testate amoebae assemblages along an elevation gradient in a brackish tidal marsh. From this study we can conclude that:

1. The lowest boundary of testate amoebae assemblage establishment (6560 tests g^{-1}) is found at flooding frequencies of 36.5% (± 0.2 m ~ MHWL). Below 0.2 m ~ MHWL, within pioneer vegetated *Scirpus maritimus* and *Phragmites australis* vegetation, testate amoebae concentrations are very poor (average 450 tests g^{-1}).

2. Within the altitudinal gradient, two testate amoebae zones (A and B), an intertidal and supratidal one, could be distinguished. Both testate amoebae zone assemblages varied with particle size of the soil. Also, the intertidal testate amoebae zone was related to the flooding frequency (from 36.5 to < 1%) and the supratidal zone to the amount of organic matter in the soil.

Table 3. Table with an overview of made transfer functions on different marsh types and protists (based on the original table of Gehrels *et al.* (2001)).

	Training set	Number of samples	Normalized sampled range	Regression model	r^2	Normalized RMSEP	References
Salt marshes (UK)	testate amoebae	52	1.01–1.36	WA-TOL	0.438	0.076	Gehrels *et al.* 2001
Freshwater tidal marsh (BE)	testate amoebae	42	0.78–1.17	PLS	0.69	0.06	Ooms *et al.* 2011
Brackish tidal marsh (BE)	testate amoebae	37	0.16–1.91	WA-PLS	0.80	0.19	This study
Salt marshes (UK)	diatoms	94	0.73–1.33	WA-PLS	0.78	0.054	Gehrels *et al.* 2001
Salt marshes (UK)	diatoms	88	~ 0.00–1.40	WA-TOL	0.72	0.214	Zong and Horton 1999 (as described in Gehrels *et al.* 2001)
Salt-marshes (Alaska)	diatoms	154	~ 0.4–2.4	WA-PLS	0.75	0.22	Hamilton and Shennan 2005
Salt marshes (UK)	foraminifera	92	0.73–1.21	PLS	0.38	0.08	Gehrels *et al.* 2001
Salt marshes (UK)	foraminifera	131	~ 0.4–1.2	WA	0.67	0.116	Horton *et al.* (1999) (as described in Gehrels *et al.* 2001)
Salt marshes (Morbihan (FR)	foraminifera	43	~ 1.45–2.15	PLS	0.52	0.12	Leorri *et al.* (2010)
Salt marshes (Basque (S)	foraminifera	59	~ 1.3–2.25	WA-PLS	0.77	0.13	Leorri *et al.* (2010)
Salt marshes (Minho-Lima (P)	foraminifera	49	~ 0.5–2.0	WA-PLS	0.39	0.42	Leorri *et al.* (2010)
Salt marshes (Sado (P)	foraminifera	22	~ 1.45–2.10	PLS	0.22	0.14	Leorri *et al.* (2010)

3. Two intermediate intertidal zones (B1 and A2) are weakly related to Elevation and have a specific species assemblage that is adapted to the more extreme irregular environment of occasional flooding.

4. The antrophogenic measure Elevation does not explain much species variation by itself. The species variation that Elevation explains is more related to the ecological variables that differentiate with elevation. For this study, the Elevation was linked with flooding and particle size for the intertidal zone and was linked with organic matter and particle size for the supratidal zone, indicating that the ecological meaning of elevation changed with elevation. This emphasizes that caution is needed when using soil protists from tidal marshes as a proxy of elevation, and thus of mean sea level. Sea level studies should be based on the protist assemblages occurring in the intertidal zone of tidal marshes, which is regularly flooded by the tides, and should better not include the supratidal zone, which is only flooded during very rare extreme conditions.

5. Transfer functions for Elevation and Normalized Elevation are made. The results point out that the testate amoebae of brackish water marshes show comparable vertical zonation as to salt and freshwater tidal marshes.

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Appendix (samples that were not used in analyses are underlined and bolt)

A. Absolute counts.

Elevation (~MHWL)	2,317	2,243	2,188	2,147	2,099	2,036	2,001	1,964	1,91	1,855	1,819	1,748	1,686	1,626	1,606	1,548	1,491	1,46	1,443	1,355	1,323
Sample number	s01	s03	s07	s09	s11	s13	s15	s17	s19	s21	s22	s24	s27	s29	s28	s30	s31	s32	s33	s34	s35
Arcella catinus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Arcella arenaria	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Assulina muscorum	3	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Campascus minutus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxis aerophila aerophila	6	2	8	2	26	1	3	1	7	0	5	4	3	12	6	6	5	0	4	3	3
Centropyxis aerophila sphagnicola	13	0	0	3	0	2	1	12	5	5	9	15	6	4	9	0	2	3	3	1	0
Centropyxis elongata	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
Centropyxis ecornis	0	0	1	0	0	0	0	0	2	0	0	2	0	0	0	0	0	2	0	0	1
Centropyxis laevigata	1	0	0	0	2	0	1	3	0	1	9	0	2	10	15	0	3	0	6	7	6
Centropyxis platystoma	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxiella arenaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corythion dubium	1	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Cyclopyxis kahli	0	4	2	1	0	0	1	6	4	4	2	1	0	2	0	0	4	0	0	0	1
Cyphoderia ampulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyphoderia littoralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Difflugia elegans parva	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia globularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia globulosa	4	2	3	16	2	0	9	11	7	12	1	11	3	0	2	0	15	0	0	2	6
Difflugia globulus	25	28	40	47	10	39	26	17	24	27	28	16	23	23	15	4	48	6	17	9	22
Difflugia lucida	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Difflugia pristis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia tenuis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diffugiella oviformis	0	1	5	1	13	0	0	2	3	0	5	3	0	18	1	6	8	2	8	3	0
Euglypha dolioliformis	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Euglypha polyepsis	2	0	0	5	0	0	3	0	3	0	0	5	2	0	1	5	0	0	0	0	7
Euglypha strigosa var. *glabra*	3	5	0	0	1	0	2	1	0	0	0	0	0	0	0	0	0	0	2	1	0
Euglypha rotunda	3	8	10	3	19	15	8	3	1	5	3	5	4	3	12	23	4	39	21	27	7
Euglypha cristata	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Heleopera petricola	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Hyalosphenia minuta	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Paraquadrula irregularis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Paulinella chromatophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiopyxis declivis	0	10	1	1	1	2	2	5	10	4	5	2	1	4	2	0	0	0	2	3	15
Pseudocorythion accutum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudocorythion wailesi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudohyalosphenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tracheuglypha dentata	15	20	25	14	39	18	24	24	37	39	32	33	34	40	39	27	7	35	39	57	51
Trinema complanatum	19	33	11	10	3	31	17	27	11	12	13	9	14	5	3	6	6	18	1	3	0
Trinema enchelys	24	21	38	31	22	33	28	17	17	32	24	14	54	14	22	56	34	15	17	14	18
Trinema lineare	20	15	5	13	19	7	21	18	17	7	11	30	2	14	20	16	14	29	28	19	12
Trinema lineare var. truncatum	0	0	0	0	1	2	0	2	0	1	0	0	1	0	1	0	0	0	1	1	0
Trinema penardi	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total counts	150	150	152	150	159	150	150	151	150	150	150	150	150	150	150	150	150	150	155	150	150

1,26	1,216	1,167	1,129	1,086	1,058	1	0,926	0,901	0,84	0,763	0,712	0,681	0,626	0,588	0,547	0,473	0,441	0,388	0,345	0,312	0,252	0,217	0,217	0,197–0,046–0,326–0,736		
s36	s37	s38	sx	s39	s40	s41	s42	s43	s16	**s08**	s10	s02	s23	s06	s05	s14	s04	**s12**	**s25**	**s44**	**s45**	**s51**	**s47**	s46	**s56**	**s62**	**s70**
1	0	0	1	4	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	3	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	2	13	8	16	32	11	23	17	2	0	8	9	6	6	5	1	7	0	0	0	2	0	0	2	2	0	0
0	2	10	8	3	0	7	13	5	11	0	0	2	3	1	6	0	6	0	0	0	0	0	0	8	0	0	1
0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	1
5	2	0	5	15	6	0	6	6	0	1	3	3	0	4	0	2	2	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	1	1	0	0	0	0	1	1	0	1	2	2	2	0	0	0	0	0	0	0	0	0	0
0	1	2	0	0	0	1	1	4	10	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	6	16	4	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	10
0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	3	10	5	12	7	9	18	12	2	2	13	8	8	3	7	2	1	0	0	0	0	0	0	16	0	0	0
30	34	43	23	30	14	27	35	18	5	1	7	2	4	0	3	5	7	0	1	0	0	0	0	36	1	0	0
0	0	3	0	1	1	1	3	4	0	1	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
0	0	0	2	0	1	1	1	0	1	0	2	0	2	0	3	2	0	0	0	0	1	0	0	4	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
0	4	0	4	1	3	3	2	8	4	0	1	0	3	1	2	2	2	0	0	0	0	0	1	2	0	0	0
0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	3	0	1	6	0	0	11	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	2	0	0	0	1	0	0	1	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0
4	13	6	11	3	17	8	1	5	15	5	11	2	10	4	11	14	11	0	0	0	0	0	0	1	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	5	11	1	14	0	25	3	12	16	3	7	3	6	11	3	4	7	1	0	0	0	0	0	6	0	0	0
0	0	0	0	0	0	0	0	0	0	0	1	3	10	11	0	8	1	0	0	0	0	3	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	9	0	0	1	0	1	3	0	0	0	0	0	0	0	0	0	0
68	48	31	45	37	49	31	34	41	41	11	52	37	30	29	19	10	16	1	0	0	1	0	0	32	0	0	2
1	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	7	0	0	1
18	19	8	12	8	6	7	7	1	7	4	12	27	33	10	40	30	30	3	0	0	1	0	1	12	0	0	1
3	17	10	20	6	12	6	2	14	22	4	21	47	32	60	35	41	39	3	0	0	0	0	0	18	0	0	0
0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
150	150	150	150	150	151	151	150	150	150	33	151	151	150	150	150	150	150	8	2	1	7	4	2	152	4	0	24

B. Error calculation (Patterson and Fishbein 1989; equation 2).

Elevation (~MHWL)	2,317	2,243	2,188	2,147	2,099	2,036	2,001	1,964	1,91	1,855	1,819	1,748	1,686	1,626	1,606	1,548	1,491
Sample number	s01	s03	s07	s10	s13	s15	s17	s21	s22	s23	s24	s28	s30	s32	s31	s33	s34
Arcella catinus	0	0	0,007	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arcella arenaria	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0
Assulina muscorum	0,011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Campascus minutus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxis aerophila aerophila	0,016	0,009	0,018	0,018	0,007	0,011	0,007	0	0,015	0,016	0,013	0,016	0,016	0	0,015	0,013	0,011
Centropyxis aerophila sphagnicola	0,023	0	0	0	0,009	0,007	0,022	0,015	0,019	0,011	0,024	0,019	0	0,011	0,009	0,011	0,007
Centropyxis elongata	0	0	0,007	0	0	0	0	0	0	0	0	0	0	0	0	0,013	0
Centropyxis ecornis	0	0	0,007	0	0	0	0	0	0	0	0,009	0	0	0,009	0	0	0
Centropyxis laevigata	0,007	0	0	0,011	0	0,007	0,011	0,007	0,019	0	0	0,024	0	0	0,011	0,015	0,017
Centropyxis platystoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Centropyxiella arenaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corythion dubium	0,007	0	0	0,007	0	0,007	0	0	0,007	0	0	0	0	0	0	0,006	0
Cyclopyxis kahli	0	0,013	0,009	0,007	0	0,007	0,016	0,013	0,009	0	0,007	0	0	0	0,013	0	0
Cyphoderia ampulla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyphoderia littoralis	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0
Difflugia globularis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia globulosa	0,013	0,009	0,011	0,023	0	0,019	0,021	0,022	0,007	0,018	0,021	0,009	0	0	0,024	0	0,009
Difflugia globulus	0,03	0,032	0,036	0,017	0,036	0,031	0,026	0,031	0,032	0,013	0,025	0,024	0,013	0,016	0,038	0,025	0,019
Difflugia lucida	0	0,007	0	0,009	0	0,011	0	0	0	0	0	0	0,007	0	0	0,006	0
Difflugia pristis	0	0	0,007	0,009	0	0	0	0	0	0,009	0	0	0	0	0	0	0
Difflugia tenuis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugia sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Difflugiella oviformis	0	0,007	0,014	0,007	0	0	0,009	0	0,015	0,011	0,011	0,007	0,016	0,009	0,018	0,018	0,011
Euglypha dolioliformis	0	0	0	0	0	0	0,007	0	0	0,009	0	0	0	0,007	0	0	0
Euglypha polyepsis	0,009	0	0	0	0	0,011	0	0	0	0	0,015	0,007	0,015	0	0	0	0
Euglypha strigosa var. *glabra*	0,011	0,015	0	0	0	0,009	0,007	0	0	0	0	0	0	0	0	0,009	0,007
Euglypha rotunda	0,011	0,018	0,02	0,021	0,024	0,018	0,011	0,015	0,011	0,02	0,015	0,022	0,029	0,036	0,013	0,027	0,031
Euglypha cristata	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0	0	0
Heleopera petricola	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0	0
Hyalosphenia minuta	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0
Paraquadrula irregularis	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0
Paulinella chromatophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plagiopyxis declivis	0	0,02	0,007	0,017	0,009	0,009	0,015	0,013	0,015	0,016	0,009	0,009	0	0	0	0,009	0,011
Pseudocorythion accutum	0	0	0	0,007	0	0	0	0	0	0,02	0	0	0	0	0	0	0
Pseudocorythion wailesi	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0
Pseudohyalosphenia	0	0	0	0,019	0	0	0	0	0	0	0	0	0	0	0	0	0
Tracheuglypha dentata	0,024	0,028	0,03	0,039	0,027	0,03	0,03	0,036	0,033	0,033	0,034	0,036	0,031	0,035	0,017	0,035	0,04
Trinema complanatum	0,027	0,034	0,021	0	0,033	0,026	0,031	0,022	0,023	0	0,019	0,011	0,016	0,027	0,016	0,006	0,011
Trinema enchelys	0,03	0,028	0,035	0,022	0,034	0,032	0,026	0,033	0,03	0,034	0,024	0,029	0,039	0,024	0,034	0,025	0,024
Trinema lineare	0,028	0,024	0,014	0,028	0,017	0,028	0,026	0,017	0,021	0,033	0,033	0,028	0,025	0,032	0,024	0,031	0,027
Trinema lineare var. *truncatum*	0	0	0	0	0,009	0	0,009	0,007	0	0	0	0,007	0	0	0	0,006	0,007
Trinema penardi	0,021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

1,46	1,443	1,355	1,323	1,26	1,216	1,167	1,086	1,058	1	0,926	0,84	0,763	0,712	0,681	0,626	0,588	0,547	0,473	0,441	0,388	0,345
s35	s36	s37	s38	s39	s40	s41	s42	s43	s46	sx	s19	s09	s11	s02	s27	s06	s05	s16	s04	s14	s29
0,007	0,007	0	0	0,013	0	0	0	0	0,011	0,007	0	0	0	0	0	0	0,007	0	0,009	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0
0	0	0	0	0	0	0	0	0	0	0	0,007	0,007	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0
0,011	0,013	0,009	0,023	0,025	0,033	0,021	0,029	0,026	0,009	0,018	0,017	0,009	0,029	0,019	0,011	0,016	0,015	0,009	0,017	0,007	0,022
0	0	0,009	0,02	0,011	0	0,017	0,023	0,015	0,018	0,018	0,015	0,011	0	0,009	0,016	0,007	0,016	0,021	0,016	0	0,013
0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0	0	0	0	0	0
0,007	0	0	0,007	0	0	0,015	0	0	0,011	0	0,009	0	0	0	0	0	0	0,007	0	0	0
0,016	0,015	0,009	0	0,024	0,016	0	0,016	0,016	0	0,015	0	0	0,009	0,011	0,009	0,013	0	0	0,009	0,009	0,02
0	0	0	0	0	0	0,007	0	0	0	0	0,007	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0
0	0	0	0,007	0	0,007	0,007	0	0	0	0	0	0,007	0,006	0,007	0	0,007	0,009	0	0,009	0,009	0
0,007	0	0,007	0,009	0	0	0,007	0,007	0,013	0,007	0	0,013	0,007	0	0	0	0,007	0,007	0,02	0,007	0	0,009
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,011	0	0,011	0,016	0	0,013	0,025	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0,017	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0
0,016	0,021	0,011	0,02	0,022	0,017	0,019	0,027	0,022	0,025	0,015	0,017	0,025	0,009	0,018	0,011	0,011	0,017	0,009	0,007	0,009	0
0,029	0,033	0,034	0,037	0,033	0,024	0,031	0,035	0,027	0,034	0,029	0,03	0,038	0,019	0,009	0,029	0	0,011	0,015	0,017	0,015	0,029
0	0	0	0,011	0,007	0,007	0,007	0,011	0,013	0	0	0	0	0	0	0	0	0	0	0,009	0	0
0	0	0	0	0	0,007	0,007	0,007	0	0,013	0,009	0	0	0	0	0	0	0,011	0,007	0	0,009	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0
0	0	0,013	0	0,007	0,011	0,011	0,009	0,018	0,009	0,013	0,011	0,007	0,022	0	0	0,007	0,009	0,013	0,009	0,009	0,027
0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0	0	0	0,007	0	0
0,017	0	0	0	0	0,007	0,016	0	0	0	0,011	0,011	0,015	0	0,009	0,009	0	0	0,021	0	0	0
0	0,007	0	0	0	0	0	0,007	0	0	0,009	0	0	0,006	0	0	0,013	0	0	0,007	0	0
0,017	0,013	0,023	0,016	0,011	0,026	0,018	0,007	0,015	0,007	0,021	0,007	0,011	0,026	0,009	0,013	0,013	0,021	0,024	0,021	0,024	0,011
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0,007	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0
0	0	0	0	0	0	0	0	0	0,007	0	0	0	0	0	0	0	0	0	0	0	0,007
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0	0
0,024	0,013	0,015	0,021	0,024	0	0,03	0,011	0,022	0,016	0,007	0,02	0,007	0,006	0,011	0,007	0,021	0,011	0,025	0,017	0,013	0,013
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,011	0	0,021	0	0	0,007	0,018	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,007	0	0	0,011	0,007	0
0,039	0,041	0,038	0,033	0,035	0,038	0,033	0,034	0,036	0,033	0,037	0,035	0,024	0,034	0,035	0,034	0,032	0,027	0,036	0,025	0,02	0,036
0	0,007	0	0,007	0	0,007	0,007	0	0,007	0,017	0	0,021	0,02	0,011	0	0,024	0	0	0	0,007	0	0,015
0,027	0,027	0,027	0,018	0,018	0,016	0,017	0,017	0,007	0,022	0,022	0,026	0,033	0,027	0,031	0,039	0,02	0,036	0,017	0,033	0,033	0,024
0,022	0,011	0,026	0,02	0,016	0,022	0,016	0,009	0,024	0,026	0,028	0,026	0,023	0,026	0,038	0,009	0,04	0,035	0,029	0,036	0,036	0,024
0	0	0	0	0	0	0	0	0	0	0	0	0	0,006	0	0,007	0	0,011	0,007	0,011	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0